Certificate of Analysis

pLVX-EF1α-mCherry-C1 Vector

Catalog No.	Amount	Lot Number
631985	10 µg	Specified on product label.

Product Information

pLVX-EF1α-mCherry-C1 is a lentiviral expression vector that can be used to generate high-titer lentivirus for transducing virtually any dividing or nondividing mammalian cell type, including primary and stem cells. The vector allows a gene-of-interest to be fused to the C-terminus of the red fluorescent protein mCherry. Expression of the fusion is driven by the human elongation factor 1 alpha (EF1α) promoter, which continues to be constitutively active even after stable integration of the vector into the host cell genome. Stable expression of the fusion allows the monitoring of a variety of cellular processes (such as differentiation in primary or stem cells) without the transgene silencing associated with CMV promoters. In addition, the vector allows efficient flow cytometric detection of stably or transiently transfected mammalian cells expressing mCherry fusions, without time-consuming drug and clonal selection.

Package Contents

- 1 tube of pLVX-EF1α-mCherry-C1 Vector (20 µl/tube)

Storage Conditions

- Store plasmids at –20°C.
- Spin briefly to recover contents.
- Avoid repeated freeze/thaw cycles.

Shelf Life

- 1 year from date of receipt under proper storage conditions.

Storage Buffer

- 10 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0)

Concentration

- 500 ng/µl

Shipping Conditions

- Dry ice (–70°C)

Product Documents

Documents for our products are available for download at **takarabio.com/manuals**
The following documents apply to this product:

- Lenti-X Lentiviral Expression Systems User Manual
- pLVX-EF1alpha-mCherry-C1 Vector Information

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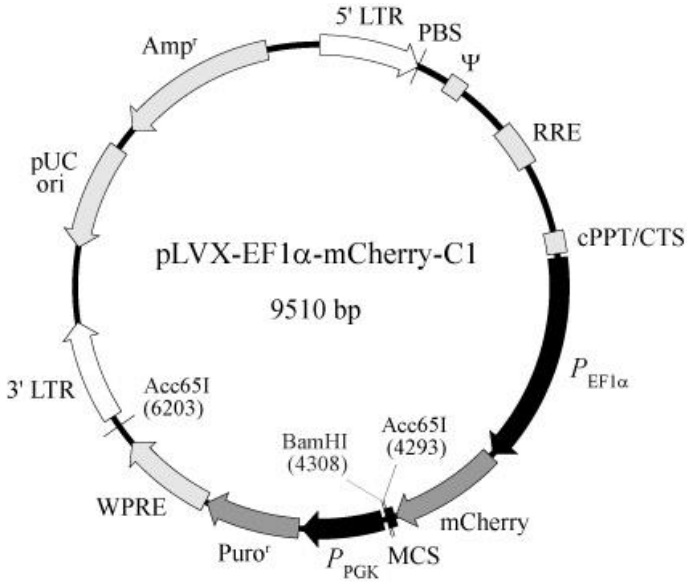

Figure 1. pLVX-EF1α-mCherry-C1 vector map.

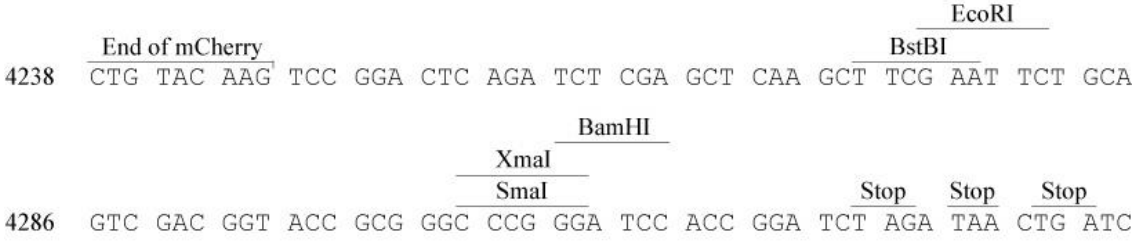

Figure 2. pLVX-EF1α-mCherry-C1 multiple cloning site (MCS).

Description

pLVX-EF1α-mCherry-C1 is an HIV-1-based, lentiviral expression vector designed to constitutively express a protein of interest fused to the C-terminus of mCherry, a mutant fluorescent protein derived from the tetrameric *Discosoma* sp. red fluorescent protein, DsRed (Nat. Biotechnol., 2004). The excitation and emission maxima of native mCherry are 587 nm and 610 nm, respectively. Stable, constitutive expression of the fusion protein is driven by the EF1α promoter ($P_{EF1\alpha}$), which continues to be constitutively active even after the vector integrates into the host cell genome (Stem Cells Dev., 2008).

pLVX-EF1α-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 RNA (J. Virol., 1999), 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 (Proc. Natl. Acad. Sci. USA, 1990). Finally, pLVX-EF1α-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 (Cell, 2000).

In addition to lentiviral elements, pLVX-EF1α-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.

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
- $P_{EF1\alpha}$ (human elongation factor 1 alpha promoter): 2185–3519
- mCherry: 3539–4246
- MCS (multiple cloning site): 4273–4312
- P_{PGK} (phosphoglycerate kinase promoter): 4336–4844
- Puro^r (puromycin resistance gene): 4865–5464
- WPRE (woodchuck hepatitis virus posttranscriptional regulatory element): 5478–6069
- 3′ LTR (3′ long terminal repeat): 6272–6908
- pUC origin of replication: 7378–8051 (complementary)
- Amp^r (ampicillin resistance gene; β-lactamase): 8196–9192 (complementary)

Additional Information

Genes cloned into the MCS must be in-frame with the mCherry coding sequence, and do not require start or stop codons. pLVX-EF1α-mCherry-C1 will constitutively express your C-terminal mCherry fusion when transduced into target cells. Before the vector can be transduced into target cells, however, it must be packaged into viral particles in HEK293T cells, using our Lenti-X™ HTX Packaging System (Cat. Nos. 631247 and 631249). 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 (Mol. Ther., 2000).

Caution!
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.

Selection of Stable Transfectants

- Selectable marker: plasmid confers resistance to puromycin.

Propagation in *E. coli*

- Suitable host strains: DH5α, HB101 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: 587 nm
- Emission: 610 nm

References

Cochrane, A. W., Chen, C. H. & Rosen, C. A. Specific interaction of the human immunodeficiency virus Rev protein with a structured region in the env mRNA. *Proc. Natl. Acad. Sci. U. S. A.* **87,** 1198–1202 (1990).

Shaner, N. C. *et al.* Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. *Nat. Biotechnol.* **22,** 1567–72 (2004).

Wang, R., Liang, J., Jiang, H., Qin, L.-J. & Yang, H.-T. Promoter-dependent EGFP expression during embryonic stem cell propagation and differentiation. *Stem Cells Dev.* **17,** 279–89 (2008).

Wu, X. *et al.* Development of a novel trans-lentiviral vector Wu, X., Wakefield, J. K., Liu, H., Xiao, H., Kralovics, R., Prchal, J. T., & Kappes, J. C. (2000). Development of a novel trans-lentiviral vector that affords predictable safety. Mol Ther, 2(1), 47–55. https: *Mol Ther* **2,** 47–55 (2000).

Zennou, V. *et al.* HIV-1 genome nuclear import is mediated by a central DNA flap. *Cell* **101,** 173–85 (2000).

Zufferey, R., Donello, J. E., Trono, D. & Hope, T. J. Woodchuck hepatitis virus posttranscriptional regulatory element enhances expression of transgenes delivered by retroviral vectors. *J. Virol.* **73,** 2886–92 (1999).

Quality Control Data

Plasmid Identity & Purity

- Digestion with the indicated restriction enzymes produced fragments of the indicated sizes on a 0.8% agarose/EtBr gel:

Enzyme(s)	Fragment(s)
BamHI	9.5 kb
Acc65I	1.9 & 7.6 kb

- Vector identity was confirmed by sequencing.
- A_{260}/A_{280}: 1.8–2.0

It is certified that this product meets the above specifications, as reviewed and approved by the Quality Department.

Notice to Purchaser

pLVX-EF1alpha-mCherry-C1 Vector

CATALOG NO.

631985

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Notice to Purchaser

STATEMENT 55

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