pLVX-IRES-Neo

Catalog No.
632181

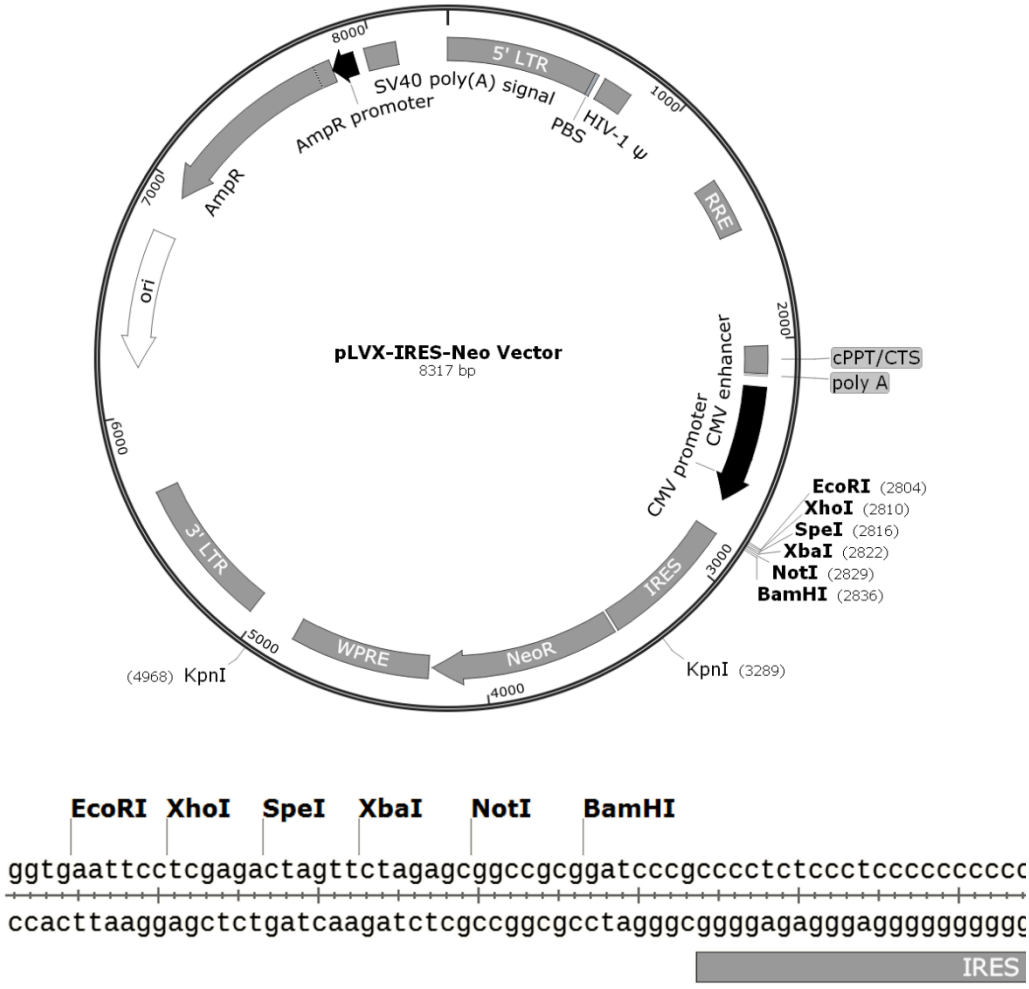

Figure 1. pLVX-IRES-Neo Vector map and multiple cloning site.

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Vector Map

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pLVX-IRES-Neo

Description

pLVX-IRES-Neo is an HIV-1-based, lentiviral expression vector that allows the simultaneous expression of your protein of interest and G418 resistance (Neo^r) in virtually any mammalian cell type, including primary cells. The vector expresses your protein of interest and Neo^r from a bicistronic mRNA transcript, allowing G418 resistance to be used as an indicator of transduction efficiency and a marker for selection.

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 Neo^r, facilitates cap-independent translation of Neo^r from an internal start site at the IRES/Neo^r junction (Jang et al. 1988).

pLVX-IRES-Neo 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 (Zufferey et al. 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 (Cochrane, Chen, and Rosen 1990). Finally, pLVX-IRES-Neo 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 (Zennou et al. 2000). 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-IRES-Neo is available as part of the Lenti-X™ Bicistronic Expression System (Neo; Cat. No. 632181). The vector is designed to constitutively coexpress your protein of interest and G418 resistance 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 Lenti-X 293T Cells (Cat. No. 632180), using our Lenti-X Packaging Single Shots (VSV-G) (Cat. Nos. 631275 and 631276). 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 (Wu et al. 2000).

Location of Features

- 5' LTR (5' long terminal repeat): 1–635
- PBS (primer binding site): 636–653
- Ψ (packaging signal): 681–806
- RRE (Rev-response element): 1303–1536
- cPPT/CTS (central polypurine tract/central termination sequence): 2028–2143
- $P_{CMV\ IE}$ (human cytomegalovirus immediate early promoter): 2505–2708
- MCS (multiple cloning site): 2805–2841
- IRES (encephalomyocarditis virus internal ribosome entry site): 2844–3417
- Neo^r (neomycin resistance gene, confers resistance to G418): 3431–4225
- WPRE (woodchuck hepatitis virus posttranscriptional regulatory element): 4239–4827
- 3' LTR (3' long terminal repeat): 5035–5668
- pUC origin of replication: 6199–6784 (complementary)
- Amp^r (ampicillin resistance gene; β-lactamase): 6955–7815 (complementary)

Selection of Stable Transductants

- Selectable marker: vector confers resistance to G418.

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

References

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