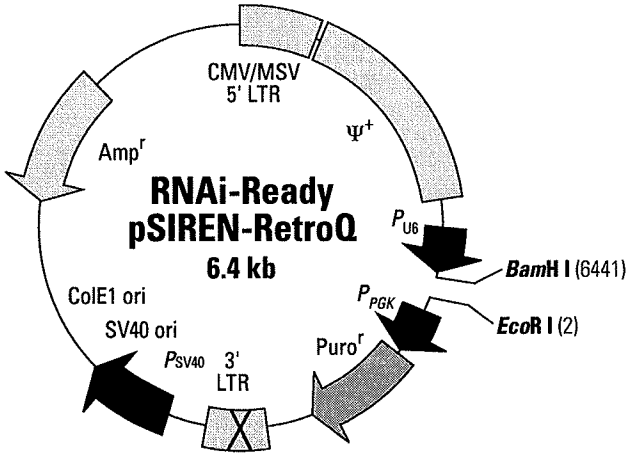

6430 6440 10 20 30 40

5'-TGTGGAAAGGACGAGGATCC[...shRNA oligo cloning site...]GAATTCTACCGGGTAGGGGAGGCGCTTTTCCCAAGGCAGT-3'

BamH I **EcoR I**

3'-ACACCTTTCCTGCTCCTAGG[...shRNA oligo cloning site...]CTTAAGATGGCCCATCCCCTCCGCGAAAAGGGTTCCGTCA-5'

Restriction Map and Cloning Site of RNAi-Ready pSIREN-RetroQ Retroviral Vector. Unique restriction sites are in bold. RNAi-Ready pSIREN-RetroQ 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 containing *Bam*H I and *Eco*R I overhangs.

Description

RNAi-Ready pSIREN-RetroQ 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 is provided as a linearized vector digested with *Bam*H I and *Eco*R I. It is used for targeted gene silencing when an oligonuceotide encoding an appropriate shRNA is ligated into the vector. You can transfect your pSIREN-RetroQ 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. The vector contains a puromycin resistance gene 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 (1–4) 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 (5) 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. RNAi-Ready pSIREN-RetroQ also contains a bacterial origin of replication and *E. coli* Amp^r gene for propagation and selection in bacteria.

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