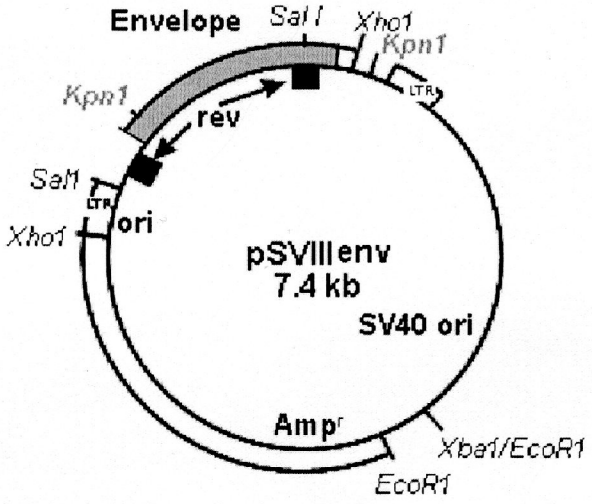
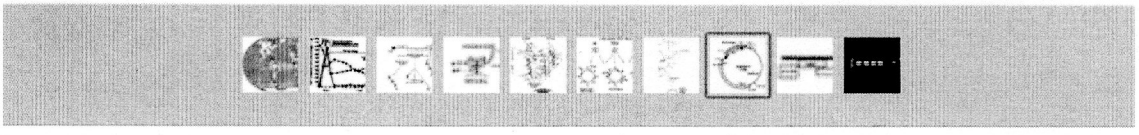
Env Expression Vector

This is a deleted provirus encoding LTRs tat + Rev + Env. Do not cotransfect with other deleted proviral constructs eg pLAIDEnv as recombination can produce live virus.





Context

The JR-CSF RT clones were made from viral RNA reverse transcribed from JR- CSF passaged 4 times through PBMCs. The Invitrogen kit SuperScript First-Strand Synthesis System for RT-PCR was used. The primer used was envN (5'- CTG CCA ATC AGG GAA GTA GCC TTG TGT -3'). After first-strand synthesis, amplification of the cDNA (PCR forward) was performed using the Invitrogen kit. The only exception to the protocol was that Roche Expand HiFidelity was used. Primers envA (5'- GGC TTA GGC ATC TCC TAT GGC AGG AAG AA -3') and envN were used. In round two of RT-PCR envB (5'- AGA AAG AGC AGA AGA CAG TGG CAA TGA -3') and envM (5'- TAG CCC TTC CAG TCC CCC CTT TTC TTT TA -3') primers were used. An EtBr 0.8% agarose gel was run to verify positive bands. Six samples were selected to be purified using a crystal violet gel and the Invitrogen PCR XL-TOPO kit for cloning. After the fragment was inserted in the TOPO vector a PCR screen was done to verify the transformation and identify clones that carried a PCRed fragment as an insert. The positive colonies were grown in 3 mL of LB + Km and then mini-prepped using the Qiagen Mini-prep Kit. The fragments were then cut out of the TOPO vector utilizing a KpnI (NEB) restriction site. The pSVIII JR-CSF was also digested with KpnI (NEB) and Antartic Phosphatase to prepare for ligating to the RT fragment. The pSVIII JR-CSF [KpnI] was ligated with the RT fragment from the TOPO vector after being cut with KpnI (see Figure 8). The ligation and transformation was verified by PCR screen and test digestion to check orientation of fragments. All clones (7) had glycerol stocks prepared and stored at -80°C. The Qiagen Mini-prep kit was used to prepare the DNA from the 7 clones.