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Dear Buzz:

Forgive me for delaying the answer to your letter but I've been snowed trying to get ready for our departure to Spain. The school year is off and running at the usual hectic pace but I'm sure things will settle down in due time. George and Bob are back and happy to be here and at work again. The new labs are under construction and should be finished by January in time for Ron Davis's arrival. The new Dean has arrived (Clayton Rich) and is beginning to make his presence felt; hopefully it will be for the good.

How are you doing in Paris? No doubt Moshe Yaniv brought you news of the lab and Stanford so I won't go into more detail now. I didn't call you and Ann on my way back from Sicily because I decided not to come into town. I arrived at Orly late one afternoon, checked into the Orly Hilton, had a marvelous dinner there (a superb restaurant, La Lousianne) and left early the next morning for here; never did see the city.

Let me get to the question you asked! We have not written the method up but it seems some one else has in a recent BBA article (not done as well as Marianne has). Enclosed are three outlines for the conditions, references and results using three different enzymes. We have never prepared any of them but have relied on begging them from others. The Neurospora enzyme (a nuisance to prepare) was an old preparation from Stu Linn that we found in a refrigerator which we have all but used up to convince ourselves that the method is useful. We also got a reasonably good supply of the Mung bean enzyme (purified) from Laskowski (he was very generous in sending me some when I asked for it) and that also works quite satisfactorily. The one we have used

most extensively, however, is the enzyme from Aspergillus, Sl. Marianne has given you the reference and the address of Ando the man who purified it. When we wrote him he was happy to send us some and I'm sure he'd do the same for you. Our supply is decreasing and I'm not sure we can spare any now. I don't know how to prepare it but if it starts with Aspergillus spores or hyphae, I'd rather not.

A word about the overall procedure. We use very hot polyoma DNA (our goal is 5×10^6 -10^7 cpm per μg) sheared to 6S (in alkaline sucrose) and denatured at 100°. We have been pushing the annealing to the limit by using C_o of $^{5x}10^{-5}$ A_{260} so that the time needed for "complete" annealing is more than a week. But at C_o of 10^{-2} to 10^{-3} A_{260} the annealing time is relatively short at moderate ionic strength. Actually in our experiments we are comparing the $C_o t$ curve for the annealing hot (P^{32}) PY DNA in the presence or absence of the cold DNA we want to test for complementary sequences. To work out the amounts of enzyme that will be needed to degrade all the non-annealed P^{32} DNA in the presence of the cellular DNA we have added cold, sheared, denatured salmon sperm DNA to the annealing mixture. We use 1.0-1.5 M NaCl in Tris, pH 7.5 at 68° for annealing and aliquots are taken at various times and digested as described by Marianne. The amount of P^{32} still acid-precipitable represents annealed sequences.

I hope these comments are useful to you. It's a very much simpler method than hydroxy apatit column separation of native and denatured DNA and should become more widely used (particularly if some enzyme manufacturer would put the enzyme on sale).

Hope Ann is enjoying Paris but then again, how can it be anything else. Say hello to Jacques, Francois Jacob, Cuzin and Gros.

Sincerely,

Paul