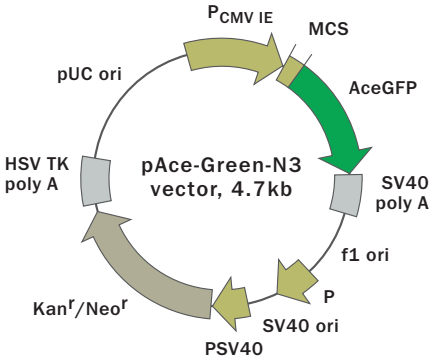
Mammalian expression vector pAce-Green-N3



For vector sequence, please visit our Web site at www.evrogen.com/support/vector-info.shtml

Product	Cat.#	Size
pAce-Green-N3	**FP106**	20 µg

Please contact your local distributor for exact prices and delivery information.

Vector type	mammalian expression vector
Reporter	AceGFP
Reporter codon usage	mammalian
Promoter for AceGFP	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic — kanamycin
	eukaryotic — neomycin (G418)
Replication	prokaryotic — pUC ori
	eukaryotic — SV40 ori

Multiple cloning site (MCS)

NheI / AflII — BglII / XhoI — SacI — HindIII — EcoRI / PstI — SalI — ApaI / SacII — SmaI/XmaI — BamHI / BspEI — AgeI — AceGFP

GCT.AGC.GCT.ACC.GGA.CTC.AGA.TCT.CGA.GCT.CAA.GCT.TCG.AAT.TCT.GCA.GTC.GAC.CCT.ACC.GCG.GGC.CCG.GGA.TCC.GGA.CCG.GTC.GCC.ACC.*ATG.AGC.AAG*

Use

- Generation of fusions to the AceGFP N-terminus

- Expression of AceGFP or its fusions in mammalian cells

Vector description

pAce-Green-N3 is an eukaryotic (mammalian) expression vector encoding the *Aequrea coerulescens* enhanced green fluorescent protein AceGFP. The vector is designed to generate AceGFP-tagged fusions or to express AceGFP in mammalian cells.

AceGFP codon usage is optimized for high expression in mammalian cells (humanized, Haas *et al.*, 1996). To increase AceGFP translation, Kozak consensus translation initiation site is generated upstream of AceGFP sequence (Kozak, 1987). Multiple cloning site (MCS) is located between $P_{CMV\ IE}$ and AceGFP coding sequence.

The vector backbone comprises immediate early promoter of cytomegalovirus ($P_{CMV\ IE}$) for protein expression, SV40 origin for replication in mammalian cells expressing SV40 T-antigen, pUC origin of replication for propagation in *E. coli* and f1 origin for single-stranded DNA production. SV40 polyadenylation signals (SV40 polyA) direct proper processing of the 3' end of the reporter mRNA.

SV40 early promoter provides neomycin resistance gene expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression in *E. coli*. Kan^r/Neo^r gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

Generation of fusions

A localization signal (or a gene of interest) should be cloned into MCS of the vector. It will be expressed as a fusion to the AceGFP N-terminus when inserted in the same reading frame as AceGFP and no intervening stop codons are present. The inserted sequence should contain an initiating ATG codon. AceGFP fusions retain fluorescent properties of the native protein allowing fusion protein localization *in vivo*.

Note: The plasmid DNA was isolated from dam^+-methylated *E.coli*. Therefore some restriction sites are bloked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a dam^- host and make fresh DNA.

Expression in mammalian cells

pAce-Green-N3 can be transfected into mammalian cells by any known transfection method. If required, stable transformants can be selected using G418 (Gorman, 1985).

Unmodified pAce-Green-N1 will express AceGFP, when transfected into eukaryotic (mammalian) cells.

Propagation in *E. coli*

Suitable host strains for propagation in *E. coli* include DH5alpha, HB101, XL1-Blue, and other general purpose strains. Plasmid incompatibility group is pMB1/ColE1. The vector confers resistance to kanamycin (30 μg/ml) to *E. coli* hosts. Copy number in *E. coli* is about 500.

Location of features

PCMV IE: 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583
MCS: 591-673
AceGFP
Kozak consensus translation initiation site: 674-684
Start codon (ATG): 681-683; Stop codon: 1398-1400
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1554-1559 & 1583-1588
mRNA 3' ends: 1592 & 1604
f1 single-strand DNA origin: 1651-2106
(packages the noncoding strand of AceGFP)
Bacterial promoter expression of Kanr gene:
-35 region: 2168-2173; -10 region: 2191-2196
Transcription start point: 2203
SV40 origin of replication: 2447-2582
SV40 early promoter
Enhancer (72-bp tandem repeats): 2280-2351 & 2352-2423
21-bp repeats: 2427-2447, 2448-2468, & 2470-2490
Early promoter element: 2503-2510
Major transcription start points: 2500, 2537, 2543 & 2548
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2631-2633; stop codon: 3423-3425
G->A mutation to remove Pst I site: 2813
C->A (Arg to Ser) mutation to remove BssH II site: 3159
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3661-3666 & 3674-3679
pUC plasmid replication origin: 4010-4653

References

Gorman C. (1985) In DNA cloning: A Practical Approach, Vol. II. Ed. D. M. Glover. (IRL Press, Oxford, U.K.), pp. 143-190.

Haas J. *et al.* (1996) Curr. Biol. 6: 315-324.

Kozak M. (1987) Nucleic Acids Res. 15:8125-8148.