Promoter-less lentiviral expression vector manual

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

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

Product Description:

Lentiviral system is a gene delivery tool using lentivectors for gene expression or knockdown. Lentivectors are HIV-1 (Human Immunodeficiency Virus 1) derived plasmids, used to generate lentiviral particles (lentivirus) that can be transduced into virtually all kinds of mammalian cell types or organs, including stem cells, primary cells and non-dividing cells both ***in vivo*** and in **cell culture** system. Particles stably integrate into the transduced cells' genome for long term expression. Therefore, lentivirus holds unique promise as gene transfer agents.

GenTarget provide several Promoterless expression vectors that contain different antibiotic markers or fluorescent-antibiotic fusion dual markers. Each vector contains a MCS (multiple cloning sites) region for sub-cloning of any desired "**Promoter-Target**" expression cassette, or any "**promoter only**" insert (dependent the vector types). Those vectors do not contain any promoters ahead of the MCS, and are ideal for promoter studies. They do contain a selection marker under RSV promoter for selecting and monitoring.

The lentivectors were engineered with several key genetic elements (WPRE, cPPT, RRE and so on) for producing high titer expression lentivirus (**see each vector schematic maps below**). In order to produce expression lentiviral particles (lentivirus), the expression lentivector has to be co-transfected with a **packaging plasmid mixture** into a virus production cell line, **293T cells**, and then the virus is collected from culture medium. GenTarget's lentivectors are fully compatible with most packaging mixtures in the marker in virus production. GenTarget also provided the packaging plasmid mixture (**Cat#: HT-pack**) and the 293T cells (**Cat#: TLV-C**) which can be purchased separately.

Each vector contains an ampicillin maker, and can be propagated in LB medium containing 100ug/ml of ampicillin. Full vector sequence was verified by sequencing analysis and can be **downloaded** from our website. (**Note**: unwanted recombination could occur during lentivector propagation because of lentivector's LTR arms. We recommend verifying your propagated lentivector by restriction digestion).

Product Content:

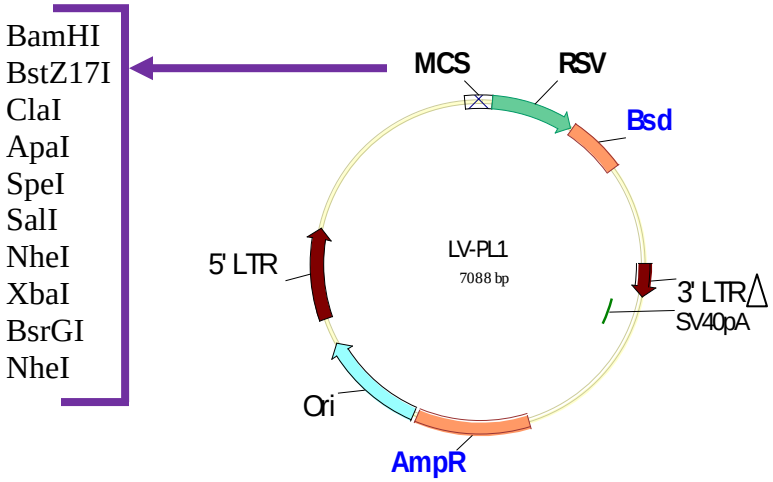
Each vector is provided as 6.0 ug/per vial x 1 vial, lyophilized, which is enough for 10 to 20 cloning processing. To reconstitute the dried vector, simply add certain 10 ~ 20 ul of DNAse free water, and gently vortex to obtain desired vector concentration. And if more DNA is needed for your own use, you can propagate the plasmid.

Key Features:

1. A MCS region contains multiple Restriction Enzyme sites for sub-cloning of "promoter-target" cassette;
2. For some vectors (see each vector for details), a common used reporter (GFP, RFP, or luciferase) is pre-installed after the MCS region, which can be sub-cloning of any "promoter only" inserts;
3. A variety of antibiotic selection markers satisfy your choices, and fluorescent fusion dual markers provide a convenient tool for tracing virus performance;
4. Engineered lentivector backbone for high titer virus production;
5. The lentivectors adapt the modification / mutation in its 3 UTR region (SIN feature), which produces only replication-incompetent lentivirus for better Bio-safety;
6. The expression lentivectors are fully compatible with most packaging mixtures in the marker in virus production;

Product schematic maps:

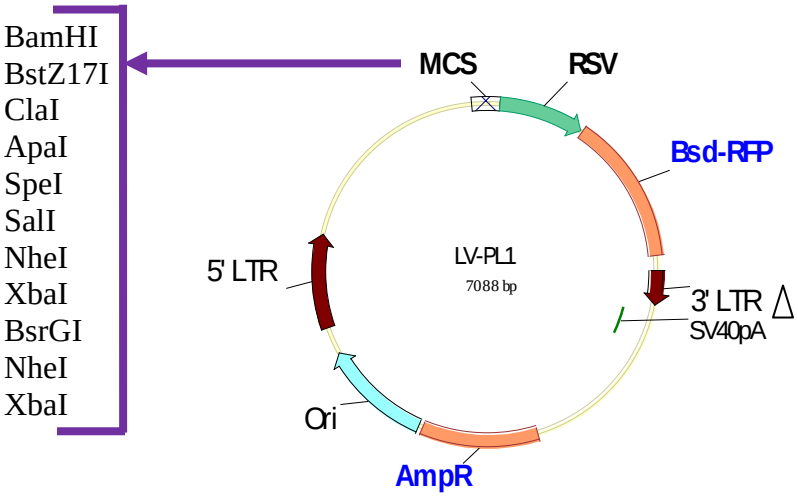
1. **Cat#: LV-PL1**:



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

GGATCCGTATACATCGATGGGCCCACTAGTGTCGACGCTAGCTCTAGATGTACAAAGTGGTGCTAGC
BamHI BstZ17I ClaI ApaI SpeI SalI NheI XbaI BsrGI NheI

2. **Cat#: LV-PL2**:



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

GGATCCGTATACATCGATGGGCCCACTAGTGTCGACGCTAGCTCTAGATGTACAAAGTGGTGCTAGC
BamHI BstZ17I ClaI ApaI SpeI SalI NheI XbaI BsrGI NheI

CGAATTCAATCTAGA--
XbaI

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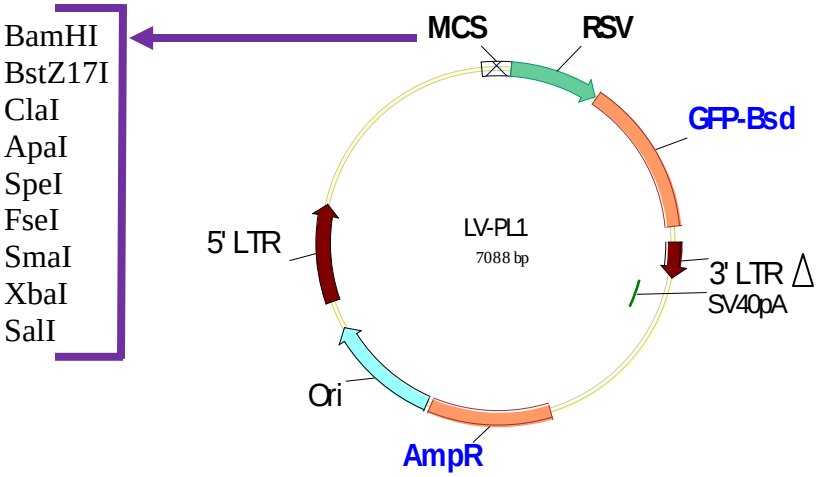
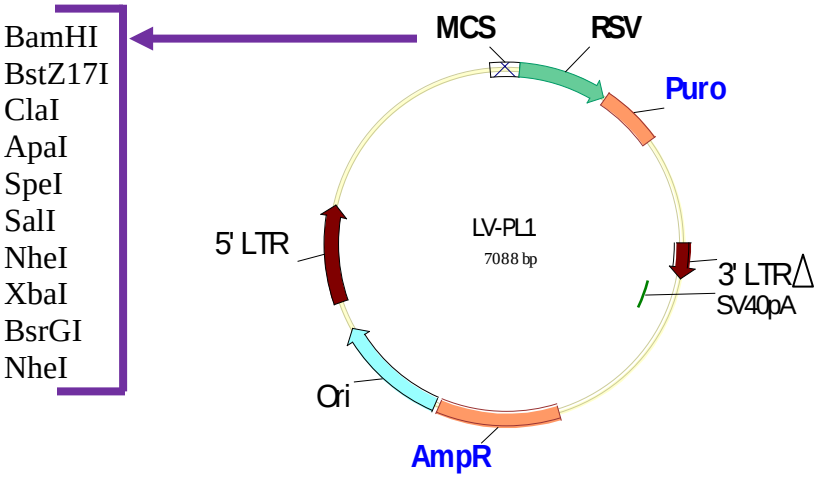
3. **Cat#: LV-PL3**:



MCS sequence (at vector position 1 ~ 54) in product LV-PL3:
GGATCCGTATACATCGATGGGCCCACTAGTGGCCGGCCCGGGTCTAGAGTCGAC
BamHI BstZ17I ClaI ApaI SpeI FseI SmaI XbaI SalI

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



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

GGATCCGTATACATCGATGGGCCCACTAGTGTCGACGCTAGCTCTAGATGTACAAAGTGGTGCTAGC
BamHI BstZ17I ClaI ApaI SpeI SalI NheI XbaI BsrGI NheI

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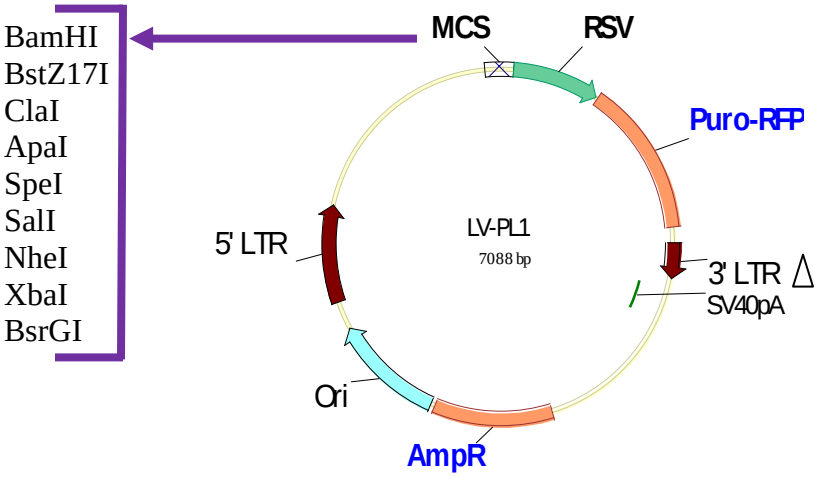
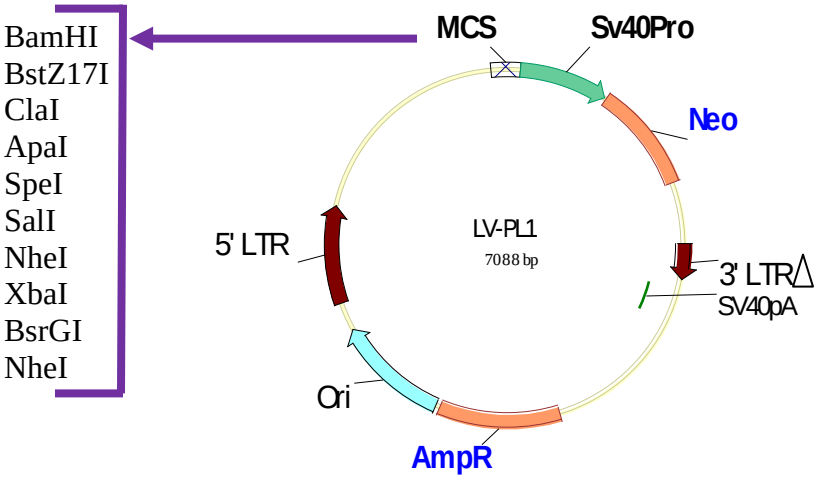
5. **Cat#: LV-PL5**:



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

GGATCCGTATACATCGATGGGCCCACTAGTGTCGACGCTAGCTCTAGATGTACA
BamHI BstZ17I ClaI ApaI SpeI SalI NheI XbaI BsrGI

6. **Cat#: LV-PL6**:



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

GGATCCGTATACATCGATGGGCCCACTAGTGTCGACGCTAGCTCTAGATGTACAAAGTGGTGCTAGC
BamHI BstZ17I ClaI ApaI SpeI SalI NheI XbaI BsrGI NheI

Cloning procedure (Guidelines for Generating Expression Lentivetor):

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 this lentivector. We highly recommend that users possess a working knowledge of molecular cloning techniques, and familiar with restriction enzyme based sub-cloning methods, as well as the lentivirus production procedure. Once the lentivector made, you need co-transfection it with **lentiviral packaging plasmids** into **293T cells**. The packaging plasmids and 293T cells are not included in this product, but you able to purchase from GenTarget or many other vendors. (For virus production, you carry out the virus production procedure according to the packaging plasmid manual.)

Sub-cloning outline:

- Lentivector preparation:

  Design and select compatible restriction enzymes between the insert (promoter of interest) and promoter-less lentivector. Open the promoter-less lentivector at its MCS region by Restriction enzyme cut, De- Phosphorylation of vector open ends by CIP or other phosphatase;

- Insert preparation:

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

- T4 ligation: ligase the promoter into lentivector;
- Plate colonies:

  Take 1~3 ul ligation mixture, transform into cloning competent cells (like DH5a, Top10, NovaBlue or the kinds). Spread cells onto LB -agar plate containing 100ug/ml of ampicillin.

- Screen the positive colonies:

  Pick a few colonies and confirm the positive colonies by restriction digestion of the purified plasmid DNAs; The insert promoter region will be verified by Sequencing analysis.

Lentivirus production:

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

Lentivirus production outline:

- One day before transfection, plate sufficient 293T cells to achieve 70-80% confluence on the day of transfection;
- Co-transfection of expression lentivector and packaging plasmid mixture into 293T cells using Transfection Reagent (such as LF2k from Invitrogen, FuGene from Roche or the your preferred transfection reagent). Follow the packaging plasmid manual for the amount of DNA to use.
- Harvest lentiviral supernatant 48-72 hours after transfection. Centrifuge 5 minutes at 1500 rpm to remove cell debris and filtrate on 0.22 μm.
- Supernatants can be directly used (simply add into any cell culture) for expression assay. Dependent upon the cell types, the expression peaks at 72 hours to one week. If the lentivector contains a fluorescent marker, you can monitor the virus performance by visulize the fluorescent signal at 48-72 hours post infection.
- For long term storage, store supernatant at -80ºC in aliquots, avoid repeated thaw/frozen cycles.

Safety Precaution:

Please use extra caution when using lentivial expression system. Lentivirus production requires Bio-safety Level 2 (BL-2) lab setting. Please refer CDC and NIH's links (see references) for more details regarding to safety guideline.

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:

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