



6560 10 20 30 40

5'- TGGAAAGGAC**GAGGATCC**[...shRNA oligo cloning site...]GAATTCTAGTATTATAATAGTAATCAATTA**CGGGGCATT**A-3'

**BamH I** **EcoR I**

3'-ACCTTTCCTGCTCCTAGG[...shRNA oligo cloning site...]CTTAAGATCAATAATTATCAT TAGTTAATGCCCCAGTAAT-5'

**Restriction Map and Cloning Site of RNAi-Ready pSIREN-RetroQ-ZsGreen Retroviral Vector.** Unique restriction sites are in bold. RNAi-Ready pSIREN-RetroQ-ZsGreen is provided as a linearized vector digested with *BamH I* and *EcoR I*. Nucleotides in gray were removed during linearization. This linearized vector is ready for ligation of an appropriate shRNA containing *BamH I* and *EcoR I* overhangs. RNAi-Ready pSIREN-RetroQ-ZsGreen is also provided as a component of the Knockout Clone & Confirm RetroQ RNAi System with Reporter (Cat. No. 632456).

### Description

RNAi-Ready pSIREN-RetroQ-ZsGreen is a self-inactivating retroviral expression vector designed to express a small hairpin RNA (shRNA) using the human U6 promoter ( $P_{U6}$ ; RNA Pol III-dependent). RNAi-Ready pSIREN-RetroQ-ZsGreen is provided as a linearized vector digested with *BamH I* and *EcoR I*. It is used for targeted gene silencing when a ds DNA oligonucleotide encoding an appropriate shRNA is ligated into the vector. You can transfect your pSIREN-RetroQ-ZsGreen 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 human U6 promoter and the shRNA.

In addition, this vector independently expresses a *Zoanthus* sp. green fluorescent protein (ZsGreen; 1), which has been engineered for brighter fluorescence (excitation maximum = 496 nm; emission maximum = 506 nm). The ZsGreen gene is positioned just downstream of the immediate early promoter of cytomegalovirus ( $P_{CMV,IE}$ ). As a result, cells transfected with this vector will express the green fluorescent protein constitutively. The ZsGreen fluorescent marker allows you to directly monitor the delivery efficiency of your gene silencing construct using either fluorescence microscopy or flow cytometry.

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

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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 may reduce the phenomenon known as promoter interference (6) and allow for 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. RNAi-Ready pSIREN-RetroQ-ZsGreen also contains a bacterial origin of replication and *E. coli* Amp<sup>r</sup> gene for propagation and selection in bacteria.

## Use

RNAi-Ready pSIREN-RetroQ-ZsGreen is used for targeted gene silencing when a ds DNA oligonucleotide encoding an appropriate shRNA is inserted. To construct recombinant pSIREN-RetroQ-ZsGreen, first design, generate, and anneal complementary shRNA oligonucleotides using the protocols in the Knockout RNAi Systems User Manual (PT3739-1). The annealed oligonucleotide should contain 5'-*Bam*H I and 3'-*Eco*R I overhangs. Then ligate the annealed oligonucleotide into RNAi-Ready pSIREN-RetroQ-ZsGreen.

Your pSIREN-RetroQ-ZsGreen 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-ZsGreen construct into a packaging cell line (see the Retroviral Gene Transfer and Expression User Manual, PT3132-1, for a list of packaging cell lines available from Clontech Laboratories, Inc.); RNA from the vector is packaged into infectious retroviral particles. 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 ZsGreen fluorescent marker in this vector allows direct monitoring of the delivery of your gene silencing construct. Use fluorescence microscopy or flow cytometry to easily detect or enrich for cells containing your recombinant shRNA vector.

## Location of Features

- CMV IE promoter ( $P_{CMV\ IE}$ ): 7–595
- ZsGreen fluorescent protein: 619–1314
- 3' MoMuLV LTR (deletion in U3): 1434–1859  
Poly A region: 1761–1766
- SV40 promoter: 2139–2425
- SV40 ori: 2360–2425
- Col E1 ori (Site of replication initiation): 3344–2745
- Ampicillin resistance gene ( $\beta$ -lactamase): 4366–3506  
Start codon (ATG): 4366–4364 stop codon (TAA): 3508–3506
- 5' LTR (CMV/MSV): 4729–5456  
Cytomegalovirus (CMV)/ mouse sarcoma virus (MSV) hybrid promoter: 4729–5239  
R region: 5312–5382  
U5 region: 5384–5456
- $\Psi^+$  (extended packaging signal): 5486–6295
- Human U6 promoter ( $P_{U6}$ ): 6311–6568  
TATA signal: 6540–6545

## Sequencing Primer Location

- U6 Forward Sequencing Primer: 6488–6511  
5'-ATGGACTATCATATGCTTACCGTA-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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2. Kinsella, T. M. & Nolan G. P. (1996) *Hum. Gene Ther.* **7**:1405–1413.
3. Ory, D. S., Neugeboren, B. A. & Mulligan, R. C. (1996) *Proc. Nat. Acad. Sci. USA* **93**:11400–11406.
4. Pear, W. S., Nolan, G. P., Scott, M. L. & Baltimore, D. (1993) *Proc. Natl. Acad. Sci. USA* **90**(18):8392–8396.
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6. 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.

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