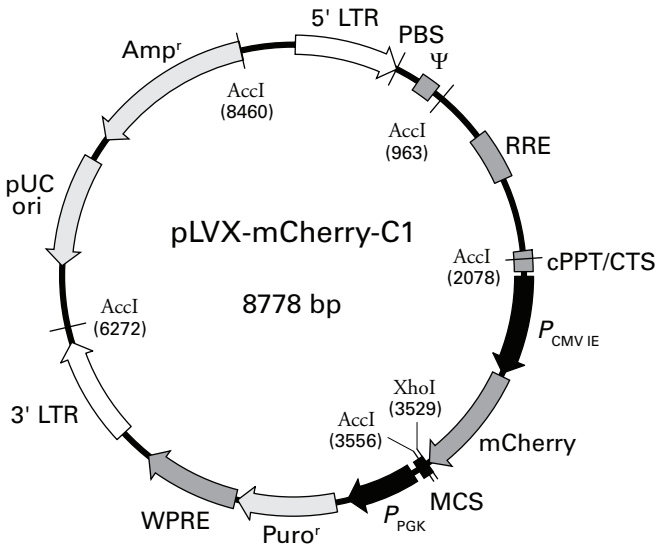
Vector Information

pLVX-mCherry-C1 Vector Information PT5091-5

Catalog No. 632561



End of mCherry — XhoI — BstBI — EcoRI

3506 CTG TAC AAG TCC GGA CTC AGA TCT CGA GCT CAA GCT TCG AAT TCT

ApaI — BamHI — SmaI/XmaI — Stop — Stop

3551 GCA GTC GAC GGT ACC GCG GGC CCG GGA TCC ACC GGA TCT AGA TAA

Stop

3596 CTG ATC

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

Description

pLVX-mCherry-C1 is an HIV-1-based, lentiviral expression vector that allows you to express your gene of interest fused to mCherry, a mutant fluorescent protein derived from the tetrameric *Discosoma sp.* red fluorescent protein, DsRed (1). The excitation and emission maxima of the native mCherry protein are 587 nm and 610 nm, respectively. Genes cloned into the multiple cloning site (MCS), located at the C-terminal end of the mCherry coding sequence, are expressed as C-terminal mCherry fusion proteins. Expression of the fusion protein is driven by the constitutively active human cytomegalovirus immediate early promoter ($P_{CMV\ IE}$), located just upstream of the mCherry coding sequence. Lentiviral particles derived from the vector allow the expression of mCherry fusion proteins in virtually any cell type, including primary cells. The unmodified vector expresses mCherry, and may be used to produce marker virus to optimize infection protocols.

pLVX-mCherry-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 (2), 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 (3). Finally, pLVX-mCherry-C1 also contains a central polypurine tract/central termination sequence element (cPPT/CTS). During target cell infection, this element creates a central DNA flap that increases nuclear import of the viral genome, resulting in improved vector integration and more efficient transduction (4).

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In addition to lentiviral elements, pLVX-mCherry-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

To construct a fusion protein, the gene of interest must be cloned into pLVX-mCherry-C1 so that it is in-frame with the mCherry coding sequence. The inserted sequence does not require an initiation codon (ATG) or a stop codon (TAA, TAG, TGA); however, if you don't want to use the stop codons downstream of the MCS (see map), you can add a stop codon to the end of your gene of interest.

The fusion protein is constitutively expressed when pLVX-mCherry-C1 is transduced into target cells. Before the vector can be transduced, 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 (5).

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/CTS (central polypurine tract/central termination sequence): 2028–2151
- $P_{CMV\ IE}$ (human cytomegalovirus immediate early promoter): 2185–2787
- mCherry fluorescent protein gene: 2807–3514
- MCS (multiple cloning site): 3528–3580
- P_{PGK} (phosphoglycerate kinase promoter): 3604–4112
- Puro^r (puromycin resistance gene): 4133–4732
- WPRE (woodchuck posttranscriptional regulatory element): 4746–5337
- 3' LTR: 5540–6176
- pUC origin of replication: 6646–7319 (complementary)
- Amp^r (ampicillin resistance gene; β-lactamase): 7464–8460 (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: ColE1
- Copy number: high

Excitation and emission maxima of mCherry

- Excitation maximum = 587 nm
- Emission maximum = 610 nm

Notes:

The vector sequence was 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 sequenced.

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. Shaner, N.C. *et al.* (2004) *Nat. Biotechnol.* **22**(12):1567–1572.
2. Zufferey, R. *et al.* (1999) *J. Virol.* **73**(4):2886–2892.
3. Cochrane, A. W. *et al.* (1990) *Proc. Natl. Acad. Sci. USA* **87**(3):1198–1202.
4. Zennou, V. *et al.* (2000) *Cell* **101**(2):173–185.
5. Wu, X. *et al.* (2000) *Mol. Ther.* **2**(1):47–55.

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