Cationic lipid-mediated co-transfection of insect cells

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Current co-transfection protocols use calcium phosphate precipitation for cultured insect (Sf-9) cells followed by multiple agarose overlays to purify recombinant baculoviruses (1). We report a more effective and convenient method that uses the cationic lipid *N*[1,1-(2,3-dioleyloxy)propoyl]-*N*,*N*,*N*,-trimethylammonium chloride, or DOTMA (2, 3), for co-transfections of Sf-9 cells and uses 96-well microtiter plates to identify and purify recombinant baculoviruses. The co-transfection method is not pH sensitive and may be used with vector DNA from mini-lysate protocols. DOTMA is sold by Gibco/BRL under the trade name Lipofectin™.

The cDNA genes for both the α- and β-chains of hemoglobin were cloned into the baculovirus transfer vector pAcYM1 (4) and verified by restriction enzyme digestion, partial DNA sequencing, and dot-blot hybridization (results not shown). One μg of CsCl-purified transfer vector for the α-chain of hemoglobin was mixed with 2μg of AcMNPV DNA and 30 μg of DOTMA in a polystyrene container. The solution was brought to a volume of 50 μl with water, mixed, and allowed to sit at room temperature for 15 minutes. The DNA/DOTMA complex was mixed with 3 ml of serum-free Grace's medium supplemented to 0.33% lactalbumin hydrolysate and 0.33% yeastolate and added to a 25-cm^2 flask containing 3×10^6 Sf-9 cells. The cells were then incubated without serum at 27°C for 3−24 hours. After this incubation, the medium was supplemented to 10% fetal bovine serum in a total volume of 6 ml and the cells incubated for a week at 27°C. 10^{-5} to 10^{-7} dilutions of the co-transfection medium were prepared and each dilution used to infect Sf-9 cells seeded in a 96-well microtiter plate at 1×10^4 cells/well. The plates were incubated at 27°C for a week. Each well was scored for the presence of viral infection and the viral titer of the co-transfection medium calculated by end-point dilution (1). We find that the viral titer from the co-transfection of Sf-9 cells using DOTMA is at least 20-fold greater than the titer obtained from the calcium phosphate precipitation method. The cells from each plate were lysed and screened with a ^{32}P-labeled probe to identify recombinant viral samples. Figure 1a shows an autoradiograph of a nitrocellulose filter from a 96-well dot-blot hybridization of a plate of cells infected with a 10^{-5} dilution of the co-transfection medium. The percentage of recombinant virus present in the co-transfection medium is estimated to be 5−10%, a 5−50-fold increase in recombinant virus over the calcium phosphate precipitation method. We find that an overnight incubation of Sf-9 cells in the presence of DOTMA provides optimal recombinant virus production. The microtiter plate screening procedure was done a second time to purify recombinant virus from wild-type virus. This process was also performed for the β-chain of hemoglobin. Purified recombinant viruses for both chains (Hα and Hβ) were used to infect 25-cm^2 culture flasks of Sf-9 cells (1). Cells were collected at 72 hours post-infection, washed, resuspended in sample buffer, and analyzed on a 15% SDS-polyacrylamide gel. Figure 1b shows that proteins identical in size to each chain are expressed in the infected cells.

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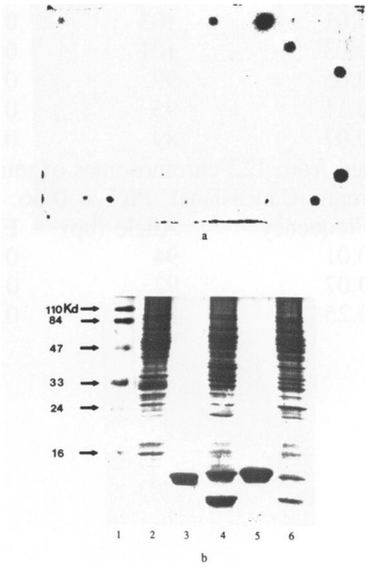

Figure 1. a. Dot-blot hybridization of cells infected with a 10^{-5} dilution of the co-transfection medium. b. 15% SDS-polyacrylamide gel. Lane 1: low MW markers; lanes 3 and 5: α- and β-chains; lanes 2, 4, and 6: uninfected cells, Hβ infected cells, and Hα infected cells.