



pRetroX-Tight-Hyg-Luc Vector Map.

Description

pRetroX-Tight-Hyg-Luc is a retroviral control vector (supplied with pRetroX-Tight-Hyg, Cat. No. 631043) that expresses firefly luciferase under the control of P_{Tight} , a modified Tet-responsive promoter. P_{Tight} consists of seven direct repeats of a 36 bp regulatory sequence that contains the 19 bp tet operator sequence (*tetO*), and a modified minimal CMV promoter (1). This vector is designed to be used as a control vector with Clontech's Advanced Inducible Expression Systems. These systems provide ready access to the inducible gene expression strategy of Gossen & Bujard, and incorporate major improvements described by Urlinger, *et al.* (2-6).

pRetroX-Tight-Hyg-Luc is optimized to eliminate promoter interference through LTR self-inactivation. The hybrid 5' LTR consists of the cytomegalovirus (CMV) type I enhancer and the mouse sarcoma virus (MSV) promoter. This promoter drives high levels of viral genome transcription in HEK 293-based packaging cell lines due, in part, to the presence of adenoviral E1A (7-8) in these cells. The self-inactivating feature of the vector is provided by a deletion in the 3' LTR enhancer region (U3). During reverse transcription of the retroviral RNA, a copy of the inactivated 3' LTR U3 region replaces the corresponding region of the 5' LTR, resulting in the inactivation of the 5' LTR CMV enhancer sequence. This mechanism can reduce the phenomenon known as promoter interference (9-10) and allow more efficient expression. In this way, pRetroX-Tight-Hyg-Luc supports high viral titers, yet eliminates potential downstream interference between the inducible P_{Tight} promoter and its adjacent viral LTR.

pRetroX-Tight-Hyg-Luc contains all of the necessary viral RNA processing elements; these include the 5' and 3' LTRs, the packaging signal (Ψ^+), and the tRNA primer binding site. For safety reasons, however, the vector lacks the structural genes (*gag*, *pol*, and *env*) necessary for retroviral particle formation and replication. pRetroX-Tight-Hyg-Luc contains a hygromycin resistance gene (Hyg^r) under the control of the murine phosphoglycerate kinase (PGK) promoter (P_{PGK}) for the selection of stable transfectants. In addition, the vector contains a ColE1 origin of replication and an *E. coli* Amp^r gene for propagation and selection in bacteria.

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Use

pRetroX-Tight-Hyg-Luc can be used as a control vector for either plasmid or retroviral expression vectors. When used as a control for retroviral expression vectors, it must be transfected into a packaging cell line, such as GP2-293; we recommend the Retro-X Universal Packaging System (Cat. No. 631530). Packaging cell lines allow you to produce infectious, replication-incompetent retroviral particles. These retroviral particles can infect a wide range of target cells, but they cannot replicate within these cells due to the absence of viral structural genes. The separate introduction and integration of the structural genes into the packaging cell line minimizes the chance of producing replication-competent virus due to recombination events during cell proliferation.

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: 656–728
- Ψ^+ (extended packaging signal): 759–1568
- P_{Tight} (modified Tet-responsive promoter): 1603–1918
- TATA box: 1880–1886
- Luciferase gene: 1935–3587
- P_{PGK} (phosphoglycerate kinase promoter): 3603–4106
- Hygromycin resistance gene (Hyg^r): 4124–5150
- 3' MMLV LTR (with a deletion in U3): 5750–6186
 - Poly A signal: 6087–6092
- P_{SV40} : 6465–6732
- SV40 origin of replication: 6686–6751
- ColE1 origin of replication: 7073
- Ampicillin resistance gene (β -lactamase): 7832–8692 (complementary)

Sequencing Primer Location

- P_{Tight} Sequencing Primer: 1579–1602
 - Primer Sequence: 5'-ATCTGAGGCCCTTTCGTCTTCACT-3'

Selection of Stable Transfectants

- Selectable marker: plasmid confers resistance to hygromycin.

Propagation in *E. coli*

- Suitable host strains: DH5 α and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 $\mu\text{g}/\text{ml}$) in *E. coli* hosts.
- *E. coli* replication origin: ColE1
- Copy number: high

Notes:

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.

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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