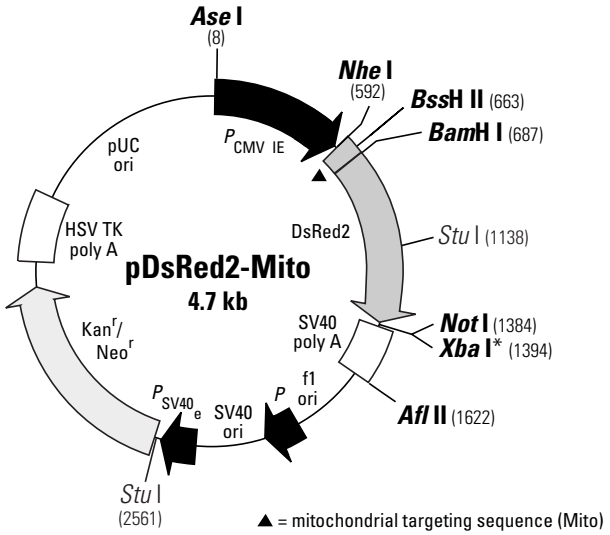
Vector Information

pDsRed2-Mito Vector Information PT3633-5

Cat. No. 632421



Restriction Map of pDsRed2-Mito. Unique restriction sites are shown in bold. The *Xba* I site (*) is methylated in the DNA provided by Clontech. If you wish to digest the vector with this enzyme, you will need to transform the vector into a *dam*⁻ host and isolate fresh DNA.

Description

pDsRed2-Mito is a mammalian expression vector that encodes a fusion of *Discosoma sp.* red fluorescent protein (DsRed2; 1, 2) and the mitochondrial targeting sequence from subunit VIII of human cytochrome c oxidase (Mito; 3, 4). The Mito sequence is fused to the 5'-end of DsRed2, a human codon-optimized DsRed variant that is engineered for faster maturation and lower non-specific aggregation (1, 5). The Mito sequence targets the Mito-DsRed2 fusion protein to the host cell's mitochondria.

To drive expression of Mito-DsRed2, this vector contains the immmediate early promoter of cytomegalovirus ($P_{CMV\ IE}$). SV40 polyadenylation signals downstream of the DsRed2 gene direct proper processing of the 3'-end of the Mito-DsRed2 mRNA. This 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—allow stably transfected eukaryotic cells to be selected using G418 (6). A bacterial promoter (*P*) upstream of this cassette drives expression of the gene encoding kanamycin resistance in *E. coli*.

Use

pDsRed2-Mito is designed for fluorescent labeling of mitochondria. The vector can be introduced into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (6). The Mito-DsRed2 fusion (excitation/emission maxima: 558 nm/583 nm) can be detected by fluorescence microscopy and by flow cytometry. Filter sets optimized for detecting DsRed by microscopy are available from Chroma Technology Corporation and Omega Optical Inc. Please see their websites (**www.chroma.com** and **www.omegafilters.com**) and the Living Colors® Vol. II User Manual, provided with this vector, for more information. To detect Mito-DsRed2-expressing cells by flow cytometry, use the instrument's argon-ion laser to excite the fluorophore at 488 nm and the FL-2 channel to detect the fluorophore's emission at 583 nm.

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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
- Mitochondrial targeting sequence from subunit VIII of human cytochrome c oxidase
  Start codon (ATG): 597–599
  End of targeting sequence: 683
- *Discosoma sp.* red fluorescent protein (DsRed2) gene
  Start codon (ATG): 705–707; stop codon: 1380–1382
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1534–1539 & 1563–1568; mRNA 3' ends: 1572 & 1584
- f1 single-strand DNA origin: 1631–2086 (packages the noncoding strand of Mito-DsRed2)
- Bacterial promoter for expression of Kan^r gene
  –35 region: 2148–2153; –10 region: 2171–2176
  Transcription start point: 2183
- SV40 origin of replication: 2427–2562
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2260–2331 & 2332–2403
  21-bp repeats: 2407–2427; 2428–2448 & 2450–2470
  Early promoter element: 2483–2489
  Major transcription start points: 2479, 2517, 2523 & 2528
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2611–2613; stop codon: 3403–3405
  G→A mutation to remove *Pst* I site: 2793
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3139
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3641–3646 & 3654–3659
- pUC plasmid replication origin: 3990–4633

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/Col E1

References

1. Living Colors DsRed2 (July 2001) *Clontechniques* **XVI**(3):2–3.
2. Matz, M. V., *et al.* (1999) *Nature Biotech.* **17**:969–973.
3. Rizzuto, R., *et al.* (1995) *Curr. Biol.* **5:**635–642.
4. Rizzuto, R., *et al.* (1989) *J. Biol. Chem.* **264:**10595–10600.
5. Haas, J., *et al.* (1996) *Curr. Biol.* **6:**315–324.
6. 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. This vector has not been completely sequenced.

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AcGFP1, DsRed, HcRed, AsRed, AmCyan, ZsGreen, ZsYellow and their variants:

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