Certificate of Analysis

pRetroX-SG2Mcyto-Red Vector

Contents

Catalog No.	Amount	Lot Number
631464	10 µg	Specified on product label.

Product Information

pRetroX-SG2Mcyto-Red is a self-inactivating retroviral vector that expresses mCherry-hGeminin (1-60), a fluorescent, ubiquitination–based, cell-cycle indicator (Fucci; 1). The vector allows you to identify cells that are transitioning between S, G2, and M phases.

Package Contents

- 20 µl pRetroX-SG2Mcyto-Red Vector (500 ng/µl)

Storage Conditions

- Store plasmids at –20°C.
- Spin briefly to recover contents.
- Avoid repeated freeze/thaw cycles.

Shelf Life

- 1 year from date of receipt under proper storage conditions.

Storage Buffer

- 10 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0)

Shipping Conditions

- Domestic: Blue ice (4°C)
- International: Dry ice (–70°C)

Product User Manuals

User manuals for Clontech products are available for download at **www.clontech.com/manuals**
The following user manual applies to this product:

- Retroviral Gene Transfer and Expression User Manual (PT3132-1).

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Avenue, Mountain View, CA 94043, USA
U.S. Technical Support: tech@clontech.com

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

(PA094131)

Vector Information

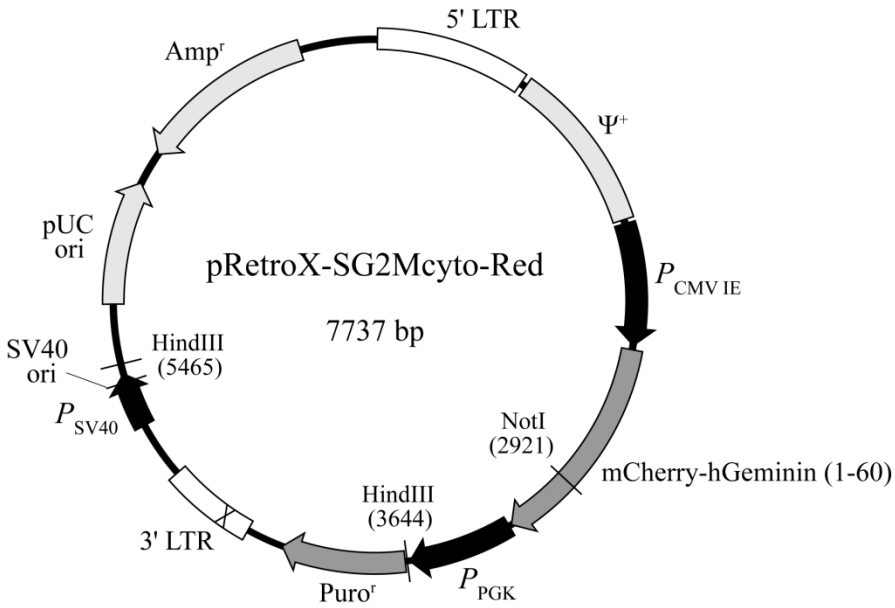

Figure 1. pRetroX-SG2Mcyto-Red Vector Map.

Description

pRetroX-SG2Mcyto-Red is a high-titer, self-inactivating retroviral vector that facilitates efficient delivery and expression of the cell cycle indicator hGeminin (1-60) tagged with the red fluorescent protein mCherry (2). Constitutive expression of the mCherry-hGeminin (1-60) fusion is driven by the cytomegalovirus immediate early promoter ($P_{CMV\ IE}$). The fusion protein is localized in the nucleus and the cytoplasm, and is present at high levels during the S, G2 and M phases of the cell cycle. At the end of M phase, Geminin is ubiquitinated, and as a result, the fusion protein is subjected to proteasomal degradation. The presence and absence of red fluorescence can be used to monitor the S, G2 and M phases of the cell cycle. mCherry is a mutant fluorescent protein derived from the tetrameric *Discosoma sp.* red fluorescent protein, DsRed (excitation and emission maxima: 587 nm and 610 nm, respectively; 3).

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 (4). The hybrid 5′ long terminal repeat (LTR) consists of the CMV type I enhancer and the murine sarcoma virus (MSV) promoter. The vector demonstrates high levels of transcription in HEK 293-based packaging cell lines due, in part, to the presence of adenoviral E1A (5–8) 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 (9) 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. pRetroX-SG2Mcyto-Red 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.

Location of Features

- 5' LTR (CMV/MSV): 1–727
- Ψ^+ (extended packaging signal): 757–1566
- $P_{CMV\ IE}$ (human cytomegalovirus immediate-early promoter): 1582–2170
- mCherry-hGeminin (1–60) (mCherry and hGeminin gene fusion): 2194–3111
- P_{PGK} (murine phosphoglycerate kinase promoter): 3123–3631
- Puro^r (puromycin resistance gene): 3652–4251
- 3' LTR (MMLV; deletion in U3): 4436–4868
- PolyA signal: 4694–4709
- P_{SV40} (SV40 promoter): 5148–5415
- SV40 origin of replication: 5369–5434
- pUC origin of replication: 5754–6353
- Amp^r (ampicillin resistance gene; β-lactamase): 6515–7375 (complementary)

Additional Information

pRetroX-SG2Mcyto-Red allows the monitoring of cell cycles in live infected cells, as well as the cell cycle phase distribution of a given cell population. Delivery by virus produced from the vector is efficient even in primary cells or cells that are difficult to transfect.

Before pRetroX-SG2Mcyto-Red 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 pRetroX-SG2Mcyto-Red lacks, allowing RNA from the vector to be packaged into non-infectious, replication-incompetent retroviral particles. For more information on generating virus, see the Retroviral Gene Transfer and Expression User Manual (PT3132-1).

NOTE: Overexpression of the fusion protein could lead to insufficient proteosomal degradation, which could prevent effective monitoring of the cell cycle. Therefore, we recommend either (i) optimizing gene transfer conditions to avoid overexpression of the fusion protein, or (ii) selecting cells that express an acceptable amount of the fusion protein.

Propagation in *E. coli*

- Recommended host strain: Stellar™ Competent Cells
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC

Excitation and emission maxima of mCherry

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

References

1. Sakaue-Sawano, A. *et al.* (2008) *Cell* **132**(3):487–498.
2. McGarry, T.J., and Kirschner, M.W. (1998). *Cell* **93**(6):1043–1053.
3. Shaner, N. C. *et al.* (2004) *Nature Biotech.* **22**(12):1567–1572.

4. Gorman, C. (1985). In DNA cloning: A practical approach, vol. II. Ed. D.M. Glover. (IRL Press, Oxford, U.K.) pp. 143–190.
5. Kinsella, T. M. & Nolan G. P. (1996) *Hum. Gene Ther.* **7**(12):1405–1413.
6. Ory, D. S. *et al.* (1996) *Proc. Nat. Acad. Sci. USA* **93**(21):11400–11406.
7. Pear, W. S. *et al.* (1993) *Proc. Natl. Acad. Sci. USA* **90**(18):8392–8396.
8. Yang, S. *et al.* (1999) *Hum. Gene Ther.* **10**(1):123–132.
9. Emerman, M. & Temin, H. M. (1984) *Cell* **39**(3 Pt 2):449–467.

Quality Control Data

Plasmid Identity & Purity

- Digestion with the indicated restriction enzymes produced fragments of the indicated sizes on a 0.8% agarose/EtBr gel:

Enzymes	Fragment Sizes
NotI	7.7 kb
HindIII	1.8 & 5.9 kb

- Vector identity was confirmed by sequencing.
- A_{260}/A_{280}: 1.8–2.0

Notice to Purchaser

This document has been reviewed and approved by the Clontech Quality Assurance Department.