



Restriction Map and Multiple Cloning Site (MCS) of pZsAmCyan1-N1 Vector. Unique restriction sites are shown in bold. The *Not I* site follows the AmCyan1 stop codon.

Description

pAmCyan1-N1 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-N1 is positioned between the immediate-early promoter of CMV ($P_{CMV IE}$) and the AmCyan1 coding sequence. Thus, genes cloned into the MCS will be expressed as fusions to the N-terminus of AmCyan1 if they are in the same reading frame as AmCyan1 and there are no intervening stop codons. The SV40 polyadenylation signals (SV40 polyA) downstream of the AmCyan1 gene 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 SV40T 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 (4). A bacterial promoter (*P*) upstream of this cassette drives expression of the Neo^r/Kan^r gene in *E. coli* host cells, which can be selected with kanamycin.

(PR093658; published September 2010)



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Use

Fusions to the N 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-N1 so that it is in frame with the AmCyan1 coding sequence, with no intervening, in-frame stop codons. The inserted gene should include the initiating ATG codon. The recombinant pAmCyan1-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) pAmCyan1-N1 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
- Multiple Cloning Site (MCS): 591–671
- *Anemonia majano* cyan fluorescent protein (AmCyan1) coding sequence
Kozak consensus translation initiation site: 672–682
Start codon (ATG): 679–681; stop codon: 1366–1368
Asn-34 to Ser mutation (A→G): 779
Lys-68 to Met mutation (A→T): 881
- SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1522–1527 & 1551–1556; mRNA 3' ends: 1560 & 1572
- f1 single-strand DNA origin: 1619–2074 (Packages the noncoding strand of AmCyan1.)
- Bacterial promoter for expression of Kan^r gene:
–35 region: 2136–2141; –10 region: 2159–2164
Transcription start point: 2171
- SV40 origin of replication: 2415–2550
- SV40 early promoter
Enhancer (72-bp tandem repeats): 2248–2319 & 2320–2391
21-bp repeats: 2395–2415, 2416–2436 & 2438–2458
Early promoter element: 2471–2477
Major transcription start points: 2467, 2505, 2511 & 2516
- Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences: Start codon (ATG): 2599–2601; stop codon: 3391–3393
G→A mutation to remove *Pst*I site: 2781
C→A (Arg to Ser) mutation to remove *Bss*H II site: 3127
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3629–3634 & 3642–3647
- pUC plasmid replication origin: 3978–4621

Propagation in *E. coli*

- Suitable host strains: DH5 α , 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. 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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