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Expression of animal virus genes using Baculovirus AcNPV

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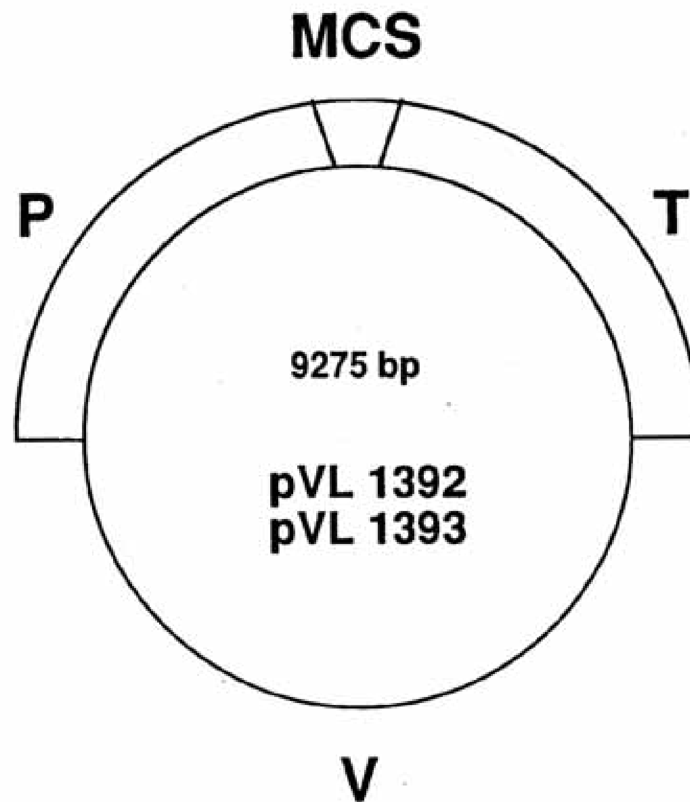
Out of the many systems employed for the expression of foreign genes, bacterial systems are by far the simplest and most efficient ones. They are relatively cheap and often give high protein yields, however, they have some drawbacks: in bacteria RNA is processed in a way different than in Eukaryota, and the post-translational modifications of proteins are much less pronounced, e.g. bacteria cannot glycosylate polypeptide chains. Therefore although eukaryotic systems are less efficient than those of bacterial origin, the foreign gene expression based on yeast or mammalian cells yields recombinant proteins more similar to their original counterparts. The system based on an insect virus, Baculovirus, while retaining most of the advantages of eukaryotic systems (for foreign gene expression), gives high yields of recombinant proteins at relatively low cost.

Baculoviruses are viral pathogens that cause a fatal disease (polyhedrosis) in insects, mainly in the members of Lepidoptera and Diptera. In nature, the virus enters the larvae *via* ingested food, it is released from its envelope in the midgut and then infects the midgut cells. Progeny viruses are released to hemolymph and they become transported to other organs. Viral particles, produced at the later stages of infection, are embedded in a protein envelope, composed mainly of a single protein called polyhedrin. Synthesis of this compound starts 24 h after the infection and continues until ultimate cell lysis. In a cell culture polyhedrin is not essential component because polyhedrin is necessary to protect viral particles in the natural environment only. The promoter of the polyhedrin gene is very potent (1mg of polyhedrin is produced per 1 ml of the insect cells in tissue culture, and the polyhedrin gene can be replaced by a foreign gene without any loss of virus viability). The size of baculovirus genome is about 130 kb and long segments (up to 25 kb) of foreign DNA can be inserted in place of the polyhedrin gene. The insertion is achieved by cloning the foreign gene into transfer vectors with baculovirus sequences (Fig.1) and subsequent co-transfection of the insect cells with plasmid and viral DNA [1, 2].

This system has been used by us for expression of the *NP* gene[1] of the influenza virus and the *gI* gene of pseudorabies virus. cDNA of the *NP* gene (1570 bp long) was cloned into *Eco*RI site of the transfer vector pVL1393 as described by Maniatis *et al.* [3]. After co-transfection with plasmid and viral DNAs, the recombinant baculoviruses were detected using biotinylated probes of the cDNA of *NP* gene. Recombinants were purified by passages in tissue culture of an insect cell line from *Spodoptera frugiperda* (Sf9). The Sf9 cells were infected with recombinant baculovirus and the samples of infected cells were collected daily until 8 days post infection. Cells were lysed and divided into cytoplasmic and nuclear fractions. Electrophoresis of proteins was performed in 12.5% SDS-polyacrylamide gels [4]. The level of NP was about 20% of all the synthesized proteins (Fig. 2). The overexpressed protein on immunoblotting reacted with the anti-NP polyclonal serum which confirmed its identity with the native NP protein. As shown in Fig. 3, most of the NP polypeptide was situated in the nucleus of the infected insect cells.

[1]Abbreviations: *NP* gene, nucleoprotein gene; AcNPV, Autographa californica Nuclear Polihedrosis Virus

A



B

MCS pVL 1392

MCS pVL 1393

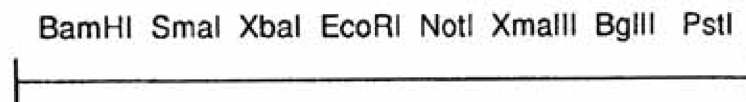


Fig. 1. The transplacement plasmids pVL 1392 and pVL 1393.
A. Plasmids were based on pUC 8 (V), contain AcNPV DNA flanking a multicloning site for foreign gene insertion, (P) promoter region of polyhedrin gene and flanking sequence, (T) terminator region of polyhedrin gene and flanking sequence, (MCS) multicloning sites. The flanking AcNPV sequences facilitate allelic replacement of the polyhedrin gene of wild type virus with the foreign gene to yield recombinant virus vectors. B. Multicloning sites for vectors pVL 1392 and pVL 1393

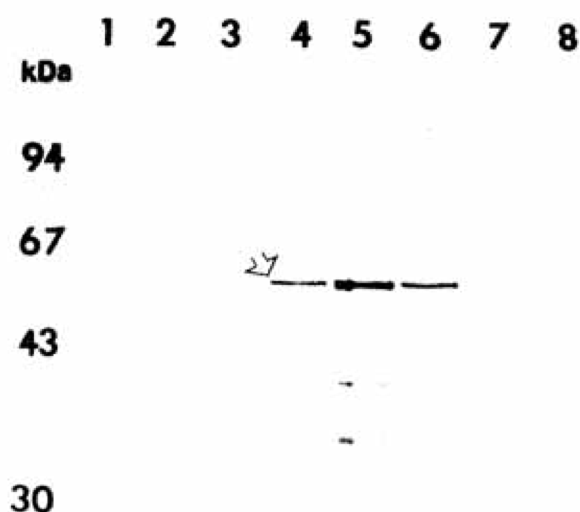


Fig. 2. Polyacrylamide gel-SDS electrophoresis of the NP protein of influenza virus produced in baculovirus.
Full length NP is marked with an arrow. 1, Molecular weight markers; 2 - 8, nuclear fractions of Sf9 cells infected with recombinant baculovirus (1 - 7 days post infection)

Fig. 3. Immunoblotting of the NP protein with rabbit polyclonal anti-NP serum.
1 - 8, Nuclear fractions of Sf9 cells infected with recombinant baculovirus (1 - 8 days post infection); 9, prestained molecular weight markers; 10 - 17, cytoplasmic fractions of infected cells (1 - 8 days post infection)

The second gene cloned to baculovirus transfer vectors was the gene coding for the gI glycoprotein of pseudorabies virus. This glycoprotein is one of the envelope proteins responsible for virulence in pigs and it is one of the candidates for a subunit vaccine. Genomic DNA of Pseudorabies virus (150 kb) was digested with *Bam*HI and the 7 kb fragment containing a few genes including *gI* gene was cloned into high copy plasmid pT7-5. Subsequently, the 2.4 kb fragment containing only *gI* gene was subcloned into *Eco*RI/*Bam*HI sites of the transfer vector pVL 1392. The recombinant baculoviruses were detected using a probe prepared by labelling the DNA segment encompassing the *gI* gene with digoxigenin. Our preliminary results indicate that the level of the recombinant protein is in this case lower than that for the NP polypeptide and we are going to attempt optimization of the conditions for its expression.

REFERENCES

1. Summers, M.D. & Smith, G.E. (1987) *A manual of methods for Baculovirus vectors and insect cell culture procedures*. Texas Agricult. Exp. Stn. Bull. **1555**.
2. Miller, L.K. (1988) *Annu. Rev. Microbiol.* **42**, 177 - 199.
3. Maniatis, T., Fritsch, E.F. & Sambrook, J. (1982) *Molecular cloning, a laboratory manual.* Cold Spring Harbor Lab.
4. Laemmli, U.K. (1970) *Nature (London)* **227**, 680 - 685.