[1976]

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From .....

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Dear Ching Juh,

As usual, no news is bad news. However I thought this might be a good time to catch you up on my work here since you and Dan have been so helpful and must be wondering.

As I mentioned in January. We made a good P⁻ amb. vector with the att deletion. I then did several test digestions on the SU40 to establish good restriction conditions. Then I digested the 2X purified 1009 under the same condition after ligation and transfection we got about 30 good recombinants. The test was replacement of the imm site to give clear plaques. I then grew these colonies up and hybridized them against cRNA-32P from SU40. This gave about 25+. Following this I prepared DNA from these + phage and ran the DNA on gels. The gel was transferred to nitrocellulose using Ed Southern's technique and hybridized against cRNA. In summary, I got no phage c̄ complete genomes. Several contained 2 or 3 fragments, but none had all four.

It would seem that the restriction reaction went too far. The technical problem was that there was no good way to know how much DNA was in the reaction. Evidently counting gave an overestimate. And there was not enough material to analyze by gel after the reaction.

I am happy that you went ahead and sent the other mutant prep. I am enclosing a gel of the two DNA's before and after 2X purification. I ended up with about .2 ml of each prep and the gel (25B) is a 20λ sample. Column 1 is 1009 purified, 2 1009 before purification, 3 is 1117 purified and 4 1117 as received. For some reason the as 1117 seemed to have a fair amount of cellular DNA. I hope that did not contaminate my "pure" words.

The second gel I am enclosing is the original and several different digests 1 - 1009 as received; 2 - 1117 as received; 3 - 1009 Hind III; 4 - 1117 - Hind III; 5 - 1009 EcoRI; 6 - 1117 EcoRI; 6-10 partial Hind III digests. Since I have a fair amount of each pure DNA, I am doing the restriction under many different conditions. I have about 35 clones that I am working up now and still can do several more restrictions. I won't begin the next until I have characterized this batch.

I have every confidence that I will have a few good clones in 1-3 weeks. However, if there is some problem with this method I would appreciate some 1117 as a back up. As my time is growing short, it would be disastrous if I didn't have a back-up prep. If you wouldn't be too abouse to one more prep, I would greatly appreciate it.

Could I prepare some of of the phage DNA's for you? I will have many types. I should be able to make a couple of them 5' and obtain fine yields.

Although the year is whizzing along, I still have plenty of time to look at translation and can carry it on after I leave.

Best Wishes to you & Dan

Thanks,

Ray