



pRetroX-Tet-On Advanced Vector Map.

Description

pRetroX-Tet-On Advanced is a Retro-X™ Q retroviral vector that expresses rtTA-Advanced, an improved version of the reverse tetracycline (Tet)-controlled transactivator protein rtTA (1–4). rtTA-Advanced is more sensitive to doxycycline (Dox) and yields lower background expression than the original rtTA. The rtTA-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 rtTA-Advanced is completely synthetic, lacks cryptic splice sites, and utilizes human codon preferences for stable expression in mammalian cells. Expression of rtTA-Advanced is driven by the powerful, constitutively active CMV promoter.

As with all of our Retro-X Q vectors, pRetroX-Tet-On 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 rtTA-Advanced (from $P_{CMV IE}$) to proceed unimpeded (5, 6). The vector also contains a neomycin resistance gene (Neo^r) that allows G418 selection of stably transduced cells. rtTA-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-On® Advanced transactivator.

pRetroX-Tet-On 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 SV40T antigen. In addition, a pUC origin of replication and an *E. coli* Amp^r gene allow for propagation and selection in bacteria.

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Use

The pRetroX-Tet-On Advanced vector is used in conjunction with Tet response vectors to create double stable Retro-XTet-On 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} ; 7, 8). In the presence of Dox, rtTA-Advanced binds to the TRE and activates expression of the gene of interest. In the absence of Dox, rtTA-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-XTet-On Advanced cell line can be found in the Retro-XTet-On Advanced Inducible Gene Expression System User manual (PT3958-1).

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
- $P_{\text{CMV IE}}$ (human cytomegalovirus immediate early promoter): 1601–2132
- rtTA Advanced: 2284–3030
- IRES (internal ribosome entry site): 3042–3631
- Neo^r (neomycin resistance gene): 3644–4438
- 3' MMLV LTR (with a deletion in U3): 4856–5281
- P_{SV40} (SV40 promoter): 5560–5827
- SV40 origin of replication: 5781–5846
- pUC origin of replication: 6168
- Amp^r (ampicillin resistance gene; β -lactamase): 6927–7787 (complementary)

Selection of Stable Transfectants

- Selectable marker: plasmid confers resistance to G418.

Propagation in *E. coli*

- Suitable host strains: DH5 α^{TM} , DH10B $^{\text{TM}}$ and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 $\mu\text{g/ml}$) in *E. coli* hosts.
- *E. coli* replication origin: pUC

Note:

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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