

February 22, 1971

Dr. David Ward  
Imperial Cancer Research Fund  
Lincoln's Inn Fields  
London, W.C.2, England

Dear David:

Enclosed are 3 tubes of E. coli B restriction endonuclease in 50% glycerol. The enzyme proved stable in 50% glycerol at  $-50^{\circ}$  for at least 10 days. This preparation is the G-200 Sephadex fraction as described by Roulland-Dussoix and Boyer in BBA 195, 219, 1969; prior to glycerol addition it was in .01 M  $KPO_4$ , pH 7 - .005 M mercaptoethanol - .001 M EDTA. Tested against high specific activity, sonicated  $^{32}P$ -T7 DNA ( $2 \times 10^5$  cpm/ $\mu$ g), it had no detectable exonuclease activity under the conditions of the B enzyme assay. To cleave SV40 DNA we use the following conditions:

SV40 DNA I - 20  $\gamma$ /ml                    6  $\lambda$   
Mix -    4  $\lambda$   
Enzyme (50% glycerol)                    10  $\lambda$   
 $36^{\circ}$  x 30 minutes  
Stop reaction by adding EDTA to .015 M

Mix: SAM .06 M in .01 N  $H_2SO_4$                     10  $\lambda$   
Tris Cl pH 7.6 1 M  $H_2SO_4$                     20  $\lambda$   
ATP .06 M    10  $\lambda$   
DTT .03 M    10  $\lambda$   
 $MgCl_2$  0.3 M                                        10  $\lambda$   
Water    40  $\lambda$

With the amount of enzyme given above 0.03  $\mu$ g DNA was converted to a heat denaturable form in 5 minutes. The reaction slows down after about 15 minutes and generally I add more enzyme after 15 minutes. Recently I have found that the formation of form III proceeds at a decreasing rate for  $> 30$  minutes and I suggest therefore that you incubate up to 1 hour.

To assay the enzyme we incubate for 5 minutes using radioactive DNA and add 5 volumes of 1 x SSC. This is heated at 100° for 2 minutes, quickly chilled in ice and passed through an S+S B-6 filter to count denatured DNA. One unit of enzyme is the amount that converts .01 µg SV40 DNA I to a denaturable form in 5 minutes.

I hope the enzyme works when it arrives and does what you need. Let me know if I can be of further help.

Best wishes,

Sincerely,

Daniel Nathans