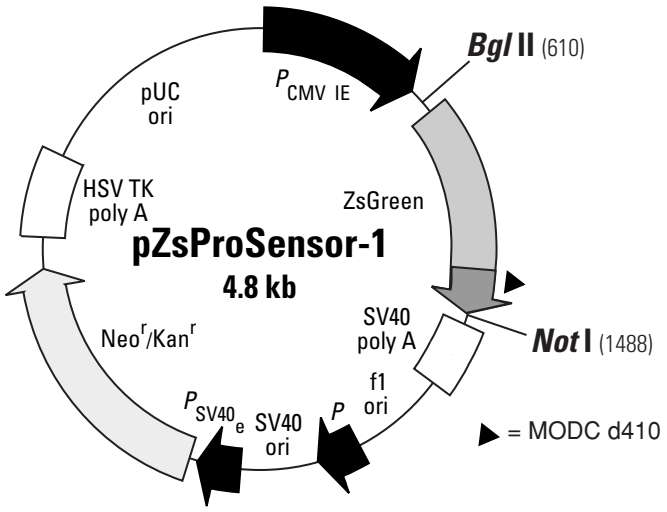

Restriction Map of pZsProSensor-1 Vector. All restriction sites shown are unique.

Description

pZsProSensor-1 is a eukaryotic expression vector designed to express ZsGreen fused to the mouse ornithine decarboxylase degradation domain (amino acids 410–461 of MODC; MODC d410). ZsGreen is the a naturally-occuring green fluorescent reef coral protein (*Zoanthus sp.*) with excitation maximum = 496 nm and emission maximum = 506 nm.

The mouse ornithine decarboxylase degradation domain (MODC d410) is fused to the C-terminus of the ZsGreen coding region. Protein fusions with the MODC d410 are targetted to the proteasome because MODC d410 contains several PEST sequences (1). As a result, the ZsGreen-MODC d410 protien fusion is highly susceptible to degradation by the proteasome.

The ZsGreen-MODC d410 fusion protein transcript is expressed from the immediate early cytomegalovirus promoter (P_{CMVIE}) in mammalian cells.

The Proteasome Sensor Vector backbone contains other sequences that help with propagation, replication and selection in host cells. The vector backbone contains an SV40 origin (SV40 ori) for replication in mammalian cells that express the SV40 T antigen, a pUC origin of replication (pUC ori) for propagation in *E. coli*, and an f1 origin (f1 ori) for single-stranded DNA production. In addition, a neomycin-resistance cassette—consisting of the SV40 early promoter (P_{SV40_e}), the neomycin/kanamycin resistance gene of Tn5 (Neo^r/Kan^r), and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK poly A) gene—allows stably-transfected eukaryotic cells to be selected using G418 (2). A bacterial promoter (*P*) upstream of this cassette drives expression of the Neo^r/Kan^r gene in *E. coli* hosts, which can be selected with kanamycin.

Use

pZsProSensor-1 Vector can be used to monitor proteasome activity in living cells. This vector is designed to express ZsGreen fused to the mouse ornithine decarboxylase degradation domain (MODC d410). When the proteasome is active in living cells, the protein will not accumulate. However, when the proteasome activity decreases—such as when proteasome inhibitors are added—the fusion protein will accumulate in cells and thus results in an increase in green flourescence under 496 nm light.

(PR2Z254; published 19 December 2002)

The recombinant Proteasome Sensor Vector can be transfected into mammalian cells using any standard transfection method. It can be used in transient as well as stable transfections. For more sensitive and homogeneous results, stable transformants can be selected using G418 (No. 8056-1, -2). We recommend selecting mammalian cell cultures in 500–1,300 µg/ml G418, depending on the cell line. Be sure to establish a kill curve for each cell line and each lot of G418 to determine the optimal selection concentration.

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
- ZsGreen-ΔMODC fusion protein
  - Start codon (ATG): 628–630; stop codon: 1483–1485
  - *Zoanthus* sp. green fluorescent protein (ZsGreen) sequence: 628–1320
  - Mouse Ornithine Decarboxylase (MODC) d410 sequence: 1326–1485
- SV40 early mRNA polyadenylation signal: 1638–1688
- f1 single-strand DNA origin: 1735–2190 (Packages the noncoding strand of ZsGreen-MODC.)
- Bacterial promoter for expression of Kan^r gene:
  - –35 region: 2252–2257; –10 region: 2275–2280
  - Transcription start point: 2287
- SV40 origin of replication: 2531–2666
- SV40 early promoter
  - Enhancer (72-bp tandem repeats): 2364–2435 & 2436–2507
  - 21-bp repeats: 2511–2531, 2532–2596 & 2598–2618
  - Early promoter element: 2587–2593
  - Major transcription start points: 2583, 2621, 2627 & 2632
- Kanamycin/neomycin resistance gene
  - Neomycin phosphotransferase coding sequences: start codon (ATG): 2715–2717; stop codon: 3507–3509
  - G→A mutation to remove *Pst* I site: 2897
  - C→A (Arg to Ser) mutation to remove *Bss*H II site: 3243
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  - Polyadenylation signals: 3745–3750 & 3758–3763
- pUC plasmid replication origin: 4094–4737

Propagation in *E. coli*

- Recommended host strain: DH5α
- Selectable marker: plasmid confers resistance to kanamycin (50 µg/ml) to *E. coli* hosts
- *E. coli* replication origin: pUC
- Copy number: ~500

References

1. Li, X. & Coffino, P. (1993) *Mol. Cell. Biol.* **13**:2377–2383.
2. Gorman, C. (1985). In *DNA Cloning: A Practical Approach, Vol. II.* Ed. D.M. Glover. (IRL Press, Oxford, U.K.) 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 Clontech Laboratories, Inc.. This vector has not been completely sequenced.