



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 nos. V496-10, V498-10, V499-10, V368-20

Version J

7 June 2010

25-0503

User Manual

Table of Contents

| | |
|--|-----------|
| Kit Contents and Storage | iv |
| Accessory Products..... | vii |
| Introduction | 1 |
| Overview..... | 1 |
| Methods..... | 5 |
| Generating an Entry Clone | 5 |
| Creating Expression Clones..... | 6 |
| Performing the LR Recombination Reaction..... | 9 |
| Transforming One Shot® Stbl3™ Competent <i>E. coli</i> | 11 |
| Analyzing Transformants | 13 |
| Expression and Analysis..... | 18 |
| Appendix | 22 |
| Recipes..... | 22 |
| Zeocin™ | 23 |
| Blasticidin..... | 25 |
| Map of pLenti4/V5-DEST | 26 |
| Map of pLenti6/V5-DEST | 27 |
| Map of pLenti6.2/V5-DEST | 28 |
| Map of pLenti6/UbC/V5-DEST | 29 |
| Features of the pLenti-DEST Vectors | 30 |
| Map of pLenti4/V5-GW/ <i>lacZ</i> | 32 |
| Map of pLenti6/V5-GW/ <i>lacZ</i> | 33 |
| Map of pLenti6.2/V5-GW/ <i>lacZ</i> | 34 |
| Map of pLenti6/UbC/V5-GW/ <i>lacZ</i> | 35 |
| Human UbC Promoter | 36 |
| Technical Support | 37 |
| Purchaser Notification | 38 |
| References | 41 |

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 v–vi. 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 37).

| Component | Catalog no. | |
|--|--|---|
| | <u>K4960-00, K4980-00, K4990-00, K367-20</u> | <u>V496-10, V498-10, 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 37).

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*(r_B⁻, m_B⁻) *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 37).

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 37).

| 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 2–3 for more information)
- SV40 or PGK promoter driving expression of Blasticidin (see page 3 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 3–4.

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 Blastcidin (see next page)
- Blastcidin (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 32–35. 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 viii.

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 Blasticidin

The pLenti6.2/V5-DEST vector contains the murine phosphoglycerate kinase-1 (PGK) promoter to drive mammalian expression of the Blasticidin 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 Blasticidin 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 37).

Continued on next page

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 37).

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 37).



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 37).

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)NNA**AT**GG

- 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 7-8 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 9–17) and **Transforming One Shot® Stbl3™ Competent *E. coli*** (pages 11–17) 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 11).
 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 vii, or visit our web site at www.invitrogen.com.

Continued on next page

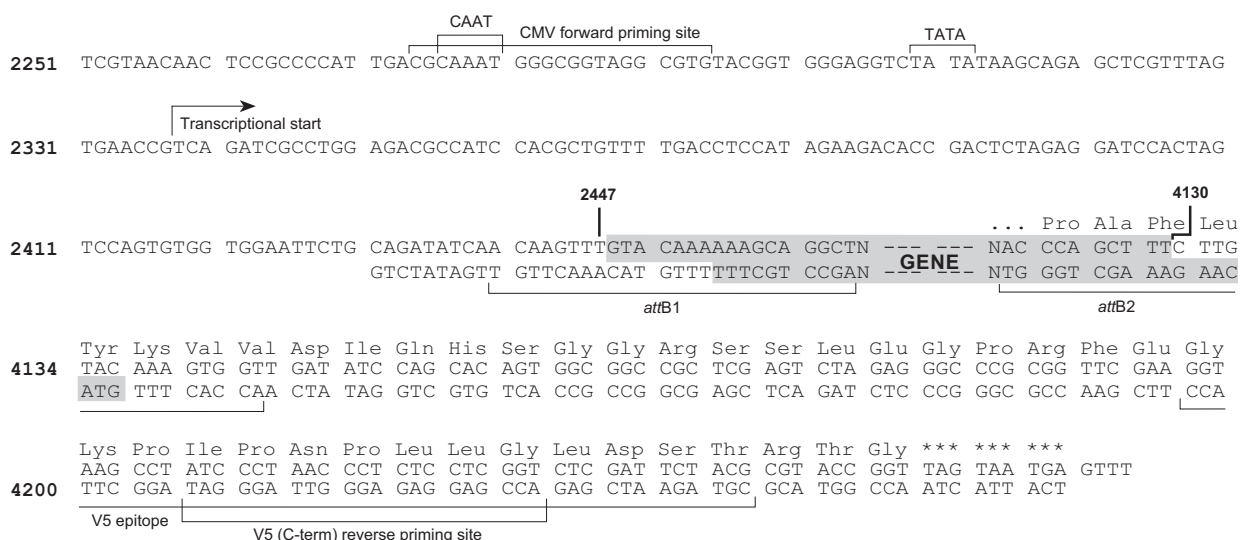
Creating Expression Clones, Continued

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.



Continued on next page

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 vii). 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 10 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 vii; 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 10) 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 vii).

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 vii) or equivalent
 - Appropriate restriction enzymes (see above)
 - E-Gels® 1.2% agarose gels (page vii) or equivalent
 - S.N.A.P.™ Midiprep Kit (page vii) or equivalent
-

Screening Colonies by Miniprep

For each transformation, pick 10–20 colonies from plates obtained after plating the transformation mix (Step 9, page 12). 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 vii)

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 7–8 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 37).

| 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 midpreps often have lower yields; therefore, a 100 ml volume of culture must be used for one DNA midprep.

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 11.
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 midprep isolation of DNA.

6. Isolate plasmid DNA using S.N.A.P.™ Midiprep Kit or equivalent (see **Important**, page 14).

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 14) 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

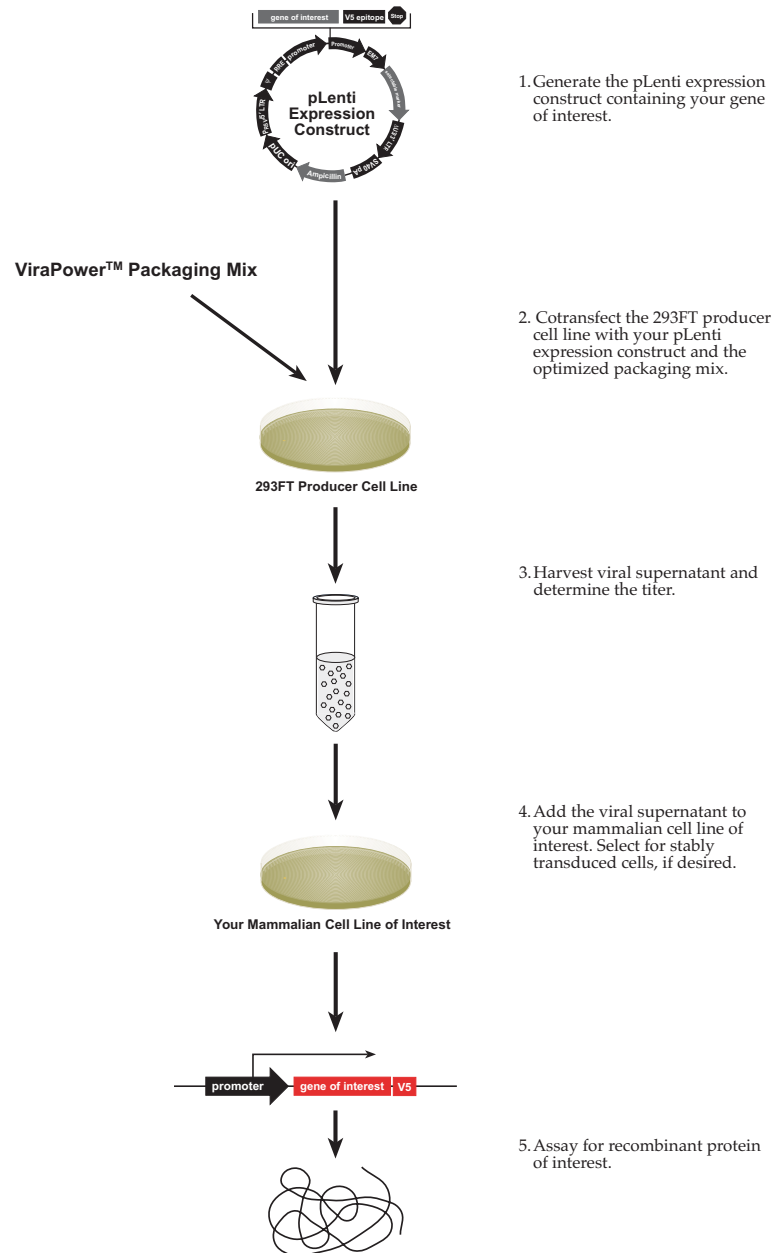
Optional: Before proceeding to generate a lentiviral stock of your pLenti-DEST expression construct, you may verify that the construct expresses the gene of interest by transfecting the plasmid directly into mammalian cells and assaying for your recombinant protein, if desired. Follow the guidelines 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 vii 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 23–25 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 37).

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 vii). **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 14 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 32–35.

| 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 vii 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 11).
 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 17).
-

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 23 and 25, 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 37).



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 vii 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™

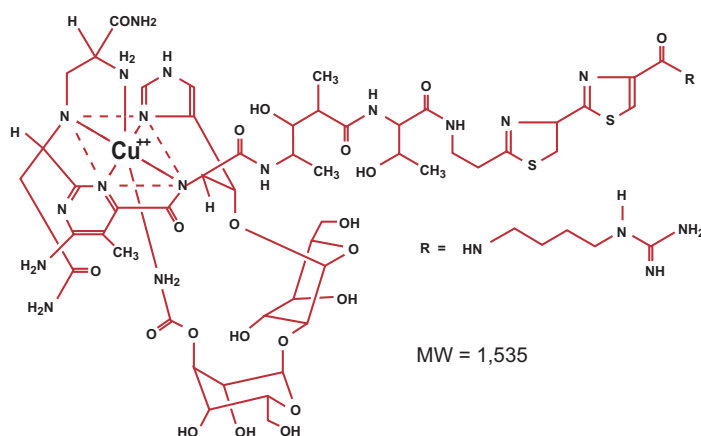
Zeocin™

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.

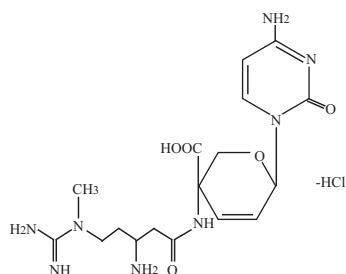
Blasticidin

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

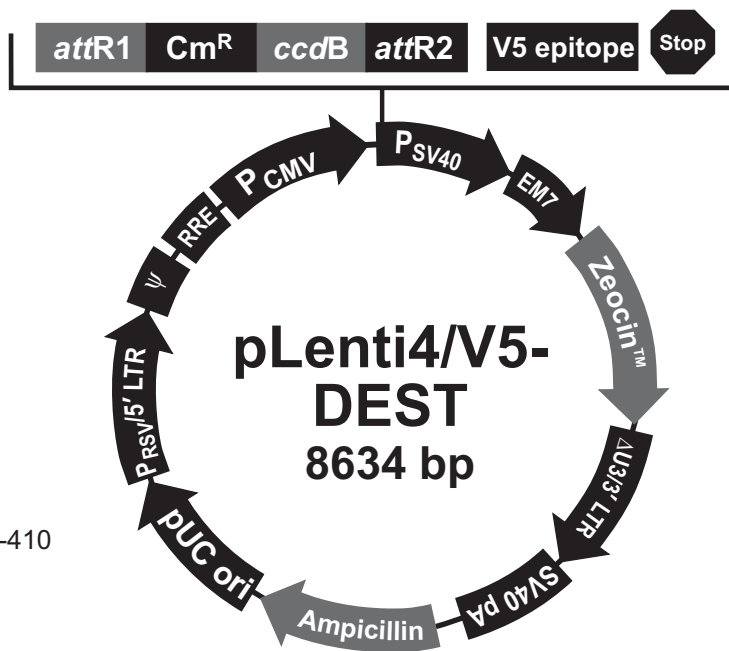
Blasticidin may be obtained separately from Invitrogen (page vii) in 50 mg aliquots. Blasticidin is soluble in water. Sterile water is generally used to prepare stock solutions of 5–10 mg/ml.

- 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 37).



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™ resistance gene: bases 4688-5062

$\Delta U3/3'$ LTR: bases 5154-5388

$\Delta U3$: bases 5154-5207

3' LTR: bases 5208-5388

SV40 polyadenylation signal: bases 5460-5591

b/a promoter: bases 6450-6548

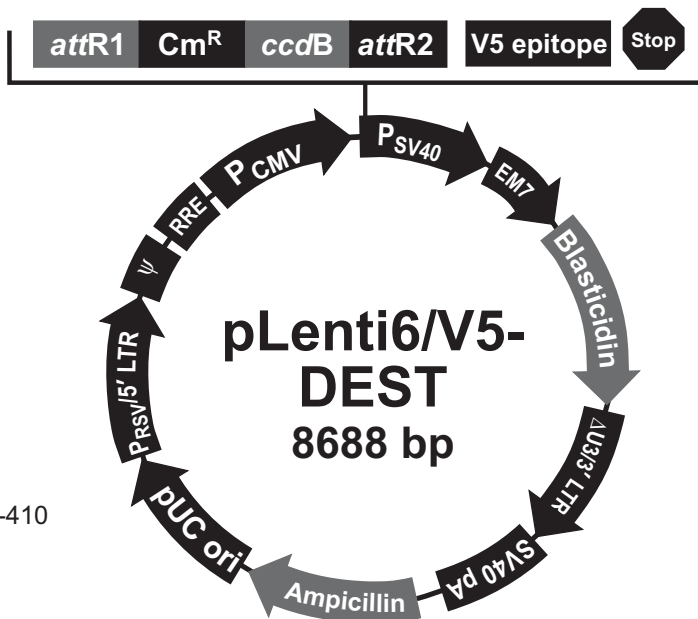
Ampicillin (*b/a*) resistance gene: bases 6549-7409

ρ UC 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 37).



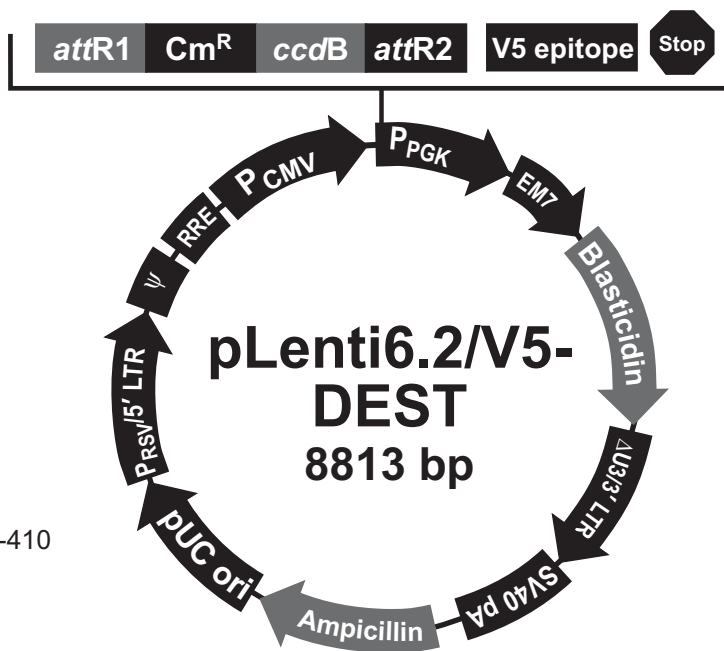
Comments for pLenti6/V5-DEST 8688 nucleotides

- 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 4657-4723
- Blasticidin resistance gene: bases 4724-5122
- $\Delta U3/3'$ LTR: bases 5208-5442
 - $\Delta U3$: bases 5208-5261
 - 3' LTR: bases 5262-5442
- SV40 polyadenylation signal: bases 5514-5645
- b/a* promoter: bases 6504-6602
- Ampicillin (*b/a*) 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 2447 and 4130. The complete sequence for pLenti6.2/V5-DEST is available from our web site at www.invitrogen.com or by contacting Technical Support (page 37).



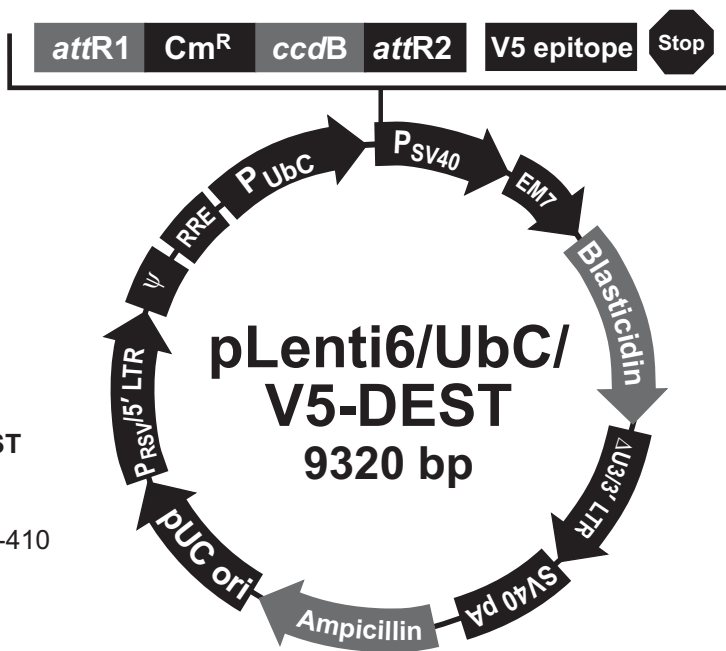
Comments for pLenti6.2/V5-DEST 8813 nucleotides

- 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 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
- PGK promoter: bases 4266-4771
- EM7 promoter: bases 4782-4848
- Blasticidin resistance gene: bases 4849-5247
- ΔU3/3' LTR: bases 5333-5567
 - ΔU3: bases 5333-5386
 - 3' LTR: bases 5387-5567
- SV40 polyadenylation signal: bases 5639-5770
- b/a promoter: bases 6629-6727
- Ampicillin (b/a) resistance gene: bases 6728-7588
- pUC origin: bases 7733-8406

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 37).



Comments for pLenti6/UbC/V5-DEST 9320 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

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). |

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> . |

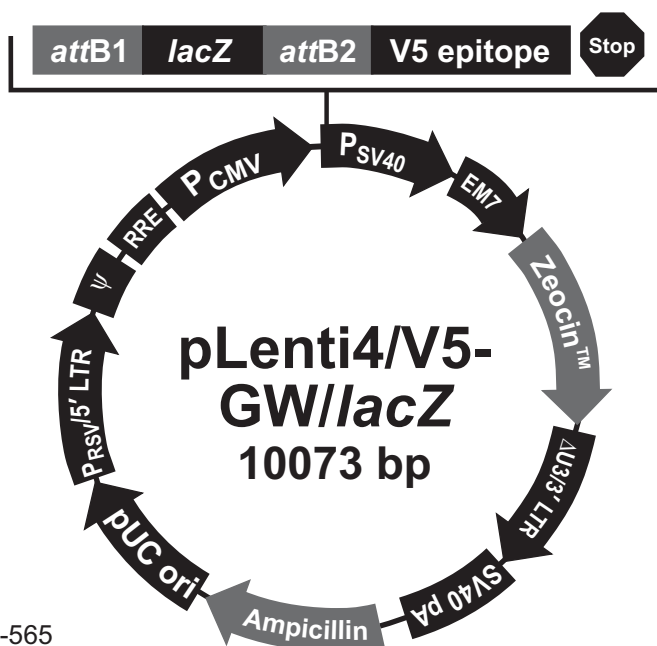
Map of pLenti4/V5-GW/lacZ

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 37).



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 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

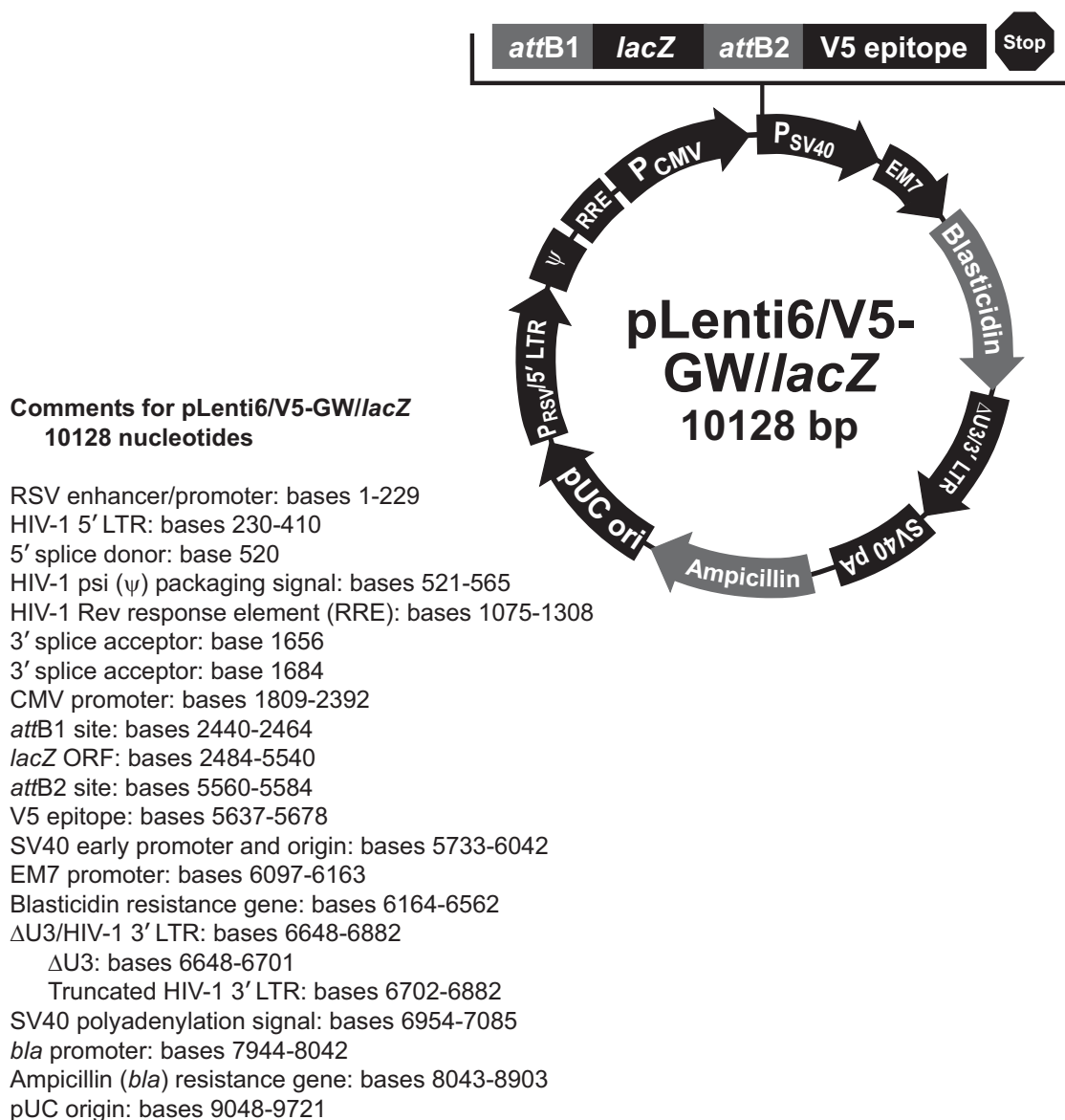
Map of pLenti6/V5-GW/lacZ

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 37).



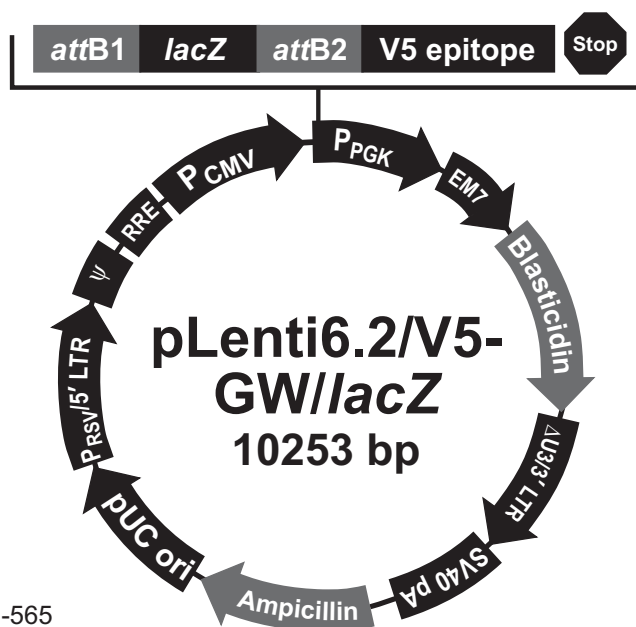
Map of pLenti6.2/V5-GW/lacZ

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 37).



Comments for pLenti6.2/V5-GW/lacZ 10253 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
PGK promoter: bases 5706-6211
EM7 promoter: bases 6222-6288
Blasticidin resistance gene: bases 6289-6687
 Δ U3/HIV-1 3' LTR: bases 6773-7007
 Δ U3: bases 6773-6826
Truncated HIV-1 3' LTR: bases 6827-7007
SV40 polyadenylation signal: bases 7079-7210
b/a promoter: bases 8069-8167
Ampicillin (*b/a*) resistance gene: bases 8168-9028
pUC origin: bases 9173-9846

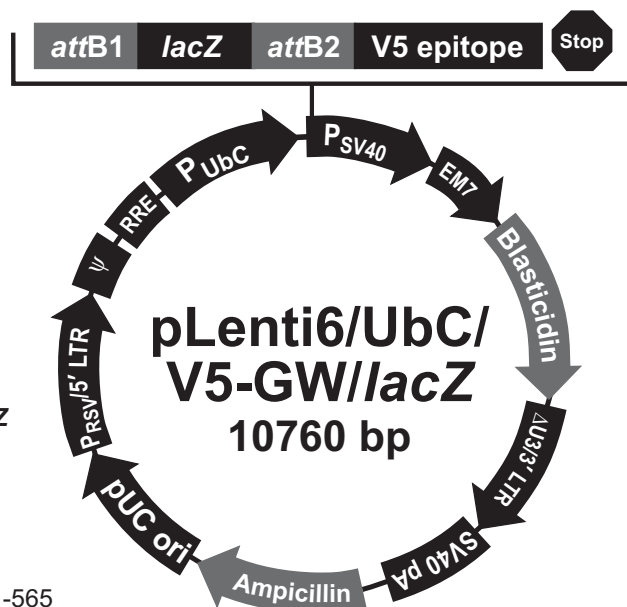
Map of pLenti6/UbC/V5-GW/lacZ

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 37).



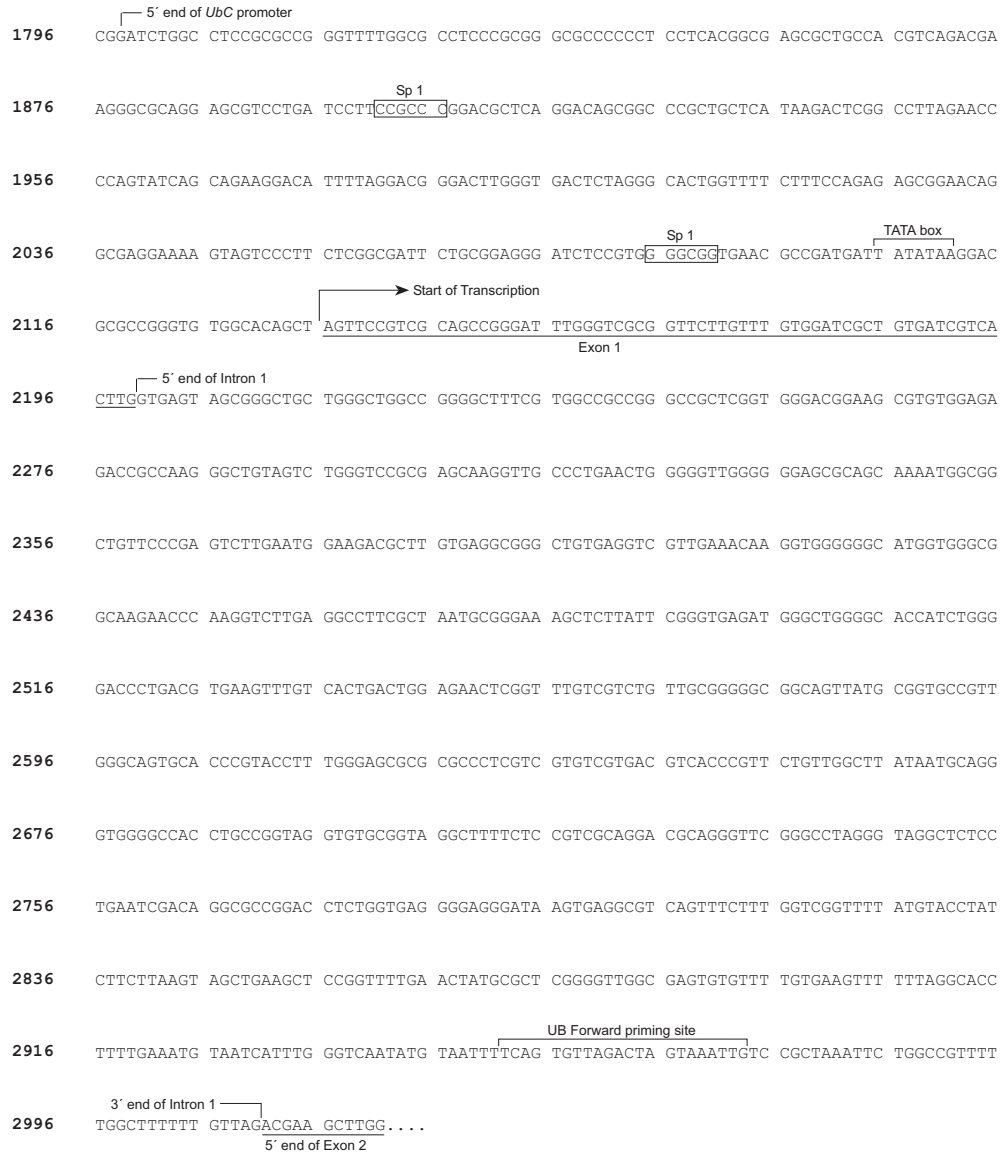
Comments for pLenti6/UbC/V5-GW/lacZ 10760 nucleotides

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
UbC promoter: bases 1798-3016
attB1 site: bases 3072-3096
lacZ ORF: bases 3116-6172
attB2 site: bases 6192-6216
V5 epitope: bases 6269-6310
SV40 early promoter and origin: bases 6365-6674
EM7 promoter: bases 6729-6795
Blasticidin resistance gene: bases 6796-7194
 Δ U3/HIV-1 3' LTR: bases 7280-7514
 Δ U3: bases 7280-7333
Truncated HIV-1 3' LTR: bases 7334-7514
SV40 polyadenylation signal: bases 7586-7717
bla promoter: bases 8576-8674
Ampicillin (*bla*) resistance gene: bases 8675-9535
pUC origin: bases 9680-10353

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).

Corporate Headquarters:

Invitrogen Corporation
5791 Van Allen Way
Carlsbad, CA 92008 USA
Tel: 1 760 603 7200
Tel (Toll Free): 1 800 955 6288
Fax: 1 760 602 6500
E-mail: tech_support@invitrogen.com

Japanese Headquarters:

Invitrogen Japan
LOOP-X Bldg. 6F
3-9-15, Kaigan
Minato-ku, Tokyo 108-0022
Tel: 81 3 5730 6509
Fax: 81 3 5730 6519
E-mail: jpinfo@invitrogen.com

European Headquarters:

Invitrogen Ltd
Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF, UK
Tel: +44 (0) 141 814 6100
Tech Fax: +44 (0) 141 814 6117
E-mail: eurotech@invitrogen.com

MSDS

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

Certificate of Analysis

The Certificate of Analysis (CofA) provides detailed quality control information for each product. The CofA is available on our website at www.invitrogen.com/cofa, and is searchable by product lot number, which is printed on each box.

Limited Warranty

Invitrogen is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about an Invitrogen product or service, contact our Technical Support Representatives.

Invitrogen warrants that all of its products will perform according to specifications stated on the certificate of analysis. The company will replace, free of charge, any product that does not meet those specifications. This warranty limits Invitrogen Corporation's liability only to the cost of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. Invitrogen reserves the right to select the method(s) used to analyze a product unless Invitrogen agrees to a specified method in writing prior to acceptance of the order.

Invitrogen makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore, Invitrogen makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, please report it to our Technical Support Representatives.

Invitrogen assumes no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose.

Purchaser Notification

Introduction

Use of the pLenti-DEST vectors is covered under a number of different licenses including those detailed below.

Limited Use Label License No. 5: Invitrogen Technology

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). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of patents owned or controlled by Invitrogen Corporation which cover this product based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Licensing Department, Invitrogen Corporation, 1600 Faraday Avenue, Carlsbad, California 92008. Phone (760) 603-7200. Fax (760) 602-6500. Email: outlicensing@invitrogen.com

Limited Use Label License No. 19: Gateway® Cloning Products

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). The purchase of this product does not convey a license under any method claims in the foregoing patents or patent applications, or to use this product with any recombination sites other than those purchased from Invitrogen Corporation or its authorized distributor. The right to use methods claimed in the foregoing patents or patent applications with this product for research purposes only can only be acquired by the use of Clonase™ purchased from Invitrogen Corporation or its authorized distributors. The buyer cannot modify the recombination sequence(s) contained in this product for any purpose. The buyer cannot sell or otherwise transfer (a) this product, (b) its components, or (c) materials made by the employment of this product or its components to a third party or otherwise use this product or its components or materials made by the employment of this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the employment of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Notwithstanding the preceding, any buyer who is employed in an academic or government institution may transfer materials made with this product to a third party who has a license from Invitrogen under the patents identified above to distribute such materials. Transfer of such materials and/or information to collaborators does not convey rights to practice any methods claimed in the foregoing patents or patent applications. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of the above patents based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that none of (i) this product, (ii) any of its components, or (iii) a method claim of the foregoing patents, was used in the manufacture of such product. Invitrogen Corporation will not assert a claim against the buyer of infringement of the above patents based upon the use of this product to manufacture a protein for sale, provided that no method claim in the above patents was used in the manufacture of such protein. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to use this product for purposes other than those permitted above, contact Licensing Department, Invitrogen Corporation, 5791 Van Allen Way, Carlsbad, California 92008. Phone (760) 603-7200.

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 38.

Continued on next page

Purchaser Notification, Continued

| | |
|---|--|
| Limited Use Label License No. 27: RNA Transfection | Use of this product in conjunction with methods for the introduction of RNA molecules into cells may require licenses to one or more patents or patent applications. Users of these products should determine if any licenses are required. |
| Limited Use Label License No. 51: Blastidicin and the Blastidicin Selection Marker | Blasticidin and the blasticidin resistance gene (<i>bsd</i>) are the subject of U.S. Patent No.5,527,701 sold under patent license for research purposes only. For information on purchasing a license to this product for purposes other than research, contact Licensing Department, Invitrogen Corporation, 5791 Van Allen Way, Carlsbad, California 92008. Phone (760) 603-7200. Fax (760) 602-6500. |
| Limited Use Label License No. 108: Lentiviral Technology | The Lentiviral Technology (based upon the lentikat™ system) is licensed from Cell Genesys, Inc., under U.S. Patent Nos. 5,686,279; 5,834,256; 5,858,740; 5,994,136; 6,013,516; 6,051,427; 6,165,782 and 6,218,187 and corresponding patents and applications in other countries for internal research purposes only. Use of this technology for gene therapy applications or bioprocessing other than for non-human research use requires a license from Cell Genesys (Cell Genesys, Inc. 342 Lakeside Drive, Foster City, California 94404). 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), including non-gene therapy research and target validation applications in laboratory animals. |
| Limited Use Label License No. 109: Retroviral Helper Lines | Retroviral helper cell lines are licensed from Wisconsin Alumni Research Foundation, under U.S. Patents and corresponding patents and applications in other countries for internal research purposes only. Use of these cell lines for Commercial Purposes requires a license from Life Technologies. |
| Limited Use Label License No. 304: Improved Transfection Reagent | This product is covered by U.S. Pat. Nos. 7,145,039, 7,166,745, 7,173,154, 7,323,594, and 7,479,573. |
| Limited Use Label License No. 317: LentiVector® Technology | This product is licensed under U.S. Pat. Nos. 5,817,491; 5,591,624; 5,716,832; 6,312,682; 6,669,936; 6,235,522; 6,924,123 and foreign equivalents from Oxford BioMedica (UK) Ltd., Oxford, UK, and is provided for use in academic and commercial in vitro and in vivo research for elucidating gene function, and for validating potential gene products and pathways for drug discovery and development, but excludes any use of LentiVector® technology for: creating transgenic birds for the purpose of producing useful or valuable proteins in the eggs of such transgenic birds, the delivery of gene therapies, and for commercial production of therapeutic, diagnostic or other commercial products not intended for research use where such products do not consist of or incorporate a lentiviral vector. Information about licenses for commercial uses excluded under this license is available from Oxford BioMedica (UK), Ltd., Medawar Centre, Oxford Science Park, Oxford OX4 4GA UK enquiries@oxfordbiomedica.co.uk or BioMedica Inc., 11622 EI Camino Real #100, San Diego CA 92130-2049 USA. LentiVector is a registered US and European Community trade mark of Oxford BioMedica plc. |
| Limited Use Label License No. 345: Gateway® Vectors | This product or one or more vectors made using this product is the subject of U.S. Patent No. 5,888,732 owned by Life Technologies Corporation. |

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

- Adra, C., Boer, P. H., and McBurney, M. W. (1987) Cloning and expression of the mouse pgk-1 gene and the nucleotide sequence of its promoter. *Gene* 60, 65-74
- Andersson, S., Davis, D. L., Dahlbäck, H., Jörnvall, H., and Russell, D. W. (1989) Cloning, Structure, and Expression of the Mitochondrial Cytochrome P-450 Sterol 26-Hydroxylase, a Bile Acid Biosynthetic Enzyme. *J. Biol. Chem.* 264, 8222-8229
- Boshart, M., Weber, F., Jahn, G., Dorsch-Häsler, K., Fleckenstein, B., and Schaffner, W. (1985) A Very Strong Enhancer is Located Upstream of an Immediate Early Gene of Human Cytomegalovirus. *Cell* 41, 521-530
- Calmels, T., Parriche, M., Burand, H., and Tiraby, G. (1991) High Efficiency Transformation of *Tolyocladium geodes* Conidiospores to Phleomycin Resistance. *Curr. Genet.* 20, 309-314
- Curradi, M., Izzo, A., Badaracco, G., and Landsberger, N. (2002) Molecular Mechanisms of Gene Silencing Mediated by DNA Methylation. *Mol. Cell. Biol.* 22, 3157-3173
- Drocourt, D., Calmels, T. P. G., Reynes, J. P., Baron, M., and Tiraby, G. (1990) Cassettes of the *Streptoalloteichus hindustanus ble* Gene for Transformation of Lower and Higher Eukaryotes to Phleomycin Resistance. *Nucleic Acids Res.* 18, 4009
- Dull, T., Zufferey, R., Kelly, M., Mandel, R. J., Nguyen, M., Trono, D., and Naldini, L. (1998) A Third-Generation Lentivirus Vector with a Conditional Packaging System. *J. Virol.* 72, 8463-8471
- Gerolami, R., Uch, R., F, Chapel, S., Bagnis, C., Brechot, C., and Mannoni, P. (2000) Gene transfer to hepatocellular carcinoma: transduction efficacy and transgene expression kinetics by using reteroviral and lentiviral vectors. *Cancer Gene Therapy* 7, 1286-1292
- Gill, D. R., Smyth, S. E., Goddard, C. A., Pringle, I. A., Higgins, C. F., Colledge, W. H., and Hyde, S. C. (2001) Increased Persistence of Lung Gene Expression Using Plasmids Containing the Ubiquitin C or Elongation Factor 1 alpha Promoter. *Gene Ther.* 8, 1539-1546
- Hamaguchi, I., Woods, N.-B., Panagopoulos, I., Andersson, E., Mikkola, H., Fahlman, C., Zufferey, R., Carlsson, L., Trono, D., and Karlsson, S. (2000) Lentivirus Vector Gene Expression during ES-Cell Derived Hematopoietic Development In Vitro. *J. Virology* 74, 10778-10784
- Hershko, A., and Ciechanover, A. (1982) Mechanisms of Intracellular Protein Breakdown. *Ann. Rev. Biochem.* 51, 335-364
- Izumi, M., Miyazawa, H., Kamakura, T., Yamaguchi, I., Endo, T., and Hanaoka, F. (1991) Blasticidin S-Resistance Gene (*bsr*): A Novel Selectable Marker for Mammalian Cells. *Exp. Cell Res.* 197, 229-233
- Kimura, M., Takatsuki, A., and Yamaguchi, I. (1994) Blasticidin S Deaminase Gene from *Aspergillus terreus* (*BSD*): A New Drug Resistance Gene for Transfection of Mammalian Cells. *Biochim. Biophys. ACTA* 1219, 653-659
- Kjems, J., Brown, M., Chang, D. D., and Sharp, P. A. (1991) Structural Analysis of the Interaction Between the Human Immunodeficiency Virus Rev Protein and the Rev Response Element. *Proc. Natl. Acad. Sci. USA* 88, 683-687
- Kozak, M. (1987) An Analysis of 5'-Noncoding Sequences from 699 Vertebrate Messenger RNAs. *Nucleic Acids Res.* 15, 8125-8148
- Kozak, M. (1990) Downstream Secondary Structure Facilitates Recognition of Initiator Codons by Eukaryotic Ribosomes. *Proc. Natl. Acad. Sci. USA* 87, 8301-8305
- Kozak, M. (1991) An Analysis of Vertebrate mRNA Sequences: Intimations of Translational Control. *J. Cell Biology* 115, 887-903
- Landy, A. (1989) Dynamic, Structural, and Regulatory Aspects of Lambda Site-specific Recombination. *Ann. Rev. Biochem.* 58, 913-949

Continued on next page

References, Continued

- Lois, C., Hong, E. J., Pease, S., Brown, E. J., and Baltimore, D. (2002) Germline Transmission and Tissue-Specific Expression of Transgenes Delivered by Lentiviral Vectors. *Science* 295, 868-872
- Luciw, P. A. (1996) in *Fields Virology* (Fields, B. N., Knipe, D. M., Howley, P. M., Chanock, R. M., Melnick, J. L., Monath, T. P., Roizman, B., and Straus, S. E., eds), 3rd Ed., pp. 1881-1975, Lippincott-Raven Publishers, Philadelphia, PA
- Malim, M. H., Hauber, J., Le, S. Y., Maizel, J. V., and Cullen, B. R. (1989) The HIV-1 Rev Trans-activator Acts Through a Structured Target Sequence to Activate Nuclear Export of Unspliced Viral mRNA. *Nature* 338, 254-257
- Marinovic, A. C., Mitch, W. E., and Price, S. R. (2000) Tools for Evaluating Ubiquitin (UbC) Gene Expression: Characterization of the Rat UbC Promoter and Use of a Unique 3' mRNA Sequence. *Biochem. Biophys. Res. Comm.* 274, 537-541
- Miller, J. H. (1972) *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
- Mulsant, P., Tiraby, G., Kallerhoff, J., and Perret, J. (1988) Phleomycin Resistance as a Dominant Selectable Marker in CHO Cells. *Somat. Cell Mol. Genet.* 14, 243-252
- Naldini, L., Blomer, U., Gage, F. H., Trono, D., and Verma, I. M. (1996) Efficient Transfer, Integration, and Sustained Long-Term Expression of the Transgene in Adult Rat Brains Injected with a Lentiviral Vector. *Proc. Natl. Acad. Sci. USA* 93, 11382-11388
- Nelson, J. A., Reynolds-Kohler, C., and Smith, B. A. (1987) Negative and Positive Regulation by a Short Segment in the 5'-Flanking Region of the Human Cytomegalovirus Major Immediate-Early Gene. *Molec. Cell. Biol.* 7, 4125-4129
- Nenoi, M., Mita, K., Ichimura, S., Cartwright, I. L., Takahashi, E., Yamaguchi, M., and Tsuji, H. (1996) Heterogeneous Structure of the Polyubiquitin Gene UbC of HeLa S3 Cells. *Gene* 175, 179-185
- Perez, P., Tiraby, G., Kallerhoff, J., and Perret, J. (1989) Phleomycin Resistance as a Dominant Selectable Marker for Plant Cell Transformation. *Plant Mol. Biol.* 13, 365-373
- Rietveld, L. E., Caldenhoven, E., and Stunnenberg, H. G. (2002) In vivo Repression of an Erythroid-Specific Gene by Distinct Corepressor Complexes. *EMBO J.* 21, 1389-1397
- Schorpp, M., Jäger, R., Schellander, K., Schenkel, J., Wagner, E. F., Weiher, H., and Angel, P. (1996) The Human Ubiquitin C Promoter Directs High Ubiquitous Expression of Transgenes in Mice. *Nuc. Acids Res.* 24, 1787-1788
- Southern, J. A., Young, D. F., Heaney, F., Baumgartner, W., and Randall, R. E. (1991) Identification of an Epitope on the P and V Proteins of Simian Virus 5 That Distinguishes Between Two Isolates with Different Biological Characteristics. *J. Gen. Virol.* 72, 1551-1557
- Takeuchi, S., Hirayama, K., Ueda, K., Sakai, H., and Yonehara, H. (1958) Blasticidin S, A New Antibiotic. *The Journal of Antibiotics, Series A* 11, 1-5
- Wulff, B. S., O'Hare, M. M., Boel, E., Theill, L. E., and Schwartz, T. W. (1990) Partial Processing of the Neuropeptide Y Precursor in Transfected CHO Cells. *FEBS Lett.* 261, 101-105
- Yamaguchi, H., Yamamoto, C., and Tanaka, N. (1965) Inhibition of Protein Synthesis by Blasticidin S. I. Studies with Cell-free Systems from Bacterial and Mammalian Cells. *J. Biochem (Tokyo)* 57, 667-677
- Yew, N. S., Przybylska, M., Ziegler, R. J., Liu, D., and Cheng, S. H. (2001) High and Sustained Transgene Expression in vivo from Plasmid Vectors Containing a Hybrid Ubiquitin Promoter. *Mol. Ther.* 4, 75-82

©2002-2008 Invitrogen Corporation. All rights reserved.

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



Corporate Headquarters

Invitrogen Corporation

5791 Van Allen Way

Carlsbad, CA 92008

T: 1 760 603 7200

F: 1 760 602 6500

E: tech_support@invitrogen.com

For country-specific contact information, visit our web site at www.invitrogen.com