GFP-LC3 Lentiviral Expression Vector

CATALOG NUMBER: LTV-801 **STORAGE:** -20ºC

Components

1. pSMPUW-GFP-Puro Control Vector (Part No. LTV-401): One tube, 10 µg at 0.25 µg/µL in TE
2. pSMPUW-GFP-LC3-Puro Expression Vector (Part No. 380102): One tube, 10 µg at 0.25 µg/µL in TE

Background

Autophagy is a lysosomal degradation pathway for cytoplasmic material, which is activated during stress conditions such as amino acid starvation or viral infection. Mammalian cells use autophagy during short periods of starvation to degrade nonessential cellular components in order to liberate nutrients for vital biosynthetic reactions. Recent results have shown that autophagy also contributes to development, growth regulation and cancer, as well as longevity.

After induction by a stress signal such as amino acid starvation, the first step in autophagy is the formation of an autophagosome. A well published autophagosome marker protein, MAP LC3, was originally identified as a microtubule associated protein and named 'microtubule-associated- protein-light-chain-3'. LC3 is a small 16-18 kDa protein that is soluble in nonstarved cells, but becomes peripherally membrane-associated during amino acid starvation. By immunoelectron microscopy, LC3 has been shown to associate to the inner and outer limiting membranes of autophagosomes, and the membrane association is mediated by a covalent conjugation to a lipid, phosphatidylethanolamine. In Western blots, two forms of LC3 are seen, LC3I and LC3II. LC3I is found in the soluble fraction, and LC3II in the pelletable membrane fraction. Both LC3I and LC3II are seen in nonstarved cells, but during autophagy induction the proportion of LC3II increases. GFP-tagged LC3 expression might be useful as an autophagy assay.

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*.

pSMPUW-GFP-LC3 is a lentiviral expression vector in which human LC-3B gene is fused in frame with GFP and the GFP-LC3 insert is cloned into pSMPUW-Puro vector (Cat.# VPK-212). A pSMPUW-GFP-Puro vector without LC3 gene is also provided as a control (Figure 1). Lentiviral supernatant can be produced by cotransfecting 293T cells (Cat.# LTV-100) with pSMPUW expression vector and a lentivirus packaging mix such as Cell Biolabs' ViraSafe™ Lentiviral Packaging System (Cat. # VPK-206). Supernatants can be used directly or purified/concentrated if needed. For long term storage, store supernatant at -80ºC in aliquots. The resulting control virus can also be used to generate GFP-LC3 and

puromycin stable cell lines, and stable clones can be selected by either green fluorescence sorting or puromycin resistance.

Related Products

1. CBA-401: pCMV-GFP-LC3 Expression Vector
2. RTV-801: pMXs- GFP-LC3 Retroviral Expression Vector
3. VPK-205: ViraSafe™ Lentiviral Packaging System, Ecotropic
4. VPK-206: ViraSafe™ Lentiviral Packaging System, Pantropic
5. VPK-107: QuickTiter™ Lentivirus Titer Kit (Lentivirus-Associated HIV p24)
6. VPK-090: ViraBind™ Lentivirus Concentration and Purification Kit
7. LTV-200: ViraDuctin™ Lentivirus Transduction Kit

pSMPUW-GFP-LC3-Puro Vector

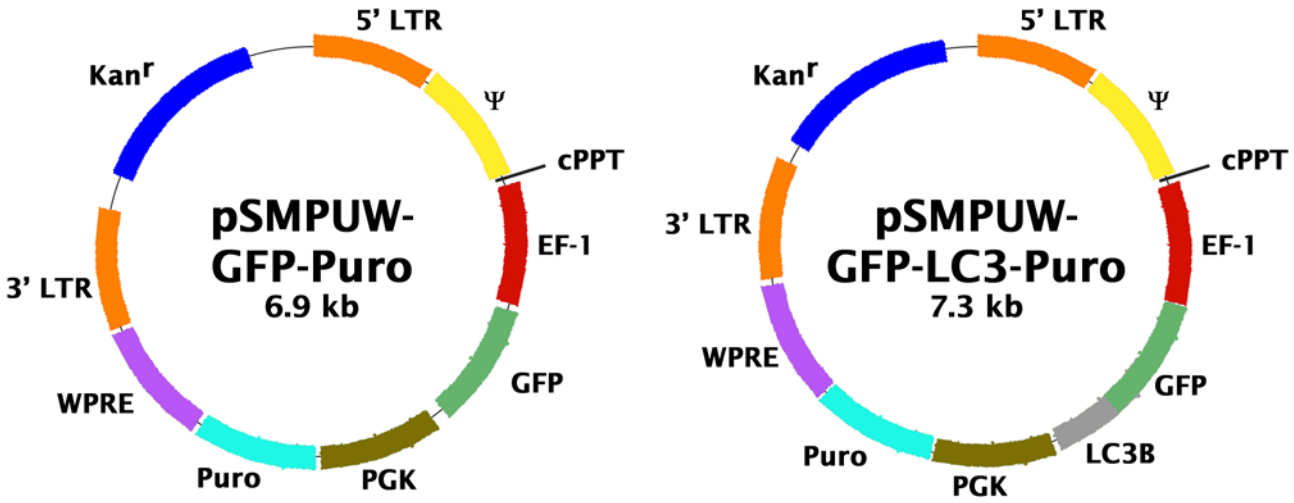

Figure 1. Schematic representation of GFP-LC3 Lentivirus Expression Vector. **Left:** pSMPUW-GFP-Puro Lentiviral Control Vector (6841 bp, **Kanamycin**-resistant). HindIII Digestion: 1331 bp + 1982 bp + 3528 bp. **Right:** pSMPUW-GFP-LC3-Puro Lentiviral Expression Vector (7249, **Kanamycin**-resistant). XhoI Digestion: 2381 bp + 4868 bp. *Note: Bacterial culture of pSMPUW vectors should be done in medium containing **10 µg/mL** Kanamycin. For maximal plasmid yield, we recommend Terrific Broth (TB) growth medium or Invitrogen's fast growing Mach1 competent cells.*

GFP-LC-3B Sequence in pSMPUW-GFP-LC3-Puro vector:

```
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 G  D  V  N  G  H  K  F  S  V  S  G  E  G  E  G  D  A  T  Y
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 Q  H  D  F  F  K  S  A  M  P  E  G  Y  V  Q  E  R  T  I  F
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CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Unique Elements of the ViraSafe™ Lentivirus Packaging System (sold separately)

Third Generation Lentivirus Packaging System	ViraSafe™ Lentivirus Packaging System
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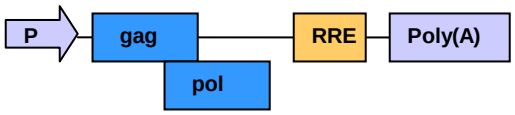	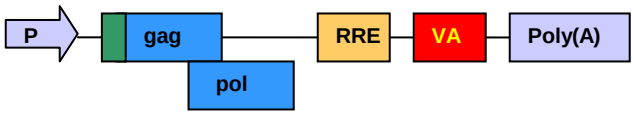
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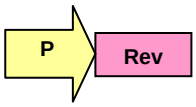	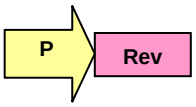
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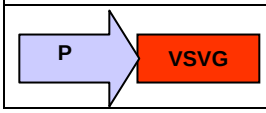	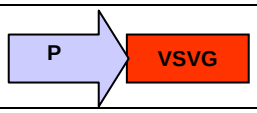

Vector Name	Element	Name	Benefits compared to 3rd Generation System
ELEMENTS ADDED			
Packaging Vector #1		Codon Wobble	• Increased safety: reduces sequence homology
		Adenovirus VA	• Increased viral titer

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 (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 4ºC 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 -80ºC 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

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