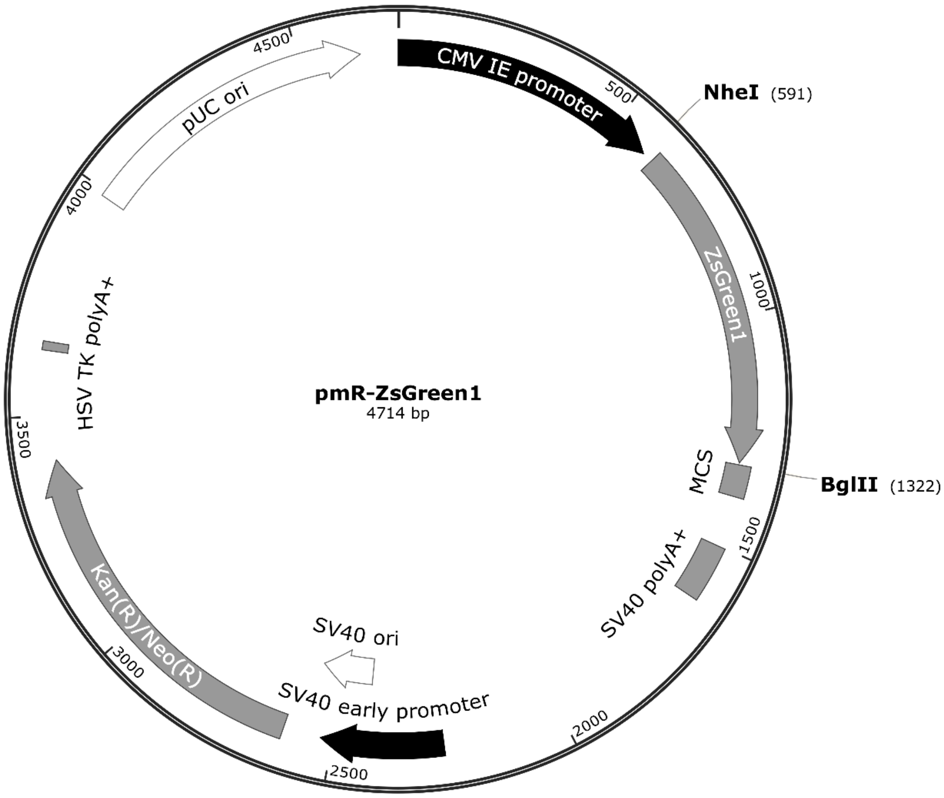
pmR-ZsGreen1 Vector

Catalog No.
632541



pMR-ZsGreen1 MCS

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                                 SacI
                         XhoI                         PstI
  ZsGreen1 Stop Codon  BglII        HindIII   EcoRI            KpnI
1311 AAACTAGTCT CAGATCTCGA GCTCAAGCTT CGAATTCTGC AGTCGACGGT
     TTTGATCAGA GTCTAGAGCT CGAGTTCGAA GCTTAAGACG TCAGCTGCCA

              SmaI
              XmaI
     KpnI   ApaI   BamHI       XbaI
1361 ACCGCGGGCC CGGGATCCAC CGGATCTAGA
     TGGCGCCCGG GCCCTAGGTG GCCTAGATCT
```

Figure 1. pmR-ZsGreen1 Vector map and multiple cloning site (MCS). *The XbaI site is methylated in the DNA provided. If you wish to digest the vector with XbaI enzyme, you will need to transform the vector into a dam-host and make fresh DNA.

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Vector Map

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pmR-ZsGreen1

Description

pmR-ZsGreen1 is a mammalian expression vector designed to constitutively express a microRNA of interest. Transfected cells can be identified by the coexpression of ZsGreen1, a human codon-optimized variant of the reef coral *Zoanthus sp.* green fluorescent protein (ZsGreen) that has been engineered for brighter fluorescence (excitation and emission maxima: 493 and 505 nm, respectively; Matz et al. 1999; Haas et al. 1996). Coexpression of ZsGreen1 and your microRNA of interest allows easy monitoring and/or selection of microRNA-expressing cells by fluorescence microscopy or flow cytometry.

The pmR-mZsGreen1 multiple cloning site (MCS) is positioned in the 3'UTR, downstream of the ZsGreen1 coding sequence. Expression of ZsGreen1 and microRNA precursors cloned into the MCS is driven by the constitutively active human cytomegalovirus immediate early promoter ($P_{CMV\ IE}$), located just upstream of the ZsGreen1 sequence. Both the fluorescent protein and the microRNA are expressed from a single mRNA transcript, which is cleaved by Drosha and Dicer to generate the mature microRNA.

Use

A small genomic fragment containing the precursor of the microRNA of interest must be isolated and cloned into pmR-ZsGreen1. This is most easily accomplished by PCR amplification from genomic DNA. We recommend including 100–300 bp of genomic DNA flanking the actual microRNA precursor to ensure efficient processing by Drosha. The orientation of the cloned microRNA precursor should be the same as that of the ZsGreen1 transcript. The sequence of the microRNA precursor and flanking genomic DNA can be obtained from a number of public databases including GenBank (http://www.ncbi.nlm.nih.gov/) and EMBL-Bank (http://www.ebi.ac.uk/embl/). The UCSC Genome Bioinformatics Site (http://genome.ucsc.edu/) hosts an easy-to-navigate genomic database with tracks for microRNAs. The Sanger Institute hosts miRBase, a compilation of known microRNA sequences (http://microrna.sanger.ac.uk/).

The pmR-ZsGreen1 vector can be transfected into mammalian cells using any standard transfection method. If desired, stable transfectants can be selected using G418. Overexpressed microRNA can be detected using our Mir-X™ miRNA qRT-PCR TB Green® Kit (Cat. Nos. 638314 and 638316). For Western analysis, the ZsGreen1 protein can be detected using either the Living Colors® Full-Length ZsGreen Polyclonal Antibody (Cat. No. 632474) or the Living Colors Anti-RCFP Polyclonal Pan Antibody (Cat. No. 632475).

Location of Features

- $P_{CMV\ IE}$ (human cytomegalovirus immediately early promoter): 1–589
- ZsGreen1 (human codon optimized): 613–1317
- MCS (multiple cloning site): 1322–1390
- SV40 early polyA+ signals: 1502–1623
- $P_{SV40\ e}$ (SV40 early promoter): 2259–2527
- SV40 ori: 2426–2561
- Kanamycin/neomycin resistance gene: 2610–3404
- HSV TK polyA+ (herpes simplex virus thymidine kinase polyadenylation signals): 3640–3658
- pUC origin of replication: 3989–4632

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) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high

Excitation and emission maxima of ZsGreen1

- Excitation maximum = 493 nm
- Emission maximum = 505 nm

References

Matz, M.V., *et al.*, Fluorescent proteins from nonbioluminescent Anthozoa species. *Nature Biotech.* **17,** 969–973 (1999).

Haas, J., *et al.*, Codon usage limitation in the expression of HIV-1 envelope glycoprotein. *Curr. Biol.* **6,** 315–324 (1996).

Gorman, C., In *DNA Cloning: A Practical Approach, Vol. II.* Ed. D. M. Glover (IRL Press, Oxford, U.K.) pp. 143–190 (1985).

Note: The vector sequence was compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Takara Bio USA, Inc. This vector has not been completely sequenced.

Notice to Purchaser

This document has been reviewed and approved by the Quality Department.