hMOPP1-GFP

EcoRI hMOPP1 1.8kb BamHI

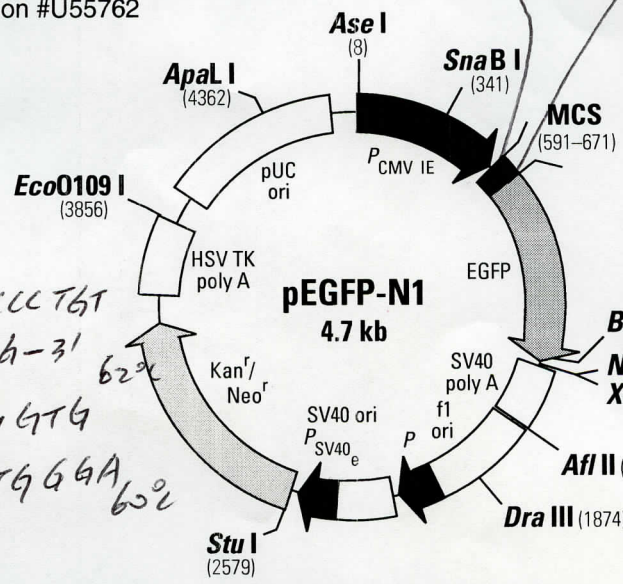
CTCGC

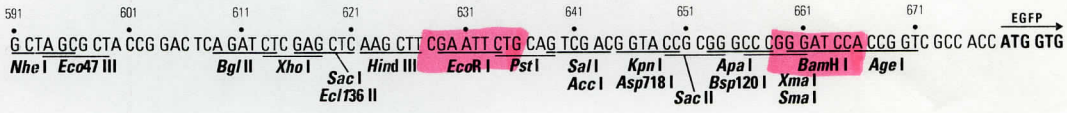
pEGFP-N1 Vector Information

PT3027-5

GenBank Accession #U55762

Catalog #6085-1





EcoRI

5': 5'-CG GAATTC AA ATG AGT GGC CCC TGT GGAG-3'

BamHI

3': 5'CG GGATCC GC CTC ACT GGG GTG CTG GGA

62°C

60°C

EGFP in frame

Restriction Map and Multiple Cloning Site (MCS) of pEGFP-N1 Vector. (Unique restriction sites are in bold.) The *Not* I site follows the EGFP stop codon. The *Xba* I site (*) is methylated in the DNA provided by CLONTECH. If you wish to digest the vector with this enzyme, you will need to transform the vector into a *dam*$^-$ and make fresh DNA.

Description:

pEGFP-N1 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-N1 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-N1 is between the immediate early promoter of CMV ($P_{CMV\ IE}$) and the EGFP coding sequences. Genes cloned into the MCS will be expressed as fusions to the N-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 virus 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-N1 backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

(PR93634; published 19 March 1999)

Xasser provided

hmcpip, D141N
hmcpip1
hmcpip1

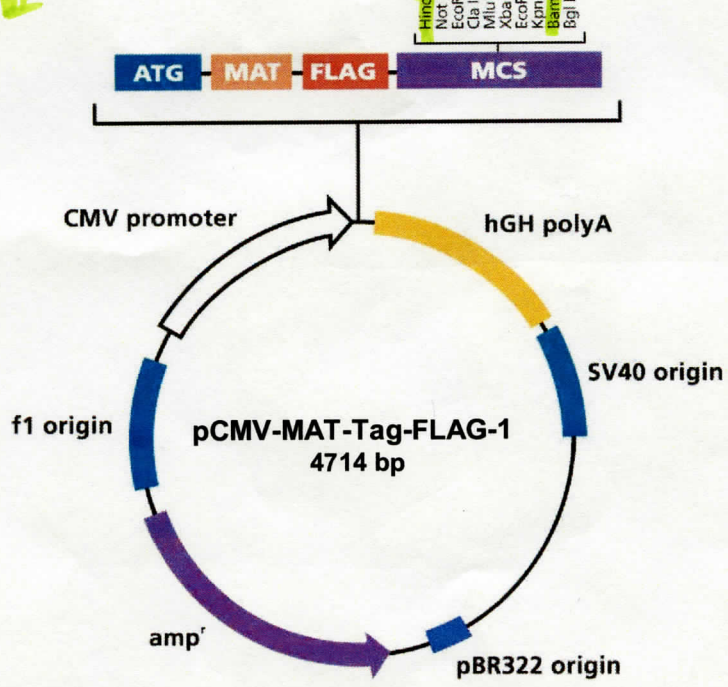
HindIII

BamHI

Human mcpip1, D141N 359

Human mcpip1, C306R 360

Human mcpip1, KK→GG 361



Multiple Cloning Site

MAT Sequence

Met	Ala	His	Asn	His	Arg	His	Lys	His	Ala

ATG GCC CAC AAC CAC CGC CAC AAG CAC GCC
TAC CGG GTG TTG GTG GCG GTG TTC GTG CGG

FLAG Peptide Sequence

Asp	Tyr	Lys	Asp	Asp	Asp	Asp	Lys

Not I EcoR I
GAC TAC AAA GAC GAT GAC GAC AAG CTT GCG GCC GCG AAT TCA
CTG ATG TTT CTG CTA CTG CTG TTC GAA CGC CGG CGC TTA AGT
Hind III

Cla I Mlu I Xba I EcoR V Kpn I BamH I Bgl II
TCG ATA GAT ACG CGT CAT CTA GAT ATC GGT ACC GGA TCC AGA TCT
AGC TAT CTA TGC GCA GTA GAT CTA TAG CCA TGG CCT AGG TCT AGA