USER GUIDE

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# Gateway® pcDNA™-DEST47 Vector

# Gateway® pcDNA™-DEST53 Vector

## Destination vectors for cloning and expression of GFP fusion proteins in mammalian cells

**Catalog numbers** 12281-010 and 12288-015

**Revision date** 1 August 2012
**Publication Part number** 25-0411

MAN0000225

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

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# Contents

# Contents and Storage

## Shipping and Storage

The Gateway® pcDNA™-DEST47 and pcDNA™-DEST53 Vectors are shipped at room temperature. Upon receipt, store the vectors at –30°C to –10°C. Products are guaranteed for 6 months from date of shipment when stored properly.

## Contents

The Gateway® pcDNA™-DEST47 and pcDNA™-DEST53 Vector components are listed below.

| Item | Concentration | Amount |
|---|---|---|
| Gateway® Destination Vector (pcDNA™-DEST47 **or** pcDNA™-DEST53) | 40 µL of vector at 150 ng/µL in TE, pH 8.0 (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) | 6 µg |
| Control Plasmid (pcDNA™/GW-47/CAT **or** pcDNA™/GW-53/CAT) | 20 µL of vector at 0.5 µg/µL in TE, pH 8.0 | 10 µg |

# Introduction

## Overview

### Description

Gateway® pcDNA™-DEST47 (7.7 kb) and pcDNA™-DEST53 (7.8 kb) vectors are derived from pcDNA™3.1/CT-GFP and pcDNA™3.1/NT-GFP, respectively, and adapted for use with the Gateway® Technology. They are designed for high-level, constitutive expression of Green Fluorescent Protein (GFP) fusion proteins in most mammalian hosts.

### Features

Gateway® pcDNA™-DEST47 and pcDNA™-DEST53 contain the following elements:

- Human cytomegalovirus immediate-early (CMV) promoter/enhancer for high-level expression in a wide range of mammalian cells.
- 'Cycle 3 mutant' of the green fluorescent protein gene (Cycle 3 GFP) for C-terminal (Gateway® pcDNA™-DEST47) or N-terminal (Gateway® pcDNA™-DEST53) fusion to the gene of interest.
- 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.
- Bovine growth hormone (BGH) 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 pUC origin for high copy replication and maintenance of the plasmid in *E. coli*.
- The ampicillin (*bla*) resistance gene for selection in *E. coli.*

For a map of Gateway® pcDNA™-DEST47, see page 16. For a map of Gateway® pcDNA™-DEST53, see page 17.

*Continued on next page*

## The Gateway® Technology

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

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. Gateway® pcDNA™-DEST47 or pcDNA™-DEST53).
3. Transfect your expression clone into the cell line of choice for transient or stable expression of your gene of interest.

For more information on the Gateway® System, refer to the Gateway® Technology with Clonase® II manual. This manual is available from **www.lifetechnologies.com/manuals** or by contacting Technical Support (page 22).

## Green Fluorescent Protein

The GFP gene used in these vectors is described in (Crameri et al., 1996). In this paper, the codon usage was optimized for expression in *E. coli* and three cycles of DNA shuffling were used to generate a mutant form of GFP that expressed well in mammalian cells and has the following characteristics:

- Excitation and emission maxima that are the same as wild-type GFP (395 nm and 478 nm for primary and secondary excitation, respectively, and 507 nm for emission).
- High solubility in *E. coli* for visual detection of transformed cells if expressed from a promoter recognized by *E. coli*. Note that there is no bacterial promoter upstream of the *att*R1 site in Gateway® pcDNA™-DEST47 or upstream of the GFP gene in Gateway® pcDNA™-DEST53.
- > 40-fold increase in fluorescent yield over wild-type GFP.

This GFP protein is subsequently referred to as Cycle 3 GFP to differentiate it from wild-type GFP.

# Methods

## Using Gateway® pcDNA™-DEST47 and pcDNA™-DEST53

**IMPORTANT!**

The Gateway® pcDNA™-DEST47 and pcDNA™-DEST53 vectors are supplied as supercoiled plasmids. Although Life Technologies 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 Gateway® Vectors

To propagate and maintain Gateway® pcDNA™-DEST47 and pcDNA™-DEST53 vectors, we recommend using 10 ng of the vector to transform One Shot® *ccd*B Survival™ 2 T1$^R$ Chemically Competent Cells (see page 21). The *ccd*B Survival™ 2 T1$^R$ *E. coli* strain is resistant to CcdB effects and can support the propagation of plasmids containing the *ccd*B gene.

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

### Entry Clone

To recombine your gene of interest into Gateway® pcDNA™-DEST47 or pcDNA™-DEST53, you should have an entry clone containing your gene of interest. For your convenience, Life Technologies offers the pENTR Directional TOPO® Cloning Kit for 5-minute cloning of your gene of interest into an entry vector (see page 21 for ordering information). For more information on entry vectors available from Life Technologies, refer to **www.lifetechnologies.com** or contact Technical Support (page 22).

For detailed information on constructing an entry clone, refer to the specific entry vector manual.

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# Using Gateway® pcDNA™-DEST47 and pcDNA™-DEST53, Continued

### Points to Consider Before Recombining into Gateway® pcDNA™-DEST47

Gateway® pcDNA™-DEST47 is a C-terminal fusion vector. 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 following this paragraph. Other sequences are possible, but the G or A at position −3 and the G at position 4 (shown in bold) illustrates the most commonly occurring sequence with strong consensus. Replacing one of the two bases at these positions provides moderate consensus, while having neither results in weak consensus. The ATG initiation codon is shown underlined.

**(G/A)**NNATG**G**

If you wish to include the V5 epitope and 6xHis tag, your gene in the entry clone should not contain a stop codon. The gene should also be designed to be in frame with the C-terminal epitope tag after recombination. Refer to the Recombination Region on page 6.

If you do NOT wish to include the V5 epitope and 6xHis tag, your gene should contain a stop codon in the entry clone.

### Points to Consider Before Recombining into Gateway® pcDNA™-DEST53

Gateway® pcDNA™-DEST53 is an N-terminal fusion vector and contains an ATG initiation codon within the context of a Kozak consensus sequence for proper initiation of translation (Kozak, 1987; Kozak, 1991; Kozak, 1990). Your gene in the entry clone should be in frame with the Cycle 3 GFP gene after recombination and should contain a stop codon. Refer to the **Recombination Region** on page 7.

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# Using Gateway® pcDNA™-DEST47 and pcDNA™-DEST53, Continued

## Gateway® LR Clonase® II Enzyme Mix

Gateway® LR Clonase® II enzyme mix combines the proprietary enzyme formulation and 5X LR Reaction Buffer previously supplied as separate components in Gateway® LR Clonase® enzyme mix into an optimized single tube format to allow easier set-up of the LR recombination reaction (see page 4 for ordering information). For detailed information on performing the LR recombination reaction using LR Clonase® II enzyme mix, refer to the Gateway® Technology with Clonase® II manual.

**Note:** You may perform the LR recombination reaction using LR Clonase® enzyme mix, if desired. To use LR Clonase® enzyme mix, follow the protocol provided with the product.

## 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® II enzyme mix (see page 21 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 with Clonase® II manual to set up the LR Clonase® reaction, transform *E. coli*, and select for the expression clone.

## 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.

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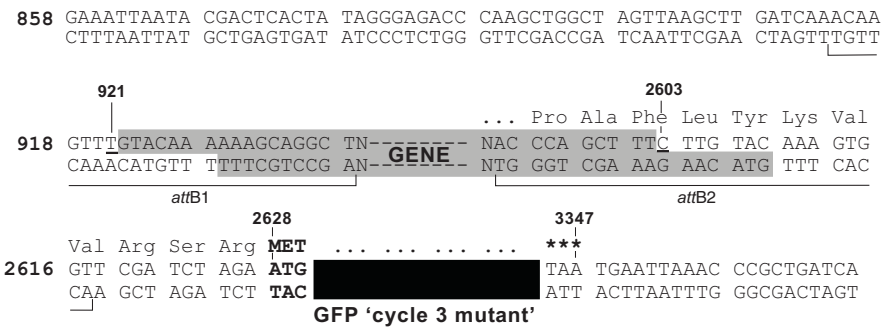
# Using Gateway® pcDNA™-DEST47 and pcDNA™-DEST53, Continued

## Recombination Region of Gateway® pcDNA™-DEST47

The recombination region of the expression clone resulting from Gateway® pcDNA-DEST47 × entry clone is shown in the following figure.

**Features of the Recombination Region:**

- Shaded regions correspond to those DNA sequences transferred from the entry clone into pcDNA™-DEST47 by recombination. Non-shaded regions are derived from the Gateway® pcDNA™-DEST47 vector.
- The underlined nucleotides flanking the shaded region correspond to bases 921 and 2603, respectively, of the pcDNA™-DEST47 vector sequence.

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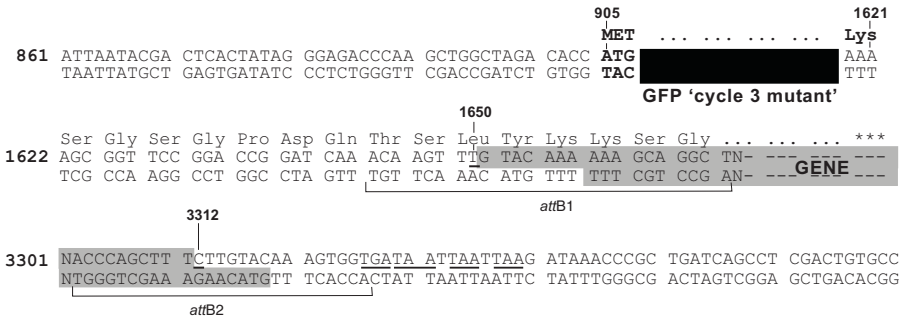
# Using Gateway® pcDNA™-DEST47 and pcDNA™-DEST53, Continued

## Recombination Region of Gateway® pcDNA™-DEST53

The recombination region of the expression clone resulting from Gateway® pcDNA™-DEST53 × entry clone is shown in the following figure.

**Features of the Recombination Region:**

- Shaded regions correspond to those DNA sequences transferred from the entry clone into Gateway® pcDNA™-DEST53 by recombination. Non-shaded regions are derived from the Gateway® pcDNA-DEST53 vector.
- In the event that your gene does not contain a stop codon, there are stop codons in all three frames downstream of the recombination site (underlined codons)
- The underlined nucleotides flanking the shaded region correspond to bases 1650 and 3312, respectively, of the Gateway® pcDNA™-DEST53 vector sequence.



# Transfection

## Introduction

This section provides general information for transfecting your expression clone into the mammalian cell line of choice. We recommend that you include a positive control vector (Gateway® pcDNA™/GW-47/CAT or pcDNA™/GW-53/CAT) and a mock transfection (negative control) to evaluate your results.

## Plasmid Preparation

After generating 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 PureLink® HiPure Plasmid Miniprep Kit (10–15 µg DNA), the PureLink® HiPure Plasmid Midiprep Kit (10–200 µg DNA), or CsCl gradient centrifugation. See page 21 for ordering information.

## 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 (see page 21 for ordering information). For more information, refer to **www.lifetechnologies.com** or contact Technical Support (page 22).

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## Positive Control

Gateway® pcDNA™/GW-47/CAT or pcDNA™/GW-53/CAT 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 a tagged chloramphenicol acetyl transferase (CAT) fusion protein that may be detected by Western blot or functional assay.

**To propagate and maintain the plasmid:**

1. Use 10 ng of the vector supplied in stock solution (0.5 µg/µL in TE, 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 50–100 µg/mL ampicillin.
3. Prepare a glycerol stock of a transformant containing plasmid for long-term storage.

# Expressing Cycle 3 GFP Fusion Proteins

## Introduction

Expression of your Cycle 3 GFP fusion protein can be performed in transiently transfected cells or stable cell lines (see page 14 for guidelines to create stable cell lines). To detect the fusion protein, you may use fluorescence, Western blot analysis, or a functional assay specific for your protein of interest. Fusion of your gene of interest to Cycle 3 GFP allows detection by fluorescence or by Western blot using GFP Antiserum (see page 12).

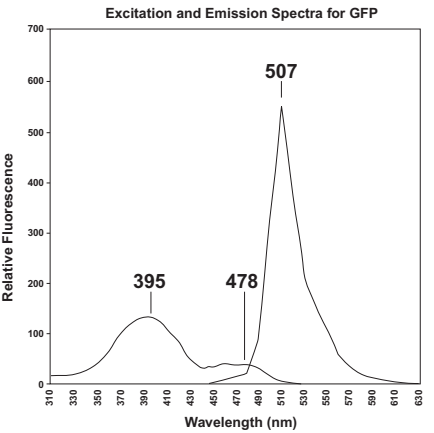
## Expressing Cycle 3 GFP Fusion Proteins

Since your Cycle 3 GFP fusion protein may express differently from the control, we recommend that you perform a time course to optimize expression of the Cycle 3 GFP fusion protein (e.g. 24, 48, 72 hours, etc. after transfection). Use one of the following techniques to evaluate expression.

## Detecting Cycle 3 GFP Fluorescence

**To detect fluorescent cells, it is important to pick the best filter set to optimize detection.** The primary excitation peak of Cycle 3 GFP is at 395 nm. There is a secondary excitation peak at 478 nm. Excitation at either of these wavelengths yields a fluorescent emission peak with a maximum at 507 nm (see the following figure). **Note that the quantum yield can vary as much as 5- to 10-fold depending on the wavelength of light that is used to excite the GFP fluorophore.**

Use of the best filter set will ensure that the optimal regions of the Cycle 3 GFP spectra are excited and passed (emitted). For best results, use a filter set designed to detect fluorescence from wild-type GFP (e.g. XF76 filter from Omega Optical, www.omegafilters.com). FITC filter sets can also be used to detect Cycle 3 GFP fluorescence. For example, the FITC filter set that we use excites Cycle 3 GFP with light from 460–490 nm, which covers the secondary excitation peak. The filter set passes light from 515–550, allowing detection of most of the Cycle 3 GFP fluorescence.

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### Note

Most media fluoresce because of the presence of riboflavin (Zylka and Schnapp, 1996) and may interfere with detection of Cycle 3 GFP fluorescence. Medium can be removed and replaced with PBS during the assay to alleviate this problem. If cells will be cultured further after assaying, remove PBS and replace with fresh medium prior to reincubation.

### Detecting Transfected Cells

After transfection, allow the cells to recover and monitor the cells by fluorescence for expression of Cycle 3 GFP. Note that the CMV promoter is a strong promoter and usually allows detection of Cycle 3 GFP by 24 hours posttransfection.

Estimate the total number of cells before assaying for fluorescence then check your plate for fluorescent cells. You can use fluorescence to estimate transfection efficiency and normalize any subsequent assay for your gene of interest.

### Detecting Fusion Proteins by Western Blot

To detect the fusion protein by Western blot, you will need an antibody to the protein of interest or an antibody to Cycle 3 GFP (see page 12). You will also need to prepare cell lysates. We recommend that you perform a time course to optimize expression of the fusion protein (e.g. 24, 48, 72 hours, *etc*. after transfection). The following cell lysis protocol is provided for your convenience. Other protocols may be suitable.

1. Wash cell monolayers (~$10^6$ cells) once with phosphate-buffered saline available from Life Technologies (see page 21 for ordering information).
2. Scrape cells into 1 mL PBS and pellet the cells at 1500 × *g* for 5 minutes.
3. Resuspend the cells in 50 µL NP-40 Cell Lysis Buffer (see page 15 for a recipe).
4. Incubate the 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.

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# Expressing Cycle 3 GFP Fusion Proteins, Continued

**Detecting Fusion Proteins by Western Blot, continued**

5. Centrifuge the resulting cell lysate at 10,000 × *g* for 10 minutes to pellet nuclei and transfer the post-nuclear lysate to a fresh tube. Assay the lysate for protein concentration.

   **Note**: Do not use assays containing Coomassie® Blue (e.g. Bradford assay) because NP-40 interferes with the binding of dye to the protein.

6. Add SDS-PAGE sample buffer (see page 15 for a recipe) to a final concentration of 1X and heat the sample at 70°C 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 Cycle 3 GFP fusion protein.

**GFP Antiserum**

GFP Antibodies are available separately for detecting your GFP fusion protein (see page 21 for ordering information). Contact Technical Support (see page 22) for more information about Anti-GFP Antibodies.

**Note**

Cycle 3 GFP will add at least 27 kDa to your protein. Remember to account for any additional amino acids located between your protein and Cycle 3 GFP. Refer to pages 6–7 for diagrams. Note that you can use Cycle 3 GFP expressed from the positive control vector as a marker.

**Assay for CAT Protein**

If you use Gateway® pcDNA™/GW-47/CAT or pcDNA™/GW-53/CAT as a positive control vector, you may assay for CAT expression using your method of choice. The *FAST* CAT® Chloramphenicol Acetyltransferase Assay Kit is available separately (see page 21 for ordering information). Other commercial kits are available for assaying CAT expression.

# Troubleshooting

## Trouble-shooting Guide

If you have trouble expressing your fusion protein, try some of the suggestions listed in the following table. Be sure to include positive and negative controls when testing for expression of your protein to ensure that the cells can express Cycle 3 GFP and that the cells were grown, transfected, and assayed correctly.

| Observation | Possible Cause | Solution |
|---|---|---|
| Recombinant protein is not detected on a Western blot | Gene of interest is out of frame with Cycle 3 GFP | Sequence your construct to confirm the protein is in frame with Cycle 3 GFP. |
| Recombinant protein is not active | Cycle 3 GFP interferes with activity or tertiary structure | Try fusing Cycle 3 GFP to the other end of the protein. For example, if you fused Cycle 3 GFP to the C-terminus (using pcDNA-DEST47) and the fusion protein is not active, try fusing Cycle 3 GFP to the N-terminus (using pcDNA-DEST53). |
| Cycle 3 GFP does not fluoresce | Fusion protein interferes with Cycle 3 GFP activity or structure | |

# Creating Stable Cell Lines

## Introduction

Gateway® pcDNA™-DEST47 and pcDNA™-DEST53 contain the neomycin resistance gene to allow selection of stable cell lines using Geneticin® Selective Antibiotic. If you wish to create stable cell lines, transfect your construct into the mammalian cell line of choice and select for foci using Geneticin® Selective Antibiotic. General guidelines are provided in the following sections.


To obtain stable transfectants, we recommend that you linearize your 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® Selective Antibiotic

Geneticin® Selective Antibiotic 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® Selective Antibiotic (Southern and Berg, 1982).

## Geneticin® Selection Guidelines

Geneticin® Selective Antibiotic is available from Life Technologies. Use as follows:

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

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 2–3 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 L.
3. Autoclave on liquid cycle for 20 minutes at 15 psi. Allow solution to cool to 55°C and add antibiotic 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 if needed, 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 |

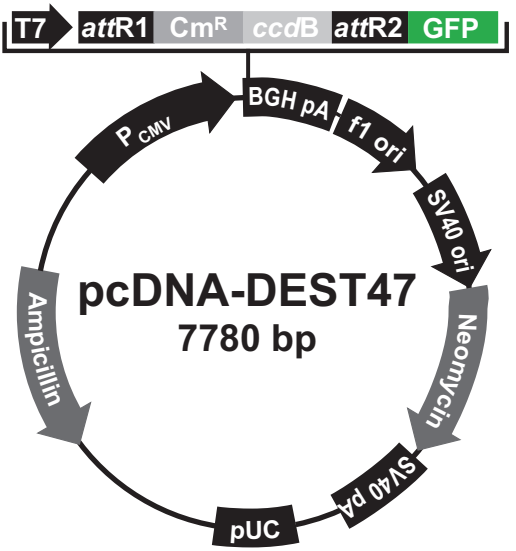
2. Bring the volume up to 90 mL with deionized water and adjust the pH to 7.8 with HCl.
3. Bring the volume up to 100 mL. Store at room temperature.

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

# Map of Gateway® pcDNA™-DEST47

## Map

The following map shows the elements of Gateway® pcDNA™-DEST47. DNA from the entry clone replaces the region between bases 921 and 2603. **The sequence of Gateway® pcDNA™-DEST47 is available at www.lifetechnologies.com or by contacting Technical Support (page 22).**




**Comments for pcDNA-DEST47**
**7780 nucleotides**

CMV promoter: bases 232-819
T7 promoter: bases 863-882
*att*R1 recombination site: bases 914-1038
Chloramphenicol resistance gene: bases 1147-1827
*ccd*B gene: bases 2147-2452
*att*R2 recombination site: bases 2493-2617
Cycle 3 GFP (C-terminal): bases 2628-3347
BGH polyadenylation region: bases 3372-3599
f1 origin: bases 3645-4073
SV40 early promoter and origin: bases 4100-4408
Neomycin resistance ORF: bases 4483-5277
SV40 early polyadenylation region: bases 5453-5583
pUC origin: bases 5966-6639
Ampicillin resistance ORF (*bla*): bases 6784-7644 (c)
*bla* promoter: bases 7645-7743 (c)
(c) = complementary strand

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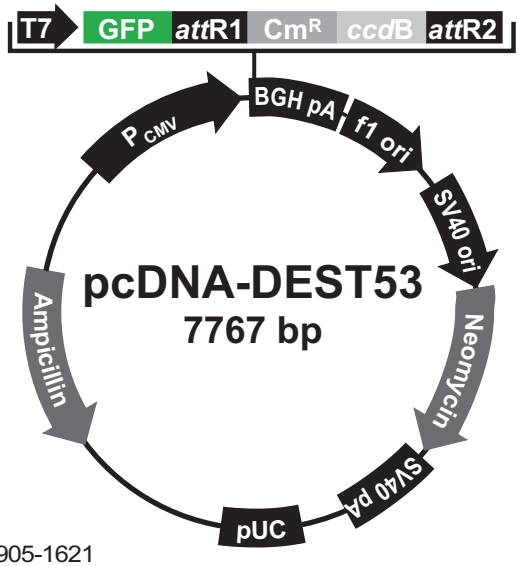
# Map of Gateway® pcDNA™-DEST53

## Map

The following map shows the elements of Gateway® pcDNA™-DEST53. DNA from the entry clone replaces the region between bases 1650 and 3312. **The sequence of Gateway® pcDNA™-DEST53 is available at www.lifetechnologies.com or by contacting Technical Support (page 22).**




**Comments for pcDNA-DEST53**
**7767 nucleotides**

CMV promoter: bases 232-819
T7 promoter: bases 863-882
Cycle 3 GFP (N-terminal): bases 905-1621
*att*R1 recombination site: bases 1643-1767
Chloramphenicol resistance gene: bases 1876-2535
*ccd*B gene: bases 2856-3161
*att*R2 recombination site: bases 3202-3326
BGH polyadenylation region: bases 3361-3588
f1 origin: bases 3634-4062
SV40 early promoter and origin: bases 4089-4397
Neomycin resistance ORF: bases 4472-5266
SV40 early polyadenylation region: bases 5440-5570
pUC origin: bases 5953-6626
Ampicillin resistance ORF (*bla*): bases 6771-7631 (c)
*bla* promoter: bases 7632-7730 (c)

(c) = complementary strand

# Features of Gateway® pcDNA™-DEST47 and pcDNA™-DEST53

## Features

Gateway® pcDNA™-DEST47 (7780 bp) and pcDNA™-DEST53 (7767 bp) contain the following elements. All features have been functionally tested and the vectors fully sequenced.

| 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). |
| T7 promoter | Allows *in vitro* transcription in the sense orientation. |
| Cycle 3 GFP ORF | Allows fusion of Cycle 3 GFP to the C-terminus **(Gateway® pcDNA™-DEST47)** or N-terminus **(Gateway® pcDNA™-DEST53)** of your protein. |
| *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. |
| Bovine growth hormone (BGH) 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). |
| SV40 polyadenylation signal | Allows efficient transcription termination and polyadenylation of mRNA. |
| pUC origin | Allows high-copy number replication and growth in *E. coli.* |
| Ampicillin resistance gene (ß-lactamase) | Allows selection of transformants in *E. coli.* |

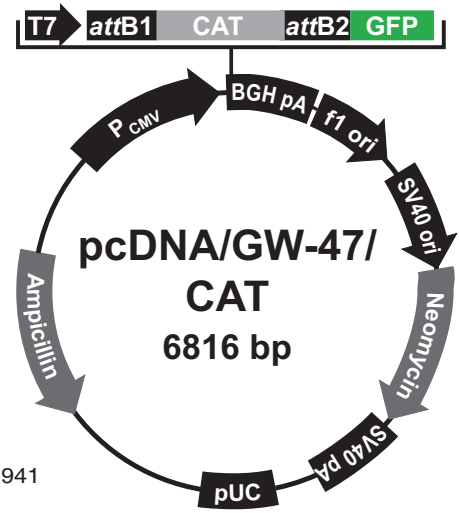
# Map of pcDNA™/GW-47/CAT

## Description

pcDNA™/GW-47/CAT (6816 bp) is a control vector expressing chloramphenicol acetyltransferase (CAT). pcDNA™/GW-47/CAT was constructed using the Gateway® LR recombination reaction between an entry clone containing the CAT gene and Gateway® pcDNA™-DEST47. CAT is expressed with a C-terminal Cycle 3 GFP fusion. The molecular weight of the fusion protein is approximately 55 kDa.

## Map of pcDNA™/GW-47/CAT

The following map shows the elements of pcDNA™/GW-47/CAT. **The sequence of pcDNA™/GW-47/CAT is available from www.lifetechnologies.com or by contacting Technical Support (page 22).**




**Comments for pcDNA/GW-47/CAT 6816 nucleotides**

CMV promoter: bases 232-819
T7 promoter: bases 863-882
*att*B1 recombination site: bases 917-941
CAT ORF: 971-1627
*att*B2 recombination site: bases 1629-1653
Cycle 3 GFP (C-terminal): bases 1664-2383
BGH polyadenylation region: bases 2408-2635
f1 origin: bases 2681-3109
SV40 early promoter and origin: bases 3136-3444
Neomycin resistance ORF: bases 3519-4313
SV40 early polyadenylation region: bases 4489-4619
pUC origin: bases 5002-5675
Ampicillin resistance ORF (*bla*): bases 5820-6680 (c)
*bla* promoter: bases 6681-6779 (c)
(c) = complementary strand

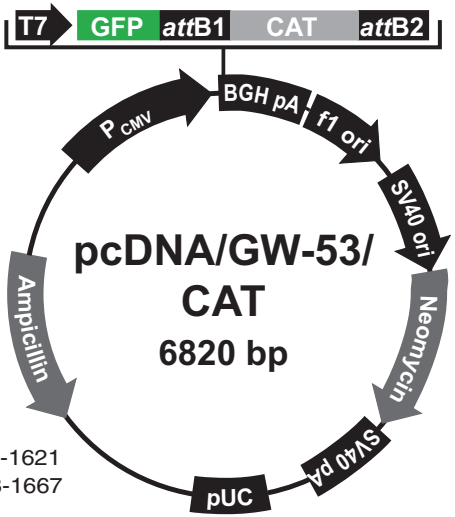
# Map of pcDNA™/GW-53/CAT

## Description

pcDNA™/GW-53/CAT (6820 bp) is a control vector expressing chloramphenicol acetyltransferase (CAT). pcDNA™/GW-53/CAT was constructed using the Gateway® LR recombination reaction between an entry clone containing the CAT gene and Gateway® pcDNA™-DEST53. CAT is expressed with an N-terminal Cycle 3 GFP fusion. The molecular weight of the fusion protein is approximately 55 kDa.

## Map of pcDNA™/GW-53/CAT

The following map shows the elements of pcDNA™/GW-53/CAT. **The sequence of pcDNA™/GW-53/CAT is available from www.lifetechnologies.com or by contacting Technical Support (page 22).**




**Comments for pcDNA/GW-53/CAT 6820 nucleotides**

CMV promoter: bases 232-819
T7 promoter: bases 863-882
Cycle 3 GFP (N-terminal): bases 905-1621
*att*B1 recombination site: bases 1643-1667
CAT ORF: bases 1697-2353
*att*B2 recombination site: bases 2355-2379
BGH polyadenylation region: bases 2414-2641
f1 origin: bases 2687-3115
SV40 early promoter and origin: bases 3142-3450
Neomycin resistance ORF: bases 3525-4319
SV40 early polyadenylation region: bases 4493-4623
pUC origin: bases 5006-5679
Ampicillin resistance ORF (*bla*): bases 5824-6684 (c)
*bla* promoter: bases 6685-6783 (c)
(c) = complementary strand

# Accessory Products

## Additional Products

Additional products that may be used with the Gateway® pcDNA™-DEST47 and pcDNA™-DEST53 vectors are available from Life Technologies. For more information, refer to **www.lifetechnologies.com** or contact Technical Support (see page 22). Ordering information is provided below.

| Product | Amount | Catalog no. |
|---|---|---|
| Gateway® LR Clonase® II Enzyme Mix | 20 reactions | 11791-020 |
| | 100 reactions | 11791-100 |
| One Shot® OmniMAX™ 2 T1$^R$ Phage-Resistant Cells | 20 reactions | C8540-03 |
| One Shot® TOP10 Chemically Competent *E. coli* | 10 reactions | C4040-10 |
| | 20 reactions | C4040-03 |
| One Shot® TOP10 Electrocomp™ *E. coli* | 10 reactions | C4040-50 |
| | 20 reactions | C4040-52 |
| One Shot® *ccd*B Survival™ 2 T1R Competent Cells | 10 reactions | A10460 |
| Lipofectamine®2000 Reagent | 1.5 mL | 11668-019 |
| | 0.75 mL | 11668-027 |
| Geneticin® Selective Antibiotic | 1 g | 11811-023 |
| | 5 g | 11811-031 |
| | 20 mL (50 mg/mL) | 10131-035 |
| | 100 mL (50 mg/mL) | 10131-027 |
| Anti-GFP Antibodies | 100 µL | A11122 |
| *FAST* CAT® Chloramphenicol Acetyltransferase Assay Kit | 1 kit | F-2900 |
| Phosphate Buffered Saline (PBS) | 500 mL | 10010-023 |

# Technical Support

## Obtaining support

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

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

## Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/support**.

## Certificate of Analysis

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**Gateway® Clone Distribution Policy**

For additional information about Life Technologies' policy for the use and distribution of Gateway® clones, see the section entitled **Gateway® Clone Distribution Policy**, page 26.

**Limited Use Label License No. 54: ULB ccdB Selection Technology**

Notice to Purchaser: This product is sold under license from the Université Libre de Bruxelles for research purposes only. ccdB selection technology is described in Bernard et al., "Positive Selection Vectors Using the F Plasmid ccdB Killer Gene" Gene 148 (1994) 71-74. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). For information on obtaining additional rights, please contact **outlicensing@lifetech.com**.

**Limited Use Label License No. 55: Cycle 3 GFP**

The 'cycle 3' mutant GFP was produced by Maxygen, Inc. using DNA shuffling technology. Commercial licensing inquiries should be directed to: Affymax Research Institute, 4001 Miranda Avenue, Palo Alto, CA 94304, U.S.A.

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# Purchaser Notification, Continued

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# Gateway® Clone Distribution Policy

## Introduction

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

## Gateway® Entry Clones

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

## Gateway® Expression Clones

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

## Additional Terms and Conditions

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

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# Notes

# Notes

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