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Dear Vittorio,

Sorry once again for taking so much time once again, Josh reminded me yesterday to keep in closer contact.... I have been thinking about our experiments and trying to accomplish something on the wet side as well. Lately, I have been grappling with the RNA experiments, and the pressure has been on to produce some results... basically been having many difficulties trying to acheive the absolute in sensitivity. Anyway, it's a lot to explain right now, but during these hard times, I feel that I've learned the useful technique of purifying DNA from low melting temp agarose.... I plan on using this for the endo expt.... I have labeled our primers and pcr'd up the region of the cutting and am about to isolate these framentsfrom sea plaque or nuseive.... then will use this purified stuff as template. If you still remember , these are the expt's you suggested. This time, I will also look at stoffel since we left out the magnesium (which is not in their provided buffer) when it did not work for us...

Concerning the paper, I have a few thoughts.....
In comparison to Dahlberg's paper, he did not show that cleavage was necessarily coupled to synthesis, but in our system cutting appears dependent on it. This may have significance if you consider that a polymerase may potentially display different activity or simple kinetics when the assay measures what happens when the enzyme is elongating from a primer which is placed immediately at the critical point (in Dahlberg's the primer was very close to or immediately at the separating strands ie... to add another base it was necessary to displace the strand or cleave it). A very potentially different scene, analogous to what we think is significant with the RNA incis with DNA as a template, is that if a polymerase gets a "running start" its kinetics or behavior in general might be different. for a reference see : Goodman, Creighton, Bloom, and Petruska "Biochemical basis of DNA replication fideltiy" Critical Reviews in Biochem. and Molec. Biol. 28(2): 83-126 (1993). You should be aware of this anyway....

Another difference with our paper which I'm sure you are aware of but I don't think that you stressed enough in the draft is that the site of cleavage in our system lies within a natural regulatory region, namely the lac operator... Also we have not yet established that the template is being cut, so far all that we have is that the newly

elongated sequence is cut.... we must check the template idea rigorously, which I am in the process of doing.

Also.... Dahlberg's effect of cutting seemed dependent on the existence of a five prime arm/end which does not seem clearly necessary in our system....
In this light, I have some concerns about how we present this activity.... our very title which says that heterogeneity in primer extension could relate to cleavage by the 5'-3' exo activity might be totally inaccurate, ie... something else in the enzyme prep might assist the pol..... I will try the system with another company's Tth pol to assure ourselves that this activity is somewhat universal.

One last thing.... I believe that we should quatitate with the phosphorimager the actual ratios of counts seen for each set of stops... we have the ability to do this very easily now.

Also, just as an aside, I was thinking that it would be nice to see if the cutting phenomena holds true if elongation is carried out on single stranded DNA of the same sequence.... might be possible if cloned into M13? maybe not, I did not check thoroughly.

Well, I might not be correct in some of my ideas, but check them out...let me know what's what.....

Ciao, Ciao!

GREG