Modification Form for Permit BIO-RRI-0055

Permit Holder: John MacDonald

Approved Personnel
(Please stroke out any personnel to be removed)

Michael Jackson
Oies Hussein
~~Hongbin Li~~
Xuanmao Chen
~~Bikram Sidhu~~
Kai Yang
Michelle Olah
Lidia Brades
Natalie Lavine

Additional Personnel
(Please list additional personnel here)

Yu-Feng Xie
Jillian Belrose
Cristi Orth
Gang Lei
Rohit Kesarwani

	Please stroke out any approved Biohazards to be removed below	**Write additional Biohazards for approval below. ***
Approved Microorganisms	E.coli; DH5 - Alpha, RosettaBlue	lentivirus
Approved Cells	[Primary] - (Rodent): Mouse Brain/. [Established] - (Human): HEK 293. (Avian) : DT - 40	
Approved Use of Human Source Material		
Approved GMO	[Plasmids] - pcDNA, pGEX	- pLB, pSMPUW (lenti expression vectors) - pRSV(Rev), pCgpV, pCMV(Eco) (lenti packaging vectors)
Approved use of Animals		
Approved Toxin(s)	Tetrodotoxin	

* *PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
** *PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.*

As the principal investigator, I have ensured that all of the personnel named on the form have been trained. I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca.

Signature of Permit Holder: _____

Classification: 2

Date of Last Biohazardous Agents Registry Form: Sep 8, 2009

Date of Last Modification (if applicable): _____ (pending Level 2 Inspection)

BioSafety Officer(s): _____ Apr. 05/10

Chair, Biohazards Subcommittee: _____

MacDonald Lab, RRI, Room 7260C-1

Proposed experimental use of lentivirus

Primary cultured neurons are notoriously difficult to transfect by traditional means (e.g. lipofectamine, $CaPO_4$, etc). Moreover, although these approaches may be successfully utilized, the efficiency achieved (usually ~2-3%) is insufficient for biochemical studies and the reliability of the procedure is too variable. In contrast, we have routinely achieved >80% efficiency using lentivirus-mediated gene transduction. We will generate lentivirus, utilizing the two expression vectors listed in the modification form, for shRNA-mediated gene silencing (pLB vector) or for overexpression of genetically modified proteins (pSMPUW) in primary murine cultured neurons. Proteins targeted for silencing include EPAC (exchange protein directly activated by cAMP), DISC1 (disrupted-in-schizophrenia 1), TRPM2 (transient receptor potential, melastatin 2) and NMDA receptor subunits (GluN1, GluN2A and GluN2B). For protein overexpression, HA- or FLAG-tagged TRPM2 will be expressed in our cultured neurons for immunostaining, immunoprecipitation and electrophysiological experiments.

Protocol for Handling Recombinant Replication-deficient Lentiviruses

Lentiviral vectors are different from the commonly used adenovirus based gene delivery systems because the gene of interest becomes stably integrated into the host cell's genome. The efficiency of lentiviral systems are due to the fact that they are actively imported into the nuclei of dividing, as well as non-dividing cells, as opposed to traditional retroviruses.

The lentiviral genome contains nine genes but only three of those are required to generate a replication-deficient virus. The three essential genes are Gag, Pol and Env and they can all be provided in trans. Gag encodes a capsid protein and Pol is required for the viral polymerase, RNase, protease and integrating functions. The Env, or, envelope gene encodes a transmembrane glycoprotein that also determines the tropism of the viral particle (ie. the specificity of the virus for a particular host cell). **In the ViraSafe Ecotropic Packaging system from Cell Biolabs, Inc. (which we will be using), the Env gene encodes a glycoprotein from Murine Leukemia Virus, thus providing a viral particle that can transduce only mouse and rat cells with high efficiency**. The remaining viral genome (*ie.* cis-elements only) are used to construct different Lentiviral cloning vectors and when the cloning vectors are transfected into packaging cell lines (usually 293 cells) also expressing the gag, pol and env protein in trans, replication-deficient Lentivirus particles can be generated that are carrying the gene of interest in the viral RNA genome.

Note! Only laboratory personnel that have been informed about safety precautions and working routines, and have permission from the person in charge are allowed to enter room 7260C-1 during Lentiviral work production. <u>This also includes cleaners and service-personnel</u>.

Principle:

All procedures for handling or manipulating Lentivirus should be carried out at Biosafety Level 2 (BL2) with the use of Containment Level 3 operational practices. All work will be done in a biological safety cabinet (BSC) by authorized personnel wearing coveralls, gloves, safety glasses and shoe covers (ie. full coverage protective clothing). Personal items (eg. purses) will not be brought into the containment room. All protective clothing will be removed upon completion of the work and left in the room or disposed of as waste (shoe covers, gloves). Protective items to be re-used will be autoclaved within room 7260C-1 using a portable autoclave. Coveralls will be kept on a coat rack within the containment room. No work with these viral vectors is permitted on the open bench.

Laboratory Facility:

The Principal Investigator has designated Room 7260C-1 for periodic lentiviral work, which contains a handwashing sink, biological safety cabinet (BSC), incubator, microscope, and CO2 source. This room is an inner lab with 2 doors between the BSC and the hallway and restricted entry to the lab. A sign stating that viral vectors are present, entry is restricted to authorized personnel, and doors are to remain closed will be posted on the laboratory door.

Working precautions for handling Lentivirus:

1. All experimental materials shall be handled with care.
2. The door to the containment room shall remain locked.
3. Within the BSC:
   a. For small quantities of low (cell lysate) and high (purified) titer Lentivirus, use sterile, aerosol barrier-containing pipette tips.
   b. For larger amounts (more than 1ml) of low titer lysates use sterile serological disposable pipettes.
   c. The maximum amount of infected growth media handled at one time should never exceed 500 ml.
4. Using a dunk tank, plastics will first be either filled (eg. pipet tips and serological pipettes) or rinsed (eg. plates and flasks) with Wescodyne Solution (20% Wescodyne/40% ethanol/40% water), drained, and then put into a high-density 4mil polyethylene plastic biohazard bag lined with a cardboard box prior to autoclaving.
5. Concentration of the viral particles will be done using Amicon Ultra-15ml 100k MWCO centrifugal filter devices. All centrifugation shall be done in closed buckets with aerosol-tight lids. Loading and unloading of samples into the sealed buckets will be done in the BSC. Buckets will be sprayed with 70% ethanol before removing from BSC.
6. Sharps shall be eliminated from experimental procedures to prevent injuries. No needles or Pasteur pipettes will be used in the production and use of lentivirus.
7. Gloves shall be worn at all times when working with viral vectors. Gloves will be sprayed with 70% ethanol and then removed by using the inside-out technique before disposing into biohazard waste to be autoclaved. Wash hands immediately after removing gloves and before

leaving work area. Never wear gloves outside of the laboratory, or touch things with gloved hands.

8. During any lentiviral work, signs and labels shall be placed to indicate each area where viral vectors are used and stored (BSC, incubators, freezer, laboratory entrance doors, etc.)

Decontamination and disposal procedures:

All materials that come in contact with viral particles must be properly decontaminated prior to disposal. Note that autoclaving of all materials will first be done within room 7260C-1 using a portable autoclave. Solid waste will then be autoclaved again through the central autoclave facility at Robarts.

1. ***Disposal/decontamination of solid waste such as, paper tissues, pipette tips, etc.***: All solid waste (including disposable plastic wares) should be discarded in biohazard bags for the appropriate treatment (autoclaving) according to institutional practices and guidelines prior to disposal.
2. ***Disposal/decontamination of liquid waste***: All liquid materials (Lentivirus-containing media, buffers, washes) should be decontaminated inside safety cabinet by addition of Sporgon Solution prior to autoclaving.
3. ***Work surfaces inside cabinets*** should be decontaminated with Sporgon Solution, followed by 70% ethanol.
4. ***Instruments, equipment*** and any other items that are not disposable and contact Lentivirus will be decontaminated with Sporgon Solution and/or autoclaved.
5. ***Routine laboratory cleaning*** will be done by lab personnel within the containment room.

Accidents:

Spills:

Effective disinfectants (10% bleach, Wescodyne or Sporgon Solution) will be made available in the laboratory at all times and for immediate use. In the event of a spill or container breakage resulting in the unintentional release of a biological agent:

(i) Place bleach soaked paper towel or absorbent on the liquid
(ii) pour a strong disinfectant solution (i.e. 10% bleach) around, but not on the spill, and mix the disinfectant with the spilled material cautiously;
(iii) evacuate the laboratory for a time expected to be sufficient for decontamination of the mixed material, normally 20 minutes;
(iv) pour a strong disinfectant solution (i.e. 10% bleach) around, but not on the spill, and mix the disinfectant with the spilled material cautiously;
(v) carefully place paper into a bag for incineration;
(vi) decontaminate all surfaces exposed to the spill with the disinfectant.

If aerosols may have been created in the spill or unintentional release, evacuate the laboratory for a time sufficient for most aerosols to settle, be dispersed, or removed by the ventilation system, usually 20-30 minutes. The use of respiratory protection should be considered for re-entry. Then proceed with items (i)-(v) above. During an emergency, the first priority is the protection of the health and safety of personnel, followed by the environment (i.e. sewer drains), followed by equipment or property.

Spills within a biological safety cabinet

- Leave the ventilation on
- All items within the cabinet should be disinfected (Walls and surfaces wiped down. equipment wiped down and/or autoclaved)
- Cover the spill area with paper towels or absorbent material
- Soak the spill area with an appropriate disinfectant (i.e. 10% bleach, Wescodyne or Sporgon Solution) Pour the disinfectant from the outside surface of the absorbent material towards the inside, surrounding the spill. Leave on for 20 to 30 minutes
- Pick up with absorbent material and place in biohazard bag to be then autoclaved
- Ventilation should run 10-15 minutes before continuing work in BSC

Spills within an incubator

- All shelves and walls within the incubator should be disinfected (walls and surfaces wiped down, and/or autoclaved)
- Cover the spill area with paper towels or absorbent material
- Soak the spill area with an appropriate disinfectant (i.e. 10% bleach, Wescodyne or Sporgon Solution) Pour the disinfectant from the outside surface of the absorbent material towards the inside, surrounding the spill. Leave on for 20 to 30 minutes (close the door of the incubator during the disinfection time)
- Pick up with absorbent material and place in biohazard bag to be then autoclaved
- Finish by wiping the incubator with 70% ethanol

Inhalation:

In case of inhalation, personnel should be directed to employee health for observation and maintained under medical surveillance. Cuts and abrasions should be treated as appropriate, according to their severity. Minor cuts should be treated with the Lab first Aid Kit (disinfectant wipe and band aid), otherwise personnel should be taken to emergency room for appropriate medical evaluation and care. Written records of all incidents should be maintained.

Eye exposure from splash or aerosol:

Rinse a minimum of 15 minutes in eye wash or flush with water. Notify the Principal Investigator or Laboratory Supervisor, who will immediately contact Workplace Health at 519-661-2047 and direct the exposed employee to appropriate medical treatment and to report the incident.

Skin exposure:

Contaminated skin should be scrubbed with germicidal soap and copious amounts of water. Notify the Principal Investigator or Laboratory Supervisor, who will immediately contact Workplace Health at 519-661-2047 and direct the exposed employee to appropriate medical treatment and to report the incident.

pSMPUW Universal Lentiviral Expression Vector (Promoterless)

CATALOG NUMBER: VPK-211 **STORAGE:** -20°C

QUANTITY AND CONCENTRATION: 10 µg at 0.25 µg/µL in TE

Background

Lentivirus vector based on the human immunodeficiency virus-1 (HIV-1) has become a promising vector for gene transfer studies. The advantageous feature of lentivirus vector is the ability of gene transfer and integration into dividing and non-dividing cells. The pseudotyped envelope with vesicular stomatitis virus envelope G (VSV-G) protein broadens the target cell range. Lentiviral vectors have been shown to deliver genes to neurons, lymphocytes and macrophages, cell types that previous retrovirus vectors could not be used. Lentiviral vectors have also proven to be effective in transducing brain, liver, muscle, and retina *in vivo* without toxicity or immune responses. Recently, the lentivirus system is widely used to integrate siRNA efficiently in a wide variety of cell lines and primary cells both *in vitro* and *in vivo*.

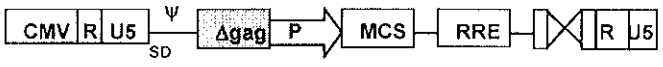
Lentivirus particles are produced from 293T cells through transient transfection of plasmids that encode for the components of the virion. Due to safety concerns regarding the infectious nature of HIV-1, recent lentiviral packaging systems have separated the viral components into 3 or 4 plasmids. However, these systems still present a small chance of generating replication-competent lentivirus upon recombination. In addition, most commercial lentiviral packaging systems provide plasmids containing the viral structure proteins in a premixed formulation, making it nearly impossible to optimize the ratio of the various plasmids for your particular experiment and host cell.

pSMPUW Universal Lentiviral Expression Vector (Promoterless) does not contain any promoter ahead of the multiple cloning sites, nor does it contain any reporter genes or antibiotic selection markers. This makes the system truly universal by allowing you to introduce your own promoter, marker or reporter that is optimal for your gene of interest or target cell. It also makes the system ideal for promoter studies. The expression vector can accommodate inserts up to 10 kb.

Related Products

1. VPK-205: ViraSafe™ Lentiviral Packaging System, Ecotropic
2. VPK-206: ViraSafe™ Lentiviral Packaging System, Pantropic
3. VPK-107: QuickTiter™ Lentivirus Titer Kit (Lentivirus-Associated HIV p24)
4. VPK-090: ViraBind™ Lentivirus Concentration and Purification Kit
5. LTV-200: ViraDuctin™ Lentivirus Transduction Kit

Unique Elements of the pSMPUW Universal Lentivirus Expression Vector

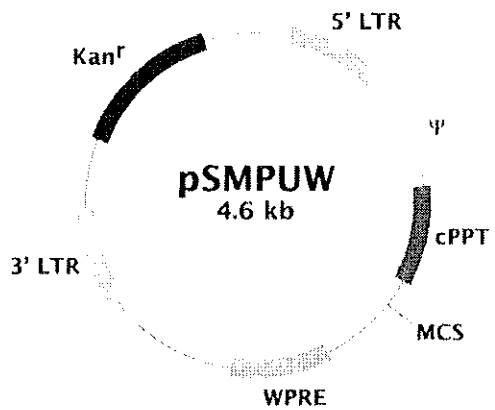
3rd Generation Lentivirus Expression Vector	pSMPUW Lentivirus Expression Vector
	

Element	Name	Benefits compared to 3rd Generation System
		ELEMENTS ADDED
cPPT	Central Polypurine Tract	• Increased gene expression levels
	Hybrid 3' LTR Poly(A)	• Increased safety: prevents read-through transcription • Increased viral titer: vector transcript more stable in packaging cells
WPRE	WPRE	• Increased viral titer
		ELEMENTS DELETED
Δgag	Gag sequence	• Increased safety: reduces sequence homology
RRE	Rev-Responsive Element	• Increased safety: reduces sequence homology

Safety Considerations

Remember that you will be working with samples containing infectious virus. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms. The ViraSafe™ Universal Lentiviral Expression System is designed to minimize the chance of generating replication-competent lentivirus, but precautions should still be taken to avoid direct contact with viral supernatants.

pSMPUW Vector



MCS: GGGGGATCCGCGGAATTCGTCGATATCAGCGTCGACAAT
BamHI EcoRI EcoRV SalI

Figure 1: pSMPUW Lentiviral Expression Vector (4632 bp, **Kanamycin**-resistant). *Note: Bacterial culture of pSMPUW vector should be done in medium containing* ***10 µg/mL*** *Kanamycin.*
EcoRI/XhoI Digestion: 1251 bp + 3381 bp

Lentivirus Production

1. One day before transfection, plate sufficient 293T cells or 293LTV cells (cat.# LTV-100) to achieve 70-80% confluence on the day of transfection.
2. Transfect cells by Calcium Phosphate or other transfection reagents.

Note: We suggest transfecting cells with FuGENE® Transfection Reagent (Roche Applied Science) or Lipofectamine™ Plus (Invitrogen). We recommend the ratio of vectors at 3:1:1:1 (pSMPUW: pCMV-VSV-G:pRSV-REV:pCgpV).

3. Harvest lentiviral supernatant 36-72 hours after transfection. Supernatant can be harvested 2 or 3 times, every 12 hours. Keep it at 4°C over the collecting period.
4. Pool the collected supernatants, centrifuge 5 minutes at 1500 rpm to remove cell debris and filtrate on 0.22 μm.
5. Supernatants can be used directly or purified/concentrated if needed. For long term storage, store supernatant at -80°C in aliquots.

Post-Packaging Considerations

Packaging your lentivirus is only the first step to ensuring successful expression of your gene. The following steps should be considered prior to infection of your host cell:

1. **Concentration and purification of your lentivirus**: Because of the latent nature of lentivirus, it is imperative that your virus be highly concentrated before infecting your host cell. Also, impurities from your viral supernatant can decrease the efficiency of infection. We recommend using Cell Biolabs' ViraBind™ Lentivirus Concentration and Purification Kit (Catalog # VPK-090). ↳add Millipore filter

2. **Measure the titer of your lentivirus**: This is an important step to ensure consistent viral transduction into your host cell. However, QPCR or stable clone counting can take as much as 1-2 weeks to perform. Traditional p24 ELISA kits can greatly overestimate your lentiviral titer. Our advanced p24 ELISA, QuickTiter™ Lentivirus Titer Kit (Catalog # VPK-107), uses exclusive technology that eliminates free p24 from your supernatant, giving you much more accurate lentiviral titers. Results are obtained in 6-18 hours.

3. **Use transduction reagents to increase infection efficiency:** Many cells are difficult to infect with lentivirus, and without supplemental reagents transduction efficiencies can be low. Reagents such as Polybrene® can help, but are often insufficient. Cell Biolabs' proprietary reagents in our ViraDuctin™ Lentivirus Transduction Kit (Catalog # LTV-200) form a super-complex with your virus to increase transduction efficiencies by promoting virus and cell interaction.

References

1. Chen, M. et al. (2002). *Nature Genetics* **32(4)**: 670-675.

2. Naldini, L., U. Blomer, P. Gallay, D. Ory, R. Mulligan, F. H. Gage, I. M. Verma, and D. Trono (1996) *Science* **272**:263-267.

3. Verma, I. M., and N. Somia (1997) *Nature* **389**:239-242

4. Kahl C. A., Marsh J., Fyffe J., Sanders D. A., and K. Cornetta (2004) *J Virol.* **78**:1421-30.

5. White S. M., Renda M., Nam N. Y., Klimatcheva E., Zhu Y., Fisk J., Halterman M., Rimel B. J., Federoff H., Pandya S., Rosenblatt J. D., and V. Planelles (1999) *J Virol.* **73**:2832-40.

6. Kafri T., van Praag H., Ouyang L., Gage F. H., and I. M. Verma (1999) *J Virol.* **73**:576-84.

Notice to Purchaser

This product is sold for research and development purposes only and is not to be incorporated into products for resale without written permission from Cell Biolabs. The patented technology is covered by a license from CHLA and University of Southern California. By the use of this product you accept the terms and conditions of all applicable Limited Use Label Licenses. You may contact our Business Development department at busdev@cellbiolabs.com for information on sublicensing this technology.

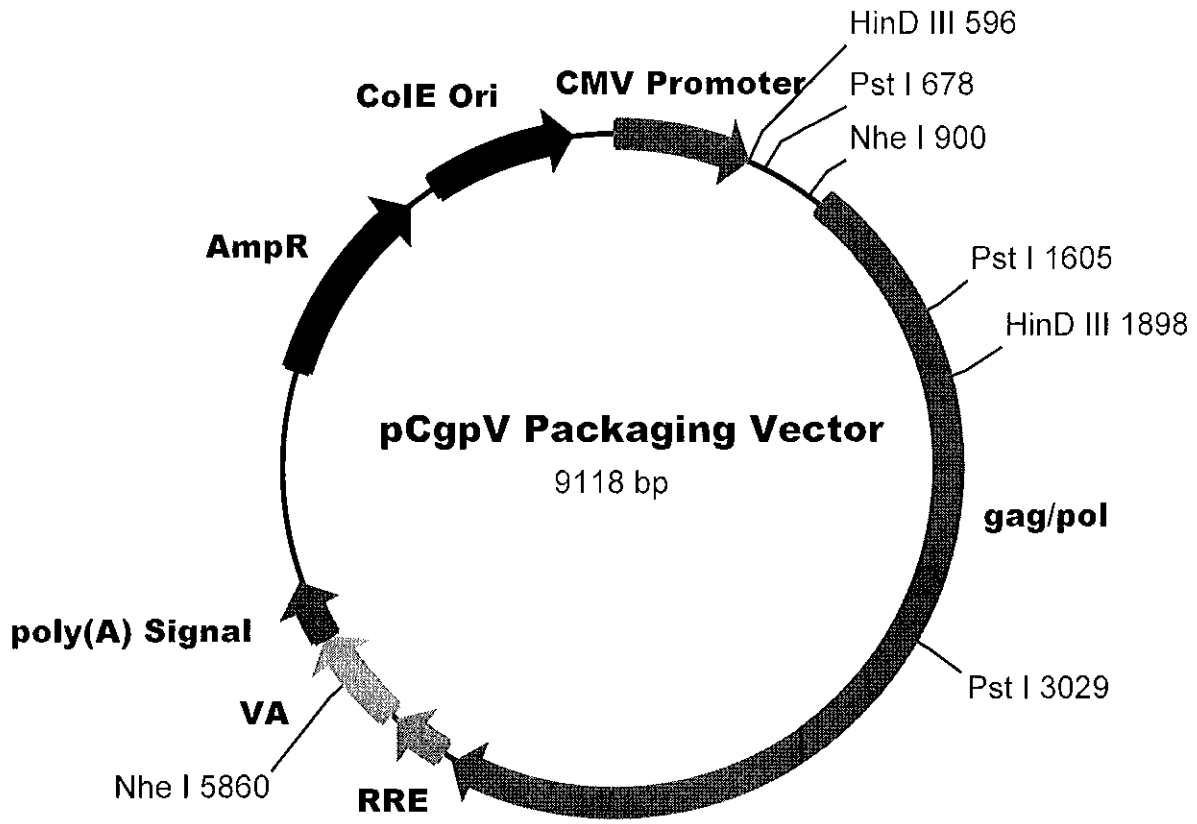
Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS 's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

This product is for RESEARCH USE ONLY; not for use in diagnostic procedures.

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com



Comments for pCgpV packaging vector

1-589bp: CMV Promoter

975-5281bp: HIV gag/pol sequence

975-2477bp: gag coding sequence
2270bp: gag/pol frameshift
2270-5281bp: pol coding sequence

5346-5578bp: HIV Rev response element (RRE)

5633-6065bp: Adenovirus VA RNA sequence

6066-6315bp: SV40 late polyadenylation signal

7273-8133bp: Amp resistance gene

8280-8919bp: ColE ori

Fragments created by PstI digest: 927bp+1424bp+6767bp=9118bp
Fragments created by NheI digest: 4158bp+4960bp=9118bp
Fragments created by Hind III digest: 1302bp+7816bp=9118bp

Browse > Stephan Kissler > Kissler et al > pLB

Print Friendly Email Add to Cart

Plasmid 11619: pLB

Gene/insert name:	None
Insert size (bp):	Unknown
Fusion proteins or tags:	GFP
Terminal:	C terminal on backbone
Vector backbone:	pLL3.7 (Search Vector Database)
Backbone manufacturer:	N/A
Type of vector:	Mammalian expression,Lentiviral,RNAi,Cre/Lox
Backbone size (bp):	8500
Cloning site 5':	HpaI
Site destroyed during cloning:	No
Cloning site 3':	XhoI
Site destroyed during cloning:	No
5' Sequencing primer:	mU6-F (List of Sequencing Primers)
Bacteria resistance:	Ampicillin
High or low copy:	High Copy
Grow in standard E. coli @ 37C:	Yes
Sequence:	View sequence
Author's Map:	View map
Plasmid Provided In:	DH5a
Principal Investigator:	Stephan Kissler
Terms and Licenses:	MTA

Comments: pLB is a modification of pLL3.7. Two genetic elements known to prevent epigenetic silencing were added. A fragment of one antirepressor element (#40) was cloned upstream of the U6 promoter and a scaffold-attached region (SAR) was cloned downstream of GFP.

Please see author's map for more detailed information.

Note: A single base pair deletion at position 11 of the U6 promoter in this plasmid does not impair the efficacy of this reagent.

Addgene has sequenced a portion of this plasmid for verification. Click here for the sequencing result.

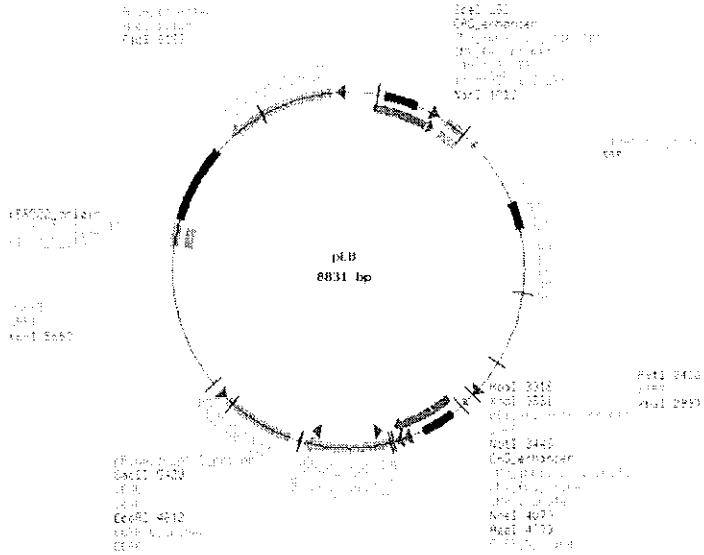
Click on map to enlarge

Plasmid Links
Author's map
Sequence
Related Plasmids
From this article
Stephan Kissler Lab Plasmids
Mammalian RNAi Tools

This is commonly requested with
pLKO.1 - TRC cloning vector
psPAX2
pLKO.1 - TRC control
pLL3.7
pMD2.G

Plasmid Cart
Your cart is empty.

Recently Viewed
pLB
Plasmid 11619



Selected features	
CAG_enhancer	318 - 605
CMV_immearly_promoter	239 - 815
CMV_fwd_primer	772 - 792
HIV-1_5_LTR	835 - 1015
truncHIV-1_3_LTR	835 - 1015
HIV-1_psi_pack	1126 - 1170
RRE	1686 - 1919
Orf frame 1	1564 - 2451
cPPT	2450 - 2465
pBluescriptKS_primer	3332 - 3348
loxP	3391 - 3424
CAG_enhancer	3544 - 3831
CMV_immearly_promoter	3489 - 4041
CMV_fwd_primer	3998 - 4018
CMV_promoter	3999 - 4068
EGFP_N_primer	4158 - 4137
EGFP	4092 - 4808

Unique restriction sites	
SpeI	252
NarI	1019
PstI	2420
XbaI	2997
HpaI	3316
XhoI	3331
NotI	3446
NheI	4070
AgeI	4079
EcoRI	4812
SacII	5423
KpnI	5650
FspI	8133

Orf frame 3	4092 - 4811
Orf frame 3	4847 - 4077
EGFP_C_primer	4745 - 4766
loxP	4831 - 4864
WPRE	4922 - 5509
Orf frame 1	5023 - 5664
pBluescriptKS_primer	5528 - 5512
cPPT	6500 - 6515
U3PPT	6500 - 6521
HIV-1_5_LTR	6837 - 7017
truncHIV-1_3_LTR	6837 - 7017
pBR322_origin	7683 - 7064
Orf frame 2	8698 - 7838
Ampicillin	8698 - 7838
AmpR_promoter	8768 - 8740

Article: In vivo RNA interference demonstrates a role for Nramp1 in modifying susceptibility to type 1 diabetes. Kissler S et al. (Nat Genet. 2006 Apr . 38(4):479-83. Pubmed)

Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication.

Also, please include the text "Addgene plasmid 11619" in your Materials and Methods section. This information allows Addgene to create a link from the plasmid page to your publication.

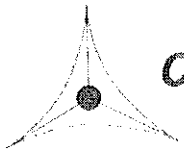
Product Manual

ViraSafe™ Lentiviral Packaging System, Ecotropic

Catalog Number

VPK-205 1 kit

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.

Introduction

Lentivirus vector based on the human immunodeficiency virus-1 (HIV-1) has become a promising vector for gene transfer studies. The advantageous feature of lentivirus vector is the ability of gene transfer and integration into dividing and non-dividing cells. Lentivirus pseudotyped with the MLV ecotropic envelope glycoprotein will only transduce mouse and rat cells with high efficiency. Lentiviral vectors have been shown to deliver genes to neurons, lymphocytes and macrophages, cell types that previous retrovirus vectors could not be used. Lentiviral vectors have also proven to be effective in transducing brain, liver, muscle, and retina *in vivo* without toxicity or immune responses. Recently, the lentivirus system is widely used to integrate siRNA efficiently in a wide variety of cell lines and primary cells both *in vitro* and *in vivo*.

Lentivirus particles are produced from 293T cells through transient transfection of plasmids that encode for the components of the virion (Figure 1). Due to safety concerns regarding the infectious nature of HIV-1, recent lentiviral packaging systems have separated the viral components into 3 or 4 plasmids. However, these systems still present a small chance of generating replication-competent lentivirus upon recombination. In addition, most commercial lentiviral packaging systems provide plasmids containing the viral structure proteins in a premixed formulation, making it nearly impossible to optimize the ratio of the various plasmids for your particular experiment and host cell.

Cell Biolabs' ViraSafe™ Lentiviral Packaging System provides a much safer method to package lentivirus, while still providing high viral titers. In addition, each plasmid is provided separately rather than in a packaging mixture. This allows you the flexibility to amplify individual plasmids and optimize the ratio of plasmids for your experiment.

Key Features of ViraSafe™ Lentiviral Packaging System:

1. Packaging Plasmids: Improve the packaging plasmid to increase performance and reduce the likelihood of recombination between vector components.
   a. Minimize HIV sequences – no accessory proteins, Tat or Rev, or LTRs
   b. Prevent overlap with vector SM by codon wobbling Gag sequences
   c. Boost particle production by incorporating adenovirus VA_I element
2. Flexible: All vectors including packaging vectors are provided separately to allow end-user to optimize the vector ratio for maximal lentivirus production.

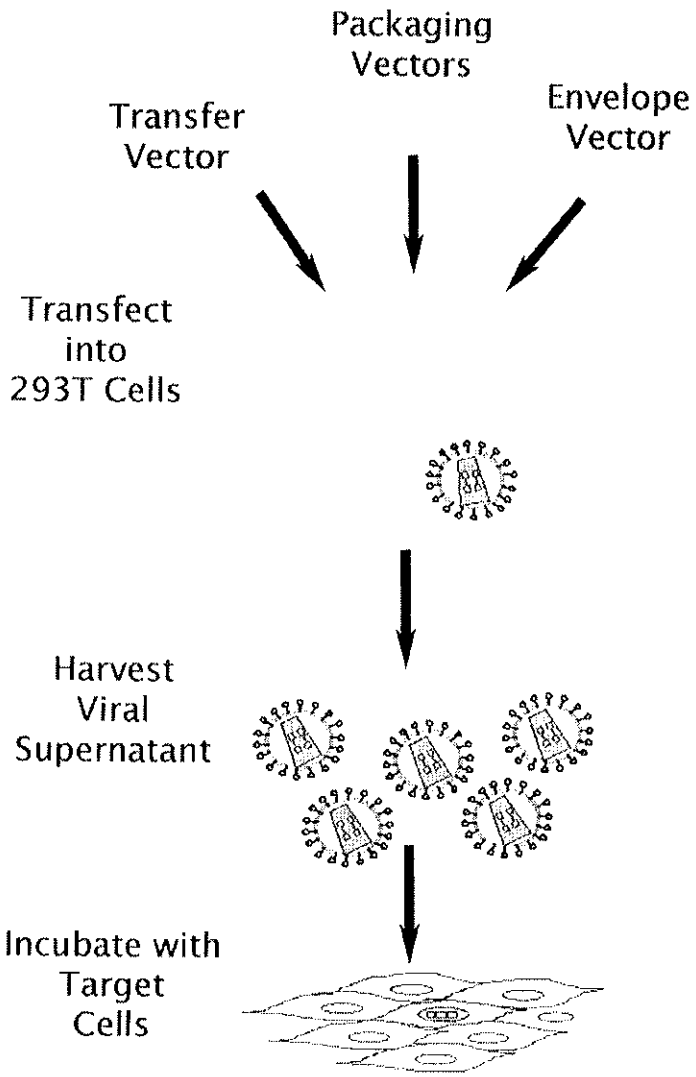
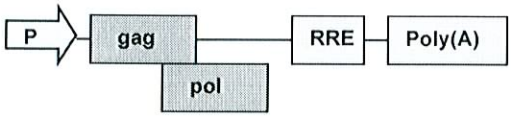
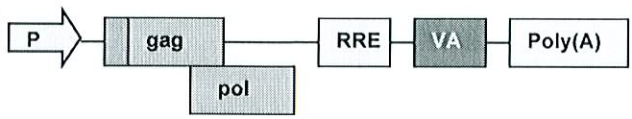
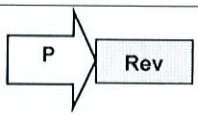
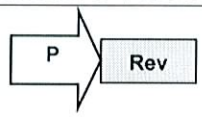
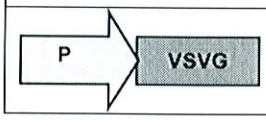
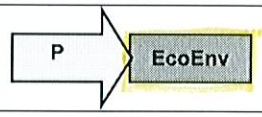


Figure 1. Lentivirus Production in 293T Cells

Related Products

1. VPK-206: ViraSafe™ Lentiviral Packaging System, Pantropic
2. VPK-107: QuickTiter™ Lentivirus Titer Kit (Lentivirus-Associated HIV p24)
3. VPK-108: QuickTiter™ Lentivirus Quantitation Kit
4. VPK-090: ViraBind™ Lentivirus Concentration and Purification Kit
5. LTV-200: ViraDuctin™ Lentivirus Transduction Kit
6. LTV-100: 293LTV Cell Line

Unique Elements of the ViraSafe™ Lentivirus Packaging System

Third Generation Lentivirus Packaging System	ViraSafe™ Lentivirus Packaging System
PACKAGING VECTOR #1	
	
PACKAGING VECTOR #2	
	
ENVELOPE VECTOR	
	

Vector Name	Element	Name	Benefits compared to 3rd Generation System
ELEMENTS ADDED			
Packaging Vector #1		Codon Wobble	• Increased safety: reduces sequence homology
		Adenovirus VA	• Increased viral titer

Kit Components

1. pRSV-Rev Packaging Vector (Part No. 320022): One 40 µL vial at 0.25 mg/mL.
2. pCMV-Eco Envelope Vector (Part No. 320026): One 40 µL vial at 0.25 mg/mL.
3. pCgpV Packaging Vector (Part No. 320024): One 40 µL vial at 0.25 mg/mL.

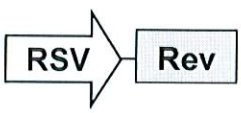


Figure 2: pRSV-Rev Packaging Vector (4180 bp, **Ampicillin**-resistant). EcoRI Digestion: 300 bp + 3880 bp

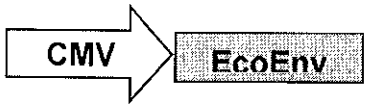


Figure 3: pCMV-Eco Envelop Vector (6763 bp, **Ampicillin**-resistant). BamHI Digestion: 777 bp + 5986 bp.

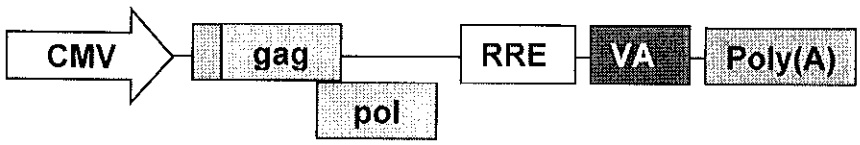


Figure 4: pCgpV Packaging Vector (9118 bp, **Ampicillin**-resistant). Pst I Digestion: 927 bp + 1424 bp + 6767 bp.

Materials Not Supplied

1. Lentiviral Transfer Vector
2. 293T cells: we recommend 293LTV Cell Line (Cat. # LTV-100) for high titer production of lentivirus.
3. Cell Culture Medium
4. Transfection Reagents

Storage

Upon receipt, store all other kit components at -20°C until their expiration dates.

Safety Considerations

Remember that you will be working with samples containing infectious virus. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms. The ViraSafe™ Universal Lentiviral Expression System is designed to minimize the chance of generating replication-competent lentivirus, but precautions should still be taken to avoid direct contact with viral supernatants.

Lentivirus Production

1. One day before transfection, plate sufficient 293T cells or 293LTV cells (cat.# LTV-100) to achieve 70-80% confluence on the day of transfection.
2. Transfect cells by Calcium Phosphate or other transfection reagents.

Note: We suggest transfecting cells with FuGENE® Transfection Reagent (Roche Applied Science) or Lipofectamine™ Plus (Invitrogen). We recommend the ratio of vectors at 3:1:1:1 (transfer vector: pCMV-Eco:pRSV-REV:pCgpV).

3. Harvest lentiviral supernatant 36-72 hours after transfection. Supernatant can be harvested 2 or 3 times, every 12 hours. Keep it at 4°C over the collecting period.
4. Pool the collected supernatants, centrifuge 5 minutes at 1500 rpm to remove cell debris and filtrate on 0.22 µm.
5. Supernatants can be used directly or purified/concentrated if needed. For long term storage, store supernatant at -80°C in aliquots.

Post-Packaging Considerations

Packaging your lentivirus is only the first step to ensuring successful expression of your gene. The following steps should be considered prior to infection of your host cell:

1. **Concentration and purification of your lentivirus**: Because of the latent nature of lentivirus, it is imperative that your virus be highly concentrated before infecting your host cell. Also, impurities from your viral supernatant can decrease the efficiency of infection. We recommend using Cell Biolabs' ViraBind™ Lentivirus Concentration and Purification Kit (Catalog # VPK-090).

2. **Measure the titer of your lentivirus**: This is an important step to ensure consistent viral transduction into your host cell. However, QPCR or stable clone counting can take as much as 1-2 weeks to perform. Traditional p24 ELISA kits can greatly overestimate your lentiviral titer. Our advanced p24 ELISA, QuickTiter™ Lentivirus Titer Kit (Catalog # VPK-107), uses exclusive technology that eliminates free p24 from your supernatant, giving you much more accurate lentiviral titers. Results are obtained in 6-18 hours.

3. **Use transduction reagents to increase infection efficiency:** Many cells are difficult to infect with lentivirus, and without supplemental reagents transduction efficiencies can be low. Reagents such as Polybrene® can help, but are often insufficient. Cell Biolabs' proprietary reagents in our ViraDuctin™ Lentivirus Transduction Kit (Catalog # LTV-200) form a super-complex with your virus to increase transduction efficiencies by promoting virus and cell interaction.

References

1. Chen, M. et al. (2002). *Nature Genetics* **32(4)**: 670-675.
2. Naldini, L., U. Blomer, P. Gallay, D. Ory, R. Mulligan, F. H. Gage, I. M. Verma, and D. Trono (1996) *Science* **272**:263-267.
3. Verma, I. M., and N. Somia (1997) *Nature* **389**:239-242
4. Kahl C. A., Marsh J., Fyffe J., Sanders D. A., and K. Cornetta (2004) *J Virol.* **78**:1421-30.

5. White S. M., Renda M., Nam N. Y., Klimatcheva E., Zhu Y., Fisk J., Halterman M., Rimel B. J., Federoff H., Pandya S., Rosenblatt J. D., and V. Planelles (1999) *J Virol.* **73**:2832-40.
6. Kafri T., van Praag H., Ouyang L., Gage F. H., and I. M. Verma (1999) *J Virol.* **73**:576-84.

Notice to Purchaser

This product is sold for research and development purposes only and is not to be incorporated into products for resale without written permission from Cell Biolabs. The patented technology is covered by a license from University of Southern California. By the use of this product you accept the terms and conditions of all applicable Limited Use Label Licenses. You may contact our Business Development department at busdev@cellbiolabs.com for information on sublicensing this technology.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

Subject: Fw: Lentivirus use
From: Ron Noseworthy <rnoseworthy@robarts.ca>
Date: Tue, 06 Apr 2010 09:33:02 -0400
To: jstanle2@uwo.ca

FYI, Ron

----- Original Message -----
From: Mike Jackson <mjackson@robarts.ca>
To: Ron Noseworthy
Sent: Tue Apr 06 09:28:49 2010
Subject: Lentivirus use

Hi Ron,

Just wished to clarify that we will not be using lentivirus for any live animal work. I had forgotten to add a statement to that effect in our biohazard modification form.

Thanks, Mike