UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

BERKELEY • DAVIS • IRVINE • LOS ANGELES • RIVERSIDE • SAN DIEGO • SAN FRANCISCO

SANTA BARBARA • SANTA CRUZ

SCHOOL OF MEDICINE
DEPARTMENT OF BIOCHEMISTRY AND BIOPHYSICS

SAN FRANCISCO, CALIFORNIA 94143
(415) 666-4324

May 26, 1977

Dr. Daphne Kamely
Office of Recombinant DNA Activities
Bldg 31, room 4A52
NIGSM, NIH
Bethesda, Maryland 20014

Dear Dr. Kamely:

We would like to undertake a project involving recombinant DNA. Under the present NIH guidelines, this project would appear to require P4 containment. We request that the Recombinant DNA Advisory Committee review the following considerations which we feel would warrant P3 containment conditions.

We propose to use polyoma virus as a vector to clone the thymidine kinase (TK) gene of pseudorabies virus (PRV) in mouse cells. DNA of a polyoma mutant temperature sensitive in an early function (ts-a) will be purified. Restriction endonuclease(s) will be used to delete a portion of the genome that codes for late function. A PRV restriction fragment that codes for TK will be identified and purified using transfection of TK^- mouse L cells to HAT resistance as an assay of activity. Recombinants of the polyoma and TK fragments will be formed using conventional procedures. TK^- L cells will be infected at the restrictive temperature and cells selected in HAT medium to obtain clones that express TK activity. These cells will be shifted to the permissive temperature and closed circular DNA isolated from the Hirt supernatant.

The following considerations apply in evaluating the biohazards of the proposed experiment:

1. PRV, a herpes virus, is not known to be pathogenic for man, but is pathogenic for pigs and cattle.
2. Viral strains with highly attenuated virulence for livestock and laboratory animals have been described and will be used if available.
3. Polyoma with a deletion of late function will be used.
4. No virions will be generated.
5. A partially defined fragment of PRV DNA will be used.
6. Mouse cells are permissive for both polyoma and PRV, hence both genomes have had the opportunity to coexist in a single host before the introduction of recombinant DNA technology.

In view of these considerations, we request permission to conduct this series of experiments under P3 containment conditions. This project will be conducted by the undersigned.

Sincerely yours,

HERBERT W. BOYER
Dept. of Biochemistry

PHILIP COFFINO
Dept. of Microbiology

HAROLD VARMUS
Dept. of Microbiology