BioVision

rev. 10/07

For research use only

pVisionGFP-N Vector

CATALOG #: 9999-20

AMOUNT: 20 µg

STORAGE CONDITIONS: -20° C

SHIPPING: Blue ice/Ice pack

APPLICATION:

Generation of Vision GFP-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 N-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-N vector will express VisionGFP when transfected into eukaryotic (mammalian) cells.

Expression in Mammalian Cells

pVisionGFP-N 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*

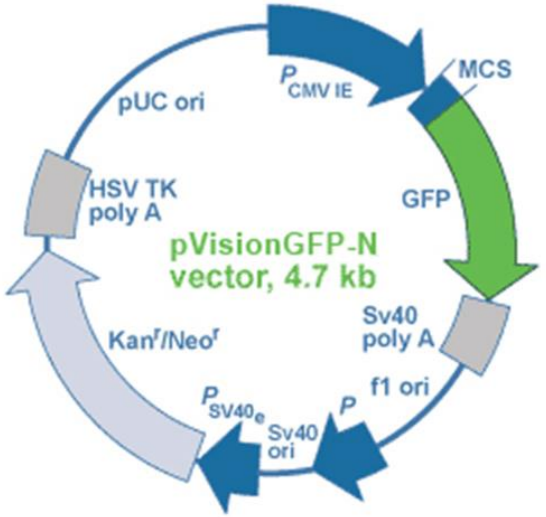
· Suitable host strains: DH5alpha, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
· Selectable marker: plasmid confers resistance to kanamycin (30 µg/ml) to *E. coli* hosts.
· *E. coli* replication origin: pUC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

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

PRODUCT DESCRIPTION:

pVisionGFP-N1 vector is a mammalian expression vector encoding green fluorescent protein, VisionGFP. pVisionGFP-N vector is designed to generate fusions to VisionGFP N-terminus for expression, localization and cellular dynamics studies or to express VisionGFP in eukaryotic (mammalian) cells. pVisionGFP-N vector carries synthetic version of the VisionGFP gene which codon usage is humanized, i.e. optimized for high expression in mammalian cells.

pVisionGFP-N 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 between $P_{CMV\ IE}$ and VisionGFP coding sequence.



Nhel Bgl II Sac I Hind III EcoR I Sal I Kpn I
GCT A.GC G.CT A.CCG.GAC.TC A.GAT. CT C. GAG. CTC. AAG.CTT. C GA.ATT. C TG.CA G. TCG.AC G.GTA. CC G.C GG. G
Eco47 III Xho I Pst I* Sac II
Apa I* BamH I Age I GFP
CC.C G G.G AT.CC A.CCG.GT C.GCC.ACC. AGC
Sma I/XmaI

BioVision Incorporated
155 S. Milpitas Boulevard, Milpitas, CA 95035 USA

Tel: 408-493-1800 | Fax: 408-493-1801
www.biovision.com | tech@biovision.com

BACKGROUND/TECHNICAL INFORMATION:

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

MCS: 591-671

VisionGFP
Kozak consensus translation initiation site: 672-682
Start codon (ATG): 679-681;
Stop codon: 1375-1377

SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1531-1536 & 1560-1565
mRNA 3' ends: 1569 & 1581

f1 single-strand DNA origin: 1628-2083
(packages the noncoding strand of VisionGFP)

Bacterial promoter expression of Kan^r gene:
-35 region: 2145-2150; -10 region: 2168-2173
Transcription start point: 2180

SV40 origin of replication: 2424-2559

SV40 early promoter
Enhancer (72-bp tandem repeats): 2257-2328 & 2329-2400
21-bp repeats: 2404-2424, 2425-2445, & 2447-2467
Early promoter element: 2480-2486
Major transcription start points: 2476, 2514, 2520 & 2525

Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2608-2610; stop codon: 3400-3402
G->A mutation to remove *Pst* I site: 2790
C->A (Arg to Ser) mutation to remove *Bss*H II site: 3136

Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3638-3643 & 3651-3656

pUC plasmid replication origin: 3987-4630

RELATED PRODUCTS:

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- Annexin V Kits & Bulk Reagents
- Caspase Assay Kits & Reagents
- Mitochondrial Apoptosis Kits & Reagents
- Nuclear Apoptosis Kits & Reagents
- Apoptosis Inducers & Inhibitors
- Apoptosis Isolation Kit

Cell Fractionation System
- Mitochondria/Cytosol Fractionation Kit
- Nuclear/Cytosol Fractionation Kit
- Membrane Protein Extraction Kit
- Cytosol/Particulate Rapid Separation Kit
- Mammalian Cell Extraction Kit
- FractionPREP Fractionation System

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- Senescence Detection Kit
- High Throughput Apoptosis/Cell Viability Assay Kits
- LDH-Cytotoxicity Assay Kit
- Bioluminescence Cytotoxicity Assay Kit
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- Akt & JNK Activity Assay Kits
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- Recombinant Adiponectin, Survivin, & Leptin
- CETP Activity Assay & Drug Discovery Kits
- Total Cholesterol Quantification Kit

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- siRNA Vectors
- Cloning Insert Quick Screening Kit
- Mitochondrial & Genomic DNA Isolation Kits
- 5 Minutes DNA Ligation Kit
- 20 Minutes Gel Staining/Destaining Kit

Antibodies & Recombinant Proteins (many)

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