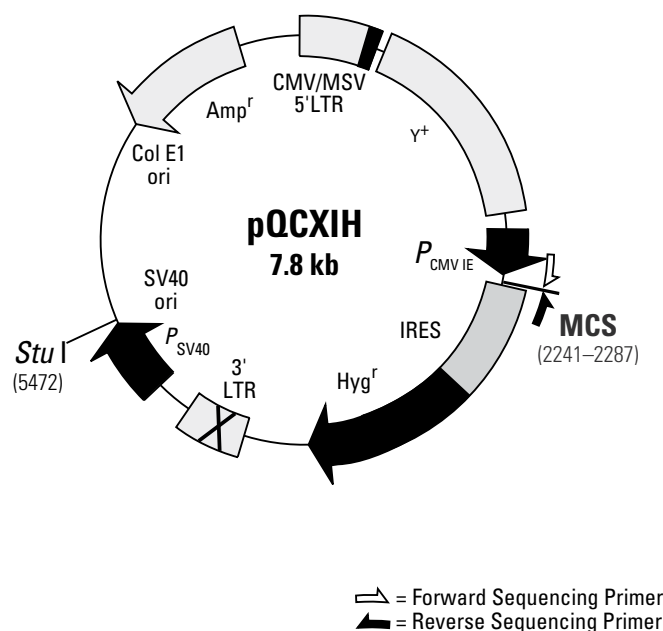


pQCXIH Vector Information

PT3668-5

Cat. No. 631516



Restriction Map and Multiple Cloning Site (MCS) of pQCXIH Vector. Unique restriction sites are in bold.

Description

pQCXIH Retroviral Vector is a self-inactivating bicistronic expression vector designed to express a target gene along with the hygromycin resistance gene (1). Upon transfection into a packaging cell line, this vector can transiently express, or integrate and stably express, a viral genomic transcript containing the CMV immediate early promoter, gene of interest, IRES and the hygromycin resistance gene (Hyg^r). The gene of interest and the hygromycin resistance gene are co-translated, via the internal ribosome entry site (IRES), as a bicistronic message in mammalian cells (2, 3).

This vector incorporates unique features including: optimization to remove promoter interference and self-inactivation. The hybrid 5' LTR consists of the cytomegalovirus (CMV) type I enhancer and the mouse sarcoma virus (MSV) promoter. This construct drives high levels of transcription in HEK 293-based packaging cell lines due, in part, to the presence of adenoviral E1A (4, 5, 6, 7) in these cells. The self-inactivating feature of the vector is provided by a deletion in the 3' LTR enhancer region (U3). During reverse transcription of the retroviral RNA, the inactivated 3' LTR is copied and replaces the 5' LTR, resulting in inactivation of the 5' LTR CMV enhancer sequences. This may reduce the phenomenon known as promoter interference (8) and allow more efficient expression.

Also included in the viral genomic transcript are the necessary viral RNA processing elements including the LTRs, packaging signal (Psi^+), and tRNA primer binding site. pQCXIH also contains a bacterial origin of replication and *E. coli* Amp^r gene for propagation and selection in bacteria.

(PR7Y2442; published January 2008)



Clontech

United States/Canada
800.662.2566

Asia Pacific
+1.650.919.7300

Europe
+33.(0)1.3904.6880

Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

Use

pQCXIH is designed to deliver and express a gene of interest, along with the hygromycin resistance marker, from a bicistronic message. The design is optimized to produce high titers via the $P_{CMV\ IE}$ in the packaging cell line. The bicistronic transcript makes it unnecessary to screen the transformants since the Hygromycin resistance is expressed in concert with the gene inserted into the multiple cloning site.

Once transfected into the packaging cell line (such as the RetroPack™ PT67 Cell Line; Cat. No. 631510), RNA from the vector is packaged into infectious, replication-incompetent retroviral particles since pQCXIH lacks structural genes (gag, pol, and env) necessary for particle formation and replication; however, these genes are stably integrated as part of the packaging cell genome. Once a high titer clone is selected, these retroviral particles can infect target cells and transmit the gene of interest but cannot replicate within these cells due to the absence of viral structural genes. The separate introduction and integration of the structural genes into the packaging cell line minimizes the chances of producing replication-competent virus due to recombination events during cell proliferation.

Location of Features

- 5' LTR (CMV/MSV): 1–728
Cytomegalovirus (CMV)/ mouse sarcoma virus (MSV) hybrid promoter: 1–511
R region: 584–654
U5 region: 655–728
- Ψ^+ (extended packaging signal): 758–1567
- Immediate early CMV promoter ($P_{CMV\ IE}$): 1601–2132
- Multiple Cloning Site: 2240–2280
- Internal ribosome entry site (IRES): 2289–2863
- Hygromycin resistance gene (Hyg^r): 2887–3921
Start codon (ATG): 2887–2889; stop codon (TAA): 3919–3921
- 3' MoMuLV LTR (deletion in U3): 4467–4892
Poly A region: 4794–4815
- SV40 promoter: 5172–5439
- SV40 ori: 5393–5458
- Col E1 ori (Site of replication initiation): 5779
- Ampicillin resistance gene (β -lactamase): 6539–7399
Start codon (ATG): 7399–7397 stop codon (TAA): 6541–6539

Sequencing Primer Locations

- pQC Seq/PCR Primers:
5' primer (2141–2164): 5'-ACGCCATCCACGCTGTTTTGACCT-3'
3' primer (2311–2334): 5'-AAGCGGCTTCGGCCAGTAACGTTA-3'

Propagation in *E. coli*

- Suitable host strains: DH5 α , DH10B, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 μ g/ml) to *E. coli* hosts.
- *E. coli* replication origin: Col E1
- Copy number: low

References

1. Julius, M. A., Yan, Q., Zheng, Z., & Kitajewski, J. (2000) *BioTechniques* **28**(4):702–707.
2. Adam, M. A., Ramesh, N., Miller, A. D. & Osborne, W. R. (1991) *J. Virol.* **65**:4985–4990.
3. Ghattas, I. R., Sanes, J. R. & Majors, J. E. (1991) *Mol. Cell Biol.* **11**:5848–5859.
4. Kinsella, T. M. & Nolan, G. P. (1996) *Hum. Gene Ther.* **7**:1405–1413.
5. Ory, D. S., Neugeboren, B. A. & Mulligan, R. C. (1996) *Proc. Nat. Acad. Sci. USA* **93**:11400–11406.
6. Pear, W. S., Nolan, G. P., Scott, M. L. & Baltimore, D. (1993) *Proc. Natl. Acad. Sci. USA* **90**(18):8392–8396.
7. Yang, S., Delgado, R., King, S. R., Woffendin, C., Barker, C. S., Yang, Z. Y., Xu, L., Nolan, G. P. & Nabel, G. J. (1999) *Hum. Gene Ther.* **10**:123–132.
8. Emerman, M. & Temin, H. M. (1984) *Cell* **39**:449–467.

Note: 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.

The viral supernatants produced by this retroviral vector could, depending on your cloned insert, contain potentially hazardous recombinant virus. Due caution must be exercised in the production and handling of recombinant retrovirus. Appropriate NIH, regional, and institutional guidelines apply.

Notice to Purchaser

Clontech products are to be used for research purposes only. They may not be used for any other purpose, including, but not limited to, use in drugs, in vitro diagnostic purposes, therapeutics, or in humans. Clontech products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without written approval of Clontech Laboratories, Inc.

For Research Use Only. Not for Use in Humans.

These products are sold under license from the Fred Hutchinson Cancer Research Center. Rights to use this product are limited to research only. No other rights are conveyed. Inquiry into the availability of a license to broader rights or the use of this product for commercial purposes should be directed to Fred Hutchinson Cancer Research Center, Technology Transfer Office, 1100 Fairview Avenue North, J6-200, Seattle, WA 98109. Purchase of this product does not grant rights to: (1) offer the materials or any derivatives thereof for resale; or (2) to distribute or transfer the materials or any derivatives thereof to third parties.

Clontech, the Clontech logo and all other trademarks are the property of Clontech Laboratories, Inc., unless noted otherwise. Clontech is a Takara Bio Company. ©2008 Clontech Laboratories, Inc.