USER GUIDE

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by life technologies™

pOG44

Flp-recombinase expression vector designed for use with the Flp-In™ System

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Not for human or animal therapeutic or diagnostic use.

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Contents

Kit Contents and Storage

Contents	40 µL of 0.5 µg/µL pOG44 in TE, pH 8.0.
Shipping and Storage	pOG44 is shipped at room temperature. Store at −30°C to −10°C.

Introduction

About the kit

Introduction

pOG44 is a 5.8 kb Flp recombinase expression vector designed for use with the Flp-In™ System (Catalog nos. K6010-01 and K6010-02). When cotransfected with the pcDNA5/FRT plasmid into a Flp-In™ mammalian host cell line, the Flp recombinase expressed from pOG44 mediates integration of the pcDNA5/FRT vector containing the gene of interest into the genome via Flp Recombination Target (FRT) sites. The vector contains the following elements:

- The human cytomegalovirus (CMV) immediate-early enhancer/promoter for high-level constitutive expression of the Flp recombinase in a wide range of mammalian cells (Andersson *et al.*, 1989; Boshart *et al.*, 1985; Nelson *et al.*, 1987).
- Synthetic intron to enhance expression of the *FLP* gene (Huang and Gorman, 1990; O'Gorman *et al.*, 1991).
- *FLP* gene encoding the Flp recombinase (Buchholz *et al.*, 1996) to mediate integration of the pcDNA5/FRT expression plasmid into the genome.

For more information about the Flp-In™ System, the pcDNA5/FRT plasmid, and generation of the Flp-In™ host cell line, refer to the Flp-In™ System manual. The Flp-In™ System manual is supplied with the Flp-In™ Complete or Core Systems, but is also available at **www.lifetechnologies.com/manuals** or by contacting Technical Support (see page 9).

FLP Gene

The *FLP* gene was originally isolated from the *Saccharomyces cerevisiae* 2μ plasmid (Broach et al., 1982; Broach and Hicks, 1980), and encodes a site-specific recombinase that is a member of the integrase family of recombinases (Argos et al., 1986). The Flp recombinase mediates a site-specific recombination reaction between interacting DNA molecules via the pairing of interacting FRT sites. For more information about site-specific recombination, refer to the next page and published reviews (Craig, 1988; Sauer, 1994).

The native *FLP* gene encodes a protein of 423 amino acids with a calculated molecular weight of 49 kDa. The *FLP* gene expressed from pOG44 encodes a temperature-sensitive Flp recombinase which carries a point mutation (flp-F70L) that results in a change in amino acid 70 from phenylalanine to leucine (Buchholz *et al.*, 1996). For more information about the properties of the flp-F70L protein, see below and Buchholz *et al.*, 1996.

Activity of the Flp Recombinase

When tested in mammalian cells, the native Flp recombinase has been shown to possess optimum recombination activity near 30°C and relatively low activity at 37°C, a result consistent with its physiological role in yeast (Buchholz et al., 1996).

The flp-F70L protein expressed from pOG44 exhibits increased thermolability at 37°C in mammalian cells when compared to the native Flp recombinase (Buchholz et al., 1996). Studies have shown that the Flp recombinase expressed from pOG44 possesses only **10%** of the activity of the native Flp recombinase at 37°C (Buchholz et al., 1996).

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Flp Recombinase-Mediated DNA Recombination

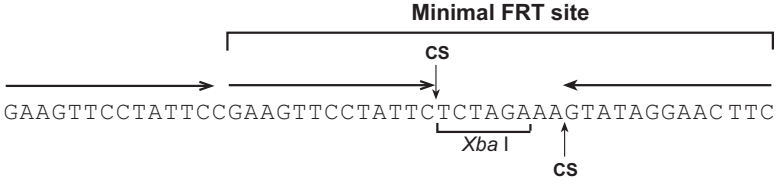
In the Flp-In™ System, integration of the pcDNA5/FRT expression construct containing your gene of interest into the genome occurs via Flp recombinase-mediated intermolecular DNA recombination. The hallmarks of Flp-mediated recombination are:

- 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.

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 the following figure). 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 (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 plasmid is used to express the gene of interest in the Flp-In™ host cell line. For more information about pFRT/*lac*Zeo, pcDNA5/FRT, and the Flp-In™ System, refer to the Flp-In™ System manual.

Generating Stable Expression Cell Lines

You will cotransfect the pOG44 plasmid and your pcDNA5/FRT construct into your Flp-In™ host cell line(s) to generate stable cell lines that express your protein of interest. Cotransfection of pOG44 and pcDNA5/FRT allows expression of Flp recombinase resulting in integration of the pcDNA5/FRT plasmid into the genome via the FRT sites. Once the pcDNA5/FRT construct has integrated into the genome, the Flp recombinase is no longer required. The continued presence of Flp recombinase would actually be detrimental to the cells because it could mediate excision of the pcDNA5/FRT construct. For this reason, **the pOG44 plasmid lacks an antibiotic resistance marker for selection in mammalian cells.** When generating stable expression cell lines, the pOG44 plasmid and, therefore, Flp recombinase expression, will gradually be lost from transfected cells as they are cultured and selected.

Methods

Using pOG44

Introduction

General guidelines to transform pOG44 into *E. coli* are provided in this section.

General Molecular Biology Techniques

For help with *E. coli* transformation, restriction enzyme analysis, and DNA biochemistry, 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 pOG44 vector including TOP10, DH5α, and JM109. We recommend that you propagate the vector in *E. coli* strains that are recombination deficient (*rec*A) and end nuclease A deficient (*end*A).

For your convenience, TOP10 is available as chemically competent or electrocompetent cells (see page 7 for ordering information).

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 pOG44 vector, transform a *rec*A, *end*A *E. coli* strain like TOP10, DH5α, JM109, or equivalent. Select transformants on LB agar plates containing 50 to 100 µg/mL ampicillin. Be sure to prepare a glycerol stock of the plasmid for long-term storage (see the following section).

Preparing a Glycerol Stock

After identifying 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 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.

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 lipids decreasing transfection efficiency. We recommend isolating plasmid DNA using the PureLink® HiPure Miniprep Kit or the PureLink® HiPure Midiprep Kit (see page 7 for ordering information).

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Note

Several Flp-In™ host cell lines which stably express the *lacZ-Zeocin*™ fusion gene and contain a single integrated FRT site are available (see page 7 for ordering information). To express your gene of interest in 293, CV-1, CHO, 3T3, BHK, or Jurkat cells, you may want to use one of the Flp-In™ host cell lines to establish your expression cell line.

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 3T3 or BHK cells. This behavior is not observed with pEF5/FRT-based expression constructs.

If you are generating Flp-In™ expression cell lines using a 3T3 or BHK 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). For more information, refer to **www.lifetechnologies.com** or call Technical Support (see page 9).

Because correct integration of your pcDNA5/FRT construct into the genome is dependent upon Flp recombinase, the expression levels of Flp recombinase in the cell will determine the efficiency of the recombination reaction. Flp recombinase levels must be sufficiently high to mediate recombination at the FRT sites (single recombination event) and overcome the low intrinsic activity of the enzyme (see page 1). We have varied the ratio of pOG44 and pcDNA5/FRT expression plasmid that we cotransfect into mammalian Flp-In™ host cells to optimize the recombination efficiency. **We recommend that you cotransfect you Flp-In™ host cell line with a ratio of *at least* 9:1 (w/w) pOG44:pcDNA5/FRT plasmid.** Note that this ratio may vary depending on the nature of the cell line. You may want to determine this ratio empirically for your cell line.

IMPORTANT!

When transfecting your Flp-In™ host cell line, be sure to use **supercoiled** pOG44 and pcDNA5/FRT plasmid DNA. Flp-mediated recombination between the FRT site on pcDNA5/FRT and the integrated FRT site in the Flp-In™ host cell line will only occur if the pcDNA5/FRT plasmid is circularized. The pOG44 plasmid should be circularized to minimize the possibility of the plasmid integrating into the genome.

Cotransfection

Once you have cloned your gene of interest into pcDNA5/FRT and have prepared clean plasmid preparations of pOG44 and your pcDNA5/FRT construct, cotransfect the plasmids into your mammalian Flp-In™ host cell line to generate your stable Flp-In™ expression cell line. We recommend that you include the appropriate positive and negative controls to help you evaluate your results. Specific guidelines and protocols for generation of the Flp-In™ expression cell line can be found in the Flp-In™ System manual.

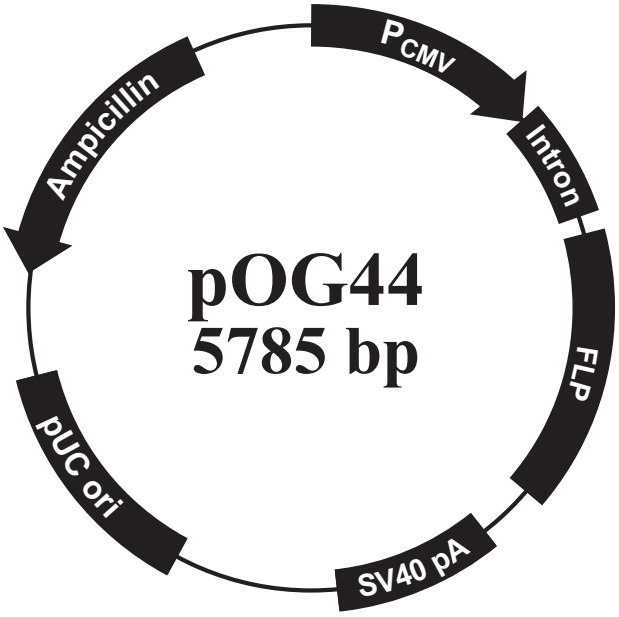
Reminder: The pcDNA5/FRT plasmid contains the hygromycin resistance gene to allow selection of transfectants using hygromycin**. The pOG44 plasmid does not contain an antibiotic resistance gene for selection in mammalian cells (see pages 5–6).**

Appendix

pOG44 Vector

Map of pOG44

pOG44 is a 5785 bp vector that expresses a temperature-sensitive Flp recombinase (flp-F70L) under the control of the human CMV promoter as previously described (O'Gorman *et al.,* 1991). The vector contains a synthetic intron to enhance expression of the *FLP* gene. Note that the vector does not contain an antibiotic resistance marker to allow stable selection in mammalian cells. The following figure summarizes the features of the pOG44 vector. **The sequence for pOG44 is available from www.lifetechnologies.com or by contacting Technical Support (see page 9).**



Comments for pOG44
5785 nucleotides

CMV promoter: bases 234-821
Synthetic intron: bases 871-1175
FLP ORF: bases 1202-2473
SV40 late polyadenylation signal: bases 2597-2732
pUC origin: bases 3327-3993 (complementary strand)
bla promoter: bases 4999-5097 (complementary strand)
Ampicillin (*bla*) resistance gene: bases 4138-4998 (complementary strand)

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Features of pOG44

The following table describes the relevant features of pOG44. All features have been functionally tested.

Feature	Benefit
Human cytomegalovirus (CMV) immediate early promoter	Allows high-level expression of the *FLP* gene (Andersson *et al.*, 1989; Boshart *et al.*, 1985; Nelson *et al.*, 1987).
Synthetic intron	Hybrid fragment which contains sequences derived from the adenovirus major late region and an IgG variable region (Huang and Gorman, 1990; O'Gorman *et al.*, 1991) and functions to enhance expression of the *FLP* gene.
FLP ORF (flp-F70L)	Encodes a temperature-sensitive Flp recombinase (Buchholz *et al.*, 1996) that mediates conservative recombination via FRT sites (O'Gorman *et al.*, 1991).
SV40 late polyadenylation signal	Allows 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 (ß-lactamase)	Allows selection of transformants in *E. coli.*

Accessory Products

Additional products

Many of the reagents used with the Flp-In™ System are available separately. See the following table for ordering information.

Product	Quantity	Catalog no.
pFRT/*lac*Zeo	20 µg	V6015-20
pFRT/*lac*Zeo2	20 µg	V6022-20
pcDNA5/FRT	20 µg	V6010-20
T7 Promoter Primer	2 µg	N560-02
Zeocin™ Selection Reagent	8 × 1.25 mL	R250-01
	50 mL	R250-05
Hygromycin B	20 mL	10687-010
PureLink® HiPure Plasmid Miniprep Kit	100 preps	K2100-03
PureLink® HiPure Plasmid Midiprep Kit	25 preps	K2100-04
One Shot® TOP10F´ (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

Flp-In™ Expression Vectors

Additional Flp-In™ expression vectors are available. For more information about the features of each vector or to download a manual for a vector, refer to **www.lifetechnologies.com/manuals** or call Technical Support (see page 9).

Product	Quantity	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

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Flp-In™ Host Cell Lines

For your convenience, Life Technologies 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 about the Flp-In™ Cell Lines, see **www.lifetechnologies.com** or call Technical Support (see page 9).

Cell Line	Quantity	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

Technical Support

Obtaining support

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Notes

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