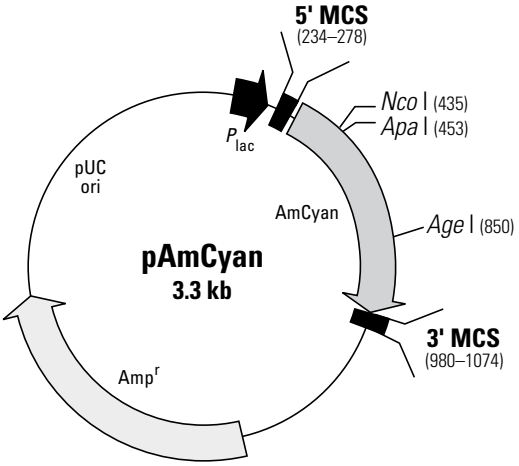
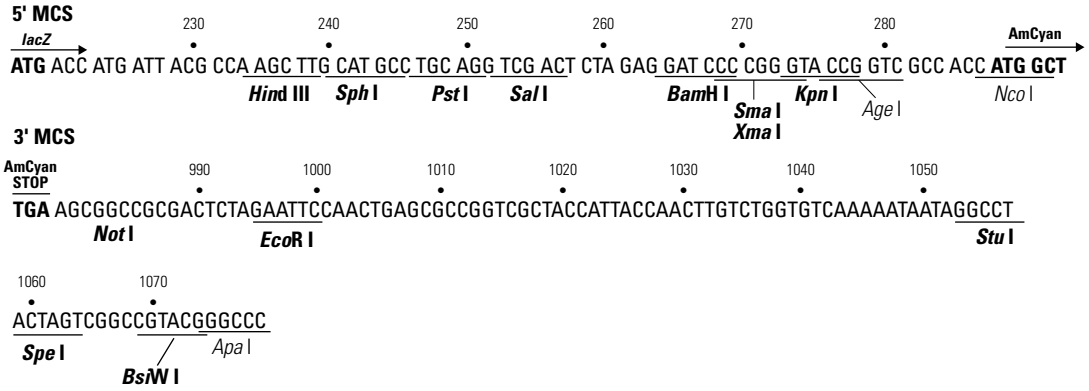
Vector Information

pAmCyan Vector Information PT3476-5

Cat. No. 632440 and sold as part of Cat. No. 630050





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

Description

pAmCyan is a pUC19-derived prokaryotic expression vector, which encodes a variant of wild-type *Anemonia majano* cyan fluorescent protein (AmCyan; 1) that has been engineered for brighter fluorescence. Two amino acid substitutions (Asn-34 to Ser; Lys-68 to Met) have been made to enhance the emission characteristics of AmCyan (excitation maximum = 458 nm; emission maximum = 489 nm).

The AmCyan gene was inserted in frame with the *lacZ* initiation codon from pUC19 so that AmCyan is expressed from the *lac* promoter (P_{lac}) in *E. coli*. The AmCyan 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 pAmCyan and subcloned into other expression vectors. An upstream sequence—located just 5' to the AmCyan gene—has been converted to a Kozak consensus translation initiation site (2) to increase the translation efficiency in eukaryotic expression systems. The pUC backbone of pAmCyan 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

pAmCyan Vector serves as a convenient source of AmCyan cDNA. The flanking MCS regions make it possible to excise the AmCyan coding sequence and insert it into other expression vectors. Alternatively, the AmCyan 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*-AmCyan fusion protein expressed in *E. coli*
  - Ribosome binding site: 206–209
  - Start codon (ATG): 217–219; stop codon: 976–978
- 5' Multiple Cloning Site (MCS): 234–278
- *Anemonia majano* cyan fluorescent protein (AmCyan) gene
  - Kozak consensus translation initiation site: 282–292
  - Start codon (ATG): 289–291; stop codon: 976–978
  - Asn-34 to Ser mutation (A→G): 389
  - Lys-68 to Met mutation (A→T; A→G): 491; 429
- 3' Multiple Cloning Site (MCS): 980–1074
- Ampicillin resistance gene
  - Promoter: –35 region: 1455–1460; –10 region: 1478–1483
  - Transcription start point: 1490
  - Ribosome binding site: 1513–1517
  - β-lactamase coding sequences:
    - Start codon (ATG): 1525–1527; stop codon: 2383–2385
    - β-lactamase signal peptide: 1525–1593
    - β-lactamase mature protein: 1594–2382
- pUC plasmid replication origin: 2533–3176

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

References

1. Matz, M. V., *et al.* (1999) *Nature Biotech.* **17**:969–973.
2. 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. This vector has not been completely sequenced.