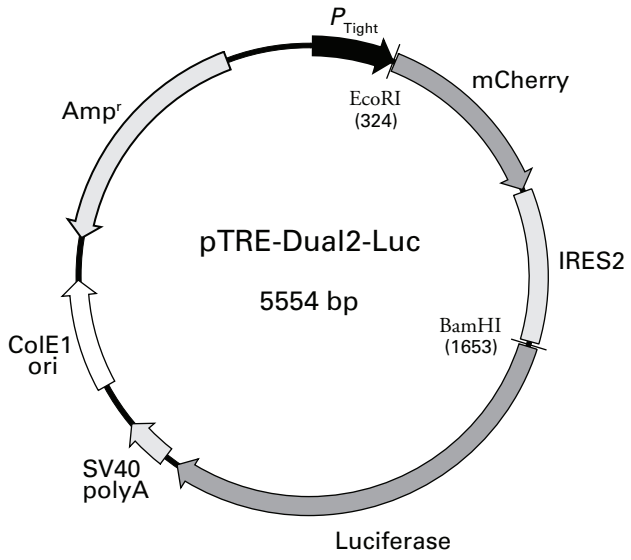
Vector Information

pTRE-Dual2-Luc Vector Information PT5039-5

Sold as part of Catalog Nos. 631112 and 631113



pTRE-Dual2Luc Vector Map.

Description

pTRE-Dual2-Luc is a tetracycline (Tet)-regulatable control vector (supplied with pTRE-Dual2) that coexpresses the red fluorescent protein mCherry and firefly luciferase 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 with our Tet-On® Advanced and Tet-Off® Advanced Inducible Gene Expression Systems (Cat. Nos. 630930 and 630934). These systems provide the inducible gene expression strategy of Gossen & Bujard, with major improvements described by Urlinger, *et al.* (2–6).

mCherry is a mutant fluorescent protein derived from the tetrameric *Discosoma sp.* red fluorescent protein, DsRed (excitation and emission maxima: 587 nm and 610 nm, respectively; 7). pTRE-Dual2-Luc allows inducible co-expression of firefly luciferase and mCherry. This ensures that a high percentage of mCherry-expressing clones also express luciferase in a doxycycline controllable manner, allowing mCherry to be used as an indicator of transfection efficiency and a marker for selection by flow cytometry. The vector also contains a ColE1 origin of replication and an ampicillin resistance gene (Amp^r) to allow for propagation and selection in *E. coli*.

Use

pTRE-Dual2-Luc is a control vector for our pTRE-Dual2 expression vector. pTRE-Dual2-Luc allows tightly regulated, doxycycline-controlled coexpression of mCherry and firefly luciferase. In order to function, the system requires the presence of a tetracycline-controlled transcriptional activator (Tet-On Advanced or Tet-Off Advanced), supplied by a stable Tet-On Advanced or Tet-Off Advanced cell line that can be created with our Tet-On Advanced or Tet-Off Advanced Inducible Gene Expression Systems (Cat. Nos. 630930 and 630934).

mCherry allows easy monitoring and/or selection of cells by fluorescence microscopy or flow cytometry (mCherry has an excitation maximum of 587 nm and an emission maximum of 610 nm). To detect luciferase, use any standard firefly luciferase assay system.

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Location of features

- P_{Tight} (modified Tet-responsive promoter): 8–321
- mCherry (human codon-optimized): 335–1045
- IRES2 (encephalomyocarditis virus internal ribosome entry site): 1052–1636
- Luciferase (firefly luciferase): 1661–3313
- SV40 polyA signal: 3367–3549
- ColE1 origin of replication: 3725–4149
- Amp^r (ampicillin resistance gene; β-lactamase): 4311–5234 (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

References

1. pTRE-Tight Vectors (April 2003) *Clontechniques* **XVIII**(3):13–14.
2. Gossen, M. & Bujard, H. (1992) *Proc. Natl. Acad. Sci USA* **89**(12):5547–5551.
3. Gossen, M., *et al.* (1995) *Science* **268**(5218):1766–1769.
4. Urlinger, S. *et al.* (2000) *Proc. Natl. Acad. Sci. USA* **97**(14):7963–7968.
5. Inducible Gene Expression Systems (January 2007) *Clontechniques* **XXII**(1):1–2.
6. Tet-On Advanced Inducible Gene Expression System (2006) *Clontechniques* **XXI**(2):1–3.
7. Shaner, N. C. *et al.* (2004) *Nature Biotech.* **22**(12):1567-1572.

Note: The vector sequence was 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.