USER GUIDE

invitrogen™
by life technologies™

pFRT/*lac*Zeo2

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

Catalog number V6022-20

Revision date 29 March 2012
Publication Part number 25-0378

MAN0000196

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

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Kit Contents and Storage

Kit Contents

20 µg pFRT/*lac*Zeo2 is supplied at 0.5 µg/µL in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 in a total volume of 40 µL.

Shipping/Storage

pFRT/*lac*Zeo2 is shipped at room temperature. Upon receipt, store the plasmid at -20°C.

Obtaining Zeocin™

Zeocin™ is used as the selection agent for the pFRT/*lac*Zeo2 plasmid and may be obtained separately (see page 17 for ordering). 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.

Product Use

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

Methods

Product Overview

Introduction

pFRT/*lac*Zeo2 is a 6.5 kb Flp recombination target (FRT) site vector designed for use with the Flp-In™ System available for purchase (see page 17). When transfected into mammalian cells, the pFRT/*lac*Zeo2 plasmid allows the generation of Flp-In™ host cell lines containing an integrated FRT site. The vector contains the following elements:

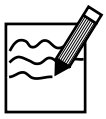
- A truncated SV40 early promoter and origin ($P_{SV40\Delta}$)for constitutive expression of the *lacZ*-Zeocin™ fusion gene in mammalian cells (see below for more information)
- FRT site for Flp recombinase-mediated integration of a Flp-In™ expression vector containing the gene of interest into the 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*Zeo2, refer to the **Appendix**, pages 15–16. 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 www.lifetechnologies.com/support or by contacting Technical Support (see page 19).

SV40Δ Promoter

The SV40 early promoter in pFRT/*lac*Zeo2 contains a 99 bp deletion from the 5′ end when compared to the wild-type SV40 early promoter. This truncated SV40 early promoter ($P_{SV40\Delta}$) is severely abrogated in its activity when compared to the wild-type SV40 early promoter (in pFRT/*lac*Zeo). The SV40Δ early promoter exhibits approximately 60-fold less activity than the wild-type SV40 early promoter in its ability to direct expression of the *lacZ-Zeocin*™ fusion gene.

To generate a Flp-In™ host cell line, you will transfect the pFRT/*lac*Zeo2 plasmid into the mammalian cell line of interest and select for stable transfectants using Zeocin™. Stable transfectants will only be selected if the *lacZ-Zeocin*™ fusion gene is expressed at suitable levels. Because of the minimal activity of the SV40Δ promoter, we reason that only stable transfectants containing FRT sites which have integrated into the most transcriptionally active genomic loci will be selected. This allows generation of Flp-In™ host cell lines which will presumably express the highest levels of the gene of interest after Flp recombinase-mediated integration of your Flp-In™ expression construct.



Note

Note that the pFRT/*lac*Zeo and pFRT/*lac*Zeo2 plasmids both contain the same elements required to propagate the vector and to generate Flp-In™ host cell lines (*i.e.* FRT site, *lacZ*-Zeocin™ fusion gene, ampicillin resistance gene). However, the vectors differ from each other in the following ways:

- SV40 early promoter used to control expression of the *lacZ*-Zeocin™ fusion gene (see above)
- Backbone of the vector

The nucleotide sequences of pFRT/*lac*Zeo and pFRT/*lac*Zeo2 are available for downloading from www.lifetechnologies.com or by calling Technical Support (see page 19).

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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*Zeo2 plasmid.

Step	Action
1	Prepare purified pFRT/*lac*Zeo2 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.

Using the Flp-In™ Host Cell Lines

You 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 page 3. For more information about Flp recombinase-mediated DNA recombination, refer to the Flp-In™ System manual or to published reviews (Craig, 1988; Sauer, 1994).

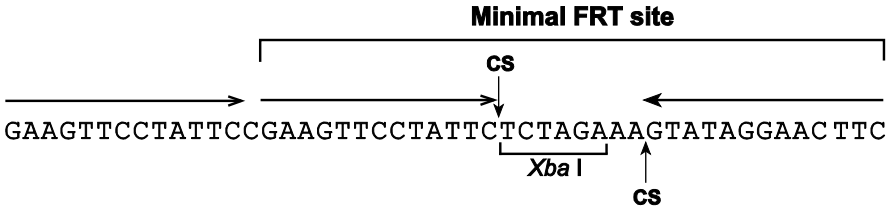
Note

The SV40Δ early promoter is only used during generation of the Flp-In™ host cell line to select for integration of pFRT/*lac*Zeo2 into the most transcriptionally active genomic locus (by directing expression of the *lacZ*-Zeocin™ fusion gene). Once the Flp-In™ host cell line is generated and you have stably integrated your pcDNA5/FRT-based construct (see above), the CMV promoter on the pcDNA5/FRT-based expression vector is used to control expression of your gene of interest.

Continued on next page

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*Zeo2 and pcDNA5/FRT-based expression vectors each contain a single FRT site. The pFRT/*lac*Zeo2 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*Zeo2

Introduction

General guidelines to transform pFRT/*lac*Zeo2 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*Zeo2 plasmid including TOP10, DH5α™, and JM109. 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 (see page 17 for ordering).

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.

Maintaining the Plasmid

To propagate and maintain the pFRT/*lac*Zeo2 vector, we recommend using the following procedure:

1. Use the supplied 0.5 µg/µL stock solution in TE buffer, pH 8.0 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 to 100 µg/mL ampicillin. For fast and easy microwaveable preparation of Low Salt LB agar containing ampicillin, imMedia™ Amp Agar is available (see page 17 for ordering). For more information, call Technical Support (see page 19).
3. Prepare a glycerol stock of each transformant containing plasmid for long-term storage (see the next page 5).

Continued on next page

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.

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

Generating Flp-In™ Host Cell Lines

Introduction

You transfect the pFRT/*lac*Zeo2 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® and pEF5/FRT/V5-DEST vectors, refer to www.lifetechnologies.com or call Technical Support (see page 19).

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 plasmid DNA using the PureLink® HiPure MiniPrep Kit (up to 30 µg DNA), the PureLink® HiPure MidiPrep Kit (up to 150 µg DNA) (see page 17 for ordering), 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 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). The Lipofectamine® 2000 Reagent for mammalian cell transfection and Neon™ Transfection System for electroporation of primary, stem cell and difficult-to-transfect cells are available (see page 17 for ordering).

Zeocin™

The pFRT/*lac*Zeo2 plasmid contains a *lacZ*-Zeocin™ fusion gene under the control of the SV40Δ 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 pages 15–16.

Continued on next page

Determining Zeocin™ Sensitivity

To successfully generate a stable cell line containing an integrated FRT site and expressing the β-galactosidase-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 pages 13–14 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

The method of Zeocin™ 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.

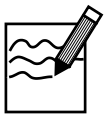
Zeocin™-resistant cells should continue to divide at regular intervals to form distinct colonies. There should not be any distinct morphological changes in Zeocin™-resistant cells when compared to cells not under selection with Zeocin™. For more information about Zeocin™ and its mechanism of action, refer to pages 13–14.

Continued on next page

Transfection Considerations

Once you have determined the appropriate Zeocin™ concentration to use for selection, you are ready to transfect the pFRT/*lac*Zeo2 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*Zeo2 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 page 9).
- **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*Zeo2 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, we recommend screening the Zeocin™-resistant clones for those expressing the highest β-galactosidase levels. Because of the minimal activity of the SV40 early promoter in pFRT/*lac*Zeo2, we expect that 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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As mentioned on page 8, we recommend that you transfect your mammalian cell line with a limiting amount of pFRT/*lac*Zeo2 plasmid. We generally use 250 ng to 2 µg of plasmid DNA per 4×10^6 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 by such means as the Neon™ Transfection System, but other methods of transfection are also suitable. Depending on the amount of pFRT/*lac*Zeo2 plasmid that you use for transfection, you may need to supplement your plasmid DNA with carrier DNA (*e.g.* salmon sperm DNA).

With the pFRT/*lac*Zeo2 plasmid, you are selecting for stable transfectants that:

1. contain a single integrated FRT site and
2. contain a single FRT site which has integrated into a highly transcriptionally active genomic locus.

Because of the more stringent selection criteria, you may obtain fewer Zeocin™-resistant clones than if you were to use the pFRT/*lac*Zeo plasmid for transfection. We generally obtain approximately 4–8 Zeocin™-resistant clones when transfecting pFRT/*lac*Zeo2 and approximately 25–50 Zeocin™-resistant clones when transfecting pFRT/*lac*Zeo into $1–5 \times 10^6$ mammalian cells.

If you wish to increase the number of Zeocin™-resistant clones that you obtain, we recommend increasing the number of cells that you transfect (by increasing the number of plates). **Do not** increase the amount of plasmid DNA that you transfect (see above).

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Possible Sites for Linearization of pFRT/*lac*Zeo2

To obtain stable transfectants, we recommend that you linearize the pFRT/*lac*Zeo2 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*Zeo2.

Enzyme	Restriction Site (bp)	Location	Supplier
Bgl II	2	Backbone	Many
Not I	15	Backbone	Many
Sal I	4160	Backbone	Many
Pst I	4170	Backbone	Many
*Eco*O109 I	4591	Backbone	Many
Xmn I	4971	Ampicillin gene	Many
ScaI I	5090	Ampicillin gene	Many
Bsa I	5505	Ampicillin gene	New England Biolabs
*Eam*1105 I	5571	Ampicillin gene	AGS*, Fermentas, Takara
Apa I	6474	Backbone	Many

*Angewandte Gentechnologie Systeme

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Selecting Stable Integrants

Once you have determined the appropriate Zeocin™ concentration to use for selection, you can generate a stable cell line with pFRT/*lac*Zeo2.

1. Transfect mammalian cells with pFRT/*lac*Zeo2 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 page 11). Select the single integrants and proceed to the next step.
8. Screen the single integrants for β-galactosidase activity (see page 12). Select those clones which exhibit the highest levels of βgalactosidase expression 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 Flp-In™ expression plasmid. **Note:** Maintain your Flp-In™ host cell line in medium containing the appropriate amount of Zeocin™ until generation of your Flp-In™ expression cell line.

Isolating Genomic DNA

Once you have obtained Zeocin™-resistant foci, you 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 is available (see page 16). Contact Technical Support for more information (see page 19).

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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*Zeo2 DNA. To label the probe, we generally use a standard random priming kit. 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*Zeo2 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*Zeo2. We generally use *Nco* I to digest genomic DNA from the Zeocin™-resistant clones. pFRT/*lac*Zeo2 contains a single *Nco* I site within the SV40 promoter.
- **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 *Nco* I 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*Zeo2 plasmid.
- DNA from multiple integrants should contain more than one hybridizing band. If the pFRT/*lac*Zeo2 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. The β-Gal Assay Kit and the β-Gal Staining Kit are available for fast and easy detection of β-galactosidase expression (see page 17 for ordering). 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™

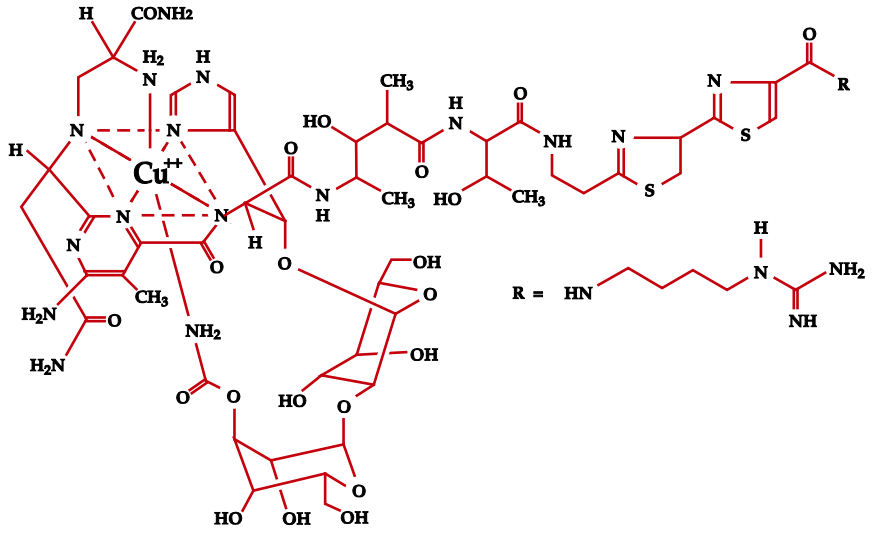
Zeocin™

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 $C_{55}H_{86}O_{21}N_{20}S_2Cu.HCl$ and the molecular weight is 1,527.5 Da. Zeocin is an HCl salt. 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.

Continued on next page

Zeocin™, Continued

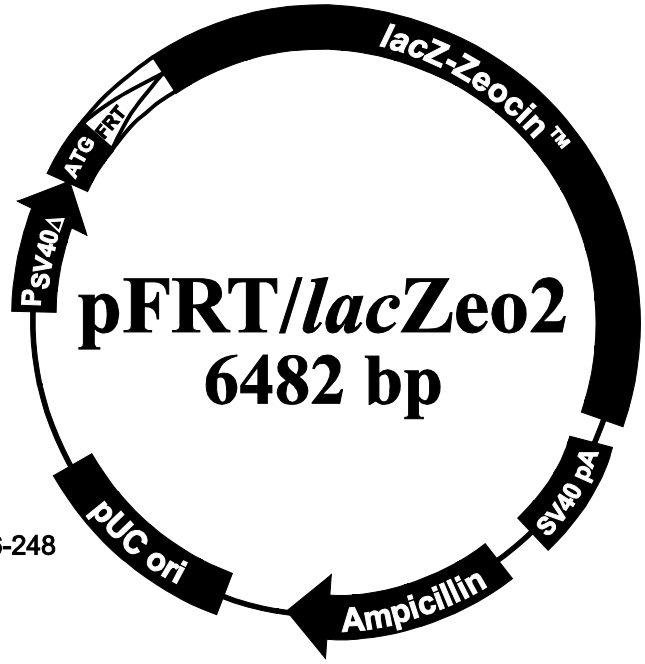
Handling Zeocin™

- 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*Zeo2 Vector

Map of pFRT/*lac*Zeo2

pFRT/*lac*Zeo2 is a 6482 bp vector that expresses a fusion protein containing β-galactosidase and the Zeocin™ resistance marker under the control of a truncated SV40 early promoter ($P_{SV40\Delta}$). 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*Zeo2 vector. The nucleotide sequence for pFRT/*lac*Zeo2 is available for downloading from www.lifetechnologies.com or by contacting Technical Support (see page 19).



Comments for pFRT/*lac*Zeo2
6482 nucleotides

SV40Δ early promoter and origin: bases 36-248
ATG initiation codon: bases 305-307
FRT site: bases 310-357
LacZ-Zeocin™ fusion gene
LacZ ORF (no ATG): bases 374-3418
Zeocin™ resistance gene (no ATG): bases 3506-3877
SV40 early polyadenylation signal: bases 4007-4137
bla promoter: bases 4684-4782
Ampicillin (*bla*) resistance gene: bases 4783-5643
pUC origin: bases 5788-6461

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Features of pFRT/*lac*Zeo2

The table below describes the relevant features of pFRT/*lac*Zeo2. All features have been functionally tested and the vector fully sequenced.

Feature	Benefit
Truncated SV40 early promoter and origin (p_{SV40})	Allows constitutive expression of the *lacZ-Zeocin*™ fusion gene in mammalian cells
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*

Accessory Products

Additional Products

Many of the reagents supplied with the pFRT/*lac*Zeo2 and other reagents suitable for use with the vector are available separately. Ordering information for these reagents is provided below. For more information, refer to www.lifetechnologies.com or call Technical Support (see page 19).

Product	Amount	Catalog no.
One Shot® TOP10 (chemically competent cells)	20 reactions	C4040-03
One Shot® TOP10 Electrocomp™ (electrocompetent cells)	20 reactions	C4040-52
Electrocomp™ TOP10 (electrocompetent cells)	20 reactions	C664-55
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
β-Gal Assay Kit	80 mL	K1455-01
β-Gal Staining Kit	1 kit	K1465-01
PureLink® HiPure MiniPrep Kit	25 preps	K2100-02
PureLink® HiPure Midiprep Kit	25 preps	K2100-04
Lipofectamine® 2000 Reagent	0.75 mL	11668-027
Neon™ Transfection System	1 each	MPK5000
Zeocin™	8 × 1.25 mL	R250-01
	50 mL	R250-05
imMedia™ Amp Agar	20 each	Q601-20
Easy-DNA™ Kit	1 kit	K1800-01

Flp-In™ Host Cell Lines

Several mammalian Flp-In™ host cell lines are available that stably express the *lacZ*-Zeocin™ fusion gene (page 17). The Flp-In™-CHO cell line expresses the *lacZ*-Zeocin™ fusion gene from pFRT/*lac*Zeo2 while other Flp-In™ cell lines express the *lacZ*-Zeocin™ fusion gene from pFRT/*lac*Zeo. Each cell line contains a single integrated FRT site as confirmed by Southern blot analysis. Maintain cell lines in medium containing Zeocin™. For more information, see www.lifetechnologies.com or call Technical Support (see page 19).

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

Continued on next page

Flp-In™ Systems

Product	Amount	Catalog no.
Flp-In™ Complete System	1 kit	K6010-01
Flp-In™ Core System	1 kit	K6010-02

Flp-In™ Expression Vectors

After you have generated your Flp-In™ host cell line using pFRT/*lac*Zeo2, you will use a Flp-In™ expression vector to express your gene of interest. Several Flp-In™ expression vectors are available (see page 17 for ordering). 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. We are always adding to our collection of Flp-In™ expression vectors. For more information about the various Flp-In™ expression vectors available, see www.lifetechnologies.com or contact Technical Support (see page 19).

Product	Amount	Catalog no.
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

Technical Support

Obtaining support

For the latest services and support information for all locations, go to www.lifetechnologies.com/support.

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Notes

Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1 760 603 7200 | Toll Free in USA 800 955 6288
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