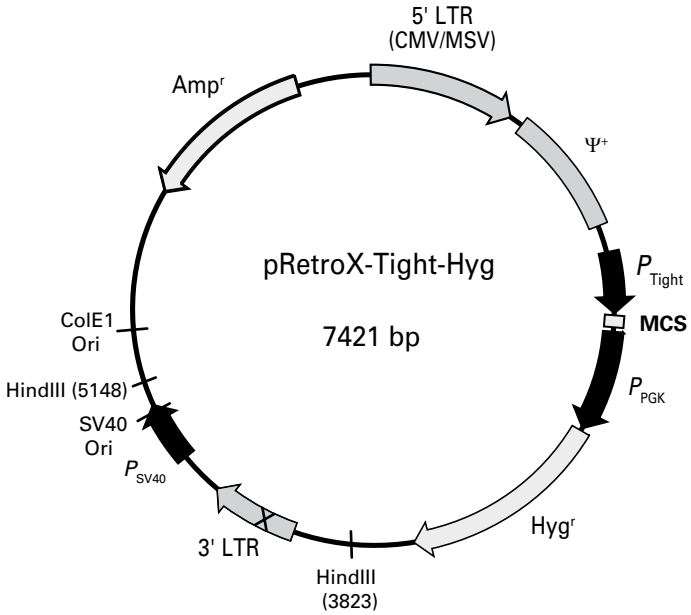
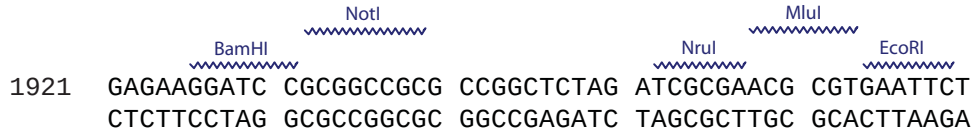
Vector Information

pRetroX-Tight-Hyg Vector Information PT3962-5

Catalog No. 631034





pRetroX-Tight-Hyg Vector Map and Multiple Cloning Site (MCS).

Description

pRetroX-Tight-Hyg is an inducible, self-inactivating, retroviral expression vector designed to express a gene of interest 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 for use with our Retro-X™ Tet-On® Advanced and Tet-Off® Advanced Inducible Expression Systems (Cat. Nos. 632104 and 632105). 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 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 supports high viral titers, yet eliminates potential downstream interference between the inducible P_{Tight} promoter and its adjacent viral LTR.

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pRetroX-Tight-Hyg 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 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.

Use

pRetroX-Tight-Hyg can be used as either a plasmid or retroviral expression vector. When used as a retroviral expression vector, 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 and transmit your gene of interest, 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
- Multiple Cloning Site (MCS): 1926–1969
- P_{PGK} (phosphoglycerate kinase promoter): 1970–2473
- Hygromycin resistance gene (Hyg^r): 2491–3517
- 3' MMLV LTR (with a deletion in U3): 4117–4553
  Poly A signal: 4454–4459
- P_{SV40}: 4832–5099
- SV40 origin of replication: 5053–5118
- ColE1 origin of replication: 5440
- Ampicillin resistance gene (β-lactamase): 6199–7059 (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: DH5α 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 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 sequence.

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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