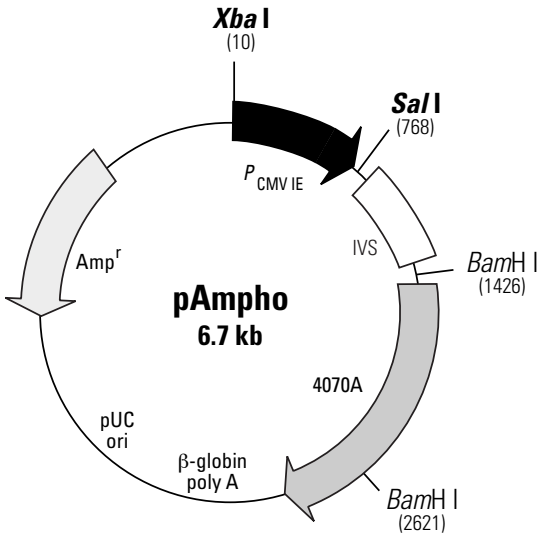
Vector Information

pAmpho Vector Information PT3750-5

Sold as part of Catalog No. 631530



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

Description

pAmpho Vector expresses the 4070A amphotropic envelope protein under the control of the CMV immediate-early promoter (1). The amphotropic envelope protein can serve as a surrogate viral envelope protein. It interacts on the host cell surface with the Ram-1 protein which acts as a viral receptor for infection (2). pAmpho 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.

Use

As part of the Retro-X™ Universal Retroviral Expression System (Cat. No. 631530), pAmpho 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 pAmpho 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 recombination events.

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Location of Features

- CMV promoter: 1–768
- Rabbit β-globin IVS: 768–1425
- Splicing signals:
  Splice donor: 792–793; splice acceptor: 1363–1364
- Amphotropic (4070A) envelope gene:
  Start codon: 1438–1440; stop codon: 3400–3402
- β-globin poly A: 3514–3519
- pUC origin of replication: 4312–4321
- Ampicillin resistance gene (β-lactamase):
  Start codon: 5937–5935; stop codon: 5078–5076

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. Miller, D. G. & Miller, A. D. (1994) *J. Virol.* **68**:8270–8276
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. This vector has not been completely sequenced.