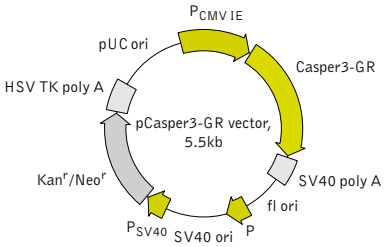
pCasper3-GR vector

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

Product	Cat.#	Size
pCasper3-GR vector	**FP971**	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-GR
Reporter codon usage	mammalian
Promoter for Casper3-GR	$P_{CMV\ IE}$
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-GR in mammalian cells under the control of CMV promoter; source of Casper3-GR coding sequence

Location of features

$P_{CMV\ IE}$: 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583
Casper3-GR
Kozak consensus translation initiation site: 672-682
Start codon (ATG): 679-681
TagRFP: 679-1374
Linker: 1375-1425
TagGFP: 1426-2130
Stop codon: 2131-2133
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 2286-2291 & 2315-2320
mRNA 3' ends: 2324 & 2336
f1 single-strand DNA origin: 2383-2838
Bacterial promoter for expression of Kan^r gene
-35 region: 2900-2905; -10 region: 2923-2928
Transcription start point: 2935
SV40 origin of replication: 3179-3314
SV40 early promoter
Enhancer (72-bp tandem repeats): 3012-3083 & 3084-3155
21-bp repeats: 3159-3179, 3180-3200 & 3202-3222
Early promoter element: 3235-3241
Major transcription start points: 3231, 3269, 3275 & 3280
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 3363-3365; Stop codon: 4155-4157
G->A mutation to remove Pst I site: 3545
C->A (Arg to Ser) mutation to remove BssH II site: 3891
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 4393-4398 & 4406-4411
pUC plasmid replication origin: 4742-5385

Vector description

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

The vector can be also used as a source of Casper3-GR coding sequence. Flanking restriction sites are convenient for excision of Casper3-GR sequence and its further insertion into other expression vectors of choice. Alternatively, Casper3-GR 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-GR vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive Casper3-GR 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