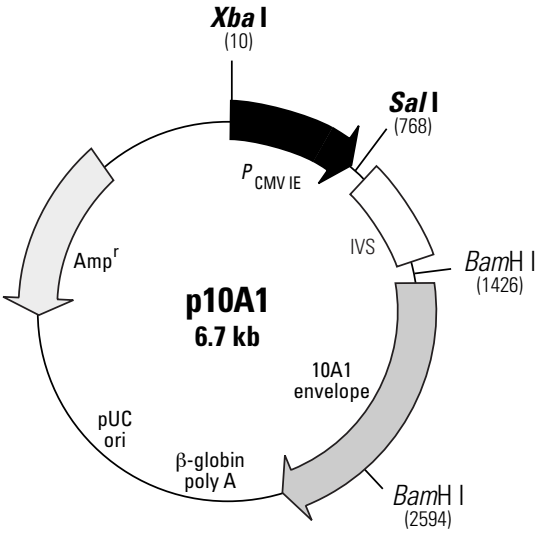
Vector Information

p10A1 Vector Information PT3748-5

Sold as part of Catalog No. 631530



Restriction Map of p10A1 Retroviral Vector. Unique restriction sites are in bold.

Description

p10A1 expresses the 10A1 envelope protein under the control of the CMV immediate-early promoter (1). Virus packaged with the 10A1 envelope protein can enter cells via two different surface molecules, the amphotropic retrovirus receptor or the GALV receptor, and therefore exhibits a broader host range than virus packaged with other envelope proteins (2, 3, 4). p10A1 includes IVS, a synthetic intron known to enhance the stability of the mRNA (5), the pUC origin of replication, and a 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), p10A1 is cotransfected with a retroviral expression vector into the GP2-293 Packaging Cell Line (6) 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, 10A1, is supplied by the p10A1 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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(PR37006; published 25 June 2003)

Location of Features

- CMV promoter: 1–768
- Rabbit β-globin IVS: 768–1425
- Splicing Signals:
  Splice donor: 792–793; splice acceptor: 1363–1364
- 10A1 envelope:
  Start codon: 1438–1440; stop codon: 3373–3375
- β-globin poly A: 3487–3492
- pUC origin of replication: 4285–4294
- Ampicillin resistance gene (β-lactamase):
  Start codon: 5910–5908; stop codon: 5051–5049

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: low

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. Miller A. D. (1996) *Proc. Natl. Acad. Sci. USA* **93**:11407–11413.
4. Han, J. Y, *et al.* (1997) *J. Virol.* **71**:8103–8108.
5. Huang, M. T. F. & Gorman, C. M. (1990) Nucleic Acids Res. **18**(4):937–947.
6. 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, Inc. This vector has not been completely sequenced.