# pFRT/*lac*Zeo

## Flp recombination target site vector designed for use with the Flp-In™ System

**Catalog no. V6015-20**

**Version B**
021402
*25-0357*

**www.invitrogen.com**
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# Important Information

## Contents

20 µg pFRT/*lac*Zeo, lyophilized in TE, pH 8.0

## Shipping/Storage

Lyophilized plasmid is shipped at room temperature and should be stored at -20°C.

## Product Specifications

The pFRT/*lac*Zeo vector is qualified by restriction enzyme digestion with specific restriction enzymes as listed below. Restriction digests must demonstrate the correct banding pattern when electrophoresed on an agarose gel (see below).

| Vector | Restriction Enzymes | Expected Results (bp) |
|---|---|---|
| pFRT/*lac*Zeo | *Kpn* I | No site |
| | *Xba* I | 8106 |

## Obtaining Zeocin™

Zeocin™ is used as the selection agent for the pFRT/*lac*Zeo plasmid and may be obtained separately from Invitrogen. For your convenience, the drug is prepared in autoclaved, deionized water and available in 1.25 ml aliquots at a concentration of 100 mg/ml. The stability of Zeocin™ is guaranteed for six months if stored at –20°C.

| Amount | Catalog no. |
|---|---|
| 1 g | R250-01 |
| 5 g | R250-05 |

## Flp-In™ Expression Vectors

After you have generated your Flp-In™ host cell line using pFRT/*lac*Zeo, you will use a Flp-In™ expression vector to express your gene of interest. Several Flp-In™ expression vectors are available from Invitrogen. Vectors include such features as C-terminal tags for purification and detection of the protein of interest and/or a secretion signal for secreted expression. For more information about Flp-In™ expression vectors available from Invitrogen, visit our World Wide Web site (www.invitrogen.com) or call Technical Service (see page 15).

| Product | Amount | Catalog no. |
|---|---|---|
| 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 |
| pEF5/FRT/V5 Directional TOPO® Expression Kit | 1 kit | K6035-01 |
| pEF5/FRT/V5-DEST Gateway™ Vector Pack | 6 µg | V6020-20 |

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# Important Information, 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, visit our World Wide Web site (www.invitrogen.com) or call Technical Service (see page 15).

| 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 |

## Accessory Products

Many of the reagents used in the Flp-In™ System are available separately from Invitrogen. See the table below for ordering information.

| Product | Amount | Catalog no. |
|---|---|---|
| pFRT/*lac*Zeo2 | 20 µg, lyophilized in TE | V6022-20 |
| pOG44 | 20 µg, lyophilized in TE | V6005-20 |
| T7 Promoter Primer | 2 µg, lyophilized in TE | N560-02 |
| Hygromycin | 1 g, liquid | R220-05 |

# Methods

# Overview

## Introduction

pFRT/*lac*Zeo is a 8.1 kb Flp recombination target (FRT) site vector designed for use with the Flp-In™ System (Catalog nos. K6010-01 and K6010-02) available from Invitrogen. When transfected into mammalian cells, the pFRT/*lac*Zeo plasmid allows the generation of Flp-In™ host cell lines containing an integrated FRT site. The vector contains the following elements:

- The SV40 early promoter and origin for high-level constitutive expression of the *lacZ-Zeocin*™ fusion gene in mammalian cells
- FRT site for Flp recombinase-mediated integration of a Flp-In™ expression vector containing the gene of interest into the Flp-In™ host cell line
- *lacZ-Zeocin*™ fusion gene for selection of stable mammalian cell lines with Zeocin™ and screening by β-galactosidase activity assay

For a map and description of the features of pFRT/*lac*Zeo, refer to the **Appendix**, pages 13-14. For more information about the Flp-In™ System, refer to the Flp-In™ System manual supplied with the Flp-In™ Complete or Core Systems. For more information about the Flp-In™ expression vectors, refer to the specific manual for each vector. The Flp-In™ System manual and the expression vector manuals are also available for downloading from our World Wide Web site (www.invitrogen.com) or by contacting Technical Service (see page 15).

## Generating Stable Flp-In™ Host Cell Lines

The table below outlines the steps necessary to generate Flp-In™ host cell lines using the pFRT/*lac*Zeo plasmid.

| Step | Action |
|---|---|
| 1 | Prepare purified pFRT/*lac*Zeo plasmid DNA and linearize the plasmid. |
| 2 | Transfect your mammalian cell line of choice and use Zeocin™ to select for stable integrants. |
| 3 | Pick and expand Zeocin™-resistant foci. |
| 4 | Prepare genomic DNA from Zeocin™-resistant foci. |
| 5 | Determine the number of integrated FRT sites in each clone using Southern blot analysis. Select clone(s) that contain a single integrated FRT site for use as Flp-In™ host cell line(s). |
| 6 | Screen single integrants for β-galactosidase activity. |

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# Overview, continued

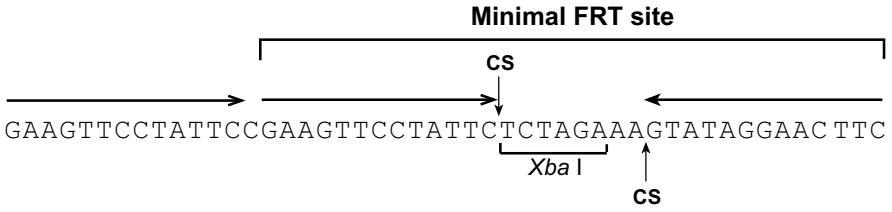
## Using the Flp-In™ Host Cell Lines

You will cotransfect the Flp-In™ host cell line with the pOG44 Flp recombinase vector and a pcDNA5/FRT-based expression plasmid containing your gene of interest to generate a Flp-In™ expression cell line. The presence of the FRT site allows your pcDNA5/FRT-based construct to stably integrate into the genome at the FRT site via Flp recombinase-mediated DNA recombination.

For more information about the FRT site, see below. For more information about Flp recombinase-mediated DNA recombination, refer to the Flp-In™ System manual or 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

In the Flp-In™ System, the pFRT/*lac*Zeo and pcDNA5/FRT vectors each contain a single FRT site. The pFRT/*lac*Zeo plasmid is used to generate the Flp-In™ host cell line and the pcDNA5/FRT-based expression plasmid is used to express the gene of interest in the Flp-In™ host cell line. For more information about pcDNA5/FRT-based expression plasmids and the Flp-In™ System, refer to the individual manual for each expression vector and the Flp-In™ System manual.

# Maintaining pFRT/*lac*Zeo

## Introduction

General guidelines to transform pFRT/*lac*Zeo into *E. coli* are provided in this section.

## General Molecular Biology Techniques

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

## *E. coli* Strain

Many *E. coli* strains are suitable for the propagation and maintenance of the pFRT/*lac*Zeo plasmid including TOP10 (Catalog no. C610-00). We recommend that you propagate the vector in *E. coli* strains that are recombination deficient (*rec*A) and endonuclease A deficient (*end*A).

For your convenience, TOP10 is available as chemically competent or electrocompetent cells from Invitrogen.

| Item | Quantity | Catalog no. |
|---|---|---|
| One Shot® TOP10 (chemically competent cells) | 21 x 50 µl | C4040-03 |
| One Shot® TOP10 Electrocomp™ (electrocompetent cells) | 21 x 50 µl | C4040-52 |
| Electrocomp™ TOP10 (electrocompetent cells) | 5 x 80 µl | C664-55 |

## Transformation Method

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

## Maintenance of Plasmid

To propagate and maintain the pFRT/*lac*Zeo vector, we recommend using the following procedure:

1. Resuspend the vector in 20 µl sterile water to prepare a 1 µg/µl stock solution. Store the stock solution at -20°C.
2. Use the stock solution to transform a *rec*A, *end*A *E. coli* strain like TOP10, DH5α, JM109, or equivalent.
3. Select transformants on LB agar plates containing 50 to 100 µg/ml ampicillin. For fast and easy microwaveable preparation of Low Salt LB agar containing ampicillin, imMedia™ Amp Agar (Catalog no. Q601-20) is available from Invitrogen. For more information, call Technical Service (see page 15).
4. Prepare a glycerol stock of each transformant containing plasmid for long-term storage (see the next page).

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# Maintaining pFRT/*lac*Zeo, continued

## Preparing a Glycerol Stock

Once you have identified the correct clone, purify the colony and make a glycerol stock for long-term storage. You should keep a DNA stock of your plasmid at -20°C.

- Streak the original colony out on an LB agar plate containing 50 µg/ml ampicillin. Incubate the plate at 37°C overnight.
- Isolate a single colony and inoculate into 1-2 ml of LB containing 50 µg/ml ampicillin.
- Grow the culture to mid-log phase ($OD_{600}$ = 0.5-0.7).
- Mix 0.85 ml of culture with 0.15 ml of sterile glycerol and transfer to a cryovial.
- Store at -80°C.

# Generating Flp-In™ Host Cell Lines

## Introduction

You will transfect the pFRT/*lac*Zeo plasmid into the mammalian cell line of choice to generate stable Flp-In™ host cell lines. Guidelines and instructions are provided in this section. Before beginning, we suggest that you review this section.

## Important

We have observed down-regulation of the viral CMV promoter and subsequent loss of gene expression when pcDNA5/FRT-based expression constructs are introduced into Flp-In™-3T3 or Flp-In™-BHK cells. If you will be cloning your gene of interest into a pcDNA5-FRT-based expression construct, we recommend that you **do not** use 3T3 or BHK cells to create your Flp-In™ host cell line.

Alternatively, if you prefer to use 3T3 or BHK cells to create your Flp-In™ host cell line, we recommend that you clone your gene of interest into a pEF5/FRT-based expression plasmid (*e.g.* pEF5/FRT/V5-D-TOPO® or pEF5/FRT/V5-DEST). Loss of gene expression due to down-regulation of the promoter is not observed in these cell lines when using pEF5/FRT-based expression constructs. For more information about the pEF5/FRT/V5-D-TOPO® or pEF5/FRT/V5-DEST vectors, refer to our Web site (www.invitrogen.com) or call Technical Service (see page 15).

## 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 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, 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 require-ments, 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). Invitrogen offers the Calcium Phosphate Transfection Kit and Lipofectamine™ 2000 Reagent for mammalian cell transfection. For more information about electroporation, refer to *Current Protocols in Molecular Biology*, Unit 9.3 (Ausubel *et al.*, 1994).

| Catalog No. | Description | Quantity |
|---|---|---|
| K2780-01 | Calcium Phosphate Transfection Kit | 75 reactions |
| 11668-027 | Lipofectamine™ 2000 Reagent | .75 ml |

## Zeocin™

The pFRT/*lac*Zeo plasmid contains a *lacZ-Zeocin*™ fusion gene under the control of the SV40 early promoter. Expression of the *lacZ-Zeocin*™ fusion gene allows selection of stable integrants using Zeocin™ antibiotic. The resulting stable integrants can then be screened by assaying for expression of β-galactosidase. For more information about preparing and handling Zeocin™, refer to the **Appendix**, pages 11-12.

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# Generating Flp-In™ Host Cell Lines, continued

## Determination of Zeocin™ Sensitivity

To successfully generate a stable cell line containing an integrated FRT site and expressing the LacZ-Zeocin™ fusion protein, you need to determine the minimum concentration of Zeocin™ required to kill your untransfected mammalian cell line. Typically, concentrations ranging from 50 to 1000 µg/ml Zeocin™ are sufficient to kill most untransfected mammalian cell lines, with the average being 100 to 400 µg/ml. We recommend that you test a range of concentrations (see protocol below) to ensure that you determine the minimum concentration necessary for your cell line. Refer to the **Appendix**, pages 11-12 for instructions on how to prepare and store Zeocin™.

1. Plate or split a confluent plate so the cells will be approximately 25% confluent. Prepare a set of 7 plates. Allow cells to adhere overnight.
2. The next day, substitute culture medium with medium containing varying concentrations of Zeocin™ ( 0, 50, 100, 250, 500, 750, and 1000 µg/ml Zeocin™).
3. Replenish the selective media every 3-4 days, and observe the percentage of surviving cells.
4. Note the percentage of surviving cells at regular intervals to determine the appropriate concentration of Zeocin™ that kills the cells within 1-2 weeks after addition of Zeocin™.

## Effect of Zeocin™ on Sensitive and Resistant Cells

Zeocin™'s method of killing is quite different from other antibiotics including hygromycin, G418, and blasticidin. **Cells do not round up and detach from the plate.** Sensitive cells may exhibit the following morphological changes upon exposure to Zeocin™:

- Vast increase in size (similar to the effects of cytomegalovirus infecting permissive cells)
- Abnormal cell shape
- Presence of large empty vesicles in the cytoplasm (breakdown of the endoplasmic reticulum and Golgi apparatus, or other scaffolding proteins)
- Breakdown of plasma and nuclear membrane (appearance of many holes in these membranes)

Eventually, these "cells" will completely break down and only "strings" of protein remain.

Zeocin™-resistant cells should continue to divide at regular intervals to form distinct colonies. There should not be any distinct morphological changes in Zeocin™-resistant cells when compared to cells not under selection with Zeocin™. For more information about Zeocin™ and its mechanism of action, refer to the **Appendix**, pages 11-12.

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# Generating Flp-In™ Host Cell Lines, continued

## Transfection Considerations

Once you have determined the appropriate Zeocin™ concentration to use for selection, you are ready to transfect the pFRT/*lac*Zeo plasmid into your mammalian cell line of choice to generate the Flp-In™ host cell line. When generating your Flp-In™ host cell line, you will need to consider the following factors:

- **Insertion of the FRT site into the genome:** Integration of the pFRT/*lac*Zeo plasmid containing the FRT site into the genome will occur randomly. Subsequent integration of the pcDNA5/FRT-based expression plasmid containing your gene of interest will occur through Flp recombinase-mediated recombination at the genomic FRT site.
- **Transfection efficiency of your cell line:** The aim of most users will be to create stable cell lines containing a single integrated FRT site ("single integrants"; see **Note** below). The probability of obtaining stable integrants containing a single FRT site or multiple FRT sites will depend upon the transfection efficiency of your cell line and the amount of DNA transfected. If you wish to increase the likelihood of obtaining single integrants, you will need to lower the transfection efficiency by limiting the amount of plasmid DNA that you transfect (see **Recommendation** on the next page).
- **Selection of foci:** You will select for stable transfectants by plating cells in medium containing Zeocin™. Zeocin™-resistant foci can then be screened by Southern blot analysis to identify single integrants. To increase the chances of obtaining single integrants, we recommend that you pick foci from plates that have been transfected with the least amount of plasmid DNA.
- **Chromosomal position effects:** Because integration of the pFRT/*lac*Zeo plasmid into the genome occurs randomly, expression levels of the *lacZ-Zeocin*™ fusion gene will be dependent on the transcriptional activity of the surrounding sequences at the integration site (*i.e.* chromosomal position effect). Once you have obtained single integrants, you may want to screen the Zeocin™-resistant clones for those expressing the highest β-galactosidase levels. Those clones expressing the highest levels of β-galactosidase should contain single FRT sites which have integrated into the most transcriptionally active regions.
- **Antibiotic concentration:** Single integrants will express only a single copy of the *lacZ-Zeocin*™ fusion gene and therefore, may be more sensitive to Zeocin™ selection than multiple integrants. If you have previously used your mammalian cell line for transfection and Zeocin™ selection, note that you may need to use lower concentrations of Zeocin™ to obtain single integrants.

**Note**

If you want to increase the expression levels of your gene of interest in the cell line of choice, you may wish to generate a Flp-In™ host cell line containing multiple integrated FRT sites. In theory, cotransfection of your pcDNA5/FRT-based construct and pOG44 into these cells will allow integration of your gene of interest into multiple genomic loci. **Note that the presence of multiple integrated FRT sites in the genome may increase the occurrence of chromosomal rearrangements or unexpected recombination events in your host cell line**.

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# Generating Flp-In™ Host Cell Lines, continued


As mentioned on the previous page, we recommend that you transfect your mammalian cell line with a limiting amount of pFRT/*lac*Zeo plasmid. We generally use 250 ng to 2 µg of plasmid DNA per 4 x $10^6$ cells for transfection, but the amount of plasmid DNA may vary due to the nature of the cell line, the transfection efficiency of your cells, and the method of transfection used. When transfecting your mammalian cell line of choice, we suggest that you try a range of plasmid DNA concentrations (*e.g.* 0.25, 0.5, 1, 2, 5 µg/ml DNA) to optimize transfection conditions for your cell line.

We generally use electroporation to transfect cells, but other methods of transfection are suitable. For a protocol to electroporate cells, refer to *Current Protocols in Molecular Biology*, Unit 9.3 (Ausubel *et al.*, 1994). Note that if you use calcium phosphate or lipid-mediated transfection methods, the amount of **total** DNA required for transfection is typically higher than for electroporation (usually between 10 and 20 µg DNA). Depending on the amount of pFRT/*lac*Zeo plasmid that you use for transfection, you may need to supplement your plasmid DNA with carrier DNA (*e.g.* salmon sperm DNA).

## Possible Sites for Linearization of pFRT/*lac*Zeo

To obtain stable transfectants, we recommend that you linearize the pFRT/*lac*Zeo plasmid 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 the ATG-FRT-*lac*Z-*Zeocin*™ cassette or other elements necessary for expression in mammalian cells. The table below lists unique sites that may be used to linearize your construct prior to transfection. Other restriction sites are possible.

**Note:** We generally use *Sca* I to linearize pFRT/*lac*Zeo.

| Enzyme | Restriction Site (bp) | Location | Supplier |
|---|---|---|---|
| *Tth*111 I | 125 | Backbone | Many |
| *Apa* I | 5617 | Backbone | Many |
| *Swa* I | 6075 | Backbone | New England Biolabs, Sigma, Takara |
| *Xmn* I | 6487 | Ampicillin gene | Many |
| *ScaI* I | 6606 | Ampicillin gene | Many |
| *Bsa* I | 7021 | Ampicillin gene | New England Biolabs |
| *Eam*1105 I | 7087 | Ampicillin gene | AGS*, Fermentas, Takara |
| *Sap* I | 8092 | Backbone | New England Biolabs |

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# Generating Flp-In™ Host Cell Lines, continued

## Selection of Stable Integrants

Once you have determined the appropriate Zeocin™ concentration to use for selection, you can generate a stable cell line with pFRT/*lac*Zeo.

1. Transfect mammalian cells with pFRT/*lac*Zeo using the desired protocol. Remember to include a plate of untransfected cells as a negative control.
2. 24 hours after transfection, wash the cells and add fresh medium to the cells.
3. 48 hours after transfection, split the cells into fresh medium. Split the cells such that they are no more than 25% confluent. If the cells are too dense, the antibiotic will not kill the cells. Antibiotics work best on actively dividing cells.
4. Incubate the cells at 37°C for 2-3 hours until they have attached to the culture dish.
5. Remove the medium and add fresh medium containing Zeocin™ at the pre-determined concentration required for your cell line.
6. Feed the cells with selective medium every 3-4 days until foci can be identified.
7. Pick at least 20 Zeocin™-resistant foci and expand each clone to test for the number of integrated FRT sites. Isolate genomic DNA and use Southern blot analysis to distinguish between single and multiple integrants (see below and the next page). Select the single integrants and proceed to the next step.
8. Screen the single integrants for β-galactosidase activity (see the next page). Select those clones which exhibit the highest levels of β-galactosidase expression (if desired) to use as your Flp-In™ host cell line(s).
9. Once you have obtained a stable Flp-In™ host cell line, you can use this cell line to isolate a stable cell line expressing your gene of interest from the pcDNA5/FRT plasmid. **Note:** The Flp-In™ host cell line should be maintained in medium containing the appropriate amount of Zeocin™ until generation of your Flp-In™ expression cell line.

## Isolation of Genomic DNA

Once you have obtained Zeocin™-resistant foci, you will need to expand the cells and isolate genomic DNA. You may use any standard protocol to isolate genomic DNA from your cells. Protocols may be found in *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994) or *Molecular Cloning: A Laboratory Manual* (Sambrook *et al.*, 1989). For easy isolation of genomic DNA, the Easy-DNA™ Kit (Catalog no. K1800-01) is available from Invitrogen. Contact Technical Service for more information (see page 15).

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### Screening Clones by Southern Blot Analysis

You can use Southern blot analysis to determine the number of integrated FRT sites present in each of your Zeocin™-resistant clones. When performing Southern blot analysis, you should consider the following factors:

- **Probe:** We recommend that you use a fragment of the *lacZ* gene (100 to 500 bp) as the probe to screen your samples. Mammalian cells do not contain an endogenous *lacZ* gene, therefore, a *lacZ* probe should allow you to identify those clones which contain pFRT/*lac*Zeo DNA. To label the probe, we generally use a standard random priming kit (*e.g.* Ambion, DECAprime II™ Kit, Catalog no. 1455). Other random priming kits are suitable.
- **Restriction digest:** When choosing a restriction enzyme to digest the genomic DNA, we recommend choosing an enzyme that cuts at a single known site outside of the *lacZ* gene in the pFRT/*lac*Zeo vector. Hybridization of the *lacZ* probe to digested DNA should then allow you to detect a single band containing the *lacZ* gene from pFRT/*lac*Zeo. We generally use *Hin*d III to digest genomic DNA from the Zeocin™-resistant clones. pFRT/*lac*Zeo contains a single *Hin*d III site within the FRT site.
- **Protocol:** You may use any Southern blotting protocol of your choice. Refer to *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994) or *Molecular Cloning: A Laboratory Manual* (Sambrook *et al.*, 1989) for detailed protocols.

### What You Should See

If you digest genomic DNA from your transfectants with *Hin*d III and use a *lacZ* fragment as a probe in your Southern analysis, you should be able to easily distinguish between single and multiple FRT integrants.

- DNA from single integrants should contain only one hybridizing band corresponding to a single copy of the integrated pFRT/*lac*Zeo plasmid.
- DNA from multiple integrants should contain more than one hybridizing band. If the pFRT/*lac*Zeo plasmid integrates into multiple chromosomal locations, the bands may be of varying sizes.

### Assay for β-Galactosidase Activity

Once you have identified single integrants, proceed to screen the clones for β-galactosidase expression. You may assay for β-galactosidase expression by activity assay using cell-free lysates (Miller, 1972) or by staining the cells for activity. Invitrogen offers the β-Gal Assay Kit (Catalog no. K1455-01) and the β-Gal Staining Kit (Catalog no. K1465-01) for fast and easy detection of β-galactosidase expression. Select those clones expressing the highest levels of β-galactosidase (if desired) to use as the host cell lines for your pcDNA5/FRT-based expression construct.

# Appendix

## Zeocin™

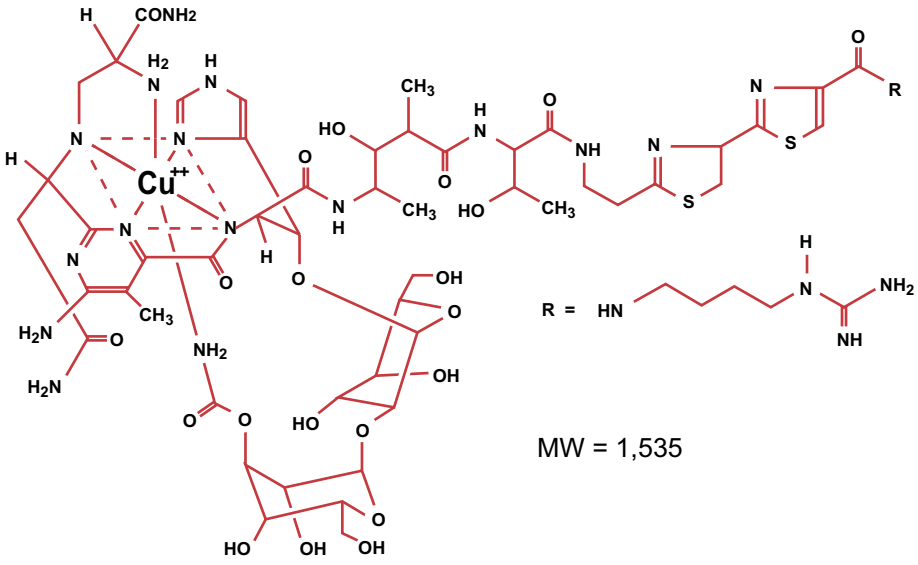
### Zeocin™

Zeocin™ is a member of the bleomycin/phleomycin family of antibiotics isolated from *Streptomyces*. Antibiotics in this family are broad spectrum antibiotics that act as strong anti-bacterial and anti-tumor drugs. They show strong toxicity against bacteria, fungi (including yeast), plants, and mammalian cells (Baron *et al.*, 1992; Drocourt *et al.*, 1990; Mulsant *et al.*, 1988; Perez *et al.*, 1989).

The Zeocin™ resistance protein has been isolated and characterized (Calmels *et al.*, 1991; Drocourt *et al.*, 1990). This protein, the product of the *Sh ble* gene (*Streptoalloteichus hindustanus* bleomycin gene), is a 13.7 kDa protein that binds Zeocin™ and inhibits its DNA strand cleavage activity. Expression of this protein in eukaryotic and prokaryotic hosts confers resistance to Zeocin™.

### Molecular Weight, Formula, and Structure

The formula for Zeocin™ is $C_{60}H_{89}N_{21}O_{21}S_3$ and the molecular weight is 1,535. The diagram below shows the structure of Zeocin™.




### Applications of Zeocin™

Zeocin™ is used for selection in mammalian cells (Mulsant *et al.*, 1988); plants (Perez *et al.*, 1989); yeast (Baron *et al.*, 1992); and prokaryotes (Drocourt *et al.*, 1990). Typically, Zeocin™ concentrations ranging from 50 to 1000 µg/ml are used for selection in mammalian cells. Before transfection, we recommend that you first test the sensitivity of your mammalian host cell to Zeocin™ as natural resistance varies among cell lines.

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# Zeocin™, continued

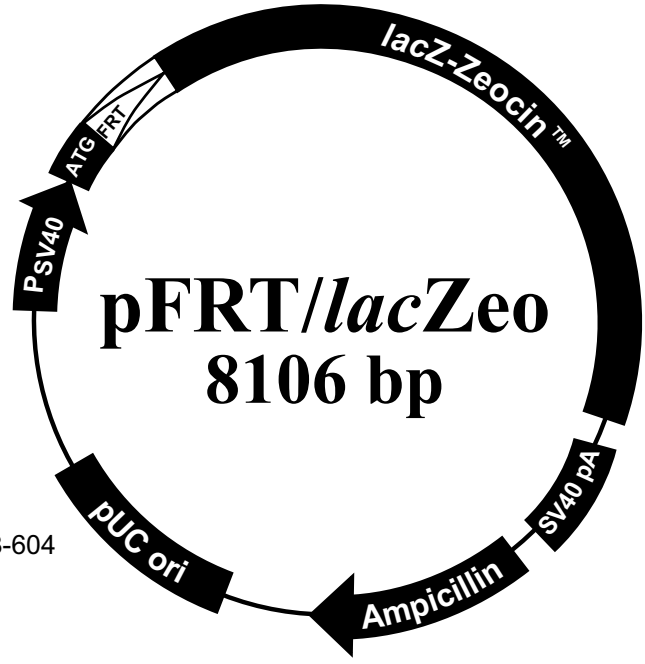
## Handling Zeocin™

- Store Zeocin™ at -20°C and thaw on ice before use.
- Zeocin™ is light sensitive. Store drug, plates, and medium containing drug in the dark.
- Wear gloves, a laboratory coat, and safety glasses or goggles when handling solutions containing Zeocin™.
- Zeocin™ is toxic. Do not ingest or inhale solutions containing the drug.

# pFRT/*lac*Zeo Vector

## Map of pFRT/*lac*Zeo

pFRT/*lac*Zeo is a 8106 bp vector that expresses a fusion protein containing β-galactosidase and the Zeocin™ resistance marker under the control of the SV40 early promoter. Note that neither the *lacZ* gene nor the Zeocin™ resistance gene contains its native ATG initiation codon. The ATG initiation codon is placed directly upstream of a FRT site and allows expression of the *lacZ-Zeocin*™ fusion gene in cells. The figure below summarizes the features of the pFRT/*lac*Zeo vector. **The complete sequence for pFRT/*lac*Zeo is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 15).**




**Comments for pFRT/*lac*Zeo**
**8106 nucleotides**

SV40 early promoter and origin: bases 278-604
ATG initiation codon: bases 609-611
FRT site: bases 614-661
LacZ-Zeocin™ fusion gene
    LacZ ORF (no ATG): bases 675-3722
    Zeocin™ resistance gene (no ATG): bases 3810-4181
SV40 early polyadenylation signal: bases 5102-5425
*bla* promoter: bases 6201-6299
Ampicillin (*bla*) resistance gene: bases 6300-7160
pUC origin: bases 7305-7978

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# pFRT/*lac*Zeo Vector, continued

## Features of pFRT/*lac*Zeo

The table below describes the relevant features of pFRT/*lac*Zeo. All features have been functionally tested and the vector fully sequenced.

| Feature | Benefit |
|---|---|
| SV40 early promoter and origin | Allows efficient, high-level expression of the *lacZ-Zeocin*™ fusion gene in mammalian cells and episomal replication in cells expressing the SV40 large T antigen |
| ATG initiation codon | Allows translation initiation of the LacZ-Zeocin™ fusion protein |
| Flp Recombination Target (FRT) site | Encodes a 34 bp (+14 bp non-essential) sequence that serves as the binding and cleavage site for Flp recombinase (Gronostajski and Sadowski, 1985; Jayaram, 1985; Senecoff *et al.*, 1985) |
| *lacZ-Zeocin*™ fusion gene | Encodes a fusion protein containing β-galactosidase and the Zeocin™ resistance marker to permit selection of stable mammalian cell lines with Zeocin™ and screening by β-galactosidase activity assay |
| SV40 early polyadenylation signal | Allows efficient transcription termination and polyadenylation of mRNA |
| *bla* promoter | Allows expression of the ampicillin (*bla*) resistance gene |
| Ampicillin (*bla*) resistance gene (β-lactamase) | Allows selection of transformants in *E. coli* |
| pUC origin | Allows high-copy number replication and growth in *E. coli* |

# Technical Service

## World Wide Web

Visit the Invitrogen Web Resource using your World Wide Web browser. At the site, you can:

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2. Follow instructions on the page and fill out all the required fields.
3. To request additional MSDSs, click the 'Add Another' button.
4. All requests will be faxed unless another method is selected.
5. When you are finished entering information, click the 'Submit' button. Your MSDS will be sent within 24 hours.

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# Technical Service, continued

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