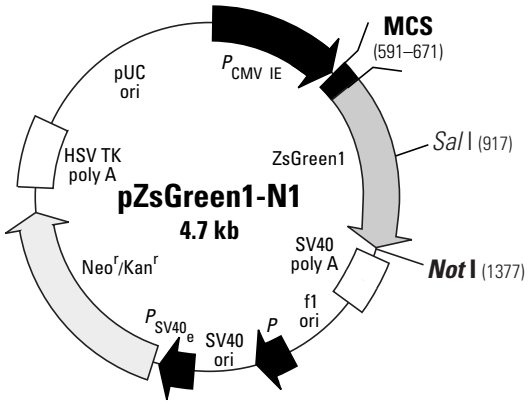
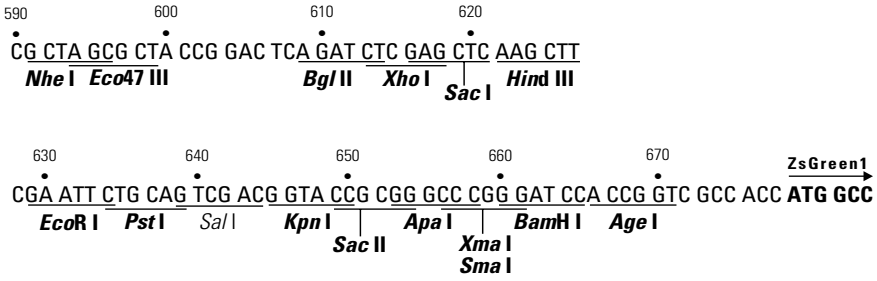
Vector Information

pZsGreen1-N1 Vector Information PT3480-5

Cat. No. 632448 and also sold as part of Cat. No. 630050





Restriction Map and Multiple Cloning Site (MCS) of pZsGreen1-N1 Vector. Unique restriction sites are shown in bold.

Description

pZsGreen1-N1 encodes a human codon-optimized variant of wild-type *Zoanthus* sp. green fluorescent protein, ZsGreen1 (1). The ZsGreen1 coding sequence contains a series of silent base-pair changes, which correspond to human codon-usage preferences, for optimal expression in mammalian cells (2). Additionally, an upstream sequence—located just 5' to the ZsGreen1 start codon—has been converted to a Kozak consensus translation initiation site (3) to further increase the translation efficiency in eukaryotic cells. A single amino acid substitution (Asn-65 to Met) has been made to enhance the emission characteristics of ZsGreen1 (excitation maximum = 496 nm; emission maximum = 506 nm).

The multiple cloning site (MCS) in pZsGreen1-N1 is positioned between the immediate-early promoter of cytomegalovirus ($P_{CMV\ IE}$) and the ZsGreen1 coding sequence. Thus, genes cloned into the MCS will be expressed as fusions to the N-terminus of ZsGreen1 if they are in the same reading frame as ZsGreen1 and there are no intervening stop codons. The SV40 polyadenylation signals (SV40 poly A) downstream of the ZsGreen1 gene direct proper processing of the 3' end of ZsGreen1 mRNA.

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_{SV40e}), 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 (4). 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.

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Use

Fusions to the N terminus of ZsGreen1 retain the fluorescent properties of the native protein allowing the localization of the fusion protein *in vivo* . The target gene should be cloned into pZsGreen1-N1 so that it is in frame with the ZsGreen1 coding sequence, with no intervening, in-frame stop codons. The inserted gene should include the initiating ATG codon. The recombinant pZsGreen1-N1 vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (available from Clontech; Cat. Nos. 631307 & 631308). 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. Unmodified (i.e., non-recombinant) pZsGreen1-N1 can also be used simply to express ZsGreen1 in a cell line of interest (e.g., as a transfection marker).

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
- Multiple Cloning Site (MCS): 591–671
- *Zoanthus* sp. green fluorescent protein (ZsGreen1) coding sequence
  - Kozak consensus translation initiation site: 672–682
  - Start codon (ATG): 679–681; stop codon: 1372–1374
  - Asn-65 to Met mutation (A→T, C→G): 875, 876
- SV40 early mRNA polyadenylation signal
  - Polyadenylation signals: 1527–1532 & 1556–1561; mRNA 3' ends: 1565 & 1577
- f1 single-strand DNA origin: 1624–2079 (Packages the noncoding strand of ZsGreen1.)
- Bacterial promoter for expression of Kan^r gene:
  - –35 region: 2141–2146; –10 region: 2164–2169
  - Transcription start point: 2176
- SV40 origin of replication: 2420–2555
- SV40 early promoter
  - Enhancer (72-bp tandem repeats): 2253–2324 & 2325–2396
  - 21-bp repeats: 2400–2420, 2421–2441 & 2443–2463
  - Early promoter element: 2476–2482
  - Major transcription start points: 2472, 2510, 2516 & 2521
- Kanamycin/neomycin resistance gene
  - Neomycin phosphotransferase coding sequences: start codon (ATG): 2604–2606; stop codon: 3396–3398
  - G→A mutation to remove *Pst* I site: 2786
  - C→A (Arg to Ser) mutation to remove *Bss*H II site: 3132
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  - Polyadenylation signals: 3634–3639 & 3647–3652
- pUC plasmid replication origin: 3983–4626

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 JM101 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (35 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/Col E1

References

1. Matz, M. V., *et al.* (1999) *Nature Biotech.* **17**:969–973.
2. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
4. 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. This vector has not been completely sequenced.

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