GFP-LC3 Retroviral Expression Vector

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

Components

1. pMX-GFP Control Vector (Part No. RTV-050): One tube, 10 µg at 0.25 µg/µL in TE
2. pMXs-GFP-LC3 Expression Vector (Part No. 380101): 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.

Retroviruses are efficient tools for delivering heritable genes into the genome of dividing cells. Cell Biolabs' pMXs retroviral vector is based on Moloney murine leukemia virus (MMLV). Transfection into a package cell line produces high-titer, replication-incompetent viruses. pMXs-GFP-LC3 is a retroviral expression vector in which human LC-3B gene is fused in frame with GFP and the GFP-LC3 insert is cloned between BamHI and NotI of pMXs vector (Cat.# RTV-010). A pMX-GFP vector without LC3 gene is also provided as a control. The vectors contain the ampicillin-resistance gene, MMLV LTRs, package signal (Figure 1).

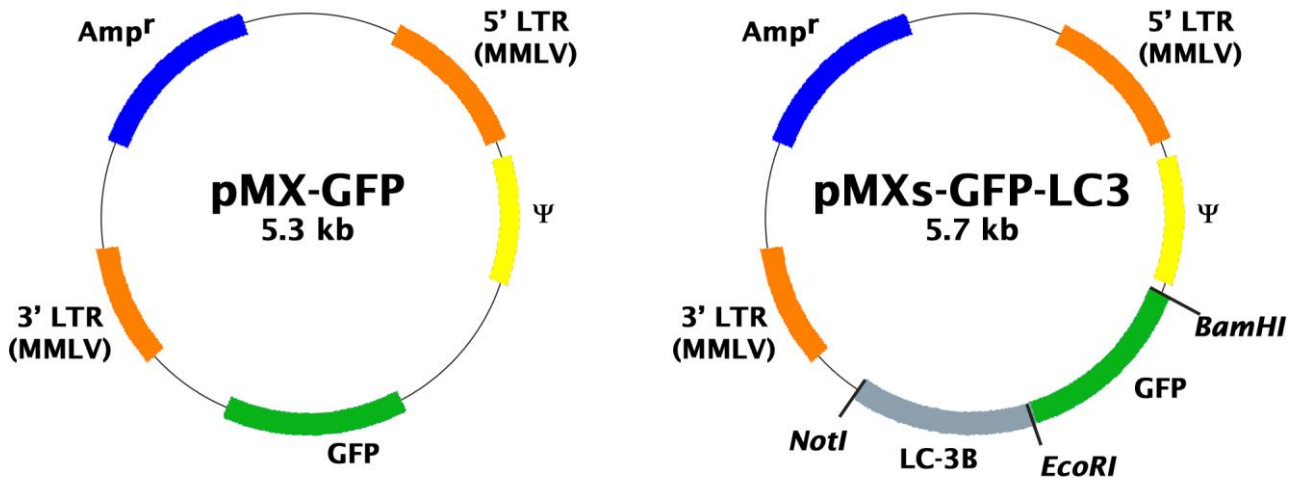

Figure 1. pMX-GFP control vector and pMXs-GFP-LC3 expression vector maps

GFP-LC-3B Sequence in pMXs-GFP-LC3 vector:

```
atggtgagcaagggcgaggagctgttcaccggggtggtgcccatcctggtcgagctggac
 M  V  S  K  G  E  E  L  F  T  G  V  V  P  I  L  V  E  L  D
ggcgacgtaaacggccacaagttcagcgtgtccggcgagggcgagggcgatgccacctac
 G  D  V  N  G  H  K  F  S  V  S  G  E  G  E  G  D  A  T  Y
ggcaagctgaccctgaagttcatctgcaccaccggcaagctgcccgtgccctggcccacc
 G  K  L  T  L  K  F  I  C  T  T  G  K  L  P  V  P  W  P  T
ctcgtgaccaccctgacctacggcgtgcagtgcttcagccgctaccccgaccacatgaag
 L  V  T  T  L  T  Y  G  V  Q  C  F  S  R  Y  P  D  H  M  K
cagcacgacttcttcaagtccgccatgcccgaaggctacgtccaggagcgcaccatcttc
 Q  H  D  F  F  K  S  A  M  P  E  G  Y  V  Q  E  R  T  I  F
ttcaaggacgacggcaactacaagacccgcgccgaggtgaagttcgagggcgacaccctg
 F  K  D  D  G  N  Y  K  T  R  A  E  V  K  F  E  G  D  T  L
gtgaaccgcatcgagctgaagggcatcgacttcaaggaggacggcaacatcctggggcac
 V  N  R  I  E  L  K  G  I  D  F  K  E  D  G  N  I  L  G  H
aagctggagtacaactacaacagccacaacgtctatatcatggccgacaagcagaagaac
 K  L  E  Y  N  Y  N  S  H  N  V  Y  I  M  A  D  K  Q  K  N
ggcatcaaggtgaacttcaagatccgccacaacatcgaggacggcagcgtgcagctcgcc
 G  I  K  V  N  F  K  I  R  H  N  I  E  D  G  S  V  Q  L  A
gaccactaccagcagaacaccccatcggcgacggccccgtgctgctgcccgacaaccac
 D  H  Y  Q  Q  N  T  P  I  G  D  G  P  V  L  L  P  D  N  H
tacctgagcacccagtccgccctgagcaaagaccccaacgagaagcgcgatcacatggtc
 Y  L  S  T  Q  S  A  L  S  K  D  P  N  E  K  R  D  H  M  V
ctgctggagttcgtgaccgccgccgggatcactctcggcatggacgagctgtacaagtac
 L  L  E  F  V  T  A  A  G  I  T  L  G  M  D  E  L  Y  K  Y
tcagatctcgagctcaagcttcgaattcccatgccgtcggagaagaccttcaagcagcgc
 S  D  L  E  L  K  L  R  I  P  M  P  S  E  K  T  F  K  Q  R
cgcaccttcgaacaaagagtagaagatgtccgacttattcgagagcagcatccaaccaaa
 R  T  F  E  Q  R  V  E  D  V  R  L  I  R  E  Q  H  P  T  K
atcccggtgataatagaacgatacaagggtgagaagcagcttcctgttctggataaaaca
 I  P  V  I  I  E  R  Y  K  G  E  K  Q  L  P  V  L  D  K  T
```

```
aagttccttgtacctgaccatgtcaacatgagtgagctcatcaagataattagaaggcgc
 K  F  L  V  P  D  H  V  N  M  S  E  L  I  K  I  I  R  R  R
ttacagctcaatgctaatcaggccttcttcctgttggtgaacggacacagcatggtcagc
 L  Q  L  N  A  N  Q  A  F  F  L  L  V  N  G  H  S  M  V  S
gtctccacaccaatctcagaggtgtatgagagtgagaaagatgaagatggattcctgtac
 V  S  T  P  I  S  E  V  Y  E  S  E  K  D  E  D  G  F  L  Y
atggtctatgcctcccaggagacgttcgggatgaaattgtcagtgtaa
 M  V  Y  A  S  Q  E  T  F  G  M  K  L  S  V  -
```

References

1. Kabeya, Y., Mizushima, N., Ueno, T., Yamamoto, A., Kirisako, T., Noda, T., Kominami, E., Ohsumi, Y., and Yoshimori, T. (2000) *EMBO J.* **19,** 5720-5728.
2. Mizushima, N., Yamamoto, A., Matsui, M., Yoshimori, T., and Ohsumi, Y. (2004) *Mol. Biol. Cell* **15,** 1101-1111.
3. Kabeya, Y., Mizushima, N., Ueno, T., Yamamoto, A., Kirisako, T., Noda, T., Kominami, E., Ohsumi, Y., and Yoshimori, T. (2000) *EMBO J.* **19,** 5720-5728.
4. Mizushima, N., Yamamoto, A., Matsui, M., Yoshimori, T., and Ohsumi, Y. (2004) *Mol. Biol. Cell* **15,** 1101-1111.

License Information

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