

Characterization of gp64 Gene and Development of Transient Expression Vector from *Bombyx mori* Nucleopolyhedrovirus

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Expression of the baculovirus major envelope glycoprotein gene (gp64) is regulated by transcription from both early and late promoters. To develop a transient expression vector under the control of gp64 gene promoter, the gp64 gene of *Bombyx mori* nucleopolyhedrovirus-K1 (BmNPV-K1) was characterized. The gp64 gene was localized at *EcoR* I-*Pst* I 7.38-kb fragment of the BmNPV-K1 genome. The *EcoR* I-*Pst* I 7.38-kb fragment was cloned and the nucleotide sequence of 2,277 bases including the coding region of gp64 gene was determined. Based on these results, transient expression vector using gp64 gene promoter was constructed and named as pBm64. *E. coli lacZ* gene was introduced into pBm64 as a reporter gene and expressed transiently in *B. mori* 5 (Bm5) cells. The expression vector transfected into the cells was maintained stably for 1 to 5 days. In order to confirm the expression of the reporter gene by gp64 promoter, recombinant virus was constructed. The recombinant virus has two independent transcription units in opposite orientations with two promoters; gp64 and polyhedrin gene promoters each initiating transcription of β -galactosidase and polyhedrin, respectively. Polyhedra formation and expression of β -galactosidase in Bm5 cells infected with the recombinant virus were observed with phase contrast microscope and *in situ* staining.