October 8, 1975

Dr. Roy Schmickel
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University of Edinburgh
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Mayfield Road
Edinburgh EH9 3JR, Scotland

Dear Roy:

We are preparing DNA of SV40 d1-1009 and will send you a preparation of Hirt-supernatant DNA ready for electrophoresis in agarose gels. For quantitation the DNA is being labeled "lightly" with ^{3}H-thymidine. The steps you outline look O.K. to me except for recovery of DNA from gels for endo R·HindIII cleavage. HindIII is very sensitive to an "inhibitor" in DNA recovered from agarose. We now dissolve the gel segment in saturated KI (potassium iodide), adsorb DNA on hydroxyapatite which has been equilibrated with saturated KI in pH 7.0-0.01 M Na phosphate, wash with saturated KI, then 0.1 M NaP, and elute DNA with 0.4 M NaP, pH 7.0. NaP is removed by extensive dialysis of DNA against 1/10 SSC.

You should have about 50 μg of d1-1009 DNA in what we send (from 20 10-cm dishes). I would be inclined to re-electrophorese the original form I d1 DNA to reduce contamination with helper and then isolate full length d1-1009 linear molecules after partial dIII digestion for coupling to λ DNA. (First determine conditions for partial digestion.) The mixture of linears and presence of an intact VP1 (or B/c) gene can be predicted from the following map of d1-1009:

As you see, several fragments will have an intact B/c (probably VP1) gene. Of course all can be cloned and readily distinguished one from the other by digestion of the λ-SV40 hybrid and especially by heteroduplex mapping.

Best of luck. Let me know if I can be of further help. Also please keep me informed of progress.

Best regards to the Murray's and to your wife.

Sincerely,

Daniel Nathans

DN:as