BioVision

rev. 11/07

For research use only

pVisionGFP-C Vector

CATALOG #: 9998-20

AMOUNT: 20 µg

STORAGE CONDITIONS: -20° C

SHIPPING: Blue ice/Ice pack

APPLICATION:

Generation of VisionGFP-tagged 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 VisionGFP C-terminus when inserted in the same reading frame as VisionGFP and no in-frame stop codons are present. VisionGFP-tagged fusions retain fluorescent properties of the native protein allowing fusion localization in vivo. Unmodified pVisionGFP-C vector will express VisionGFP when transfected into eukaryotic (mammalian) cells.

Expression in Mammalian Cells

pVisionGFP-C can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of VisionGFP or VisionGFP-tagged fusions in many cell types. If required, stable transformants can be selected using G418.

Propagation in *E. coli*

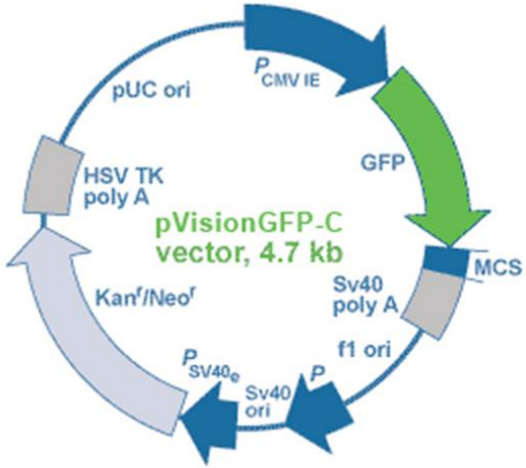
· Suitable host strains: DH5alpha, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
· Selectable marker: plasmid confers resistance to kanamycin (30 µg/ml) to *E. coli* hosts.
· *E. coli* replication origin: pUC
· Copy number: ~500
· Plasmid incompatibility group: pMB1/ColE1

FOR RESEARCH USE ONLY! Not to be used on humans.

PRODUCT DESCRIPTION:

pVisionGFP-C1 vector is a mammalian expression vector encoding green fluorescent protein, VisionGFP. pVisionGFP-C vector is designed to generate fusions to VisionGFP C-terminus for expression, localization and cellular dynamics studies or to express VisionGFP in eukaryotic (mammalian) cells. pVisionGFP-C vector carries synthetic version of the VisionGFP gene which codon usage is humanized, i.e. optimized for high expression in mammalian cells.

pVisionGFP-C vector backbone contains immediate early promoter of cytomegalovirus ($P_{CMV\ IE}$) for protein expression, SV40 origin for replication in mammalian cells, pUC origin of replication for propagation in *E. coli,* and f1 origin for single-stranded DNA production. 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.* To increase VisionGFP mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of VisionGFP coding sequence. Multiple cloning site (MCS) is located VisionGFP coding sequence and SV40 polyadenylation signal (SV40 poly A).



MCS
Bgl II Sac I EcoR I Sal I Kpn I Apa I*
GGT. GAA. GAA. AGA. TCT. CGA. GCT. CAA. GCT. TCG. AAT. TCT. GCA. GTC. GAC. GGT. ACC. GCG. GGC. CCG.
Xho I Hind III Pst I* Sac II Sma I/Xma II
BamH I Stops
GGA. TCC. ACC. GGA. TCT. AGA. TAA. CTG. ATC. A
Xba I# Bcl I#

*Not unique site. #Sites are blocked by methylation.

Note: This vector has not been completely sequenced.

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BACKGROUND/TECHNICAL INFORMATION:

Location of Features:
$P_{CMV\ IE}$: 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583

VisionGFP
Kozak consensus translation initiation site: 606-616
Start codon (ATG): 613-615; Stop codon: 1378-1380
Last amino acid in VisionGFP: 1306-1308

MCS: 1309-1394

SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1520-1525 & 1549-1554
mRNA 3' ends: 1558 & 1570

f1 single-strand DNA origin: 1617-2072 (Packages the noncoding strand of VisionGFP.)

Bacterial promoter for expression of Kan^r gene
-35 region: 2134-2139; -10 region: 2157-2162
Transcription start point: 2169

SV40 origin of replication: 2413-2548

SV40 early promoter
Enhancer (72-bp tandem repeats): 2246-2317 & 2318-2389
21-bp repeats: 2393-2413, 2414-2434, & 2436-2456
Early promoter element: 2469-2475
Major transcription start points: 2465, 2503, 2509 & 2514

Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2597-2599; stop codon: 3389-3391
G->A mutation to remove *Pst* I site: 2779
C->A (Arg to Ser) mutation to remove *Bss*H II site: 3125

Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3627-3632 & 3640-3645

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