



Restriction Map of pTet-Off-Advanced Vector. Unique restriction sites are in bold

Description

pTet-Off-Advanced expresses an improved version of the Tet (tetracycline)-controlled transactivator protein called tTA-Advanced (1–4). It is expressed to higher levels than the original tTA used in the Tet-Off[®] System (2). The tTA-Advanced protein is a fusion of amino acids 1–207 of the Tet repressor (TetR) and 39 amino acids containing three minimal "F"-type transcriptional activation domains (ADs) from the VP16 protein of herpes simplex virus. The gene is fully synthetic, lacks cryptic splice sites in the mRNA, and utilizes human codon preferences for stable expression in mammalian cells.

Use

The pTet-Off-Advanced Vector is used to develop stable Tet-Off Advanced cell lines, which are hosts for a tetracycline-controlled gene expression system. Once a vector containing a gene of interest under the control of a Tet-responsive element (e.g., TRE-Tight or TRE2) is transfected into a Tet-Off Advanced stable cell line, tTA-Advanced binds to the TRE in the absence of doxycycline (Dox, a tetracycline derivative), and activates transcription of the gene of interest to a very high level. In the presence of Dox, tTA-Advanced is unable to bind the TRE in a Tet-responsive promoter and the system is inactive. Additional information on TRE-containing vectors and protocols describing how to construct a Tet-Off Advanced cell line can be found in the Tet-Off Advanced Inducible Gene Expression System User Manual (PT3945-1).



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Location of Features

- Fragment containing P_{CMV} : 86–677
- tTA-Advanced: 775–1521
- Fragment containing the SV40 poly A signal: 1544–1977
- Col E1 origin of replication: 2344–2987
- Ampicillin resistance gene:
 β -lactamase coding sequences: 3994–3135
- Neomycin/kanamycin resistance gene: 6201–5407
- SV40 promoter (P_{SV40}) controlling expression of the neomycin/kanamycin resistance gene: 6865–6522.

Propagation in *E. coli*

- Suitable host strains: DH5 α and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 μ g/ml) in *E. coli* hosts.
- *E. coli* replication origin: Col E1

References

1. Inducible Gene Expression Systems (January 2007) *Clontechiques* XXII(1):1–2.
2. Urlinger, S., *et al.* (2000) *Proc. Natl. Acad. Sci. USA* 97(14):7963–7968.
3. Gossen, M. & Bujard, H. (1992) *Proc. Natl. Acad. Sci. USA* 89(12):5547–5551.
4. Gossen, M., *et al.* (1995) *Science* 268(5218):1766–1769.

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.

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