

## Development of an HIV-1-dependent expression vector with the Cre / loxP system

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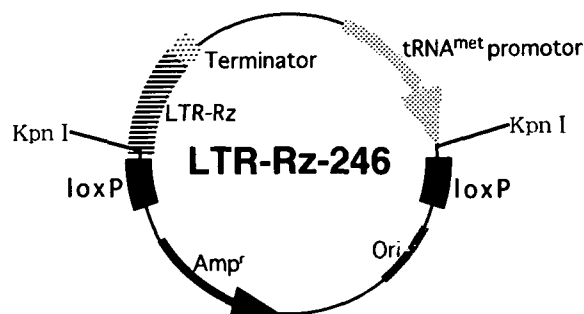
### ABSTRACT

Previously, we used the human methionine tRNA promoter as an expression cassette for hammerhead ribozymes. The tRNA promoter driven ribozyme was targeted against the LTR portion of the HIV-1 NL4-3 strain. We constructed VSV-G-pseudotyped MuLV-based vectors expressing the ribozyme. The ribozyme expressing retrovirus vector strongly suppressed gag p24 antigen production in freshly HIV-1 infected MT-4 cells. In this study, the potential of such a molecular genetic intervention was examined by using the Cre-loxP recombination system. Site-specific excision of HIV-1 was achieved by using this model system with an acute infection. These studies represent one step toward the development of a novel antiviral strategy for the treatment of AIDS.

### INTRODUCTION

A novel antiviral strategy has employed the use of antiviral genes that are delivered to uninfected cells as either RNA or DNA and provide intracellular protection from HIV-1 infection (1). Antiviral genes include those encoding antisense molecules, ribozymes, transdominant proteins, and intracellular antibodies. Current antiviral strategies, both

chemical and genetic, target steps in virus replication leading to the integration of proviral DNA or those following the establishment of the integrated provirus; however, a strategy to target proviral DNA directly has not been reported. One such strategy takes advantage of the Cre-loxP recombination system of bacteriophage P1, which has been utilized extensively in the excision of DNA both *in vitro* and *in vivo* (2-9). The results reported in this study demonstrate that the expression of Cre provides intracellular inhibition of replication of a recombinant LTR-Rz containing loxP and highlight its potential as an effective antiviral agent against HIV-1 infection.

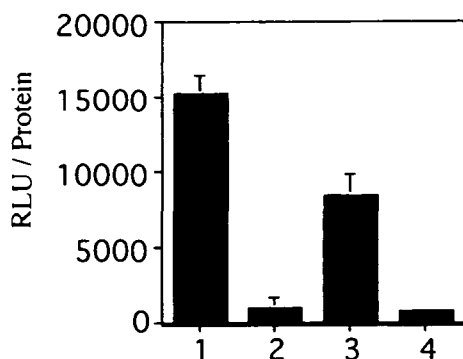


**Figure 1.** Schematic structure of the plasmid used in this study.

## MATERIALS AND METHODS

pBS185 (Cre Expression Vector) and pBS246 (loxP<sup>2</sup> Cassette Vector) were purchased from GIBCO. BRL. These plasmids were isolated from *E.coli* XL2-blue and were purified on a JETSTAR Plasmid Purification System (GENOMED Inc.). LTR-Rz and pBS246 were digested with Kpn I, and after BAP treatment, the pBS246 fragment was ligated with the LTR-Rz fragment (Figure 1).

COS cells were grown in Dulbecco's modified Eagle's medium (D-MEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) at 37°C in a 5% CO<sub>2</sub> atmosphere. Plasmids expressing the ribozymes, the luciferase gene, and other parts of the HIV-1 genome (except *env* and *nef*), were co-transfected into COS cells. After 2 days, the luciferase activities in these cells were measured. Concurrently, COS cells (2 × 10<sup>4</sup> cells / 500 μl) were prepared and transfected with the 1. LTR-Rz & control vector (L6), the 2. LTR-Rz-246 & L6 vector, and the 3. LTR-Rz-246 & pBS185 vector with the FuGENE™ 6 transfection reagent (Roche Diagnostics K.K.). After 24hrs, the medium was removed, fresh medium was added, and the cells were transfected with 1.3. NL-luc or 2. pBS185 & NL-luc. The next day, the COS cells were harvested and the luciferase activity was measured.



**Figure 2.** Luciferase activities after Cre/loxP homologous recombination in COS cells. 1. Control, 2. LTR-Rz, 3. LTR-Rz-246 + pBS185, 4. LTR-Rz-246 & pBS185.

## RESULTS AND DISCUSSION

At first, the cDNA inserted between the loxP sequence required promoter activation to synthesize the Cre protein by recombination, so we examined its expression. The gene encoding luciferase was co-transfected with the Cre expression vector (pBS185) into COS cells, and the luciferase activity after two days was measured. We confirmed that it functioned as a plasmid DNA from the cDNA inserted between the loxP sequence in the Cre protein, and that its quantity depended on the promoter activation (data not shown.). Next, as shown in figure 2, when the ribozyme expression vector was inserted between the loxP sequence and transfected in the COS cells, the luciferase activity was increased. Therefore, the Cre / loxP system has the function of the molecule switch, and can be used as a system to express a gene of interest.

## ACKNOWLEDGEMENT

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