



## Promoter-less Lentiviral Expression Vector

Cat#	Product Name	Amounts
LV-PL1	Promoterless MCS lentivector ( <b>Bsd</b> )	6 ug, lyophilized plasmid DNA
LV-PL2	Promoterless MCS lentivector ( <b>Bsd-RFP</b> )	6 ug, lyophilized plasmid DNA
LV-PL3	Promoterless MCS lentivector ( <b>GFP-Bsd</b> )	6 ug, lyophilized plasmid DNA
LV-PL4	Promoterless MCS lentivector ( <b>Puro</b> )	6 ug, lyophilized plasmid DNA
LV-PL5	Promoterless MCS lentivector ( <b>Puro-RFP</b> )	6 ug, lyophilized plasmid DNA
LV-PL6	Promoterless MCS lentivector ( <b>Neo</b> )	6 ug, lyophilized plasmid DNA

**Storage:** Store at -20°C after reconstituted with DNAase free water. Stable for >12 months.

### Product Description:

GenTarget's lentivector system is Human Immunodeficiency Virus-1 (HIV) based plasmids for gene expression and knockdown. The lentivectors are used to generate lentiviral particles (lentivirus) that can be transduced into almost all kinds of mammalian cells, including stem cells, primary cells, and non-dividing cells both *in vivo* and *in vitro*. Lentiviral Particles stably integrate into the transduced cells' genome for long term expression, making it a great gene transfer agent.

GenTarget provides several Promoterless expression vectors containing a variety of antibiotic markers or fluorescent-antibiotic fusion dual markers. Each vector contains a multiple cloning site (MCS) region for sub-cloning of any "**Promoter-Target**" expression cassette, or "**promoter only**" insert, depending on vector types. These vectors do not contain any promoters ahead of the MCS, and are ideal for promoter studies. They contain a selection marker under the RSV promoter for selection and monitoring.

These lentivectors were engineered with several key genetic elements (WPRE, cPPT, RRE) for producing high titer expression lentivirus (**see vector schematic maps below**). In order to produce expression lentiviral particles (lentivirus), the lentivector must be co-transfected with a **packaging plasmid mixture (CAT#: HT-Pack)** into the virus production **293T cell line**, after incubation, the virus-containing supernatant is collected. GenTarget's lentivectors are fully compatible with most packaging mixtures on the market. GenTarget also provides packaging plasmid mixture (**Cat#: HT-pack**) and 293T cells (**Cat#: TLV-C**).



Each vector contains an ampicillin maker and can be propagated in LB medium containing 100 µg/ml of ampicillin. The full vector sequence was verified by sequence analysis and can be [downloaded](#) from our website.

**Note:** Unwanted recombination can occur during lentivector propagation due to the lentivectors' LTR arms; therefore, we recommend verifying your propagated lentivector by restriction digestion.

## Product Contents:

Each vector is provided as 6.0 µg/per vial x 1 vial, lyophilized. This amount is sufficient for 10 to 20 cloning processes. To reconstitute the dried vector, simply add 10 - 20 µl of DNase-free water to obtain the desired vector concentration and gently vortex. The plasmid can be propagated if more DNA is needed for another purpose.

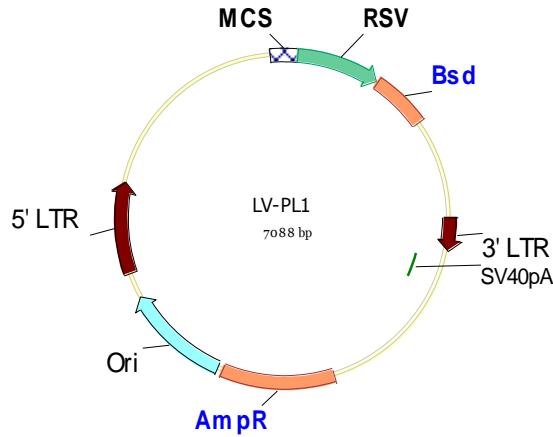
## Key Features:

- An MCS region containing multiple Restriction Enzyme sites for sub-cloning of a "promoter-target" cassette
- In some vectors a commonly used reporter (GFP, RFP, or Luciferase) is pre-installed after the MCS region; the reporter can be used in a sub-clone of any "promoter only" inserts
- A variety of antibiotic selection markers is available including fluorescent-antibiotic fusion dual markers for convenient tracing of viral performance
- Engineered lentivector backbone for high titer virus production
- A modification / mutation in the 3' UTR region (SIN feature), which produces only replication-incompetent lentivirus for enhanced Bio-safety
- These lentivectors are fully compatible with most packaging mixtures on the market

## Product schematic maps:

### 1. Cat#: LV-PL1:

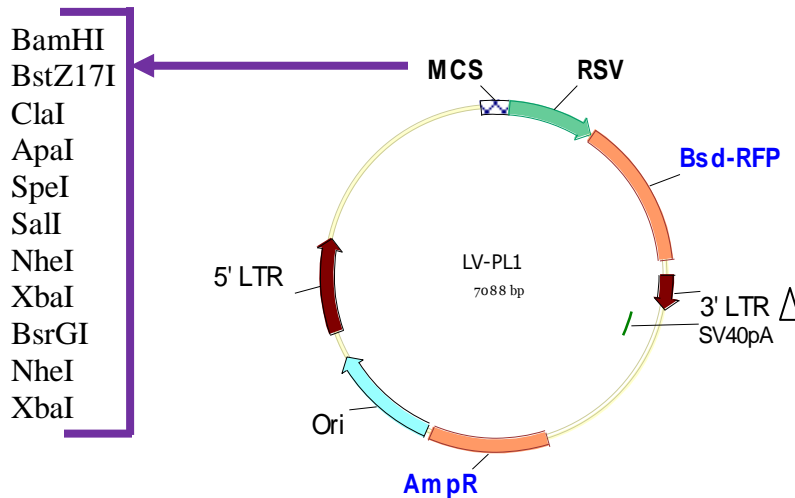
BamHI  
BstZ17I ←  
ClaI  
ApaI  
SpeI  
SalI  
NheI  
XbaI  
BsrGI  
NheI



MCS sequence (at vector position 1 ~ 67) in product LV-PL1:

GGATCC **GTATACATCGAT** GGGCCC ACTAGT **GTCGACGCTAGC** TCTAGATGTACAAAGTGGT **GCTAGC**  
 BamHI **BstZ17I** ClaI **ApaI** SpeI **Sall** NheI **XbaI** BsrGI NheI

2. Cat#: LV-PL2:



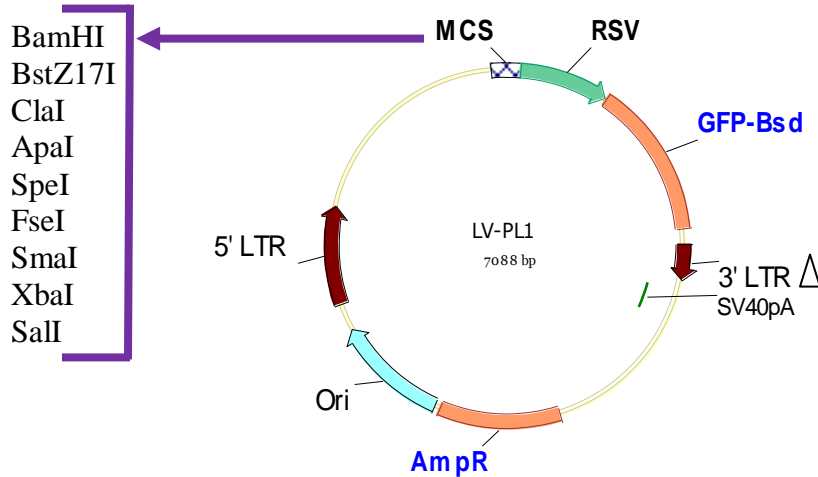
MCS sequence (at vector position 1 ~ 82) in product LV-PL2:

GGATCC **GTATACATCGAT** GGGCCC ACTAGT **GTCGACGCTAGC** TCTAGATGTACAAAGTGGT **GCTAGC**  
 BamHI **BstZ17I** ClaI **ApaI** SpeI **Sall** NheI **XbaI** BsrGI NheI

CGAATTCAAT**TCTAGA**--  
**XbaI**



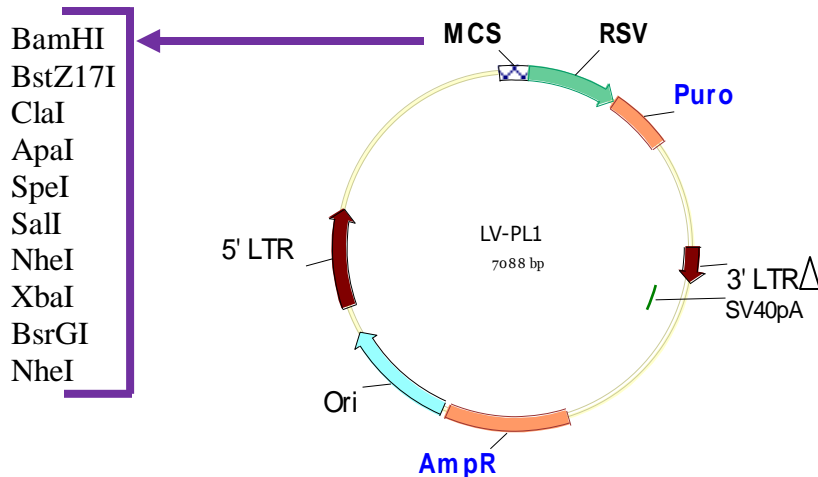
### 3. Cat#: LV-PL3:



#### MCS sequence (at vector position 1 ~ 54) in product LV-PL3:

GGATCCGTATACATCGATGGGCCCACTAGTGGCCGGCCCGGGTCTAGAGTCCGAC  
 BamHI BstZ171 ClaI ApaI SpeI FseI SmaI XbaI SalI

### 4. Cat#: LV-PL4:

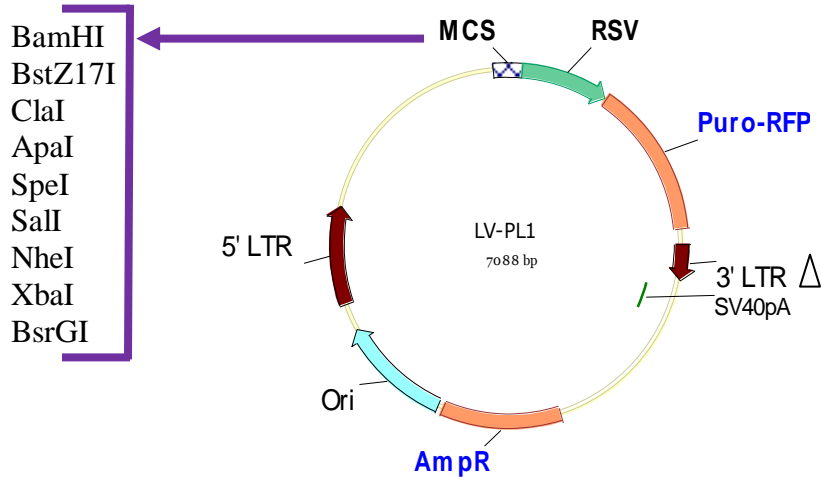


#### MCS sequence (at vector position 1 ~ 67) in product LV-PL4:

GGATCCGTATACATCGATGGGCCCACTAGTGTCGACGCTAGCTCTAGATGTACAAAGTGGTGCTAGC  
 BamHI BstZ171 ClaI ApaI SpeI SalI NheI XbaI BsrGI NheI



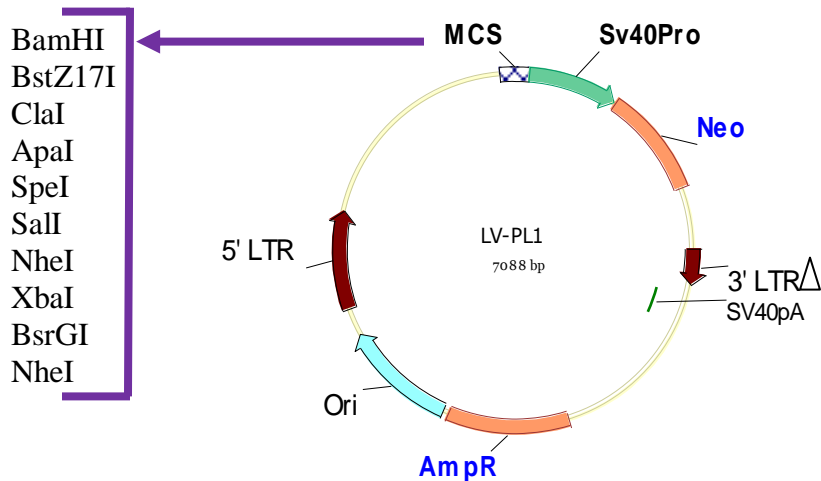
## 5. Cat#: LV-PL5:



MCS sequence (at vector position 1 ~ 54) in product LV-PL5:

GGATCCGTATACATCGATGGGCCCACTAGTGTCGACGCTAGCTCTAGATGTACA  
 BamHI BstZ171 ClaI ApaI SpeI SalI NheI XbaI BsrGI

## 6. Cat#: LV-PL6:



MCS sequence (at vector position 1 ~ 67) in product LV-PL6:

GGATCCGTATACATCGATGGGCCCACTAGTGTCGACGCTAGCTCTAGATGTACAAAGTGGTGCTAGC  
 BamHI BstZ171 ClaI ApaI SpeI SalI NheI XbaI BsrGI NheI



## Cloning procedure (Guidelines for Generating Expression Lentivector):

The Promoterless lentiviral expression vector is designed to help you create a lentivirus to deliver and express a gene of interest from a promoter of your choice. The first step is to sub-clone the promoter into the lentivector. We strongly recommend that users possess a working knowledge of molecular cloning techniques and be familiar with restriction enzyme based sub-cloning methods as well as the lentivirus production procedure. Once the lentivector is made, you will need to co-transfect it with **lentiviral packaging plasmids** into **293T cells**. The packaging plasmids and 293T cells are not included with this product, but you may purchase them from GenTarget.

**NOTE:** Carry out the virus production procedure according to the packaging plasmid manual.

### Sub-cloning outline:

#### **Lentivector preparation:**

- Design and select compatible restriction enzyme sites between the insert (promoter of interest) and promoterless lentivector.
- Open the promoterless lentivector at its MCS region by restriction enzyme cutting and dephosphorylate the vector open ends by CIP or another phosphatase.

#### **Insert preparation:**

- Select the "promoter-target" of your choice.
- Cut the insert by the same restriction enzymes or overhung compatible enzymes as used in the lentivector preparation (above).
- If the promoter and the target are sub-cloned separately in two steps, make sure that your promoter contains all necessary components (such as TATA box, enhancer, and a transcription initiation site) and that the promoter region does not contain ATG (to avoid unwanted transcription start).

**NOTE:** if the promoter and the target are sub-cloned in two steps, you have to sequentially open the lentivector in two position to accommodate the promoter and the target.

**T4 ligation:** ligate the promoter into the lentivector.

#### **Plate the Colonies:**



- Transform 1-3  $\mu$ l ligation mixture into cloning competent cells (such as DH5a, Top10, or NovaBlue).
- Spread cells onto an LB -agar plate containing 100  $\mu$ g/ml ampicillin.

### **Screen the positive colonies:**

- Pick a few colonies and confirm those that are positive by restriction digestion of the purified plasmid DNAs.
- The insert promoter region may be verified by sequencing analysis.

### **Lentivirus production:**

To obtain expression ready lentivirus, the generated expression lentivector must be co-transfected with packaging plasmid mixture (**Cat#:** [HT-pack](#) ) into 293T cells (**Cat#:** [TLV-C](#)). GenTarget's lentivector is fully compatible with other vendors' packaging plasmids for virus production and can be packaged with either the 2nd or the 3rd packaging system. The packaging plasmids and 293T cells are not included in this product, but they are available separately from GenTarget or may be obtained from other vendors.

### **Lentivirus production outline:**

#### **Day 0:**

- One day before transfection, plate sufficient 293T cells to achieve 70-80% confluence on the day of transfection

#### **Day 1**

Co-transfect the expression lentivector and the packaging plasmid mixture into 293T cells using Transfection Reagent (such as LF2k from Invitrogen, FuGene from Roche, or your preferred transfection reagent). Follow the packaging plasmid manual for the amount of DNA to use.

#### **Day 2 or 3**

- Harvest lentiviral supernatant 48-72 hours after transfection. Centrifuge 5 minutes at 1500 rpm to remove cell debris and filter at 0.22  $\mu$ m.
- Supernatants can be directly used (simply add into any cell culture) for expression assay. Depending upon cell type, the expression will peak after 72 hours to one week. If the lentivector contains a fluorescent marker, you can monitor the virus performance by visualizing the fluorescent signal at 48-72 hours post-infection.
- Store supernatants long-term at -80  $^{\circ}$ C in aliquots; avoid repeated freeze/thaw cycles.



## Warranty and purchase terms:

1. This product is warranted to meet its quality as described when used in accordance with its instructions. GenTarget disclaims any implied warranty of this product for any particular application. In no event shall GenTarget be liable for any incidental or consequential damages in connection with this product. GenTarget's sole remedy for breach of this warranty should be, at GenTarget's option, to replace the product.
2. The passing, giving, re-producing, or re-selling of this product is prohibited without written permission from GenTarget, Inc.
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## Safety Precaution:

GenTarget lentivectors adapt must advanced lentiviral safety features (using the third generation vectors with self-inactivation SIN-3UTR), and the generated lentivirus is replication incompetent. However, please use extra caution when using lentiviral particles. Use the lentiviral particles in Bio-safety II cabinet. Wear glove all the time at handling Lentiviral particles! Please refer CDC and NIH's guidelines for more details regarding to safety issues.

## References:

- NIH Guidelines for [Biosafety Considerations for Research with Lentiviral Vectors](#). (Link).
- [CDC guidelines for Lab Biosafety levels](#) (Link).
- J Virol. 2004; **78**:1421-30.
- J Virol. 2000 November; 74(22): 10778–10784.

## Warranty and purchase terms:

**This product is for research use only.** It is warranted to meet its quality as described when used in accordance with its instructions. GenTarget disclaims any implied warranty of this product for particular application. In no event shall GenTarget be liable for any incidental or consequential damages in connection with the products. GenTarget's sole remedy for breach of this warranty should be, at GenTarget's option, to replace the products.





**Related products:** GenTarget's premade lentivirus products

<b>Product Category</b>	<b>Product Description (please click category name to see product's pages)</b>
<a href="#">Human, mouse or rat ORFs</a>	Premade lentivirus expressing a <b>human, mouse or rat</b> gene with RFP-Blastididin fusion dual markers.
<a href="#">Fluorescent markers</a>	Premade lentivirus express human codon optimized fluorescent protein, <b>GFP / RFP / CFP / BFP / YFP</b> .
<a href="#">Luciferase expression</a>	Premade lentivirus for all kinds of luciferase protein expression: <b>firefly and Renilla</b> with different antibiotic selection markers.
<a href="#">CRE recombinase</a>	Premade lentivirus for expressing <b>nuclear permeant CRE</b> recombinase with different fluorescent and antibiotic markers.
<a href="#">LoxP ColorSwitch</a>	Premade lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" cassette, used to monitor the CRE recombination event in vivo.
<a href="#">CRISPR /hu CAS9</a>	Premade lentivirus express humanized wild-type <b>Cas9</b> endonuclease for genomic editing with <b>CRISPR</b>
<a href="#">TetR inducible expression repressor</a>	Premade lentivirus expressing <b>TetR</b> (tetracycline regulator) protein, the repressor protein for the inducible expression system.
<a href="#">iPS factors</a>	Premade lentivirus for human and mouse iPS ( <b>Myc, NANOG, OCT4, SOX2, FGF4</b> ) factors with different fluorescent and antibiotic markers
<a href="#">T-antigen Expression</a>	Express <b>SV40 large T antigen</b> with different selection markers
<a href="#">Cell Organelle imaging</a>	Premade lentivirus for cell organelle imaging. The fluorescent marker <b>GFP/RFP/CFP was sub-cellular localized</b> in different cell organelle for living cell imaging.
<a href="#">LacZ expression</a>	Express different full length <b><math>\beta</math>-galactosidase (lacZ)</b> with different selection markers
<a href="#">Anti-miRNA lentivirus</a>	Pre-made lentivirus expression a specific <b>anti-miRNA</b> cassette.
<a href="#">Fluorescent-ORF fusion</a>	Pre-made lentivirus expression a " <b>GFP/RFP/CFP-ORF</b> " fusion target.
<a href="#">Pre-made shRNA lentivirus</a>	Premade shRNA lentivirus for knockdown a specific genes ( <b>P53, LacZ, Luciferase</b> and more).
<a href="#">microRNA and anti-microRNA</a>	Premade lentivirus expression human or mouse <b>precursor miRNA</b> . And <b>anti-miRNA</b> lentivector and virus for human and mouse miRNA.



<a href="#">lentivirus</a>	
<a href="#">Negative control lentiviruses</a>	Premade <b>negative control lentivirus with different markers:</b> serves as the negative control of lentiviruses treatment, for validation of the specificity of any lentivirus target expression effects.
<a href="#">Other Enzyme expression</a>	Ready-to-use lentivirus, expressing <b>specific enzymes</b> with different selection markers.