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by life technologies™

pLenti6.3/V5–DEST and pLenti7.3 /V5–DEST Gateway® Vector Kits

Gateway®–adapted destination vectors for cloning and high–level expression in mammalian cells using the ViraPower™ HiPerform™ Lentiviral Expression Systems

Catalog nos. V533-06, V534-06

Rev. Date: 2 June 2010
Manual part no. A10292

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User Manual

Table of Contents

Kit Contents and Storage

Types of Kits

This manual is supplied with the following products.

Product	Catalog no.
ViraPower™ HiPerform™ Lentiviral Gateway® Expression Kit	K5330-00
ViraPower™ HiPerform™ Lentiviral FastTiter™ Gateway® Expression Kit	K5340-00
pLenti6.3/V5–DEST Gateway® Vector Kit	V533-06
pLenti7.3/V5–DEST Gateway® Vector Kit	V534-06

System Components

The following table shows the components associated with the pLenti6.3/V5–DEST Gateway Vector and the pLenti7.3/V5–DEST Gateway Vector Kits, listed above.

Components	Quantity	Catalog No.	
		V533-06	V534-06
pLenti6.3/V5–DEST Gateway® Vector	40 µl at 150 ng/µl in TE Buffer, pH 8.0	✓	
pLenti6.3/V5-GW/*lacZ* Control Vector	20 µl at 0.5 µg/µl in TE Buffer, pH 8.0	✓	
pLenti7.3/V5–DEST Gateway® Vector	40 µl at 150 ng/µl in TE Buffer, pH 8.0		✓
pLenti7.3/V5-GW/*lacZ* Control Vector	20 µl at 0.5 µg/µl in TE Buffer, pH 8.0		✓
One Shot® Stbl3™ Chemically Competent *E. coli*	20 reactions	✓	✓

Shipping/Storage

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

Item	Shipping	Storage
pLenti6.3/V5–DEST Gateway® Vector	Dry ice	-20°C
pLenti6.3/V5-GW/*lacZ* Control Vector	Dry ice	-20°C
pLenti7.3/V5–DEST Gateway® Vector	Dry ice	-20°C
pLenti7.3/V5-GW/*lacZ* Control Vector	Dry ice	-20°C
One Shot® Stbl3™ Chemically Competent *E. coli*	Dry ice	-80°C

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

The following reagents are included with the One Shot® Stbl3™ Chemically Competent *E. coli* kit. Transformation efficiency is ≥ 1 x 10^8 cfu/µg plasmid DNA. There are enough reagents for 20 reactions. **Store at –80°C.**

Reagent	Composition	Quantity
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
Stbl3™ Cells	--	21 x 50 µl
pUC19 Control DNA	10 pg/µl in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8	50 µl

Genotype of Stbl3™ Cells

F$^-$ *mcr*B *mrr hsd*S20(r_B^-, m_B^-) *rec*A13 *sup*E44 *ara*-14 *gal*K2 *lac*Y1 *pro*A2 *rps*L20(Str^R) *xyl*-5 λ$^-$ *leu mtl*-1

Note: This strain is *end*A1+

293FT Cell Line

Each ViraPower™ HiPerform™ Lentiviral Expression Kit includes the 293FT producer cell line. The 293FT Cell Line is supplied as one vial containing 3 x 10^6 frozen cells in 1 ml Freezing Medium. **Upon receipt, store in liquid nitrogen.**

For instructions to thaw, culture, and maintain the 293FT Cell Line, see the 293FT Cell Line manual, included with the ViraPower™ HiPerform™ Lentiviral Expression Kit. To download the manual, visit our web site at www.invitrogen.com, or contact **Technical Support** (page 27).

Intended Use

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

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Accessory Products

Additional Products

The products listed 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 27).

Item	Quantity	Catalog no.
PureLink™ HiPure Plasmid Midiprep Kit	25 reactions 50 reactions	K2100-04 K2100-05
PureLink™ Quick96 Plasmid Kit	4 x 96 preps 24 x 96 preps	K2110-04 K2110-24
PureLink™ HQ Plasmid Miniprep Kit	100 reactions	K2100-01
One Shot® Stbl3™ Chemically Competent *E. coli*	20 x 50 µl	C7373-03
Vivid Colors™ pLenti6.3/V5-GW/EmGFP Expression Control Vector	40 µl @ 0.5 µg/µl in TE Buffer, pH 8.0	V370-06
Lipofectamine™ 2000 Reagent	0.75 ml 1.5 ml	11668-027 11668-019
Ampicillin	5 g	Q100-16
Blasticidin	50 mg	R210-01
Gateway® LR Clonase™ II Enzyme Mix	20 reactions 100 reactions	11791-020 11791-100
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
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

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ViraPower™ HiPerform™ Lentiviral Expression Products

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

Product	Quantity	Catalog no.
ViraPower™ HiPerform™ Lentiviral TOPO® Expression Kit	1 kit	K5310-00
ViraPower™ HiPerform™ Lentiviral FastTiter™ TOPO® Expression Kit	1 kit	K5330-00
pLenti6.3/V5-TOPO® TA Cloning Kit	1 kit	K5315-20
pLenti7.3/V5-TOPO® TA Cloning Kit	1 kit	K5325-20
Vivid Colors™ pLenti6.3-GW/EmGFP Expression Control Vector	40 µl @ 0.5 µg/µl in TE Buffer, pH 8.0	V370-06
ViraPower™ Lentiviral Support Kit	20 reactions	K4970-00
ViraPower™ Lentiviral Packaging Mix	60 reactions	K4975-00
293FT Cell Line	3 x 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. They are designed to allow high–level expression of recombinant fusion proteins in dividing and non–dividing mammalian cells using Invitrogen's ViraPower™ HiPerform™ Lentiviral Expression Systems. The pLenti6.3 and pLenti7.3 DEST vectors contain two new elements (WPRE and cPPT) to yield cell–specific, high performance results. The **WPRE** (Woodchuck Posttranscriptional Regulatory Element) from the woodchuck hepatitis virus, is placed directly downstream of the gene of interest, allowing for increased transgene expression(Zufferey *et al.*, 1998), with more cells expressing your gene of interest. **cPPT** (Polypurine Tract) from the HIV-1 integrase gene, increases the copy number of lentivirus integrating into the host genome (Park, 2001) and allows for a two-fold increase in viral titer. Both WPRE and cPPT together, produce at least a four-fold increase in protein expression. In addition, the pLenti7.3/V5–DEST Gateway® Vector (Catalog no. V534–06) allows for an accurate titer of functional lentivirus in just **two days** using EmGFP.

The pLenti6.3/V5–DEST and pLenti7.3/V5–DEST vectors are also available with:

- Human cytomegalovirus (CMV) immediate early promoter to control expression of the gene of interest (see pages 2–3 for more information) in both vectors
- SV40 promoter driving expression of Blasticidin (pLenti6.3/V5–DEST), or EmGFP (pLenti7.3/V5–DEST) (see page 3 for more information)
- Blasticidin (Izumi *et al.*, 1991; Kimura *et al.*, 1994; Takeuchi *et al.*, 1958; Yamaguchi *et al.*, 1965) resistance gene for stable transduction and selection in *E. coli* and mammalian cells (pLenti6.3/V5–DEST Gateway® vector, only) **or**
- Emerald Green Fluorescent Protein (EmGFP, derived from *Aequorea Victoria* GFP, pLenti7.3/V5–DEST Gateway® vector only) which allows for fluorescence detection by flow cytometry and quick-screen of transient expression. pLenti7.3/V5–DEST vectors can achieve viral titers in only **2** days.

Additional Information

For more information about the ViraPower™ HiPerform™ Lentiviral Expression Systems (Catalog nos: K5330-00and K5340-00), please review the System manual. For more information on Gateway® Technology, visit our web site at www.invitrogen.com, or contact **Technical Support** (page 27).

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Features of the Vectors

The HiPerform™ pLenti6.3/V5–DEST and pLenti7.3/V5–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 (ΔU3) 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)
- Polypurine Tract from HIV (cPPT) for increased viral titer (Park et al., 2001)
- Human CMV 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, *att*R1 and *att*R2, downstream of the CMV promoter for recombinational cloning of the gene of interest from an entry clone
- Chloramphenicol resistance gene (Cm^R) located between the two *att*R sites for counterscreening
- The *ccd*B gene located between the *att*R sites for negative selection
- C-terminal V5 epitope for detection of the recombinant protein of interest (Southern *et al.*, 1991)
- Woodchuck Posttranscriptional Regulatory Element (WPRE) for increase transgene expression (Zufferey et al., 1999)
- SV40 promoter driving expression of Blasticidin (see next page)
- Blasticidin (Izumi *et al.*, 1991; Kimura *et al.*, 1994; Takeuchi *et al.*, 1958; Yamaguchi *et al.*, 1965) resistance gene for selection in *E. coli* and mammalian cells (pLenti6.3/V5–DEST vector, only)
- Emerald Green Fluorescent Protein (EmGFP, derived from *Aequorea Victoria* GFP) for fluorescence detection and quick screen transient expression (pLenti7.3/V5–DEST vector, only)
- 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 25–26. A control lentiviral expression vector (Vivid Colors™ pLenti6.3/V5–GW/EmGFP) containing Emerald Green Fluorescent Protein (EmGFP) is available separately from Invitrogen (page vi).

CMV Promoter

The pLenti6.3/V5–DEST and pLenti7.3/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.

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Promoter Driving Blasticidin

The pLenti6.3/V5–DEST vector contains the SV40 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 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.* pLenti6.3/V5–DEST).
3. Use your expression clone in the ViraPower™ HiPerform™ Lentiviral Expression System (see below).

For detailed information about the Gateway® Technology, refer to the Gateway® Technology with Clonase™ II manual which is available from our Web site (www.invitrogen.com) or by contacting **Technical Support** (page 27).

The ViraPower™ HiPerform™ Lentiviral Expression System

The ViraPower™ HiPerform™ Lentiviral Expression System (Catalog nos: K5330-00 and K5340-00) 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™ HiPerform™ 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™ HiPerform™ Lentiviral Expression System, you will:

1. Create an expression clone in either the pLenti6.3/V5–DEST vector if you are using Gateway® technology, or the pLenti7.3/V5–TOPO® vector if you are using the TOPO® Cloning technology.
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 (pLenti6.3 and pLenti7.3 vectors) or generate a stable cell line using Blasticidin selection (pLenti6.3/V5–DEST, **only**).

For more information about the ViraPower™ HiPerform™ Lentiviral Expression System, the ViraPower™ Packaging Mix, and the biosafety features of the System, refer to the ViraPower™ HiPerform™ 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 www.invitrogen.com or by contacting **Technical Support** (page 27).

Methods

Generating an Entry Clone

Introduction

To recombine your DNA sequence of interest into pLenti6.3/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 (www.invitrogen.com) or by contacting **Technical Support** (page 27).

If you wish to express a human gene of interest in a pLenti–DEST vector, you may use an Ultimate™ Human Open Reading Frame (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 27).

Points to Consider Before Recombining into a pLenti–DEST Vector

pLenti6.3/V5–DEST and pLenti7.3/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 an ATG initiation codon in the context of a Kozak translation initiation sequence for proper initiation of translation in mammalian cells (Kozak, 1987; Kozak, 1990; Kozak, 1991). An example of a Kozak consensus sequence is **(G/A)**NNATG**G**. Other sequences are possible, but the G or A at position –3 (shown in bold) and the G at position +4 (shown in bold) are the most critical for function. The ATG initiation codon is underlined.
- 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 page 6 for a diagram of the recombination region of the pLenti6.3/V5–DEST and pLenti7.3/V5–DEST Gateway® vectors.

Creating Expression Clones

Introduction

After you have generated an entry clone, 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** (page 7) and **Transforming One Shot® Stbl3™ Competent *E. coli*** (page 9) before beginning.

Experimental Outline

To generate an expression clone, you will:

1. Perform an LR recombination reaction using the *att*L–containing entry clone and the *att*R–containing pLenti6.3/V5–DEST Gateway® 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* (page 9).
3. Analyze expression clones using restriction enzyme analysis and sequencing.

Important

We do not recommend that you propagate the pLenti6.3/V5–DEST or the pLenti7.3/V5–DEST Gateway® vectors in *E. coli* due to the possibility of unwanted recombination. The amount of each plasmid supplied is sufficient for 40 reactions.

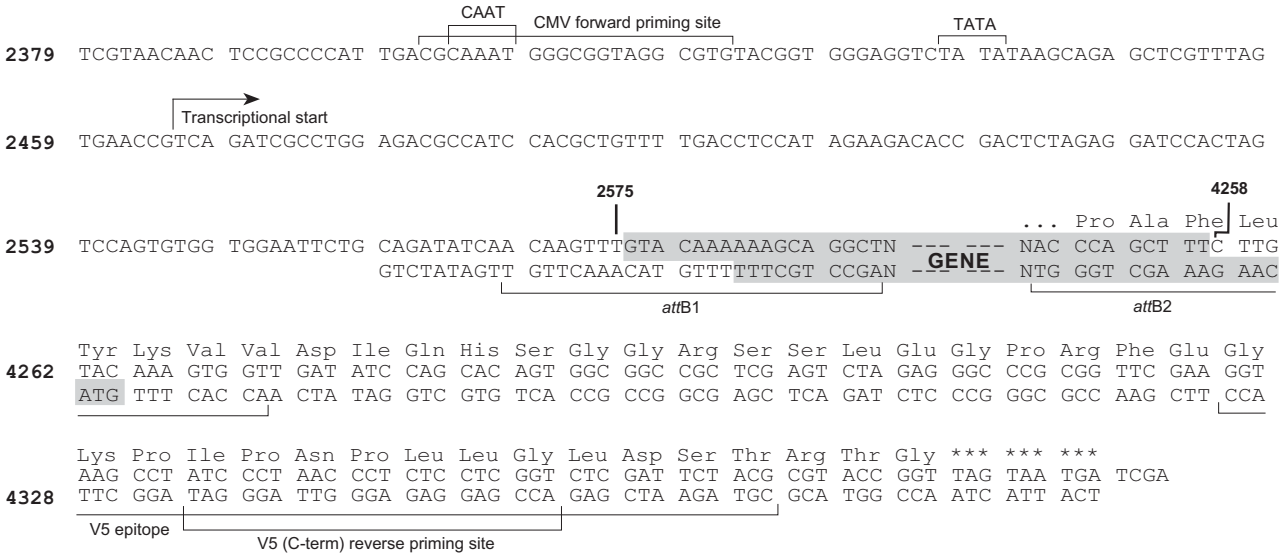
Continued on next page

Recombination Region of the pLenti6.3/V5–DEST and the pLenti7.3/V5–DEST Gateway® Vectors

The Gateway® recombination region of the expression clone resulting from pLenti6.3/V5–DEST × entry clone and pLenti7.3/V5–DEST × entry clone is shown below.

Features of the Gateway® Recombination Region:

- Shaded regions correspond to those DNA sequences transferred from the entry clone into the pLenti6.3/V5–DEST vector and the pLenti7.3/V5–DEST by recombination.
- Non-shaded regions are derived from the pLenti6.3/V5–DEST and the pLenti7.3/V5–DEST Gateway® Vectors.
- Bases 2447 and 4130 of the pLenti6.3/V5–DEST and pLenti7.3/V5–DEST sequence are marked.



Performing the LR Recombination Reaction

Introduction

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

LR Clonase™ II Enzyme Mix

To catalyze the LR recombination reaction, you will use LR Clonase™ II enzyme mix, which is available separately from Invitrogen (page vi). 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 under **Setting up the LR Recombination Reaction** (next page) to perform the LR recombination reaction using LR Clonase™ II enzyme mix.

Materials Needed

You will need the following materials:

- Purified plasmid DNA of your entry clone (50–150 ng/µl in TE, pH 8.0)
- pLenti–DEST vector (supplied with the kit)
- LR Clonase™ II enzyme mix (available separately, page vi; 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)

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Setting up the LR Recombination Reaction

Follow the procedure below 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* included with the kit. The transformation efficiency of One Shot® Stbl3™ Chemically Competent *E. coli* is ≥ 1 x 10^8 cfu/µg 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 *ccd*A gene and will prevent negative selection with the *ccd*B gene.

Materials Needed

You will need the following materials:

- LR recombination reaction (from Step 7, previous page)
- One Shot® Stbl3™ Chemically Competent *E. coli* (supplied with the kit, 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)
- 42°C water bath
- LB Amp plates containing 100 µg/ml Ampicillin (two for each transformation; warm at 37°C for 30 minutes before use)
- 37°C shaking and non–shaking incubator

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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 2–3 µl of the LR recombination reaction (from Step 7, page 8) 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 1ml 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 retransform 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

Do **not** use PCR screening of clones 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.

Analyzing Transformants

To analyze transformants:

1. Pick 10–20 ampicillin–resistant colonies from plating the transformation mix. Culture cells overnight.
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 to generate a starter culture.
7. Inoculate the starter culture into LB–ampicillin and grow.
8. Isolate plasmid DNA using a midiprep kit (see **Important**, next page) for lentivirus production.

Continued on next page

Important

Stbl3™ *E. coli* is wild type for endonuclease 1 (*end*A1+). 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 *end*A1+ *E. coli* strains.

We recommend using the PureLink™ HQ Mini Plasmid Purification Kit and preparing lentiviral plasmid DNA using PureLink™ MidiPrep Kits (page vi).

Materials Needed

You will need the following materials:

- LB medium containing 100 µg/ml ampicillin
- PureLink™ HQ Mini Plasmid Purification Kit (page vi) or equivalent
- Appropriate restriction enzymes
- E-Gels® 1.2% agarose gels (page vi) or equivalent

Screening Colonies by Miniprep

For each transformation:

1. Pick 10–20 colonies from plates obtained after plating the transformation mix (Step 9, page 10). Culture colonies overnight in LB medium containing 100 µg/ml ampicillin.
2. 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.
3. Perform restriction digestion on plasmid DNA, then analyze the digested DNA on 0.8% or 1.2% agarose gels to confirm the correct clones.

Restriction Digestion

To confirm that no rearrangement in the LTR regions of the plasmid has taken place, perform restriction digestion 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.

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 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 vi)

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 page 6 or the locations of the primer binding sites in each vector.

Note: For your convenience, Invitrogen has a custom primer synthesis service. For more information, see our Web site (www.invitrogen.com) or contact **Technical Support** (page 27).

Vectors	Primer	Sequence
pLenti6.3/V5–DEST and pLenti7.3/V5–DEST	CMV forward primer	5′–CGCAAATGGGCGGTAGGCGTG–3′
	V5(C–term) reverse primer	5′–ACCGAGGAGAGGGTTAGGGAT–3′

Continued on next page

Isolating Lentiviral Plasmid DNA

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

1. Dilute 1 µl of miniprep plasmid DNA from a positive clone 1:500 in TE.
2. Use 1 µl of this diluted DNA to retransform into One Shot® Stbl3™ Chemically Competent Cells as described on page 9.
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 50–100 ml volume for large scale or midiprep isolation of DNA.
6. Isolate plasmid DNA using the PureLink™ MidiPrep Kit (see **Important**, page 12).
7. Perform restriction analysis (see page 12) 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 PureLink™ MidiPrep Kit.

Important: Do not use miniprep plasmid DNA for lentivirus production.

Continued on next page

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 is not required.

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. To verify expression of your gene of interest:

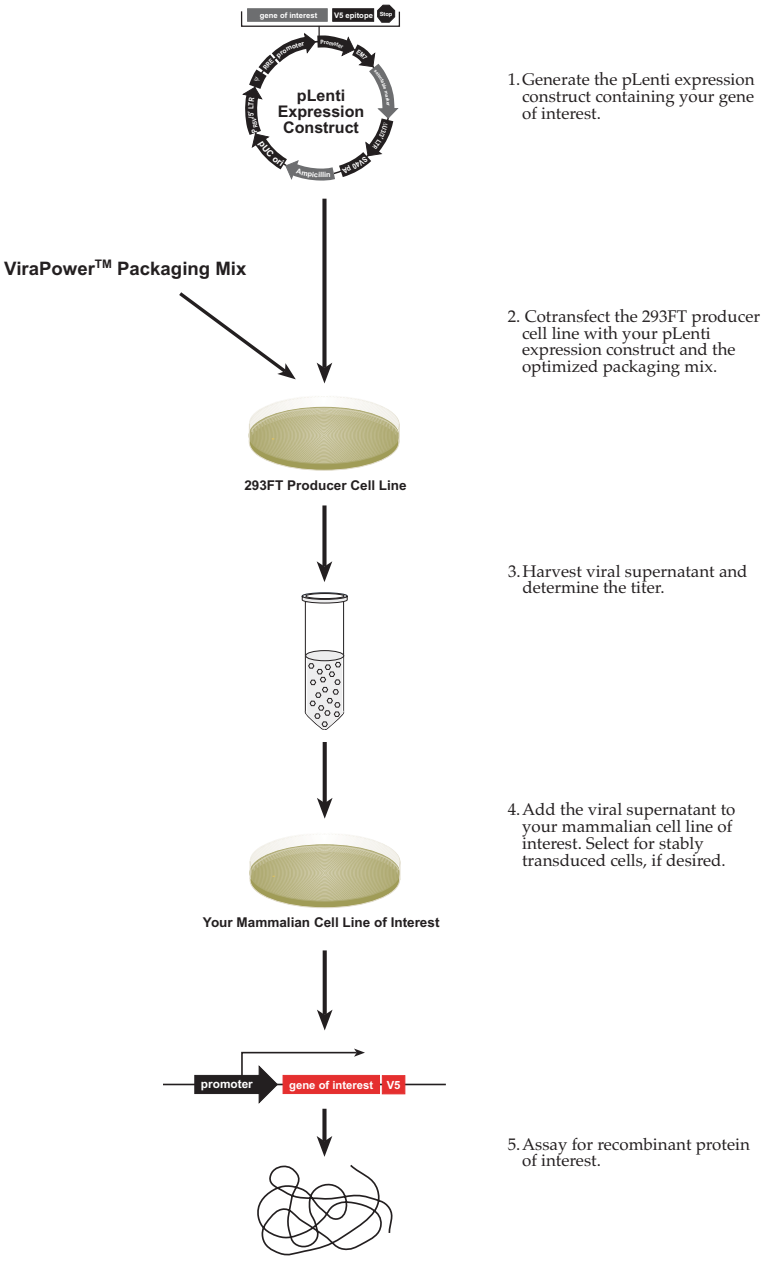
- Use an easy-to-transfect, dividing mammalian cell line (*e.g.* HEK 293 or COS–7).
- Use a transfection reagent that allows high-efficiency transfection; we recommend using Lipofectamine™ 2000 Reagent.

  Note: Lipofectamine™ 2000 is supplied with the ViraPower™ HiPerform™ Lentiviral Gateway® Expression Kits, but is also available separately from Invitrogen (page vi).
- 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™ HiPerform™ 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).



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Materials Needed

To express your gene of interest from the pLenti–DEST construct using Invitrogen's ViraPower™ HiPerform™ Lentiviral Expression Kits (Catalog nos: K5330-00 and K5340-00), you will need the following reagents that are supplied with the Expression kits:

- 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 for selection of stably transduced mammalian cells (pLenti6.3/V5–DEST vector **only**, see the **Appendix**, page 21 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™ HiPerform™ Lentiviral Expression System manual. Both manuals are available for downloading from www.invitrogen.com or by contacting **Technical Support** (page 27).

Obtaining Reagents

The 293FT Cell Line and the ViraPower™ Lentiviral Support Kits containing the ViraPower™ Packaging Mix, Lipofectamine™ 2000, and selection agent are included with the ViraPower™ HiPerform™ Lentiviral Gateway® Expression Kit and the ViraPower™ HiPerform™ Lentiviral FastTiter™ Gateway® Expression Kit (catalog nos: K5330–00 and K5340–00, respectively). These reagents are also available to order separately from Invitrogen (see page vi).

Continued on next page

Plasmid Preparation

Before expressing your recombinant fusion protein using the ViraPower™ HiPerform™ 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 PureLink™ Midiprep DNA Isolation Kit (page vi). **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 12 for details.

Positive Controls

A positive control vector is included with each pLenti–DEST vector for use as an expression control in the ViraPower™ HiPerform™ 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 **Appendix**, pages 25–26.

Vector	Positive Control
pLenti6.3/V5–DEST	pLenti6.3/V5–GW/*lacZ*
pLenti7.3/V5–DEST	pLenti7.3/V5–GW/*lacZ*

A control lentiviral expression vector (pLenti6.3/V5–GW/EmGFP) containing Emerald Green Fluorescent Protein (EmGFP) to optimize transfection and virus production is available separately from Invitrogen. For ordering information, see page vi or visit www.invitrogen.com.

Propagating the Control Plasmids

To propagate and maintain the control plasmids:

1. Transform 10 ng of the stock solution to transform One Shot® Stbl3™ *E. coli* (see page 9).
2. Select transformants on selective plates containing 100 µg/ml ampicillin.
3. Propagate the plasmid in LB containing 100 µg/ml ampicillin.
4. Prepare a glycerol stock of a transformant containing plasmid for long–term storage.

Continued on next page

Producing Viral Stocks

Refer to the ViraPower™ HiPerform™ 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 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 (pLenti7.3/V5–DEST) or use Blasticidin to select for stably transduced cells (pLenti6.3/V5–DEST). Before generating your stably transduced cell line, we recommend that you generate a kill curve to determine the minimum concentration of Blasticidin required to kill your untransduced host cell line. For guidelines to generate a kill curve, refer to the ViraPower™ HiPerform™ Lentiviral Expression System manual. For instructions to prepare and handle Blasticidin, see **Appendix**, page 21.

Transducing Mammalian Cells

Refer to the ViraPower™ HiPerform™ 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 Blasticidin selection (pLenti6.3/V5–DEST vector, only).

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 www.invitrogen.com or call **Technical Support** (page 27). See page vii for ordering information.

Note

The C–terminal peptide containing the V5 epitope and the *att*B2 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 pLenti6.3/V5–GW/*lacZ* and pLenti7.3/V5–GW/*lacZ* control lentiviral constructs 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 (see page vi for ordering details of the above products) 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.

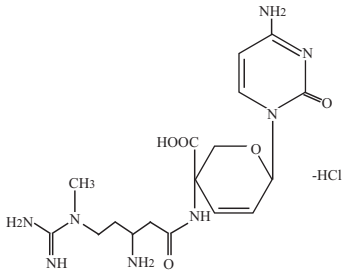
Blasticidin (pLenti6.3/V5–DEST Vector, Only)

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 $C_{17}H_{26}N_8O_5$-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 fume hood.

Preparing and Storing Stock Solutions

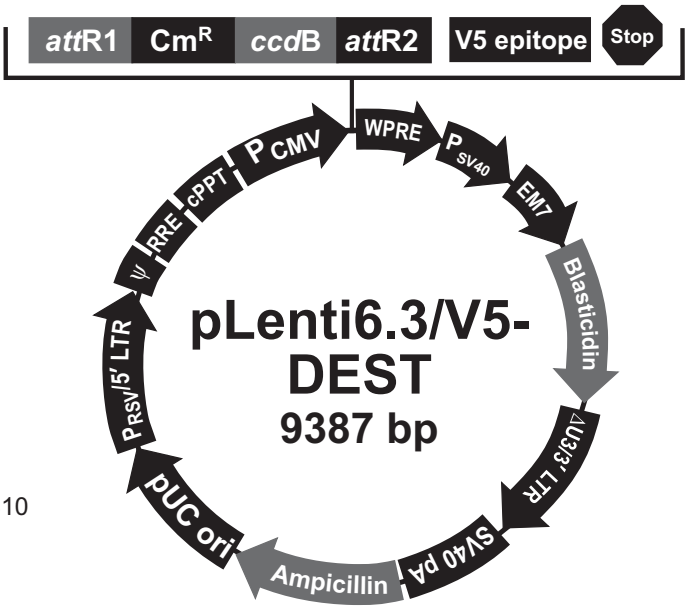
Blasticidin may be obtained separately from Invitrogen (page vi) in 50 mg aliquots. Blasticidin is soluble in water. Sterile water is generally used to prepare stock solutions of 5 to 10 mg/ml.

- 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°C for long–term storage or store at 4°C for short–term storage.
- Aqueous stock solutions are stable for 1-2 weeks at 4°C and 6–8 weeks at–20°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°C for up to 2 weeks.
- Medium containing Blasticidin may be stored at 4°C for up to 2 weeks.

Map of pLenti6.3/V5–DEST

Map of pLenti6.3/V5–DEST

The map below shows the elements of pLenti6.3/V5–DEST Gateway® vector. DNA from the entry clone replaces the region between bases 2447 and 4130. **The complete sequence for pLenti6.3/V5–DEST Gateway® vector is available from our web site (www.invitrogen.com) or by contacting Technical Support (see page 27)**



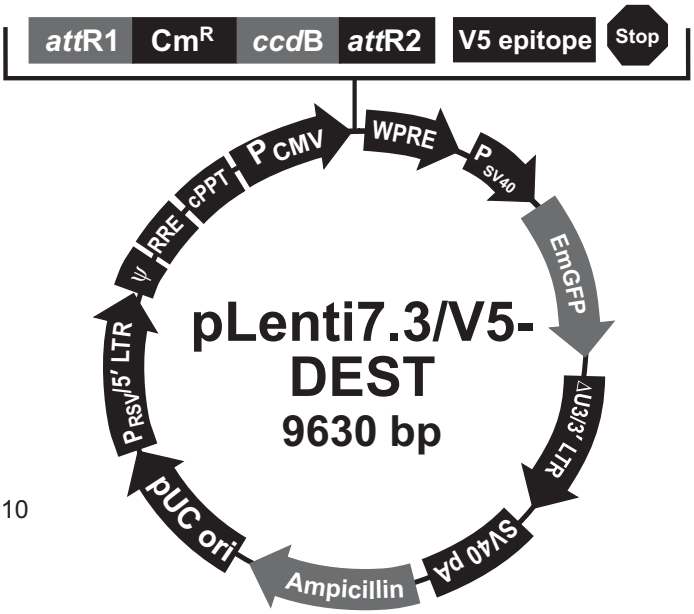
Comments for pLenti6.3/V5-DEST
9387 nucleotides

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
cPPT: bases 1801-1923
CMV promoter: bases 1935-2519
*att*R1 site: bases 2568-2692
Chloramphenicol resistance gene (Cm^R): bases 2801-3460
*ccd*B gene: bases 3802-4107
*att*R2 site: bases 4148-4272
V5 epitope: bases 4325-4366
WPRE: bases 4385-4982
SV40 promoter: bases 4993-5301
EM7 promoter: bases 5356-5422
Blasticidin resistance gene: bases 5423-5821
ΔU3/3′ LTR: bases 5907-6141
ΔU3: bases 5907-5960
3′ LTR: bases 5961-6141
SV40 polyadenylation signal: bases 6213-6344
bla promoter: bases 7203-7301
Ampicillin (*bla*) resistance gene: bases 7302-8162
pUC origin: bases 8307-8980

Map of pLenti7.3/V5–DEST

Map of pLenti7.3/V5–DEST

The map below shows the elements of pLenti7.3/V5–DEST Gateway® vector. DNA from the entry clone replaces the region between bases 2447 and 4130. **The complete sequence for pLenti7.3/V5–DEST Gateway® vector is available from our web site (www.invitrogen.com) or by contacting Technical Support (see page 27).**



Comments for pLenti7.3/V5-DEST
9630 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
cPPT: bases 1801-1923
CMV promoter: bases 1935-2519
*att*R1 site: bases 2568-2692
Chloramphenicol resistance gene (Cm^R): bases 2801-3460
*ccd*B gene: bases 3802-4107
*att*R2 site: bases 4148-4272
V5 epitope: bases 4325-4366
WPRE: bases 4385-4982
SV40 promoter: bases 4993-5301
EmGFP: bases 5360-6079
ΔU3/3′ LTR: bases 6150-6384
ΔU3: bases 6150-6203
3′ LTR: bases 6151-6384
SV40 polyadenylation signal: bases 6456-6587
bla promoter: bases 7446-7544
Ampicillin (*bla*) resistance gene: bases 7545-8405
pUC origin: bases 8550-9223

Features of the pLenti6.3 and pLenti7.3-DEST Vectors

Features of the Vectors

The pLenti6.3/V5–DEST (9387 bp), pLenti7.3/V5–DEST (9630 bp), Gateway® vectors contain the following elements. Features have been functionally tested.

Feature	Benefit
Rous Sarcoma Virus (RSV) enhancer/promoter	Allows Tat–independent production of viral mRNA (Dull *et al.*, 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 *et al.*, 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 *et al.*, 1991; Malim *et al.*, 1989)
Polypurine Tract from HIV (cPPT)	Provides for increased viral titer (Park, 2001)
CMV promoter	Permits high-level, constitutive expression of the gene of interest (Andersson *et al.*, 1989; Boshart *et al.*, 1985; Nelson *et al.*, 1987)
*att*R1 and *att*R2 sites	Bacteriophage –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
*ccd*B gene	Permits negative selection of the plasmid
V5 epitope	Allows detection of the recombinant fusion protein by Anti–V5 Antibodies (Southern *et al.*, 1991)
Woodchuck Posttranscriptional Regulatory Element (WPRE)	Provides for increased transgene expression (Zufferey *et al.*, 1998)
SV40 early promoter and origin	Allows high-level expression of the selection marker and episomal replication in cells expressing the SV40 large T antigen
EM7 promoter	Synthetic prokaryotic promoter for expression of the selection marker in *E. coli*
Blasticidin (*bsd*) resistance gene	Permits selection of stably transduced mammalian cell lines (Kimura *et al.*, 1994)
Emerald Green Fluorescent Protein (EmGFP)	Allows for fluorescence detection by flow cytometry and quick-screen transient expression. Can achieve viral titers in only **2** days
ΔU3/HIV–1 truncated 3′ LTR	Allows viral packaging but self-inactivates the 5′ LTR for biosafety purposes (Dull *et al.*, 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
bla promoter	Allows expression of the ampicillin resistance gene
Ampicillin resistance gene (β–lactamase)	Allows selection of the plasmid in *E. coli*.
pUC origin	Permits high–copy replication and maintenance in *E. coli*.

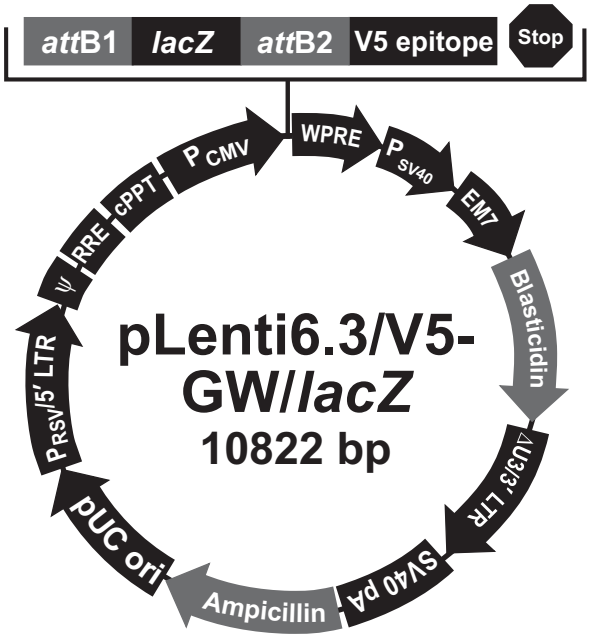
Map of pLenti6.3/V5–GW/*lacZ*

Description

pLenti6.3/V5–GW/*lacZ* is a 10822 bp control vector expressing β–galactosidase, and was generated using the Gateway® LR recombination reaction between an entry clone containing the *lacZ* gene and pLenti6.3/V5–DEST. β–galactosidase is expressed as a C–terminal V5 fusion protein with a molecular weight of approximately 121 kDa.

Map of pLenti6.3/V5–GW/*lacZ*

The map below shows the elements of pLenti6.3/V5–GW/*lacZ*. **The complete sequence of the vector is available from our web site (www.invitrogen.com) or by contacting Technical Support (see page 27).**



Comments for pLenti6.3/V5-GW/*lacZ*
10822 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 1655
3′ splice acceptor: base 1683
cPPT: bases 1800-1922
CMV promoter: bases 1934-2518
*att*B1 site: bases 2567-2591
lacZ ORF: bases 2606-5670
*att*B2 site: bases 5682-5706
V5 epitope: bases 5759-5800
WPRE: bases 5819-6416
SV40 promoter: bases 6427-6736
EM7 promoter: bases 6791-6857
Blasticidin resistance gene: bases 6858-7256
ΔU3/HIV-1 3′ LTR: bases 7342-7576
ΔU3: bases 7342-7395
Truncated HIV-1 3′ LTR: bases 7396-7576
SV40 polyadenylation signal: bases 7648-7779
bla promoter: bases 8638-8736
Ampicillin (*bla*) resistance gene: bases 8737-9587
pUC origin: bases 9742-10415

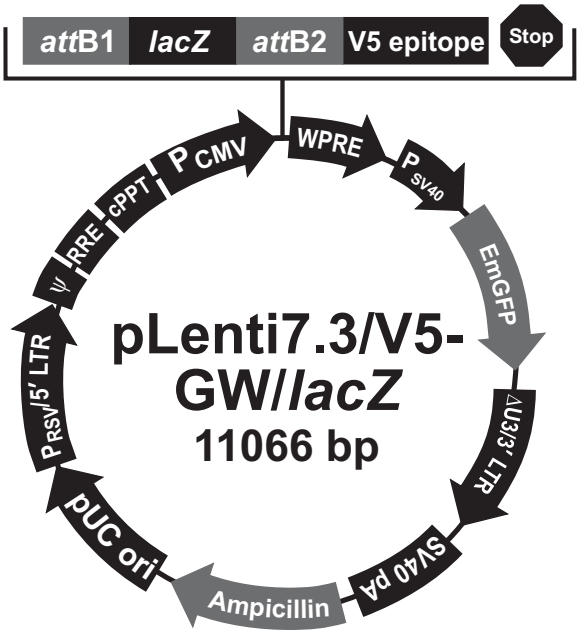
Map of pLenti7.3/V5–GW/*lacZ*

Description

pLenti7.3/V5–GW/*lacZ* is an 11066 bp control vector expressing β–galactosidase, and was generated using the Gateway® LR recombination reaction between an entry clone containing the *lacZ* gene and pLenti7.3/V5–DEST. β–galactosidase is expressed as a C–terminal V5 fusion protein with a molecular weight of approximately 121 kDa.

Map of pLenti7.3/V5–GW/*lacZ*

The map below shows the elements of pLenti7.3/V5–GW/*lacZ*. **The complete sequence of the vector is available from our Web site (www.invitrogen.com) or by calling Technical Support (see page 27).**



Comments for pLenti7.3/V5-GW/*lacZ*
11066 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
cPPT: bases 1801-1923
CMV promoter: bases 1935-2519
*att*B1 site: bases 2568-2592
lacZ ORF: bases 2608-5672
*att*B2 site: bases 5684-5708
V5 epitope: bases 5761-5802
WPRE: bases 5821-6418
SV40 promoter: bases 6429-6738
EmGFP: bases 6796-7515
ΔU3/3′ LTR: bases 7586-7820
    ΔU3: bases 7586-7639
    3′ LTR: bases 7640-7820
SV40 polyadenylation signal: bases 7892-8023
bla promoter: bases 8882-8980
Ampicillin (*bla*) resistance gene: bases 8981-9841
pUC origin: bases 9986-10659

Technical Support

Web Resources

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

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

Contact Us

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SDS

SDSs (Safety Data Sheets) are available on our web site at www.invitrogen.com/sds.

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.

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Purchaser Notification

Introduction

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

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 31.

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Purchaser Notification, Continued

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Purchaser Notification, Continued

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Gateway® Clone Distribution Policy

Introduction

The information supplied in this section is intended to provide clarity concerning 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 *att*L1 and *att*L2 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 *att*B1 and *att*B2 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.

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Notes

Notes

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