基本信息

启动子:	CMV promoter
平台编号:	bio-110326
复制子:	pUC ori，f1 ori
终止子:	SV40 poly(A) signal
质粒分类:	哺乳系列质粒；哺乳荧光质粒；哺乳绿色质粒
质粒大小:	4733bp
质粒标签:	C-EGFP
原核抗性:	Kan，50μg/ml
筛选标记:	Neo/G418
克隆菌株:	DH5α大肠杆菌
培养条件:	37℃，有氧，LB
表达宿主:	293T 等哺乳细胞
培养条件:	37℃，有氧，5%CO2
诱导方式:	无须诱导，瞬时表达
5'测序引物:	pEGFP-N-5：TGGGAGGTCTATATAAGCAGAG
3'测序引物:	pEGFP-N-3: CGTCGCCGTCCAGCTCGACCAG

质粒简介

　　pEGFP-N1 encodes a red-shifted variant of wild-type GFP　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 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. Sequences flanking EGFP have been converted to a Kozak consensus translation initiation site　to further increase the translation efficiency in eukaryotic cells. The MCS in pEGFP-N1 is between the immediate early promoter of CMV 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, consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes
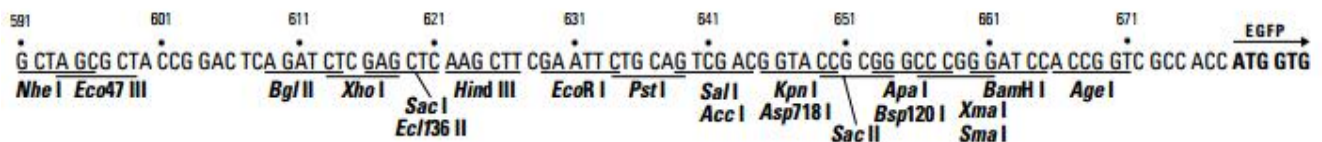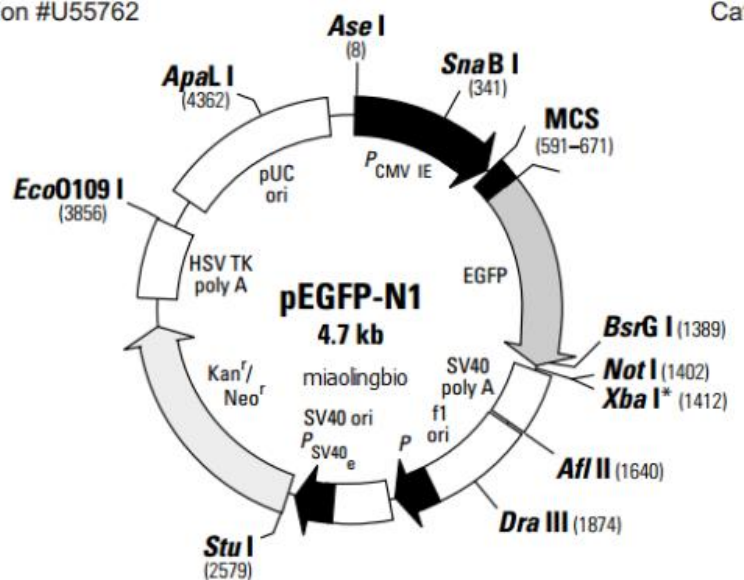
simplex virus thymidine kinase (HSV TK) gene, allows stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette expresses kanamycin resistance in E. coli. The pEGFP-N1 backbone also provides a pUC origin of replication for propagation in E. coli and an f1 origin for single-stranded DNA production.

Fusions to the N terminus of EGFP retain the fluorescent properties of the native protein allowing the localization of the fusion protein in vivo . The target gene should be cloned into pEGFP-N1 so that it is in frame with the EGFP coding sequences, with no intervening in-frame stop codons. The inserted gene should include the initiating ATG codon. The recombinant EGFP vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 . pEGFP-N1 can also be used simply to express EGFP in a cell line of interest (e.g., as a transfection marker).

质粒图谱

pEGFP-N1 Vector Information
GenBank Accession #U55762

pEGFP-N1
4.7 kb
miaolingbio


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591        601        611        621        631        641        651        661        671                    EGFP
G CTA GCG CTA CCG GAC TCA GAT CTC GAG CTC AAG CTT CGA ATT CTG CAG TCG ACG GTA CCG CGG GCC CGG GAT CCA CCG GTC GCC ACC ATG GTG
Nhe I  Eco47 III    Bgl II  Xho I      Hind III   EcoR I  Pst I   Sal I   Kpn I      Apa I      BamH I   Age I
                            Sac I                          Acc I  Asp718 I  Bsp120 I  Xma I
                            Ecl136 II                              Sac II            Sma I
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质粒序列

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LOCUS       Exported                4733 bp ds-DNA     circular SYN
30-AUG-2016
DEFINITION  synthetic circular DNA
ACCESSION   .
VERSION     .
KEYWORDS    Untitled 7
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SOURCE      synthetic DNA construct
  ORGANISM  synthetic DNA construct
REFERENCE   1  (bases 1 to 4733)
  AUTHORS   .
  TITLE     Direct Submission
  JOURNAL   Exported Tuesday, August 30, 2016 from SnapGene Viewer
3.1.4

FEATURES             Location/Qualifiers
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early
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arrow
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origin of
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