



## 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  $P_{CMV IE}$  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) AmphiPack293, EcoPack2-293, or Pantropic System), RNA from the vector is packaged into infectious, replication-incompetent 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
- $\Psi^+$  (extended packaging signal): 758–1567
- Immediate early CMV promoter ( $P_{CMV IE}$ ): 1601–2132
- Multiple Cloning Site (MCS): 2239–2287
- Internal ribosome entry site (IRES): 2289–2862
- Puromycin resistance gene ( $Pur^r$ ): 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 ( $\beta$ -lactamase): 6800–5940
  - Start codon (ATG): 6800–6798      stop codon (TAA): 5940–5942

## Sequencing Primer Locations

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

## Propagation in *E. coli*

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

## 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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