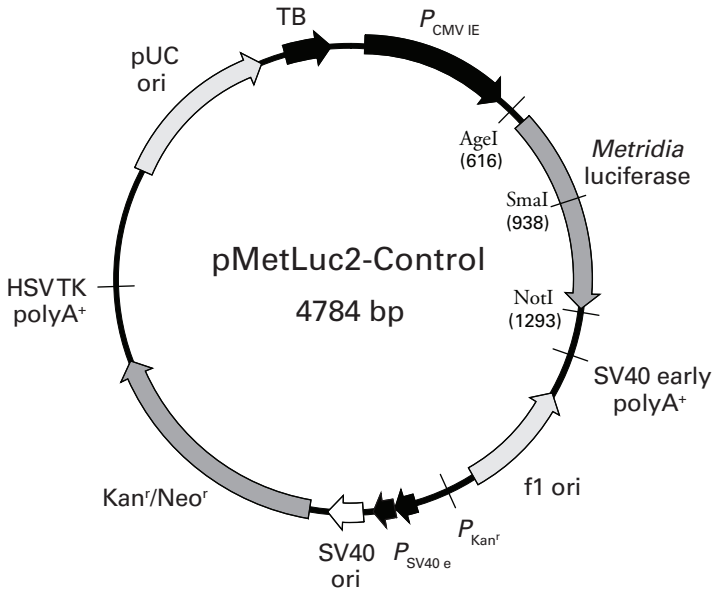
Vector Information

pMetLuc2-Control Vector Information

PT4060-5

Cat. Nos. 631729
631735
631743
631745



pMetLuc2-Control Vector Map.

Description

The pMetLuc2-Control vector encodes a sequence-optimized, secreted luciferase from the marine copepod *Metridia longa*. The 24 kDa *Metridia* luciferase (MetLuc) contains a 17 amino acid, N-terminal signal peptide that allows the protein to be efficiently secreted into the cell culture medium (1), making it easy to detect without cell lysis.

Expression of MetLuc is driven by the constitutively active cytomegalovirus immediate early promoter ($P_{CMV\ IE}$). 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 largeT 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.*

Use

The pMetLuc2-Control vector can be used as a positive control for the constitutive expression and secretion of MetLuc in experiments using the pMetLuc2-Reporter vector. pMetLuc2-Control can also be used to monitor the inhibitory effect of drugs, such as Brefeldin A, on protein secretion.

The pMetLuc2-Control Vector can be transfected into mammalian cells using any standard transfection method. Stable transfectants can be selected using G418 when required (3). The presence of MetLuc in the cell culture medium can be easily detected by adding luciferase substrate to a small aliquot of the medium and analyzing the sample with a luminometer.

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Location of features

- $P_{CMV\ IE}$ (human cytomegalovirus immediate early promoter): 1–589
- *Metridia* luciferase (*Metridia longa* secreted luciferase): 628–1287
- SV40 early polyA$^+$ signals: 1443–1448 and 1472–1477
- f1 origin of replication: 1540–1995 (complementary)
- P_{Kan^r} (bacterial promoter for Kan^r gene expression): 2057–2085
- $P_{SV40\ e}$ (SV40 early promoter and enhancer sequences): 2169–2379
- SV40 origin of replication: 2336–2474
- Kan^r/Neo^r (Tn5 kanamycin/neomycin resistance gene):
  - Neomycin phosphotransferase coding sequence: 2520–3314
  - G→A mutation to remove *Pst* I site: 2702
  - C→A (Arg→Ser) mutation to remove *Bss*H II site: 3048
- HSV TK polyA$^+$ (herpes simplex virus thymidine kinase polyadenylation signals): 3550–3555 & 3563–3568
- pUC origin of replication: 3899–4542
- TB (Transcription Blocker): 4572–4725

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.