

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

Description

pQCXIP Retroviral Vector is a bicistronic expression vector designed to express a target gene along with the puromycin 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 puromycin resistance gene (Pur'). The gene of interest and the puromycin resistance gene are co-transcribed, 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. pQCXIP also contains a bacterial origin of replications and *E. coli* Amp^r gene for propagation and selection in bacteria.



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pQCXIP **Vector Information**

Use

pQCXIP is designed to deliver and express a gene along with the puromycin resistance marker from a bicistronic message. The design is optimized to produce high titers via the $\dot{P}_{\text{CMV-IE}}$ in the packaging cell line. The bicistronic transcript makes it unnecessary to screen the transformants since the puromycin 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) AmphoPack293, EcoPack2-293, or Pantropic System), RNA from the vector is packaged into infectious, replicationincompetent retroviral particles since pQCXIP 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 ($P_{\text{CMV IE}}$): 1601–2132

Multiple Cloning Site (MCS): 2239–2287

Internal ribosome entry site (IRES): 2289-2862

Puromycin resistance gene (Pur): 2898–3494

Start codon (ATG): 2895-2897; stop codon (TGA): 3492-3494

3' MoMuLV LTR (deletion in U3): 3868–4293

Poly A region: 4195-4216 SV40 promoter: 4573–4840

SV40 ori: 4794–4859

Col E1 ori (Site of replication initiation): 5180

Ampicillin resistance gene (β-lactamase): 6800–5940

Start codon (ATG): 6800-6798 stop codon (TAA): 5940-5942

Sequencing Primer Locations

pQC Seg/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: Col E1
- Copy number: low

Protocol No. PT3669-5 Clontech Laboratories, Inc. www.clontech.com Version No. PR7Y2443 pQCXIP Vector Information

References

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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.

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