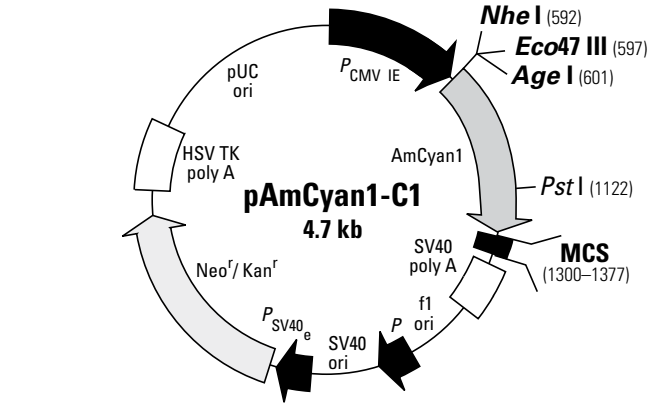
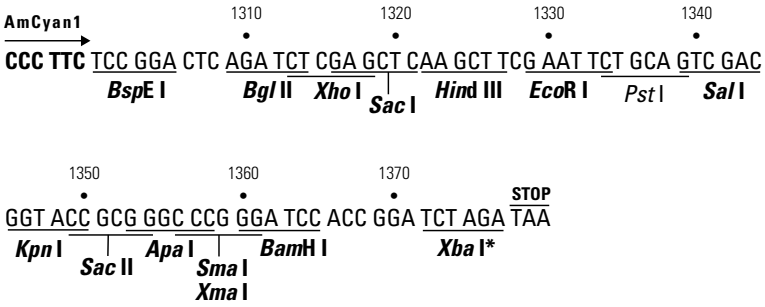
Vector Information

pAmCyan1-C1 Vector Information PT3478-5

Cat. No. 632441 and also sold as part of Cat. No. 630050





Restriction Map and Multiple Cloning Site (MCS) of pAmCyan1-C1. All sites shown are unique. The *Xba* I site (*) is methylated in the DNA provided by CLONTECH. If you wish to digest the vector with these enzymes, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Description

pAmCyan1-C1 encodes a human codon-optimized variant of wild-type *Anemonia majano* cyan fluorescent protein, AmCyan1 (1). The AmCyan1 coding sequence contains a series of silent base-pair changes, which correspond to human codon-usage preferences, for optimal expression in mammalian cells (2). Additionally, an upstream sequence—located just 5' to the AmCyan1 start codon—has been converted to a Kozak consensus translation initiation site (3) to further increase the translation efficiency in eukaryotic cells. Two amino acid substitutions (Asn-34 to Ser; Lys-68 to Met) have been made to enhance the emission characteristics of AmCyan1 (excitation maximum = 458 nm; emission maximum = 489 nm).

The multiple cloning site (MCS) in pAmCyan1-C1 is positioned between the AmCyan1 coding sequence and a pair of SV40 polyadenylation signals (SV40 poly A). Thus, genes cloned into the MCS will be expressed as fusions to the C-terminus of AmCyan1 if they are in the same reading frame as AmCyan1 and there are no intervening stop codons. Expression of AmCyan1 is driven by the cytomegalovirus immediate-early promoter ($P_{CMV\ IE}$). The SV40 poly A signals downstream of the MCS direct proper processing of the 3' end of AmCyan1 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 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_{SV40_e}), 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 (4). 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 C terminus of AmCyan1 retain the fluorescent properties of the native protein allowing the localization of the fusion protein *in vivo*. The target gene should be cloned into pAmCyan1-C1 so that it is in frame with the AmCyan1 coding sequence, with no intervening, in-frame stop codons. The recombinant pAmCyan1-C1 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) pAmCyan1-C1 can also be used simply to express AmCyan1 in a cell line 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
- *Anemonia majano* cyan fluorescent protein (AmCyan1) coding sequence
  Kozak consensus translation initiation site: 606–616
  Start codon (ATG): 613–615
  Asn-34 to Ser mutation (A→G): 713
  Lys-68 to Met mutation (A→T): 815
- Multiple Cloning Site (MCS): 1300–1377
  Stop codon: 1378–1380
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1520–1525 & 1549–1554; mRNA 3' ends: 1558 & 1570
- f1 single-strand DNA origin: 1617–2072 (Packages the noncoding strand of AmCyan1.)
- Bacterial promoter for expression of Kan^r gene
  –35 region: 2134–2139; –10 region: 217–2162
  Transcription start point: 2169
- SV40 origin of replication: 2413–2548
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2246–2317 & 2318–2389
  21-bp repeats: 2393–2413, 2414–2434 & 2436–2456
  Early promoter element: 2469–2475
  Major transcription start points: 2465, 2503, 2509 & 2514
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2597–2599; stop codon: 3389–3391
  G→A mutation to remove *Pst* I site: 2779
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3125
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3627–3632 & 3640–3545
- pUC plasmid replication origin: 3976–4619

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/Col E1

References

1. Matz, M. V., *et al.* (1999) *Nature Biotech.* **17**:969–973.
2. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
4. 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. This vector has not been completely sequenced.

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