# Efficacy of Gene Transfer of Recombinant Baculovirus Vector

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ABSTRACT

A novel recombinant baculovirus vector system containing coding genes for polyhedron promoter, vesicular stomatitis virus G (VSVG), polyA, cytomegalovirus (CMV) promoter, enhanced green fluorescent protein (EGFP), and protein transduction domain (PTD) was constructed. We applied this recombinant baculovirus vector into cells and murine tissues and compared efficacy of gene transfer and expression of this recombinant baculovirus vector system with control vector system. From this result, we confirmed that this novel recombinant baculovirus vector system was very effective than control vector system.

Keyword

enhanced green fluorescent protein, protein transduction domain, vesicular stomatitis virus G

## Ⅰ. INTRODUCTION

Recombinant baculoviruses are widely used to express heterologous genes in insect cells cultured. The baculovirus expression vector system is particularly advantageous for many application field and specialized media, transfection reagents, and vectors have been developed in response to recent advances in insect cell culture and molecular biology methods [1]. Since 1983, baculovirus system is one of the most powerful eukaryotic vector systems for recombinant protein expression [1]. Baculovirus system has significant benefits in view of safety, large-scale, and high level of gene expression.

Recently, specific proteins, called PTDs, have been identified as carriers for the efficient delivery of proteins that do not permeate living cells [2]. Although the mechanism is unknown, transduction occurs in receptor- and transporter-independent manners, which appears to target the lipid bilayer directly [3]. PTDs include the peptides derived from the basic domain of HIV-1 Tat, the homeodomain of *Drosophila Antennapedia* and the HSV VP22 transcription factor. The short Tat peptide, YGRKKRRQRRR (residues 47-57) is sufficient for the intracellular transduction and subcellular localization [4, 5]. This domain can deliver a wide variety of proteins, ranging in size from 15 to 120 kDa, across the plasma membrane by a mechanism referred to as protein transduction [4].

In this study, we constructed a recombinant baculovirus vector system containing PTD and compared efficacy of gene transfer and expression in cells and murine tissues.

## II. MATERIALS AND METHODS

### *A. cell culture*

The insect cell line, Sf9, was grown in Grace's medium (Invitrogen, USA), supplemented with 10% fetal bovine serum (FBS; HyClone, USA). The human hepatoma cell line, Huh7, the human cervical carcinoma line, HeLa, and the human glioblastoma, A172, were grown in Dulbecco's modified Eagle medium (DMEM; Gibco BRL, USA), supplemented with 10% FBS and 4 mM glutamine. The cultures were maintained at 37℃ in a humidified atmosphere of 95% air/ 5% $CO_2$ [2].

### *B. Construction of vector expressing fusion protein*

In order to construct the plasmid with the HIV-1 Tat fused to the C-terminus of EGFP (pEGFP-Tat), two oligonucleotides were synthesized and annealed to generate a double stranded oligonucleotide encoding the 11 amino acids from the basic domain of the HIV-1 Tat.

The sequences were (top strand) 5'-GATCTAGAAGCAGCGACAGAGGCGAAGAAGGACGG TATTAACT-3' and (bottom strand) 5' -ATCTTCGTCGCTGTCTCCGCTTCTTCCTGCCATAATTG ACAGCT-3' [2]. The double stranded oligonucleotide was inserted into pCR *2.1* (Invitrogen, USA) in order to generate pCR 2.1-Tat. Second, the EGFP gene sequence was amplified using PCR from pEGFP-N1 (Invitrogen, USA), with the sense primer 5'-AGAATCCGCTAGCGCTACCGGTCGCCACCCATGG -3' and antisense primer 5'-GAAGATCTCTTGTACAGCTCGTCCAT-3' [2]. The *Eco*RV/*Bgl*II EGFP fragment of the PCR product and the *Eco*RV /*Bgl*II Tat fragment of pCR 2.1-Tat were subcloned into the *Nde*I/*Bam*HI sites of pET-15b Clontech, USA), generating pEGFP-Tat. The ABI Prism automated sequencing method was used to confirm the nucleotide sequences of all the PCR products.

*C. Transduction of the fusion protein into cells*

When the cells were 70% confluent, the culture medium was replaced with fresh medium containing 10% FBS, and the EGFP or EGFP-Tat added to the growth medium, to a final concentration of 0.5 μM. The cells were then sampled at the times shown or after at least 10 min.

*D. Fluorescence microscopy*

The cultured cells were grown on glass coverslips, and then infected with the recombinant baculovirus (m.o.i. 10) for 1 h. Forty eight hour after infection, the cells were washed three times with 1 ml of PBS (pH 6.4), sealed in GelMount (Biomedia, USA), then viewed on an fluorescencemicroscope (Carl Zeiss, Germany) and recorded with an Axio camera (Carl Zeiss, Germany).

*E. Western blot analysis*

The Sf9 cells were infectedwith Bac-EGFP, Bac-EGFP-Tat m.o.i. (multiplicity ofinfection) 10, in 6-well plates. After 48 h, the cells were lysed in a Laemmli buffer (125 mM Tris, 2% sodium dodecyl sulfate [SDS], 5% 2-mercaptoethanol, 10% glycerol and 0.001% bromophenol blue, pH 6.8) and heated to 100℃ for 5 min prior to electrophoresis. All the samples were run on SDS-10% polyacrylamide gel electrophoresis. The VSV-G-specific monoclonal antibody was obtained from Roche Molecular Biochemicals. The presence of the VSV-G protein was detected using an ECL Western blotting analysis system (Amersham Bioscience, Sweden).

## III. RESULTS AND CONCLUSIONS

We constructed novel recombinant baculovirus vector system. A peptide (RKKRRQRRR), derived from the HIV-1 Tat basic domain fused to the C-terminus of EGFP as recombinant fusion proteins, was produced to facilitate the visualization of the PTDs in cell cultures. A control EGFP expression vector was also constructed by inserting the coding sequence for EGFP into the pET15b expression vector.

We compared expression of VSVG of recombinant baculovirus cotaining Tat and without Tat (A: EGFP expressed by VSVG using flourecense microscope, B: EGFP expressed by VSVG using Western blot hybridization) in Fig. 1.

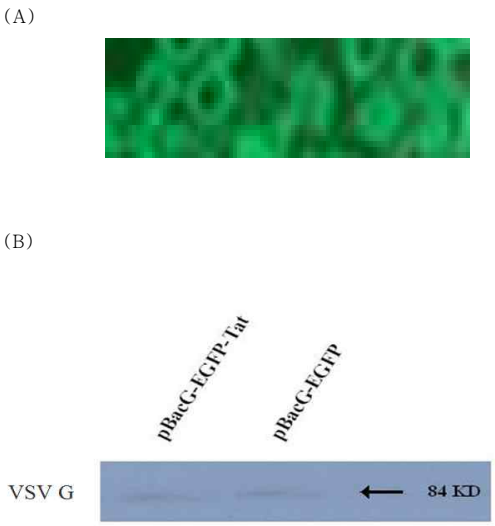


Fig. 1. Expression of VSVG of recombinant baculovirus cotaining Tat and without Tat (A: EGFP expressed by VSVG using flourecense microscope, B: EGFP expressed by VSVG using Western blot hybridization)

We confirmed transfection efficiency of pBac-VSVG-EGFP-PTD was higher than pBac-EGFP in murine pancreas, lung, and kidney tissues (Fig. 2).

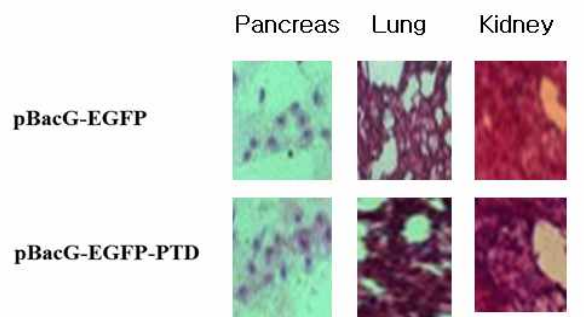


Fig. 2. Expression of EGFP of recombinant baculoviruses cotaining PTD and without PTD genes infected into mouse organs (pancreas, lung, and kidney)

From this results, we confirmed this novel recombinant baculovirus vector system was superior to other control vector system.

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## REFERENCES

[1] The Bac-to-Bac Baculovirus Expression System,"Guide to baculovirus expression vector systems (BEVS) and insect cell culture techniques,"*Invitrogen Life Technolgies* pp. 1, 2012.

[2] J. S. Yoon, Y. T. Jung, S. K. Hong, S. H. Kim, M. C. Shin, D. G. Lee, W. S. Min, S. Y. Paik, "Charateristics of HIV-Tat protein transduction domain,"*J. Microbiol.* vol. 42, pp. 328-335, 2004.

[3] S. R. Schwarze, and S. F. Dowdy,"In vivo protein transduction: Intracellular delivery of biologically active proteins, compounds and DNA,"*Trends. Pharmacol. Sci.* vol. 21, pp. 45-48, 2000.

[4] E. Vives, P. Brodin, and B. Lebleu. "A truncated HIV-1 Tat protein basic domain rapidly translocates through the plasma membrane and accumulates in the cell nucleus," *J. Biol. Chem.* vol. 272, pp. 16010-16017, 1997.

[5] H. Nagahara, A. M. Vocero-Akbani, E. L. Snyder, A. Ho, D. G. Latham, N. A. Lissy, M. Becker-Hapak, S. A. Ezhevsky, and S. F. Dowdy, "Transduction of full-length TAT fusion proteins into mammalian cells: TAT-p27Kip1 induces cell migration," *Nature Medicine* 4, 1449-1452, 1998.