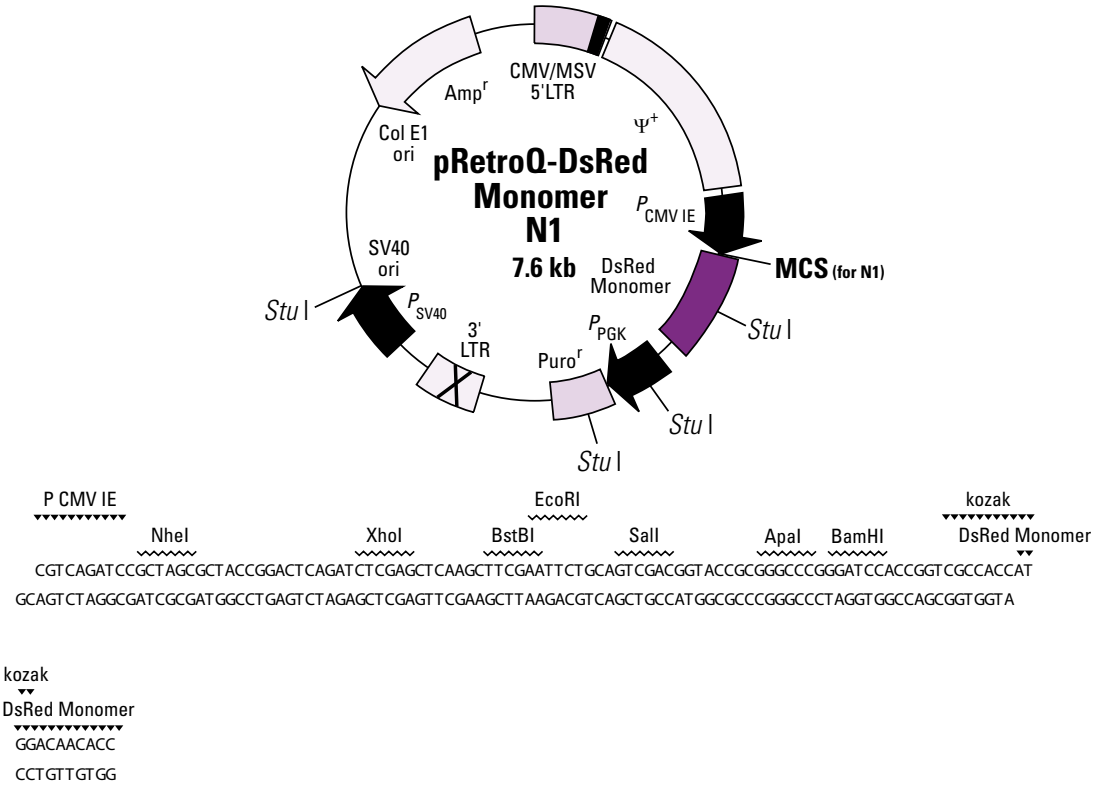
Vector Information

pRetroQ-DsRed Monomer-N1 Vector Information PT3881-5

Catalog No. 632507



Restriction Map and Multiple Cloning Site (MCS) of pRetroQ-DsRed Monomer-N1 Vector. All sites shown are unique.

Description

pRetroQ-DsRed Monomer-N1 is a high-titer, self-inactivating retroviral vector that facilitates efficient delivery and expression of DsRed-monomer (DsRed.M1) as well as N terminal fusions of DsRed monomer to target cells. DsRed.M1 is a monomeric mutant derived from the tetrameric *Discosoma* sp. red fluorescent protein DsRed (1).

DsRed-Monomer contains forty-five amino acid substitutions. When DsRed-Monomer is expressed in mammalian cell cultures, red fluorescent cells can be detected by either fluorescence microscopy or flow cytometry 12–16 hours after transfection or 24–48 hours after infection (DsRed-Monomer excitation and emission maxima = 557 nm and 592 nm, respectively. The DsRed-Monomer coding sequence is human codon-optimized for high expression in mammalian cells (2) The MCS in pRetroQ-DsRed Monomer-N1 lies between the immediate early promoter of CMV (PCMV IE) and the DsRed-Monomer coding sequences. Genes cloned into the MCS are expressed as fusions to the N-terminus of DsRed Monomer when they are in the same reading frame as DsRed Monomer 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 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-

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DsRed Monomer-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 T antigen.

Use

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

Once pRetroQ-DsRed Monomer-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-DsRed Monomer-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): 1–728
  - Cytomegalovirus (CMV)/mouse sarcoma virus (MSV) hybrid promoter: 1–511
  - R region: 584–654
  - U5 region: 655–728
- ψ+ (extended packaging signal): 758–1567
- Immediate early CMV promoter ($P_{CMV\ IE}$): 1583–2171
- Multiple Cloning Site: 2173–2247
- Human codon-optimized DsRed-Monomer gene: 2261–2938
  - Start codon (ATG): 2261–2263
  - Stop codon: 2936–2938
- PGK promoter: 2958–3466
- Puromycin resistance gene (Puro^r): 3487–4086
- 3' MoMuLV LTR (deletion in U3): 4271–4703
  - PolyA region: 4529–4544
- SV40 promoter: 4983–5250
- SV40 ori: 5204–5269
- ColE1 plasmid replication region
  - Site of replication initiation: 5590
- Ampicillin resistance gene (β-lactamase): 6350–7210 (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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