pmRi-mCherry

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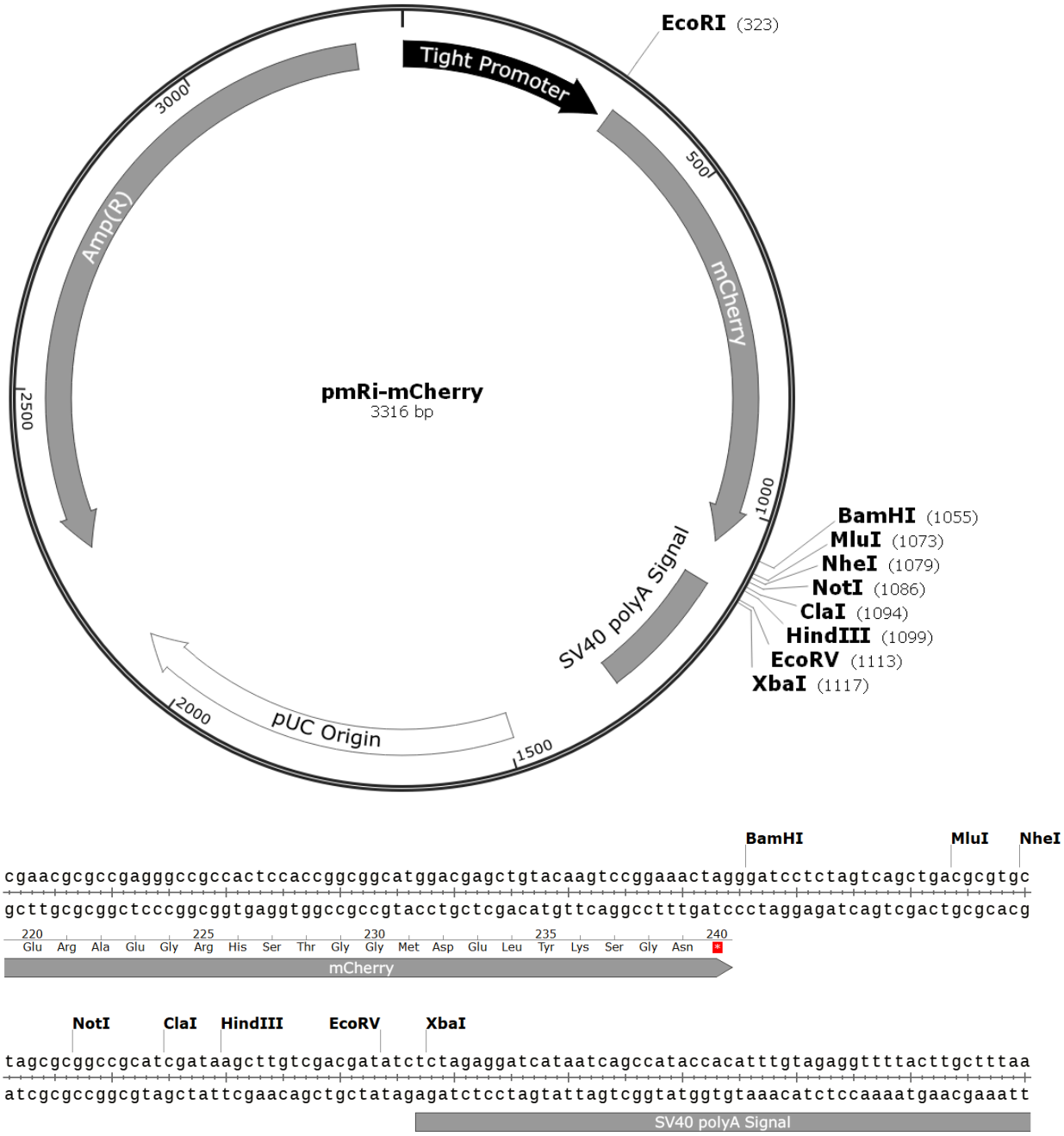

Figure 1. pmRi-mCherry vector map and multiple cloning site (MCS).

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Description

pmRi-mCherry 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 mCherry, a mutant fluorescent protein derived from the tetrameric *Discosoma sp.* Red fluorescent protein, DsRed (Shaner et al., 2004). The pmRi-mCherry multiple cloning site (MCS) is positioned in the 3' UTR, downstream of the mCherry 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 mCherry and your microRNA of interest allows easy monitoring and/or selection of microRNA-expressing cells by fluorescence microscopy or flow cytometry. The excitation and emission maxima of the native mCherry protein are 587 nm and 610 nm, respectively. The vector also contains a pUC origin of replication and an ampicillin resistance gene (Amp') for propagation and selection in *E. coli*.

Use

pmRi-mCherry allows tightly regulated, doxycycline (Dox)-controlled coexpression of a microRNA of interest and mCherry. 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-mCherry 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 (Red; Cat. No. 631118).

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® DsRed Polyclonal Antibody (Cat. No. 632496) or the Monoclonal Antibody (Cat. Nos. 632392 and 632393).

Location of Features

- P_{Tight} (modified Tet-responsive promoter): 3–318
- mCherry (human codon-optimized): 335–1054
- MCS (multiple cloning site): 1055–1122
- SV40 polyA signal: 1117–1317
- ColE1 origin of replication: 1491–2090
- Amp^r (ampicillin resistance gene; β-lactamase): 2252–3247 (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
- Plasmid incompatibility group: pMB1/ColE1

Excitation and emission maxima of mCherry

- Excitation maximum = 587 nm
- Emission maximum = 610 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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