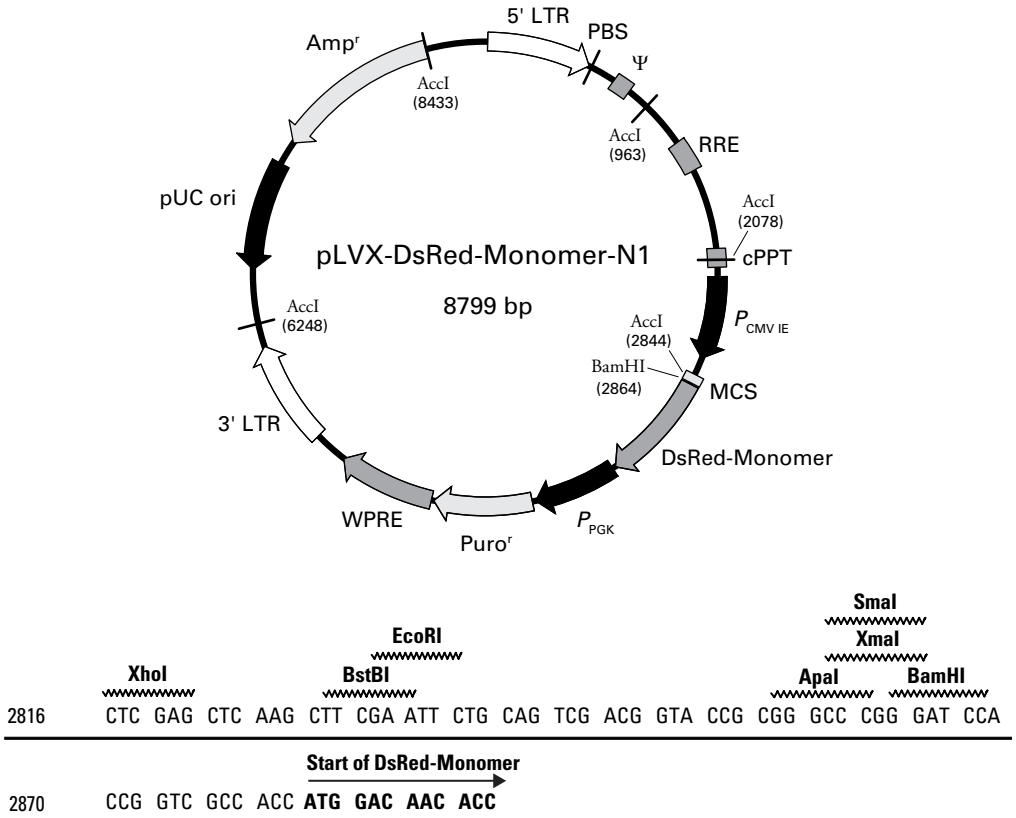
Vector Information

pLVX-DsRed-Monomer-N1 Vector Information PT3992-5

Catalog No. 632152



pLVX-DsRed-Monomer-N1 Vector Map and Multiple Cloning Site (MCS).

Description

pLVX-DsRed-Monomer-N1 is an HIV-1-based, lentiviral expression vector that allows you to express your gene of interest fused to DsRed-Monomer, a monomeric mutant of the *Discosoma* sp. red fluorescent protein. Genes cloned into the multiple cloning site (MCS), located upstream of the DsRed-Monomer coding sequence, are expressed as N-terminal fusions of the DsRed-Monomer 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 MCS. Lentiviral particles derived from the vector allow the expression of DsRed-Monomer fusion proteins in virtually any cell type, including primary cells. The unmodified vector expresses DsRed-Monomer, and may be used to produce marker virus to optimize infection protocols.

pLVX-DsRed-Monomer-N1 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-DsRed-Monomer-N1 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-DsRed-Monomer-N1 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-DsRed-Monomer-N1 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™ HTX Packaging System (Cat. Nos. 631247 and 631249). 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
- $P_{CMV\ IE}$ (human cytomegalovirus immediate early promoter): 2185–2788
- MCS (multiple coning site): 2816–2868
- DsRed-Monomer (*Discosoma* sp. red fluorescent protein monomer): 2882–3559
- P_{PGK} (phosphoglycerate kinase promoter): 3579–4087
- Puro^r (puromycin resistance gene): 4108–4707
- WPRE (woodchuck posttranscriptional regulatory element): 4721–5312
- 3' LTR: 5516–6152
- pUC origin of replication: 6622–7292 (complementary)
- Amp^r (ampicillin resistance gene; β-lactamase): 7437–8433 (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

Excitation and emission maxima of DsRed-Monomer

- Excitation maximum = 557 nm
- Emission maximum = 592 nm

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

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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.

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The DsRed Monomer and the Fruit Fluorescent Proteins are covered by one or more of the following U.S. Patents: 7,157,566; 7,393,923; 7,005,511 and 7,250,298.

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