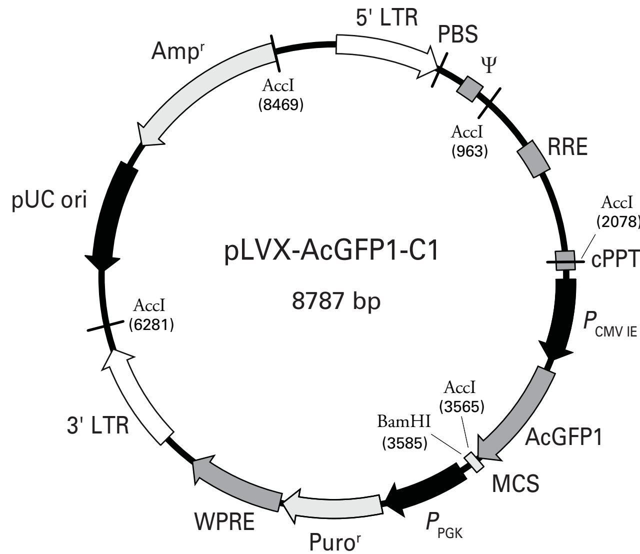
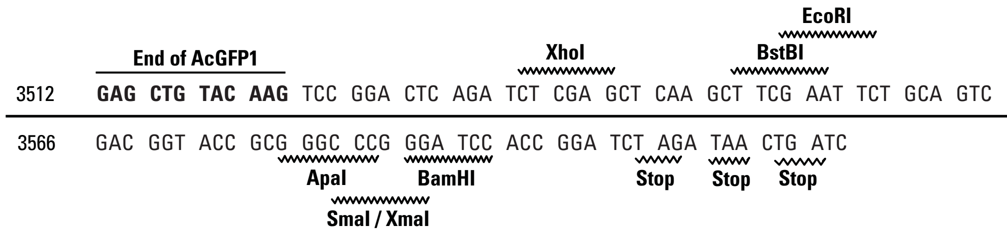
pLVX-AcGFP1-C1 Vector Information

PT4001-5

Catalog Nos. 632155





pLVX-AcGFP1-C1 Vector Map and Multiple Cloning Site (MCS).

Description

pLVX-AcGFP1-C1 is an HIV-1-based, lentiviral expression vector that allows you to express your gene of interest fused to AcGFP1, a green fluorescent protein derived from *Aequorea coerulescens*. Genes cloned into the multiple cloning site (MCS), located at the C-terminal end of the AcGFP1 coding sequence, are expressed as C-terminal fusions of the AcGFP1 protein. Expression of the fusion protein is driven by the constitutively active human cytomegalovirus immediate early promoter ($P_{CMV\ IE}$) located just upstream of the AcGFP1 coding sequence. Lentiviral particles derived from the vector allow the expression of AcGFP1 fusion proteins in virtually any cell type, including primary cells. The unmodified vector expresses AcGFP1, and may be used to produce marker virus to optimize infection protocols.

pLVX-AcGFP1-C1 contains all of the viral processing elements necessary for the production of replication-incompetent lentivirus, as well as elements to improve viral titer, transgene expression, and overall vector function. The woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) promotes RNA processing events and enhances nuclear export of viral and transgene RNA (1), leading to increased viral titers from packaging cells, and enhanced expression of your gene of interest in target cells. In addition, the vector includes a Rev-response element (RRE), which further increases viral titers by enhancing the transport of unspliced viral RNA out of the nucleus (2). Finally, pLVX-AcGFP1-C1 also contains a central polypurine tract (cPPT) element that increases nuclear importation of the viral genome during target cell infection, resulting in improved vector integration and more efficient transduction (3).

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In addition to lentiviral elements, pLVX-AcGFP1-C1 contains a puromycin resistance gene ($Puro^r$) under the control of the murine phosphoglycerate kinase (PGK) promoter (P_{PGK}) for the selection of stable transductants. The vector also contains a pUC origin of replication and an *E. coli* ampicillin resistance gene (Amp^r) for propagation and selection in bacteria.

Use

pLVX-AcGFP1-C1 constitutively expresses your gene of interest from $P_{CMV\ IE}$ when transduced into target cells. Before the vector can be transduced into cells, however, it must be transfected into 293T packaging cells with our Lenti-X™ HT Packaging System (Cat. Nos. 632160 and 632161). This packaging system allows you to safely produce high titer, infectious, replication-incompetent, VSV-G pseudotyped lentiviral particles that can infect a wide range of cell types, including non-dividing and primary cells (4).

Location of Features

- 5' LTR: 1–635
- PBS (primer binding site): 636–653
- Ψ (packaging signal): 685–822
- RRE (Rev-response element): 1303–1536
- cPPT(central polypurine tract): 2028–2151
- P_{CMV} (human cytomegalovirus immediate early promoter): 2185–2787
- AcGFP1 (*Aequorea coerulescens* green fluorescent protein): 2807–3604
- MCS (multiple cloning site): 3537–3591
- P_{PGK} (phosphoglycerate kinase promoter): 3613–4121
- $Puro^r$ (puromycin resistance gene): 4142–4741
- WPRE (woodchuck posttranscriptional regulatory element): 4755–5346
- 3' LTR: 5549–6185
- pUC origin of replication: 6655–7328 (complementary)
- Amp^r (ampicillin resistance gene; β-lactamase): 7473–8469 (complementary)

Selection of Stable Transfectants

- Selectable marker: plasmid confers resistance to puromycin.

Propagation in *E. coli*

- Suitable host strains: DH5α, DH10B and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high

Notes:

The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech. This vector has not been completely sequence.

The viral supernatants produced by this lentiviral vector could contain potentially hazardous recombinant virus. Due caution must be exercised in the production and handling of recombinant lentivirus. Appropriate NIH, regional, and institutional guidelines apply.

References

1. Zufferey, R. *et al.* (1999) *J. Virol.* **73**(4):2886–2892.
2. Cochrane, A. W. *et al.* (1990) *Proc. Natl. Acad. Sci. USA* **87**(3):1198–1202.
3. Zennou, V. *et al.* (2000) *Cell* **101**(2):173–185.
4. Wu, X. *et al.* (2000) *Mol. Ther.* **2**(1):47–55. 8. Pear, W.S. *et al.* (1993) *Proc. Natl. Acad. Sci. USA* **90**(18):8392-8396.

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