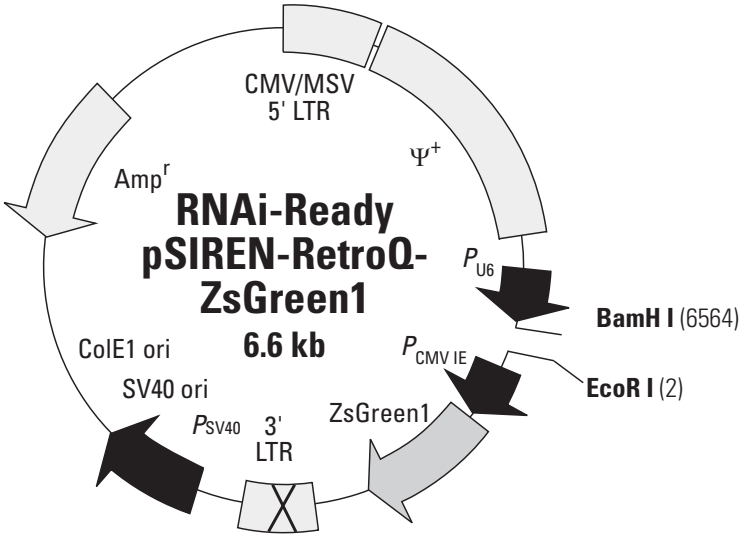
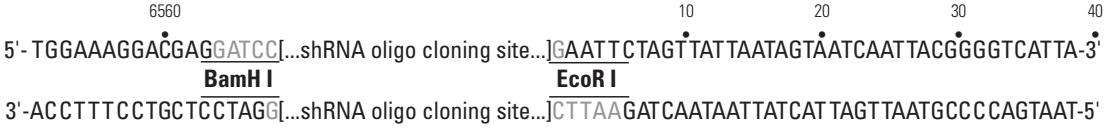
Vector Information

RNAi-Ready pSIREN-RetroQ-ZsGreen1 Vector Information PT3777-5

Cat. No. 632455





Restriction Map and Cloning Site of RNAi-Ready pSIREN-RetroQ-ZsGreen1 Retroviral Vector. Unique restriction sites are in bold. RNAi-Ready pSIREN-RetroQ-ZsGreen1 is provided as a linearized vector digested with BamHI and EcoRI. Nucleotides in gray were removed during linearization. This linearized vector is ready for ligation of an appropriate shRNA containing BamHI and EcoRI overhangs.

Description

RNAi-Ready pSIREN-RetroQ-ZsGreen1 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-ZsGreen1 is provided as a linearized vector digested with BamHI and EcoRI. 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-ZsGreen1 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 (ZsGreen1; 1), which has been engineered for brighter fluorescence (excitation maximum = 496 nm; emission maximum = 506 nm). The ZsGreen1 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 ZsGreen1 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 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

Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

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-ZsGreen1 also contains a bacterial origin of replication and *E. coli* Amp^r gene for propagation and selection in bacteria.

Use

RNAi-Ready pSIREN-RetroQ-ZsGreen1 is used for targeted gene silencing when a ds DNA oligonucleotide encoding an appropriate shRNA is inserted. To construct recombinant pSIREN-RetroQ-ZsGreen1, 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'-BamHI and 3'-EcoRI overhangs. Then ligate the annealed oligonucleotide into RNAi-Ready pSIREN-RetroQ-ZsGreen1.

Your pSIREN-RetroQ-ZsGreen1 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-ZsGreen1 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 ZsGreen1 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
- ZsGreen1 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 (β-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
- Ψ^+ (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: 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

References

1. Matz, M. V., *et al.* (1999) *Nature Biotech.* **17**:969–973.
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.
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**:123–132.
6. Emerman, M. & Temin, H. M. (1984) *Cell* **39**:449–467.

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.