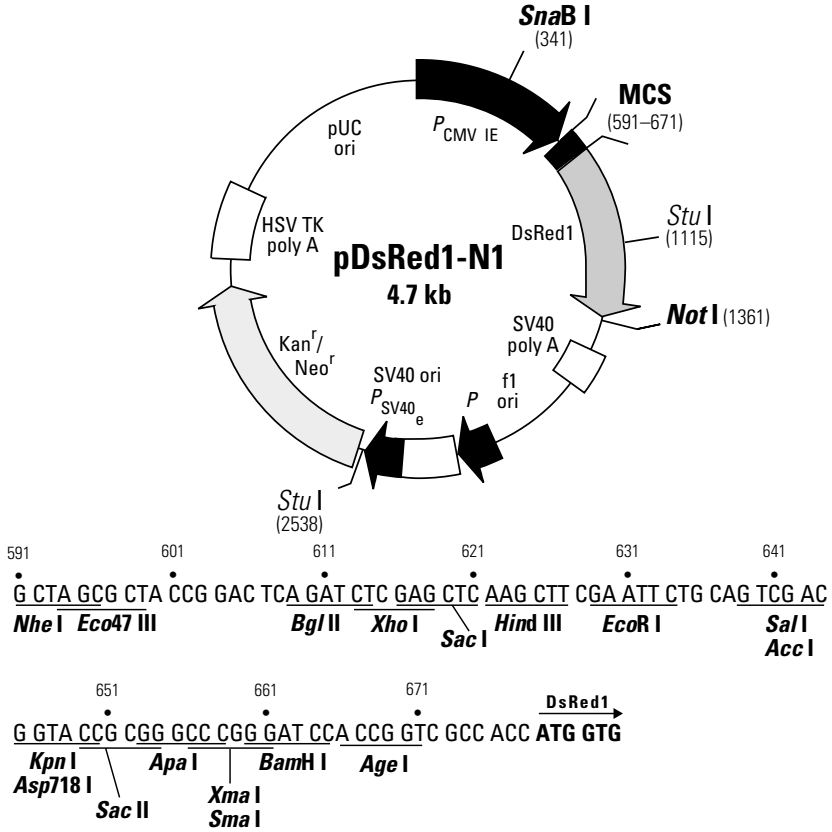

Restriction Map and Multiple Cloning Site (MCS) of pDsRed1-N1 Vector. Unique restriction sites are in bold. The *Not* I site follows the DsRed1 stop codon.

Description

pDsRed1-N1 encodes a novel red fluorescent protein (RFP; 1) that has been optimized for high expression in mammalian cells (excitation maximum = 558 nm; emission maximum = 583 nm). RFP was 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). Sequences upstream of DsRed1 have been converted to a Kozak consensus translation initiation site (3) to increase translation efficiency in eukaryotic cells. The MCS is between the immediate early promoter of CMV ($P_{CMV\ IE}$) and the DsRed1 coding sequence. Genes cloned into the MCS as described below are expressed as fusions to the N-terminus of DsRed1. SV40 polyadenylation signals downstream of the DsRed1 gene direct proper processing of the 3' end of the DsRed1 mRNA. The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 T antigen. A neomycin-resistance cassette (Neo^r) allows stably transfected eukaryotic cells to be selected using G418. This cassette consists 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. A bacterial promoter upstream of the cassette confers kanamycin resistance to *E. coli*. The pDsRed1-N1 backbone also has a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use

Fusions to the N terminus of DsRed1 typically do not alter the fluorescence properties of native DsRed1, allowing *in vivo* localization of the fusion protein.The target gene should be cloned into pDsRed1-N1 in frame with the DsRed1 coding sequence, with no intervening in-frame stop codons. The inserted gene should include an initiating ATG codon. Recombinant pDsRed1-N1 can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418 (4). Unmodified pDsRed1-N1 can also be used to express DsRed1 in a cell line of interest (*e.g.*, for use as a transfection marker).

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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
- MCS: 591–671
- *Discosoma sp.* Red Fluorescent Protein (DsRed1) gene
  Kozak consensus translation initiation site: 672–682
  Start codon (ATG): 679–681; Stop codon: 1357–1359
  Insertion of Val at position 2: 682–684
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1511–1516 & 1540–1545; mRNA 3' ends: 1549 & 1561
- f1 single-strand DNA origin: 1608–2063 (Packages the noncoding strand of DsRed1.)
- Bacterial promoter for expression of Kan^r gene:
  –35 region: 2125–2130; –10 region: 2148–2153
  Transcription start point: 2160
- SV40 origin of replication: 2404–2539
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2237–2308 & 2309–2380
  21-bp repeats: 2384–2404, 2405–2425 & 2427–2447
  Early promoter element: 2460–2466
  Major transcription start points: 2456, 2494, 2500 & 2505
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences: start codon (ATG): 2588–2590; stop codon: 3380–3382
  G→A mutation to remove *Pst* I site: 2770
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3116
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3618–3623 & 3631–3636
- pUC plasmid replication origin: 3967–4610

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 (30 μg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ≈500
- Plasmid incompatibility group: pMB1/ColE1

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.