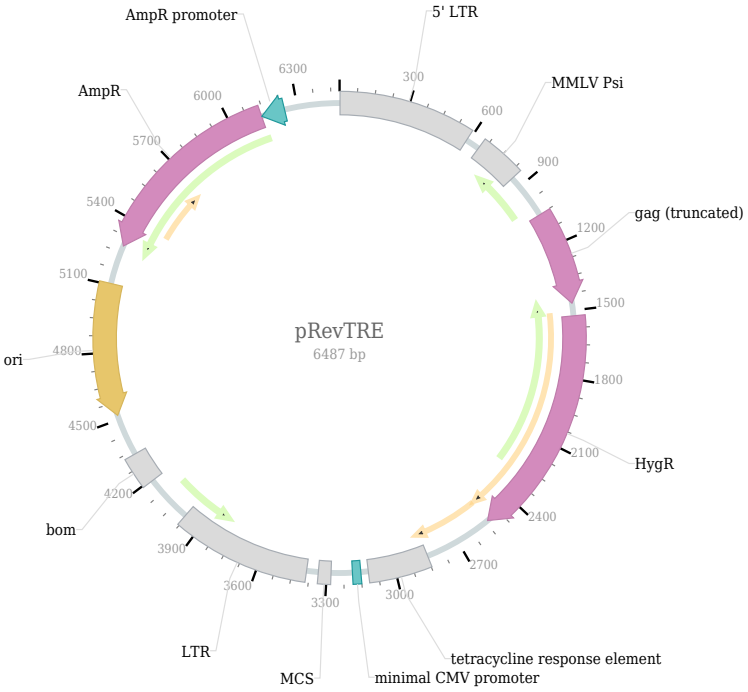
pRevTRE

Vector ID	V007373
Antibiotic Resistance	Ampicillin
Length	6487 bp
Vector Type	Tetracycline Regulatory System

Product Information

Replication Origin	ori
Selection Marker	Hygromycin
Promoter	LTR
Cloning Method	Enzyme digestion and ligation
Comment	pRevTRE is a retroviral Tet response vector that expresses a gene of interest from the Tetresponse 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 (Hygr) gene for antibiotic selection in mammalian cells. The TRE is derived from vectors described previously (2, 3). pRevTRE also includes the E. coli Ampr gene for antibiotic selection in bacteria.The complete RevTet-Off and RevTet-On Systems also include the control vector pRevTRELuc, which was constructed by cloning the firefly luciferase gene into the Hind III/Cla I sites in the MCS of pRevTRE.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 RevTetTM 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 Tetresponse 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 >105 cfu/ml should be produced to achieve high-level induction.

Vector Map



Plasmid Resuspension Protocol

1. Centrifuge at 5,000×g for 5 min.
2. Carefully open the tube and add sterile water to dissolve the DNA: add 20 µl for 5 µg plasmid, and 100 µl for 100 µg plasmid.
3. Close the tube and incubate for 10 minutes at room temperature.
4. Briefly vortex the tube and then do a quick spin to concentrate the liquid at the bottom. Speed is less than 5000×g.
5. Store the plasmid at -20 ℃.