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# 재조합 베쿨로바이러스 벡터의 제조와 감염

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## Construction and Transfection of Recombinant Baculovirus Vectors

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### 요 약

베쿨로바이러스 벡터가 uroplakin II promoter, polyhedron promoter, vesicular stomatitis virus G (VSVG), enhanced green fluorescent protein (EGFP), protein transduction domain (PTD) 등의 유전자로 재조합 되었다. 이렇게 재조합된 베쿨로바이러스 벡터들은 여러가지 세포주에 감염을 시켰다. 우리는 이 재조합 벡터와 다른 대조 벡터를 비교하여 유전자 전달과 유전자 발현을 비교하였다. 그 결과 이 재조합 베쿨로바이러스 벡터는 대조 벡터에 비하여 유전자 전달과 유전자 발현의 효율이 우수한 것으로 나타났다.

### ABSTRACT

Baculovirus vectors were recombined using uroplakin II promoter, polyhedron promoter, vesicular stomatitis virus G (VSVG), enhanced green fluorescent protein (EGFP), protein transduction domain (PTD) gene and so on. These novel recombinant vectors were infected into various cell lines. We performed and analyzed gene transfer and gene expression of these recombinant vectors comparison with other control vectors. From this result, we identified that these recombinant vectors have higher efficient gene transfer and expression of than control vector.

### Keyword

baculovirus, protein transduction domain, uroplakin II promoter, vesicular stomatitis virus G

### I . INTRODUCTION

Baculoviruses have double-stranded, circular, and supercoiled DNA molecules in a rod-shaped capsid. They are the most prominent viruses known to transfer the genes. Recombinant baculoviruses are widely used to express heterologous genes in cultured insect cells [1].

Recombinant baculoviruses can serve as

gene-transfer vectors for expression of recombinant proteins in a wide range of mammalian cell types. More over, by inclusion of a dominant selectable marker in the viral vector, cell lines can be derived that stably express recombinant genes. The baculoviruses are a family of large rod-shaped viruses that can be divided

to two genera: nucleopolyhedroviruses (NPV) and granuloviruses (GV). While GVs contain only one nucleocapsid per envelope, NPVs contain either single (SNPV) or multiple (MNPV) nucleocapsids per envelope. The enveloped virions are further occluded in granulin matrix in GVs and polyhedrin for NPVs. Moreover, GV have only single virion per granulin occlusion body while polyhedra can contain multiple embedded virions [2]. Baculoviruses have very species-specific tropisms among the invertebrates with over 600 host species having been described. Immature (larval) forms of moth species are the most common hosts, but these viruses have also been found infecting sawflies, mosquitoes, and shrimp. Although baculoviruses are capable of entering mammalian cells in culture [3], they are not known to be capable of replication in mammalian or other vertebrate animal cells.

Baculovirus expression in insect cells represents a robust method for producing recombinant glycoproteins [4], [5]. Baculovirus-produced proteins are currently under study as therapeutic cancer vaccines with several immunologic advantages over proteins derived from mammalian sources [6]. They have a restricted range of hosts that they can infect that is typically restricted to a limited number of closely related insect species. Because baculoviruses are not harmful to humans, they are considered a safe option for use in research applications.

We reconstructed with useful genes of polyhedron promoter, vesicular stomatitis virus G (VSVG), polyA, uroplakin II promoter, enhanced green fluorescent protein (EGFP), and protein transduction domain (PTD). This reconstructed vector was infected into various cell lines and tissues. We investigated gene transfer and gene expression of this reconstructed vector in comparison to other vectors and recognized that this reconstructed vector was higher effective than any other control vector.

## II. MATERIALS AND METHODS

### 2.1. cell culture

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°C in a humidified atmosphere of 95% air/ 5% CO<sub>2</sub> [7].

### 2.2. 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'-ATCTTCGTCGCTGTCTCCGCTTCTTCTGCCATAATTG ACAGCT-3' [6]. 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' [3]. The *EcoRV*/*Bgl*II EGFP fragment of the PCR product and the *EcoRV*/*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.

### 2.3. Transduction of the fusion protein

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.

### 2.4. 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 (Biomedica, USA), then viewed on an fluorescence microscope (Carl Zeiss, Germany) and recorded with an Axio camera (Carl Zeiss, Germany).

### III. RESULTS AND CONCLUSIONS

Baculovirus vector was reconstructed in this study. These vectors were included genes of polyhedron promoter, vesicular stomatitis virus G (VSVG), polyA, uroplakin II promoter, enhanced green fluorescent protein (EGFP), and protein transduction domain (PTD). 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.

We reconstructed with diverse genes including polyhedron promoter, vesicular stomatitis virus G (VSVG), polyA, uroplakin II promoter, enhanced green fluorescent protein (EGFP), and protein transduction domain (PTD) (Fig. 1).

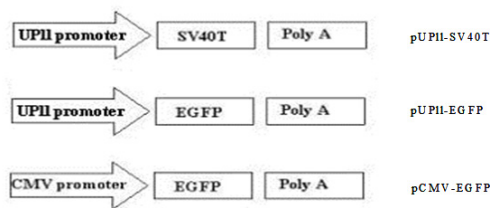


Fig. 1. Recombined baculovirus vectors constructed with diverse genes including uroplakin II promoter.

We reconstructed four baculovirus vectors with pBac-EGFP, pBacG-EGFP-PTD, pBacG-hTERT, and pBacG-hTERT-PTD.

Transduction efficiency of reconstructed baculovirus vectors (pBac-EGFP and pBacG-EGFP-PTD) were compared in infected cell lines (NIH293, 293T, SNU46, Huh7, HepG2, and HFF) (Fig. 2).

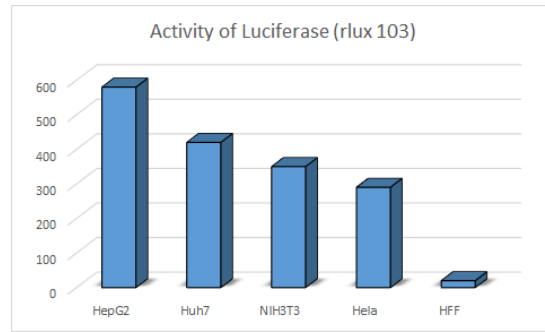


Fig. 2. Determination of gene expression of cell lines by recombined baculovirus (uroplakin II promoter-EGFP) in infected cell lines (HepG2, Huh7, NIH3T3, HeLa, and HFF).

Gene expression efficiency of recombined baculovirus vector with uroplakin II were higher in infected cell lines HepG2, Huh7, NIH3T3, HeLa than HFF. Among them, In HepG2 cell line, gene expression efficiency of recombined baculovirus vector was higher than in any other cell lines.

In this study, we recombined baculovirus vectors with genes of uroplakin II promoter, vesicular stomatitis virus G (VSVG), enhanced green fluorescent protein (EGFP), and protein transduction domain (PTD) and polyA. This recombined vector was infected into various cell lines. We investigated gene transfer and gene expression of this recombined vector in comparison to control vector and recognized that this recombined vector was higher effective than control vector.

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