Invitrogen™
life technologies

Instruction Manual

pT-REx-DEST Gateway™ Vectors

Destination vectors for use with a tetracycline-regulated expression system

Catalog nos. 12301-016, 12302-014

Version C
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25-0529

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Important Information

Shipping and Storage

The pT-REx-DEST Gateway™ Vectors 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 pT-REx-DEST Gateway™ Vector components are listed below.

Item	Concentration	Volume
Gateway™ Destination Vector (pT-REx-DEST30 **or** pT-REx-DEST31)	lyophilized in TE, pH 8.0	6 µg
Control Plasmid (pT-REx/GW-30/*lac*Z **or** pT-REx/GW-31/*lac*Z)	lyophilized in TE, pH 8.0	10 µg

Quality Control

The pT-REx-DEST Gateway™ vectors as well as the corresponding Control Plasmids are qualified by restriction endonuclease digestion. pT-REx-DEST30 and pT-REx-DEST31 are further qualified in a recombination assay using Gateway™ LR Clonase™ Enzyme Mix. The *ccd*B gene is assayed by transformation using an appropriate *E. coli* strain.

Accessory Products

T-REx™ Cell Lines

Invitrogen offers four mammalian cell lines that stably express the Tet repressor from the pcDNA™6/TR plasmid and should be maintained in medium containing blasticidin. Ordering information is provided below.

Cell Line	Source	Catalog no.
T-REx™-293	Human embryonic kidney	R710-07
T-REx™-HeLa	Human cervical adenocarcinoma	R714-07
T-REx™-CHO	Chinese hamster ovary	R718-07
T-REx™-Jurkat	Human T-cell leukemia	R722-07

Additional Products

Listed below are additional products that may be used with the pT-REx-DEST30 and pT-REx-DEST31 vectors.

Product	Amount	Catalog no.
Gateway™ LR Clonase™ Enzyme Mix	20 reactions	11791-019
One Shot® TOP10 Chemically Competent Cells	10 reactions	C4040-10
	20 reactions	C4040-03
One Shot® TOP10 Electrocompetent Cells	10 reactions	C4040-50
	20 reactions	C4040-52
pcDNA™6/TR	20 µg, lyophilized	V1025-20
Lipofectamine™ 2000 Reagent	.75 ml	11668-027
	1.5 ml	11668-019
Blasticidin	50 mg	R210-01
Geneticin®	1 g	11811-023
	5 g	11811-031
	20 ml (50 mg/ml)	10131-035
	100 ml (50 mg/ml)	10131-027
Tetracycline	5 g	Q100-19

Methods

Overview

Description

The pT-REx-DEST vectors have been adapted for use with the Gateway™ Technology. These vectors allow high-level tetracycline-regulated expression of the gene of interest in mammalian cells expressing the Tet repressor and are designed for use with the T-REx™ System available from Invitrogen (Catalog nos. K1030-01 and K1030-02).

Features

The pT-REx-DEST vectors contain the following elements:

- Hybrid promoter consisting of human cytomegalovirus immediate-early (CMV) promoter/enhancer and tetracycline operator 2 ($TetO_2$) sites for tetracycline-regulated expression in a wide range of mammalian cells
- N-terminal polyhistidine (6xHis) tag for detection and purification **(pT-REx-DEST31 only)**
- Two recombination sites, *att*R1 and *att*R2, downstream of the CMV 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
- SV40 polyadenylation sequence for proper termination and processing of the transcript
- f1 intergenic region for production of single-strand DNA in F plasmid-containing *E. coli*
- SV40 early promoter and origin for expression of the neomycin resistance gene and stable propagation of the plasmid in mammalian hosts expressing the SV40 large T antigen
- Neomycin resistance gene for selection of stable cell lines
- The ampicillin (*bla*) resistance gene for selection in *E. coli*
- The pUC origin for high copy replication and maintenance of the plasmid in *E. coli*

For a map of pT-REx-DEST30, see page 16. For a map of pT-REx-DEST31, see page 17.

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Overview, continued

The Gateway™ Technology

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

1. Clone your gene of interest into a Gateway™ entry vector to create an entry clone.
2. Generate an expression clone by performing an LR recombination reaction between the entry clone and a Gateway™ destination vector (*e.g.* pT-REx-DEST30 or pT-REx-DEST31).
3. Co-transfect your expression clone and pcDNA™6/TR into the cell line of choice for tetracycline-regulated expression of the gene of interest.

For more information on the Gateway™ System, refer to the Gateway™ Technology Manual. This manual is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (page 21).

The T-REx™ System

The pT-REx-DEST vectors are designed to be used with the T-REx™ System. These vectors contain two tetracycline operator 2 ($TetO_2$) sites within the human CMV promoter for tetracycline-regulated expression of your gene of interest (Yao *et al.*, 1998). The $TetO_2$ sequences serve as binding sites for 4 Tet repressor molecules (comprising two Tet repressor homodimers) and confer tetracycline-responsiveness to your gene of interest. In the T-REx™ System, the Tet repressor is expressed from the pcDNA™6/TR plasmid.

In the absence of tetracycline, expression of your gene of interest is repressed by the binding of Tet repressor homodimers to the $TetO_2$ sequences. Addition of tetracycline to the cells derepresses the hybrid CMV/$TetO_2$ promoter and allows expression of your gene of interest.

For more details about the $TetO_2$ sequences, see the next page. For more information about pcDNA™6/TR and the Tet repressor, refer to the T-REx™ System manual. This manual is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (page 21).

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Overview, continued

Tet Operator Sequences

The promoters of bacterial *tet* genes contain two types of operator sequences, O_1 and O_2, that serve as high affinity binding sites for the Tet repressor (Hillen and Berens, 1994; Hillen *et al.*, 1983). Each O_1 and O_2 site binds to one Tet repressor homodimer. While Tet repressor homodimers bind to both *tet* operators with high affinity, studies have shown that the affinity of the Tet repressor homodimer for O_2 is three- to five-fold higher than for O_1 (Hillen and Berens, 1994).

Tet operators have been incorporated into heterologous eukaryotic promoters to allow tetracycline-regulated gene expression in mammalian cells (Gossen and Bujard, 1992; Yao *et al.*, 1998). In the T-REx™ System, two copies of the O_2 operator sequence ($TetO_2$) are inserted into the strong CMV promoter of the pT-REx-DEST vectors to allow regulated expression of your gene of interest by tetracycline. For more information about *tet* operators, refer to Hillen and Berens (1994).

Yao *et al.* (1998) have recently demonstrated that the location of *tet* operator sequences in relation to the TATA box of a heterologous promoter is critical to the function of the *tet* operator. Regulation by tetracycline is only conferred upon a heterologous promoter by proper spacing of the $TetO_2$ sequences from the TATA box (Yao *et al.*, 1998). For this reason, the first nucleotide of the $TetO_2$ operator sequence has been placed 10 nucleotides after the last nucleotide of the TATA element in the CMV promoter for the pT-REx-DEST vectors (see diagrams on pages 6-7).

In other tetracycline-regulated systems, the $TetO_2$ sequences are located upstream of the TATA element in the promoter of the inducible expression vector (Gossen and Bujard, 1992). These systems differ substantially from the T-REx™ System in that they use regulatory molecules composed of the Tet repressor fused to a viral transactivation domain. The presence of viral transactivation domains appears to overcome the requirement for specific positioning of the $TetO_2$ sequences in relation to the TATA box of the heterologous promoter. However, the presence of viral transactivation domains has been found to have deleterious effects in some mammalian cell lines.

Using the pT-REx-DEST Vectors

Important

The pT-REx-DEST vectors are supplied as supercoiled plasmids. Although Invitrogen has previously recommended using a linearized destination vector for more efficient recombination, further testing has found that linearization of these vectors is **NOT** required to obtain optimal results for any downstream application.

Propagating the pT-REx-DEST Vectors

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

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

Resuspending the pT-REx-DEST Vectors

Before you perform the LR Clonase™ reaction, resuspend pT-REx-DEST30 or pT-REx-DEST31 to 50-150 ng/µl in sterile water.

Entry Clone

To recombine your gene of interest into pT-REx-DEST30 or pT-REx-DEST31, you should have an entry clone containing your gene of interest. For your convenience, Invitrogen offers the pENTR Directional TOPO® Cloning Kit (Catalog no. K2400-20) for 5-minute cloning of your gene of interest into an entry vector. For more information on entry vectors available from Invitrogen, refer to our Web site (www.invitrogen.com) or contact Technical Service (page 21).

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 the pT-REx-DEST Vectors, continued

Points to Consider Before Recombining into pT-REx-DEST30

Your insert should contain a Kozak consensus sequence with an ATG initiation codon for proper initiation of translation (Kozak, 1987; Kozak, 1991; Kozak, 1990). An example of a Kozak consensus sequence is provided below. Other sequences are possible, but the G or A at position -3 and the G at position +4 are the most critical for function (shown in bold). The ATG initiation codon is shown underlined.

(G/A)NNATG**G**

Your gene in the entry clone should also contain a stop codon. Refer to the **Recombination Region** on the next page.

Points to Consider Before Recombining into pT-REx-DEST31

pT-REx-DEST31 is an N-terminal fusion vector and contains an ATG codon within the context of a Kozak consensus sequence (Kozak, 1987; Kozak, 1991; Kozak, 1990). Your gene in the entry clone should be in frame with the polyhistidine (6xHis) tag after recombination and should contain a stop codon. Refer to the **Recombination Region** on page 7.

Note: If your gene in the entry clone contains its own Kozak consensus sequence with an ATG initiation codon, proper initiation of translation will still occur immediately upstream of the polyhistidine (6xHis) tag. Infrequently, initiation of translation may also occur at the second Kozak consensus sequence, resulting in expression of a small amount of native, untagged protein.

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 vi 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 the pT-REx-DEST Vectors, continued

Confirming the Expression Clone

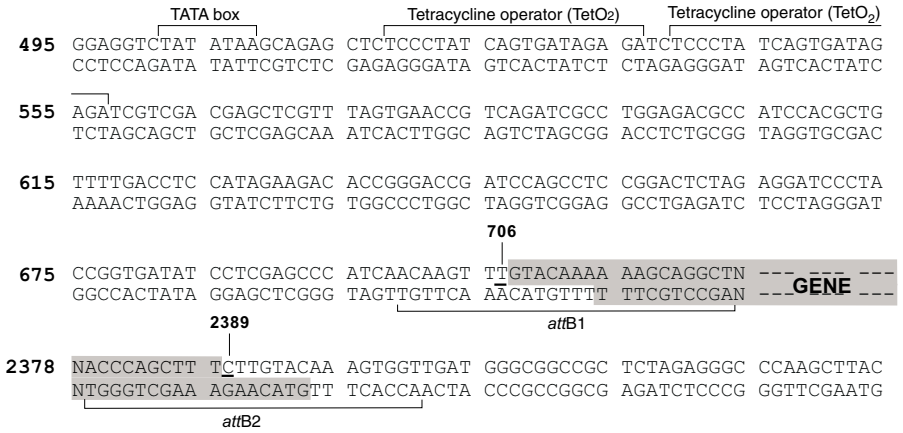
The *ccd*B gene mutates at a very low frequency, resulting in a very low number of false positives. True expression clones will be ampicillin-resistant and chloramphenicol-sensitive. Transformants containing a plasmid with a mutated *ccd*B gene will be both ampicillin- and chloramphenicol-resistant. 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 of pT-REx-DEST30

The recombination region of the expression clone resulting from pT-REx-DEST30 × entry clone is shown below.

Features of the Recombination Region:

- Shaded regions correspond to those DNA sequences transferred from the entry clone into pT-REx-DEST30 by recombination. Non-shaded regions are derived from the pT-REx-DEST30 vector.
- The underlined nucleotides flanking the shaded region correspond to bases 706 and 2389, respectively, of the pT-REx-DEST30 vector sequence.



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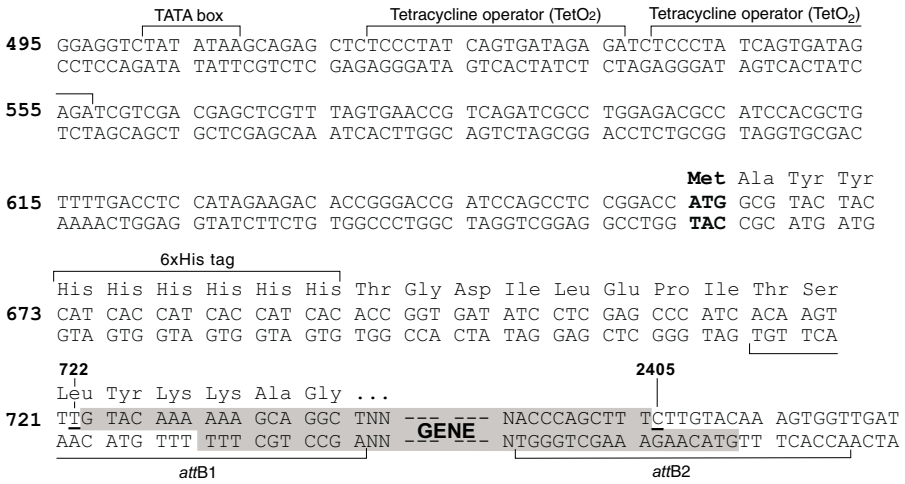
Using the pT-REx-DEST Vectors, continued

Recombination Region of pT-REx-DEST31

The recombination region of the expression clone resulting from pT-REx-DEST31 × entry clone is shown below.

Features of the Recombination Region:

- Shaded regions correspond to those DNA sequences transferred from the entry clone into pT-REx-DEST31 by recombination. Non-shaded regions are derived from the pT-REx-DEST31 vector.
- The underlined nucleotides flanking the shaded region correspond to bases 722 and 2405, respectively, of the pT-REx-DEST31 vector sequence.



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.

Transfection

Introduction

This section provides general information for cotransfecting your expression clone and pcDNA™6/TR into the mammalian cell line of choice. We recommend that you include a positive control vector (pT-REx/GW-30/*lac*Z or pT-REx/GW-31/*lac*Z) and a mock transfection (negative control) in your experiments to evaluate your results.

Four T-REx™ cell lines which stably express the Tet repressor are available from Invitrogen (see page vi for ordering information). If you wish to assay for tetracycline-inducible expression of your gene of interest in one of these cell lines, you may want to use an Invitrogen T-REx™ cell line as your host. For more information, refer to our Web site (www.invitrogen.com) or contact Technical Service (page 21).

Methods of Transfection

For established cell lines (*e.g.* HeLa), consult original references or the supplier of your cell line for the optimal method of transfection. We recommend that you follow exactly the protocol for your cell line. Pay particular attention to medium requirements, when to pass the cells, and at what dilution to split the cells. Further information is provided in *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994).

Methods for transfection include calcium phosphate (Chen and Okayama, 1987; Wigler *et al.*, 1977), lipid-mediated (Felgner *et al.*, 1989; Felgner and Ringold, 1989) and electroporation (Chu *et al.*, 1987; Shigekawa and Dower, 1988). If you wish to use a cationic lipid-based reagent for transfection, we recommend using Lipofectamine™ 2000 Reagent available from Invitrogen (see page vi for ordering information). For more information on transfection reagents available from Invitrogen, refer to our Web site (www.invitrogen.com) or contact Technical Service (page 21).

Note

Your gene of interest will be constitutively expressed if you transfect your pT-REx-DEST construct into mammalian host cells that do not contain the pcDNA™6/TR plasmid.

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Transfection, continued

Positive Control

pT-REx/GW-30/*lac*Z or pT-REx/GW-31/*lac*Z is provided as a positive control vector for mammalian cell transfection and expression (see pages 19-20 for maps) and may be used to optimize recombinant protein expression levels in your cell line. These vectors allow expression of β-galactosidase which may be detected by Western blot or functional assay.

To propagate and maintain the plasmid:

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

Important

Because tetracycline-regulated expression in the T-REx™ System is based on a repression/derepression mechanism, the amount of Tet repressor that is expressed in the host cell line from pcDNA™6/TR will determine the level of transcriptional repression of the Tet operator sequences in your pT-REx-DEST construct. Tet repressor levels should be sufficiently high to suitably repress basal level transcription. Based on our experience, we recommend that you cotransfect your mammalian host cell line with a ratio of **at least 6:1** (w/w) pcDNA™6/TR:pT-REx-DEST construct. You may want to try varying ratios to optimize repression and expression for your particular cell line and your gene of interest.

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Transfection, continued

Cotransfection and Induction with Tetracycline

General guidelines are provided below to cotransfect your pT-REx-DEST construct (or positive control plasmid) and pcDNA™6/TR into your cell line of interest and to induce expression of your protein of interest with tetracycline. Refer to the T-REx™ System manual for more information on transfection and the preparation and handling of tetracycline.

- Use cells that are approximately 60% confluent for transfection.
- Cotransfect pcDNA™6/TR and your pT-REx-DEST construct at a ratio of 6:1 (w:w) into the cell line of choice using your preferred method. Absolute amounts of plasmid will vary depending on the method of transfection and the cell line used.
- After transfection, add fresh medium and allow the cells to recover for 24 hours before induction.
- Remove medium and add fresh medium containing the appropriate concentration of tetracycline to the cells. In general, we recommend that you add tetracycline to a final concentration of 1 µg/ml (5 µl of a 1 mg/ml stock solution per 5 ml of medium) to the cells and incubate the cells for 24 hours at 37°C to obtain maximal induction of your protein of interest.
- Harvest the cells and assay for expression of your gene of interest.

Expression and Analysis

Introduction

Expression of your gene of interest from the expression clone can be performed in transiently transfected cells or stable cell lines (see page 13 for guidelines to create stable cell lines). To detect expression of your recombinant protein by Western blot analysis, you may use an antibody to the protein of interest or an antibody to the polyhistidine (6xHis) tag (pT-REx-DEST31 only). A cell lysis protocol for Western blot analysis is provided below. Other protocols are suitable.

Preparation of Cell Lysates

To lyse cells:

1. Wash cell monolayers (~5 x 10^5 to 1 x 10^6 cells) once with phosphate-buffered saline (PBS, available from Gibco™, Catalog no. 10010-023).
2. Scrape cells into 1 ml PBS and pellet the cells at 1500 x g for 5 minutes.
3. Resuspend in 50 µl Cell Lysis Buffer (see page 14 for a recipe). Other cell lysis buffers are suitable. Vortex.
4. Incubate cell suspension at 37°C for 10 minutes to lyse the cells. **Note:** You may prefer to lyse the cells at room temperature or on ice if degradation of your protein is a potential problem.
5. Centrifuge the cell lysate at 10,000 x g for 10 minutes at +4°C to pellet nuclei and transfer the supernatant to a fresh tube. Assay the lysate for protein concentration. Do not use protein assays utilizing Coomassie Blue or other dyes. NP-40 interferes with the binding of the dye with the protein.
6. Add SDS-PAGE sample buffer (see page 15 for a recipe) to a final concentration of 1X and boil the sample for 5 minutes.
7. Load 20 µg of lysate onto an SDS-PAGE gel and electrophorese. Use the appropriate percentage of acrylamide to resolve your recombinant protein.

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Expression and Analysis, continued

Polyacrylamide Gel Electrophoresis

To facilitate separation and visualization of your protein by polyacrylamide gel electrophoresis, a wide range of pre-cast NuPAGE® and Novex® Tris-Glycine polyacrylamide gels and electrophoresis apparatus are available from Invitrogen. For more information, refer to our Web site (www.invitrogen.com) or contact Technical Service (page 21).

Detecting Recombinant Proteins

To detect expression of your recombinant protein by Western blot analysis, you may use an antibody to the protein of interest or an antibody to the polyhistidine (6xHis) tag (pT-REx-DEST31 only).

Assay for β-galactosidase

If you use pT-REx/GW-30/*lac*Z or pT-REx/GW-31/*lac*Z as a positive control, you may assay for β-galactosidase expression by Western blot analysis or activity assay using cell lysates (Miller, 1972). Invitrogen offers β-Gal Antiserum (Catalog no. R901-25), 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.

Note

The N-terminal polyhistidine (6xHis) tag in pT-REx-DEST31 will add approximately 3 kDa to your protein.

Purification of Recombinant Fusion Proteins

The presence of the N-terminal polyhistidine (6xHis) tag in pT-REx-DEST31 allows for the use of a metal-chelating resin such as ProBond™ to purify your fusion protein. The ProBond™ Purification System (Catalog no. K850-01) and bulk ProBond™ resin (Catalog no. R801-01) are available from Invitrogen. Refer to the ProBond™ Purification System manual for protocols to purify your fusion protein. Invitrogen also offers Ni-NTA Agarose (Catalog no. R901-01) for purification of proteins containing a polyhistidine (6xHis) tag. **Note:** Other metal-chelating resins and purification methods are suitable.

Creating Stable Cell Lines

Introduction

The pT-REx-DEST vectors contain the neomycin resistance gene to allow selection of stable cell lines using Geneticin®. If you wish to create stable cell lines, transfect your construct into the mammalian cell line of choice and select for foci using Geneticin®. General guidelines are provided below.

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

Geneticin®

Geneticin® blocks protein synthesis in mammalian cells by interfering with ribosomal function. It is an aminoglycoside, similar in structure to neomycin, gentamycin, and kanamycin. Expression in mammalian cells of the bacterial aminoglycoside phosphotransferase gene (APH), derived from Tn*5*, results in detoxification of Geneticin® (Southern and Berg, 1982).

Geneticin® Selection Guidelines

Geneticin® is available from Invitrogen (see page vi for ordering information). Use as follows:

1. Prepare Geneticin® in a buffered solution (*e.g.* 100 mM HEPES, pH 7.3).
2. Use 100 to 1000 µg/ml of Geneticin® in complete medium.
3. Calculate concentration based on the amount of active drug.
4. Test varying concentrations of Geneticin® on your cell line to determine the concentration that kills your cells (kill curve). Cells differ in their susceptibility to Geneticin®.

Cells will divide once or twice in the presence of lethal doses of Geneticin® Selective Antibiotic, so the effects of the drug take several days to become apparent. Complete selection can take from 2 to 3 weeks of growth in selective medium.

Appendix

Recipes

LB (Luria-Bertani) Medium and Plates

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

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

LB agar plates

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

Cell Lysis Buffer

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

1. This solution can be prepared from the following common stock solutions. For 100 ml, combine

1 M Tris base	5 ml
5 M NaCl	3 ml
Nonidet P-40	1 ml

2. Bring the volume up to 90 ml with deionized water and adjust the pH to 7.8 with HCl.
3. Bring the volume up to 100 ml. Store at room temperature.

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

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Recipes, continued

4X SDS-PAGE Sample Buffer

1. Combine the following reagents:

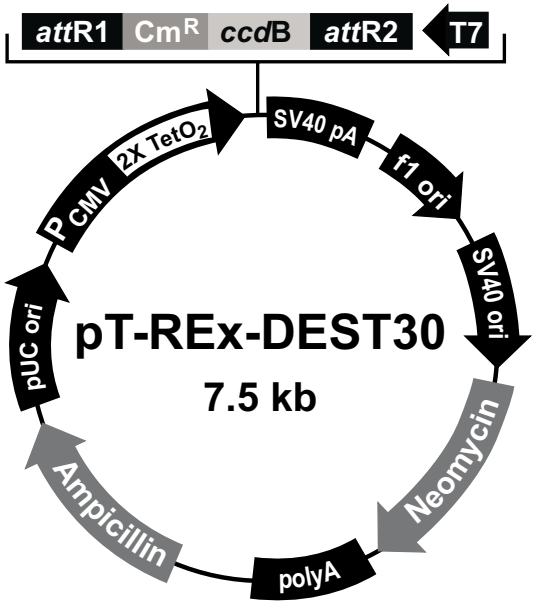
0.5 M Tris-HCl, pH 6.8	5 ml
Glycerol (100%)	4 ml
β-mercaptoethanol	0.8 ml
Bromophenol Blue	0.04 g
SDS	0.8 g

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

Map of pT-REx-DEST30

Map

The map below shows the elements of pT-REx-DEST30. DNA from the entry clone replaces the region between bases 706 and 2389. **The complete sequence of pT-REx-DEST30 is available from our Web site (www.invitrogen.com) or by contacting Technical Service (page 21).**



Comments for pT-REx-DEST30
7544 nucleotides

CMV promoter: bases 1-588
    TATA box: bases 502-508
    Tetracycline operator (2X $TetO_2$) sequence: bases 518-557
*att*R1 recombination site: bases 699-823
Chloramphenicol resistance gene: bases 932-1591
*ccd*B gene: bases 1933-2238
*att*R2 recombination site: bases 2279-2403
T7 promoter: bases 2464-2483 (complementary strand)
SV40 polyadenylation region: bases 2915-3045
f1 origin: bases 3175-3603
SV40 early promoter and origin: bases 3790-4098
Neomycin resistance ORF: bases 4157-4951
Polyadenylation region: bases 5015-5063
Ampicillin (*bla*) resistance ORF: bases 5474-6334
pUC origin: bases 6479-7152

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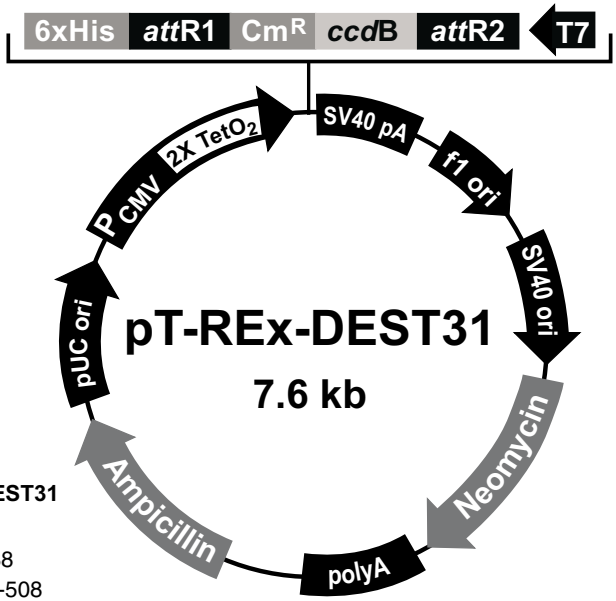
Map of pT-REx-DEST31

Map

The map below shows the elements of pT-REx-DEST31. DNA from the entry clone replaces the region between bases 722 and 2405. **The complete sequence of pT-REx-DEST31 is available from our Web site (www.invitrogen.com) or by contacting Technical Service (page 21).**



Comments for pT-REx-DEST31
7559 nucleotides

CMV promoter: bases 1-588
    TATA box: bases 502-508
    Tetracycline operator (2X $TetO_2$) sequence: bases 518-557
6xHis tag: bases 673-690
*att*R1 recombination site: bases 715-839
Chloramphenicol resistance gene: bases 948-1607
*ccd*B gene: bases 1949-2254
*att*R2 recombination site: bases 2295-2419
T7 promoter: bases 2479-2498 (complementary strand)
SV40 polyadenylation region: bases 2930-3060
f1 origin: bases 3190-3618
SV40 early promoter and origin: bases 3805-4113
Neomycin resistance ORF: bases 4172-4966
Polyadenylation region: bases 5030-5078
Ampicillin (*bla*) resistance ORF: bases 5489-6349
pUC origin: bases 6494-7167

Features of the pT-REx-DEST Vectors

Features

pT-REx-DEST30 (7544 bp)and pT-REx-DEST31 (7559 bp) contain the following elements. All features have been functionally tested.

Feature	Benefit
Human cytomegalovirus (CMV) immediate-early promoter/enhancer	Allows efficient, high-level expression of your recombinant protein (Andersson *et al.*, 1989; Boshart *et al.*, 1985; Nelson *et al.*, 1987)
Tetracycline operator (O_2) sequences	Two tandem 19 nucleotide repeats which serve as binding sites for Tet repressor homodimers (Hillen and Berens, 1994)
N-terminal polyhistidine tag **(pT-REx-DEST31 only)**	Allows purification of recombinant proteins on metal-chelating resin such as ProBond™ Allows detection of recombinant fusion protein using antibodies against the polyhistidine tag
*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
T7 promoter (complementary strand)	Allows efficient *in vitro* transcription in the antisense orientation
SV40 polyadenylation signal	Allows efficient transcription termination and polyadenylation of mRNA (Goodwin and Rottman, 1992)
f1 origin	Allows rescue of single-stranded DNA
SV40 early promoter and origin	Allows high-level expression of the neomycin resistance gene and episomal replication in cells expressing the SV40 large T antigen
Neomycin resistance gene	Allows selection of stable transfectants in mammalian cells (Southern and Berg, 1982)
Polyadenylation signal	Allows efficient transcription termination and polyadenylation of mRNA
Ampicillin resistance gene (β-lactamase)	Allows selection of transformants in *E. coli*
pUC origin	Allows high-copy number replication and growth in *E. coli*

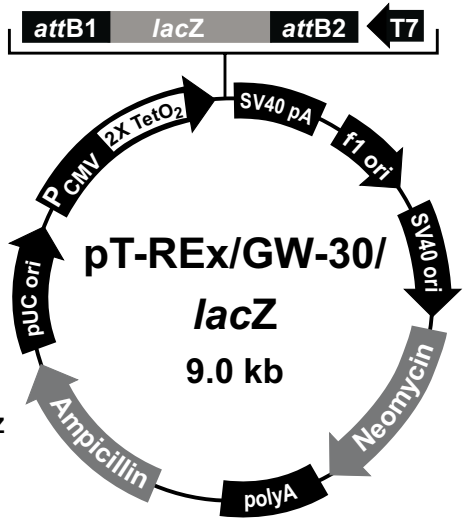
Map of pT-REx/GW-30/*lacZ*

Description

pT-REx/GW-30/*lac*Z is an 8999 bp control vector containing the gene for β-galactosidase. pT-REx/GW-30/*lac*Z was constructed using the Gateway™ LR recombination reaction between an entry clone containing the *lac*Z gene and pT-REx-DEST30. The molecular weight of β-galactosidase is approximately 116 kDa.

Map of pT-REx/GW-30/*lacZ*

The map below shows the elements of pT-REx/GW-30/*lac*Z. **The complete sequence of pT-REx/GW-30/*lac*Z is available from our Web site (www.invitrogen.com) or by contacting Technical Service (page 21).**



Comments for pT-REx/GW-30/*lac*Z
8999 nucleotides

CMV promoter: bases 1-588
TATA box: bases 502-508
Tetracycline operator (2X $TetO_2$) sequence: bases 518-557
*att*B1 recombination site: bases 699-723
*lac*Z ORF: bases 743-3799
*att*B2 recombination site: bases 3834-3858
T7 promoter: bases 3919-3938 (complementary strand)
SV40 polyadenylation region: bases 4370-4500
f1 origin: bases 4630-5058
SV40 early promoter and origin: bases 5245-5553
Neomycin resistance ORF: bases 5612-6406
Polyadenylation region: bases 6470-6518
Ampicillin (*bla*) resistance ORF: bases 6929-7789
pUC origin: bases 7934-8607

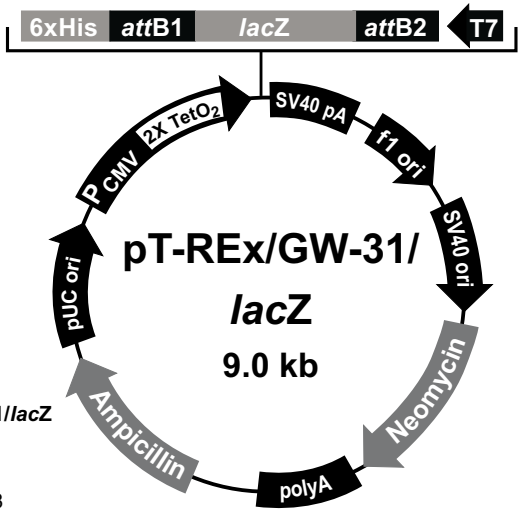
Map of pT-REx/GW-31/*lacZ*

Description

pT-REx/GW-31/*lac*Z is a 9015 bp control vector containing the gene for β-galactosidase. pT-REx/GW-31/*lac*Z was constructed using the Gateway™ LR recombination reaction between an entry clone containing the *lac*Z gene and pT-REx-DEST31. β-galactosidase is expressed as a fusion to the N-terminal 6xHis tag. The molecular weight of the fusion protein is approximately 120 kDa.

Map of pT-REx/GW-31/*lacZ*

The map below shows the elements of pT-REx/GW-31/*lac*Z. **The complete sequence of pT-REx/GW-31/*lac*Z is available from our Web site (www.invitrogen.com) or by contacting Technical Service (page 21).**



Comments for pT-REx/GW-31/*lac*Z
9015 nucleotides

CMV promoter: bases 1-588
TATA box: bases 502-508
Tetracycline operator (2X $TetO_2$) sequence: bases 518-557
6xHis tag: bases 673-690
*att*B1 recombination site: bases 715-739
*lac*Z ORF: bases 759-3815
*att*B2 recombination site: bases 3850-3874
T7 promoter: bases 3934-3953 (complementary strand)
SV40 polyadenylation region: bases 4385-4515
f1 origin: bases 4645-5073
SV40 early promoter and origin: bases 5260-5568
Neomycin resistance ORF: bases 5627-6421
Polyadenylation region: bases 6485-6533
Ampicillin (*bla*) resistance ORF: bases 5489-6349
pUC origin: bases 7949-8622

Technical Service

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- Obtain citations for Invitrogen products
- Request catalog and product literature

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http://www.invitrogen.com

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Technical Service, continued

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5. When you are finished entering information, click the 'Submit' button. Your MSDS will be sent within 24 hours.

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