pLVX-MetLuc Control Vector Information

PT4423-5

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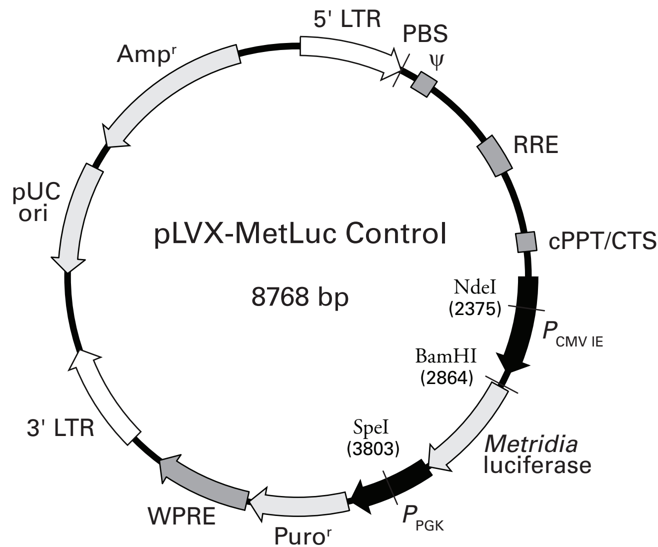

pLVX-MetLuc Control Vector Map.

Description

pLVX-MetLuc Control is an HIV-1-based, lentiviral vector that constitutively expresses *Metridia* luciferase (MetLuc), a secreted reporter protein that can be easily detected in the medium surrounding the cells. The vector can be used as a control to monitor the constitutive expression and secretion of *Metridia* luciferase in your cell type of interest.

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). Expression of *Metridia* luciferase is driven by the constitutively active human cytomegalovirus immediate early promoter ($P_{CMV\ IE}$), located just upstream of the *Metridia* luciferase coding sequence.

pLVX-MetLuc Control 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-MetLuc Control 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 Control 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.

(PR093648; published 3 September 2010)

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Use

The pLVX-MetLuc Control vector is available as part of the Lenti-X™ Ready-To-Glow™ Secreted Luciferase Reporter System (Cat. No. 631746). Lentiviral particles derived from the vector allow you to monitor the constitutive expression and secretion of *Metridia* luciferase in your cell type of interest. Secretion of *Metridia* luciferase can be monitored by adding chemiluminescent substrate to a sample of the cell medium. The vector can also be used to monitor the inhibitory effect of drugs, like Brefeldin A, on protein secretion. 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
- $P_{CMV\ IE}$ (human cytomegalovirus immediate early promoter): 2185–2788
- *Metridia longa* secreted luciferase (human codon optimized): 2881–3537
- P_{PGK} (phosphoglycerate kinase promoter): 3548–4056
- $Puro^r$ (puromycin resistance gene): 4077–4676
- WPRE (woodchuck hepatitis virus posttransctiptional regulatory element): 4690–5281
- 3' LTR: 5485–6121
- pUC origin of replication: 6591–7261 (complementary)
- Amp^r (ampicillin resistance gene; β-lactamase): 7406–8402 (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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TS.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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