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Short communication

A simplified method for the extraction of baculoviral DNA for PCR analysis: A practical application

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Abstract

There are two major strategies to genetically modify baculoviruses. One uses a bacmid-based system which replicates in *Escherichia coli* using a bacterial origin of replication. The other employs a transfer vector and viral DNA which are co-transfected into insect cells and utilise host enzyme-mediated homologous recombination. Putative recombinants are then typically screened by plaque assay. The bacmid system is more convenient, but it requires a number of complex construction and isolation steps to obtain the correct bacmid genome. Generally, the transfer vector method is preferable when only a small number of genetic modifications are required.

In this study a rapid and reliable method was developed to extract baculovirus DNA for PCR analysis from cultured insect cells. Briefly, viral DNA was isolated in three steps: SDS lysis, chloroform extraction and ethanol precipitation. The method was tested for direct screening of recombinant viruses in plaque assays. Contrary to previous reports, baculovirus DNA was isolated directly from viral plaques and successfully analysed by PCR. No prior amplification of the virus by passage in tissue culture was necessary. The major advantage of this method was a reduction in assay times from a few days to a few hours. Moreover, this method is very convenient for detecting baculoviruses in cell culture: cross-contamination within viral stocks, monitoring mixed viral infection and confirmation of viral genomic integrity.

Keywords: Baculovirus; Plaque assay; DNA extraction; PCR

The *Baculoviridae* are a family of large, enveloped DNA viruses characterised by rod-shaped nucleocapsids and relatively large double-stranded DNA genomes. Baculoviruses are infectious only to arthropods, with the vast majority of permissive species falling within the Order *Lepidoptera*, Class *Insecta*. Baculovirus genomes range from approximately 80–180 kbp and contain two genera, *Nucleopolyhedrovirus* and *Granulovirus* (Theilmann et al., 2005). A distinctive characteristic of the *Baculoviridae* is the production of two structurally and functionally distinct virion phenotypes during the infection cycle. Initially, budded viruses are produced and, at late stages of infection, virions are embedded in a quasi-crystalline protein matrix forming the occlusion bodies. Budded viruses are produced when nucleocapsids bud through the plasma membrane of the midgut epithelial cells to initiate secondary infections within the infected animal (Blissard, 1996; Granados and Lawler, 1981; Keddie et al., 1989). The latter phenotype is required to spread the virus in nature via oral infection of insects. Occlusion-derived viruses are released from the occlusion body by the alkaline environment within the midgut lumen of the larva and subsequently initiate primary infection of the mature columnar epithelial cells of the midgut.

Wild-type baculoviruses are highly specific insecticides with no toxicity for non-target organisms (Granados and Federici, 1986). Nevertheless, their application as microbial pesticides has not met their potential to control pests in crops, forests and pastures, with the exception of the soybean caterpillar nuclear polyhedrosis virus (*Anticarsia gemmatalis* multinucleopolyhedrovirus or AgMNPV), which is used on approximately one million hectares annually in Brazil. Problems that have limited expansion of baculovirus use include narrow host range, slow-killing speed, technical and economical difficulties for in vitro

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commercial production, timing of application based on frequent host population monitoring and variability of field efficacy due to climatic conditions. Farmers' attitudes toward pest control, which have been based on the application of fast-killing chemical insecticides, have also contributed to its slow acceptance as a biocontrol agent (Moscardi, 1999).

One approach to increase the speed of kill of wild-type baculoviruses is the modification of genes involved in developmental processes within the insect and/or the expression of insect specific toxins, insect hormones or enzymes (Bonning and Hammock, 1996). Genetic manipulation of baculoviruses is simpler and more straightforward when it is performed in susceptible insect cell lines. For those baculoviruses that can replicate in susceptible cell lines, two major strategies have been developed for the construction and purification of recombinants. One of the methods uses a baculovirus shuttle vector (bacmid) that can replicate in *E. coli* as a plasmid and infect susceptible lepidopteran cell lines. To date, this strategy has been developed for a few baculovirus species, of which the AcMNPV-derived system is the most widespread (Airenne et al., 2003; Leusch et al., 1995; Luckow et al., 1993; Pijlman et al., 2003; Wang et al., 2003). Nevertheless, the major drawbacks of this method are the large number of complex construction steps required to make the vectors and the subsequent screening process needed to isolate the correct bacmid genome. If only a small number of genetic modifications to the wild-type baculovirus are required, the bacmid method represents a less than optimal investment in time and energy. Thus, in a considerable number of cases, a classical homologous recombination strategy will be the method of choice. The classical method employed to introduce changes in a baculoviral genome, is based on the co-transfection of insect cells with viral DNA and a transfer plasmid containing the gene to be inserted flanked by viral sequences. Inside the cell, host enzymes mediate homologous recombination between the viral sequences in the transfer vector and the identical sequences in the viral genome (Fig. 1). An allelic replacement reaction involves two distinct and independent homologous recombination events: one in each of the two regions of viral DNA flanking the foreign gene in the transfer plasmid. If both recombination events occur, a double recombinant is obtained. Nevertheless, the most common type of recombinant virus found in virus stock following co-transfections, is the single cross-over recombinant in which the entire plasmid DNA has integrated into the viral genome. Subsequent screening of the co-transfection cell culture supernatant for the presence of recombinant viruses is a tedious and time-consuming task, because the desired recombinant represents 0.1–1% of the total virus population. In this context, a critical and limiting step is initial screening, usually done by plaque assay, in which putative recombinant plaques are identified visually and purified by additional rounds of plaque assay (Kitts and Possee, 1993; O'Reilly et al., 1992). Because single and double cross-over recombinants both express the heterologous gene, a high percentage of the plaques picked in the first and subsequent rounds of purification do not contain the desired recombinant DNA.

When screening a co-transfection supernatant by plaque assay, PCR analysis of DNA extracted directly from plaque picks can reduce the time and reagents required for subsequent steps. Previous studies that attempted PCR amplification of viral DNA extracted directly from a plaque pick were unsuccessful (Malitschek and Schartl, 1991; O'Reilly et al., 1992; Webb et al., 1991). Typically, the viral titre was too low to be used directly in PCR assays (Malitschek and Schartl, 1991; O'Reilly et al., 1992), or the eluted viral DNA was packaged in such a way as

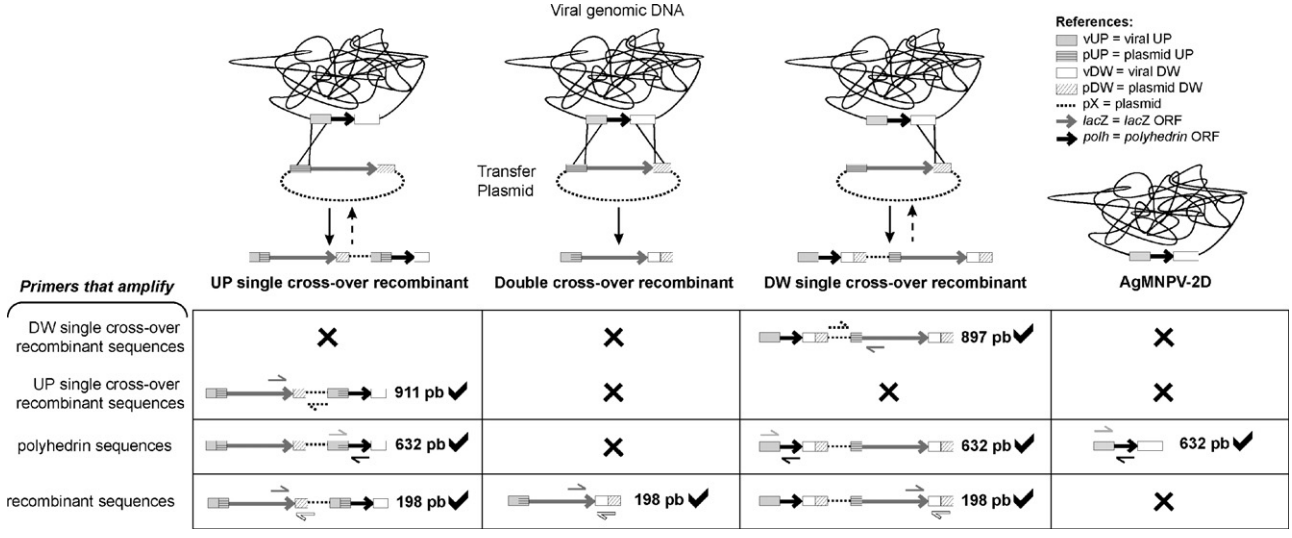

Fig. 1. PCR identification of parental and alternative recombinant genomes: example of an adequate PCR analysis for putative recombinants. The upper part of the figure indicates the different species that result from a co-transfection assay with viral genomic DNA (the polyhedrin ORF is coloured in black) and a transfer plasmid carrying the lacZ ORF (in grey) flanked by polyhedrin upstream (UP) and downstream (DW) non-coding sequences. A double cross-over recombination will give rise to a true allelic replacement. Nevertheless, the probability of only one homologous recombination event occurring is much higher. Thus, the most common type of recombinant virus found in virus stock is the single cross-over recombinant, in which the entire plasmid DNA has integrated into the viral genome. In the lower part of the figure, the diagram shows a typical PCR analysis performed on the different plaque picks to determine their nature (either recombinant or parental). *Note*: the same pattern was used for the primers and their homologous sequences in the different viral species (also see Table 1). The sizes of the primers are not proportional.