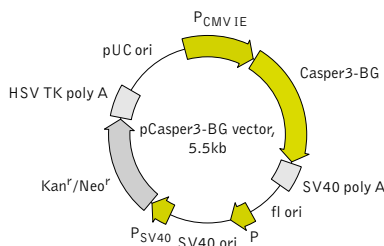


pCasper3-BG vector

The vector sequence has been compiled using the information from sequence databases, published literature, and other sources, together with partial sequences obtained by Evrogen. This vector has not been completely sequenced.



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

Location of features

P_{CMV IE}: 1-589
 Enhancer region: 59-465
 TATA box: 554-560
 Transcription start point: 583
 Casper3-BG
 Kozak consensus translation initiation site: 672-682
 Start codon (ATG): 679-681
 TagBFP: 679-1374
 Linker: 1375-1424
 TagGFP2: 1425-2151
 Stop codon: 2149-2151
 SV40 early mRNA polyadenylation signal
 Polyadenylation signals: 2304-2309 & 2333-2338
 mRNA 3' ends: 2342 & 2354
 f1 single-strand DNA origin: 2401-2856
 Bacterial promoter for expression of Kan^r gene
 -35 region: 2918-2923; -10 region: 2941-2946
 Transcription start point: 2953
 SV40 origin of replication: 3197-3332
 SV40 early promoter
 Enhancer (72-bp tandem repeats): 3030-3101 & 3102-3173
 21-bp repeats: 3177-3197, 3198-3218 & 3220-3240
 Early promoter element: 3253-3259
 Major transcription start points: 3249, 3287, 3293 & 3298
 Kanamycin/neomycin resistance gene
 Neomycin phosphotransferase coding sequences:
 Start codon (ATG): 3381-3383; Stop codon: 4173-4175
 G->A mutation to remove Pst I site: 3563
 C->A (Arg to Ser) mutation to remove BssH II site: 3909
 Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
 Polyadenylation signals: 4411-4416 & 4424-4429
 pUC plasmid replication origin: 4760-5403

Product	Cat.#	Size
pCasper3-BG vector	FP970	20 µg
The price does not include delivery. The price varies in different countries. Please contact your local distributor for exact prices and delivery information.		
Vector type	mammalian expression vector	
Reporter	Casper3-BG	
Reporter codon usage	mammalian	
Promoter for Casper3-BG	P _{CMV IE}	
Host cells	mammalian	
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)	
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori	
Use	Expression of fluorescent caspase-3 apoptosis sensor Casper3-BG in mammalian cells under the control of CMV promoter; source of Casper3-BG coding sequence	

Vector description

pCasper3-BG is a mammalian expression vector encoding a fluorescent sensor Casper3-BG. To increase mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of the Casper3-BG sequence [Kozak 1987].

The vector can be also used as a source of Casper3-BG coding sequence. Flanking restriction sites are convenient for excision of Casper3-BG sequence and its further insertion into other expression vectors of choice. Alternatively, Casper3-BG coding sequence can be amplified by PCR.

Note: The plasmid DNA was isolated from dam⁺-methylated *E. coli*. Therefore some restriction sites are blocked 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.

The vector backbone contains 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 poly A) direct proper processing of the 3'-end of the reporter mRNA.

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

Expression in mammalian cells

pCasper3-BG vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive Casper3-BG expression in many cell types. If required, stable transformants can be selected using G418 [Gorman 1985].

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.

References

- Gorman (1985). "High efficiency gene transfer into mammalian cells." In: *DNA cloning: A Practical Approach, Vol. II*. Ed. by Glover. (IRL Press, Oxford, U.K.) Pp. 143-190.
- Kozak (1987) "An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs." *Nucleic Acids Res*, 15 (20): 8125-8148 / pmid: 3313277

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