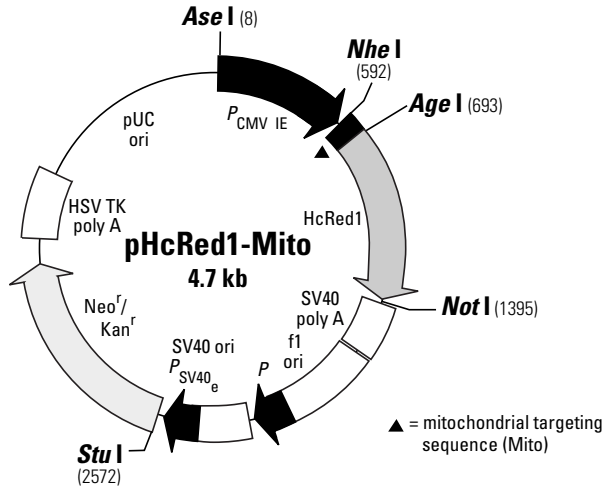
Vector Information

pHcRed1-Mito Vector Information PT3734-5

Cat. No. 632434



Restriction map of pHcRed1-Mito. Unique restriction sites are in bold.

Description

pHcRed1-Mito encodes the far-red fluorescent protein HcRed1 fused with a mitochondrial targeting sequence from the precursor protein of human cytochrome C oxidase subunit VIII (1, 2). HcRed1, whose excitation and emission maxima occur at 588 nm and 618 nm ±4 nm, respectively, is a fluorescent variant of a chromoprotein found in the reef coral *Heteractis crispa* (3). The HcRed1 coding sequence has been human codon-optimized for efficient translation in mammalian cells (4).

Expression of the HcRed1 fusion is driven by the immediate early promoter of cytomegalovirus ($P_{CMV\ IE}$). SV40 polyadenylation signals downstream of the HcRed1 gene direct proper processing of the 3' end of the HcRed1-Mito mRNA transcript. The vector also contains an SV40 origin for replication in any mammalian cell line that expresses the SV40 T-antigen, a pUC origin of replication for propagation in *E. coli*, and an f1 origin for single-stranded DNA production. A neomycin resistance cassette—consisting of the SV40 early promoter (P_{SV40e}), the neomycin/kanamycin resistance gene of Tn5 (Neo^r/Kan^r), and polyadenylation signals from the herpes simplex virus thymidine kinase (HSV TK poly A) gene—allows stably transfected eukaryotic cells to be selected using G418 (5). A bacterial promoter (*P*) upstream of this cassette drives expression of the gene encoding kanamycin resistance in *E. coli*.

Use

pHcRed1-Mito is designed for fluorescent labeling of mitochondria. The plasmid can be introduced into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (5). pHcRed1-Mito is not intended as a cloning vector; however, the backbone does contain unique restriction sites upstream and downstream of the HcRed1-Mito sequence which permit excision of the HcRed1-Mito sequence.

Clontech

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

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

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Location of Features

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  Enhancer region: 59–465; TATA box: 554–560; transcription start point: 583
  C→G mutation to remove *Sac* I site: 569
- HcRed1-Mito fusion: 597–1391
  Start codon: 597–599
  Mitochondrial targeting sequence: 597–683
  Start of HcRed1 coding sequence (ATG): 705–707
  Insertion of Val at position 2: 708–710
  Stop codon: 1389–1391
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1545–1550 & 1574–1579; mRNA 3' ends: 1583 &1595
- f1 single-strand DNA origin: 1642–2097 (Packages the noncoding strand of HcRed1-Mito.)
- Bacterial promoter for expression of Kan^r gene.
  –35 region: 2159–2164; –10 region: 2182–2187; Transcription start point: 2194
- SV40 origin of replication: 2438–2573
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2271–2342, 2343–2414
  21-bp repeats: 2418–2438, 2439–2459 & 2461–2481
  Early promoter element: 2494–2500
  Major transcription start points: 2490, 2528, 2534 & 2539
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2622–2624; stop codon: 3414–3416
  G→A mutation to remove *Pst* I site: 2834
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3150
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3652–3658 & 3665–3670
- pUC plasmid replication origin: 4001–4644

Propagation in *E. coli*

- Suitable host strains: DH5α, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid, such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (50 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/ColE1

References

1. Rizzuto, R., *et al.* (1995) *Curr. Biol.* **5**:635–642.
2. Rizzuto, R., *et al.* (1989) *J. Biol. Chem.* **246**:10595–10600.
3. Gurskaya, N. G., *et al.* (2001) *FEBS Letters* **507**:16–20.
4. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
5. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II,* Ed. Glover, D. M. (IRL Press, Oxford, UK), pp. 143–190.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech Laboratories, Inc. This vector has not been completely sequenced.