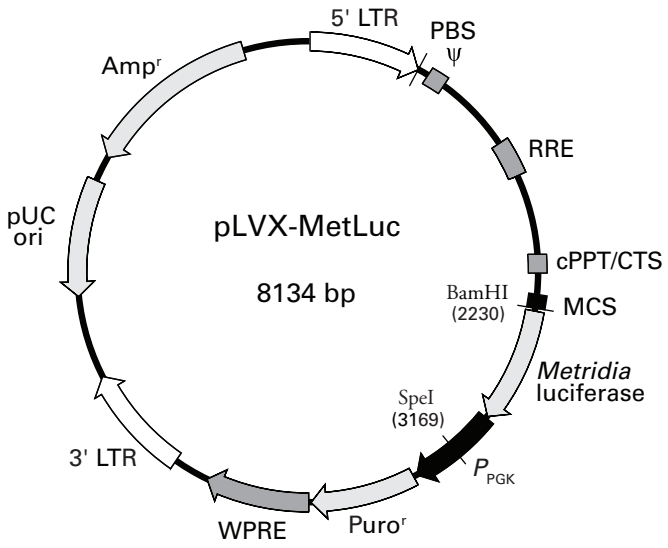
Vector Information

pLVX-MetLuc Vector Information PT4422-5

Sold as part of Cat. No. 631746



EcoRI Bsp120I
BstBI ApaI BamHI

2191 AAGCTTCGAA TTCTGCAGTC GACGGTACCG CGGGCCCGGG ATCCCGCGAC
TTCGAAGCTT AAGACGTCAG CTGCCATGGC GCCCGGGCCC TAGGGCGCTG

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

Description

pLVX-MetLuc is a promoterless, HIV-1-based, lentiviral vector that allows the functional analysis of different promoters and promoter/enhancer combinations inserted into its multiple cloning site (MCS). The vector encodes *Metridia* luciferase (MetLuc), a secreted reporter protein that can be easily detected in the medium surrounding the cells.

Metridia luciferase is a naturally secreted luciferase from the marine copepod *Metridia longa*. The human codon-optimized *Metridia* luciferase gene encodes a 219 amino acid (24 kDa) polypeptide that includes a 17 amino acid N-terminal signal peptide necessary for secretion (1). When a functional promoter is cloned into the MCS, located immediately upstream of the reporter, *Metridia* luciferase is expressed and secreted into the surrounding medium, where it is easily detected by the addition of a chemiluminescent substrate.

Lentiviral particles derived from pLVX-MetLuc allow you to monitor the activity of your promoter of interest in virtually any cell type, even primary cells. pLVX-MetLuc 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

(PR093647; published 3 September 2010)

Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

of unspliced viral RNA out of the nucleus (3). Finally, pLVX-MetLuc 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).

In addition to lentiviral elements, pLVX-MetLuc contains a puromycin resistance gene (Puro^r) under the control of the murine phosphoglycerate kinase 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

The pLVX-MetLuc vector, available as part of the Lenti-X™ Ready-To-Glow™ Secreted Luciferase Reporter System (Cat. No. 631746), can be used to monitor promoter activity in live cells as well as *in vivo*. A promoter must be cloned into the MCS, located upstream of the *Metridia* luciferase coding sequence. Without the addition of a functional promoter, the vector will not express MetLuc. Lentiviral particles derived from the vector allow you to monitor your promoter of interest in virtually any cell type, even primary cells. If required, stable transfectants can be selected using puromycin.

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
- MCS (multiple cloning site): 2195–2234
- *Metridia* luciferase (human codon optimized): 2247–2903
- P_{PGK} (phosphoglycerate kinase promoter): 2914–3422
- Puro^r (puromycin resistance gene): 3443–4042
- WPRE (woodchuck hepatitis virus posttransctiptional regulatory element): 4056–4647
- 3' LTR: 4851–5487
- pUC origin of replication: 5957–6627 (complementary)
- Amp^r (ampicillin resistance gene; β-lactamase): 6772–7768 (complementary)

Propagation in *E. coli*

- Recommended host strains: DH5α™ and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC

References

1. Markova, S.V. *et al.* (2004) *J. Bio. Chem.* **279**(5):3212-3217.
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.

Note: 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.

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S.V. Markova, S. Golz, L.A. Frank, B. Kalthof, and E.S. Vysotski (2004): Cloning and Expression of cDNA for a Luciferase from the marine copepod *Metridia longa. J. Biol.Chem.* **279**:3212-3117.

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