pEGFP-C1 NoDAMURA B2

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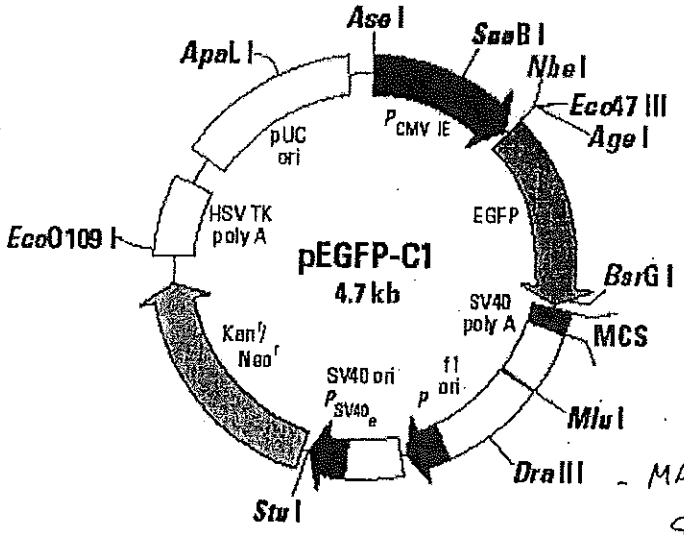
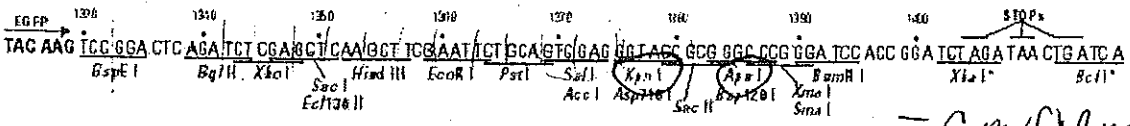


Annotated Map/MCS (pdf)
Sequence (text)
Sequence (pdf)
Restriction Digest (pdf)
Description
Use
Feature Locations
Primer Sites
Propagation
References
License Statement

- Made by: Chris Sullivan
- 8/1/03
- Subcloned KpnI-ApaI fragment from pcDNA3.1PURO NoDAB2 into same sites.

Click here to view the MCS for this vector.

Restriction Map and Multiple Cloning Site of pEGFP-C1. (Unique restriction sites are in bold.) The *Xba* I and *Bcl* I sites (*) are 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.



- Confirmed by IFA.

TOP

Description

pEGFP-C1 encodes a red-shifted variant of wild-type GFP (1–3) which has been optimized for brighter fluorescence and higher expression in mammalian cells. (Excitation maximum = 488 nm; emission maximum = 507 nm.) pEGFP-C1 encodes the GFPmut1 variant (4) which contains the double-amino-acid substitution of Phe-64 to Leu and Ser-65 to Thr. The coding sequence of the EGFP gene contains more than 190 silent base changes which correspond to human codon-usage preferences (5). Sequences flanking EGFP have been converted to a Kozak consensus translation initiation site (6) to further increase the translation efficiency in eukaryotic cells. The MCS in pEGFP-C1 is between the EGFP coding sequences and the SV40 poly A. Genes cloned into the MCS will be expressed as fusions to the C-terminus of EGFP if they are in the same reading frame as EGFP and there are no intervening stop codons. SV40 polyadenylation signals downstream of the EGFP gene direct proper processing of the 3' end of the EGFP mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T-antigen. A neomycin resistance cassette (neo^r), consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex thymidine kinase (HSV TK) gene, allows stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli*. The pEGFP-C1 backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

>gb|AF174533.1| Nodamura virus RNA1 segment, complete sequence
Length=3204

Score = 740 bits (820), Expect = 0.0
Identities = 413/415 (99%), Gaps = 0/415 (0%)
Strand=Plus/Plus

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Query  85    ATGACAAACATGTCATGCGCTTACGAGCTAATCAAGTCACTTCCAGCCAAGCTGGAGCAG  144
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2744  ATGACAAACATGTCATGCGCTTACGAGCTAATCAAGTCACTTCCAGCCAAGCTGGAGCAG  2803

Query  145   CTGGCTCAGGAGACGCAAGCAACGATCCAAACGCTCATGATCGCGGATCCCAACGTCAAC  204
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2804  CTGGCTCAGGAGACGCAAGCAACGATCCAAACGCTCATGATCGCGGATCCCAACGTCAAC  2863

Query  205   AAGGATCTGCGAGCGTTCTGCGAGTTCCTGACCGTACAGCACCAGCGGGCGTATCGAGCG  264
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2864  AAGGATCTGCGAGCGTTCTGCGAGTTCCTGACCGTGCAGCACCAGCGGGCGTATCGAGCG  2923

Query  265   ACGAACAGCCTGCTCATCAAACCGCGAGTCGCAGCAGCGCTTCGCGGGGAGGAGCTGGAC  324
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2924  ACGAACAGCCTGCTCATCAAACCGCGAGTCGCAGCAGCGCTTCGCGGGGAGGAGCTGGAC  2983

Query  325   CTGGGCGAGGCGGACGTCGCCGCCCGGGTCCGCCAGCTAAAACAACAGCTGGCGGAGCTC  384
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2984  CTGGGCGAGGCGGACGTCGCCGCCCGGGTCCGCCAGCTAAAACAACAGCTGGCGGAGCTC  3043

Query  385   GAGATGGAAATCAAGCCAGGGCACCAACAGGTGGCCCAAGTAAGCGGCAGGCGGAAGGCC  444
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3044  GAGATGGAAATCAAGCCAGGGCACCAACAAGTGGCCCAAGTAAGCGGCAGGCGGAAGGCC  3103

Query  445   GCAGCCGCAGCTCCCGTGGCCCAGCTGGGTCGCGTGGGCGTGGTAAATGAGTGAT  499
             |||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3104  GCAGCCGCAGCTCCCGTGGCCCAGCTGGGTCGCGTGGGCGTGGTAAATGAGTGAT  3158
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arrows indicate 2 silent point mutations