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DEPARTMENT OF BIOCHEMISTRY

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Dr. Renato Dulbecco
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P.O. Box 1809
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Dear Renato:

I am sorry that it has taken so long to send you this material but Marianne was ill and it took some time for her to get it together.

I am including the protocol for preparing very highly labeled P^{32} polyoma DNA. It is obviously applicable to SV40 DNA as well. I think it is quite straight forward but if there are any questions don't hesitate to let me know.

The two photographs show the data for two different experiments and should give you an idea of the kind of data the method provides. I think we can further reduce the concentration of P^{32} DNA to the order of $2\text{-}3 \times 10^{-5}$ A_{260}; that would increase the sensitivity of detecting unlabeled complementary sequences. The xerox copies show the protocol and data for Cot-16. I enclose it to illustrate the ingredients of the annealing mixture and the schedule for taking samples used in that experiment. After the samples are removed from the annealing mixture they are diluted in the S_1 enzyme digestion buffer and frozen. After all the samples are collected, they are incubated with the enzyme at 37° for an hour and then acid precipitated. The amount of P^{32} which remains acid precipitable is a measure of the amount of DNA which is annealed. Also attached is the computer printout for calculating the curves and the Cot 1/2 for the three samples of Cot-16.

More recently, we've handled the data somewhat differently and have plotted it in a linear way. Cot-16 is replotted using only the time points up to 24 hours or less. You can see the points (unfortunately we did not collect enough early points because the experiment was not designed for this purpose) yield a nice straight line which enables us to calculate the second-order rate constant for annealing. These second-order rate constants yield Cot 1/2 values which are in good agreement with the predicted values.

The advantage of this kind of plot is that we can use the short time intervals, even up to an hour to determine the rate constants. (See Graph 1). A second experiment using the same technique of handling the data gave equally good results (Graph 2) . The second set of computer output shows how the program calculates from the same points what we call a Wetmur Plot. (after Wetmur and Davidson). The program gives us the values for the theoretical second-order line which best fits the experimental points.

We do have some of the Taka-diastase powder from which the S_1 enzyme can be prepared (see enclosed reprint). If you want some I can send you a little but it is commercially available(Enzyme Development Corporation, 2 Penn Plaza, New York, New York , 10001).

We have finally succeeded in making the SV40 λ dvgal hybrid duplex circles; the yield is quite good, about 25%, and I hope now to make sufficient quantities for some interesting biological experiments. We do have some now and we plan to try to transfer 3T3 with them. We have also synthesized SV40 dimer circles by fusing monomeric linears and we shall test their infectivity and transforming activity as well. It should be interesting to make SV40 dimer from viruses having different genotypes. I am hopeful I can find other interesting DNA's to insert into SV40 that can broaden our usefulness of this approach.

I hope all is well at the Salk. Please give my very best to Maureen, Margarite and all the rest of the people there.

With best regards,

Sincerely,

Paul Berg

(Signed and sent in Dr. Berg's absence)

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