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# An efficient procedure for the isolation of recombinant baculovirus

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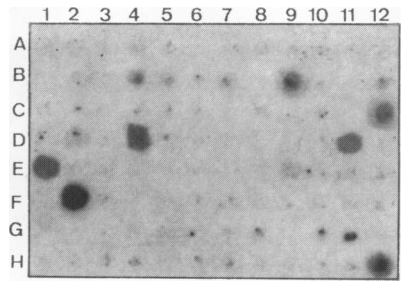
The baculovirus vector system is becoming widely used for the expression of genes in insect cells (1). A number of methods for selecting recombinant virus encoding the gene of interest have been described (2,3). Here we present an efficient and simple alternative.
The transfer vector pAcgD267, vector pAc373 (2), containing a glycoprotein D (gD) DNA fragment of herpes simplex virus type 1 (HSV-1), encoding amino acids 1 to 242 of the mature protein, was cotransfected with Autographa californica nuclear polyhedrosis virus (AcNPV) DNA into Spodoptera frugiperda Sf21 cells. After one week extracellular virus was harvested and the titer was determined. Since the recombination percentage is between 0.1% and 5% (2) a minimum of 1000 virus particles has to be screened to detect at least 1 recombinant. Of a 96-wells tray, containing $10^4$ cells/well 95 wells were infected with 20 pfu (plaque forming units) of the extracellular virus and well 96 with 150 pfu as a control. One week post infection the cells were lysed, blotted and hybridized with a gD probe. The culture medium of cells that hybridized (6 wells) were combined and used for a second screening, shown in the Figure. This time the 95 wells were infected with 1 pfu/well. As recombinant virus stock medium was used from cells, that hybridized with the gD probe and that did not contain occlusion bodies, which indicates the absence of wild-type virus (Well D11 in the Figure). The correct position of the gD fragment was confirmed by restriction-enzyme analysis, followed by Southern hybridization.
Since the generally used visual selection (2) for recombinant virus is difficult and even complicated by the fact that occlusion-body negative variants are frequently present in an AcNPV population, this may result in pursuing false-positives. The method described here does not have this disadvantage. An additional advantage of the present method over others (2,3) is that only two cycles of screening are necessary to obtain recombinant virus. This limits the possibility of integration of chromosomal DNA into the viral DNA (4). Finally, this method works equally well with relatively low transfection efficiencies and recombination percentages.

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