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2nd August 1971

Dr. Dan Nathans,
Department of Microbiology,
The Johns Hopkins University,
725 N. Wolfe Street,
Baltimore, Maryland 21205,
U. S. A.

Dear Dan,

I'm sorry for the long delay in answering your letter of June 24th but I was out of London for the last few weeks. I would very much like to provide you with a simple procedure to strand separate SV40 DNA. However, although we had some limited success with the Szybalski method using poly X, we have not been able to get consistently reproducible results. The separations achieved are extremely dependent on the poly X molecular weight (or some other unknown property). The first preparation of poly X (obtained from A.M. Michelson) worked nicely at the start but then deteriorated on aging. When I rechecked the S20 it had fallen from 13S to 8S. Michelson had no more poly X so I tried several commercial preps. - none worked and none had S20s greater than 9S. I got some poly phos. from Grunberg-Manago but could not synthesize material of greater than 10S and the separations were incomplete as judged by hybridization studies. I will include our method and early results if you should like to try the procedure and if you can get some good poly X (which I don't have at the moment).

A number of other people have tried synthetic polymers for SV40 or Polyoma but without success (Kubinski and Rose, P.N.A.S. 57, 1720, 1967; Rüst, B.B.R.C. ?, 455, 1970; Shatkin and Sambrook; Crawford and Berg (unpublished experiments). The polymers tested include poly G, UG, IG, U, A and C. I also tried Iodo C, Bromo U, Bromo UG and diaminopurine - all without success. Although poly X has given some limited separation of SV40 DNA it does not work with polyoma DNA.

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If you can find a source of very high molecular weight poly X and would like to give it a try our conditions are as follows:

1-2 μg SV40 Form II DNA in 0.1 ml of 1 x SSC + 2^{-5} μg poly X.
Heat at 100° for 90' - quick cool in ice and add to CsCl gradient.
A typical separation (with the original high molecular weight stuff) is shown below.

I'm sorry I can't be more helpful. Your B restriction enzyme worked fine but without the poly X step, I'm afraid I have not been able to put it to use on the originally planned experiments.

Best wishes.

Sincerely,

David Ward

