



Vivid Colors™ pcDNA™6.2/EmGFP and YFP-DEST Gateway® Vectors

Gateway®-adapted destination vectors for
expression of N- and C-terminal EmGFP and
YFP fusion proteins in mammalian cells

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Kit Contents and Storage

Types of Products

This manual is supplied with the following products:

Product	Cat. no.
Vivid Colors™ pcDNA™ 6.2/C-EmGFP-DEST Gateway® Vector	V355-20
Vivid Colors™ pcDNA™ 6.2/N-EmGFP-DEST Gateway® Vector	V356-20
Vivid Colors™ pcDNA™ 6.2/C-YFP-DEST Gateway® Vector	V357-20
Vivid Colors™ pcDNA™ 6.2/N-YFP-DEST Gateway® Vector	V358-20

Shipping and Storage

The Vivid Colors™ pcDNA™ 6.2/EmGFP and YFP-DEST Gateway® Vectors are shipped at room temperature. Upon receipt, store at -20°C.

Kit Contents

Each Vivid Colors™ Gateway® vector includes a destination vector and a corresponding expression control plasmid containing the CAT gene as listed below. All vectors are supplied in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0.

Item	Concentration	Quantity
Vivid Colors™ pcDNA™ 6.2/N-EmGFP-DEST Gateway® Vector	150 ng/μL (40 μL total)	6 μg
pcDNA™ 6.2/N-EmGFP/GW/CAT control vector	0.5 μg/μL (20 μL total)	10 μg
Vivid Colors™ pcDNA™ 6.2/N-YFP-DEST Gateway® Vector	150 ng/μL (40 μL total)	6 μg
pcDNA™ 6.2/N-YFP/GW/CAT control vector	0.5 μg/μL (20 μL total)	10 μg
Vivid Colors™ pcDNA™ 6.2/C-EmGFP-DEST Gateway® Vector	150 ng/μL (40 μL total)	6 μg
pcDNA™ 6.2/C-EmGFP/GW/CAT control vector	0.5 μg/μL (20 μL total)	10 μg
Vivid Colors™ pcDNA™ 6.2/C-YFP-DEST Gateway® Vector	150 ng/μL (40 μL total)	6 μg
pcDNA™ 6.2/C-YFP/GW/CAT control vector	0.5 μg/μL (20 μL total)	10 μg

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

Introduction

Product Overview

Description of the System

Vivid Colors™ pcDNA™6.2/EmGFP and YFP-DEST vectors combine the ease and flexibility of Gateway® recombination based cloning with the brightness of Emerald Green Fluorescent Protein (EmGFP) and Yellow Fluorescent Protein (YFP) derived from *Aequorea victoria* GFP.

Users can easily make an EmGFP or YFP N- or C-terminally tagged mammalian expression clone by performing an LR recombination reaction between a Gateway® entry vector containing the gene of choice and a Vivid Colors™ pcDNA™6.2/EmGFP or YFP-DEST Gateway® Vector. After transfection of the expression clone into mammalian cells, the fluorescent-tagged protein of interest can be identified by fluorescence detection methods for localization experiments. The protein of interest can also be analyzed by Western blot.

The Vivid Colors™ pcDNA™6.2/EmGFP or YFP-DEST Gateway® Vectors are supplied with either an N- or C-terminal tagged EmGFP or YFP/GW/CAT plasmid that serves as a control for transfection efficiency of the expression clone into the target cells, as well as a control for expression of the gene of interest.

Continued on next page

Product Overview, Continued

Features of the Vectors

The Vivid Colors™ pcDNA™ 6.2/EmGFP or YFP-DEST Gateway® Vectors contain the following elements:

- Human cytomegalovirus immediate-early (CMV) promoter/enhancer for high-level expression in a wide range of mammalian cells
- Two recombination sites, *attR1* and *attR2*, downstream of the CMV promoter for recombinational cloning of the gene of interest from an entry clone
- Emerald Green Fluorescent Protein (EmGFP) or Yellow Fluorescent Protein (YFP) derived from *Aequorea victoria* GFP for N- or C-terminal fusion to the gene of interest for fluorescent detection
- The V5 epitope tag for detection of recombinant protein using Anti-V5 antibodies (optional on N-terminal fusion vectors only)
- CAT gene located between the two *attR* sites for counterselection
- The *ccdB* gene located between the two *attR* sites for negative selection
- The Herpes Simplex Virus thymidine kinase polyadenylation signal for proper termination and processing of the recombinant 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 Blasticidin resistance gene and stable propagation of the plasmid in mammalian hosts expressing the SV40 large T antigen
- Blasticidin 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 maps and features of the pcDNA™ 6.2/EmGFP or YFP-DEST Gateway® Vectors, see pages 28-33.

Continued on next page

Product Overview, Continued

Green Fluorescent Protein (GFP)

Green Fluorescent Protein (GFP) is a naturally occurring bioluminescent protein derived from the jellyfish *Aequorea victoria* (Shimomura *et al.*, 1962). GFP emits fluorescence upon excitation, and the gene encoding GFP contains all of the necessary information for posttranslational synthesis of the luminescent protein. GFP is often used as a molecular beacon because it requires no species-specific cofactors for function, and the fluorescence is easily detected using fluorescence microscopy and standard filter sets. Commonly, GFP is fused to a protein of interest, and upon expression, the localization of the fusion protein can be detected in cells. GFP can also function as a reporter gene downstream of a promoter of interest.

GFP and Spectral Variants

Modifications have been made to the wild-type GFP to enhance its expression in mammalian systems. These modifications include amino acid substitutions that correspond to the codon preference for mammalian use, and mutations that increase the brightness of the fluorescence signal, resulting in “enhanced” GFP (Zhang *et al.*, 1996). Mutations have also arisen or have been introduced into GFP that further enhance and shift the spectral properties of GFP such that these proteins will emit fluorescent color variations (reviewed in Tsien, 1998). The Emerald GFP (EmGFP) and Yellow Fluorescent Protein (YFP) are such variants of enhanced GFP.

Continued on next page

Product Overview, Continued

EmGFP and YFP

The EmGFP and YFP variants have been described in a published review (Tsien, 1998) and are summarized in the table below. The amino acid mutations are represented by the single letter abbreviation for the amino acid in the consensus GFP sequence, followed by the codon number and the single letter amino acid abbreviation for the substituted amino acid.

Fluorescent Protein	GFP Mutations*
EmGFP	S65T, S72A, N149K, M153T, I167T
YFP	S65G, S72A, K79R, T203Y

*Mutations listed are as described in the literature. When examining the actual sequence, the vector codon numbering starts at the first amino acid **after** the initiation methionine of the fluorescent protein, so that mutations appear to be increased by one position. For example, the S65T mutation actually occurs in codon 66 of EmGFP.

EmGFP and YFP Fluorescence

The fluorescent proteins from the Vivid Colors™ pcDNA™ 6.2/EmGFP and YFP-DEST vectors have the following excitation and emission wavelengths, as published in the literature (Tsien, 1998):

Fluorescent Protein	Excitation (nm)	Emission (nm)
EmGFP	487	509
YFP	514	527

Filter Sets for Detecting EmGFP or YFP Fluorescence

Both the EmGFP and YFP can be detected with standard FITC filter sets. However, for optimal detection of the fluorescence signal, you may use a filter set which is optimized for detection within the excitation and emission ranges for each of the fluorescent proteins.

These filter sets are available from Omega Optical (www.omegafilters.com), and Chroma (www.chroma.com).

Continued on next page

Product Overview, Continued

Gateway® Technology

Gateway® Technology is a universal cloning method that takes advantage of the site-specific recombination properties of bacteriophage lambda (Landy, 1989).

The Gateway® Technology enables rapid and highly efficient transfer of DNA sequences into multiple vector systems for protein expression and functional analysis while maintaining orientation and reading frame. Gateway® Technology also permits the use and expression of multiple types of DNA sequences (*e.g.* PCR products, cDNA clones, or restriction fragments).

For more information on the Gateway® Technology, refer to the Gateway® Technology with Clonase® II manual. This manual is available for downloading from www.lifetechnologies.com or by contacting Technical Support (page 36).

Experimental Outline

To express your gene of interest with an N- or C- terminal EmGFP or YFP fusion peptide in mammalian cells using Gateway® Technology, you will perform the following steps:

1. Generate an entry clone containing your gene of interest
2. Generate an expression clone by performing an LR recombination reaction between the entry clone and a Vivid Colors™ pcDNA™6.2/EmGFP or YFP-DEST vector.
3. Transfect your expression clone into the mammalian cell line of choice for transient or stable expression of your gene of interest.

The following sections of this manual provide instructions and guidelines for these steps.

Methods

Generating an Entry Clone

Introduction

To recombine your gene of interest into the Vivid Colors™ pcDNA™ 6.2/EmGFP or YFP-DEST Gateway® Vectors, you will first need an entry clone containing the gene of interest. This section provides guidelines for obtaining or generating an entry clone.

Experimental Approach

There are several ways to create an entry clone containing your gene of interest. The following methods are described in detail in the Gateway® Technology with Clonase® II manual.

- TOPO® clone your gene of interest into one of several available TOPO® charged entry clones.
 - Use conventional cloning with restriction enzymes and sub-clone your gene of interest into a supercoiled pENTR™ vector
 - Purchase one of our Ultimate™ Human ORF or Mouse ORF clones
 - Transfer your gene of interest from an existing Gateway® expression vector by performing a BP recombination reaction with a pDONR™ vector.
-

Entry Vectors

Many entry vectors are available for purchase to facilitate the generation of entry clones (see table below for vector recommendations). Refer to the manual for the specific entry vector you are using for detailed instructions to construct an entry clone. To choose an entry vector, visit www.lifetechnologies.com or contact Technical Support (page 36).

Entry Vector	Cat. no.
pENTR™/D-TOPO®	K2400-20
pENTR™/SD/TOPO®	K2420-20
pENTR™/TEV/D-TOPO®	K2525-20

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Generating an Entry Clone, Continued

Ultimate™ ORF

To express a human or mouse gene of interest, we recommend using an Ultimate™ Human ORF or Mouse ORF Clone. Each Ultimate™ ORF Clone is a fully sequenced clone provided in a Gateway® entry vector that is ready-to-use in an LR recombination reaction with pcDNA™6.2/EmGFP-DEST or pcDNA™6.2/YFP-DEST vectors.

For more information about Ultimate™ ORF Clones, visit www.lifetechnologies.com or contact Technical Support (page 36).

Designing Your Fusion Construct

Vivid Colors™ pcDNA™6.2/EmGFP or YFP-DEST vectors are fusion vectors, designed to provide an N- or C-terminal fusion to your gene of choice. However, some features of the EmGFP or YFP-DEST vectors are dependent upon the design of the entry clone insert:

- The pcDNA™6.2/C-EmGFP-DEST and pcDNA™6.2/C-YFP-DEST vectors require a Kozak consensus sequence at the beginning of your gene of interest.
- The pcDNA™6.2/N-EmGFP and pcDNA™6.2/N-YFP-DEST vectors contain the V5 epitope, which will be expressed at the C-terminal end of the fusion protein if your gene of interest does not have a stop codon.

These features are discussed below and summarized on the following page.

Kozak Consensus Sequence

If you are making an expression clone using the pcDNA™6.2/C-EmGFP-DEST or pcDNA™6.2/C-YFP-DEST vector, for proper initiation of translation, your gene of interest must contain a Kozak translation initiation sequence with an ATG initiation codon (Kozak, 1987; Kozak, 1991; Kozak, 1990). An example of a Kozak consensus sequence is provided below. The ATG initiation codon is shown underlined.

(G/A)NNATGN

Other sequences are possible, but G or A at position -3 (shown in bold) is critical for a functional Kozak sequence. At position +4, any of the four nucleotides can be present to form part of the Kozak sequence.

Continued on next page

Generating an Entry Clone, Continued

V5 Epitope

The V5 epitope is a 14 amino acid epitope (GKPIP_NPLLDST) derived from the P and V proteins of the SV5 paramyxovirus (Southern *et al.*, 1991). If you are making an expression clone using the pcDNA™ 6.2/N-EmGFP-DEST or pcDNA™ 6.2/N-YFP-DEST vector, you may express the V5 epitope located at the C-terminal end of the fusion by not including a stop codon in your gene of interest. Fusion proteins containing the V5 epitope can be detected using specific antibodies (see page 35 for ordering information).

Points to Consider Before Recombining

To obtain the fusion protein that you want from the expression clone, consider the options below before designing your entry clone. For more information on the recombination regions of the vectors, refer to pages 10–11.

To create an expression clone using...	And you wish to...	Then your insert...
pcDNA™ 6.2/N-EmGFP-DEST or pcDNA™ 6.2/N-YFP-DEST	Include the N-terminal fluorescent fusion and NOT the V5 epitope	Should contain a stop codon
pcDNA™ 6.2/N-EmGFP-DEST or pcDNA™ 6.2/N-YFP-DEST	Include the N-terminal fluorescent fusion and the V5 epitope	Should not contain a stop codon (it will be in frame with the V5 epitope).
pcDNA™ 6.2/C-EmGFP-DEST or pcDNA™ 6.2/C-YFP-DEST	Express your protein of interest fused to EmGFP or YFP at the C-terminus	<ul style="list-style-type: none"> • Should contain a Kozak consensus sequence • Should not contain a stop codon

Creating an Expression Clone

Introduction

After you have generated an entry clone, you will perform the LR recombination reaction to transfer your gene of interest into a Vivid Colors™ pcDNA™ 6.2/EmGFP or YFP-DEST vector to create an expression clone. To ensure that you obtain the best results, we recommend that you read this section and the next section entitled **Performing the LR Recombination Reaction** (page 12) before beginning.

Experimental Outline

To generate an expression clone, you will:

1. Perform an LR recombination reaction using the *attL*-containing entry clone and the *attR*-containing pcDNA™ 6.2/EmGFP or YFP-DEST vector of choice.
 2. Use the reaction mixture to transform a suitable *E. coli* host (see page 12).
 3. Select for expression clones (refer to pages 10–11 for diagrams of the recombination region of the resulting expression clones).
-

Propagating the Vectors

To propagate and maintain the pcDNA™ 6.2/EmGFP or YFP-DEST vectors, we recommend using the supplied vector to transform One Shot® *ccdB* Survival™ T1^R Chemically Competent *E. coli* (see page 34 for ordering). The *ccdB* Survival™ T1^R *E. coli* strain is resistant to CcdB effects and supports the propagation of plasmids containing the *ccdB* gene. To maintain the integrity of the vector, select for transformants in media containing 50-100 µg/mL ampicillin and 15-30 µg/mL chloramphenicol.

Note: Do not use general *E. coli* cloning strains including TOP10 or DH5α™ for propagation and maintenance of the Vivid Colors™ pcDNA™ 6.2/EmGFP or YFP-DEST vectors, as these strains are sensitive to CcdB effects.

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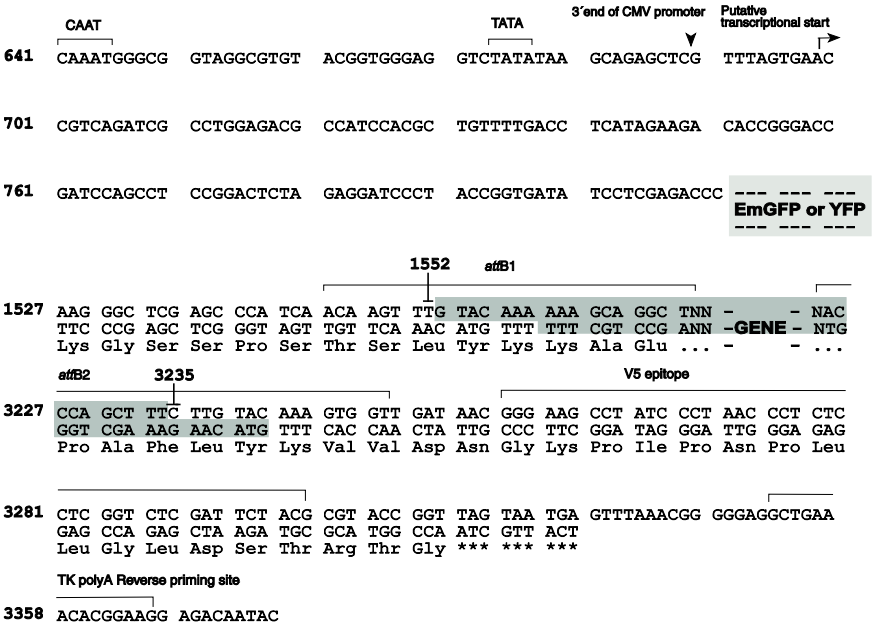
Creating an Expression Clone, Continued

Recombination Region for pcDNA™ 6.2/ N-EmGFP-DEST and pcDNA™ 6.2/ N-YFP-DEST

The recombination region of the expression clone resulting from Vivid Colors™ pcDNA™ 6.2/N-EmGFP-DEST × entry clone or pcDNA™ 6.2/N-YFP-DEST × entry clone is shown below.

Features of the Recombination Region:

- Shaded regions between the *attB* sites correspond to DNA sequences transferred from the entry clone into pcDNA™ 6.2/N-EmGFP-DEST or pcDNA™ 6.2/N-YFP-DEST by recombination. Non-shaded regions are derived from the pcDNA™ 6.2/N-EmGFP-DEST or pcDNA™ 6.2/N-YFP-DEST vectors.
- The overlined nucleotides flanking the shaded region correspond to bases 1552 and 3235 of the pcDNA™ 6.2/N-EmGFP-DEST vector or the pcDNA™ 6.2/N-YFP-DEST vector sequence.



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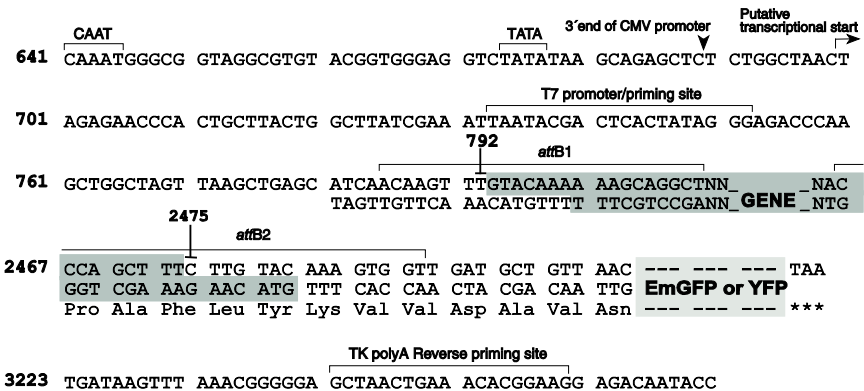
Creating an Expression Clone, Continued

Recombination Region for pcDNA™ 6.2/C-EmGFP-DEST and pcDNA™ 6.2/C-YFP-DEST

The recombination region of the expression clone resulting from Vivid Colors™ pcDNA™ 6.2/C-EmGFP-DEST × entry clone or pcDNA™ 6.2/C-YFP-DEST × entry clone is shown below.

Features of the Recombination Region:

- Shaded regions between the *attB* sites correspond to DNA sequences transferred from the entry clone into pcDNA™ 6.2/C-EmGFP-DEST or pcDNA™ 6.2/C-YFP-DEST by recombination. Non-shaded regions are derived from the pcDNA™ 6.2/C-EmGFP-DEST or pcDNA™ 6.2/C-YFP-DEST vectors.
- The overlined nucleotides flanking the shaded region correspond to bases 792 and 2475 of the pcDNA™ 6.2/C-EmGFP-DEST or pcDNA™ 6.2/C-YFP-DEST vector sequence.



Performing the LR Recombination Reaction

Introduction

Once you have obtained an entry clone containing your gene of interest, you may perform an LR recombination reaction between the entry clone and a Vivid Colors™ pcDNA™ 6.2/EmGFP or YFP-DEST vector. Then you can use the reaction mixture to transform a suitable *E. coli* host (see below) and select for an expression clone. We recommend including a positive control (pENTR™-gus; included with LR Clonase® II) and a negative control (omitting the LR Clonase® II enzyme mix) in your experiment to help you evaluate your results.

E. coli Host

You may use any *E. coli* strain containing *recA* and *endA* including TOP10, DH5α™, or equivalent for transformation (see page 34 for ordering information). **Do not** use the LR reaction mixture to transform *E. coli* strains that contain the F' episome (e.g. TOP10F'). These strains contain the *ccdA* gene and will prevent negative selection with the *ccdB* gene.

LR Clonase® II Enzyme Mix

LR Clonase® II enzyme mix is available separately for purchase (page 34) to catalyze the LR recombination reaction. The LR Clonase® II enzyme mix combines the proprietary enzyme formulation and 5X LR Clonase® Reaction Buffer previously supplied as separate components with LR Clonase® enzyme mix into an optimized single-tube format for easier set-up of the LR recombination reaction. Use the protocol provided on the next page to perform the LR recombination reaction using the LR Clonase® II enzyme mix.

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. **Do not** use the protocol for LR Clonase® II enzyme mix as reaction conditions differ.

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Performing the LR Recombination Reaction, Continued

Materials Needed

- Purified plasmid DNA of your entry clone (50-150 ng/ μ L in TE, pH 8.0)
- Vivid Colors™ pcDNA™ 6.2/EmGFP or YFP-DEST Gateway® Vector (150 ng/ μ L in TE, pH 8.0)
- LR Clonase® II enzyme mix (page 34; keep at -20°C until immediately before use)
- pENTR™-gus (supplied with LR Clonase® II enzyme mix; use as a control for the LR reaction; 50 ng/ μ L)
- TE Buffer, pH 8.0 (10 mM Tris-HCl, pH 8.0, 1 mM EDTA)
- 2 $\mu\text{g}/\mu\text{L}$ Proteinase K solution (supplied with LR Clonase® II enzyme mix; thaw and keep on ice until use)
- Appropriate competent *E. coli* host (see previous page) and growth medium for expression
- S.O.C. Medium
- LB agar plates containing 100 $\mu\text{g}/\text{mL}$ ampicillin

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Performing the LR Recombination Reaction, Continued

LR Recombination Reaction

Follow this procedure to perform the LR reaction between your entry clone and the destination vector. To include a negative control, set up a second sample reaction, but omit the LR Clonase® II enzyme mix.

1. Add the following to 1.5 mL microcentrifuge tubes at room temperature and mix.

Component	Sample	Positive Control
Entry clone (50–150 ng/reaction)	1–7 µL	–
Destination vector (150 ng/µL)	1 µL	1 µL
pENTR™-gus (50 ng/µL)	–	2 µL
TE Buffer, pH 8.0	to 8 µL	5 µL

2. Remove the LR Clonase® II enzyme mix from –20°C and thaw on ice (~ 2 minutes).
3. Vortex the LR Clonase® II enzyme mix briefly twice (2 seconds each time).
4. To each sample above, add 2 µL of LR Clonase® II enzyme mix. Mix well by pipetting up and down.
Reminder: Return LR Clonase® II enzyme mix to –20°C immediately after use.
5. Incubate reactions at 25°C for 1 hour.
Note: Extending the incubation time to 18 hours typically yields more colonies.
6. Add 1 µL of the Proteinase K solution to each reaction. Incubate for 10 minutes at 37°C.
7. Use 1 µL of the LR recombination reaction to transform a suitable *E. coli* host (follow the manufacturer's instructions) and select for expression clones.
Note: You may store the LR reaction at –20°C for up to 1 week before transformation, if desired.

Selection of Expression Clones

What You Should See

If you use *E. coli* cells with a transformation efficiency of $\geq 1 \times 10^8$ cfu/ μ g, the LR reaction should yield > 5,000 colonies if the entire reaction is transformed and plated.

Confirming the Expression Clone

The *ccdB* 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 *ccdB* gene will be both ampicillin- and chloramphenicol-resistant. To check your putative expression clone, test for growth on LB plates containing 30 μ g/mL chloramphenicol. A true expression clone will not grow in the presence of chloramphenicol.

Sequencing

To confirm that your gene of interest is in frame with the N- or C-terminal fluorescent protein sequence, you may sequence your expression construct. We suggest using the following primer sequences. Refer to the diagrams on pages 10-11 for the location of the primer binding sites.

Vector	Primer	Sequence
pcDNA™ 6.2/N-EmGFP-DEST or N-YFP-DEST	TK polyA reverse	5'-CTTCCGTGTTTCAGTTAGC-3'
pcDNA™ 6.2/C-EmGFP-DEST or C-YFP-DEST	T7 Promoter Primer	5'-TAATACGACTCACTATAGGG-3'

For your convenience, we offer a custom primer synthesis service. For more information, visit www.lifetechnologies.com or contact Technical Support (page 36).

Transfecting Cells

Introduction

This section provides general information for transfecting your expression clone into the mammalian cell line of choice. We recommend that you include the positive control vector (pcDNA™ 6.2/EmGFP/GW/CAT or pcDNA™ 6.2/YFP/GW/CAT, below) and a mock transfection (negative control) in your experiments to evaluate your results.

Positive Control

Vivid Colors™ pcDNA™ 6.2/EmGFP/GW/CAT or YFP/GW/CAT vectors are provided as a positive control for mammalian cell transfection and expression (see pages 32-33 for maps) and may be used to optimize recombinant protein expression levels in your cell line. These vectors allow expression of an N- or C-terminally tagged chloramphenicol acetyl transferase (CAT) fusion protein that may be detectable by fluorescence microscopy, Western blot, or functional assay.

To propagate and maintain the control plasmid:

1. Use the 0.5 µg/µL stock solution to transform a *recA*, *endA* *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.
-

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 clean and free of contamination 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® HQ Mini Plasmid Purification Kit (page 34). Other methods of obtaining high quality plasmid DNA may be suitable.

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Transfecting Cells, Continued

Linearizing the Plasmid

Although we recommend linearizing your pcDNA™6.2/EmGFP or YFP expression clone before stable transfection to decrease the chances of vector integration that disrupts necessary elements (see next page), it is not necessary to do so for transient transfection. However, if you wish to do so, make sure to cut at a unique site that is not located within a critical element or within your gene of interest. Restriction site information for plasmid vectors is available at www.lifetechnologies.com.

Methods of Transfection

For established cell lines, consult the 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). For high efficiency transfection in a broad range of mammalian cell lines, we recommend using Lipofectamine® 2000 Reagent (page 34) available for purchase. For more information about Lipofectamine® 2000 and other transfection reagents, visit www.lifetechnologies.com or contact Technical Support (page 36).

Recombinant Protein Expression

In general, recombinant protein can be visualized by UV fluorescence at 24 hours post transfection. However for some cell lines, protein may be detected in as little as 6–8 hours after transfection.

Creating Stable Cell Lines

Introduction

The Vivid Colors™ pcDNA™ 6.2/EmGFP and YFP-DEST vectors contain the Blasticidin resistance gene to allow selection of stable cell lines. If you wish to create stable cell lines, transfect your expression construct into the mammalian cell line of choice and select for stable transfectants using Blasticidin. General information and guidelines are provided below.

Linearizing the Plasmid

To obtain stable transfectants, we recommend that you linearize your pcDNA™ 6.2/EmGFP or YFP expression 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. Cut at a unique site that is not located within a critical element or within your gene of interest. Restriction site information for plasmid vectors is available at www.lifetechnologies.com.

Blasticidin

Blasticidin S HCl is a nucleoside antibiotic isolated from *Streptomyces griseochromogenes* which inhibits protein synthesis in both prokaryotic and eukaryotic cells (Takeuchi *et al.*, 1958; Yamaguchi *et al.*, 1965). Resistance is conferred by expression of either one of two blasticidin S deaminase genes: *bsd* from *Aspergillus terreus* (Kimura *et al.*, 1994) or *bsr* from *Bacillus cereus* (Izumi *et al.*, 1991). These deaminases convert blasticidin S to a nontoxic deaminohydroxy derivative (Izumi *et al.*, 1991). Blasticidin is available separately for purchase (see page 34 for ordering information). To prepare Blasticidin see the Appendix, (page 27).

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Creating Stable Cell Lines, Continued

Determining Blasticidin Sensitivity

To successfully generate a stable cell line expressing your protein of interest, you first need to determine the minimum concentration of Blasticidin required to kill your untransfected host cell line. Test a range of concentrations to ensure that you determine the minimum concentration necessary for your cell line (see protocol below). Refer to page 27 for instructions on how to prepare and store Blasticidin.

1. Prepare 6 plates of cells so that each plate will be approximately 25% confluent.
2. Replace the growth medium with fresh growth medium containing a range of Blasticidin concentrations: 0, 1, 3, 5, 7.5, and 10 $\mu\text{g}/\text{mL}$.
3. Replenish the selective media every 3–4 days, and observe the percentage of surviving cells.
4. Count the number of viable cells at regular intervals to determine the appropriate concentration of antibiotic that kills your cells within 1–3 weeks after addition of Blasticidin.

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Creating Stable Cell Lines, Continued

Generating Stable Cell Lines

Once you have determined the appropriate Blasticidin concentration to use for selection, you can generate a stable cell line expressing your Vivid Colors™ pcDNA™6.2/EmGFP or YFP expression construct.

1. Transfect the mammalian cell line of interest with the pcDNA™6.2/EmGFP or YFP expression construct using your transfection method of choice.
2. 24 hours after transfection, wash the cells and add fresh growth medium without Blasticidin.
3. 48 hours after transfection, split the cells into fresh growth medium without Blasticidin 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 growth medium and replace with fresh growth medium containing Blasticidin at the predetermined concentration required for your cell line (see previous page).
6. Feed the cells with selective media every 3–4 days until Blasticidin-resistant colonies can be identified.
7. Pick at least 10 Blasticidin-resistant colonies and expand them to assay for recombinant protein expression.

Refer to page 27 for instructions on how to prepare and store Blasticidin.

Detecting Vivid Colors™ Fusion Proteins

Introduction

You can perform detection and analysis of your recombinant protein of interest from the expression clone in either transiently transfected cells or stable cell lines. Once you have transfected your expression clone into mammalian cells, you may:

- Detect protein expression and localization directly in cells by fluorescence microscopy or other methods that use light excitation and detection of emission. See below for recommended fluorescence microscopy filter sets.
 - Detect protein expression by Western blot of cell lysates using an antibody. See the table on the next page for suggested antibodies.
-

Filters for use with EmGFP YFP

Both the EmGFP and YFP can be detected with standard FITC filter sets. However, for optimal detection of the fluorescence signal, you may use a filter set which is optimized for detection within the excitation and emission ranges for each of the fluorescent proteins. These filter sets are listed in the table below:

Fluorescent Protein	Filter Set for Fluorescence Microscopy
EmGFP	Omega XF100
YFP	Chroma 41028 Omega XF1042

For information on obtaining these filter sets, contact Omega Optical, Inc. (www.omegafilters.com) or Chroma Technology Corporation (www.chroma.com).

Continued on next page

Detecting Vivid Colors™ Fusion Proteins, Continued

Antibodies for Detecting Recombinant Protein

You may detect expression of your recombinant fusion protein by western blot analysis using an antibody against your protein of interest, or by using antibodies against the appropriate epitope available for purchase (see page 35 for ordering information).

Note: You can use an antibody against the V5 epitope **only** for pcDNA™6.2/N-EmGFP-DEST and YFP-DEST if you have included the V5 epitope in your fusion construct. See page 8 for details on this option.

Preparing Cell Lysates for Western Blot Detection

To detect your fusion protein by western blot, you will need to prepare a cell lysate from transfected cells. A sample protocol is provided below. Other protocols may be suitable. To lyse cells:

1. Wash cell monolayer ($\sim 5 \times 10^5$ to 1×10^6 cells) once with phosphate-buffered saline (PBS).
 2. Scrape cells into 1 mL PBS and pellet the cells at $1500 \times g$ for 5 minutes.
 3. Resuspend in 50 μ L Cell Lysis Buffer (see page 26 for a recipe). Other cell lysis buffers may be suitable. Vortex.
 4. To lyse cells, perform 3 freeze thaw cycles by incubating the samples in a dry ice/ethanol bath for 2 minutes, then incubating the sample in a 37°C water bath for one minute. Perform this step 3 times to ensure complete cell lysis without protein degradation.
 5. Centrifuge the cell lysate at $10,000 \times g$ for 10 minutes at 4°C to pellet nuclei and transfer the supernatant to a fresh tube. Assay the lysate for protein concentration.
Note: 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 26 for a recipe) to a final concentration of 1X and boil the sample for 5 minutes.
 7. Load 20 μ g of lysate onto an SDS-PAGE gel and electrophorese. Use the appropriate percentage of acrylamide to resolve your fusion protein.
-

Continued on next page

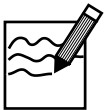
Detecting Vivid Colors™ Fusion Proteins, Continued

Polyacrylamide Gel Electrophoresis

To facilitate separation and visualization of your recombinant fusion protein by polyacrylamide gel electrophoresis, a wide range of pre-cast NuPAGE® and Novex® Tris-Glycine polyacrylamide gels and electrophoresis apparatus are available for purchase. For more information, visit www.lifetechnologies.com or contact Technical Support (page 36).

Detecting Recombinant Fusion Proteins

To detect expression of your recombinant fusion protein by western blot analysis, you may use an antibody to your protein of interest, an Anti-GFP antibody available for purchase (see page 22) or an Anti-V5 antibody if your fusion protein contains this epitope (see page 8 for this option). The ready-to-use WesternBreeze® Chromogenic Kits and WesternBreeze® Chemiluminescent Kits are available for purchase to facilitate detection of antibodies by colorimetric or chemiluminescent methods (see page 34 for ordering information).



Note

The N or C-terminal peptide fusion containing EmGFP or YFP will add approximately 27 kDa to your protein. The fusion peptide including the V5 epitope will add approximately 4 kDa to your protein, if it is included in your fusion construct.

Detecting CAT Protein

If you use the provided positive control vector in your experiment, you may assay for CAT expression using your method of choice. CAT Antiserum is also available separately for purchase (see page 34 for ordering information). Other commercial kits are available for assaying CAT expression. The molecular weight of the GFP or YFP/CAT fusion protein is approximately 57 kDa.

Troubleshooting

Introduction

The table below lists some potential problems solutions that may help you troubleshoot protein expression with a Vivid Colors™ pcDNA™ 6.2/EmGFP or YFP vector construct. For more information on troubleshooting within the Gateway® system, refer to the Gateway® Technology with Clonase® II manual. This manual is available from www.lifetechnologies.com or by contacting Technical Support (page 36).

Problem	Possible Cause	Solution
No fluorescence signal detected after transfecting control or experimental expression clone	Incorrect filters used to detect fluorescence	Be sure to use the recommended filter sets for detection of fluorescence (see page 21).
Transfected control give fluorescent signal, but experimental expression clone does not	Fusion protein interferes with EmGFP or YFP	Try fusing EmGFP or YFP to the other end (N- or C- terminal) of your protein.
Recombinant protein is not detectable with specific antibodies	EmGFP or YFP interferes with fusion protein	Try fusing EmGFP or YFP to the other end (N- or C- terminal) of your protein.
	No Kozak consensus sequence added to C-terminal fusions	Make sure a Kozak consensus sequence is present (see page 7).
	Gene of interest not in frame with fluorescent protein	Make sure gene of interest is in frame with fluorescent protein (see pages 10–11).
Recombinant protein is not localizing correctly within cells	EmGFP or YFP interferes with fusion protein	Try fusing EmGFP or YFP to the other end (N- or C- terminal) of your protein.
	Mutation in localization signals or critical elements in your gene of interest	Confirm sequence and reclone if needed.

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 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.

Continued on next page

Recipes, Continued

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 PMSE, 1 μ M leupeptin, or 0.1 μ M aprotinin before use.

4X SDS-PAGE Sample Buffer

1. Combine the following reagents:

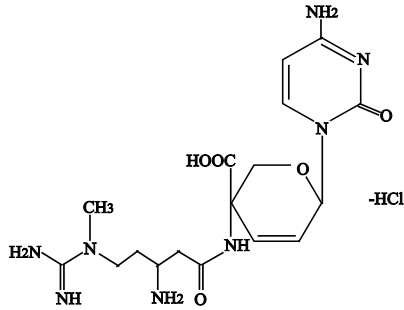
0.5 M Tris-HCl, pH 6.8	5 mL
Glycerol (100%)	4 mL
β -mercaptoethanol	0.8 mL
Bromophenol Blue	0.04 g
SDS	0.8 g

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

Blasticidin

Molecular Weight, Formula, and Structure

The formula for Blasticidin S is $C_{17}H_{26}N_8O_5 \cdot HCl$, and the molecular weight is 458.9. The diagram below shows the structure of Blasticidin.



Handling Blasticidin

Always wear gloves, mask, goggles, and protective clothing (e.g. a laboratory coat) when handling Blasticidin. Weigh out Blasticidin and prepare solutions in a hood.

Preparing and Storing Stock Solutions

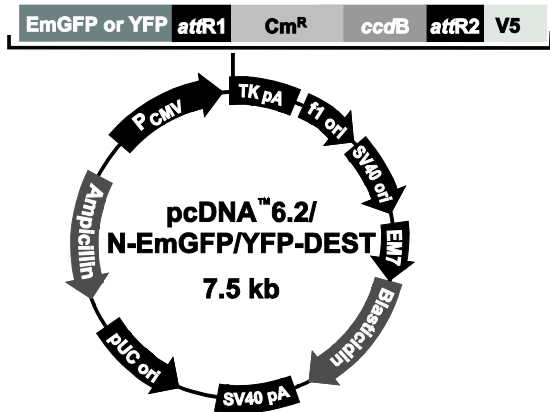
Blasticidin may be obtained separately in 50 mg aliquots (see page 34). Blasticidin is soluble in water. Use sterile water to prepare stock solutions of 5–10 mg/mL.

- Dissolve Blasticidin in sterile water and filter-sterilize the solution.
 - Aliquot solution in small volumes suitable for one time use (see next to last point below) and freeze at $-20^{\circ}C$ for long-term storage or store at $4^{\circ}C$ for short-term storage.
 - Aqueous stock solutions are stable for 1–2 weeks at $4^{\circ}C$ and 6–8 weeks at $-20^{\circ}C$.
 - pH of the aqueous solution should be 7.0 to prevent inactivation of Blasticidin.
 - Do not subject stock solutions to freeze/thaw cycles (**do not store in a frost-free freezer**).
 - Upon thawing, use what you need and store the thawed stock solution at $4^{\circ}C$ for up to 2 weeks.
 - Medium containing Blasticidin may be stored at $4^{\circ}C$ for up to 2 weeks.
-

Map of pcDNA™ 6.2/N-EmGFP/YFP-DEST

Map of pcDNA™ 6.2/N-EmGFP/YFP-DEST

The map below shows the elements of the Vivid Colors™ pcDNA™/6.2 N-terminal EmGFP/YFP-DEST vectors. DNA from the entry clone replaces the region between bases 1552 and 3235 after performing the LR reaction. The nucleotide sequences of these vectors are available for downloading from www.lifetechnologies.com or by contacting Technical Support (page 36).



Comments for:	pcDNA™ 6.2/ N-EmGFP-DEST 7528 nucleotides	pcDNA™ 6.2/ N-YFP-DEST 7530 nucleotides
CMV promoter:	102-689	102-689
EmGFP/YFP:	813-1529	813-1529
attR1 site:	1545-1669	1545-1669
Chloramphenicol resistance (CmR) gene:	1778-2437	1778-2437
ccdB gene:	2779-3084	2779-3084
attR2 site:	3125-3249	3125-3249
V5 epitope:	3257-3298	3257-3298
TK polyA reverse priming site:	3332-3350	3332-3350
TK polyadenylation signal:	3325-3596	3325-3596
f1 origin:	3632-4091	3632-4091
SV40 early promoter and origin:	4085-4393	4087-4395
EM7 promoter:	4448-4514	4450-4516
Blasticidin resistance gene:	4521-4913	4517-4915
SV40 early polyadenylation signal:	5071-5201	5073-5203
pUC origin (c):	5584-6257	5586-6259
Ampicillin (<i>bla</i>) resistance gene (c):	6402-7262	6404-7264
<i>bla</i> promoter (c):	7257-7361	7259-7363

(c) = complementary strand

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Features of pcDNA™ 6.2/N-EmGFP/YFP-DEST, Continued

Features of pcDNA™ 6.2/ N-EmGFP/YFP- DEST

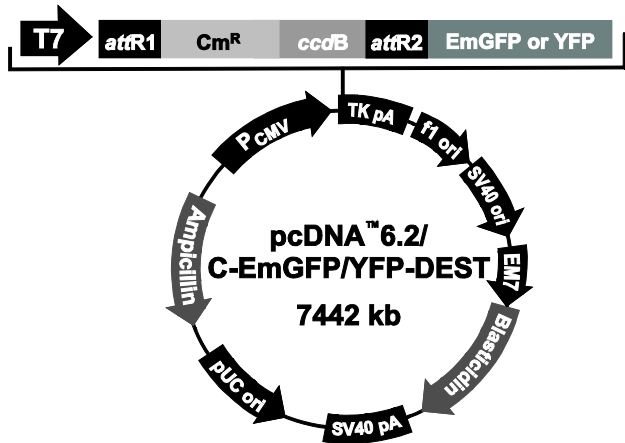
Vivid Colors™ pcDNA™ 6.2/N-terminal EmGFP-DEST (7528 bp) and YFP-DEST (7530 bp) vectors contain the following elements. All features have been functionally tested and the vectors have been fully sequenced.

Feature	Benefit
Human cytomegalovirus (CMV) immediate-early promoter/enhancer	Allows efficient, high-level expression of your recombinant protein (Andersson <i>et al.</i> , 1989; Boshart <i>et al.</i> , 1985; Nelson <i>et al.</i> , 1987)
<i>attR1</i> and <i>attR2</i> sites	Allows recombinational cloning of the gene of interest from an entry clone
<i>ccdB</i> gene	Allows negative selection of plasmid
CAT gene	Allows counterselection of plasmid
EmGFP or YFP	N- or C- terminal fusion to the gene of interest for fluorescent detection
V5 epitope	Allows detection of recombinant fusion protein with Anti-V5 antibodies (Southern <i>et al.</i> , 1991)
Herpes Simplex Virus Thymidine Kinase (TK) polyadenylation signal	Allows efficient transcription termination and polyadenylation of mRNA (Cole and Stacy, 1985)
f1 origin	Allows rescue of single-stranded DNA
SV40 early promoter and origin	Allows efficient, high-level expression of the Blasticidin resistance gene and episomal replication in cells expressing the SV40 large T antigen
EM7 promoter	Allows expression of the Blasticidin resistance gene in <i>E. coli</i>
Blasticidin (<i>bsd</i>) resistance gene	Allows selection of stable transfectants in mammalian cells (Kimura <i>et al.</i> , 1994)
SV40 early polyadenylation signal	Allows efficient transcription termination and polyadenylation of mRNA
pUC origin	Allows high-copy number replication and growth in <i>E. coli</i>
Ampicillin (<i>bla</i>) resistance gene (β -lactamase)	Allows selection of transformants in <i>E. coli</i>

Map of pcDNA™ 6.2/C-EmGFP/YFP-DEST

Map of pcDNA™ 6.2/C-EmGFP/YFP-DEST

The map below shows the elements of Vivid Colors™ pcDNA™ 6.2/C-terminal EmGFP and YFP-DEST vectors. DNA from the entry clone replaces the region between bases 792 and 2475 after performing the LR reaction. The nucleotide sequences of these vectors are available for downloading from www.lifetechnologies.com or by contacting Technical Support (page 36).



Comments for	pcDNA™ 6.2/C-EmGFP/YFP-DEST 7442 nucleotides
CMV promoter:	102-689
T7 promoter/priming site:	733-752
attR1 site:	785-909
Chloramphenicol resistance (Cm ^R) gene:	1018-1667
ccdB gene:	2019-2324
attR2 site:	2365-2489
EmGFP/YFP:	2503-3222
TK polyA reverse priming site:	3244-3262
TK polyadenylation signal:	3237-3508
f1 origin:	3544-4003
SV40 early promoter and origin:	3999-4307
EM7 promoter:	4362-4428
Blastidicin resistance gene:	4429-4827
SV40 early polyadenylation signal:	4985-5115
pUC origin (c):	5498-6171
Ampicillin (<i>bla</i>) resistance gene (c):	6316-7176
<i>bla</i> promoter (c):	7171-7275

(c) = complementary strand

Continued on next page

Features of pcDNA™ 6.2/C-EmGFP/YFP-DEST

Features of pcDNA™ 6.2/C-EmGFP/YFP-DEST

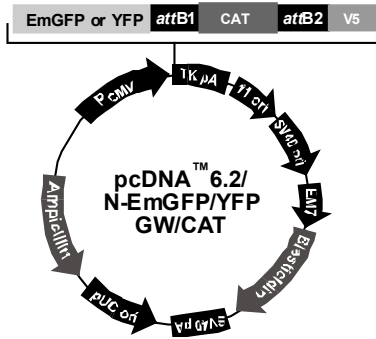
Vivid Colors™ pcDNA™ 6.2/C-terminal EmGFP-DEST (7442 bp) and YFP-DEST (7442 bp) vectors contain the following elements. All features have been functionally tested, and the vectors have been fully sequenced.

Feature	Benefit
Human cytomegalovirus (CMV) immediate-early promoter/enhancer	Allows efficient, high-level expression of your recombinant protein (Andersson <i>et al.</i> , 1989; Boshart <i>et al.</i> , 1985; Nelson <i>et al.</i> , 1987)
T7 promoter/priming site	Allows <i>in vitro</i> transcription in the sense orientation and sequencing through the insert
<i>attR1</i> and <i>attR2</i> sites	Allows recombinational cloning of the gene of interest from an entry clone
<i>ccdB</i> gene	Allows negative selection of plasmid
CAT gene	Allows counterselection of plasmid
EmGFP or YFP	N- or C-terminal fusion to the gene of interest for fluorescent detection
TK polyA reverse priming site	Allows sequencing of the insert
Herpes Simplex Virus Thymidine Kinase (TK) polyadenylation signal	Allows efficient transcription termination and polyadenylation of mRNA (Cole and Stacy, 1985)
f1 origin	Allows rescue of single-stranded DNA
SV40 early promoter and origin	Allows efficient, high-level expression of the Blasticidin resistance gene and episomal replication in cells expressing the SV40 large T antigen
EM7 promoter	Allows expression of the Blasticidin resistance gene in <i>E. coli</i>
Blasticidin (<i>bsd</i>) resistance gene	Allows selection of stable transfectants in mammalian cells (Kimura <i>et al.</i> , 1994)
SV40 early polyadenylation signal	Allows efficient transcription termination and polyadenylation of mRNA
pUC origin	Allows high-copy number replication and growth in <i>E. coli</i>
Ampicillin (<i>bla</i>) resistance gene (β -lactamase)	Allows selection of transformants in <i>E. coli</i>

Map of pcDNA™ 6.2/N-EmGFP or YFP/GW/CAT

Map of pcDNA™ 6.2/N-EmGFP or YFP/GW/CAT

The map below shows the elements of Vivid Colors™ pcDNA™ 6.2/N-terminal EmGFP and YFP/GW/CAT vectors. The plasmid was generated by performing a LR recombination reaction between an entry vector containing the CAT gene and the pcDNA™ 6.2/N-EmGFP or YFP-DEST vector. The nucleotide sequences of these vectors are available for downloading from www.lifetechnologies.com or by contacting Technical Support (page 36).

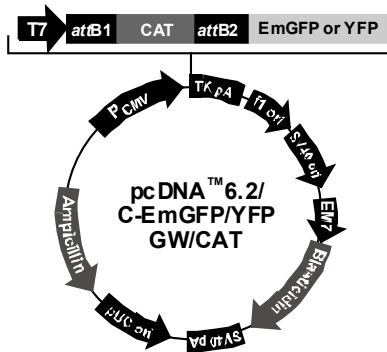


Comments for:	pcDNA™ 6.2/ N-EmGFP/GW/CAT 6560 nucleotides	pcDNA™ 6.2 / N-YFP/GW/CAT 6562 nucleotides
CMV promoter:	102-689	102-689
EmGFP/YFP:	813-1529	813-1529
<i>attB1</i> site:	1545-1569	1545-1569
CAT gene:	1599-2258	1599-2258
<i>attB2</i> site:	2257-2281	2257-2281
V5 epitope:	2289-2330	2289-2330
TK polyA reverse priming site:	2364-2382	2364-2382
TK polyadenylation signal:	2357-2628	2357-2628
f1 origin:	2664-3121	2664-3123
SV40 early promoter and origin:	3117-3425	3119-3427
EM7 promoter:	3480-3546	3482-3548
Blasticidin resistance gene:	3547-3945	3549-3947
SV40 early polyadenylation signal:	4103-4233	4105-4235
pUC origin (c):	4616-5289	4618-5291
Ampicillin (<i>bla</i>) resistance gene (c):	5434-6294	5436-6296
<i>bla</i> promoter (c):	6289-6393	6291-6395
(c) = complementary strand		

Map of pcDNA™ 6.2/C-EmGFP or YFP/GW/CAT

Map of pcDNA™ 6.2/C-EmGFP or YFP/GW/CAT

The map below shows the elements of Vivid Colors™ pcDNA™ 6.2/C-terminal EmGFP and YFP/GW/CAT vectors. The plasmid was generated by performing a LR recombination reaction between an entry vector containing the CAT gene and the pcDNA™ 6.2/C-EmGFP or YFP-DEST vector. The nucleotide sequences of these vectors are available for downloading from www.lifetechnologies.com or by contacting Technical Support (page 36).



Comments for:	pcDNA™ 6.2/ C-EmGFP/GW/CAT 6448 nucleotides	cDNA™ 6.2/ C-YFP/GW/CAT 6448 nucleotides
CMV promoter:	102-689	102-689
T7 promoter/priming site:	733-752	733-752
attB1 site:	785-809	785-809
CAT gene:	813-1472	813-1472
attB2 site:	1471-1495	1471-1495
EmGFP/YFP:	1509-2228	1509-2228
TK polyA reverse priming site:	2250-2268	2250-2268
TK polyadenylation signal:	2243-2514	2243-2514
f1 origin:	2550-3009	2550-3009
SV40 early promoter and origin:	3005-3313	3005-3313
EM7 promoter:	3368-3434	3368-3434
Blasticidin resistance gene:	3435-3833	3435-3833
SV40 early polyadenylation signal:	3991-4121	3991-4121
pUC origin (c):	4504-5177	4504-5177
Ampicillin (<i>bla</i>) resistance gene (c):	5322-6182	5322-6182
<i>bla</i> promoter (c):	6177-6281	6177-6281

(c) = complementary strand

Accessory Products

Additional Products

Additional products that may be used with Vivid Colors™ pcDNA™ 6.2/EmGFP and YFP-DEST vectors are available for purchase. Ordering information is provided below.

Product	Quantity	Cat. no.
Ultimate™ ORF Clones	1 glycerol stock	HORF01 MORF01
Gateway® LR Clonase® II Enzyme Mix	20 reactions 100 reactions	11791-020 11791-100
One Shot® TOP10 Chemically Competent Cells	10 reactions 20 reactions	C4040-10 C4040-03
OneShot® TOP10 Electrocompetent Cells	10 reactions 20 reactions	C4040-50 C4040-52
OneShot® <i>ccdB</i> Survival™ T1 ^R Chemically Competent Cells	10 reactions	C7510-03
PureLink® HQ Plasmid Miniprep Purification Kit	100 preps	K2100-01
Lipofectamine® 2000	0.75 mL 1.5 mL	11668-027 11668-019
Blasticidin S HCl	50 mg	R210-01
CAT Antiserum	50 µL	R902-25
Anti-GFP Antibody; rabbit polyclonal sera	100 µL	A-6455
Anti-GFP Antibody; rabbit IgG fraction	100 µL	A-11122
Anti-V5 Antibody	50 µL	R960-25
Anti-V5-HRP Antibody	50 µL	R961-25
Anti-V5-AP Antibody	125 µL	R962-25
WesternBreeze® Chemiluminescent Kit Anti-Rabbit	1 kit	WB7106
WesternBreeze® Chromogenic Kit Anti-Rabbit	1 kit	WB7105

Accessory Products, Continued

Detecting Recombinant Protein

Expression of your recombinant fusion protein can be detected using an antibody to the appropriate epitope. The table below describes the antibodies available for purchase for detection of fusion proteins expressed from the Vivid Colors™ pcDNA™ 6.2 vectors as appropriate.

Antibody	Description	Recognition	Cat. no
Anti-GFP	Rabbit polyclonal antiserum	Recognizes GFP and derivatives	A6455
Anti-GFP	Rabbit polyclonal IgG	Recognizes GFP and derivatives	A11122
Anti-V5	Mouse monoclonal IgG _{2a}	Detects 14 amino acid V5 epitope	R960-25
Anti-V5 HRP	Mouse monoclonal IgG _{2a} conjugated to HRP	Detects 14 amino acid V5 epitope	R961-25
Anti-V5-AP	Mouse monoclonal IgG _{2a} conjugated to AP	Detects 14 amino acid V5 epitope	R692-25

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

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.lifetechnologies.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

Continued on next page

Technical Support, Continued

Limited Warranty

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Limited Use Label License ULB ccdB Selection Technology

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 licensing information for use in other than research, please contact: outlicensing@lifetech.com or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

Limited Use Label License GFP

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Gateway® Clone Distribution Policy

Introduction

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

Gateway® Entry Clones

Invitrogen understands that Gateway® entry clones, containing *attL1* and *attL2* sites, may be generated by academic and government researchers for the purpose of scientific research. Invitrogen agrees that such clones may be distributed for scientific research by non-profit organizations and by for-profit organizations without royalty payment to Invitrogen.

Gateway® Expression Clones

Invitrogen also understands that Gateway® expression clones, containing *attB1* and *attB2* sites, may be generated by academic and government researchers for the purpose of scientific research. Invitrogen agrees that such clones may be distributed for scientific research by academic and government organizations without royalty payment to Invitrogen. Organizations other than academia and government may also distribute such Gateway® expression clones for a nominal fee (\$10 per clone) payable to Invitrogen.

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 Invitrogen 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 Invitrogen's 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 Invitrogen's licensing department at 760-603-7200.

References

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