pmRi-ZsGreen1

Catalog No.
631121

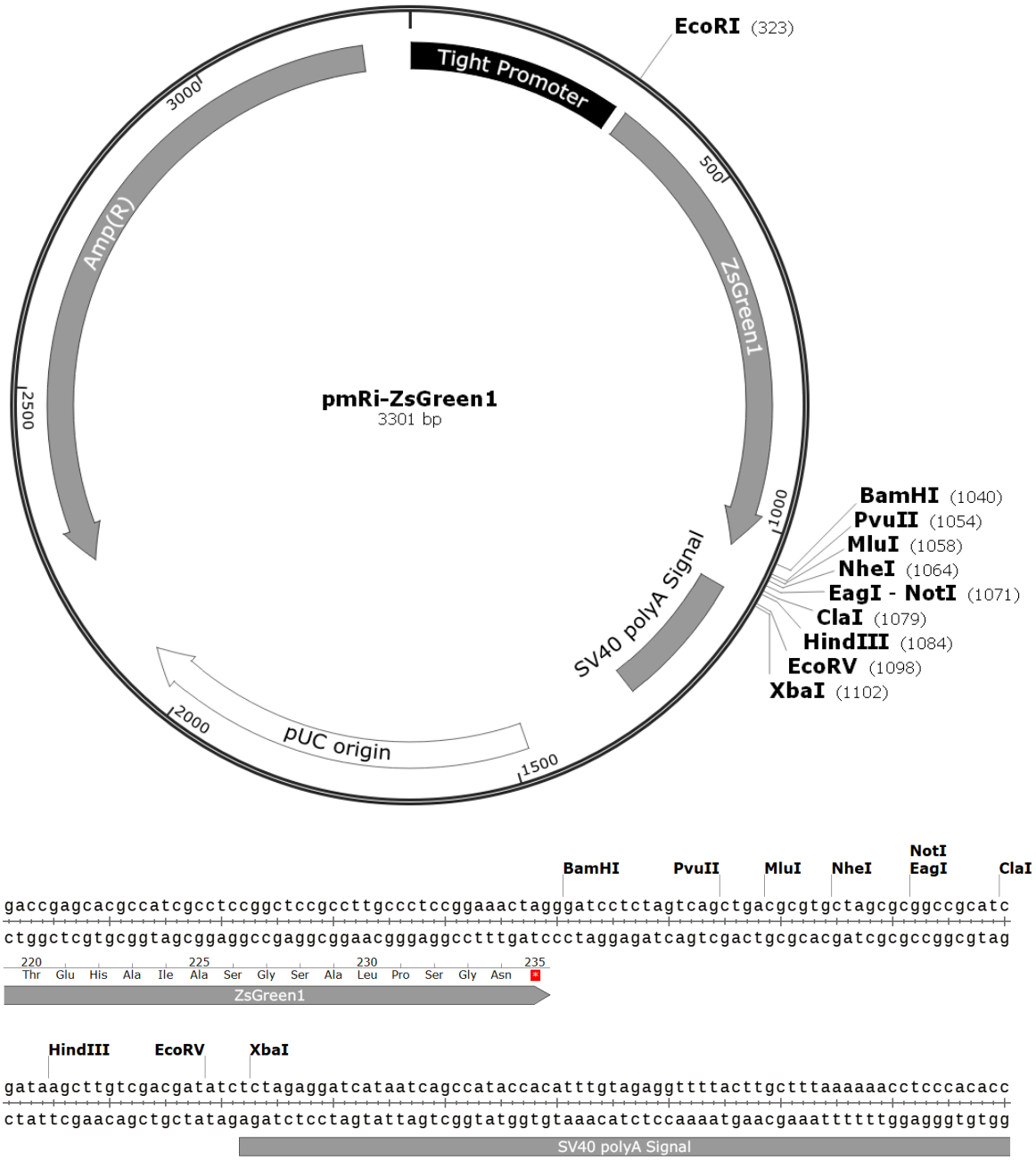

Figure 1. pmRi-ZsGreen1 vector map and multiple cloning site (MCS).

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Description

pmRi-ZsGreen1 is a tetracycline (Tet)-regulatable, mammalian expression vector designed to express a microRNA of interest under the control of $P_{Tight'}$, a modified Tet-responsive promoter. P_{Tight} consists of a modified minimal CMV promoter, and seven direct repeats of a 36 bp regulatory sequence that contains the 19 bp tet operator sequence (*tetO*; 1). This vector is designed to be used in conjunction with Tet-On or Tet-Off transactivators (Gossen & Bujard, 1992; Gossen et al., 1995; Urlinger et al., 2000), which are supplied by numerous vectors and vector systems available from Takara Bio.

Induced expression in 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; Haas, Park, & Seed, 1996; Matz et al., 1999). The pmRi-ZsGreen1 multiple cloning site (MCS) is positioned in the 3' UTR, downstream of the ZsGreen1 coding 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. 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 vector also contains a pUC origin of replication and an ampicillin resistance gene (Amp') for propagation and selection in *E. coli*.

Use

pmRi-ZsGreen1 allows tightly regulated, doxycycline (Dox)-controlled coexpression of a microRNA of interest and ZsGreen1. A small genomic fragment containing the precursor of the microRNA of interest must be isolated and cloned into the vector. 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/).

pmRi-ZsGreen1 can be transfected into mammalian cells using any standard transfection method. If desired, stable transfectants can be obtained by cotransfecting the vector with one of the linear selection markers supplied with the vector and selecting on medium containing the appropriate antibiotic. Dox-regulated expression requires the presence of a tetracycline-controlled transactivator (Tet-On or Tet-Off), supplied in numerous vectors and vector systems available from Takara Bio. Alternatively, a complete system is available in the Mir-X™ Inducible miRNA System (Green; Cat. No. 631120).

Overexpressed microRNA can be detected using Takara Bio's Mir-X™ mi RNA qRT-PCR TB Green® Kit (Cat. Nos. 638314 and 638316). For Western analysis, the mCherry protein can be detected using either the Living Colors® Full-Length ZsGreen Polyclonal Antibody Polyclonal Antibody (Cat. No. 632496) or the Monoclonal Antibody (Cat. Nos. 632474) or the Living Colors Anti-RCFP Polyclonal Pan Antibody (Cat. No. 632475).

Location of Features

- P_{Tight} (modified Tet-responsive promoter): 3–318
- ZsGreen1 (human codon-optimized): 335–1039
- MCS (multiple cloning site): 1040–1107
- SV40 polyA signal: 1102–1302
- ColE1 origin of replication: 1476–2075

- Amp^r (ampicillin resistance gene; β-lactamase): 2237–3232 (complementary)

Propagation in *E. coli*

- Recommended host strain: DH5α, HB101, and other general-purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: ColE1
- Copy number: Low
- Plasmid incompatibility group: pMB1/ColE1

Excitation and emission maxima of ZsGreen1

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

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.

References

Gossen, M., & Bujard, H. (1992). Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proceedings of the National Academy of Sciences*, *89*(12), 5547–5551. https://doi.org/10.1073/PNAS.89.12.5547

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