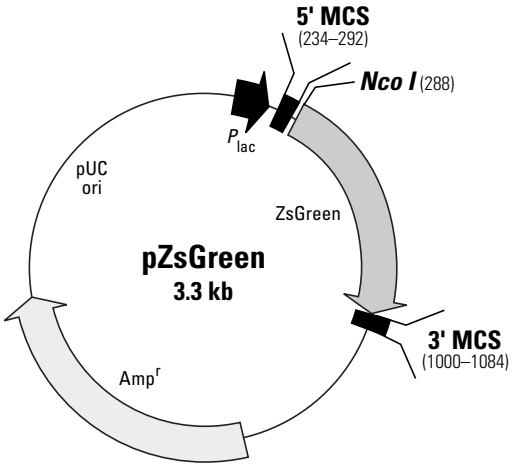
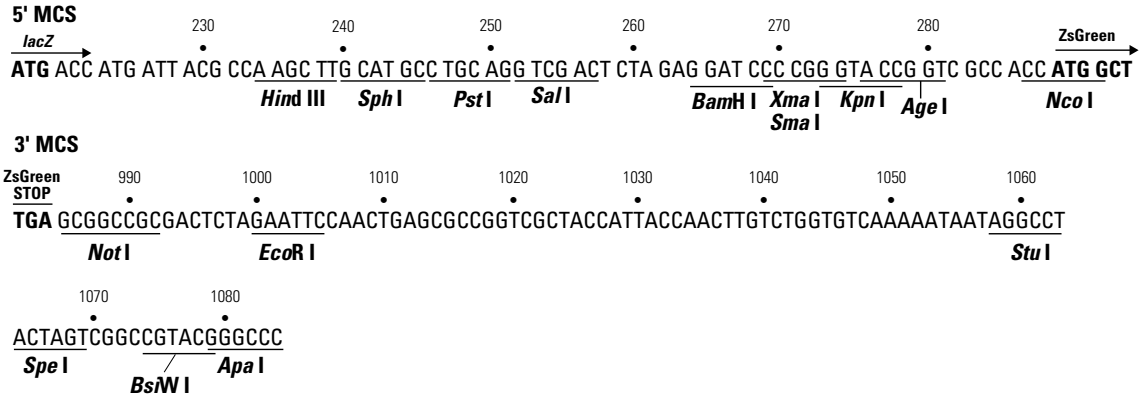
Vector Information

pZsGreen Vector Information PT3486-5

Cat. No. 632446 and also sold as part of Cat. No. 630050





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

Description

pZsGreen is a pUC19-derived prokaryotic expression vector, which encodes a variant of wild-type *Zoanthus* sp. green fluorescent protein (ZsGreen; 1) that has been engineered for brighter fluorescence. A single amino acid substitution (Asn-65 to Met) has been made to enhance the emission characteristics of ZsGreen (excitation maximum = 496 nm; emission maximum = 506 nm).

The ZsGreen gene was inserted in frame with the *lacZ* initiation codon from pUC19 so that ZsGreen is expressed from the *lac* promoter (P_{lac}) in *E. coli.* The ZsGreen coding sequence is flanked by distinct multiple cloning sites (MCS) at the 5' and 3' ends so that the gene can be readily excised from pZsGreen and subcloned into other expression vectors. An upstream sequence—located just 5' to the ZsGreen gene—has been converted to a Kozak consensus translation initiation site (2) to increase the translation efficiency in eukaryotic expression systems. The pUC backbone of pZsGreen provides a high-copy-number origin of replication (pUC ori) and an ampicillin resistance gene (Amp^r) for propagation and selection in *E. coli*.

Use

pZsGreen Vector serves as a convenient source of ZsGreen cDNA. The flanking MCS regions make it possible to excise the ZsGreen coding sequence and insert it into other expression vectors. Alternatively, the ZsGreen coding sequence can be amplified by PCR.

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Location of features

- *lac* promoter: 95–178
  - CAP binding site: 111–124
  - –35 region: 143–148; –10 region: 167–172
  - Transcription start point: 179
  - *lac* operator: 179–199
- *lacZ*-ZsGreen fusion protein expressed in *E. coli*
  - Ribosome binding site: 206–209
  - Start codon (ATG): 217–219; stop codon: 982–984
- 5' Multiple Cloning Site (MCS): 234–292
- *Zoanthus* sp. green fluorescent protein (ZsGreen) gene
  - Kozak consensus translation initiation site: 282–292
  - Start codon (ATG): 289–291; stop codon: 982–984
  - Asn-65 to Met mutation (A→T; C→G): 485; 486
  - Val-79 to Ala mutation (T→C): 527
- 3' Multiple Cloning Site (MCS): 1000–1084
- Ampicillin resistance gene
  - Promoter: –35 region: 1460–1465; –10 region: 1483–1488
  - Transcription start point: 1495
  - Ribosome binding site: 1518–1522
  - β-lactamase coding sequences:
    - Start codon (ATG): 1530–1532; stop codon: 2388–2390
    - β-lactamase signal peptide: 1530–1598
    - β-lactamase mature protein: 1599–2387
- pUC plasmid replication origin: 2538–3181

Propagation in *E. coli*

- Recommended host strain: JM109
- Selectable marker: plasmid confers resistance to ampicillin (50 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. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.

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.