Seminar Minutes of 9-30-74

I. Ron: Genes were separated on the basis of size after treatment of B. subtilis DNA with R_I endonuclease, which cuts the DNA at specific sequences that may be randomly located. (The occurrence of these specific sequences in some DNA and not in others indicates an evolutionary relationship & certain amino acids are more likely to be represented in these sequences than others.)

1. Electrophoresis of the R_I digested DNA on agarose gel for 12-16 hours, gave a good banding pattern, as seen by fluorescence and photography. There were 3 separate R_I endonuclease digestions of B. subtilis 1070 DNA & the banding patterns of the 3 agarose gel electrophoretic runs were similar.
2. Dushko ran a densitometry. The densitometer gave the same pattern but didn't detect the bands as clearly as does the eye (which accentuates edges more readily).
3. To find out if there is room for improvement of experimental resolution, use enough of a clean single species marker DNA & get its banding pattern; this will provide some information about electrophoretic, densitometer, & photographic resolutions.
4. A discussion of photographic problems followed. The agar gel tube is cylindrical, causing optical problems - one cannot focus on a sharp image since there is alot of scattered light coming out, light gets scattered up from bits of agar, tilting of disc (one looks down at discs of emitting material) results in blurring of band width; therefore, do a scan across the middle & not around the edges. It would be preferable to have a thin slab rather than a cylindrical tube. Try making the gels in thin capillaries where the cross section of the tubing is rectangular rather than cylindrical. The main objective is to get a thinner section, so use gels with a smaller cross section, thereby reducing the dimensions through which all the light scattering occurs. Also, in using thinner sections, less material would have to be used. One may also try quartz tubes. It may be possible to get higher magnification of photography; try close-up attachment & other lenses.
5. There is not a linear relationship between densitometry & DNA concentration. The level of densitometry background was compared to the peaks of bands. The background (i.e., interbands) is as much as 80-90% of the "peaks" of bands. The bands & also the interbands may contain alot more species of DNA than was thought, or the interbands may represent diffusion of bands - unresolved lobes of bands.
6. Suggestions: run the densitometry much more slowly, so as not to run into instrument rise-time problems; do a blank run without DNA; determine the residual fluorescence of the uncomplexed ethidium bromide (i.e., blank using buffer with eth. br.).
7. In the previous experiment it was shown that Tryp-Tyr biological activity is found in the heavier side of the gel, localized in the second band (which is the first large one). In this experiment, in each case the sliced out top 1 cm of electrophoresed R_I digested B.s. 1070 DNA, was reelectrophoresed, by being placed on top of fresh gel.
   a. Using the first sliced out cm, the electrophoresis ran overnight at 20 volts total (low voltage being necessary for good separation and resolution); in the morning no bands were seen. The new gel shrank about 2 cm apart from the applied gel piece, presumably before any DNA diffusion into the new gel could occur.
   b. The second cm of fluorescent material was put on top of the new gel, & 200 volts was applied for 10 minutes, the gel then being checked for fluorescent bands. None were seen. The buffer with which the new gel was prepared, onto which the gel slice was placed, lacked Eth. Br. Presumably, it took awhile for the

Eth. Br. of the superimposed gel slice to diffuse into the new gel.

c. The third cm of material having 5 bands was applied over the new gel with 150 volts for 13 minutes followed by low voltage for several hours. The bands were observed diffusing down into the new gel & then the same bands disappeared. Several hours later, the bands reappeared. A 60% improvement was obtained by this rebanding since the 5 bands were spread over 1.6 cm instead of the previous 1 cm. Other material migrated much faster (thus the 5 bands were cleaner)

8. The first 2.1 cm of fluorescing material from the third gel was cut into slices which were mashed & incubated as previously described to let the DNA diffuse out. This DNA extract of donor 1070 B.s. was mixed with competent cells of recipient B.s. 863. The His_1 marker survived R_I endonuclease digestion & was tested. The plating tested for Tryp-Tyr transformation (by use of plates with amino acids minus Tryp & Tyr plus shikimic acid) and for His_1 transformation biological activity (by use of plates with AAs minus His). The Tryp marker was found in the 3rd-5th slice, most of the counts occurring later, at the lighter end. The His_1 marker had background of histidine + transformants until the 16th mm where the marker appeared. There was an excellent separation of the 2 markers - by biological activity almost 100%, & on the gel by a 1.5 cm distance of one from the other.
9. A gel run will be done selecting for $Hist_2$ which is located between Tryp & Tyr. Competent cells are now being made to test for other markers that are resistant to R_I endonuclease digestion, & that may be localized in the gel.
10. Using replica plating, im all the Tryp-Tyr transformants checked from gels with R_I treated DNA only one was able to grow on plates with AAs minus phenylalanine. The measureable linkage between Aro_2 and Tryp is destroyed.
11. In the sheared material, linkage between Tryp-Tyr and Phe (Aro_2 marker) did not disappear but did decrease as M.W. of fragments decreased, as expected.
12. It would be useful to acquire sets of mutants that change the M.W. of the fragments; then we would have a very good means of measuring the actual distance (in daltons) between markers.
13. It is necessary to overcome the problem of gel shrinkage. Agar gel is preferable to other gels because it is structurally strong & has high diffusibility, being an open structure. pH, salt concentration, & temp. have profound influences on the shrinkage of gels. Melt the agar by slow boiling in a water bath for 15 mins. to one hour; this is safer than higher temperatures for shorter times. Avoid the use of the sterilizer which may hydrolyze the agar giving unreliable results in its use.
14. Continue work on gel electrophoresis technology, refining the methods, before going on to use other strains to see if there are obvious strain differences. Improve the densitometry technique, calibrate it with a monospecific DNA to know the limits of resolution, quantitate the work with gels, improve the gel slicing technique (perhaps the freezing microtome may be adapted for accurate slicing of frozen segments if frozen DNA can be used), do more careful measurements of biological activity, check band spreading, clean up the DNA by rerunning it for rebanding, as described above.
15. Currently SB512 (B. globigii) is being digested to see if 512 banding pattern is different from that of B.s. There is no data on pure hybrid DNA at this time (135-1, a hybrid strain between subtilis & globigii).