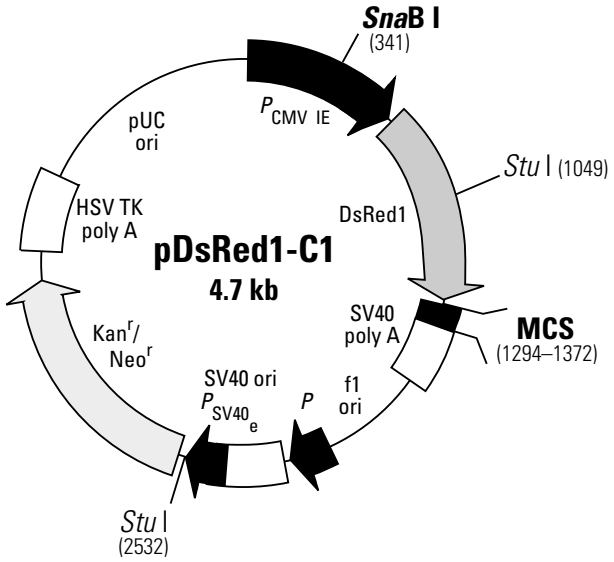

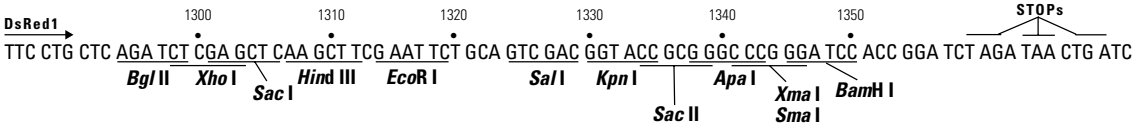

Restriction Map and Multiple Cloning Site (MCS) of pDsRed1-C1. Unique restriction sites are in bold.

Description:

pDsRed1-C1 encodes a novel red fluorescent protein (DsRed1; 1) that has been optimized for high expression in mammalian cells (excitation maximum = 558 nm; emission maximum = 583 nm.). Red fluorescent protein was originally isolated from an IndoPacific sea anemone relative, *Discosoma sp.*; DsRed1's coding sequence contains 144 silent base pair changes, which correspond to human codon-usage preferences for high expression in mammalian cells (2). A nucleotide sequence upstream of DsRed1 has been converted to a Kozak consensus translation initiation site (3) to further increase the translation efficiency in eukaryotic cells. The multiple cloning site (MCS) in pDsRed1-C1 is positioned between the DsRed1 coding sequence and the SV40 polyadenylation signal (SV40 poly A). Genes cloned into the MCS will be expressed as fusions to the C-terminus of DsRed1 if they are in the same reading frame as DsRed1 and there are no intervening stop codons. SV40 poly A signals downstream of the MCS direct proper processing of the 3' end of mRNA transcripts. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T-antigen. A neomycin resistance cassette (Neo^r), consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK) gene, allows stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli*. The pDsRed1-C1 backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use:

pDsRed1-C1 can be used to construct fusions to the C-terminus of DsRed1. If a fusion construct retains the fluorescent properties of the native DsRed1 protein, its expression and localization *in vivo* can be monitored by fluorescence microscopy or flow cytometry. The target gene should be cloned into pDsRed1-C1 so that it is in frame with the DsRed1 coding sequences, with no intervening in-frame stop codons. The recombinant DsRed1 vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (4). pDsRed1-C1 can also be used simply to express DsRed1 in a cell line of interest (e.g., as a cotransfection marker).

(PR01605; published 15 December 1999)

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
- *Discosoma sp.* human codon-optimized Red Fluorescent Protein (DsRed1) gene
  Kozak consensus translation initiation site: 606–616
  Start codon (ATG): 613–615; Stop codon: 1363–1365
  Insertion of Val at position 2: 616–618
  Last amino acid in DsRed1: 1288–1290
- MCS: 1294–1369
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1505–1510 & 1534–1539; mRNA 3' ends: 1543 & 1655
- f1 single-strand DNA origin: 1602–2057 (Packages the noncoding strand of DsRed1.)
- Bacterial promoter for expression of Kan^r gene
  –35 region: 2119–2124; –10 region: 2142–2147
  Transcription start point: 2154
- SV40 origin of replication: 2398–2533
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2231–2302 & 2303–2374
  21-bp repeats: 2378–2398, 2399–2419, & 2421–2441
  Early promoter element: 2454–2460
  Major transcription start points: 2450, 2488, 2494 & 2499
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2582–2584; stop codon: 3374–3376
  G→A mutation to remove *Pst* I site: 2764
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3110
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3612–3617 & 3625–3630
- pUC plasmid replication origin: 3961–4604

Primer Locations:

- DsRed1-N Sequencing Primer (#6482-1): 816–796
- DsRed1-C Sequencing Primer (#6483-1): 1208–1231

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 (25 μg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ≈500
- Plasmid incompatibility group: pMB1/Col E1

References:

1. Matz, M. V., *et al.* (1999) *Nature Biotech.* **17**:969–973.
2. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
4. 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.