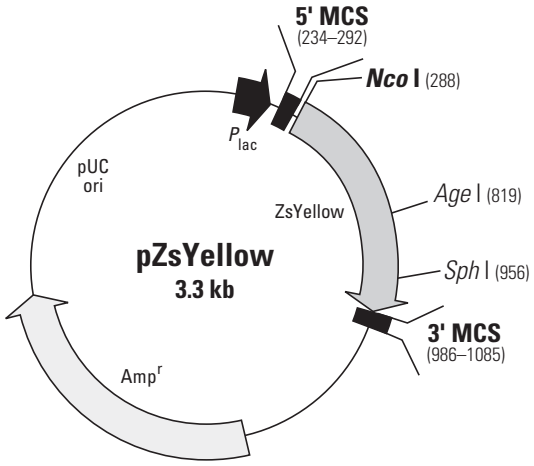
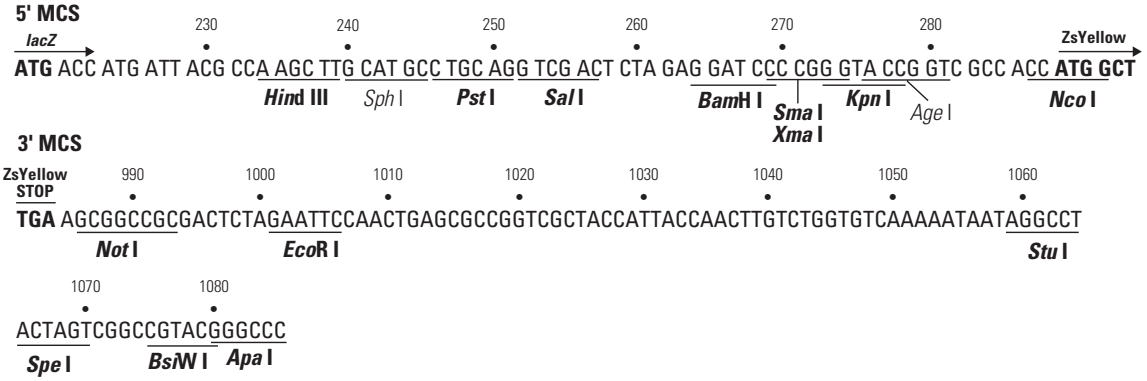
Vector Information

pZsYellow Vector Information PT3481-5

Cat. No 632443 and also sold as part of Cat. No. 630050





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

Description

pZsYellow is a pUC19-derived prokaryotic expression vector, which encodes a variant of wild-type *Zoanthus* sp. yellow fluorescent protein (ZsYellow) that has been engineered for brighter fluorescence. Wild-type ZsYellow cDNA was originally cloned from a *Zoanthus* species of coral, native to the Indian and Pacific oceans (1). A single amino acid substitution (Met-128 to Val) has been made to enhance the emission characteristics of ZsYellow (excitation maximum = 529 nm; emission maximum = 539 nm).

The ZsYellow gene was inserted in frame with the *lacZ* initiation codon from pUC19 so that ZsYellow is expressed from the *lac* promoter (P_{lac}) in *E. coli.* The ZsYellow coding sequence is flanked by distinct multiple cloning sites (MCS) at the 5' and 3' ends so that the gene can be easily excised from pZsYellow and subcloned into other expression vectors. An upstream sequence—located just 5' to the ZsYellow 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 pZsYellow 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

pZsYellow Vector serves as a convenient source of the ZsYellow cDNA. The flanking MCS regions make it possible to excise the ZsYellow coding sequence and insert it into other prokaryotic or eukaryotic expression vectors. Alternatively, the ZsYellow 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*-ZsYellow 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. yellow fluorescent protein (ZsYellow) gene
  - Kozak consensus translation initiation site: 282–292
  - Start codon (ATG): 289–291; stop codon: 982–984
  - Met-128 to Val mutation (A→G): 673
- 3' Multiple Cloning Site (MCS): 986–1085
- Ampicillin resistance gene
  - Promoter: –35 region: 1461–1466; –10 region: 1484–1489
  - Transcription start point: 1496
  - Ribosome binding site: 1519–1523
  - β-lactamase coding sequences:
    - Start codon (ATG): 1531–1533; stop codon: 2389–2391
    - β-lactamase signal peptide: 1531–1599
    - β-lactamase mature protein: 1600–2388
- pUC plasmid replication origin: 2539–3182

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.