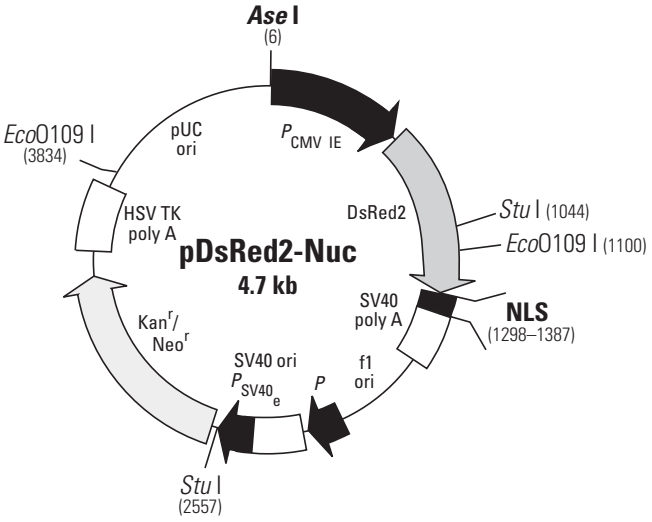
Vector Information

pDsRed2-Nuc Vector Information PT3641-5

Cat. No. 632408



Plasmid map and nuclear localization signal of pDsRed2-Nuc. NLS = three tandem repeats of the nuclear localization signal from simian virus large T-antigen. Unique restriction sites are shown in bold.

Description

pDsRed2-Nuc is a mammalian expression vector that encodes *Discosoma sp.* red fluorescent protein (DsRed2; 1, 2) fused with three copies of the nuclear localization signal (NLS) of the simian virus 40 large T-antigen (3, 4). The NLS sequences are fused to the 3'-end of DsRed2—a human codon-optimized DsRed variant engineered for faster maturation and lower non-specific aggregation (1, 5). The reiteration of the NLS sequence significantly increases the efficiency with which the DsRed2 fusion translocates into the nucleus of mammalian cells (6).

To drive expression of the DsRed2 fusion, 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-NLS mRNA transcript. To further increase the translational efficiency of the DsRed2 fusion, upstream sequences have been converted to a Kozak consensus translation initiation site (7). 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 (8). A bacterial promoter (*P*) upstream of this cassette drives expression of the gene encoding kanamycin resistance in *E. coli*.

Use

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

(PR063554; published 29 June 2010)

Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

Location of features

- Human cytomegalovirus (CMV) immediate early promoter: 1–587
  Enhancer region: 57–463; TATA box: 552–553; transcription start point: 583
  C→G mutation to remove *Sac* I site: 567
- *Discosoma sp.* red fluorescent protein (DsRed2) gene
  Kozak consensus translation initiation site: 604–614
  Start codon (ATG): 611–613 Stop codon: 1388–1390
  Last amino acid in DsRed2 coding region: 1283–1285
- Tandem repeat of the nuclear localization signal (NLS): 1298–1387
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1530–1535 & 1559–1564; mRNA 3' ends: 1568 & 1580
- f1 single-strand DNA origin: 1627–2082 (Packages the noncoding strand of DsRed2-Nuc.)
- Bacterial promoter for expression of Kan^r gene.
  –35 region: 2144–2149; –10 region: 2167–2172
  Transcription start point: 2179
- SV40 origin of replication: 2423–2558
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2256–2327 & 2328–2399
  21-bp repeats: 2403–2423, 2424–2444 & 2446–2466
  Early promoter element: 2479–2485
  Major transcription start points: 2475, 2513, 2519 & 2524
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2607–2609; stop codon: 3399–3401
  G→A mutation to remove *Pst* I site: 2789
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3135
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3637–3642 & 3650–3655
- pUC plasmid replication origin: 3986–4629

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

1. Living Colors DsRed2 (July 2001) *CLONTECHniques* **XVI**(3):2–3.
2. Matz, M. V., *et al.* (1999) *Nature Biotech.* **17**:969–973.
3. Kalderon, D., *et al.* (1984) *Cell* **39**:499–509.
4. Lanford, R. E., *et al.* (1986) *Cell* **46**:575–582.
5. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
6. Fischer-Fantuzzi, L. & Vesco, C. (1988) *Mol. Cell. Biol.* **8**:5495–5503.
7. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
8. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II,* Ed. Glover, D. M. (IRL Press, Oxford, UK), pp. 143–190.