Lentiviral Vectors for Gene Expression

Plasmids for Transient and Stable Gene Expression

Catalog number: cDNA-pLV##
Size: 10 μg plasmid DNA
Storage Temperature: -20°C

November 2012

BIOSETTIA
A Better Solution in Life Science
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Product List

pLV-cDNA Gene Expression Vector	Catalog #
pLV-EF1α-MCS-IRES-Puro	cDNA-pLV01
pLV-EF1α-MCS-IRES-Hyg	cDNA-pLV02
pLV-EF1α-MCS-IRES-Bsd	cDNA-pLV03
pLV-EF1α-MCS-IRES-Neo	cDNA-pLV04
pLV-EF1α-MCS-IRES-GFP	cDNA-pLV05
pLV-EF1α-MCS-IRES-RFP	cDNA-pLV06
pLV-EF1α-MCS-IRES-GFP-Bsd	cDNA-pLV07
pLV-EF1α-MCS-IRES-RFP-Bsd	cDNA-pLV08
pLV-EF1α-MCS-IRES-RFP-Puro	cDNA-pLV09
pLV-EF1α-MCS-IRES-RFP-Neo	cDNA-pLV10
pLV-EF1α-MCS-IRES-GFP-Puro	cDNA-pLV11

Product Description

1. The pLV-cDNA vector is a self-inactivated (SIN) vector carrying a deletion in the U3 region of the 3' LTR, which eliminates the promoter activity of LTR. The self-inactivating deletion provides an additional level of safety by preventing the integrated viral genome from generating full-length lentiviral RNA. The pLV-cDNA vector can be used in transiently expressing gene of interest by transfection and long-term expression while the cells are transduced by packaged lentivirus.

2. Lentiviral transduction is one of the most effective delivery system for stable gene expression. Unlike the retroviral system, the lentiviral integration is cell cycle independent. The genetic materials encoded by the lentivirus can be efficiently delivered into both dividing and non-dividing cells. The gene of interest is integrated into the host chromosome, thus the inserted gene is stably expressed in transduced cell lines.

3. The human EF1α promoter on the vector is a house-keeping gene promoter. It is unlikely to be silenced by methylation in vitro and in vivo.

4. The delivery efficiency and promoter activity can be monitored by internal ribosomal entry sequence (IRES) expressing selectable markers from the same (EF1α) promoter.

5. The selectable markers: GFP-Bsd, RFP-Bsd, GFP-Puro, RFP-Puro, and RFP-Neo are fluorescent proteins translationally fused to drug-resistant genes. The transfected and transduced cells are resistance to corresponding antibiotic selection and show green (or red) fluorescence as well.

6. The selection markers are expressed via internal ribosomal entry sequence (IRES). In general, the level of marker gene expression via IRES is about 7-10 folds lower than the same marker gene expressed directly from the promoter. So, the antibiotic selection favors transduced cells harboring higher copy numbers of lenti-cDNA integration and results in higher level of GOI expression in pLV-cDNA transduced cells than the cells transduced with other expression vectors using two separated promoters to express GOI and selection marker.

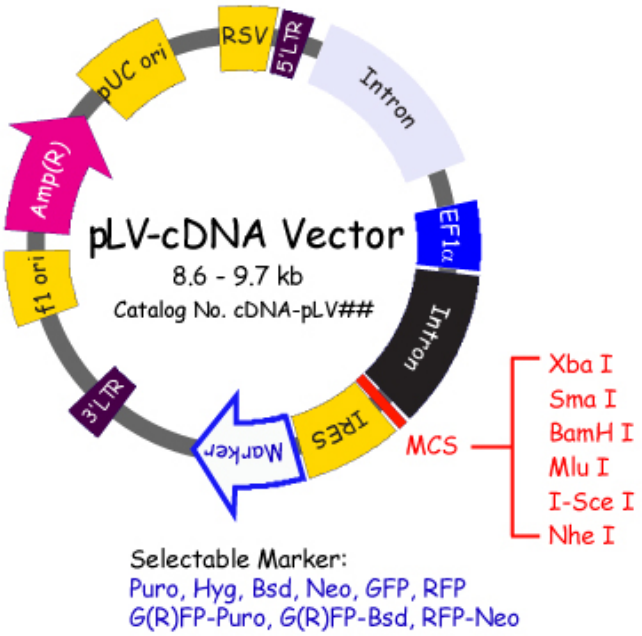

Figure 1. pLV-cDNA Gene Expression Vector System.

Product Components

Each lentiviral gene expression vector kit provides:

40 µl	pLV-cDNA vector (250 ng/µl)
20 µl	EF1a-forward-primer, TTGGATCTTGGTTCATTCTC (10 µM)
20 µl	IRES-reverse-primer, AATAACATATAGACAAACGC (10 µM)

Store the lentiviral gene expression vector, sequencing primers at -20°C.

Protocol

Clone Gene of Interest into pLV-cDNA Vector

Follow standard cloning protocol to PCR amplify the gene of interest (GOI) and clone into the multiple cloning site (MCS) of the pLV-cDNA vectors (Fig. 2).

Note: 1. An ATG start codon is required for GOI expression.

2. A Kozak consensus sequence (GCCACC) before start codon is highly recommended.

3. The inserted fragment or GOI should not contain a polyadenylation signal sequence.

4. The compatible cloning sites should be considered if necessary. For example, the *Avr* II and *Spe* I sites are compatible with *Xba* I and *Nhe* I sites; the *Bgl* II and *Bcl* I sites are compatible with *Bam*H I site; the *Asc* I and *Bss*H II sites are compatible with *Mlu* I site.

5. For restriction digesting lentiviral vector, less plasmid DNA, more restriction enzymes, higher reaction volume, and shorter reaction time are recommended. For example, we usually add 1-2 µg of plasmid DNA in a 50 µl reaction mix including 20 units of enzyme and incubate at 37°C for less than 2 hours.

6. Due to high frequency of homologous recombination occurred in lentiviral vector in some bacterial strains (e.g. TOP10, XL-1 Blue and XL-10 Gold), we recommend using LV101 (Biosettia), DH5α or Stbl3 (Invitrogen) for pLV-cDNA plasmid transformation.

7. In some cases, the bacterial transformants or correct recombinants hardly grow at 37°C because the bacterial strain is less tolerant to overexpression of GOI. We suggest that using different bacterial strain for transformation and/or growing the transformants at room temperature might be helpful.

8. The bigger size of GOI inserted into pLV-cDNA vector, the lower titer of lentivirus will be generated. The size of insertion should not be over 6 kb.

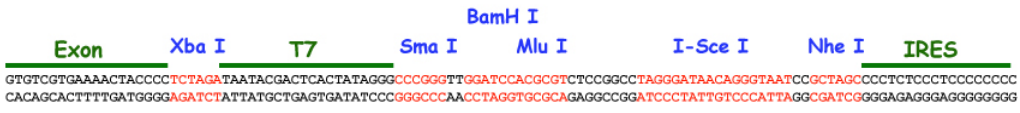

Figure 2. The multiple cloning sites (MCS) sequence in pLV-cDNA vectors.

Plasmid DNA preparation and screening for insertions.

1. Inoculate single colonies from pLV-cDNA plate into 6 ml of LB medium with 100 µg/ml of ampicillin in a 15-ml tube. Grow bacterial culture at 37°C for 20 hours in a floor shaker with 300 rpm.

2. Spin down whole 6-ml bacterial culture in a desktop centrifuge (e.g. Sorvall RT6000) at 4,000 rpm for 10 min. Discard the supernatant and start plasmid DNA purification.

Note: We have tested that growing bacteria in 6 ml of LB medium and shaking in a floor shaker with 300 rpm at 37°C for 20 hours gives a better yield of plasmid DNA. We recommend using bacteria strain LV101 or Stabl3 and Favorgen nucleic acid purification kits (http://biosettia.com/php/products-nucleic-acid-purification) to prepare high-yield and good-quality plasmid DNA for future experiments such as transfection and lentiviral production. In general, the DNA yield is around 30-50 μg for each miniprep. The yield and quality of miniprep plasmid DNA purified by using Favorgen plasmid DNA extraction mini kit (Catalog # FAPDE 001) is sufficient for generating 30 ml of lentivirus.

3. To confirm the insertion of GOI in the pLV-cDNA vector, we recommend that the diagnostic digestion be performed with *Sac* I plus one restriction site in MCS, such as *Nhe* I site.

Lentiviral production from pLV-cDNA plasmid

Production of lenti-cDNA viral stocks requires packaging of the lentiviral genomic RNA transcribed from pLV-cDNA vector with HIV-1 *gag*, *pol*, and *rev* gene products and vesicular stomatitis virus G (VSV-G) protein encoded by helper plasmids. In general, at least two helper plasmids are required, with one plasmid expressing the Gag-Pol polyprotein and an accessory protein Rev and the other expressing VSV-G as envelop protein to increase cell tropism. Nevertheless, Gag-Pol and Rev proteins can be expressed from separated plasmids as well. Therefore, a three-plasmid system or a four-plasmid system can be used to generate lenti-cDNA viral stocks, depending on the source and nature of the helper plasmids. In the three-plasmid system, pLV-cDNA is cotransfected with two helper plasmids (Gag-Pol + Rev and VSV-G) into cells, while in the four-plasmid system; pLV-cDNA is cotransfected with three helper plasmids (Gag-Pol, Rev and VSV-G). It is generally considered to be safer to produce the lentiviral stocks with more helper plasmids, due to the reduced chance of recombination among all vectors that generates replication-competent viruses.

The manufacturers of transfection reagents, the suppliers of lentiviral packaging constructs, and many academic laboratories have provided protocols for producing lentiviral stocks. The following procedure is provided as an example only. We produce lenti-cDNA viral stocks in 293T cells using the transfection conditions summarized in a table below.

	10-cm plate		6-well plate	
	3-plasmid system	4-plasmid system	3-plasmid system	4-plasmid system
pLV-cDNA vector	9 μg		1.5 μg	
Gag-Pol + Rev expression vector[1]	6 μg		1.0 μg	
Gag-Pol expression vector[2]		4.5 μg		0.75 μg
Rev expression vector[3]		1.8 μg		0.3 μg
VSV-G expression vector[4]	3 μg	2.7 μg	0.5 μg	0.45 μg
Total plasmid DNA	18 μg		3.0 μg	
Lipofectamine™ 2000	45 μl		7.5 μl	
Total Opti-MEM	3 ml		0.5 ml	
293T cells / vol. of medium	1.0×10^7/5ml		1.7×10^6/1ml	

Below we have listed the commonly used vectors for lentiviral packaging.

[1] For example: pCMV-deltaR8.91 (TRC), psPAX2 (Addgene)

[2] For example: pMDLg/pRRE (Addgene), pLP1 (Invitrogen), pPACKH1-GAG (SBI)

[3] For example: pRSV-REV (Addgene), pLP2 (Invitrogen), pPACKH1-REV (SBI)

[4] For example: pMD.G (TRC), pMD2.G (Addgene), pCMV-VSV-G (Addgene), pVSV-G (SBI), pLP/VSVG (Invitrogen)

Note: The transfection reagent Lipofectamine™ 2000 (LF2000, Invitrogen) is preferred for transfection. The average lentiviral titers in our preparations are around 5 x 10^6 - 5 x 10^7 infection units per ml (IU/ml) when titered with 293T cells.
Suggestion: We recommend using Biosettia Pre-mixed Lentiviral Packaging System (Catalog # pLV-PACK-500) for optimal lentiviral production.

Day 0: Seed **6.0 × 10^6** (1.0 × 10^6) 293T cells in a **10-cm plate** (6-well plate), so that the cell density will be around **1.0 × 10^7** (1.7 × 10^6) at the time of transduction.

Day 1: Gently mix **45.0** (7.5) µl LF2000 and **1.5** (0.25) ml Opti-MEM medium and incubate at room temperature for 5 minutes. Meanwhile, gently mix **18.0** (3.0) µg in total of pLV-cDNA vector and helper plasmids mixture into **1.5** (0.25) ml Opti-MEM medium (Invitrogen).

Gently mix DNA and LF2000, incubate at room temperature for 20 minutes to allow DNA and lipid to form complexes. In the meantime, replace the overnight culture medium with **5.0** (1.0) ml DMEM + 10% FBS without antibiotics. Add the **3.0** (0.5) ml DNA-LF2000 complexes to 293T cells.

Note: We have noticed that fetal bovine serum purchased from different manufacturers may affect the attachment of 293T cells to the bottom of tissue culture plates, resulting in variation in the efficiency of lentiviral production. We recommend switching to a different brand of FBS if 293T cells disattach from plates during lentiviral production.

Day 2: Replace the media containing the DNA-LF2000 complexes with **10.0** (2.0) ml complete medium at 12-16 hours post-transfection.

Day4: Collect supernatants at 48 hours post-transfection and transfer media to a polypropylene storage tube. Spin the virus-containing media at 1300 rpm for 5 minutes to pellet any 293T cells that were carried over during collection. Carefully transfer the supernatant to a sterile polypropylene storage tube.

Note: Lentiviral stock may be stored at 4 °C for up to 5 days, but should be aliquoted and frozen at -80 °C for long-term storage.
Suggestion: To reduce the number of freeze and thaw cycles, aliquot lentiviral stock to smaller tubes before storage at -80 °C.

Titering the lentiviral stocks

It is important to titer the lentiviral stocks in the cell line of interest to produce consistent results using the equivalent multiplicity of infection (MOI) in experiments. Knowing MOI will help you to control the copy number of lenti-cDNA integrated into the chromosomes of the cells of interest. While titering virus with antibiotic selection, the titering procedure includes selection of stably transduced cells with the corresponding antibiotics and counting the antibiotic-resistant cell colonies. Alternatively, a flow cytometry can be used to determine the viral titer by measuring the number of green (or red) fluorescent cells. If you are using both methods to titer your lentiviruses, please keep in mind that the titers may be different due to sensitivities of FACS machine and cell lines resistant to drug selection.

Please be aware the lenti-cDNA virus titer may be underestimated when antibiotic selection or flow cytometry is deployed to determine the titer. For example, the transduced cells with single integration event may not be selected due to low level of selection marker expression by IRES.

A. Determination of lentiviral titers by antibiotics selection.

Day 0: Seed the cells of your choice in a 6-well plate so that the cell density that will be ~25-50% confluent at the time of transduction.

Day 1: Thaw lentiviral stock, gently mix virus and then prepare 2 ml 10-fold serial dilutions ranging from 10^{-3} to 10^{-7} in complete medium containing 5-8 μg/ml polybrene.

Remove the medium from previous day and add 2 ml fresh dilutions into each well.

Suggestion: Leave one well uninfected for mock control. Spin transduction in a desktop centrifuge (e.g. Sorvall RT6000) at 1,000 × g for 30-60 min at room temperature may be necessary if titering virus in cell lines other than 293T.

Day 2: Replace the medium containing virus and polybrene with 2 ml of complete medium.

Day 3-4: Replace medium with fresh medium containing antibiotic to select for stably transduced cells.

Note: At least 48 hours of transduction allows lenti-cDNA to integrate into the host genome. The optimal antibiotic concentration varies from cell line to cell line.

Suggestion: A pilot experiment should be performed to determine the minimum concentration of antibiotics required to kill the untransduced cells before this experiment.

Day 5-6: Replace medium with 2 ml fresh medium containing antibiotic every 2 days.

Day 7-8: Allow antibiotic-resistant colonies to form in dilution wells. No live cells should be growing in the mock control well.

Note: The number of days required for the formation of visible colonies may vary among different cell lines.

Wash wells twice with 2 ml PBS

Stain cells with 1 ml 0.5% crystal violet solution in 20% ethanol and incubate for 30 minutes at room temperature.

Wash wells with distilled water by submerging the plate in a tray full of water, and repeat the wash one more time.

Dry the plate and count the number of blue-stained colonies.

The titer should be the average colony number times the dilution factor.

Note: The transduction efficiency varies from cell line to cell line. The lentiviral titer in the cell line of your interest may be lower (sometimes more than 10-fold) or higher than the virus titer in commonly used cell lines such as 293T cells.

B. Determination of lentiviral titers by flow cytometry.

Day 0: Seed the cell of your choice in a 6-well plate so that the cell density that will be ~25-50% confluent at the time of transduction.

Note: The number of cells seeded in the well is required to calculate lentiviral titer later.

Day 1: Thaw lentiviral stock, gently mix virus and then prepare 2 ml 10-fold serial dilutions ranging from 10^{-1} to 10^{-4} in complete medium containing 5-8 μg/ml polybrene.

Remove the medium from previous day and add 2 ml fresh dilutions into each well.

Suggestion: Leave one well uninfected for mock control. Spin transduction in a desktop centrifuge (e.g. Sorvall RT6000) at 1,000 × g for 30-60 min at room temperature may be necessary if titering virus in cell lines other than 293T.

Day 2: Replace the medium containing virus and polybrene with 2 ml of complete medium.

Day 3-4: Follow your lab protocol to collect and resuspend cells for flow cytometry to determine the percentage of green or red fluorescent cells.

Note: At least 48 hours of transduction allows lenti-cDNA to integrate into the host genome.

The titer should be the average number of live fluorescent cells times the dilution factor.

Note: The transduction efficiency varies from cell line to cell line. The lentiviral titer in the cell line of your interest may be lower (sometimes more than 10-fold) or higher than the virus titer in commonly used cell lines such as 293T cells.

Example: 1×10^5 cells were seeded on Day 0 and the cell doubling time is around 24 hours. The FACS data showed 5% and 40% of green fluorescence cells in the 10^{-3} and 10^{-2} dilution wells, respectively. The lentiviral titer is calculated by multiplying the fraction of transduced cells by 2×10^5 (the expected number of cells in the well on Day 1, the time of transduction), and by the dilution factor.

$0.05 \times (2 \times 10^5) \times 10^3 = 1 \times 10^7$

$0.4 \times (2 \times 10^5) \times 10^2 = 8 \times 10^6$

The average lentiviral titer is 9×10^6 IU/ml

Lentiviral Transduction for Gene Expression.

Day 0: Seed cells at appropriate density.

Suggestion: Plate cells so that cell density will be ~10-25% confluent at the time of transduction.

Day 1: Transduction. Remove the medium from the tissue culture plate by aspiration and replace it with fresh complete medium containing 5-8 μg/ml polybrene. Gently mix lentivirus with pipette tip, and add appropriate amount of virus to each well.

Note: (1) Polybrene may be toxic to some cell lines. (2) The non-concentrated and non-purified lentiviral stock collected from 293T supernatant may contain substances affecting the target cell growth, especially when a large volume of low-titer lentiviral stock is added. In case the lentiviral stock inhibits cell growth, concentration and purification of the viruses may be required. (See below for a protocol of virus concentration) Alternatively, incubation time for transduction can be shortened to hours. For example, the virus-containing medium may be replaced with fresh medium after one hour of transduction.

Suggestion: Transduce cells at multiplicity of infection (MOI) = 1, 5, 10, 25, and 50 to determine the optimal expression level. Spin transduction in a desktop centrifuge (e.g. Sorvall RT6000) at 1,000 × g for 30-60 min at room temperature helps increase of transduction efficiency.

Day 2: Replace the transduction medium with fresh complete medium to remove lentivirus and polybrene.

Day 3-4: Select transduced cells (>50% confluence is recommended) with medium containing appropriate antibiotics or by flow cytometry to sort out fluorescence-positive cells if necessary.

Note: The optimal antibiotic concentration varies from cell line to cell line.

Suggestion: A pilot experiment should be performed to determine the minimum concentration of antibiotic required to kill the untransduced cells before this experiment.

Day 6+: Analysis of transduced cells.

Suggestion: Expand the culture of cell lines stably expressing GOI and store the cell line stocks in liquid nitrogen before analyzing the cells.

Optional: Concentration of lentivirus by ultracentrifugation

1. Filter lentivirus through a 0.45 μm filter.

2. Centrifuge at 25,000 rpm for 90 minutes in a SW-28 or SW-41 rotor.

3. Discard the supernatant and use a Pasteur pipette with an attached P100 tip to carefully remove the remaining medium.

4. Gently resuspend viral pellet in 1/100 volume of DMEM. Let the virus suspension sit for overnight at 4° C.

5. On the following day, mix gently, aliquot and freeze virus.

Safety Guidelines for Working with Lenti-cDNA.

The recombinant lentiviruses have been designated as Level 2 organisms by NIH and CDC. A Biosafety Level 2 (BSL-2) facility is required in order to work with lentiviruses. The information of Biosafety in Microbiological and Biomedical Laboratories (BMBL) can be downloaded from the following link:
http://www.cdc.gov/od/ohs/biosfty/bmbl5/BMBL_5th_Edition.pdf
Please be aware that you are working with media containing lentiviral particles which could transduce human cells.

Related Products

cDNA Templates.

cDNA Template	Catalog #
human fetal kidney cell HEK-293 cDNA	cDNA-hsa-01
human myelogenous leukemia cell line K-562 cDNA	cDNA-hsa-02
human lung fibroblast cell MRC-5 cDNA	cDNA-hsa-03
human hippocampus cDNA	cDNA-hsa-04
human medulla cDNA	cDNA-hsa-05
human testis cDNA	cDNA-hsa-06
human fetal liver cDNA	cDNA-hsa-07
human fetal brain cDNA	cDNA-hsa-08
human fetal lung and spleen cDNA	cDNA-hsa-09
human fetal brain, liver and heart cDNA	cDNA-hsa-10
human adult brain cDNA	cDNA-hsa-11
human adult leukocyte cDNA	cDNA-hsa-12
human adult liver and skin cDNA	cDNA-hsa-13
human adult pancreas and spleen cDNA	cDNA-hsa-14
human adult brain, lung and testis cDNA	cDNA-hsa-15
human adult heart, skeletal muscle and breast cDNA	cDNA-hsa-16
human adult colon, kidney and stomach cDNA	cDNA-hsa-17
human adult breast tumor and lung tumor cDNA	cDNA-hsa-18
human adult stomach tumor and colon tumor cDNA	cDNA-hsa-19
mouse brain cDNA	cDNA-mmu-01
mouse kidney cDNA	cDNA-mmu-02
mouse stem cell cDNA	cDNA-mmu-03
mouse stomach cDNA	cDNA-mmu-04
mouse placenta cDNA	cDNA-mmu-05
mouse testis cDNA	cDNA-mmu-06
mouse E14 embryonic stem cell cDNA	cDNA-mmu-07
mouse muscle cDNA	cDNA-mmu-08
mouse heart cDNA	cDNA-mmu-09
mouse fetal brain cDNA	cDNA-mmu-10
rat liver cDNA	cDNA-rno-01
rat brain cDNA	cDNA-rno-02

Lentiviral Packaging Mix.

Pre-mixed Plasmids for Lentiviral Packaging	Catalog #
Lentiviral Packaging Mix. 500 µg	pLV-PACK-500
Lentiviral Packaging Mix. 1000 µg	pLV-PACK-1000

Nucleic Acid Purification Kits

Plasmid DNA Purification Kits	Catalog #
Plasmid DNA Extraction Mini Kit (100)	FAPDE 001
Plasmid DNA Extraction Mini Kit (300)	FAPDE 001-1
Plasmid DNA Extraction Midi Kit (25)	FAPDE 002
Plasmid DNA Extraction Midi Kit (50)	FAPDE 002-1
Plasmid DNA Extraction Maxi Kit (10)	FAPDE 003
Plasmid DNA Extraction Maxi Kit (25)	FAPDE 003-1
EndoToxinFree Plasmid DNA Extraction Maxi Kit (10)	FAPDE 003-EF

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