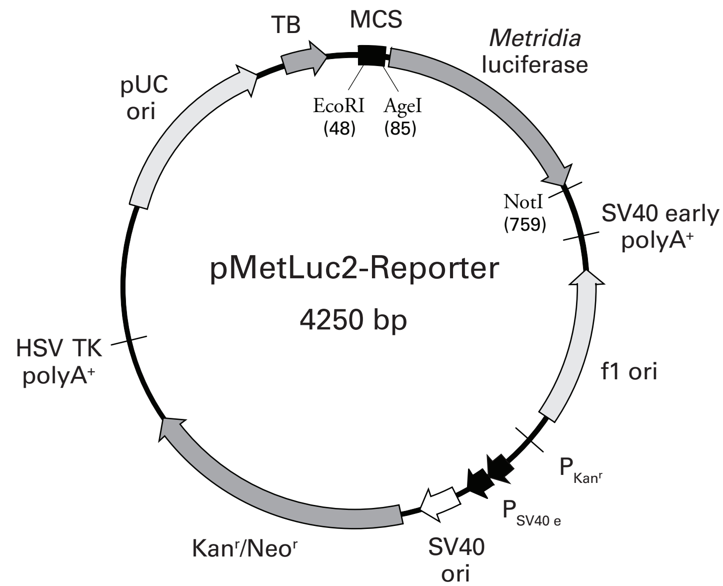
pMetLuc2-Reporter Vector Information

PT4059-5

Cat. No. 631729 & 631735

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                   SacI
           XhoI                           PstI                    SacII          BamHI
    BglII             HindIII    EcoRI              SalI                ApaI               AgeI
27  AGATCTCGAG  CTCAAGCTTC  GAATTCTGCA  GTCGACGGTA  CCGCGGGCCC  GGGATCCACC  GGTC
    TCTAGAGCTC  GAGTTCGAAG  CTTAAGACGT  CAGCTGCCAT  GGCGCCCGGG  CCCTAGGTGG  CCAG
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pMetLuc2-Reporter Vector Map and Multiple Cloning Site (MCS).

Description

pMetLuc2-Reporter is a promoter reporter vector that allows the analysis of promoter function in cell-based assays. The vector encodes a sequence-optimized, secreted luciferase from the marine copepod *Metridia longa*. The 24 kDa *Metridia* luciferase (MetLuc) reporter protein contains a 17 amino acid, N-terminal signal peptide that allows efficient secretion of the reporter (1). When a functional promoter is cloned into the MCS, located upstream of the MetLuc reporter gene, MetLuc is expressed and secreted into the medium surrounding the transfected cells.

SV40 polyadenylation signals downstream of the MetLuc gene direct proper processing of the 3' end of the MetLuc mRNA. The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 large T antigen, a pUC origin of replication for propagation in *E. coli*, and an f1 origin for single-stranded DNA production. A neomycin resistance cassette (Neo^r) allows stably transfected eukaryotic cells to be selected using G418. This cassette consists of the SV40 early promoter, the Tn5 kanamycin/neomycin resistance gene, and polyadenylation signals from the herpes simplex virus thymidine kinase (HSV TK) gene. The vector also contains a synthetic transcription blocker (TB), composed of adjacent polyadenylation and transcription pause sites, that reduces background readthrough transcription (2). A bacterial promoter (P_{Kan^r}) upstream of the cassette allows kanamycin resistance in *E. coli.*

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Use

The pMetLuc2-Reporter vector is used to monitor the activity of promoters cloned into the MCS, located upstream of the *Metridia longa* luciferase coding sequence. Upon induction, functional promoters will drive the expression of secreted luciferase, while nonfunctional promoters will not. The presence of the luciferase can be easily detected by adding luciferase substrate to a small aliquot of the culture medium and analyzing the sample in a luminometer. Promoter function can be quantified by the relative intensity of the bioluminescent signal.

The pMetLuc2-Reporter Vector can be transfected into mammalian cells using any standard transfection method. Stable transfectants can be selected using G418 when required (3).

Location of features

- MCS (multiple cloning site): 27–89
- *Metridia* luciferase (*Metridia longa* secreted luciferase): 97–753
- SV40 early polyA$^+$ signals: 909–914 and 938–943
- f1 origin of replication: 1006–1461 (complementary)
- P_{Kan^r} (bacterial promoter for Kan^r gene expression): 1523–1551
- $P_{SV40\ e}$ (SV40 early promoter and enhancer sequences): 1635–1863
- SV40 origin of replication: 1802–1940
- Kan^r/Neo^r (Tn5 kanamycin/neomycin resistance gene):
  - Neomycin phosphotransferase coding sequence: 1986–2780
  - G→A mutation to remove *Pst* I site: 2168
  - C→A (Arg→Ser) mutation to remove *Bss*H II site: 2514
- HSV TK polyA$^+$ (herpes simplex virus thymidine kinase polyadenylation signals): 3016–3021 & 3029–3034
- pUC origin of replication: 3365–4008
- TB (Transcription Blocker): 4038–4191

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
- Plasmid incompatibility group: pMB1/Col E1

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.

References

1. Markova, S.V. *et al.* (2004) *J. Bio. Chem.* **279**(5):3212-3217.
2. Eggermont, J. & Proudfoot, N. (1993) *EMBO J.* **12**(6):2539–2548.
3. Gorman, C. (1985) DNA cloning: A Practical Approach, Vol. II. Ed. D. M. Glover. (IRL Press Oxford, U.K.), pp. 143-190.