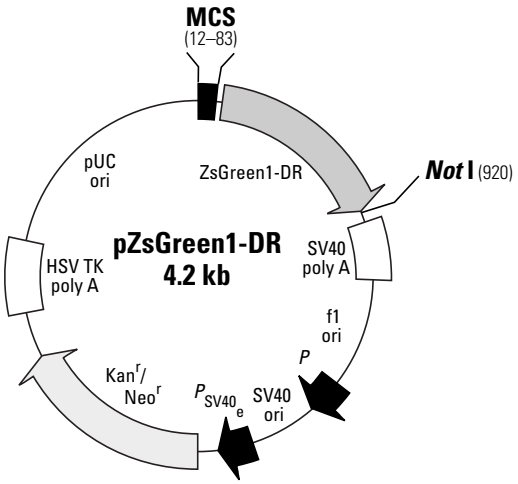
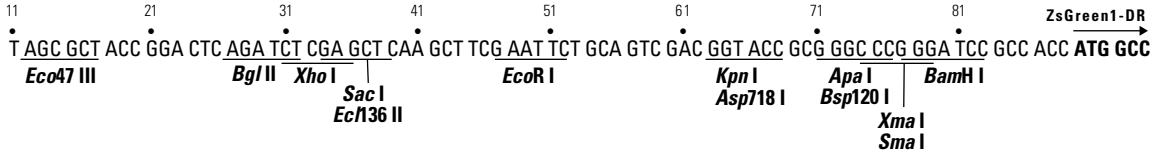
Vector Information

pZsGreen1-DR Vector Information PT3719-5

Cat. No. 632428





Restriction Map and Multiple Cloning Site (MCS) of pZsGreen1-DR. All sites shown are unique. The *Not* I site follows the ZsGreen1-DR stop codon.

Description

pZsGreen1-DR is a promoterless vector that encodes ZsGreen1-DR, a destabilized variant of *Zoanthus sp.* green fluorescent protein (ZsGreen; 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). In contrast to the original protein, ZsGreen1-DR has a short half-life, making it well suited for studies that require rapid reporter turnover. This destabilized variant was constructed by fusing the C-terminus of the protein to amino acid residues 422–461 of mouse ornithine decarboxylase (MODC), one of the most short-lived proteins in mammalian cells (3). This region of MODC contains a PEST sequence that targets the protein for degradation, resulting in rapid protein turnover (3,4). Three point mutations in this sequence further reduce the protein half-life (3). Sequences upstream of ZsGreen1-DR have been converted to a Kozak consensus translation initiation site (5) to enhance 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).

pZsGreen1-DR can be used to monitor transcription from different promoters and promoter/enhancer combinations inserted into the multiple cloning site (MCS), located upstream of the ZsGreen1-DR coding sequence. Downstream SV40 polyadenylation signals direct proper processing of the 3' end of the ZsGreen1-DR mRNA. The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 large T antigen, a pUC origin of replication for propagation in *E. coli*, and an f1 origin for single-stranded DNA production. A neomycin-resistance cassette (Neo^r) allows stably transfected eukaryotic cells to be selected using G418. This cassette consists of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK) gene. A bacterial promoter upstream of the cassette expresses kanamycin resistance in *E. coli*.

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Use

ZsGreen1-DR can be used as an *in vivo* reporter of gene expression. Because of its rapid turnover rate, its expression from a promoter of interest provides a more accurate assessment of the promoter's activity over time than does the more stable ZsGreen1. Promoter/enhancer elements should be inserted in the MCS upstream of the ZsGreen1-DR coding sequence. **Without the addition of a functional promoter, this vector will not express ZsGreen1-DR**. The recombinant pZsGreen1-DR vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (6).

Location of features

- MCS: 12–83
- Destabilized *Zoanthus sp.* Green Fluorescent Protein (ZsGreen1-DR) gene
  - Kozak consensus translation initiation site: 83–93
  - Start codon (ATG): 90–92; Stop codon: 915–917
  - Asn-65 to Met mutation (A→T, C→G): 286, 287
  - Mouse ornithine decarboxylase PEST sequence: 795–917
- SV40 early mRNA polyadenylation signal
  - Polyadenylation signals: 1070–1075 & 1099–1104
  - mRNA 3' ends: 1108 & 1120
- f1 single-strand DNA origin: 1167–1622
  - (Packages noncoding strand of ZsGreen1-DR.)
- Ampicillin resistance (β-lactamase) promoter
  - –35 region: 1684–1689; –10 region: 1707–1712
  - Transcription start point: 1719
- SV40 origin of replication: 1963–2098
- SV40 early promoter
  - Enhancer (72-bp tandem repeats): 1794–1867 & 1868–1939
  - 21-bp repeats: 1943–1963, 1964–1984 & 1986–2006
  - Early promoter element: 2019–2025
  - Major transcription start points: 2015, 2053, 2059 & 2064
- Kanamycin/neomycin resistance gene
  - Neomycin phosphotransferase coding sequences:
  - Start codon (ATG): 2147–2149; stop codon: 2939–2941
  - G→A mutation to remove *Pst* I site: 2329
  - C→A (Arg→Ser) mutation to remove *Bss*H II site: 2675
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  - Polyadenylation signals: 3177–3182 & 3190–3195
- pUC plasmid replication origin: 3526–4169

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: ~500
- Plasmid incompatibility group: pMB1/Col E1

Excitation and emission maxima of ZsGreen1

- Excitation maximum = 496 nm
- Emission maximum = 506 nm

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. Li, X., *et al.* (1998) *J. Biol. Chem.* **273**:34970–34975.
4. Rechsteiner, M., *et al.* (1990) *Semin. Cell Biol.* **1**:433–440.
5. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
6. 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.