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A new method to propagate defective HSV-1 vectors

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Defective HSV-1 vectors have great potential (1) because of the capability of HSV-1 to infect and persist in postmitotic cells, including neurons, in adult animals. The current method to initiate propagation of a HSV-1 virus stock is to transfect cells with a mixture of vector DNA and HSV-1 DNA (2,3). Preparation of HSV-1 DNA is laborious compared to preparation of HSV-1 virus stock and transfection of cells with HSV-1 DNA is inefficient compared to infection with HSV-1 virus. To aid in exploiting the potential of HSV-1 vectors, a simpler and more efficient procedure to propagate HSV-1 vectors, using HSV-1 virus instead of HSV-1 DNA, was developed: $1.5X10^5$ CV1 monkey fibroblasts were seeded on a 60mm plate. The following day the cells were transfected (4) with an 0.5ml calcium phosphate coprecipitate containing 1ug HSV-1 vector DNA and 9 ug salmon sperm DNA. Four hours later the cells were shocked with 15% glycerol(5). Following a 24 hour incubation at 37C, $1.5X10^6$ PFU of HSV-1 in 100 ul was added to each plate for 1 hour at room temperature. The virus inoculum was aspirated and the plates were washed once with 5ml of medium. Semiconfluent plates of CV1 cells which were not exposed to either virus or DNA were trypsinized. 5ml of medium, containing $5X10^6$ recently trypsinized CV1 cells, was added to complete the monolayer. Following incubation for 1.5 hours at 37C, to allow the CV1 cells to attach to the plates, the medium was removed and replaced with either fresh liquid medium or methocel. After incubation for 3 days virus was harvested or after incubation for five days plaques were visualized with neutral red. Virus stocks were subsequently passaged at a 1:2 dilution on CV1 cells. This procedure will aid in propagating many different defective HSV-1 vectors and in evaluating the properties of different strains and mutants of HSV-1 as helper virus.

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