

Restriction Map of pEco Retroviral Vector. Unique restriction sites are in bold.

Description

pEco Vector expresses the ecotropic envelope protein under the control of the CMV immediate-early promoter (1). The ecotropic envelope protein can serve as a surrogate viral envelope protein (2). It interacts on the host cell surface with the ecotropic receptor protein, murine cationic amino acid transporter-1 (MCAT-1) which facilitates rat or mouse host cell infection. pEco includes IVS, a synthetic intron known to enhance the stability of the mRNA (3), the pUC origin of replication, and the bacterial ampicillin resistance (Amp^{r)} gene for propagation and antibiotic selection in bacteria.

As part of the Retro-X[™] Universal Retroviral Expression System (Cat. No. 631530), pEco is cotransfected with a retroviral expression vector into the GP2-293 Packaging Cell Line (4) to produce infectious, replication-incompetent retrovirus. The genes encoding the viral *gag* and *pol* proteins are stably integrated into GP2-293, while the *env* gene is supplied by the pEco Vector. Although the resulting virus can infect target cell lines and transmit a gene-of-interest, it cannot replicate because target cell lines lack the viral structural and polymerase/integrase genes. The separate introduction and integration of the viral genes into the packaging cell line and the use of minimal viral sequences in the vector minimize the chance of producing replication-competent virus due to

Use



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recombination events.

Location of Features

- CMV promoter: 1–768
- Rabbit β-globin IVS: 768–1425
- Splicing signals
 - Splice Donor: 792–793; splice acceptor: 1363–1364
- Ecotropic envelope gene: Start codon: 1438–1440; stop codon: 3508–3510
- β-globin poly A: 3541–3546
- pUC origin of replication: 4339–4348
- Ampicillin resistance gene (β-lactamase):
 - Start codon: 5964–5962; stop codon: 5101–5103

Propagation in E. coli

- Suitable host strains: DH5α, Fusion-Blue[™], and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) to E. coli hosts.
- E. coli replication origin: pUC
- Copy number: high

References

- 1. Yee, J. K., et al. (1994) Proc. Natl. Acad. Sci. USA 91:9564–9568.
- 2. Albritton, L. M., et al. (1989) Cell 57(4):659–666.
- 3. Huang, M. T. F. & Gorman, C. M. (1990) Nucleic Acids Res. 18(4):937–947.
- 4. Witte, O. N. & Baltimore, D. (1977) Cell 11:505–511.

Notes: Due caution must be exercised in the production and handling of recombinant retrovirus. Appropriate NIH, regional, and institutional guidelines apply.

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 Laboratories. This vector has not been completely sequenced.

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This document has been reviewed and approved by the Clontech Quality Assurance Department.