Seminar Minutes of 9-23-74

I. J.L.: Discussion about Trace Elements

1. A footnote in Medical Research, 1950, states that the chemicals in use at that time were sufficiently contaminated that it was not necessary to add additional trace elements for the growth of microorganisms.
2. The citrate in the Spizizen Minimal Medium, with its high chelating ability, must provide trace elements.
3. Recommendation of review by Hutner, Ann.Rev. of Microbio., 1972, concerning trace metal nutrition for microorganisms.
4. Trace elements and chelating agents must be considered together.
5. The development of competence in E. coli and B. subtilis may have something more to do with trace elements than has been considered.
6. Resins can be used to get metal free suspensions.
7. Agar is a very efficient carrier of metal elements.
8. The main difference between complete and synthetic media may be in the trace metals.

II. Ron: Transformation in B. subtilis 1023

1. B. subtilis 1070 (thymidine$^-$) was grown in H^3 -thymidine (the labelled thymidine is incorporated into its DNA), the DNA was extracted and treated with R_I endonuclease which fragments the DNA.
2. Gel electrophoresis, using TEB buffer with ethidium bromide as fluorescent DNA label, was employed for 16 hours at 20 volts, to separate the DNA fragments of various sizes.
3. The first 2 cm of the fluorescent segment of the gel strip, that had 8 bands, was cut into 20 1mm slices; each slice was mashed with buffer into a suspension which was incubated at 37° for 2 hours, and the agar was then removed by centrifugation. This method of recovering the DNA in the supernatant favors retrieval of small pieces of DNA, which diffuse out of the gel. The big pieces may be trapped in the agar.
4. This DNA extract of donor (B.subtilis 1070 wild for all markers except thymidine) was mixed with the recipient, B.s. 1023 Aro_2^- $Tryp_2^-$ His_2^- Tyr_1^- Cys^- Lys^- which was first treated for competency.
5. Each DNA extract from an agar slice was tested for its ability to transform the tryp$^-$ to tryp$^+$ on plates having amino acids but minus tryptophane and having shikimic acid to by-pass the Aro_2 mutation. In a second experiment the selection was for tryp$^+$ TYR$^+$, the plates provided lacking both.
6. The absolute number of Transformants (count of colonies) against the slice # was counted, as well as the counts of radioactivity in the supernatant from each slice extraction.
7. A sharp early peak was obtained for biological activity indicating that the tryp$^+$ marker had indeed survived R_I endonuclease digestion and transformed tryp$^-$ cells to tryp$^+$. Almost all of the DNA transformation biological activity was from the first 3 slices, giving one sharp peak - the heaviest fraction - and very little elsewhere.
8. The peaks of radioactivity counts are spread out, indicating that most of the DNA is elsewhere in lighter smaller fractions. Peaks of transformations and counts do not correlate.
9. The control (to compare material of approximately comparable M.W. as shown by sucrose gradients) was sheared DNA (instead of R_I treated DNA), the sheering being accomplished by passage of the DNA through a #30 syringe. Such DNA fragmentation occurs randomly somewhere near the middle. When sheared DNA was electrophoresed (showing no bands), sliced, DNA extracted in the supernatant from each gel slice and incubated with the recipient cells, the plotting of # of transformants against slice # gave a broad spread instead of a sharp clear peak and also alot more counts than in the R_I digested material. The # of transformants & the

\# of counts mirror one another.

10. During the electrophoretic run the gels shrank by about 30%; this was not because of sterilization of the gel material by heat prior to use, for non-sterilized material shrank fust as much as the sterilized.
11. An enrichment of specific activity for tryp-tyr (# of transformants per quantity of DNA) was obtained in R_I treated DNA, compared to #30-sheared DNA.
12. Suggestions:
    a. Try to use the extreme markers in the analysis, markers within the $Tryp_2$ His Tyr segment but closer to its outer edges, e.g., $Tryp_7$ & inh.
    b. Look for other markers that survive $R_I$digestion.
    c. Attempt to correlate banding pattern with biological activity. Do the bands correspond to DNA fragments of different size and biologic activity? The problem is that as soon as the gel is cut into slices, the banding pattern is destroyed (the cutting has been at 1mm segments & not according to band position).
    e. Convert this analytic technique to a preparative technique.
    f. Use 1/4 of gel slice for radioactivity counts and 3/4 of each slice for biologic activity; this way the count measurement will not be slanted in favor of small DNA pieces as are the counts from the mashed gel supernatant.
    g. Use higher DNA concentrations (currently 3 γ at a time is used).
    h. Go back to using slabs and improve that technique because one can load a slab with 20 times the amount of DNA.
    i. Use thicker agar & run it longer electrophoretically for better separation of heavier and light fractions.
    j. Try longer gels and run the electrophoresis longer.
13. The characteristics that are changing in the bands are: the M.W.s of the segments, the ratio of denatured to non-denatured segments, the proportion of sticky to non-sticky ended DNA, the density (G-C content)..
14. The ultimate goal: The highest specific activity is desired, the isolation and separation of segments of the genetic map resulting in gene purification, at present to remove everything that is not Tryp-Tyr.

III. Millie: Transfection with strain MR631 tryptophane suppressor in E. coli

1. Using λ DNA and untreated cells, and L Broth complete medium, transfection could not be obtained; no plaques were seen.
   Using competency treated cells, there was a low efficiency of transfection.
2. Using the synthetic medium Tryptophan-supplemented H Medium of Gussin (1966), as described by Yamamoto et al, J. Mol. Biol. 58:103-115 (1971), again no plaques were obtained with untreated cells.
   There was transfection with competency treated cells, but the transfectability was lower than what is usually obtained using λ on E. coli C600 r^+m^+.