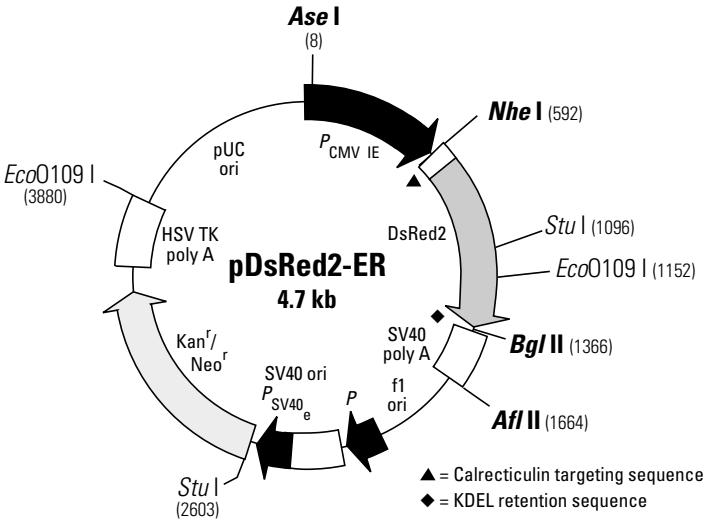

Plasmid Map of pDsRed2-ER. Unique restriction sites are shown in bold.

Description

pDsRed2-ER is a mammalian expression vector that encodes a fusion consisting of *Discosoma sp.* red fluorescent protein (DsRed2; 1, 2); the endoplasmic reticulum (ER) targeting sequence of calreticulin (3), fused to the 5' end of DsRed2; and the ER retention sequence, KDEL (4, 5), fused to the 3' end of DsRed2—a human codon-optimized (6) DsRed variant engineered for faster maturation and lower non-specific aggregation.

To drive expression of DsRed2, 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 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 (7). A bacterial promoter (*P*) upstream of this cassette drives expression of the gene encoding kanamycin resistance in *E. coli*.

Use

pDsRed2-ER is designed for fluorescent labeling of the endoplasmic reticulum in living cells (8, 9). The vector can be introduced into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (7). DsRed2 (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-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.

(PR1X546; published 31 October 2001)

Location of features

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  Enhancer region: 59–465; TATA box: 554–560
  Transcription start point: 583
- Calreticulin signal sequence: 597–647
- *Discosoma sp.* red fluorescent protein (DsRed2) gene: 663–1337
  KDEL coding sequence (in frame with DsRed2): 1350–1361
  Stop codon: 1362–1364
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1576–1581 & 1605–1610; mRNA 3' ends: 1614 & 1626
- f1 single-strand DNA origin: 1673–2128 (packages the noncoding strand of DsRed2-ER)
- Bacterial promoter for expression of Kan^r gene
  –35 region: 2190–2195; –10 region: 2213–2218
  Transcription start point: 2225
- SV40 origin of replication: 2469–2604
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2302–2373 & 2374–2445
  21-bp repeats: 2449–2469, 2470–2490 & 2492–2512
  Early promoter element: 2525–2531
  Major transcription start points: 2521, 2559, 2565 & 2570
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2653–2655; stop codon: 3445–3447
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3683–3688 & 3696–3701
- pUC plasmid replication origin: 4032–4675

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

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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.