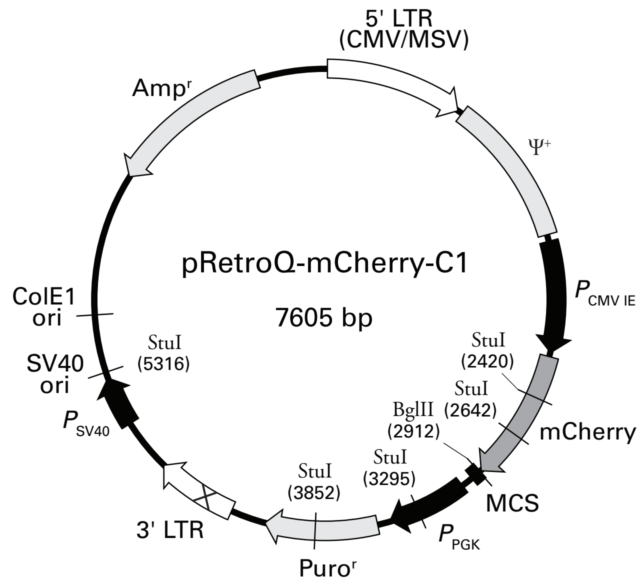
pRetroQ-mCherry-C1 Vector Information

PT5097-5

Catalog No. 632567

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                                         BglII                                  EcoRI
        End of mCherry                            XhoI                  BstBI
2893    CTG  TAC  AAG  TCC  GGA  CTC  AGA  TCT  CGA  GCT  CAA  GCT  TCG  AAT  TCT  GCA

         SalI                    ApaI         BamHI                  Stop   Stop   Stop
2938    GTC  GAC  GGT  ACC  GCG  GGC  CCG  GGA  TCC  ACC  GGA  TCT  AGA  TAA  CTG  ATC
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pRetroQ-mCherry-C1 Vector Map and Multiple Cloning Site (MCS).

Description

pRetroQ-mCherry-C1 is a high-titer, self-inactivating retroviral vector that facilitates efficient delivery and expression of mCherry, or C-terminal mCherry fusions, to target cells. mCherry is a mutant fluorescent protein derived from the tetrameric *Discosoma sp.* red fluorescent protein, DsRed (1). The excitation and emission maxima of the native mCherry protein are 587 nm and 610 nm, respectively.

The multiple cloning site (MCS) in pRetroQ-mCherry-C1 is located just downstream of the mCherry coding sequence. Genes cloned into the MCS are expressed as C-terminal mCherry fusion proteins when they are in the same reading frame as mCherry 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 repeats (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.

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Additionally, the viral genomic transcript contains the necessary viral RNA processing elements, including the LTRs, packaging signal (ψ^+), and tRNA primer-binding site. pRetroQ-mCherry-C1 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.

Use

pRetroQ-mCherry-C1 is designed to efficiently deliver and express C-terminal mCherry fusions into primary cells or cells that are difficult to transfect. C-terminal mCherry fusion proteins retain the fluorescent properties of the native protein, allowing the *in vivo* localization of the fusion protein. The gene of interest should be cloned into pRetroQ-mCherry-C1 so that it is in frame with the mCherry coding sequence. The inserted sequence does not require an initiation codon (ATG) or a stop codon (TAA, TAG, TGA); however, if you don't want to use the stop codons downstream of the MCS (see map), you can add a stop codon to the end of your gene of interest. The recombinant mCherry vector can be transduced or transfected into mammalian cells. If required, stable transformants can be selected using puromycin. pRetroQ-mCherry-C1 can also be used simply to express mCherry in a cell line of interest (e.g., as an infection marker).

Before pRetroQ-mCherry-C1 can be transduced into mammalian cells, it must be 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)). The packaging cell line supplies the viral structural genes (gag, pol, and env) necessary for particle formation and replication that pRetroQ-mCherry-C1 lacks, allowing RNA from the vector to be packaged into non-infectious, replication-incompetent retroviral particles. Once a high-titer supernatant is produced, these retroviral particles can infect target cells and transmit the gene of interest, but they cannot replicate within the target 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 caused by recombination events during cell proliferation.

Location of features

- 5' LTR (CMV/MSV): 1–728
- Ψ^+ (extended packaging signal): 757–1566
- $P_{CMV\ IE}$ (human cytomegalovirus immediate-early promoter): 1582–2170
- mCherry fluorescent protein gene: 2194–2901
  - Start codon: (ATG): 2194–2196
  - Last codon: 2899–2901
- MCS (multiple cloning site): 2911–2967
- P_{PGK} (PGK promoter): 2991–3499
- Puro^r (puromycin resistance gene): 3520–4119
- 3' LTR (MMLV; deletion in U3): 4304–4736
  - PolyA signal: 4562–4577
- P_{SV40} (SV40 promoter): 5016–5283
- SV40 origin of replication: 5237–5302
- ColE1 origin of replication: 5623
- Amp^r (ampicillin resistance gene; β-lactamase): 6383–7243 (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

Excitation and emission maxima of mCherry

- Excitation maximum = 587 nm
- Emission maximum = 610 nm

Notes:
The vector sequence was 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.

References

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