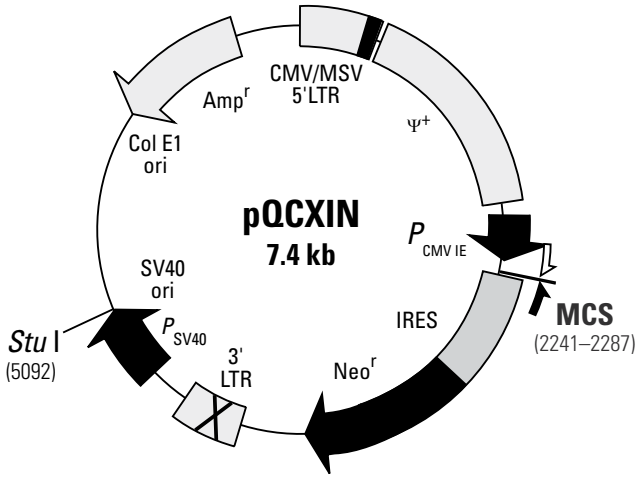
Vector Information

pQCXIN Retroviral Vector Information PT3667-5

Cat. No. 631514



= Forward Sequencing Primer
= Reverse Sequencing Primer

2240 2250 2260 2270 2280

CGCGGCCGCACCGGTAGGCCTCGTACGCTTAATTAACGGATCCGGAATTCC

***Not* I** ***Age* I** ***Bsi* WI** ***Pac* I** ***Bam*H I** ***Eco*R I**

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

Description

pQCXIN Retroviral Vector is a bicistronic expression vector designed to express a target gene along with the neomycin selection marker (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 neomycin resistance gene (Neo^r). The gene of interest and the neomycin resistance gene are co-translated, via the internal ribosome entry site (IRES), from 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. pQCXIN also contains a bacterial origin of replication and *E. coli* Amp^r gene for propagation and selection in bacteria.

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Use

pQCXIN is designed to deliver and express a gene along with the neomycin 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 neomycin resistance is expressed in concert with the DNA inserted into the multiple cloning site.

Once transfected into the packaging cell line (such as the RetroPack™ PT67 Cell Line (Cat. No.631510) AmphoPack293, EcoPack2-293, or Pantropic System), RNA from the vector is packaged into infectious, replication-incompetent retroviral particles since pQCXIN 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 introductioin and integration of the structural genes into the packaging cell line minimizes the chances of producing replication-competent virus due to recomination 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
- Cytomegalovirus (CMV) immediate early promoter ($P_{CMV\ IE}$): 1601–2132
- Multiple Cloning Site (MCS): 2238–2287
- Internal ribosome entry site (IRES): 2289–2862
- Neomycin resistance gene (Neo^r): 2876–3670
- 3' MoMuLV LTR (deletion in U3): 4087–4512
  Poly A signal: 4415–4420
  cleavage site: 4435–4436
- SV40 promoter: 4792–5059
- SV40 ori: 5013–5078
  Site of replication initiation
- Col E1 ori (Site of replication initiation): 5399
- Ampicillin resistance gene (β-lactamase): 7019–6159
  Start codon (ATG): 7019–7017 stop codon (TAA): 6161–6159

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: DH5α, DH10B, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: ColE1
- Copy number: low

References

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4. Kinsella, T. M. & Nolan G. P. (1996) *Hum. Gene Ther.* **7**:1405–1413.
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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 sequences obtained by Clontech Laboratories, Inc. This vector has 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.