



pLenti4/V5-DEST, pLenti6/V5-DEST, pLenti6.2/V5-DEST and pLenti6/UbC/V5-DEST Gateway[®] Vector Kits

Gateway[®]-adapted destination vectors for cloning and high-level expression in mammalian cells using the ViraPower[™] Lentiviral Expression System

Catalog numbers V496-10, V498-10, V499-10, V368-20

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About this guide

IMPORTANT! Before using this product, read and understand the information in the “Safety” appendix in this document.

Changes from previous version

Revision	Date	Description
A.0	March 2015	<ul style="list-style-type: none">• Changed revision schema from previous “Version I” and reset to A.0• Updated pLenti6/V5-DEST cloning site information and vector map to reflect corrected vector sequence

Kit Contents and Storage

Types of Kits

This manual is supplied with the following products.

Product	Catalog no.
ViraPower™ Lentiviral Gateway® Expression Kit	K4960-00
ViraPower™ II Lentiviral Gateway® Expression Kit	K367-20
ViraPower™ Zeo Lentiviral Gateway® Expression Kit	K4980-00
ViraPower™ UbC Lentiviral Gateway® Expression Kit	K4990-00
pLenti6/V5-DEST Gateway® Vector Kit	V496-10
pLenti4/V5-DEST Gateway® Vector Kit	V498-10
pLenti6/UbC/V5-DEST Gateway® Vector Kit	V499-10
pLenti6.2/V5-DEST Gateway® Vector Kit	V368-20

Kit Components

The ViraPower™ Gateway® Kits include the components listed in the table below. For a detailed description of the reagents supplied with the ViraPower™ Gateway® vectors and the One Shot® Stbl3™ Chemically Competent *E. coli*, see pages vi–vii. For a detailed description of the reagents supplied with the ViraPower™ Bsd or Zeo Lentiviral Support Kits, or instructions to culture and maintain the 293FT Cell Line, see the ViraPower™ Lentiviral Expression System or 293FT Cell Line manuals, respectively. Both manuals are supplied with the ViraPower™ Gateway® Expression Kits, but are also available for downloading from our web site at www.invitrogen.com or by contacting **Technical Support** (page 42).

Component	Catalog no.	
	<u>K4960-00, K4980-00,</u> <u>K4990-00, K367-20</u>	<u>V496-10, V498-10,</u> <u>V499-10, V368-20</u>

ViraPower™ Lentiviral Gateway® Vectors	√	√
One Shot® Stbl3™ Chemically Competent <i>E. coli</i>	√	√
ViraPower™ Bsd or Zeo Lentiviral Support Kit	√	
293FT Cell Line	√	

Continued on next page

Kit Contents and Storage, Continued

Shipping/Storage The ViraPower™ Gateway® Kits are shipped as described below. Upon receipt, store each item as detailed below.

Note: Catalog nos. V496–10, V498–10, V499–10 and V368–20 include Box 1 and Box 2 **only**.

Box	Component	Shipping	Storage
1	ViraPower™ Lentiviral Gateway® Vectors	Wet Ice	–20°C
2	One Shot® Stbl3™ Chemically Competent <i>E. coli</i>	Dry ice	–80°C
3	ViraPower™ Bsd or Zeo Lentiviral Support Kit	Blasticidin: Room temperature All other reagents: Wet ice	Blasticidin: –20°C (Bsd Kit only; shipped in a separate box) ViraPower™ Packaging: Mix: –20°C Lipofectamine™ 2000: 4°C Zeocin: –20°C, protected from light (Zeo Kit only)
4	293FT Cell Line	Dry ice	Liquid nitrogen

ViraPower™ Lentiviral Gateway® Vectors

Each catalog number contains the following vectors. All vectors are supplied in aliquot detailed below. **Store the vectors at –20°C.**

Catalog nos.	Vector	Quantity	Composition (supplied as)
V496–10 and K4960–00	pLenti6/V5-DEST	6 µg	40 µl of 150 ng/µl vector in 10 mM Tris-HCl, 1mM EDTA, pH 8.0
	pLenti6/V5-GW/ <i>lacZ</i> control	10 µg	20 µl of 0.5 µg/µl vector in 10 mM Tris-HCl, 1mM EDTA, pH 8.0
V498–10 and K4980–00	pLenti4/V5-DEST	6 µg,	40 µl of 150 ng/µl vector in 10 mM Tris-HCl, 1mM EDTA, pH 8.0
	pLenti4/V5-GW/ <i>lacZ</i> control	10 µg	20 µl of 0.5 µg/µl vector in 10 mM Tris-HCl, 1mM EDTA, pH 8.0
V499–10 and K4990–00	pLenti6/UbC/V5-DEST	6 µg,	40 µl of 150 ng/µl vector in 10 mM Tris-HCl, 1mM EDTA, pH 8.0
	pLenti6/UbC/V5-GW/ <i>lacZ</i> control	10 µg	20 µl of 0.5 µg/µl vector in 10 mM Tris-HCl, 1mM EDTA, pH 8.0
V368–20 and K367–20	pLenti6.2/V5-DEST	6 µg,	40 µl of 150 ng/µl vector in 10 mM Tris-HCl, 1mM EDTA, pH 8.0
	pLenti6.2/V5-GW/ <i>lacZ</i> control	10 µg	20 µl of 0.5 µg/µl vector in 10 mM Tris-HCl, 1mM EDTA, pH 8.0

Continued on next page

Kit Contents and Storage, Continued

ViraPower™ Lentiviral Support Kit

The ViraPower™ Lentiviral Gateway® Expression Kits (Catalog nos. K4960-00, K4980-00, and K4990-00) and ViraPower™ II Lentiviral Gateway® Expression Kit (Catalog no. K367-20) includes the ViraPower™ Bsd or Zeo Lentiviral Support Kit, as appropriate and the 293FT Cell Line to facilitate production of replication-incompetent lentiviral stocks. For a detailed description of the reagents provided in the ViraPower™ Bsd or Zeo Lentiviral Support Kits and their use, refer to the ViraPower™ Lentiviral Expression System manual. For a detailed description of the 293FT Cell Line and instructions to culture and maintain the cells, refer to the 293FT Cell Line manual. Both manuals are supplied with the ViraPower™ Lentiviral Gateway® Expression Kits, but are also available for downloading from our web site at www.invitrogen.com or by contacting **Technical Support** (page 42).

One Shot® Stbl3™ Chemically Competent *E. coli*

The following reagents are included with the One Shot® Stbl3™ Chemically Competent *E. coli* kit (Box 2). Transformation efficiency is $\geq 1 \times 10^8$ cfu/ μ g plasmid DNA. **Store Box 2 at -80°C .**

Reagent	Composition	Quantity
Stbl3™ Cells	--	21 × 50 μ l
S.O.C. Medium	2% Tryptone 0.5% Yeast Extract 10 mM NaCl 2.5 mM KCl 10 mM MgCl ₂ 10 mM MgSO ₄ 20 mM glucose	6 ml
pUC19 Control DNA	10 pg/ μ l in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8	50 μ l

Genotype of Stbl3™ Cells

F⁻ *mcrB mrr hsdS20*(*rB⁻, mB⁻*) *recA13 supE44 ara-14 galK2 lacY1 proA2 rpsL20*(Str^R)
xyl-5 λ^- leu mtl-1

Note: This strain is *endA1+*

293FT Cell Line

The ViraPower™ Lentiviral Gateway® Expression Kits (Catalog nos. K4960–00, K4980–00, and K4990–00) and ViraPower™ II Lentiviral Gateway® Expression Kit (Catalog no. K367–20) includes the ViraPower™ Bsd or Zeo Lentiviral Support Kit, as appropriate and the 293FT Cell Line to facilitate production of replication-incompetent lentiviral stocks. For a detailed description of the reagents provided in the ViraPower™ Bsd or Zeo Lentiviral Support Kits and their use, refer to the ViraPower™ Lentiviral Expression System manual. For a detailed description of the 293FT Cell Line and instructions to culture and maintain the cells, refer to the 293FT Cell Line manual. Both manuals are supplied with the ViraPower™

Lentiviral Gateway® Expression Kits, but are also available for downloading from our web site at www.invitrogen.com or by contacting **Technical Support** (page 42).

Accessory Products

Additional Products

The products below may be used with the pLenti-DEST vectors. For more information, visit our web site at www.invitrogen.com or contact **Technical Support** (page 42).

Product	Quantity	Catalog no.
pLenti6/V5-DEST Gateway® Vector Kit	6 µg (supplied as 40 µl of 150 ng/µl vector in 10 mM Tris-HCl, 1mM EDTA, pH 8.0)	V496-10
pLenti4/V5-DEST Gateway® Vector Kit	6 µg (supplied as 40 µl of 150 ng/µl vector in 10 mM Tris-HCl, 1mM EDTA, pH 8.0)	V498-10
pLenti6/Ubc/V5-DEST Gateway® Vector Kit	6 µg (supplied as 40 µl of 150 ng/µl vector in 10 mM Tris-HCl, 1mM EDTA, pH 8.0)	V499-10
pLenti6.2/V5-DEST Gateway® Vector Kit	6 µg (supplied as 40 µl of 150 ng/µl vector in 10 mM Tris-HCl, 1mM EDTA, pH 8.0)	V368-20
Gateway® LR Clonase™ II Enzyme Mix	20 reactions 100 reactions	11791-020 11791-100
Ampicillin	5 g	Q100-16
Blasticidin	50 mg	R210-01
Zeocin™	1 g 5 g	R250-01 R250-05
One Shot® Stbl3™ Chemically Competent <i>E. coli</i>	20 × 50 µl	C7373-03
S.N.A.P.™ Midiprep DNA Isolation Kit	20 reactions	K1910-01
PureLink™ HQ Plasmid Miniprep Kit	100 preps	K2100-01
PureLink™ HiPure Plasmid Miniprep Kit	25 preps	K2100-02
PureLink™ HiPure Plasmid Midiprep Kit	25 preps	K2100-04
AccuPrime™ Pfx SuperMix	200 reactions	12344-040
E-Gel® 1.2% Starter Pak (6 gels + Powerbase™)	1 kit	G6000-01
E-Gel® 1.2% 18 Pak	18 gels	G5018-01
Lipofectamine™ 2000 Reagent	0.75 ml 1.5 ml	11668-027 11668-019
β-gal Antiserum, rabbit IgG fraction	500 µl	A11132
β-Gal Assay Kit	1 kit	K1455-01
β-Gal Staining Kit	1 kit	K1465-01

Continued on next page

Accessory Products, Continued

ViraPower™ Lentiviral Expression Products

The pLenti-DEST vectors are designed for use with the ViraPower™ Lentiviral Expression System available from Invitrogen. Ordering information for other ViraPower™ lentiviral support products and expression kits is provided below.

Item	Quantity	Catalog no.
ViraPower™ Lentiviral Directional TOPO® Expression Kit	1 kit	K4950-00
Vivid Colors™ pLenti6.2-GW/EmGFP Expression Control Vector	20 µg plasmid DNA	V369-20
ViraPower™ Bsd Lentiviral Support Kit	20 reactions	K4970-00
ViraPower™ Zeo Lentiviral Support Kit	20 reactions	K4985-00
ViraPower™ Lentiviral Packaging Mix	60 reactions	K4975-00
293FT Cell Line	3 × 10 ⁶ cells	R700-07

Detection of Recombinant Protein

Expression of your recombinant fusion protein can be detected using an antibody to the V5 epitope (see table below). Horseradish peroxidase (HRP) or alkaline phosphatase (AP)-conjugated antibodies allow one-step detection using chemiluminescent or colorimetric detection methods. A fluorescein isothiocyanate (FITC)-conjugated antibody allows one-step detection in immunofluorescence experiments. The amount of antibody supplied is sufficient for 25 western blots or 25 immunostaining reactions, as appropriate.

Item	Quantity	Catalog no.
Anti-V5 Antibody	50 µl	R960-25
Anti-V5-HRP Antibody	50 µl	R961-25
Anti-V5-AP Antibody	125 µl	R962-25
Anti-V5-FITC Antibody	50 µl	R963-25

Introduction

Overview

Introduction

The pLenti-DEST vectors are destination vectors adapted for use with the Gateway® Technology, and are designed to allow high-level expression of recombinant fusion proteins in dividing and non-dividing mammalian cells using Invitrogen's ViraPower™ Lentiviral Expression System.

Depending on the vector chosen, the pLenti-DEST vectors are available with:

- Human cytomegalovirus (CMV) immediate early promoter or the human ubiquitin C (UbC) promoter to control expression of the gene of interest (see pages 2–4 for more information)
- SV40 or PGK promoter driving expression of Blasticidin (see page 4 for more information)
- Zeocin™ or Blasticidin resistance gene for selection in *E. coli* and mammalian cells

See the table below for a list of the available pLenti-DEST vectors, and the promoters and selection marker present in each vector.

Vector	Promoter Driving Gene of Interest	Promoter Driving Selection Marker in Mammalian Cells	Selection Marker
pLenti4/V5-DEST	CMV	SV40	Zeocin™
pLenti6/V5-DEST	CMV	SV40	Blasticidin
pLenti6.2/V5-DEST	CMV	PGK	Blasticidin
pLenti6/UbC/V5-DEST	UbC	SV40	Blasticidin

For More Information

For more information about the Gateway® Technology and the ViraPower™ Lentiviral Expression System, refer to pages 4–5.

Continued on next page

Overview, Continued

Features of the Vectors

The pLenti-DEST vectors contain the following elements:

- Rous Sarcoma Virus (RSV) enhancer/promoter for Tat-independent production of viral mRNA in the producer cell line (Dull *et al.*, 1998)
- Modified HIV-1 5' and 3' Long Terminal Repeats (LTR) for viral packaging and reverse transcription of the viral mRNA (Dull *et al.*, 1998; Luciw, 1996)
Note: The U3 region of the 3' LTR is deleted (Δ U3) and facilitates self-inactivation of the 5' LTR after transduction to enhance the biosafety of the vector (Dull *et al.*, 1998)
- HIV-1 psi (Ψ) packaging sequence for viral packaging (Luciw, 1996)
- HIV Rev response element (RRE) for Rev-dependent nuclear export of unspliced viral mRNA (Kjems *et al.*, 1991; Malim *et al.*, 1989)
- Human CMV or UbC promoter for constitutive expression of the gene of interest from a viral or cellular promoter, respectively (see below and the next page for more information)
- Two recombination sites, *attR1* and *attR2*, downstream of the CMV or UbC promoter for recombinational cloning of the gene of interest from an entry clone
- Chloramphenicol resistance gene (Cm^{R}) located between the two *attR* sites for counterscreening
- The *ccdB* gene located between the *attR* sites for negative selection
- C-terminal V5 epitope for detection of the recombinant protein of interest (Southern *et al.*, 1991)
- SV40 or PGK promoter driving expression of Blasticidin (see next page)
- Blasticidin (Izumi *et al.*, 1991; Kimura *et al.*, 1994; Takeuchi *et al.*, 1958; Yamaguchi *et al.*, 1965) or Zeocin[™] (Drocourt *et al.*, 1990; Mulsant *et al.*, 1988) resistance gene for selection in *E. coli* and mammalian cells
- Ampicillin resistance gene for selection in *E. coli*
- pUC origin for high-copy replication of the plasmid in *E. coli*

A control plasmid containing the *lacZ* gene is included with each pLenti-DEST vector for use as a positive expression control in the mammalian cell line of choice. For more information, see pages 37–40. A control lentiviral expression vector (pLenti6.2-GW/EmGFP) containing Emerald Green Fluorescent Protein (EmGFP) is available separately from Invitrogen. For ordering information, see page x.

CMV Promoter

The pLenti4/V5-DEST, pLenti6/V5-DEST and pLenti6.2/V5-DEST vectors contain the human CMV immediate early promoter to allow high-level, constitutive expression of the gene of interest in mammalian cells (Andersson *et al.*, 1989; Boshart *et al.*, 1985; Nelson *et al.*, 1987). Although highly active in most mammalian cell lines, activity of the viral promoter can be down-regulated in some cell lines due to methylation (Curradi *et al.*, 2002), histone deacetylation (Rietveld *et al.*, 2002), or both.

Continued on next page

Overview, Continued

UbC Promoter

The pLenti6/UbC/V5-DEST vector contains the human UbC promoter to allow constitutive, but more physiological levels of expression from the gene of interest in mammalian cells (Marinovic *et al.*, 2000). When compared to the CMV promoter, the UbC promoter is generally 2-4 fold less active. The UbC promoter is not down-regulated, making it useful for transgenic studies (Gill *et al.*, 2001; Lois *et al.*, 2002; Marinovic *et al.*, 2000; Schorpp *et al.*, 1996; Yew *et al.*, 2001).

Promoter Driving Blastocidin

The pLenti6.2/V5-DEST vector contains the murine phosphoglycerate kinase-1 (PGK) promoter to drive mammalian expression of the Blastocidin selection marker. In some mammalian cell types, the activity of viral promoters such as SV40 may become significantly reduced over time due to promoter silencing from methylation (Curradi *et al.*, 2002) or histone deacetylation (Rietveld *et al.*, 2002). The PGK promoter is a ubiquitous housekeeping promoter (Adra *et al.*, 1987) that has been shown to promote long-term persistent expression from lentiviral vectors (Hamaguchi *et al.*, 2000). The PGK promoter in the pLenti6.2/V5-DEST vector may provide consistent Blastocidin expression in cells that may be susceptible to promoter silencing, such as undifferentiated embryonic stem (ES) cells (Gerolami *et al.*, 2000; Hamaguchi *et al.*, 2000).

The Gateway® Technology

Gateway® Technology 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 in mammalian cells using the Gateway® Technology, simply:

1. Clone your gene of interest into a Gateway® entry vector of choice 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.* pLenti4/V5-DEST, pLenti6/V5-DEST, pLenti6.2/V5-DEST, or pLenti6/UbC/V5-DEST).
3. Use your expression clone in the ViraPower™ Lentiviral Expression System (see next page).

For detailed information about the Gateway® Technology, refer to the Gateway® Technology with Clonase™ II manual which is available from our web site at www.invitrogen.com or by contacting **Technical Support** (see page 42).

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Overview, Continued

The ViraPower™ Lentiviral Expression System

The ViraPower™ Lentiviral Expression System facilitates highly efficient, *in vitro* or *in vivo* delivery of a target gene to dividing and non-dividing mammalian cells using a replication-incompetent lentivirus. Based on the lentikat™ system developed by Cell Genesys (Dull *et al.*, 1998), the ViraPower™ Lentiviral Expression System possesses features which enhance its biosafety while allowing high-level gene expression in a wider range of cell types than traditional retroviral systems. To express your gene of interest in mammalian cells using the ViraPower™ Lentiviral Expression System, you will:

1. Create an expression clone in one of the pLenti-DEST vectors.
2. Cotransfect your expression clone and the ViraPower™ Packaging Mix into the 293FT Cell Line to produce lentivirus.
3. Use your lentiviral stock to transduce the mammalian cell line of choice.
4. Assay for “transient” expression of the recombinant protein or generate a stable cell line using Blasticidin or Zeocin™ selection, as appropriate.

For more information about the ViraPower™ Lentiviral Expression System, the ViraPower™ Packaging Mix, and the biosafety features of the System, refer to the ViraPower™ Lentiviral Expression System manual. For more information about the 293FT Cell Line, refer to the 293FT Cell Line manual. Both manuals are available for downloading from our web site at www.invitrogen.com or by contacting **Technical Support** (page 42).

Methods

Generating an Entry Clone

Introduction

To recombine your DNA sequence of interest into pLenti4/V5-DEST, pLenti6/V5-DEST, pLenti6.2/V5-DEST, or pLenti6/Ubc/V5-DEST, you will need to generate an entry clone containing the DNA sequence of interest. Many entry vectors are available from Invitrogen to facilitate generation of entry clones (see table below for a representative list).

Entry Vector	Catalog no.
pCR8 [®] /GW/TOPO [®]	K2500–20
pENTR [™] /D-TOPO [®]	K2400–20
pENTR [™] /SD/D-TOPO [®]	K2420–20
pENTR [™] /TEV/D-TOPO [®]	K2525–20

Once you have selected an entry vector, refer to the manual for the specific entry vector you are using for instructions to construct an entry clone. All entry vector manuals are available for downloading from our web site at www.invitrogen.com or by contacting **Technical Support** (page 42).



If you wish to express a human gene of interest in a pLenti-DEST vector, you may use an Ultimate[™] Human ORF (hORF) Clone available from Invitrogen. The Ultimate[™] hORF Clones are fully sequenced clones provided in a Gateway[®] entry vector that is ready to use in a recombination reaction with a pLenti-DEST vector. For more information about the Ultimate[™] hORF Clones available, visit our web site at www.invitrogen.com/clones or contact **Technical Support** (page 42).

Points to Consider Before Recombining into a pLenti-DEST Vector

pLenti4/V5-DEST, pLenti6/V5-DEST, pLenti6.2/V5-DEST and pLenti6/Ubc/V5-DEST allow fusion of your gene of interest to a C-terminal tag. Remember that your gene of interest in the entry clone must:

- contain a Kozak consensus sequence with an ATG initiation codon for proper initiation of translation (Kozak, 1987; Kozak, 1990; Kozak, 1991). An example of a Kozak consensus sequence is provided below. Other sequences are possible, but the G or A at position –3 and the G at position +4 (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)NNATGG

- Be in frame with the C-terminal tag after recombination
- **NOT** contain a stop codon if you want to express a fusion protein

Refer to pages 8–10 for diagrams of the recombination region of each pLenti-DEST vector.

Creating Expression Clones

Introduction

After you have generated an entry clone, you will perform the LR recombination reaction to transfer the gene of interest into the pLenti-DEST vector to create your expression clone. To ensure that you obtain the best possible results, we recommend that you read this section and the sections entitled **Performing the LR Recombination Reaction** (pages 11–20) and **Transforming One Shot® Stbl3™ Competent *E. coli*** (pages 13–20) 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 pLenti4/V5-DEST, pLenti6/V5-DEST, pLenti6.2/V5-DEST, or pLenti6/Ubc/V5-DEST vector.
Note: Both the entry clone and the destination vector should be supercoiled.
 2. Use the reaction mixture to transform One Shot® Stbl3™ Competent *E. coli* (see page 13).
 3. Analyze expression clones using restriction enzyme analysis and sequencing.
-

Vector Information

The pLenti4/V5-DEST, pLenti6/V5-DEST, pLenti6.2/V5-DEST, or pLenti6/Ubc/V5-DEST vectors are supplied as 6 µg of supercoiled plasmid in 40 µl of 150 ng/µl vector in 10 mM Tris-HCl, 1mM EDTA, pH 8.0



Important

We do not recommend that you propagate the pLenti4/V5-DEST, pLenti6/V5-DEST, pLenti6.2/V5-DEST, or pLenti6/Ubc/V5-DEST vectors in *E. coli* due to the possibility of unwanted recombination. The amount of each plasmid supplied is sufficient for 40 reactions. To purchase these plasmids separately, refer to page ix, or visit our web site at www.invitrogen.com.

Continued on next page

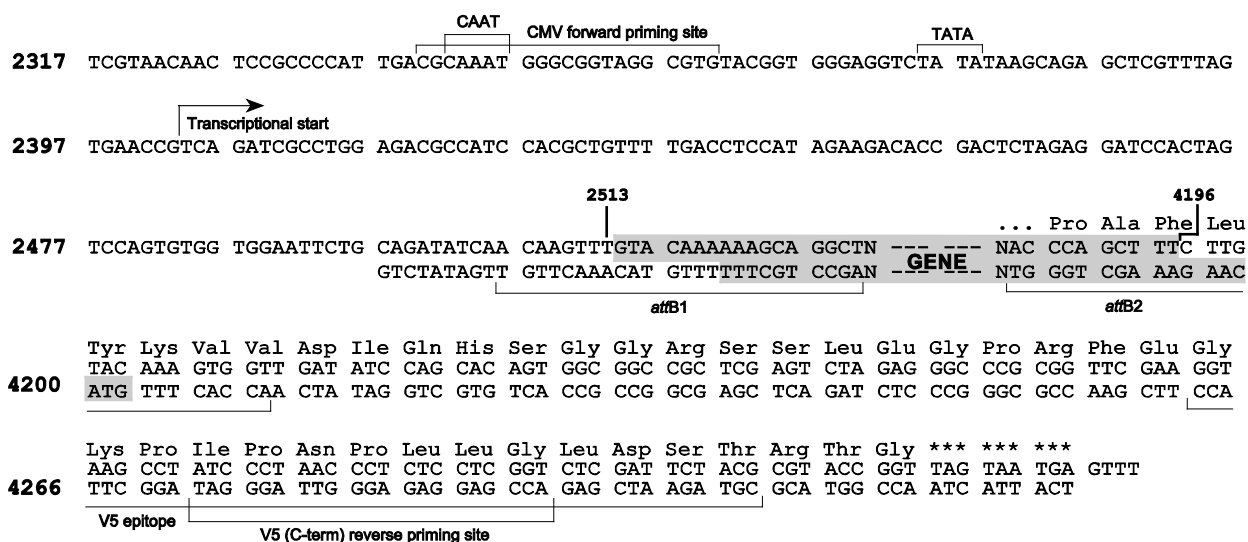
Creating Expression Clones, Continued

Recombination Region of pLenti4/V5-DEST, pLenti6/V5-DEST, and pLenti6.2/V5-DEST

The recombination region of the expression clone resulting from pLenti4/V5-DEST × entry clone, pLenti6/V5-DEST × entry clone, or pLenti6.2/V5-DEST × entry clone is shown below.

Features of the Recombination Region:

- Shaded regions correspond to those DNA sequences transferred from the entry clone into the pLenti4/V5-DEST, pLenti6/V5-DEST or pLenti6.2/V5-DEST vector by recombination.
- Non-shaded regions are derived from the pLenti4/V5-DEST, pLenti6/V5-DEST or pLenti6.2/V5-DEST vector.
- Bases 2447 and 4130 of the pLenti4/V5-DEST, pLenti6/V5-DEST or pLenti6.2/V5-DEST sequence are marked.



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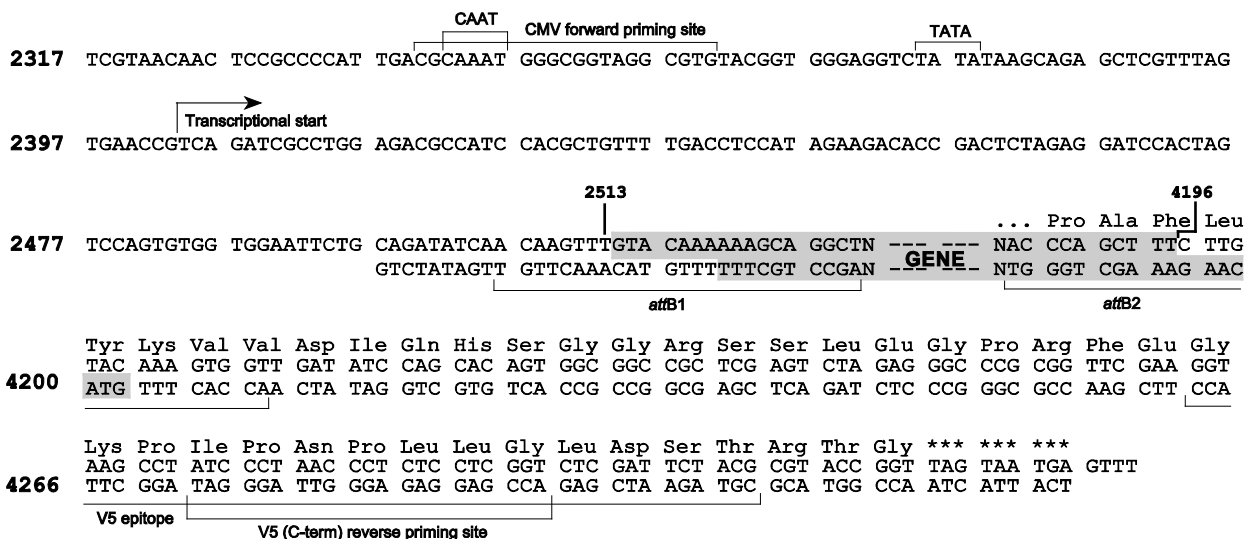
Creating Expression Clones, Continued

Recombination Region of pLenti6.2/V5-DEST

The recombination region of the expression clone resulting pLenti6.2/V5-DEST × entry clone is shown below.

Features of the Recombination Region:

- Shaded regions correspond to those DNA sequences transferred from the entry clone into the pLenti6.2/V5-DEST vector by recombination.
- Non-shaded regions are derived from the pLenti6.2/V5-DEST vector.
- Bases 2513 and 4196 of the pLenti6.2/V5-DEST sequence are marked.



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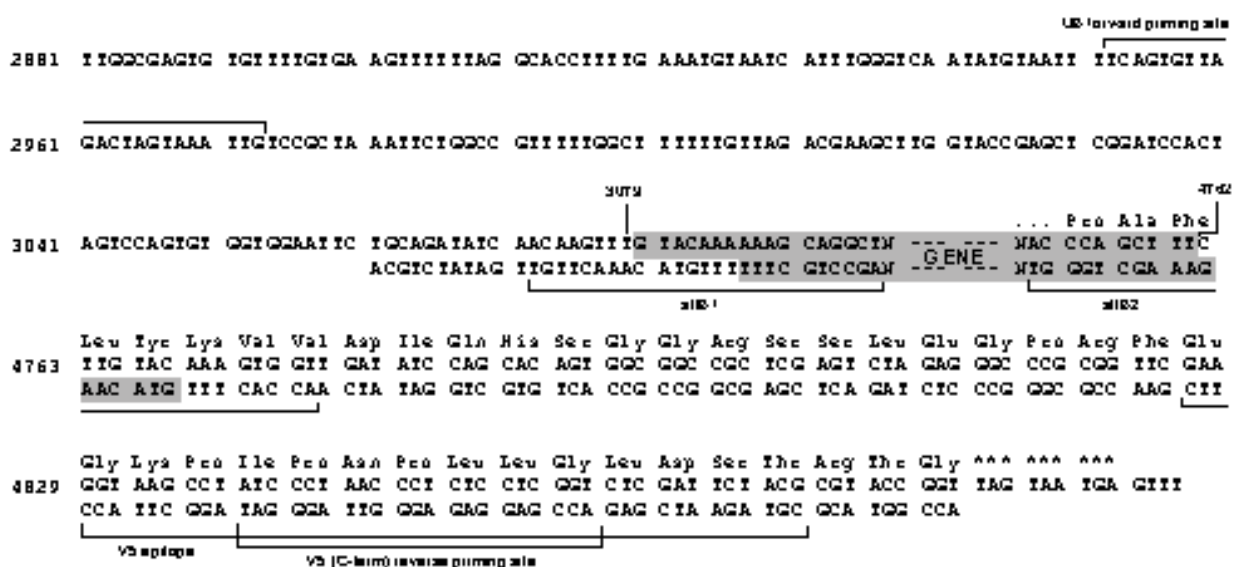
Creating Expression Clones, Continued

Recombination Region of pLenti6/UbC/V5-DEST

The recombination region of the expression clone resulting from pLenti6/UbC/V5-DEST × entry clone is shown below. Note that this diagram does not contain the complete sequence of the UbC promoter. For a diagram of the UbC promoter and a description of its features, see the **Appendix**, page 41.

Features of the Recombination Region:

- Shaded regions correspond to those DNA sequences transferred from the entry clone into the pLenti6/UbC/V5-DEST vector by recombination.
- Non-shaded regions are derived from the pLenti6/UbC/V5-DEST vector.
- Bases 3079 and 4762 of the pLenti6/UbC/V5-DEST sequence are marked.



Performing the LR Recombination Reaction

Introduction

Follow the guidelines and instructions in this section to perform the LR recombination reaction using your entry clone containing the gene of interest and the pLenti-DEST vector. We recommend including a negative control (no LR Clonase™ II) in your experiment to help you evaluate your results.

LR Clonase™ II Enzyme Mix

To catalyze the LR recombination reaction, you will use LR Clonase™ II enzyme mix, which is available separately from Invitrogen (page ix). The LR Clonase™ II enzyme mix combines the proprietary enzyme formulation and 5X LR Clonase™ Reaction Buffer previously supplied as separate components in LR Clonase™ enzyme mix into an optimized single-tube format for easier set-up of the LR recombination reaction. Use the protocol provided on page 12 to perform the LR recombination reaction using LR Clonase™ II enzyme mix.

Materials Needed

You will need the following items:

- Purified plasmid DNA of your entry clone (50–150 ng/μl in TE, pH 8.0)
 - pLenti-DEST vector (supplied with the kit, Box 1)
 - LR Clonase™ II enzyme mix (page ix; keep at –20°C until immediately before use)
 - TE Buffer, pH 8.0 (10 mM Tris-HCl, pH 8.0, 1 mM EDTA)
 - 2 μg/μl Proteinase K solution (supplied with the LR Clonase™ II enzyme mix; thaw and keep on ice until use)
-

Continued on next page

Performing the LR Recombination Reaction, Continued

Setting Up the LR Recombination Reaction

Follow this procedure to perform the LR recombination reaction between your entry clone and the pLenti-DEST vector. If you want to include a negative control, set up a separate reaction but omit the LR Clonase™ II enzyme mix.

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

Component	Sample
Entry clone (50-150 ng/reaction)	1-7 µl
Destination vector (150 ng/µl)	1 µl
TE Buffer, pH 8.0	to 8 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 the 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 the reaction 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. Proceed to **Transforming One Shot® Stbl3™ Competent *E. coli***, next page.
Note: You may store the LR reaction at -20°C for up to 1 week before transformation, if desired.
-

Transforming One Shot® Stbl3™ Competent *E. coli*

Introduction

Follow the instructions in this section to transform the LR recombination reaction into One Shot® Stbl3™ Chemically Competent *E. coli* (Box 2) included with the kit. The transformation efficiency of One Shot® Stbl3™ Chemically Competent *E. coli* is $\geq 1 \times 10^8$ cfu/ μ g plasmid DNA.



Important

For optimal results, we recommend using Stbl3™ *E. coli* for transformation as this strain is particularly well-suited for use in cloning unstable DNA such as lentiviral DNA containing direct repeats. Note that transformants containing unwanted recombinants are generally **not** obtained when Stbl3™ *E. coli* are used for transformation.

Do not transform the LR recombination reaction into *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.

Materials Needed

You will need the following items:

- LR recombination reaction (from Step 7, previous page)
 - One Shot® Stbl3™ Chemically Competent *E. coli* (supplied with the kit, Box 2; one vial per transformation; thaw on ice immediately before use)
 - LB Medium, pre-warmed to 37°C
Note: You may use S.O.C. Medium provided with the kit in place of LB Medium for cell recovery
 - pUC19 positive control (if desired to verify the transformation efficiency; supplied with the kit, Box 2)
 - 42°C water bath
 - LB Amp plates containing 100 μ g/ml ampicillin (two for each transformation; warm at 37°C for 30 minutes before use)
 - 37C shaking and non-shaking incubator
-

Continued on next page

Transforming One Shot[®] Stbl3[™] Competent *E. coli*, Continued

One Shot[®] Stbl3[™] Transformation Procedure

Use this procedure to transform the LR recombination reaction into One Shot[®] Stbl3[™] Chemically Competent *E. coli*.

1. Thaw on ice, one vial of One Shot[®] Stbl3[™] chemically competent cells for each transformation.
 2. Add 2 to 3 μl of the LR recombination reaction (from Step 7, page 12) into a vial of One Shot[®] Stbl3[™] cells and mix gently. **Do not mix by pipetting up and down.** For the pUC19 control, add 10 pg (1 μl) of DNA into a separate vial of One Shot[®] cells and mix gently.
 3. Incubate the vial(s) on ice for 30 minutes.
 4. Heat-shock the cells for 30 seconds at 42°C without shaking.
 5. Remove the vial(s) from the 42°C water bath and place them on ice for 2 minutes.
 6. Transfer cells gently to a sterile, 15-ml tube containing 1 ml of pre-warmed LB Medium.
 7. Cap the tube(s) tightly and shake horizontally at 37°C for 1 hour at 225 rpm in a shaking incubator.
 8. Spread 100 μl of the transformation mix on a pre-warmed LB-Amp plate and incubate overnight at 37°C. For the pUC19 control, dilute the transformation mix 1:10 into LB Medium (*e.g.* add 100 μl of the transformation mix to 900 μl of LB Medium) and plate 25–100 μl .
 9. Store the remaining transformation mix at 4°C. Plate out additional cells the next day, if desired.
 10. Proceed to **Analyzing Transformants**, next page.
-

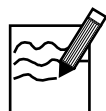
Analyzing Transformants

Introduction

We recommend analyzing the transformants using both restriction digestion and sequencing or PCR analysis as described below. This allows you to confirm the presence of the insert as well as ensure the absence of any aberrant lentiviral vector recombination between the LTRs.

You will screen colonies by performing miniprep DNA isolation and restriction analysis to validate the clones. You may also perform PCR analysis and/or sequencing of your clones to determine that your insert is in the correct orientation and is in-frame with the V5 epitope tag.

After verifying the correct clones, you will use the miniprep DNA to re-transform Stbl3™ *E. coli*. You will then isolate plasmid DNA for transfection and lentivirus production. Plasmid DNA for transfection into 293FT cells must be very clean and free from contaminants and salts, and should be isolated by midiprep.



Note

PCR screening of clones should never be used in place of restriction analysis. For example, clones that contain both correct and aberrantly recombined DNA may look positive by PCR but may not be optimal for lentivirus production.

Experimental Outline

1. For each transformation, pick 10–20 ampicillin-resistant colonies from plating the transformation mix. Culture cells overnight in LB medium containing 100 µg/ml ampicillin.
 2. Isolate plasmid DNA for each colony using a miniprep kit (see **Important**, next page).
 3. Analyze the plasmids by restriction analysis to confirm the presence and orientation of your insert as well as the integrity of the vector.
 4. **Optional:** Sequence the plasmids or perform PCR to determine that your gene of interest is in frame with the C-terminal V5 epitope tag.
 5. Retransform One Shot® Stbl3™ Chemically Competent *E. coli* separately with the validated clones.
 6. Inoculate LB-ampicillin with a fresh colony and grow for 6–8 hours to generate a starter culture.
 7. Inoculate the starter culture into at least 100 ml LB-ampicillin and grow for 18 hours.
 8. Isolate plasmid DNA using a midiprep kit (see **Important**, next page) for lentivirus production.
-

Continued on next page

Analyzing Transformants, Continued



Important

Stbl3™ *E. coli* is wild type for endonuclease 1 (*endA1+*). When performing plasmid DNA isolation with commercially available kits, ensure that Solution I of the Lysis buffer (often called Resuspension Buffer) contains 10 mM EDTA to inactivate the endonuclease to avoid DNA nicking and vector degradation. Alternatively, follow the instructions included in the plasmid purification kits for *endA1+* *E. coli* strains.

We recommend using the PureLink™ HQ Mini Plasmid Purification Kit and preparing lentiviral plasmid DNA using S.N.A.P.™ Midiprep Kits (page ix).

Restriction Digest

To confirm that no rearrangement in the LTR regions of the plasmid has taken place, perform restriction digests using a combination of *Afl* II and *Xho* I. *Afl* II sites are present in both LTRs. The *Xho* I site is present in the plasmid backbone at the 3' end of the insert. Assuming there are no *Afl* II or *Xho* I sites in the insert, 3 DNA fragments are generated from the *Afl* II + *Xho* I digest. Any unexpected DNA fragments are a result of LTR recombination.

If *Afl* II and/or *Xho* I sites are present in the insert, you can use a restriction enzyme or a combination of enzymes that cut once in the vector and once in the insert. The complete restriction enzyme maps of vectors are available at www.invitrogen.com.

Materials Needed

You will need the following items:

- LB medium containing 100 µg/ml ampicillin
 - PureLink™ HQ Mini Plasmid Purification Kit (page ix) or equivalent
 - Appropriate restriction enzymes (see above)
 - E-Gels® 1.2% agarose gels (page ix) or equivalent
 - S.N.A.P.™ Midiprep Kit (page ix) or equivalent
-

Screening Colonies by Miniprep

For each transformation, pick 10–20 colonies from plates obtained after plating the transformation mix (Step 9, page 14). Culture them overnight in LB medium containing 100 µg/ml ampicillin.

Isolate plasmid DNA using PureLink™ HQ Mini Plasmid Purification Kit or equivalent (see **Important**, above). The typical yield of pLenti DNA with PureLink™ HQ Mini Plasmid Purification Kit is ~ 5–7 µg, which is lower than the average DNA yield using this purification kit.

Perform restriction digests on plasmid DNA, and analyze the digested DNA on 0.8% or 1.2% agarose gels to confirm the correct clones.

What You Should See

Depending on the restriction sites you are using, you should be able to determine the number and size of bands you should obtain from your digestion. Agarose gel analysis should show the correct digestion pattern indicating proper recombination into the lentiviral vector. Additional or unexpected bands indicate aberrant recombination of the lentiviral vector.

Continued on next page

Analyzing Transformants, Continued

Analyzing Transformants by PCR

Use the protocol below (or any other suitable protocol) to analyze positive transformants using PCR. For PCR primers, use a primer such as the V5(C-term) Reverse primer (see below for sequence) and a primer that hybridizes within your insert. You will have to determine the amplification conditions. If you are using this technique for the first time, we recommend performing restriction analysis in parallel. Artifacts may be obtained because of mispriming or contaminating template.

Materials Needed:

AccuPrime™ *Pfx* SuperMix (page ix)

Appropriate forward and reverse PCR primers (20 μM each)

Procedure:

1. For each sample, aliquot 48 μl of AccuPrime™ *Pfx* SuperMix into a 0.5 ml microcentrifuge tube. Add 1 μl each of the forward and reverse PCR primer.
 2. Pick 10–20 colonies that have been analyzed by restriction digest with *Afl* II and *Xho* I (see previous page) and resuspend them individually in 50 μl of SuperMix containing PCR primers (remember to make a patch plate to preserve the colonies for further analysis).
 3. Incubate reaction for 10 minutes at 94°C to lyse cells and inactivate nucleases.
 4. Amplify for 20–30 cycles.
 5. For the final extension, incubate at 72°C for 10 minutes. Store at 4°C.
 6. Visualize PCR products by agarose gel electrophoresis.
-

Sequencing

To confirm that your gene of interest is in frame with the C-terminal tag, you may sequence your expression construct to confirm that your gene is cloned in the correct orientation and in frame with the V5 epitope. We recommend using the following primers to help you sequence your expression construct. Refer to the diagrams on pages 8–10 for the locations of the primer binding sites in each vector.

Note: For your convenience, Invitrogen has a custom primer synthesis service. For more information, visit our web site at www.invitrogen.com or contact **Technical Support** (page 42).

Vector	Primer	Sequence
pLenti4/V5-DEST, pLenti6/V5-DEST, or pLenti6.2/V5-DEST	CMV forward primer	5'-CGCAAATGGGCGGTAGGCGTG-3'
	V5(C-term) reverse primer	5'-ACCGAGGAGAGGGTTAGGGAT-3'
pLenti6/UbC/V5-DEST	UB forward primer	5'-TCAGTGTTAGACTAGTAAATTG-3'
	V5(C-term) reverse primer	5'-ACCGAGGAGAGGGTTAGGGAT-3'

Continued on next page

Analyzing Transformants, Continued

Isolating Lentiviral Plasmid DNA

This protocol provides general steps to retransform Stbl3™ *E. coli* and perform isolation of plasmid DNA for lentivirus production. pLenti plasmid DNA midipreps often have lower yields; therefore, a 100 ml volume of culture must be used for one DNA midiprep.

1. Dilute 1 µl of miniprep plasmid DNA from a positive clone 1:500 in TE.
2. Use 1 µl of this diluted DNA to retransform into One Shot® Stbl3™ Chemically Competent Cells as described on page 13.
3. Plate approximately one-tenth of the transformation on LB plates containing 100 µg/ml ampicillin and incubate at 37°C overnight.
4. Pick 1 colony and culture in 2–3 ml LB medium containing 100 µg/ml ampicillin for 6–8 hours at 37°C to obtain a starter culture.
5. Inoculate the entire volume of the starter culture into LB medium containing 100 µg/ml ampicillin and culture at 37°C overnight.

Note: Use a 100 ml volume for large scale or midiprep isolation of DNA.

6. Isolate plasmid DNA using S.N.A.P.™ Midiprep Kit or equivalent (see **Important**, page 16).

Note: For best results using the S.N.A.P.™ Midiprep Kit, split the 100 ml culture into two 50-ml tubes and process as if they were separate samples. Run both samples over the same DNA binding column B, (*i.e.* perform two spins) and treat as a single DNA prep in subsequent steps.

7. Perform restriction analysis (see page 16) to confirm the presence of the insert.
8. Use the purified plasmid DNA from the positive clone for producing the lentivirus and to check protein expression (optional, see next page).

Note: Typical DNA yield should be ~300–400 µg and the O.D._{260/280} ratio should be between 1.8 and 2.1.

DNA Isolation Guidelines

Once you have generated and validated your clone you will isolate plasmid DNA for transfection. Plasmid DNA for transfection into eukaryotic cells must be very clean and free from contamination with phenol and sodium chloride. Contaminants will kill the cells, and salt will interfere with lipid complexing, decreasing transfection efficiency. We recommend isolating lentiviral plasmid DNA using the S.N.A.P.™ Midiprep Kit.

Important: Do not use mini-prep plasmid DNA for lentivirus production.

Continued on next page

Analyzing Transformants, Continued

Maintaining the Expression Clone

Once you have generated your expression clone, maintain and propagate the plasmid in LB medium containing 100 µg/ml ampicillin. Addition of Blasticidin or Zeocin™ is not required.

Preparing a Glycerol Stock for Long-Term Storage

Once you have identified the correct clone, be sure to purify the colony and make a glycerol stock for long term storage. We recommend that you store a stock of plasmid DNA at -20°C.

1. Streak the original colony out for single colonies on LB plates containing 100 µg/ml ampicillin.
 2. Isolate a single colony and inoculate into 1–2 ml of LB containing 100 µg/ml ampicillin.
 3. Grow until culture reaches stationary phase.
 4. Mix 0.85 ml of culture with 0.15 ml of sterile glycerol and transfer to a cryovial.
 5. Store at -80°C.
-

Verifying Expression of Recombinant Protein

Optional: Before proceeding to generate a lentiviral stock of your pLenti-DEST expression construct, you may verify that the construct expresses the gene of interest by transfecting the plasmid directly into mammalian cells and assaying for your recombinant protein, if desired. Follow the guidelines below:

- Use an easy-to-transfect, dividing mammalian cell line (*e.g.* HEK 293 or COS-7).
- Use a transfection reagent that allows high-efficiency transfection; we recommend using Lipofectamine™ 2000 Reagent.

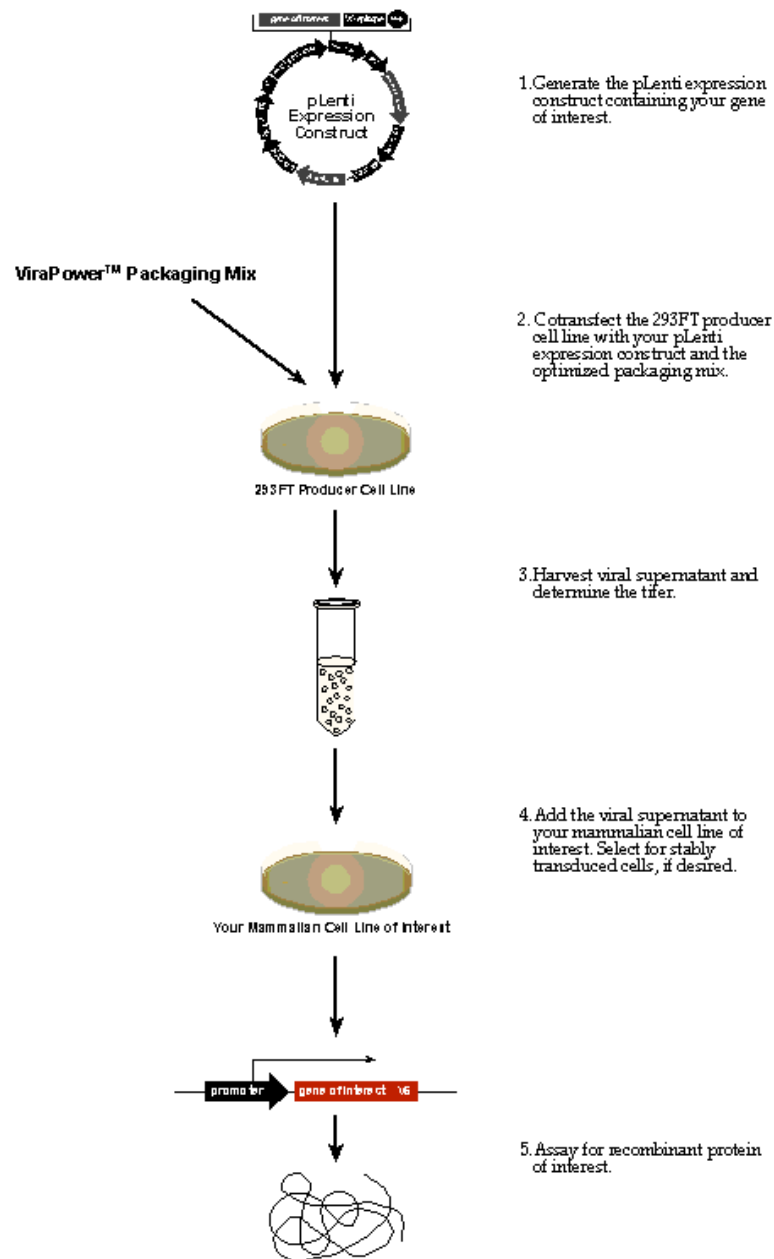
Note: Lipofectamine™ 2000 is supplied with the ViraPower™ Lentiviral Gateway® Expression Kits, but is also available separately from Invitrogen (see page ix for ordering information).

- Follow the manufacturer's instructions for the transfection reagent you are using to perform plasmid transfection. If you are using Lipofectamine™ 2000, follow the instructions included with the product.
-

Expression and Analysis

Introduction

Once you have obtained purified plasmid DNA of your pLenti-DEST expression construct, you are ready to use Invitrogen's ViraPower™ Lentiviral Expression System to produce a viral stock, which may then be used to transduce your mammalian cell line of choice to express your recombinant protein (see experimental outline below).



Continued on next page

Expression and Analysis, Continued

Materials Needed

To express your gene of interest from the pLenti-DEST construct using Invitrogen's ViraPower™ Lentiviral Expression System, you will need to have the following reagents:

- 293FT Cell Line for producing maximized levels of virus (Naldini *et al.*, 1996). This cell line is derived from 293F cells and stably expresses the SV40 large T antigen for enhanced virus production.
- ViraPower™ Packaging Mix. When cotransfected with the pLenti-DEST expression construct into the 293FT producer cell line, this optimized mixture of plasmids supplies the viral proteins in *trans* that are required to create viral particles.
- Transfection reagent for efficient delivery of the ViraPower™ Packaging Mix and the pLenti-DEST expression construct to 293FT cells. We recommend using Lipofectamine™ 2000 Reagent for optimal transfection efficiency.
- Blasticidin or Zeocin™, as appropriate for selection of stably transduced mammalian cells (see the **Appendix**, pages 27–29 for more information).
- **Optional:** Control lentiviral expression vector (see next page).

For more information about the 293FT Cell Line, see the 293FT Cell Line manual. For more information about the ViraPower™ Packaging Mix, refer to the ViraPower™ Lentiviral Expression System manual. Both manuals are available for downloading from our web site at www.invitrogen.com or by contacting **Technical Support** (page 42).

Obtaining Reagents

The 293FT Cell Line and the ViraPower™ Lentiviral Support Kits containing the ViraPower™ Packaging Mix, Lipofectamine™ 2000, and selection agent are available from Invitrogen (see below for ordering information).

Item	Catalog No.
ViraPower™ Bsd Lentiviral Support Kit	K4970–00
ViraPower™ Zeo Lentiviral Support Kit	K4985–00
293FT Cell Line	R700–07

Continued on next page

Expression and Analysis, Continued

Plasmid Preparation

Before expressing your recombinant fusion protein using the ViraPower™ Lentiviral Expression System, you must prepare purified plasmid DNA. Plasmid DNA for transfection into eukaryotic cells must be clean and free of contamination with phenol or sodium chloride. Contaminants will kill the cells, and salt may interfere with lipid complexing, decreasing transfection efficiency. We recommend isolating plasmid DNA using the S.N.A.P.™ Midiprep DNA Isolation Kit (page ix). **Do not use miniprep DNA to generate lentivirus.**

Note: We recommend performing restriction analysis to verify the integrity of your expression construct after plasmid preparation. See page 16 for details.

Positive Controls

A positive control vector is included with each pLenti-DEST vector for use as an expression control in the ViraPower™ Lentiviral Expression System (see table below). In each vector, β -galactosidase is expressed as a C-terminally tagged fusion protein that may be easily detected by western blot or functional assay. For details, see the **Appendix**, pages 37–40.

Vector	Positive Control
pLenti4/V5-DEST	pLenti4/V5-GW/ <i>lacZ</i>
pLenti6/V5-DEST	pLenti6/V5-GW/ <i>lacZ</i>
pLenti6.2/V5-DEST	pLenti6.2/V5-GW/ <i>lacZ</i>
pLenti6/UbC/V5-DEST	pLenti6/UbC/V5-GW/ <i>lacZ</i>

A control lentiviral expression vector (pLenti6.2-GW/EmGFP) containing Emerald Green Fluorescent Protein (EmGFP) to optimize transfection and virus production is available separately from Invitrogen. For ordering information, see page ix or visit www.invitrogen.com.

Propagating the Control Plasmids

To propagate and maintain the pLenti vectors:

1. We recommend using 10 ng of your vector to transform OneShot® Stbl3™ *E coli* (see page 13).
 2. Select transformants on selective plates containing 100 μ g/ml ampicillin.
 3. Propagate the plasmid in LB containing 100 μ g/ml ampicillin.
 4. Prepare a glycerol stock of a transformant containing plasmid for long-term storage (see page 20).
-

Continued on next page

Expression and Analysis, Continued

Producing Viral Stocks

Refer to the ViraPower™ Lentiviral Expression System manual for detailed guidelines and protocols to:

- Cotransfect your pLenti-DEST expression construct and the ViraPower™ Packaging Mix into the 293FT Cell Line to generate a lentiviral stock.
 - Determine the titer of your viral stock.
-

Determining Antibiotic Sensitivity

Once you have produced a lentiviral stock with a suitable titer, you will use this stock to transduce your lentiviral construct into the mammalian cell line of choice. You may assay for transient expression of your recombinant protein or use Zeocin™ or Blasticidin, as appropriate to select for stably transduced cells. Before generating your stably transduced cell line, we recommend that you generate a kill curve to determine the minimum concentration of Zeocin™ or Blasticidin required to kill your untransduced host cell line. For guidelines to generate a kill curve, refer to the ViraPower™ Lentiviral Expression System manual. For instructions to prepare and handle Zeocin™ or Blasticidin, see the **Appendix**, pages 27 and 29, respectively.

Transducing Mammalian Cells

Refer to the ViraPower™ Lentiviral Expression System manual for instructions and guidelines to:

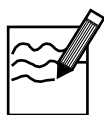
- Transduce your lentiviral construct into the mammalian cell line of interest at the appropriate multiplicity of infection (MOI).
 - Generate stable cell lines using Zeocin™ or Blasticidin selection.
-

Detecting Recombinant Fusion Proteins

To detect expression of your recombinant fusion protein, you may perform:

- Western blot analysis using the Anti-V5, Anti-V5-HRP, or Anti-V5-AP antibodies available from Invitrogen or an antibody to your protein
- Immunofluorescence using an Anti-V5-FITC antibody
- Functional analysis

For more information about the Anti-V5 antibodies, visit our web site at www.invitrogen.com or contact **Technical Support** (page 42).



Note

The C-terminal peptide containing the V5 epitope and the *attB2* site will add approximately 4.5 kDa to the size of your protein.

Assay for β -galactosidase Activity

The β -galactosidase protein expressed from the Lenti4/V5-GW/*lacZ*, Lenti6/V5-GW/*lacZ*, Lenti6.2/V5-GW/*lacZ*, Lenti6/Ubc/V5-GW/*lacZ* control lentiviral construct is approximately 121 kDa in size. You may assay for β -galactosidase expression by western blot, using cell-free lysates (Miller, 1972), or by staining. Invitrogen offers a β -gal Antiserum, β -Gal Assay Kit, and the β -Gal Staining Kit (refer to page ix for ordering information) for detection of β -galactosidase.

Appendix

Recipes

LB (Luria-Bertani) Medium

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. Allow solution to cool to ~55°C and add antibiotic, if desired.
 4. Store at 4°C.
-

LB Plates Containing Ampicillin

Follow the instructions below to prepare LB agar plates containing ampicillin.

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

Zeocin™

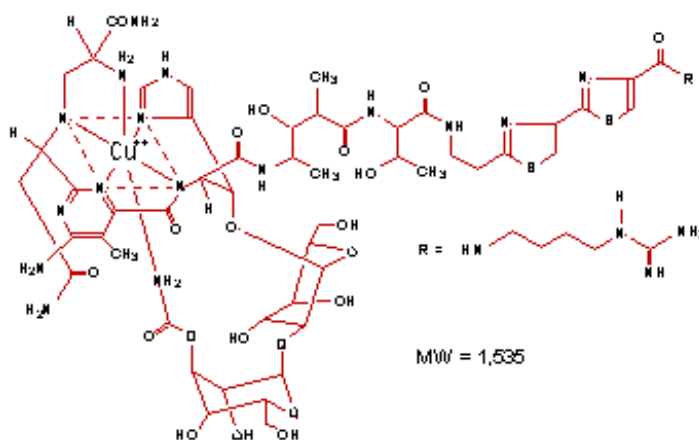
Zeocin™

Zeocin™ belongs to a family of structurally related bleomycin/phleomycin-type antibiotics isolated from *Streptomyces*. Antibiotics in this family are broad spectrum antibiotics that act as strong antibacterial and anti-tumor drugs. They show strong toxicity against bacteria, fungi (including yeast), plants, and mammalian cells (Baron *et al.*, 1992; Drocourt *et al.*, 1990; Mulsant *et al.*, 1988; Perez *et al.*, 1989).

The Zeocin™ resistance protein has been isolated and characterized (Calmels *et al.*, 1991; Drocourt *et al.*, 1990). This protein, the product of the *Sh ble* gene (*Streptoalloteichus hindustanus* bleomycin gene), is a 13.7 kDa protein that binds Zeocin™ and inhibits its DNA strand cleavage activity. Expression of this protein in eukaryotic and prokaryotic hosts confers resistance to Zeocin™.

Molecular Weight, Formula, and Structure

The formula for Zeocin™ is $C_{60}H_{89}N_{21}O_{21}S_3$ and the molecular weight is 1,535. The diagram below shows the structure of Zeocin™.



Applications of Zeocin™

Zeocin™ is used for selection in mammalian cells (Mulsant *et al.*, 1988); plants (Perez *et al.*, 1989); yeast (Baron *et al.*, 1992); and prokaryotes (Drocourt *et al.*, 1990). Suggested concentrations of Zeocin™ for selection in mammalian cell lines and *E. coli* are listed below:

Organism	Zeocin™ Concentration and Selective Medium
<i>E. coli</i>	25–50 µg/ml in Low Salt LB medium*
Mammalian Cells	50–1000 µg/ml (varies with cell line)

*Efficient selection requires that the concentration of NaCl be no more than 5 g/L (<90 mM).

Continued on next page

Zeocin™, Continued

Handling Zeocin™

- **High salt and acidity or basicity inactivate Zeocin™.** Therefore, we recommend that you reduce the salt in bacterial medium and adjust the pH to 7.5 to keep the drug active. Note that the pH and salt concentration do not need to be adjusted when preparing tissue culture medium containing Zeocin™.
 - Store Zeocin™ at –20°C and thaw on ice before use.
 - Zeocin™ is light sensitive. Store the drug, and plates or medium containing drug, in the dark at 4°C. Culture medium containing Zeocin™ may be stored at 4°C protected from exposure to light for up to 1 month.
 - Wear gloves, a laboratory coat, and safety glasses or goggles when handling Zeocin™-containing solutions.
 - Zeocin™ is toxic. Do not ingest or inhale solutions containing the drug.
-

Preparing and Storing Zeocin™

Zeocin™ is supplied in autoclaved, deionized water in 1.25 ml aliquots at a concentration of 100 mg/ml. The stability of Zeocin™ is guaranteed for six months, if stored at –20°C protected from exposure to light.

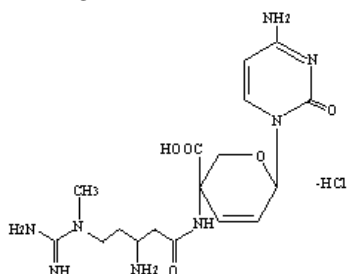
Blasticidin

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 non-toxic deaminohydroxy derivative (Izumi *et al.*, 1991).

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

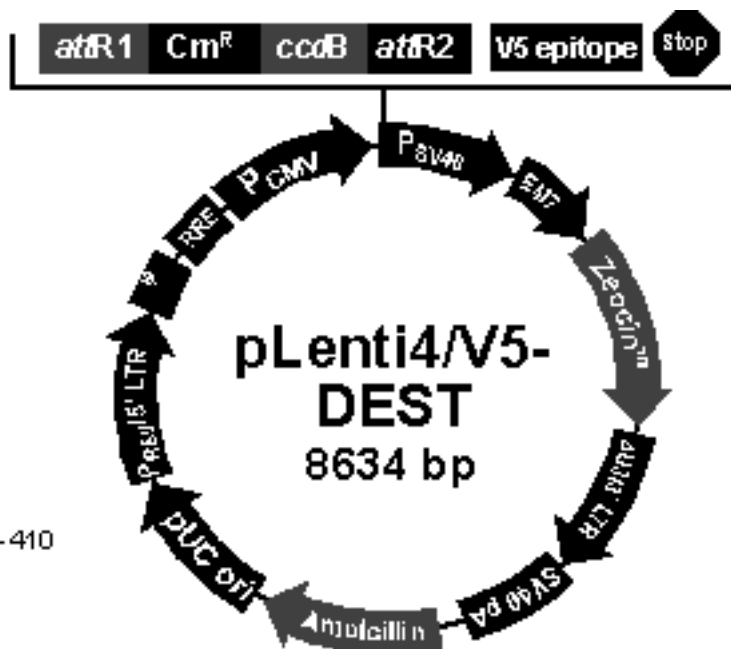
Blasticidin may be obtained separately from Invitrogen (page ix) in 50 mg aliquots. Blasticidin is soluble in water. Sterile water is generally used to prepare stock solutions of 5–10 mg/ml.

- Dissolve Blasticidin in sterile water and filter-sterilize the solution.
 - Aliquot 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 pLenti4/V5-DEST

Map of pLenti4/V5-DEST

The map below shows the elements of pLenti4/V5-DEST. DNA from the entry clone replaces the region between bases 2447 and 4130. The complete sequence for pLenti4/V5-DEST is available from our web site at www.invitrogen.com or by contacting Technical Support (page 42).



Comments for pLenti4/V5-DEST
8634 nucleotides

RSV/5' LTR hybrid promoter: bases 1-410

RSV promoter: bases 1-229

HIV-1 5' LTR: bases 230-410

5' splice donor: base 520

HIV-1 psi (Ψ) packaging signal: bases 521-565

HIV-1 Rev response element (RRE): bases 1075-1308

3' splice acceptor: base 1656

3' splice acceptor: base 1684

CMV promoter: bases 1809-2392

attR1 site: bases 2440-2564

Chloramphenicol resistance gene (Cm^R): bases 2673-3332

ccdB gene: bases 3674-3979

attR2 site: bases 4020-4144

V5 epitope: bases 4197-4238

SV40 early promoter and origin: bases 4293-4602

EM7 promoter: bases 4621-4687

Zeocin^R resistance gene: bases 4688-5062

ΔU3/3' LTR: bases 5154-5388

ΔU3: bases 5154-5207

3' LTR: bases 5208-5388

SV40 polyadenylation signal: bases 5460-5591

bla promoter: bases 6450-6548

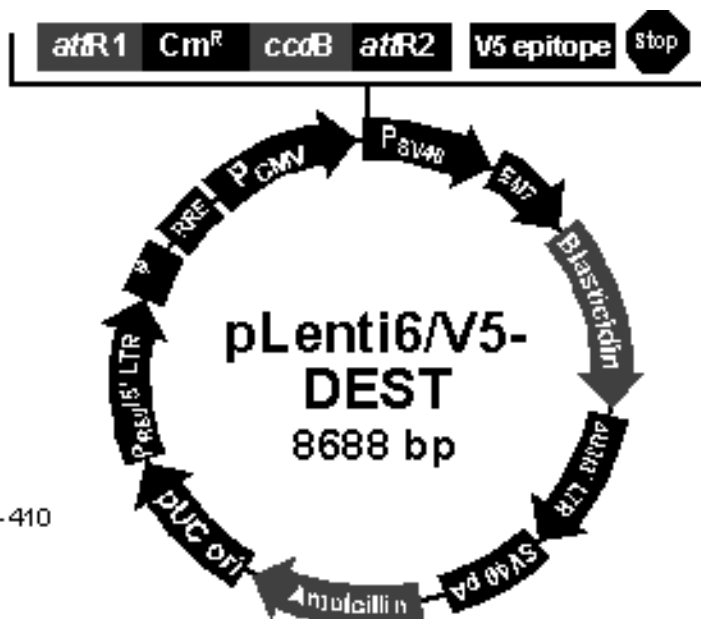
Ampicillin (*bla*) resistance gene: bases 6549-7409

pUC origin: bases 7554-8227

Map of pLenti6/V5-DEST

Map of pLenti6/V5-DEST

The map below shows the elements of pLenti6/V5-DEST. DNA from the entry clone replaces the region between bases 2447 and 4130. The complete sequence for pLenti6/V5-DEST is available from our web site at www.invitrogen.com or by contacting Technical Support (page 42).



Comments for pLenti6/V5-DEST
8688 nucleotides

RSV5' LTR hybrid promoter: bases 1-410

RSV promoter: bases 1-229

HIV-1 5' LTR: bases 230-410

5' splice donor: base 520

HIV-1 psi (Ψ) packaging signal: bases 521-565

HIV-1 Rev response element (RRE): bases 1075-1308

3' splice acceptor: base 1656

3' splice acceptor: base 1684

CMV promoter: bases 1809-2392

attR1 site: bases 2440-2564

Chloramphenicol resistance gene (Cm^R): bases 2673-3332

ccdB gene: bases 3674-3979

attR2 site: bases 4020-4144

V5 epitope: bases 4197-4238

SV40 early promoter and origin: bases 4293-4602

EM7 promoter: bases 4657-4723

Blasticidin resistance gene: bases 4724-5122

Δ U3/3' LTR: bases 5208-5442

Δ U3: bases 5208-5261

3' LTR: bases 5262-5442

SV40 polyadenylation signal: bases 5514-5645

bla promoter: bases 6504-6602

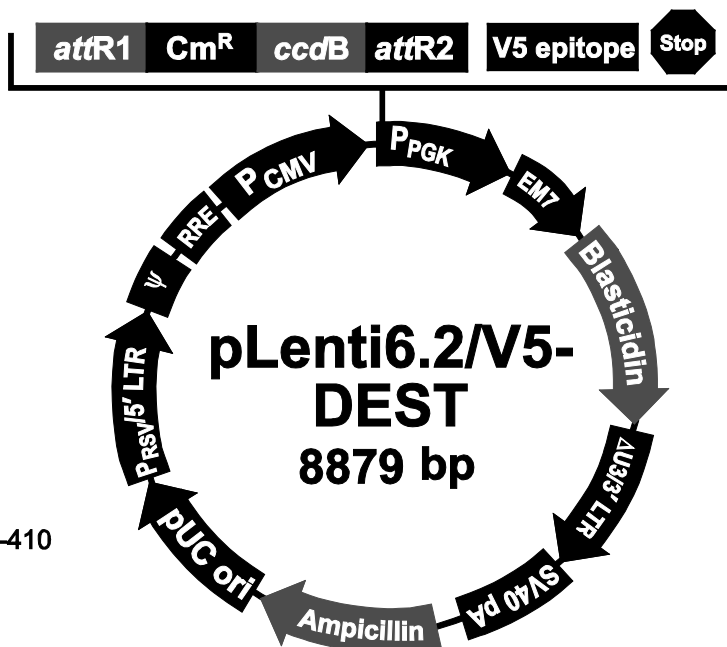
Ampicillin (*bla*) resistance gene: bases 6603-7463

pUC origin: bases 7608-8281

Map of pLenti6.2/V5-DEST

Map of pLenti6.2/V5-DEST

The map below shows the elements of pLenti6.2/V5-DEST. DNA from the entry clone replaces the region between bases 2513 and 4196. The complete sequence for pLenti6.2/V5-DEST is available from our web site at www.invitrogen.com or by contacting Technical Support (page 42).



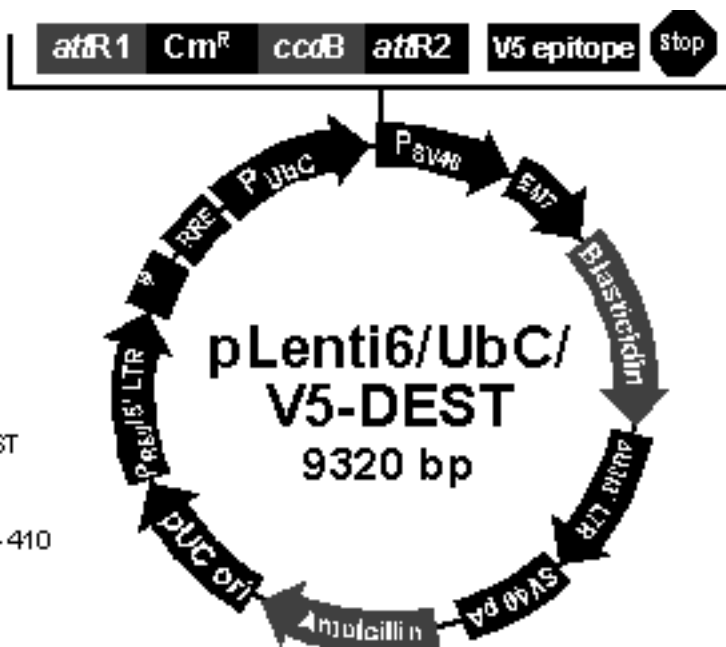
Comments for pLenti6.2/V5-DEST 8813 nucleotides

RSV/5' LTR hybrid promoter: bases 1-410
RSV promoter: bases 1-229
HIV-1 5' LTR: bases 230-410
5' splice donor: base 520
HIV-1 psi (ψ) packaging signal: bases 521-565
HIV-1 Rev response element (RRE): bases 1075-1308
3' splice acceptor: base 1656
3' splice acceptor: base 1684
CMV promoter: bases 1874-2458
attR1 site: bases 2506-2630
Chloramphenicol resistance gene (*Cm^R*): bases 2739-3398
ccdB gene: bases 3740-4045
attR2 site: bases 4086-4210
V5 epitope: bases 4263-4304
PGK promoter: bases 4332-4837
EM7 promoter: bases 4848-4914
Blasticidin resistance gene: bases 4915-5313
 Δ U3/3' LTR: bases 5333-5633
 Δ U3: bases 5399-5452
3' LTR: bases 5453-5633
SV40 polyadenylation signal: bases 5705-5835
bla promoter: bases 6695-6793
Ampicillin (*bla*) resistance gene: bases 6794-7654
pUC origin: bases 7799-8472

Map of pLenti6/UbC/V5-DEST

Map of pLenti6/UbC/V5-DEST

The map below shows the elements of pLenti6/UbC/V5-DEST. DNA from the entry clone replaces the region between bases 3079 and 4762. The complete sequence for pLenti6/UbC/V5-DEST is available from our web site at www.invitrogen.com or by contacting Technical Support (page 42).



Comments for pLenti6/UbC/V5-DEST
9320 nucleotides

RSV ψ 5' LTR hybrid promoter: bases 1-410

RSV promoter: bases 1-229

HIV-1 5' LTR: bases 230-410

5' splice donor: base 520

HIV-1 ψ (ψ) packaging signal: bases 521-565

HIV-1 Rev response element (RRE): bases 1075-1308

3' splice acceptor: base 1656

3' splice acceptor: base 1684

UbC promoter: bases 1798-3016

attR1 site: bases 3072-3196

Chloramphenicol resistance gene (Cm^R): bases 3305-3964

ccdB gene: bases 4306-4611

attR2 site: bases 4652-4776

V5 epitope: bases 4829-4870

SV40 early promoter and origin: bases 4925-5234

EM7 promoter: bases 5289-5355

Blasticidin resistance gene: bases 5356-5754

$\Delta U3/3'$ LTR: bases 5840-6074

$\Delta U3$: bases 5840-5893

3' LTR: bases 5894-6074

SV40 polyadenylation signal: bases 6146-6277

bla promoter: bases 7136-7234

Ampicillin (*bla*) resistance gene: bases 7235-8095

pUC origin: bases 8240-8913

Features of the pLenti-DEST Vectors

Features of the Vectors

The pLenti4/V5-DEST (8634 bp), pLenti6/V5-DEST (8688 bp), pLenti6.2/V5-DEST (8813 bp) and pLenti6/UbC/V5-DEST (9320 bp) vectors contain the following elements. Features have been functionally tested and the vectors have been fully sequenced.

Feature	Benefit
Rous Sarcoma Virus (RSV) enhancer/promoter	Allows Tat-independent production of viral mRNA (Dull <i>et al.</i> , 1998).
HIV-1 truncated 5' LTR	Permits viral packaging and reverse transcription of the viral mRNA (Luciw, 1996).
5' splice donor and 3' acceptors	Enhances the biosafety of the vector by facilitating removal of the ψ packaging sequence and RRE such that expression of the gene of interest in the transduced host cell is no longer Rev-dependent (Dull <i>et al.</i> , 1998).
HIV-1 psi (ψ) packaging signal	Allows viral packaging (Luciw, 1996).
HIV-1 Rev response element (RRE)	Permits Rev-dependent nuclear export of unspliced viral mRNA (Kjems <i>et al.</i> , 1991; Malim <i>et al.</i> , 1989).
CMV promoter (pLenti4/V5-DEST, pLenti6/V5-DEST, and pLenti6.2/V5-DEST only)	Permits high-level, constitutive expression of the gene of interest (Andersson <i>et al.</i> , 1989; Boshart <i>et al.</i> , 1985; Nelson <i>et al.</i> , 1987).
Human ubiquitin C (UbC) promoter (pLenti6/UbC/V5-DEST only)	Permits constitutive expression of the gene of interest in a broad range of mammalian cell types (Hershko & Ciechanover, 1982; Schorpp <i>et al.</i> , 1996; Wulff <i>et al.</i> , 1990).
<i>attR1</i> and <i>attR2</i> sites	Bacteriophage λ -derived DNA recombination sequences that permit recombinational cloning of the gene of interest from a Gateway® entry clone (Landy, 1989).
Chloramphenicol resistance gene (Cm ^R)	Allows counterscreening of the plasmid.
<i>ccdB</i> gene	Permits negative selection of the plasmid.
V5 epitope	Allows detection of the recombinant fusion protein by Anti-V5 Antibodies (Southern <i>et al.</i> , 1991).
SV40 early promoter and origin (pLenti4/V5-DEST, pLenti6/V5-DEST, and pLenti6/UbC/V5-DEST only)	Allows high-level expression of the selection marker and episomal replication in cells expressing the SV40 large T antigen.
PGK promoter (pLenti6.2/V5-DEST only)	Allows high-level expression of the selection marker in mammalian cell lines.
EM7 promoter	Synthetic prokaryotic promoter for expression of the selection marker in <i>E. coli</i> .
Blasticidin (<i>bsd</i>) resistance gene (pLenti6/V5-DEST, pLenti6.2/V5-DEST and pLenti6/UbC/V5-DEST only)	Permits selection of stably transduced mammalian cell lines (Kimura <i>et al.</i> , 1994).

Zeocin™ resistance (<i>Sh ble</i>) gene (pLenti4/V5-DEST only)	Permits selection of stably transduced mammalian cell lines (Drocourt <i>et al.</i> , 1990; Mulsant <i>et al.</i> , 1988).
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Continued on next page

Features of the pLenti-DEST Vectors, Continued

Feature	Benefit
Δ U3/HIV-1 truncated 3' LTR	Allows viral packaging but self-inactivates the 5' LTR for biosafety purposes (Dull <i>et al.</i> , 1998). The element also contains a polyadenylation signal for transcription termination and polyadenylation of mRNA in transduced cells.
SV40 polyadenylation signal	Allows transcription termination and polyadenylation of mRNA.
<i>bla</i> promoter	Allows expression of the ampicillin resistance gene.
Ampicillin resistance gene (β -lactamase)	Allows selection of the plasmid in <i>E. coli</i> .
pUC origin	Permits high-copy replication and maintenance in <i>E. coli</i> .

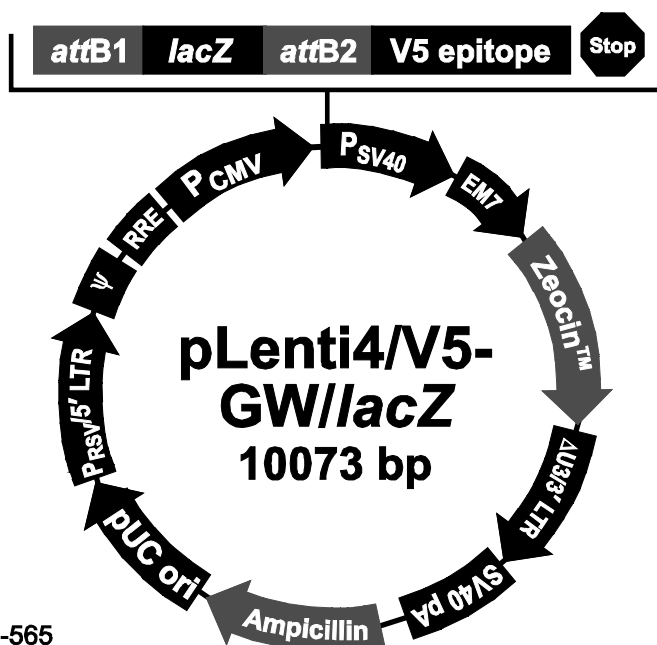
Map of pLenti4/V5-GW/lacZ

Description

pLenti4/V5-GW/lacZ is a 10073 bp control vector expressing β -galactosidase, and was generated using the Gateway® LR recombination reaction between an entry clone containing the *lacZ* gene and pLenti4/V5-DEST. β -galactosidase is expressed as a C-terminal V5 fusion protein with a molecular weight of approximately 121 kDa.

Map of pLenti4/V5-GW/lacZ

The map below shows the elements of pLenti4/V5-GW/lacZ. The complete sequence of the vector is available from our web site at www.invitrogen.com or by contacting Technical Support (page 42).



Comments for pLenti4/V5-GW/lacZ 10073 nucleotides

RSV enhancer/promoter: bases 1-229
HIV-1 5' LTR: bases 230-410
5' splice donor: base 520
HIV-1 psi (ψ) packaging signal: bases 521-565
HIV-1 Rev response element (RRE): bases 1075-1308
3' splice acceptor: base 1656
3' splice acceptor: base 1684
CMV promoter: bases 1809-2392
attB1 site: bases 2440-2464
lacZ ORF: bases 2484-5540
attB2 site: bases 5560-5584
V5 epitope: bases 5637-5678
SV40 early promoter and origin: bases 5733-6041
EM7 promoter: bases 6060-6126
Zeocin™ resistance gene: bases 6127-6501
 Δ U3/HIV-1 3' LTR: bases 6593-6827
 Δ U3: bases 6593-6646
 Truncated HIV-1 3' LTR: bases 6647-6827
SV40 polyadenylation signal: bases 6899-7030
bla promoter: bases 7889-7987
Ampicillin (*bla*) resistance gene: bases 7988-8848
pUC origin: bases 8993-9666

Map of pLenti6/V5-GW/lacZ

Description

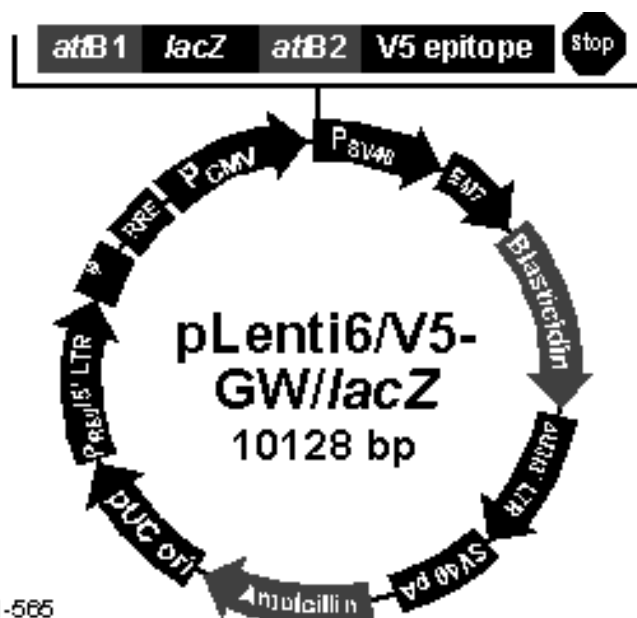
pLenti6/V5-GW/lacZ is a 10128 bp control vector expressing β -galactosidase, and was generated using the Gateway® LR recombination reaction between an entry clone containing the *lacZ* gene and pLenti6/V5-DEST. β -galactosidase is expressed as a C-terminal V5 fusion protein with a molecular weight of approximately 121 kDa.

Map of pLenti6/V5-GW/lacZ

The map below shows the elements of pLenti6/V5-GW/lacZ. The complete sequence of the vector is available from our web site at www.invitrogen.com or by contacting Technical Support (page 42).

Comments for pLenti6/V5-GW/lacZ
10128 nucleotides

RSV enhancer/promoter: bases 1-229
HIV-1 5' LTR: bases 230-410
5' splice donor: base 520
HIV-1 psi (ψ) packaging signal: bases 521-565
HIV-1 Rev response element (RRE): bases 1075-1308
3' splice acceptor: base 1656
3' splice acceptor: base 1684
CMV promoter: bases 1809-2392
atB1 site: bases 2440-2464
lacZ ORF: bases 2484-5540
atB2 site: bases 5560-5584
V5 epitope: bases 5637-5678
SV40 early promoter and origin: bases 5733-8042
EM7 promoter: bases 6097-6163
Blasticidin resistance gene: bases 6164-6562
 Δ U3/HIV-1 3' LTR: bases 6648-6882
 Δ U3: bases 6648-6701
Truncated HIV-1 3' LTR: bases 6702-6882
SV40 polyadenylation signal: bases 6954-7085
bla promoter: bases 7944-8042
Ampicillin (*bla*) resistance gene: bases 8043-8903
pUC origin: bases 9048-9721



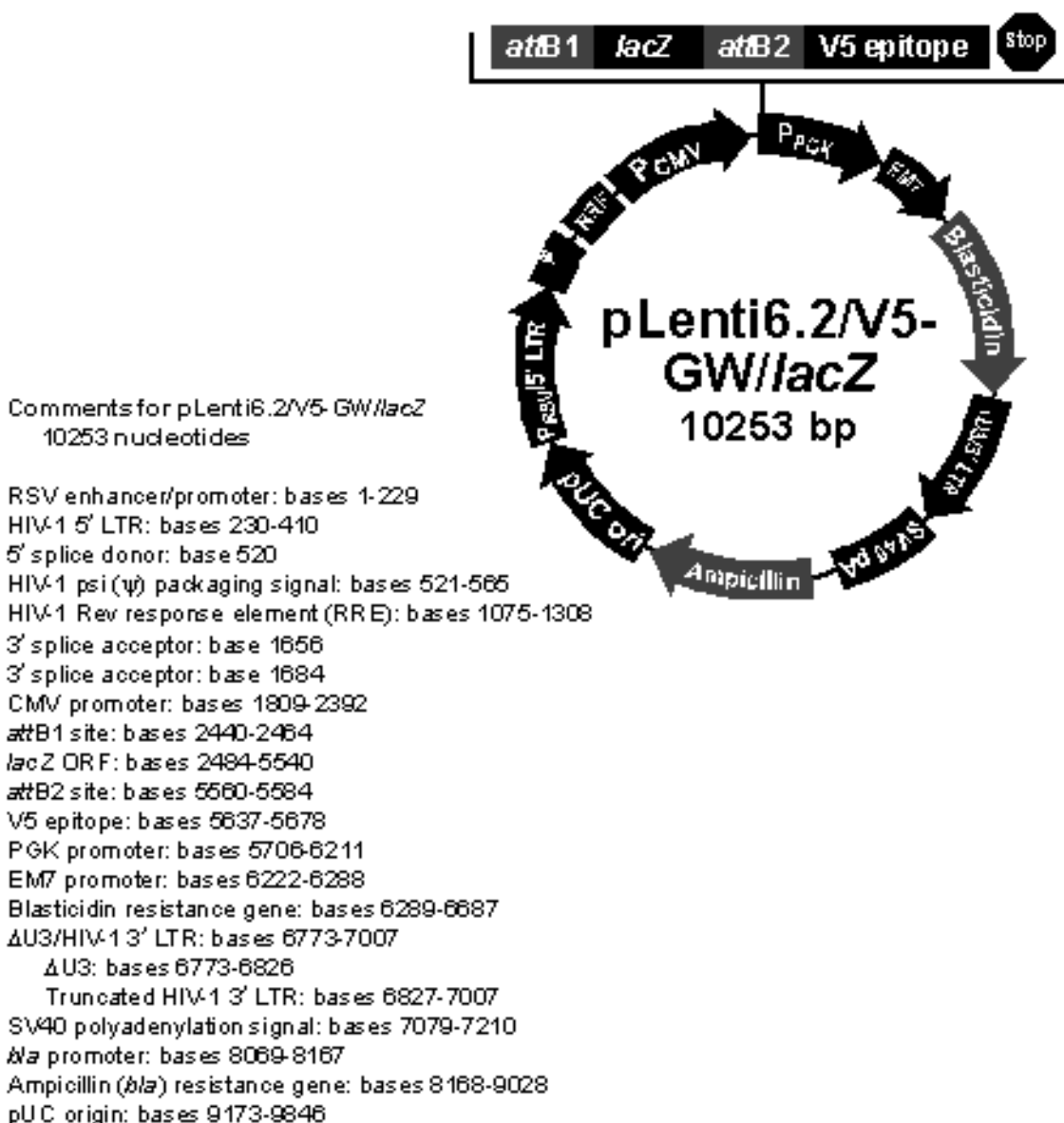
Map of pLenti6.2/V5-GW/lacZ

Description

pLenti6.2/V5-GW/lacZ is a 10253 bp control vector expressing β -galactosidase, and was generated using the Gateway® LR recombination reaction between an entry clone containing the *lacZ* gene and pLenti6.2/V5-DEST. β -galactosidase is expressed as a C-terminal V5 fusion protein with a molecular weight of approximately 121 kDa.

Map of pLenti6.2/V5-GW/lacZ

The map below shows the elements of pLenti6.2/V5-GW/lacZ. The complete sequence of the vector is available from our web site at www.invitrogen.com or by contacting Technical Support (page 42).



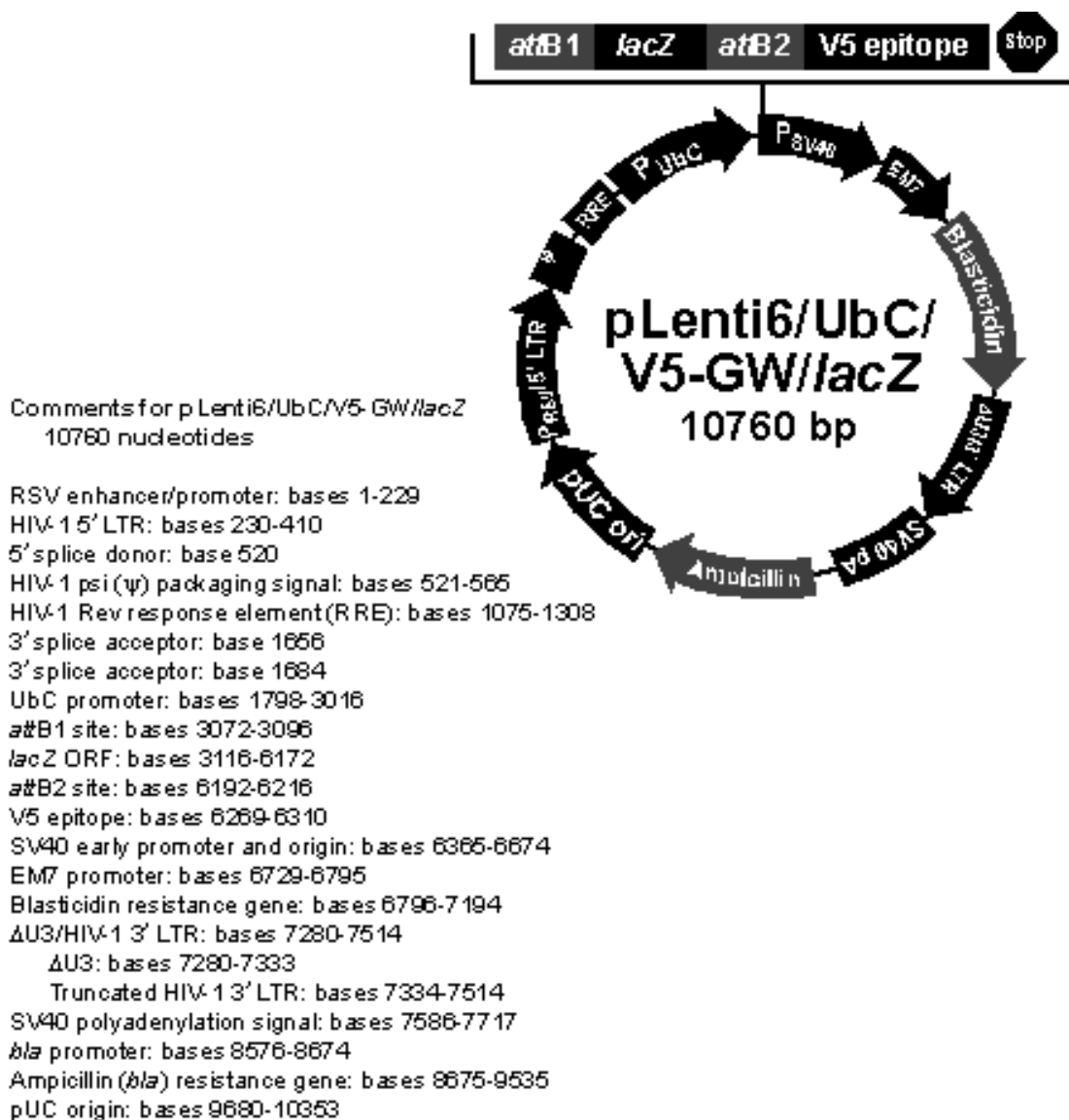
Map of pLenti6/UbC/V5-GW/lacZ

Description

pLenti6/UbC/V5-GW/lacZ is a 10760 bp control vector expressing β -galactosidase, and was generated using the Gateway[®] LR recombination reaction between an entry clone containing the *lacZ* gene and pLenti6/UbC/V5-DEST. β -galactosidase is expressed as a C-terminal V5 fusion protein with a molecular weight of approximately 121 kDa.

Map of pLenti6/ UbC/V5-GW/lacZ

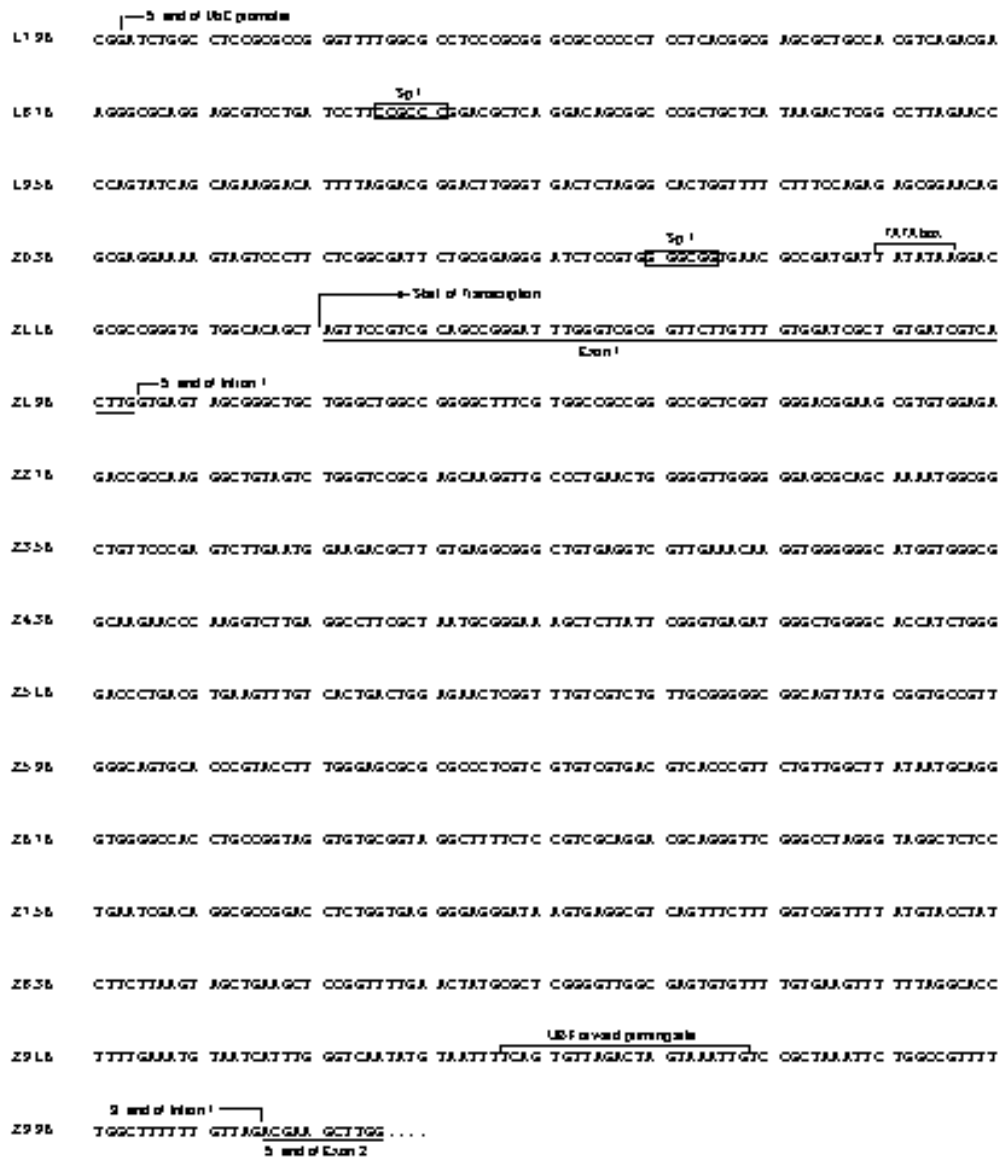
The map below shows the elements of pLenti6/UbC/V5-GW/lacZ. The complete sequence of the vector is available from our web site at www.invitrogen.com or by contacting Technical Support (page 42).



Human UbC Promoter

Description

The human ubiquitin C (UbC) promoter (in pLenti6/UbC/V5-DEST) allows high-level expression of recombinant protein in most mammalian cell lines (Wulff *et al.*, 1990) and in virtually all tissues tested in transgenic mice (Schorpp *et al.*, 1996). The diagram below shows the features of the UbC promoter as described by Neno *et al.*, 1996 .



Technical Support

Web Resources



Visit the Invitrogen web site at www.invitrogen.com for:

- Technical resources, including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, etc.
 - Complete technical support contact information.
 - Access to the Invitrogen Online Catalog.
 - Additional product information and special offers.
-

Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our web site (www.invitrogen.com).

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MSDS

MSDSs (Material Safety Data Sheets) are available on our web site at www.invitrogen.com/msds.

Certificate of Analysis

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**Gateway[®] Clone
Distribution Policy**

For additional information about Invitrogen's policy for the use and distribution of Gateway[®] clones, see the section entitled **Gateway[®] Clone Distribution Policy**, page 45.

Continued on next page

Purchaser Notification, Continued

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Promoter**

The use of the CMV promoter is covered under U.S. Patent Nos. 5,168,062 and 5,385,839 owned and licensed by the University of Iowa Research Foundation and is sold for research use only. Commercial users must obtain a license to these patents directly from the University of Iowa Research Foundation (UIRF), 214 Technology Innovation Center, Iowa City, Iowa 52242. For further information, please contact the Associate Director of UIRF, at 319-335-4546.

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Blasticidin Selection
Marker**

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Marker**

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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.

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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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