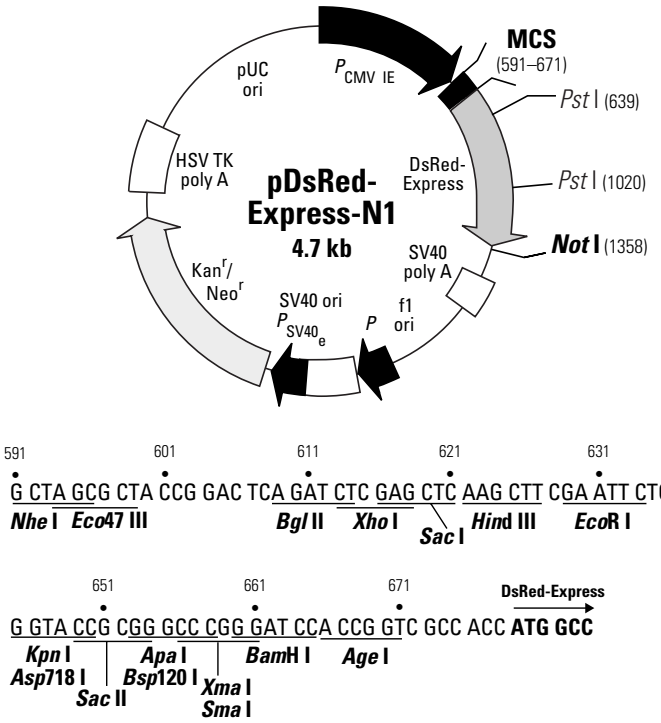
Vector Information

pDsRed-Express-N1 Vector Information PT3725-5

Cat. No. 632429



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

Description

pDsRed-Express-N1 is a mammalian expression vector that encodes DsRed-Express, a variant of *Discosoma sp.* red fluorescent protein (DsRed; 1). DsRed-Express contains nine amino acid substitutions (listed on page 2), which improve the solubility of the protein and reduce the time from transfection to detection of red fluorescence (2). In addition, these substitutions reduce the level of residual green emission (2). When DsRed-Express is expressed in mammalian cell cultures, red-emitting cells can be detected by either fluorescence microscopy or flow cytometry 8–12 hours after transfection (DsRed-Express excitation and emission maxima = 557 nm and 579 nm, respectively). Although DsRed-Express most likely forms the same tetrameric structure as wild-type DsRed, DsRed-Express displays a reduced tendency to aggregate (2). The DsRed-Express coding sequence is human codon-optimized for high expression in mammalian cells (3).

The multiple cloning site (MCS) in pDsRed-Express-N1 is positioned between the immediate early promoter of CMV ($P_{CMV\ IE}$) and the DsRed-Express coding sequence. Genes cloned into the MCS are expressed as fusions to the N-terminus of DsRed-Express if they are in the same reading frame as DsRed-Express and there are no intervening stop codons. Sequences upstream of DsRed-Express have been converted to a Kozak consensus translation initiation site to increase translation efficiency in eukaryotic cells (4). SV40 polyadenylation signals downstream of the DsRed-Express gene direct proper processing of the 3' end of the DsRed-Express mRNA. The vector backbone contains an SV40 origin for replication in mammalian cells expressing 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 (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*.

(PR33592; published 7 March 2003)

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

Use

pDsRed-Express-N1 can be used to construct fusions to the N-terminus of DsRed-Express. If a fusion construct retains the fluorescent properties of the native DsRed-Express protein, its expression can be monitored by flow cytometry and its localization *in vivo* can be determined by fluorescence microscopy. The target gene should be cloned into pDsRed-Express-N1 so that it is in frame with the DsRed-Express coding sequence, with no intervening in-frame stop codons. The inserted gene should include an initiating ATG codon. Recombinant pDsRed-Express-N1 can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418 (5). pDsRed-Express-N1 can also be used as a cotransfection marker; the unmodified vector will express DsRed-Express.

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 (DsRed-Express) gene
  - Kozak consensus translation initiation site: 672–682
  - Start codon (ATG): 679–681; Stop codon: 1354–1356
  - CGC→GCC (Arg-2 to Ala) mutation: 682–684
  - AAG→GAG (Lys-5 to Glu) mutation: 691–693
  - AAC→GAC (Asn-6 to Asp) mutation: 694–696
  - ACC→TCC (Thr-21 to Ser) mutation: 739–741
  - CAC→ACC (His-41 to Thr) mutation: 799–801
  - AAC→CAG (Asn-42 to Gln) mutation: 802–804
  - GTG→GCC (Val-44 to Ala) mutation: 808–810
  - TGC→TCC (Cys-117 to Ser) mutation: 1027–1029
  - ACC→GCC (Thr-217 to Ala) mutation: 1327–1329
- SV40 early mRNA polyadenylation signal
  - Polyadenylation signals: 1508–1513 & 1537–1542; mRNA 3' ends: 1546 & 1558
- f1 single-strand DNA origin: 1605–2060 (Packages the noncoding strand of DsRed-Express.)
- Bacterial promoter for expression of Kan^r gene:
  - –35 region: 2122–2127; –10 region: 2145–2150
  - Transcription start point: 2157
- SV40 origin of replication: 2401–2536
- SV40 early promoter
  - Enhancer (72-bp tandem repeats): 2234–2305 & 2306–2377
  - 21-bp repeats: 2381–2401, 2402–2422 & 2424–2444
  - Early promoter element: 2457–2463
  - Major transcription start points: 2453, 2491, 2497 & 2502
- Kanamycin/neomycin resistance gene
  - Neomycin phosphotransferase coding sequences: Start codon (ATG): 2585–2587; Stop codon: 3377–3379
  - G→A mutation to remove *Pst* I site: 2767
  - C→A (Arg to Ser) mutation to remove *Bss*H II site: 3113
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  - Polyadenylation signals: 3615–3620 & 3628–3633
- pUC plasmid replication origin: 3964–4607

Sequencing primer locations

- DsRed1-N Sequencing Primer (Cat. No. 632387; 5'-GTACTGGAACTGGGGGGACAG-3'): 879–859

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 the JM109 or XL1-Blue strains..
- 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

Excitation and emission maxima of DsRed-Express

- Excitation maximum = 557 nm
- Emission maximum = 579 nm

References

1. Matz, M. V., *et al.* (1999) *Nature Biotech.* **17**:969–973.
2. Bevis, B. J. & Glick B. S. (2002) *Nature Biotech.* **20**:83–87.
3. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
4. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
5. 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 Laboratories, Inc. This vector has not been completely sequenced.