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by life technologies™

pEF5/FRT/V5-DEST Gateway™ Vector

A destination vector for cloning and expression in mammalian cells using the Flp-In™ System

Catalog no. V6020-20

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Manual part no. 25-0477

MAN0000266

User Manual

Contents

Important Information

Shipping and Storage

pEF5/FRT/V5-DEST and pEF5/FRT/V5/GW-CAT 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 pEF5/FRT/V5-DEST Gateway™ Vector components are listed below.

Item	Concentration	Amount
pEF5/FRT/V5-DEST Vector	lyophilized in TE, pH 8.0	6 µg
pEF5/FRT/V5/GW-CAT Control Plasmid	lyophilized in TE, pH 8.0	10 µg

Intended Use

For research use only. Not intended for human or animal diagnostic or therapeutic uses.

Accessory Products

Additional Products

Additional products that may be used with pEF5/FRT/V5-DEST are available from Invitrogen. Ordering information is provided below.

Product	Amount	Catalog no.
Hygromycin	1 g	R220-05
Zeocin™	1 g	R250-01
	5 g	R250-05
Gateway™ LR Clonase™ Enzyme Mix	20 reactions	11791-019
pFRT/*lac*Zeo	20 µg, lyophilized in TE	V6015-20
pFRT/*lac*Zeo2	20 µg, lyophilized in TE	V6022-20
pOG44	20 µg, lyophilized in TE	V6005-20
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
CAT Antiserum*	50 µL	R902-25

*The amount supplied is sufficient to perform 25 Western blots using 10 mL working solution per reaction.

Flp-In™ Expression Vectors

Additional Flp-In™ expression vectors are available from Invitrogen. For more information about each vector, refer to our Web site (www.invitrogen.com) or contact Technical Support (page 16).

Product	Amount	Catalog no.
pEF5/FRT/V5 Directional TOPO® Expression Kit	1 kit	K6035-01
pcDNA5/FRT™	20 µg, lyophilized in TE	V6010-20
pcDNA5/FRT/V5-His™ TOPO® TA Expression Kit	1 kit	K6020-01
pSecTag/FRT/V5-His TOPO® TA Expression Kit	1 kit	K6025-01

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Accessory Products, continued

Flp-In™ Host Cell Lines

For your convenience, Invitrogen has available several mammalian Flp-In™ host cell lines that stably express the *lacZ-Zeocin*™ fusion gene from pFRT/*lac*Zeo or pFRT/*lac*Zeo2. Each cell line contains a single integrated FRT site as confirmed by Southern blot analysis. The cell lines should be maintained in medium containing Zeocin™. For more information, refer to our Web site (www.invitrogen.com) or contact Technical Support (page 16).

Cell Line	Amount	Catalog no.
Flp-In™-293	3 x 10^6 cells, frozen	R750-07
Flp-In™-CV-1	3 x 10^6 cells, frozen	R752-07
Flp-In™-CHO	3 x 10^6 cells, frozen	R758-07
Flp-In™-BHK	3 x 10^6 cells, frozen	R760-07
Flp-In™-3T3	3 x 10^6 cells, frozen	R761-07
Flp-In™-Jurkat	3 x 10^6 cells, frozen	R762-07

Detection of Recombinant Proteins

Expression of your recombinant fusion protein can be detected using Anti-V5 antibodies available from Invitrogen. Horseradish peroxidase (HRP) or alkaline phosphatase (AP)-conjugated antibodies allow one-step detection using colorimetric or chemiluminescent detection methods. Fluorescein isothiocyanate (FITC)-conjugated antibodies allow one-step detection in immunofluorescence experiments.

The amount of antibody supplied is sufficient for 25 Western blots or 25 immunostaining reactions (FITC-conjugated antibody only).

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-V5-FITC Antibody		R963-25

Methods

Overview

Description

pEF5/FRT/V5-DEST is a 7.5 kb vector designed to allow high-level, constitutive expression of the gene of interest in a variety of mammalian hosts using the Flp-In™ System. Once pEF5/FRT/V5-DEST is recombined with an appropriate entry clone, the resulting expression clone may also be used for transient expression of your gene of interest. For more information on the Gateway™ Technology and the Flp-In™ System, see the next page.

Features

pEF5/FRT/V5-DEST contains the following elements:

- Human EF-1α promoter for high-level expression across a wide range of mammalian cells (see page 12 for a diagram)
- 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
- *ccd*B gene located between the two *att*R sites for negative selection
- V5 epitope tag for detection
- Bovine growth hormone (BGH) polyadenylation sequence for proper termination and processing of the recombinant transcript
- FLP Recombination Target (FRT) site for Flp recombinase-mediated integration of the vector into the Flp-In™ host cell line (see pages 3-4 for more information)
- Hygromycin resistance gene for selection of stable cell lines (see important note on page 4)
- 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 pEF5/FRT/V5-DEST, see page 13. For more information on the EF-1α promoter, see page 12.

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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™ 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.* pEF5/FRT/V5-DEST).
3. Transfect your expression clone into the cell line of choice for transient expression of your gene of interest. If you wish to constitutively express your gene of interest using the Flp-In™ System, see below.

For more information on the Gateway™ Technology, refer to the Gateway™ Technology Manual. This manual is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Support (page 16).

The Flp-In™ System

The Flp-In™ System allows integration and expression of your gene of interest in mammalian cells at a specific genomic location. To use the Flp-In™ System:

1. Introduce a Flp Recombination Target (FRT) site into the genome of the mammalian cell line of choice.
2. Cotransfect an expression vector containing your gene of interest (*e.g.* pEF5/FRT/V5-DEST expression clone) and a vector expressing Flp recombinase (pOG44) into the Flp-In™ host cell line. The Flp recombinase facilitates integration of the vector containing your gene of interest into the genome via Flp recombinase-mediated DNA recombination at the FRT site (O'Gorman *et al.*, 1991).

For more information about the Flp-In™ System, the pOG44 plasmid, and generation of the Flp-In™ host cell line, refer to the Flp-In™ System manual. This manual is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Support (page 16).

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Overview, continued

Flp Recombinase-Mediated DNA Recombination

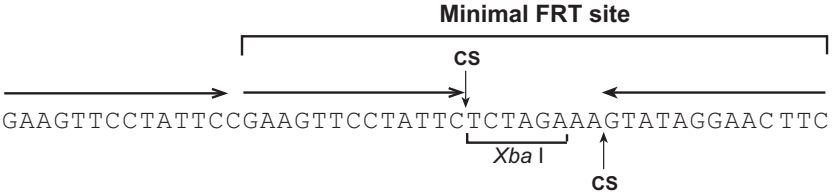
In the Flp-In™ System, integration of your pEF5/FRT/V5-DEST expression construct into the genome occurs via Flp recombinase-mediated intermolecular DNA recombination. The hallmarks of Flp-mediated recombination are listed below.

- Recombination occurs between specific FRT sites (see below) on the interacting DNA molecules
- Recombination is conservative and requires no DNA synthesis; the FRT sites are preserved following recombination and there is minimal opportunity for introduction of mutations at the recombination site
- Strand exchange requires only the small 34 bp minimal FRT site (see below)

For more information about the Flp recombinase and conservative site-specific recombination, refer to published reviews (Craig, 1988; Sauer, 1994).

FRT Site

The FRT site, originally isolated from *Saccharomyces cerevisiae*, serves as a binding site for Flp recombinase and has been well-characterized (Gronostajski and Sadowski, 1985; Jayaram, 1985; Sauer, 1994; Senecoff *et al.*, 1985). The minimal FRT site consists of a 34 bp sequence containing two 13 bp imperfect inverted repeats separated by an 8 bp spacer that includes an *Xba* I restriction site (see figure below). An additional 13 bp repeat is found in most FRT sites, but is not required for cleavage (Andrews *et al.*, 1985). While Flp recombinase binds to all three of the 13 bp repeats, strand cleavage actually occurs at the boundaries of the 8 bp spacer region (see figure below) (Andrews *et al.*, 1985; Senecoff *et al.*, 1985).



CS = cleavage site

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Overview, continued

FRT Site in pEF5/FRT/V5-DEST

The pEF5/FRT/V5-DEST vector contains a single FRT site immediately upstream of the hygromycin resistance gene for Flp recombinase-mediated integration and selection of the pEF5/FRT/V5-DEST construct following cotransfection of the vector (with pOG44) into a Flp-In™ mammalian host cell line. The FRT site serves as both the recognition and cleavage site for the Flp recombinase and allows recombination to occur immediately adjacent to the hygromycin resistance gene. The Flp recombinase is expressed from the pOG44 plasmid. For more information about pOG44, refer to the pOG44 manual or the Flp-In™ System manual.

Important

The hygromycin resistance gene in pEF5/FRT/V5-DEST lacks a promoter and an ATG initiation codon; therefore, transfection of the pEF5/FRT/V5-DEST plasmid alone into mammalian host cells will not confer hygromycin resistance to the cells. The SV40 promoter and ATG initiation codon required for expression of the hygromycin resistance gene are integrated into the genome (in the Flp-In™ host cell line) and are only brought into the correct proximity and frame with the hygromycin resistance gene through Flp recombinase-mediated integration of pEF5/FRT/V5-DEST at the FRT site. For more information about the generation of the Flp-In™ host cell line and for details on the Flp-In™ System, refer to the Flp-In™ System manual.

Note

You may transfect the expression clone (the resulting vector from the Gateway™ LR recombination reaction between an entry clone and pEF5/FRT/V5-DEST) into the mammalian cell line of choice for transient expression of your gene of interest.

Using pEF5/FRT/V5-DEST

Important

The pEF5/FRT/V5-DEST 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 pEF5/FRT/V5-DEST

If you wish to propagate and maintain pEF5/FRT/V5-DEST, 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.

Entry Clone

To recombine your gene of interest into pEF5/FRT/V5-DEST, 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 Support (page 16).

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 pEF5/FRT/V5-DEST, continued

Points to Consider Before Recombining

Your insert should contain a Kozak translation initiation sequence with an ATG initiation codon for proper initiation of translation (Kozak, 1987; Kozak, 1991; Kozak, 1990). An example of a Kozak consensus sequence is provided below. 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)NN<u>ATG</u>**G**

If you wish to include the V5 epitope 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 V5 epitope after recombination. Refer to the **Recombination Region** on the next page.

If you **DO NOT** wish to include the V5 epitope tag, be sure that your gene contains a stop codon in the entry clone.

Resuspending pEF5/FRT/V5-DEST

Before you perform the LR Clonase™ reaction, resuspend pEF5/FRT/V5-DEST to 50-150 ng/µL in sterile water.

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 a *rec*A *end*A *E. coli* strain (*e.g.* TOP10 or DH5α), and select for the expression clone.

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Using pEF5/FRT/V5-DEST, continued

Confirming the Expression Clone

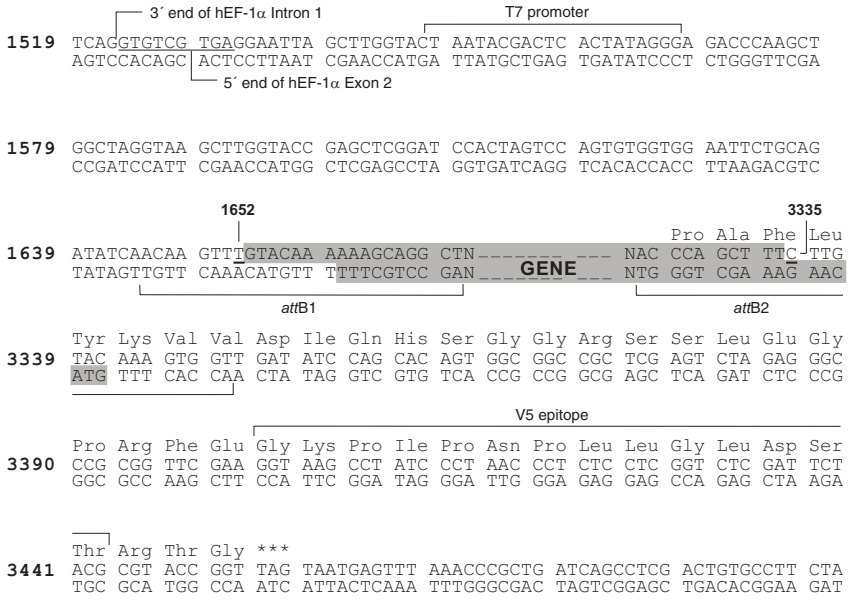
The *ccd*B gene mutates at a very low frequency, resulting in a very low number of false positives. True expression clones will be ampicillin-resistant and chloramphenicol-sensitive. Transformants containing a plasmid with a mutated *ccd*B gene will be both ampicillin- and chloramphenicol-resistant. 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 pEF5/FRT/V5-DEST × entry clone is shown below.

Features of the Recombination Region:

- Shaded regions correspond to DNA sequences transferred from the entry clone into pEF5/FRT/V5-DEST by recombination. Non-shaded regions are derived from the pEF5/FRT/V5-DEST vector.
- The underlined nucleotides flanking the shaded region correspond to bases 1652 and 3335, respectively, of the pEF5/FRT/V5-DEST vector sequence.



Generating Stable Flp-In™ Expression Cell Lines

Introduction

This section provides general information for cotransfecting your expression clone and pOG44 plasmids into your mammalian Flp-In™ host cell line to generate your stable Flp-In™ expression cell line. We recommend that you include the pEF5/FRT/V5/GW-CAT positive control vector and a mock transfection (negative control) to evaluate your results. Specific guidelines and protocols as well as detailed information about pOG44 and generation of the Flp-In™ host cell line can be found in the Flp-In™ System manual.

Note: If you wish to assay for transient expression of your gene of interest, you may transfect the expression clone directly into the mammalian cell line of choice.

Flp-In™ host cell lines which stably express the *lacZ-Zeocin*™ fusion gene from pFRT/*lac*Zeo or pFRT/*lac*Zeo2 and which contain a single integrated FRT site are available from Invitrogen (see page **Error! Bookmark not defined.** for ordering information). For more information on these cell lines, refer to our Web site (www.invitrogen.com) or contact Technical Support (page 16).

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.

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Generating Stable Flp-In™ Expression Cell Lines, continued

Positive Control

pEF5/FRT/V5/GW-CAT is provided as a positive control vector for mammalian cell transfection and expression (see page 15 for a map) and may be used to assay for recombinant protein expression levels in your Flp-In™ host cell line. Cotransfection of the positive control vector and pOG44 into your Flp-In™ host cell line allows you to generate a stable cell line expressing chloramphenicol acetyl transferase (CAT) at the same genomic locus as your gene of interest. If you have several different Flp-In™ host cell lines, you may use the pEF5/FRT/V5/GW-CAT control vector to compare protein expression levels between the various cell lines.

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.

Determination of Hygromycin Sensitivity

The pEF5/FRT/V5-DEST vector contains the hygromycin resistance gene (Gritz and Davies, 1983) for selection of transfectants with the antibiotic, hygromycin B (Palmer *et al.*, 1987). Before generating a stable cell line expressing your protein of interest (Flp-In™ expression cell line), we recommend that you generate a kill curve to determine the minimum concentration of hygromycin required to kill your untransfected Flp-In™ host cell line. Generally, concentrations between 10 and 400 µg/mL hygromycin are required for selection of most mammalian cell lines. For instructions to handle and store hygromycin B and for general guidelines on performing a kill curve, refer to the Flp-In™ System manual.

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Generating Stable Flp-In™ Expression Cell Lines, continued

Important

REMINDER: Remember that the hygromycin resistance gene in pEF5/FRT/V5-DEST lacks a promoter and an ATG initiation codon; therefore, transfection of the pEF5/FRT/V5-DEST plasmid alone into mammalian host cells will **not** confer hygromycin resistance to the cells. The SV40 promoter and ATG initiation codon required for expression of the hygromycin resistance gene are integrated into the genome (in the Flp-In™ host cell line) and can only be brought into the correct proximity and frame with the hygromycin resistance gene through Flp recombinase-mediated integration of pEF5/FRT/V5-DEST at the FRT site.

Methods of Transfection

For established cell lines (*e.g.* HeLa, CHO), 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 lipid-based reagent for transfection of adherent or suspension cells, we recommend using Lipofectamine™ 2000 Reagent available from Invitrogen (Catalog no. 11668-027). For more information on transfection reagents available from Invitrogen, refer to our Web site (www.invitrogen.com) or contact Technical Support (page 16).

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Generating Stable Flp-In™ Expression Cell Lines, continued

Detection of Recombinant Fusion Proteins

If you have recombined the gene of interest in frame with the V5 epitope, you may detect expression of your recombinant fusion protein by Western blot analysis using Anti-V5 antibodies available from Invitrogen (see page **Error! Bookmark not defined.** for ordering information). 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 tag. The ready-to-use WesternBreeze™ Chromogenic and WesternBreeze™ Chemiluminescent Kits are available from Invitrogen to facilitate detection of antibodies by colorimetric or chemiluminescent methods. For more information, refer to our Web site (www.invitrogen.com) or contact Technical Support (page 16).

Assay for CAT Protein

If you use pEF5/FRT/V5/GW-CAT as a positive control vector, you may assay for CAT expression using your method of choice. Note that CAT is fused to the C-terminal V5 epitope tag, so you can use Western blot analysis and an Anti-V5 antibody to detect expression of CAT. CAT Antiserum is also available separately from Invitrogen (see page v for ordering information). Other commercial kits are available for assaying CAT expression. The molecular weight of the CAT fusion protein is approximately 30 kDa.

Note

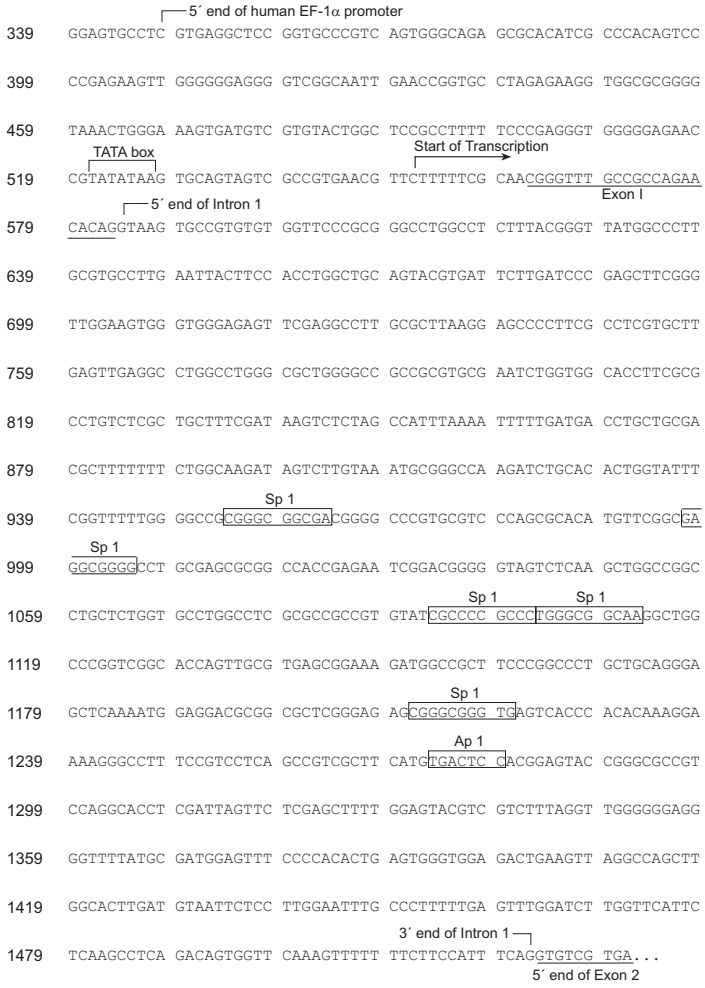
The C-terminal peptide tag containing the V5 epitope will add approximately 5 kDa to the size of your protein.

Appendix

Human EF-1α Promoter

Description

The diagram below shows the features of the human EF-1α promoter (Mizushima and Nagata, 1990) used in the pEF5/FRT/V5-DEST vector. Features are marked as described in Uetsuki *et al.*, 1989.



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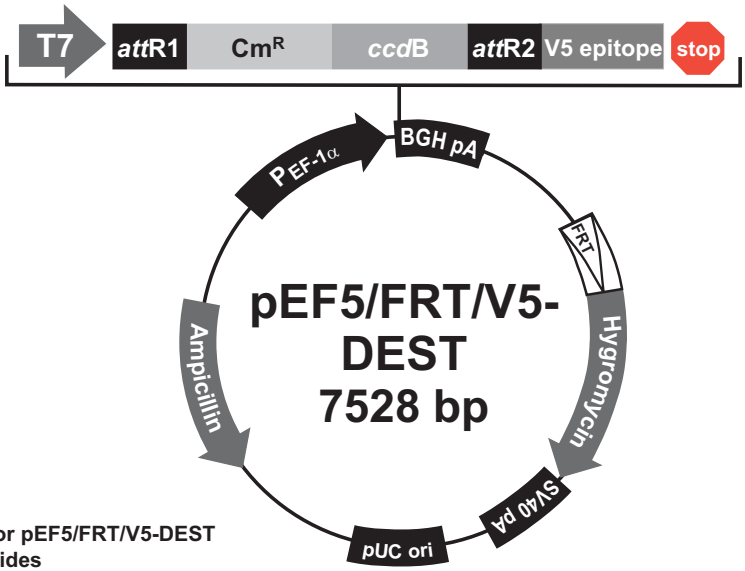
Map and Features of pEF5/FRT/V5-DEST

Map of pEF5/FRT/V5-DEST

The map below shows the elements of pEF5/FRT/V5-DEST. DNA from the entry clone replaces the region between bases 1652 and 3335. **The complete sequence of pEF5/FRT/V5-DEST is available from our Web site (www.invitrogen.com) or by contacting Technical Support (page 16).**



Comments for pEF5/FRT/V5-DEST
7528 nucleotides

EF-1α promoter: bases 348-1531
T7 promoter: bases 1548-1567
*att*R1 recombination site: bases 1645-1769
Chloramphenicol resistance gene: bases 1878-2537
*ccd*B gene: bases 2879-3184
*att*R2 recombination site: bases 3225-3349
V5 epitope: bases 3402-3443
BGH polyadenylation signal: bases 3487-3711
FRT site: bases 3994-4041
Hygromycin resistance gene (no ATG): bases 4049-5069
SV40 early polyadenylation signal: bases 5201-5331
pUC origin: bases 5714-6387
bla promoter: bases 7393-7491 (complementary strand)
Ampicillin (*bla*) resistance gene: bases 6532-7392 (complementary strand)

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Map and Features of pEF5/FRT/V5-DEST, continued

Features of pEF5/FRT/V5-DEST

pEF5/FRT/V5-DEST (7528 bp) contains the following elements. All features have been functionally tested.

Feature	Benefit
Human elongation factor 1α (hEF-1α) promoter	Allows overexpression of your recombinant protein 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 your recombinant protein with Anti-V5 antibodies (Southern *et al.*, 1991)
Bovine growth hormone (BGH) polyadenylation signal	Allows efficient transcription termination and polyadenylation of mRNA (Goodwin and Rottman, 1992)
Flp Recombination Target (FRT) site	Encodes a 34 bp (+14 bp of non-essential) sequence that serves as the binding and cleavage site for Flp recombinase (Gronostajski and Sadowski, 1985; Jayaram, 1985; Senecoff *et al.*, 1985)
Hygromycin resistance gene (no ATG)	Allows selection of stable transfectants in mammalian cells (Gritz and Davies, 1983) when brought in frame with a promoter and an ATG initiation codon through Flp recombinase-mediated recombination
SV40 early polyadenylation signal	Allows efficient transcription termination and polyadenylation of mRNA
pUC origin	Allows high-copy number replication and growth in *E. coli*
bla promoter	Allows expression of the ampicillin (*bla*) resistance gene
Ampicillin (*bla*) resistance gene	Permits selection of transformants in *E. coli*

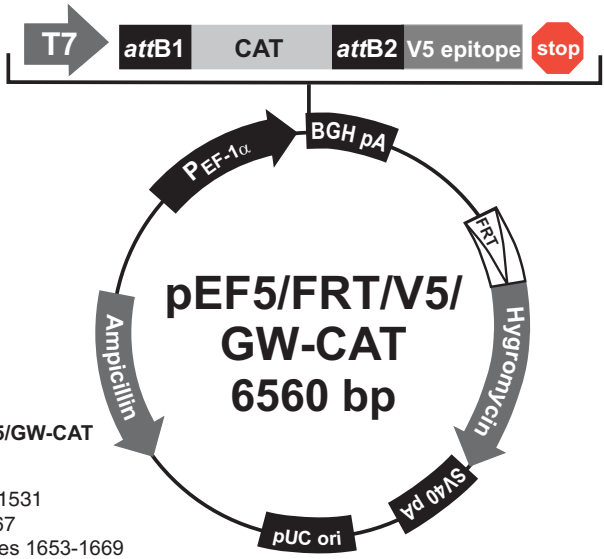
Map of pEF5/FRT/V5/GW-CAT

Description

pEF5/FRT/V5/GW-CAT is a 6560 bp control vector expressing chloramphenicol acetyltransferase (CAT). pEF5/FRT/V5/GW-CAT was constructed using the Gateway™ LR recombination reaction between an entry clone containing the CAT gene and pEF5/FRT/V5-DEST. CAT is expressed as a fusion to the V5 epitope tag. The molecular weight of the fusion protein is approximately 30 kDa.

Map of pEF5/FRT/V5/GW-CAT

The map below shows the elements of pEF5/FRT/V5/GW-CAT. **The complete sequence of pEF5/FRT/V5/GW-CAT is available from our Web site (www.invitrogen.com) or by contacting Technical Support (page 16).**



Comments for pEF5/FRT/V5/GW-CAT
6560 nucleotides

EF-1α promoter: bases 348-1531
T7 promoter: bases 1548-1567
*att*B1 recombination site: bases 1653-1669
CAT ORF: bases 1699-2355
*att*B2 recombination site: bases 2356-2381
V5 epitope: bases 2434-2475
BGH polyadenylation signal: bases 2519-2743
FRT site: bases 3026-3073
Hygromycin resistance gene (no ATG): bases 3081-4101
SV40 early polyadenylation signal: bases 4233-4363
pUC origin: bases 4746-5419
bla promoter: bases 6425-6523 (complementary strand)
Ampicillin (*bla*) resistance gene: bases 5564-6424 (complementary strand)

Technical Support

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 at www.invitrogen.com/sds.

Certificate of Analysis

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Technical Support, continued

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Introduction

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Purchaser Notification, continued

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Gateway® Clone Distribution Policy

Introduction

The information supplied in this section is intended to provide clarity concerning Invitrogen's policy for the use and distribution of cloned nucleic acid fragments, including open reading frames, created using Invitrogen's commercially available Gateway® Technology.

Gateway® Entry Clones

Invitrogen understands that Gateway® entry clones, containing *att*L1 and *att*L2 sites, may be generated by academic and government researchers for the purpose of scientific research. Invitrogen agrees that such clones may be distributed for scientific research by non-profit organizations and by for-profit organizations without royalty payment to Invitrogen.

Gateway® Expression Clones

Invitrogen also understands that Gateway® expression clones, containing *att*B1 and *att*B2 sites, may be generated by academic and government researchers for the purpose of scientific research. Invitrogen agrees that such clones may be distributed for scientific research by academic and government organizations without royalty payment to Invitrogen. Organizations other than academia and government may also distribute such Gateway® expression clones for a nominal fee ($10 per clone) payable to Invitrogen.

Additional Terms and Conditions

We would ask that such distributors of Gateway® entry and expression clones indicate that such clones may be used only for research purposes, that such clones incorporate the Gateway® Technology, and that the purchase of Gateway® Clonase™ from Invitrogen is required for carrying out the Gateway® recombinational cloning reaction. This should allow researchers to readily identify Gateway® containing clones and facilitate their use of this powerful technology in their research. Use of Invitrogen's Gateway® Technology, including Gateway® clones, for purposes other than scientific research may require a license and questions concerning such commercial use should be directed to Invitrogen's licensing department at 760-603-7200.

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Notes

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