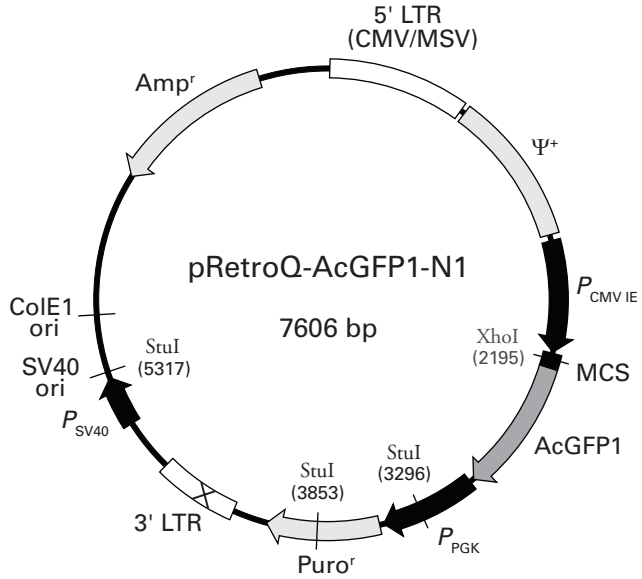
Vector Information

pRetroQ-AcGFP1-N1 Vector Information PT3879-5

Catalog No. 632505



EcoRI

NheI XhoI BstBI

2160 CCG CTA GCG CTA CCG GAC TCA GAT CTC GAG CTC AAG CTT CGA ATT CTG

SalI ApaI BamHI Start of AcGFP1

2218 CAG TCG ACG GTA CCG CGG GCC CGG GAT CCA CCG GTC ATG GTG AGC

pRetroQ-AcGFP1-N1 Vector Map and Multiple Cloning Site (MCS).

Description

pRetroQ-AcGFP1-N1 is a high-titer, self-inactivating retroviral vector that facilitates efficient delivery and expression of AcGFP1, as well as N terminal fusions of AcGFP1, to target cells. AcGFP1 is the green fluorescent protein from *Aequorea coerulescens* (AcGFP1; Excitation maximum = 475 nm; emission maximum = 505 nm).

The coding sequence of the AcGFP1 gene contains silent base changes, which correspond to human codon-usage preferences (1). The MCS in pRetroQAcGFP1-N1 lies between the immediate early promoter of CMV ($P_{CMV\ IE}$) and the AcGFP1 coding sequences. Genes cloned into the MCS are expressed as fusions to the N-terminus of AcGFP1 when they are in the same reading frame as AcGFP1 and there are no intervening stop codons.

The RetroQ retroviral vector backbone incorporates several unique features. This vector contains a puromycin resistance cassette (Puro^r) driven by the PGK promoter (P_{PGK}) for selection of positively-infected cells (2).The hybrid 5′ long terminal repeat (LTR) consists of the CMV type I enhancer and the murine sarcoma virus (MSV) promoter. This vector demonstrates high levels of transcription in HEK 293-based packaging cell lines due, in part, to the presence of adenoviral E1A (3–6) 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 CMV enhancer sequences. This can reduce the phenomenon known as promoter interference (7) and allow more efficient expression.

Additionally, the viral genomic transcript contains the necessary viral RNA processing elements, including the LTRs, packaging signal (ψ^+), and tRNA primer binding site. pRetroQ-AcGFP1-N1 contains a bacterial origin of replication, an *E. coli* Amp^r gene for propagation and selection in bacteria, and an SV40 origin for replication in mammalian cells expressing the SV40 large T antigen.

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Use

pRetroQ-AcGFP1-N1 is designed to efficiently deliver and express fusions to the N terminus of AcGFP1 into primary cells or cells that are difficult to transfect. Fusions to the N terminus of AcGFP1 retain the fluorescent properties of the native protein, allowing the *in vivo* localization of the fusion protein. The target gene should be cloned into pRetroQ AcGFP1-N1 so that it is in frame with the AcGFP1 coding sequences, with no intervening in-frame stop codons. The inserted gene should include the initiating ATG codon. The recombinant AcGFP1 vector can be infected or transfected into mammalian cells. If required, stable transformants can be selected using puromycin. pRetroQ-AcGFP1-N1 can also be used simply to express AcGFP1 in a cell line of interest (e.g., as an infection marker).

Once pRetroQ-AcGFP1-N1 is transfected into a packaging cell line (such as the RetroPack™ PT67 Cell line (Cat. No. 631510), AmphoPack™ -293 (Cat. No. 631505), EcoPack™ 2-293 (Cat. No. 631507), Pantropic Expression System (Cat. No. 631512), or Retro-X™ Universal Packaging System (Cat. No. 631530)), RNA from the vector is packaged into non-infectious, replication-incompetent retroviral particles, since pRetroQ-AcGFP1-N1 lacks the structural genes (gag, pol, and env) necessary for particle formation and replication. These genes, however, are stably integrated as part of the packaging cell genome. Once a high-titer supernatant is produced, 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): 1–727
  - Cytomegalovirus (CMV)/mouse sarcoma virus (MSV) hybrid promoter: 1–510
  - R region: 583–653
  - U5 region: 654–727
- ψ^+ (extended packaging signal): 757–1566
- $P_{CMV\ IE}$ (cytomegalovirus immediate early promoter): 1582–2170
- MCS (multiple cloning cite): 2172–2246
- AcGFP1 (*Aequorea coerulescens* green fluorescent protein gene): 2254–2970
  - Start codon: (ATG): 2254–2256
  - Stop codon: 2968–2970
- P_{PGK} (PGK promoter): 2992–3500
- Puro^r (puromycin resistance gene): 3521–4120
- 3' LTR (MMLV; deletion in U3): 4305–4737
  - PolyA signal: 4563–4578
- P_{SV40} (SV40 promoter): 5017–5284
- SV40 origin of replication: 5238–5303
- ColE1 origin of replication: 5624
- Amp^r (ampicillin resistance gene; β-lactamase): 6384–7244 (complementary)

Propagation in *E. coli*

- Suitable host strains: DH5α™, Fusion Blue, and other general-purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: ColE1
- Copy number: low

NOTE: 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.

References

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