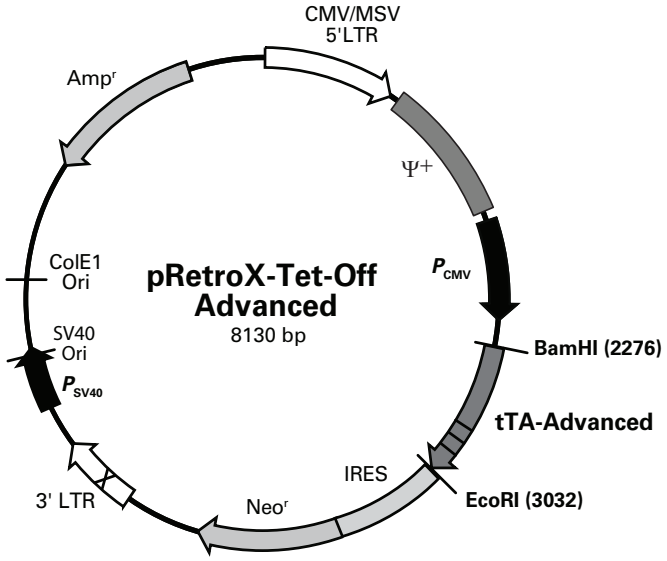
Vector Information

pRetroX-Tet-Off Advanced Vector Information PT3969-5

Catalog No. 632105



pRetroX-Tet-Off Advanced Vector Map.

Description

pRetroX-Tet-Off Advanced is a Retro-X™ Q retroviral vector that expresses tTA-Advanced, an improved version of the tetracycline(Tet)-controlled transactivator protein tTA (1-4). The tTA-Advanced protein is a fusion of the Tet repressor (TetR) DNA-binding domain, and three minimal "F"-type transcriptional activation domains from the herpes simplex virus VP16 protein. The gene encoding tTA-Advanced is completely synthetic, lacks cryptic splice sites, and utilizes human codon preferences for stable expression in mammalian cells. Expression of tTA-Advanced is driven by the powerful, constitutively active CMV promoter.

As with all of our Retro-X Q vectors, pRetroX-Tet-Off Advanced uses LTR self-inactivation to eliminate promoter interference. In LTR self-inactivation, the mechanism of viral integration is used to introduce a deletion into the 5′ LTR. As a result of this mutation, the 5′ LTR CMV/ MSV promoter, which drives high expression of the complete viral genome in packaging cells, is inactive in stably transduced target cells. This allows the expression of tTA-Advanced (from P_{CMV}) to proceed unimpeded (5, 6). The vector also contains a neomycin resistance gene (Neo^r) that allows G418 selection of stably transduced cells. tTA-Advanced and the Neo^r marker are coexpressed from a bicistronic transcript containing an internal ribosome entry site (IRES). This ensures that a high frequency of G418 resistant clones express the Tet-Off® Advanced transactivator.

pRetroX-Tet-Off Advanced contains all of the necessary viral RNA processing elements; these include the 5′ and 3′ LTRs, a packaging signal (Ψ$^+$), and a tRNA primer binding site. The vector also contains an SV40 origin of replication for plasmid propagation in mammalian cells that express SV40 T antigen, as well as a ColE1 origin of replication and an *E. coli* Amp^r gene for propagation and selection in bacteria.

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Use

The pRetroX-Tet-Off Advanced vector is used in conjunction with Tet response vectors to create double stable, Retro-X Tet-Off Advanced cell lines. Such cell lines are essentially Doxycycline (Dox)-controlled gene expression systems in which the Tet response vector expresses a gene of interest under the control of a Tet-responsive element (TRE; e.g. P_{Tight} or P_{TRE2}; 7). In the absence of Dox, tTA-Advanced binds to the TRE and activates expression of the gene of interest. In the presence of Dox, tTA-Advanced is unable to bind to the TRE, and the system is inactive. Additional information on TRE-containing vectors, and protocols describing how to construct a Retro-X Tet-Off Advanced cell line can be found in the Retro-X Tet-Off Advanced Inducible Gene Expression System User manual (PT3959-1).

Location of Features

- 5' LTR (CMV/MSV): 1–728
  U3 region, containing a Cytomegalovirus (CMV)/ mouse sarcoma virus (MSV) hybrid promoter: 1–584
  R region: 585–655
  U5 region: 656–727
- Ψ^+ (extended packaging signal): 759–1568
- P_{CMV} (Cytomegalovirus promoter): 1601–2132
- tTA Advanced: 2284–3027
- IRES (Internal Ribosome Entry Site): 3039–3612
- Neo^r (Neomycin resistance gene): 3625–4419
- 3' MMLV LTR (with a deletion in U3): 4837–5262
- P_{SV40}: 5541–5808
- SV40 origin of replication: 5762–5827
- ColE1 origin of replication: 6149
- Ampicillin resistance gene (β-lactamase): 6908–7768

Selection of Stable Transfectants

- Selectable marker: plasmid confers resistance to G418 (neomycin).

Propagation in *E. coli*

- Suitable host strains: DH5α™, DH10B™ and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: ColE1
- Copy number: high

Notes:

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.

The viral supernatants produced by this retroviral vector could contain potentially hazardous recombinant virus. Due caution must be exercised in the production and handling of recombinant retrovirus. Appropriate NIH, regional, and institutional guidelines apply.

References

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4. Urlinger, S. *et al.* (2000) *Proc. Natl. Acad. Sci. USA* **97**(14):7963-7968.
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7 pTRE-Tight Vectors (April 2003) *Clontechniques* **XVIII**(3):13-14.

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