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From Roy Schmickel

Ext. .....

Sept 24, 1975

Dear Dan,

I have settled in Edinburgh and the family is quite pleased. I am doing some learning experiments with recombination and transfection. The lab is certainly well equipped for all of the λ work and there seem to be good provisions for EM heteroduplexing. However, there are no cell culture facilities readily available on this campus. There probably will be cell culture opportunity in the MRC cytogenetics unit which is across town at Western General Hospital. It will probably take time to work out the arrangements, and I believe I can grow the SV40 there.

For these reasons, your offer to supply the dl 1009 is not only generous but will greatly facilitate the project. Ken had planned to talk to you at Madrid, but evidently you were not there at the same time. Could you send the DNA directly to me?

Since spending the day at your lab with you, I have had a chance to go over the papers you gave me and to think about the project in more detail. I would appreciate it if you would look at the outline of the protocol and see if you think it is reasonable.

1. Separate the DNA of form I 1009 from the ts helper form I on 1.4% agarose / Eth Br. as in Plate III, JMB 89 – Lai & Nathans

✓ 2. Localize the Form I dl 1009 c̄ 360 A° light and cut from agarose

Electrophorese DNA from agarose in dialysis membrane sac.

NO 3. Conc. this DNA by alcohol ppt.

✓ 4. Establish conditions for single cut Hind III digestion

✓ 5. Establish conditions do partial digestion and B) again

✓ 6. A) Using these conditions do partial digestion and B) again electrophorese on 1.4% agarose/ Eth Br.

7. Localize DNA by 360 A° light and cut out full length dl 1009 linears. (Standards could be made by limit digestion of dl 1009 with EcoRI). Separate from 1.4% agarose by electrophoresis into membrane sac.

8. This DNA to be used for ligase reaction and transfection

9. Pick plaques + make stocks

10. Prepare DNA from stocks

11. Remove inserted DNA by Hind III digestion + electrophoresis and isolate inserted DNA

12. Do heteroduplex mapping c̄ wild SV40 - wild type linears

13. Select stock which has deletion loop .2 units from end

Others will be ≈.4 from end

This raises some questions: How much DNA should be applied to gels to end up c̄ 3μgm for the restriction digestion? Could you draw a diagram of electrophoresis sac for separating the DNA? Do you think it would be more economical to skip the 2nd electrophoresis (Step 6 B) and use the partial digest directly for recombination? Partial digestion would generate 16 different molecules whereas selecting for full length linears of dl 1009 would only comprise 4 different molecules.

Thanks for your help. I certainly like the idea for the project and am anxious to proceed.

Sincerely

Roy