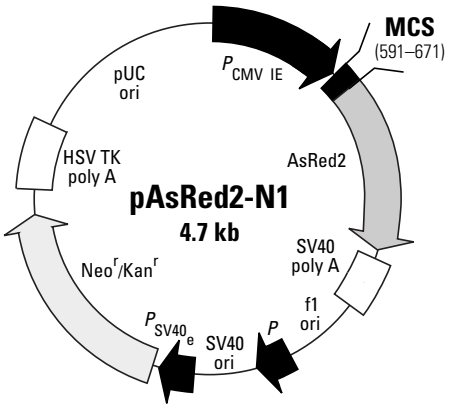
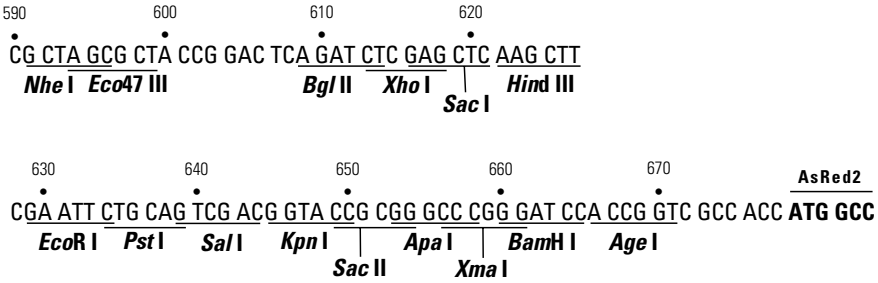
Vector Information

pAsRed2-N1 Vector Information PT3672-5

Cat. No. 632449 and sold as part of Cat. No. 630050





Restriction Map and Multiple Cloning Site (MCS) of pAsRed2-N1 Vector. All sites shown are unique.

Description

pAsRed2-N1 encodes AsRed2, a variant of *Anemonia sulcata* red fluorescent protein (1, 2). AsRed2 has been engineered for brighter fluorescence (Clontech Laboratories, Inc., unpublished data). The AsRed2 coding sequence also contains a series of silent base-pair changes, which correspond to human codon-usage preferences, for optimal expression in mammalian cells (3). Additionally, an upstream sequence—located just 5' to the AsRed2 start codon—has been converted to a Kozak consensus translation initiation site (4) to further increase the translation efficiency in eukaryotic cells.

The multiple cloning site (MCS) in pAsRed2-N1 is positioned between the immediate-early promoter of cytomegalovirus ($P_{CMV\ IE}$) and the AsRed2 coding sequence. Thus, genes cloned into the MCS will be expressed as fusions to the N-terminus of AsRed2 if they are in the same reading frame as AsRed2 and there are no intervening stop codons. The SV40 polyadenylation signals (SV40 poly A) downstream of the AsRed2 gene direct proper processing of the 3' end of AsRed2 mRNA.

The vector backbone contains an SV40 origin (SV40 ori) for replication in mammalian cells that express the SV40 T antigen, a pUC origin of replication (pUC ori) for propagation in *E. coli*, and an f1 origin (f1 ori) for single-stranded DNA production. In addition, 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 (5). A bacterial promoter (*P*) upstream of this cassette drives expression of the Neo^r/Kan^r gene in *E. coli* hosts, which can be selected with kanamycin.

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Use

Fusions to the N terminus of AsRed2 retain the fluorescent properties of the native protein allowing the localization of the fusion protein *in vivo* (AsRed2 excitation maximum = 576 nm; AsRed2 emission maximum = 592 nm). The target gene should be cloned into pAsRed2-N1 so that it is in frame with the AsRed2 coding sequence, with no intervening, in-frame stop codons. The inserted gene should include the initiating ATG codon. The recombinant pAsRed2-N1 vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (available from Clontech; Cat. Nos. 631307 & 631308). We recommend selecting mammalian cell cultures in 500–1,300 µg/ml G418, depending on the cell line. Be sure to establish a kill curve for each cell line and each lot of G418 to determine the optimal selection concentration. Unmodified (i.e., non-recombinant) pAsRed2-N1 can also be used simply to express AsRed2 in cells of interest (e.g., as a transfection marker).

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
- Multiple Cloning Site (MCS): 591–671
- *Anemonia sulcata* red fluorescent protein (AsRed2) coding sequence
  - Kozak consensus translation initiation site: 672–682
  - Start codon (ATG): 679–681; stop codon: 1375–1377
  - Phe-4 (7*) to Leu mutation (C→G): 690
  - Lys-12 (15*) to Arg mutation (A→G): 713
  - Phe-35 (38*) to Leu mutation (T→C): 781
  - Thr-68 (70*) to Ala mutation (A→G): 880
  - Phe-84 (88*) to Leu mutation (T→C): 928
  - Ala-143 (148*) to Ser mutation (G→T): 1105
  - Lys-163 (170*) to Glu mutation (A→G): 1165
  - Met-202 (208*) to Leu mutation (A→C): 1282
  - *Numbering based on *Aequorea victoria* GFP according to the sequence alignment described in Ref. 2.
  - C→G mutation to remove *Xho* I site: 783
- SV40 early mRNA polyadenylation signal
  - Polyadenylation signals: 1531–1536 & 1560–1565; mRNA 3' ends: 1569 & 1581
- f1 single-strand DNA origin: 1628–2083 (Packages the noncoding strand of AsRed2.)
- Bacterial promoter for expression of Kan^r gene:
  - –35 region: 2145–2150; –10 region: 2168–2173
  - Transcription start point: 2180
- SV40 origin of replication: 2424–2559
- SV40 early promoter
  - Enhancer (72-bp tandem repeats): 2257–2328 & 2329–2400
  - 21-bp repeats: 2404–2424, 2425–2445 & 2447–2467
  - Early promoter element: 2480–2486
  - Major transcription start points: 2476, 2514, 2520 & 2525
- Kanamycin/neomycin resistance gene
  - Neomycin phosphotransferase coding sequences: start codon (ATG): 2608–2610; stop codon: 3400–3402
  - G→A mutation to remove *Pst* I site: 2790
  - C→A (Arg to Ser) mutation to remove *Bss*H II site: 3136
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  - Polyadenylation signals: 3638–3643 & 3651–3656
- pUC plasmid replication origin: 3987–4630

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 JM101 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/Col E1

References

1. Matz, M. V., *et al.* (1999) *Nature Biotech.* **17**:969–973.
2. Lukyanov, K. A., *et al.* (2000) *J. Biol. Chem.* **275**:25879–25882.
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.