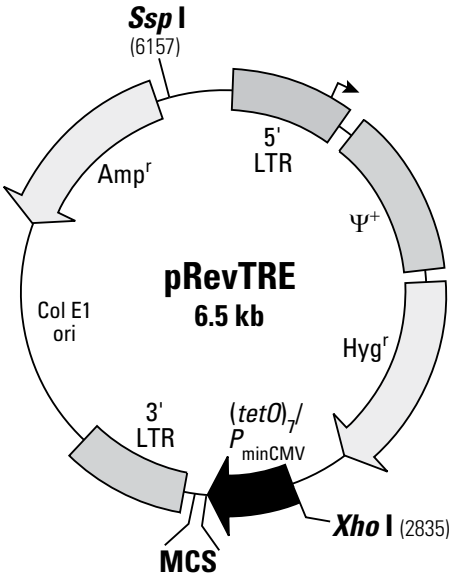
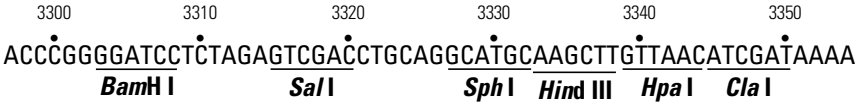
Vector Information

pRevTRE Vector Information

PT3215-5

Catalog No. 631002





Restriction map and multiple cloning site (MCS) of pRevTRE Vector. All restriction sites shown are unique.

Description:

pRevTRE is a retroviral Tet response vector that expresses a gene of interest from the Tet-response element (TRE). This vector is derived from pLNCX, a retroviral vector created using elements of Moloney murine leukemia virus (MoMuLV) and Moloney murine sarcoma virus (MoMuSV) as described (1). The TRE contains seven direct repeats of the *tetO* operator sequence, upstream of a minimal CMV promoter, which can be bound by the tTA and rtTA transactivators. The 5' viral LTR controls expression of the transcript that contains Ψ^+ (the extended viral packaging signal) and the hygromycin resistance (Hyg^r) gene for antibiotic selection in mammalian cells. The TRE is derived from vectors described previously (2, 3). pRevTRE also includes the *E. coli* Amp^r gene for antibiotic selection in bacteria.

The complete RevTet-Off and RevTet-On Systems also include the control vector pRevTRE-Luc, which was constructed by cloning the firefly luciferase gene into the *Hin*d III/*Cla* I sites in the MCS of pRevTRE.

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Use:
pRevTRE can be used to establish inducible Tet Systems via retrovirus-mediated gene transfer (4). Retroviral gene transfer allows the highly efficient transduction of virtually all dividing cell types. The RevTet™ Systems are also suitable for establishing transgenic animals. In combination with the pRevTet-On or pRevTet-Off regulatory vector, a gene of interest can be inducibly expressed at high levels in response to varying concentrations of tetracycline (Tc) or Tc derivatives such as doxycycline (Dox). tTA and rtTA bind to the Tet-response element (TRE) and activate transcription from the minimal promoter in the absence or presence of Dox, respectively. pRevTRE lacks the viral genes *gag*, *pol*, and *env,* which are supplied by the packaging cell line. It can be transfected into a high titer packaging cell line and thereby mediate production of infectious, replication-incompetent retroviral particles (1, 6–7). The transcript produced by the pRevTRE construct is recognized by the viral structural proteins expressed in a packaging cell line and packaged into infectious retroviral particles. Because the RNA transcript packaged in these particles does not contain the viral genes, it cannot replicate in the target cells that it infects.

The level of induction in cell populations infected with this vector depends on the efficiency of infection, the site of integration, and the titer of the virus. Viral supernatants with titers >10^5 cfu/ml should be produced to achieve high-level induction.
Note: Clone in frame with the ATG at vector position 3329, within the *Sph* I site (if cloning downstream of it) of the vector MCS, so that translation of your gene of interest will not be affected.

Location of features:
- 5' MoMuSV LTR: 1–589
- Ψ^+ (extended packaging signal): 659–1468
- Hygromycin resistance gene: 1510–2544
  Start codon: 1510–1512; stop codon 2542–2544
- TRE ([*tetO*]$_7$/P_{minCMV}): 2833–3276
- MCS: 3277–3354
- 3' MoMuLV LTR: 3390–3983
- Ampicillin resistance (β-lactamase) gene: 6139–5279
  Start codon: 6139–3137; stop codon 5277–5279

Propagation in *E. coli*:
- Suitable host strains: DH5α, HB101, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (50 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: Col E1
- Copy number: low copy

References:
1. Miller, A. D. & Rosman, G. J. (1989) *BioTechniques* **7**:980–990.
2. Tet Expression Systems and Cell Lines (July 1996) *Clontchniques* **XI**(3):2–5.
3. Gossen, M. & Bujard, H. (1992) *Proc. Natl. Acad. Sci. USA* **89**:5547–5551.
4. RevTet-On & RevTet-Off Systems (April 1998) *Clontechniques* **XIII**(2):8–9.
5. *Retroviruses* (1997) CSHL Press.
6. *Current Protocols in Molecular Biology* (1996) Supplement 36, Section III

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, depending on your cloned insert, 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.

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