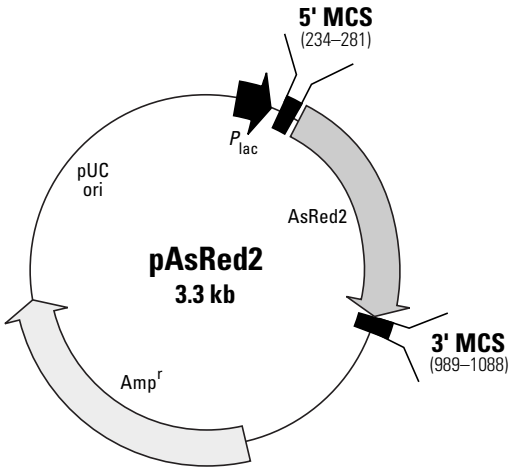
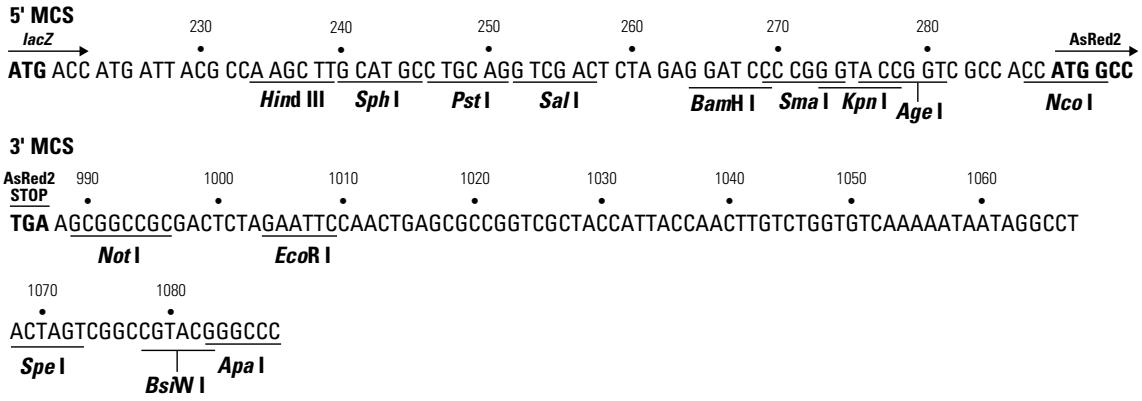
Vector Information

pAsRed2 Vector Information PT3702-5

Cat. No. 632451 and sold as part of Cat. No. 630050





Restriction Map and Multiple Cloning Site (MCS) of pAsRed2 Vector. Unique restriction sites are shown in bold.

Description

pAsRed2 is a pUC19-derived prokaryotic expression vector that encodes AsRed2, a variant of *Anemonia sulcata* red fluorescent protein (1, 2). AsRed2 has been engineered for brighter fluorescence (Clontech Laboratories, Inc., unpublished data). In addition, the AsRed2 coding sequence contains a series of silent base-pair changes, which correspond to human codon-usage preferences for optimal expression in mammalian cells (3). An upstream sequence—located just 5' to the AsRed2 start codon—has been converted to a Kozak consensus translation initiation site to further increase the translation efficiency in eukaryotic cells (4).

The AsRed2 coding sequence is flanked by distinct multiple cloning sites (MCS) at the 5' and 3' ends so that the gene can be readily excised from pAsRed2 and subcloned into other expression vectors. The AsRed2 gene was inserted in frame with the *lacZ* initiation codon from pUC19. Therefore, in *E. coli*, AsRed2 is expressed from the *lac* promoter (P_{lac}) as a fusion with several additional amino acids, including the first five amino acids of the *lacZ* protein. However, if you excise the AsRed2 coding sequence using a restriction site in the 5' MCS, the resulting fragment will encode the native (i.e., non-fusion) AsRed2 protein. The pUC backbone of pAsRed2 provides a high-copy-number origin of replication (pUC ori) and an ampicillin resistance gene (Amp^r) for propagation and selection in *E. coli*.

Use

pAsRed2 Vector serves as a convenient source of AsRed2 cDNA. Alternatively, the AsRed2 coding sequence can be amplified by PCR.

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Location of features

- *lac* promoter: 95–178
  - CAP binding site: 111–124
  - –35 region: 143–148; –10 region: 167–172
  - Transcription start point: 179
  - *lac* operator: 179–199
- *lacZ*-AsRed2 fusion protein expressed in *E. coli*
  - Ribosome binding site: 206–209
  - Start codon (ATG): 217–219; stop codon: 985–987
- 5' Multiple Cloning Site (MCS): 234–281
- *Anemonia sulcata* red fluorescent protein (AsRed2) gene
  - Kozak consensus translation initiation site: 282–292
  - Start codon (ATG): 289–291; stop codon: 985–987
  - Phe-4 (7*) to Leu mutation (C→G): 300
  - Lys-12 (15*) to Arg mutation (A→G): 323
  - Phe-35 (38*) to Leu mutation (T→C): 391
  - Thr-68 (70*) to Ala mutation (A→G): 490
  - Phe-84 (88*) to Leu mutation (T→C): 538
  - Ala-143 (148*) to Ser mutation (G→T): 715
  - Lys-163 (170*) to Glu mutation (A→G): 775
  - Met-202 (208*) to Leu mutation (A→C): 892
  - *Numbering based on *Aequorea victoria* GFP according to the sequence alignment described in Ref. 2.
  - C→G mutation to remove *Xho* I site: 393
- 3' Multiple Cloning Site (MCS): 989–1088
- Ampicillin resistance (β-lactamase) gene
  - Promoter: –35 region: 1464–1469; –10 region: 1487–1492
  - β-lactamase coding sequences:
    - Start codon (ATG): 1534–1536; stop codon: 2392–2394
- pUC plasmid replication origin: 2542–3185

Propagation in *E. coli*

- Recommended host strain: JM109
- Selectable marker: plasmid confers resistance to ampicillin (50 µg/ml) to *E. coli* hosts
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/Col E1

Excitation & emission maxima of AsRed2

- Excitation maximum = 576 nm
- Emission maximum = 592 nm

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.

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.