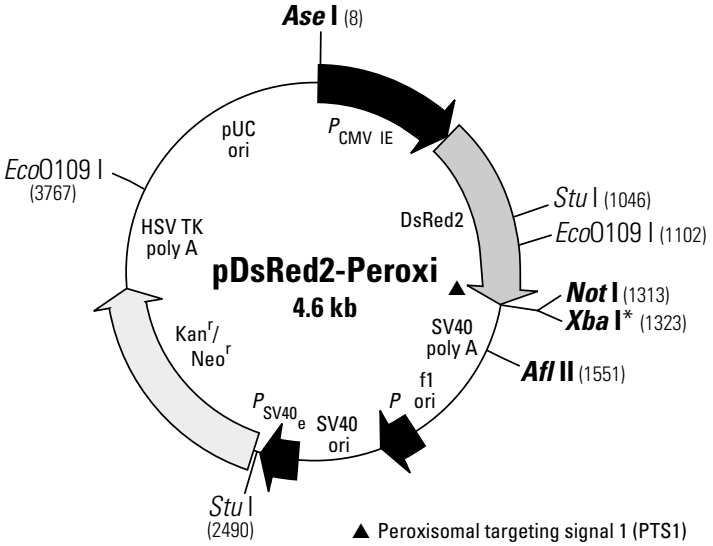

Restriction Map of pDsRed2-Peroxi Vector. Unique restriction sites are shown in bold. The *Not* I site follows the PTS1 stop codon. The *Xba* I site (*) is methylated in the DNA provided by CLONTECH. If you wish to digest the vector with this enzyme, you will need to transform the vector into a *dam*⁻ strain and make fresh DNA.

Description

pDsRed2-Peroxi is a mammalian expression vector that encodes a fusion of *Discosoma sp.* red fluorescent protein (DsRed2; 1, 2) and the peroxisomal targeting signal 1 (PTS1). The PTS1 sequence is fused to the 3'-end of DsRed2, a DsRed variant engineered for faster maturation and lower non-specific aggregation. The PTS1 sequence encodes the tripeptide SKL, which targets the DsRed2-PTS1 fusion protein to the matrix of peroxisomes (3–6).

To drive expression of DsRed2-PTS1, this vector contains the immediate early promoter of cytomegalovirus ($P_{CMV\ IE}$). SV40 polyadenylation signals downstream of the DsRed2 gene direct proper processing of the 3'-end of the DsRed2-PTS1 mRNA transcript. Because it encodes DsRed2, a gene variant that uses human-preferred codons (7), the DsRed2-PTS1 transcript is suited for efficient translation in mammalian cells. To further increase the translational efficiency of DsRed2-PTS1, upstream sequences have been converted to a Kozak consensus translation initiation site (8). 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—allow stably transfected eukaryotic cells to be selected using G418 (9). A bacterial promoter (*P*) upstream of this cassette drives expression of the gene encoding kanamycin resistance in *E. coli*.

Use

pDsRed2-Peroxi is designed for fluorescent labeling of peroxisomes. The vector can be introduced into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (9). The DsRed2-PTS1 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 DsRed2-PTS1-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.

(PR19170; published 10 September 2001)

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
- DsRed2-PTS1 gene fusion
  Start codon (ATG): 613–615; Stop codon: 1309–1311
  Kozak consensus translation initiation site: 606–616
  Discosoma sp. Red Fluorescent Protein (DsRed2) gene: 613–1287
  Peroxisomal Targeting Signal (PTS1): 1300–1308
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1463–1468 & 1492–1497; mRNA 3' ends: 1501 & 1513
- f1 single-strand DNA origin: 1560–2015 (Packages the noncoding strand of DsRed2-PTS1.)
- Bacterial promoter for expression of Kan^r gene:
  –35 region: 2077–2082; –10 region: 2100–2105; Transcription start point: 2112
- SV40 origin of replication: 2356–2491
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2188–2260 & 2261–2332
  21-bp repeats: 2336–2356, 2357–2377 & 2379–2399
  Early promoter element: 2412–2418; Major transcription start points: 2408, 2446, 2452 & 2457
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences: start codon (ATG): 2540–2542; stop codon: 3332–3335
  G→A mutation to remove *Pst* I site: 2722
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3068
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3570–3575 & 3583–3588
- pUC plasmid replication origin: 3919–4562

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 JM101 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. Living Colors DsRed2 (July 2001) *CLONTECHniques* **XVI**(3):2–3.
2. Matz, M. V., *et al.* (1999) *Nature Biotech.* **17**:969–973.
3. Gould, S. J., *et al.* (1989) *J. Cell Biol.* **108**:1657–1664.
4. Gould, S. J., *et al.* (1990) *EMBO J.* **9**:85–90.
5. Monosov, E. Z., *et al.* (1996) *J. Histochem. Cytochem.* **44**:581–589.
6. Wiemer, E. A., *et al.* (1997) *J. Cell Biol.* **136**:71–80.
7. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
8. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
9. Gorman, C. (1985). In *DNA cloning: A practical approach, vol. II.* Ed. D. M. Glover. (IRL Press, Oxford, U.K.) 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.