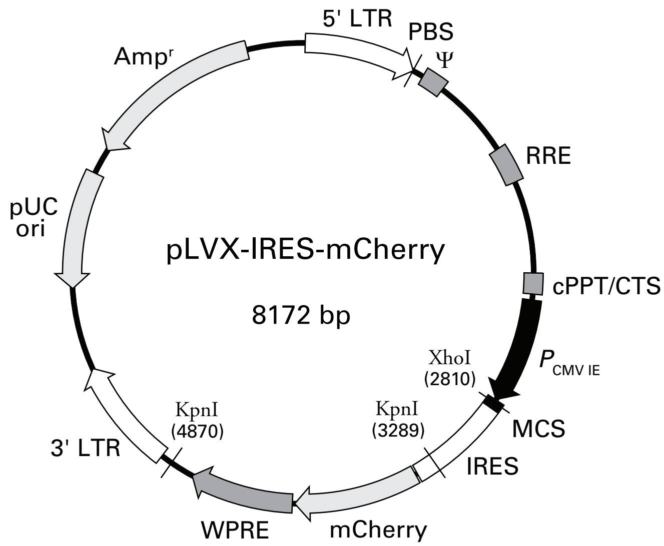
Vector Information

Catalog No. 631237

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                     XhoI                         NotI
           EcoRI                 SpeI     XbaI               BamHI
2801   GTGAATTCCT CGAGACTAGT TCTAGAGCGG CCGCGGATCC
       CACTTAAGGA GCTCTGATCA AGATCTCGCC GGCGCCTAGG
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pLVX-IRES-mCherry Vector Map and Multiple Cloning Site (MCS).

Description

pLVX-IRES-mCherry is an HIV-1-based, lentiviral expression vector that allows the simultaneous expression of your protein of interest and mCherry in virtually any mammalian cell type, including primary cells. mCherry is a mutant fluorescent protein derived from the tetrameric *Discosoma sp.* red fluorescent protein, DsRed (1). The vector expresses the two proteins from a bicistronic mRNA transcript, allowing mCherry to be used as an indicator of transduction efficiency and a marker for selection by flow cytometry.

Expression of the bicistronic transcript is driven by the constitutively active human cytomegalovirus immediate early promoter ($P_{CMV\ IE}$) located just upstream of the MCS. An encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES), positioned between the MCS and mCherry, facilitates cap-independent translation of mCherry from an internal start site at the IRES/mCherry junction (1).

pLVX-IRES-mCherry 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 RNA (2), leading to increased viral titers from packaging 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-IRES-mCherry 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). The vector also contains a pUC origin of replication and an *E. coli* ampicillin resistance gene (Amp^r) for propagation and selection in bacteria.

(PR973291; published 31 July 2009)

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Use

pLVX-IRES-mCherry is designed to constitutively coexpress your protein of interest and mCherry from $P_{CMV\ IE}$ when transduced into mammalian cells. Before it can be transduced into target cells, the vector must be packaged into viral particles in HEK293T cells, using our Lenti-X™ HT Packaging System (Cat. Nos. 632160 and 632161). This packaging system allows the safe production of high titer, infectious, replication-incompetent, VSV-G pseudotyped lentiviral particles that can infect a wide range of cell types, including nondividing and primary cells (5).

The presence of mCherry allows transductants to be visualized by fluorescence microscopy and sorted by flow cytometry with standard FITC filter sets (mCherry has an excitation maximum of 587 nm and an emission maximum of 610 nm).

Location of Features

- 5' LTR (5' long terminal repeat): 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
- MCS (multiple cloning site): 2803–2840
- IRES (encephalomyocarditis virus internal ribosome entry site): 2842–3416
- mCherry: 3417–4127
- WPRE (woodchuck hepatitis virus posttranscriptional regulatory element): 4141–4732
- 3' LTR (3' long terminal repeat): 4935–5571
- pUC origin of replication: 6040–6713 (complementary)
- Amp^r (ampicillin resistance gene; β-lactamase): 6858–7854 (complementary)

Selection of Transductants

- Marker: mCherry

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

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) *Nature Biotech.* **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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