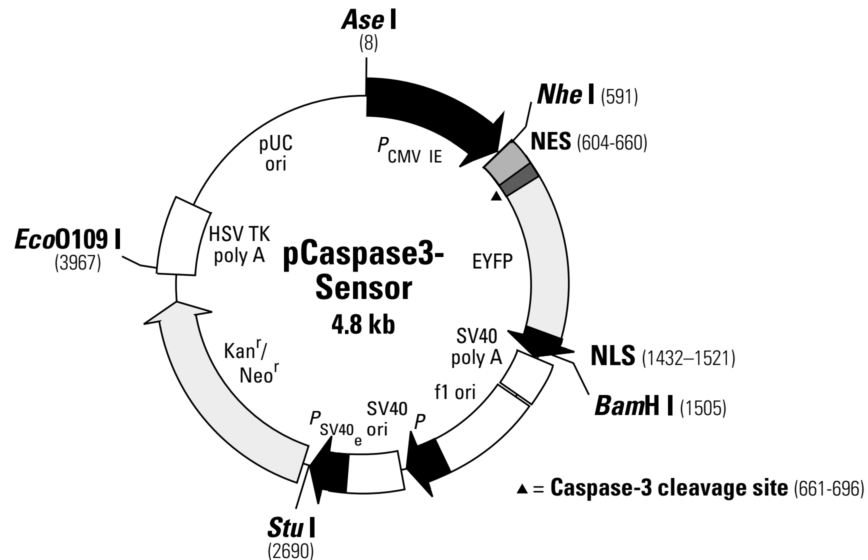

Map of pCaspase3-Sensor. NLS = three tandem repeats of the nuclear localization signal from simian virus large T-antigen. NES = Dominant nuclear export signal of MAPkinase kinase. All restriction sites shown are unique.

Description

pCaspase3-Sensor Vector can be used to detect the onset of caspase-3 activity in mammalian cells. This vector encodes the enhanced yellow-green variant (EYFP) of the *Aequorea victoria* green fluorescent protein (GFP) fused at the 3' end to three copies of the nuclear localization signal (NLS) of the simian virus 40 large T-antigen (1,2). At the 5' end the gene contains a sequence encoding the nuclear export signal (NES) of the Map Kinase Kinase (MAPKK;3). The NES is separated from the EYFP coding region by a 36-nucleotide sequence encoding the region of Poly (ADP-ribose) polymerase (PARP) cleaved by caspase-3. The complete coding sequence for this fusion protein is human codon-optimized (4).

Because the NES of MAPKK dominates the NLS, the full-length fluorescent fusion protein distributes to the cytosol. If caspase-3 is activated, the NES will be cleaved from the fusion protein and the truncated EYFP-NLS fusion will translocate to the nucleus via the NLS. The translocation of the fluorescent protein from the cytosol to the nucleus indicates caspase-3 activity at a cellular level.

The fluorescence excitation maximum of EYFP is 513 nm; the emission spectrum has a peak at 527 nm (in the yellow-green region). The E_m of EYFP at 513 nm is 36,500 $cm^{-1}M^{-1}$ and its quantum yield is 0.63 (5), resulting in a bright fluorescent signal. The fluorescence intensity is roughly equivalent to that of EGFP.

The vector contains an SV40 origin of replication and a neomycin resistance (Neo^r) gene for selection (using G418) in eukaryotic cells. A bacterial promoter (*P*) upstream of Neo^r expresses kanamycin resistance in *E. coli*. The vector backbone also provides a pUC19 origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production. The pCaspase3-Sensor Vector can be introduced into mammalian cells using any standard transfection method. If desired, stable transfectants can be selected using G418 (6).

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Use

pCaspase3-Sensor can be used to detect the onset of caspase-3 activity in mammalian cells. This vector allows visual monitoring of caspase-3 activity in the cell via fluorescence microscopy. If the fluorescent fusion protein encoded by the pCaspase3-Sensor vector is in the cytosol, caspase-3 is not active. However, if caspase-3 becomes active, the dominant nuclear export signal (NES) is cleaved from the fusion protein and the truncated fluorescent protein will become localized to the nucleus via the nuclear localization signal.

Location of features

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  Enhancer region: 59–465; TATA box: 554–560; transcription start point: 583
  C→G mutation to remove *Sac* I site: 569
- Nuclear export signal (NES) of Map Kinase Kinase (MAPKK): 604–660
- Caspase-3 cleavage site: 661–696
- Enhanced yellow fluorescent protein (EYFP) gene: 697–1431
- Tandem repeat of the nuclear localization signal (NLS): 1432–1521
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1664–1669 & 1693–1698; mRNA 3' ends: 1702 & 1714
- f1 single-strand DNA origin: 1680–2135 (Packages the noncoding strand of pCaspase3-sensor.)
- Bacterial promoter for expression of Kan^r gene.
  –35 region: 2278–2283; –10 region: 2301–2306
  Transcription start point: 2313
- SV40 origin of replication: 2557–2692
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2390–2461 & 2462–2533
  21-bp repeats: 2537–2557, 2558–2578 & 2580–2600
  Early promoter element: 2613–2619
  Major transcription start points: 2609, 2647, 2653 & 2658
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2741–2743; stop codon: 3533–3535
  G→A mutation to remove *Pst* I site: 2923
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3269
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3771–3776 & 3784–3789
- pUC plasmid replication origin: 4120–4763

Primer Locations

- EGFP-N Sequencing Primer (#6479-1): 760–739

Propagation in *E. coli*

- Suitable host strains: DH5α, 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 (50 μg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: High
- Plasmid incompatibility group: pMB1/ColE1

References

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4. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
5. Ormö, M., *et al.* (1996) *Science* **273**: 1392–1395.
6. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II,* Ed. Glover, D. M. (IRL Press, Oxford, UK), pp. 143–190.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by BD Biosciences Clontech. This vector has not been completely sequenced.

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