12 September 1972

Dr. Ian Macpherson
Imperial Cancer Research Fund
Lincoln's Inn Fields
London, England

Dear Dr. Macpherson:

I have enclosed copies of the first experiment performed with the DNA's you have sent to us. The protocols are as described in the preprint I sent you earlier: 5 ng of slowly-reassociating double-stranded ^{3}H DNA made by Schmidt-Ruppin RSV polymerase is reassociated in the presence of 4 mg/ml of cell DNA. Assays for extent of reannealing of the viral DNA are performed with hydroxyapatite (Fig. 1) or single strand specific nuclease (Fig. 2) Some of the results conform to expectation — namely, DNA from salmon sperm and normal BHK cells (data not shown here) do not effect reannealing, whereas SR-transformed cells appear to have about 1 copy per diploid cell. However, the other data is very perplexing — the HSV transformed cells appear to have onecopy and the Bryan-transformed have none. One obvious possibility is that the DNA's from these two lines were confused at some point in our lab or yours. Since BHK itself is negative and we have seen no cross reaction between avian and mammalian tumor virus nucleotide sequences heretofore, it seems very improbable to me that the HSV/BHK is truly positive. (The experiments have been done twice with hydroxyapatite assay, with identical result.) Of course, the Bryan-transformed line could be negative, thus SR- accounting for its tendency to revert, and that would be very interesting. But in view of the confusion, I asked you in my telegram to send another batch of HSV/BHK DNA, as well as revertant DNA. It will obviously be important to look at another preparation of DNA from Bryan-transformed cells with a low percentage of revertants.

Needless to say, I am unhappy about these difficulties. Unfortunately, repetition of tedious preparations and assays on both our parts will be required to rectify matters. If you have any ideas about why HSV/BHK might be positive, please forward them.

Regards,

Harold E. Varmus
Assistant Professor
Department of Microbiology

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encl.