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A universal retroviral vector for efficient constitutive expression of exogenous genes

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We have constructed a plasmid pXT1 which can be used to derive replication-defective retroviruses capable of efficient constitutive expression of foreign genes in embryonic as well as adult cells. This plasmid is based on pXm5 (used to derive the retrovirus N2 (ref. 1)), and has the LTRs and part of the *gag* region of Moloney Murine Leukaemia Virus. A selectable neomycin (*neo*) gene, conferring G418 resistance, is under the control of the LTR, which although normally repressed in embryonic cells (2), can become transcriptionally active under selective conditions (3). An internal herpes simplex virus thymidine kinase (TK) promoter is used for expression of the exogenous gene; this promoter is active in embryonic stem cells (4) and in a wide variety of tissues in transgenic mice (5).

Infectious, helper-free replication-defective retroviruses can be obtained either by direct transfection onto the Ψ2 packaging cell line (6), or via transfection of the amphotrophic cell line PA317 (7), and infection of Ψ2 cells . Titres of viral supernatants on NIH3T3 fibroblasts range from 1-5x10^5 G418^R cfu/ml (Ψ2 transfection) to 1-5x10^6 G418^R cfu/ml (Ψ2 infection). Titres on embryonic stem (EC or ES) cells are 10^3-10^5 fold lower (depending on cell line) due to repression of the viral LTR. Viruses based on pXT1, carrying a transforming gene under the control of the TK promoter, have been used to infect fibroblasts, EC and ES cells, bone marrow and primary embryonic cerebellum cultures; in all cases both *neo* and exogenous genes are efficiently expressed.

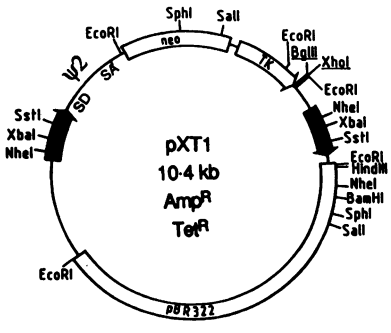

Fig.1 pXT1 was derived from pXm5 by conversion of the *Xho*I site to a *Bgl*II site using the self-annealed *Sal*I-*Bgl*II-*Xho*I oligonucleotide adaptor

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5' TCGACAGATCTGC 3'
       GTCTAGACGAGCT
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and insertion of the herplex simplex virus TK promoter (8) as an 825-bp *Bam*HI-*Bgl*II fragment into the new *Bgl*II site. This step destroyed the *Bam*HI site at the 5' end of the promoter, but retained unique *Bgl*II and *Xho*I sites for insertion of genes downstream. The viral packaging sequence (Ψ2) and the splice donor (SD) and cryptic splice acceptor (SA) sites are indicated; the viral LTRs are shaded.

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