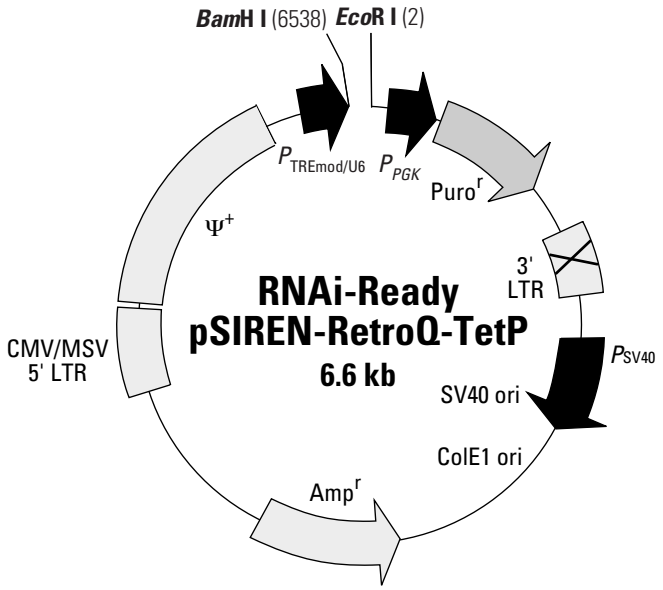

Restriction Map and Cloning Site of RNAi-Ready pSIREN-RetroQ-TetP Retroviral Vector. All restriction sites shown are unique. RNAi-Ready pSIREN-RetroQ-TetP is provided as a linearized vector digested with *Bam*H I and *Eco*R I. Nucleotides in gray were removed during linearization. This linearized vector is ready for ligation of an appropriate shRNA oligo containing *Bam*H I and *Eco*R I overhangs.

Description

RNAi-Ready pSIREN-RetroQ-TetP is a self-inactivating retroviral expression vector designed to express a ds short hairpin RNA (shRNA) under the control of the modified Tet-responsive promoter ($P_{TREmod/u6}$) derived from the P_{TREmod} and the human U6 promoter (P_{U6}). RNAi-Ready pSIREN-RetroQ-TetP is provided as a linearized vector digested with *Bam*H I and *Eco*R I. It is used for targeted and inducible gene silencing when a DNA oligonucleotide encoding an appropriate shRNA is ligated into the vector. shRNA expression is controlled by the tetracycline suppressor tTS (1). The P_{TREmod} consists of seven direct repeats of a 36 bp sequence that contains the 19 bp tet operator sequence (tetO). You can transfect your pSIREN-RetroQ-TetP construct as a plasmid expression vector, or—upon transfection into a packaging cell line—this vector can transiently express, or integrate and stably express a viral genomic transcript containing the $P_{TREmod/u6}$ promoter and the shRNA. The vector contains a puromycin resistance gene (Puro^r) under the control of the murine phosphoglycerate kinase (PKG) promoter (P_{PGK}) for the selection of stable transfectants.

This retroviral vector is optimized to eliminate promoter interference through 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 (2–5) 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 mechanism may reduce the phenomenon known as promoter interference (6, 7) and allow more efficient expression. Viral infection of host cells with recombinant pSIREN-RetroQ-TetP is the preferred delivery method.

(PR611410; published 13 February 2006)

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. RNAi-Ready pSIREN-RetroQ-TetP also contains a bacterial origin of replication and *E. coli* Amp^r gene for propagation and selection in bacteria.

Use

RNAi-Ready pSIREN-RetroQ-TetP is used for targeted and inducible gene silencing when a DNA oligonucleotide encoding an appropriate shRNA is inserted. To construct recombinant pSIREN-RetroQ-TetP-shRNA constructs, first design, generate, and anneal complementary shRNA oligonucleotides using the protocols in the Knockout Inducible RNAi Systems User Manual (PT3810-1). The annealed oligonucleotide should contain 5'-*Bam*H I and 3'-*Eco*R I sites. Then ligate the annealed oligonucleotide into the RNAi-Ready pSIREN-RetroQ-TetP vector.

Your pSIREN-RetroQ-TetP construct can be transfected as a plasmid expression vector to screen for functional shRNA oligonucleotides. For gene silencing experiments using viral delivery, transfect the pSIREN-RetroQ-TetP construct into a packaging cell line (such as the Retro-X™Universal Packaging System [Cat. No. 631512]). RNA from the vector is packaged into infectious retroviral particles. These infectious particles are replication-incompetent since pSIREN-RetroQ-TetP 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. These retroviral particles can infect a wide range of target cells and transmit the shRNA 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.

pSIREN-RetroQ-TetP-Luc Vector contains a validated luciferase shRNA oligonucleotide insert. When tested in HEK 293 cells plated at 1 x 10^5 per well and transfected with a 2:1:1 ratio of ptTS-Neo:pSIREN-RetroQ-TetP-Luc: pCMV-Luc, we consistently observe a 60–75% knockdown of luciferase activity with 48 hr of 1 µg/ml Dox induction. This control vector allows you to directly monitor the cloning efficiency of your shRNA insert into the pSIREN-RetroQ-TetP Vector.

Location of Features

- PGK promoter (P_{PGK}): 2–510
- Puromycin resistance gene ($Puro^r$): 531–1130
- 3' MoMuLV LTR (deletion in U3): 1311–1736
  Poly A region: 1562–1577
- SV40 promoter: 2016–2302
- SV40 ori: 2237–2302
- Col E1 ori (site of replication initiation): 3221–2622
- Ampicillin resistance gene (β-lactamase): 4243–3383
  Start codon (ATG): 4243–4241 Stop codon (TAA): 3385–3383
- 5' LTR (CMV/MSV): 4606–5333
  Cytomegalovirus (CMV)/ mouse sarcoma virus (MSV) hybrid promoter: 4606–5116
  R region: 5189–5259
  U5 region: 5261–5333
- Ψ^+ (extended packaging signal): 5363–6172
- P_{tight} Tet-responsive promoter: 6188–6476
  Tet responsive element (TRE_{mod}): 6188–6415
  Location of seven tetO 19-mers: 6190–6290 ; 6211–6229; 6247–6265; 6282–6300 ;6318–6336; 6354–6372 & 6389–6407
- U6 promoter (P_{U6}): 6470–6536
- TATA box: 6514–6519

Sequencing Primer Location

- U6 Forward Sequencing Primer: 6105-6132
  5'-CTTGAACCTCCTCGTTCGACCCCGCCTC-3'

Selection of Stable Transfectants

- Selectable marker: plasmid confers resistance to puromycin.

Propagation in *E. coli*

- Suitable host strains: DH5α, DH10B, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: Col E1
- Copy number: low

References

1. Freundlieb S., Schirra-Muller, C. & Bujard. H. (1999) J. Gene Med. **1**(1):4–12.
2. Kinsella, T. M. & Nolan G. P. (1996) Hum. Gene Ther. **7**(18):1405–1413.
3. Ory, D. S., Neugeboren, B. A. & Mulligan, R. C. (1996) Proc. Nat. Acad. Sci. USA **93**(21):11400–11406.
4. Pear, W. S., Nolan, G. P., Scott, M. L. & Baltimore, D. (1993) Proc. Natl. Acad. Sci. USA **90**(18):8392–8396.
5. 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**(1):123–132.
6. Barton, G.M. & Medzhitov R. (2002) Proc. Natl. Acad. Sci. USA **99**(23):14943–14945.
7. Emerman, M. & Temin, H. M. (1984) Cell **39**(3 pt. 2):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. 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.