Dear Josh and Esther,

10-28-57

The Pseudomonas cultures were shipped about a week ago. I apologise for the delay, but upon opening the envelopes I discovered that some required re-lyophilizing before they could be shipped. The little vial of Kinetin included in the package was to have been shipped with Bob's yeast cultures to Rubbo, but I forgot to include it, so sent it with the Pseudomonas instead.

The crosses for the heterozygotes (Lac v, Gal +/-) you requested are under way, though it may take a while to get suitable diploids.

The problems with the diploids and their peculiar segregants (those which appeared to cross with both Lac_{1a} & Lac_{1b} testers) may turn out to be due to the testing conditions. It appears at this point that I have no very good indicator for Lac_1^{W112}. That is, an Hfr which will consistently produce papillae when cross-brushed with strains carrying Lac_1^{W112} and not with strains carrying Lac_1^{Y87} or Lac_1^{Y53}. The Hfr M- Lac_1^{Y87} almost always has given a negative reaction with W112 or its auxtrophic derivatives under conditions used for testing segregants from the diploids. This may be due to: 1) the auxotrophic markers. Newton has data indicating W3120 (the Lac_1^{Y87} Hfr M-) recombines consistently and well with F- Lac_1^{W112} prototrophs. He has no data regarding auxotrophs, however, and W112 and its derivatives used in making the diploids are TLB_1^-. 2) the Medium. Complete medium would probably be the best to use from the standpoint that then ideally only the recombination of Lac markers would determine the reaction. Unfortunately it is somewhat messy to score because of reversions of Lac_{1a} and relatively weak fermentation reactions. M-lac+methionine would be ideal, except for potential variability in recombination due to the different auxotrophic markers of the segregants from the diploids. Also, for some reason, on M-lac+ meth. W3120 seems to produce even fewer papillae due to recombination.

I used W3146 as the indicator for Lac_1^{W112} in the results I sent you last time. This was due partly to my misunderstanding Newton's data, and partly to the fact that this strain gave the best reactions with controls. Although the results were not very strong or consistent, in general, on M-lac+meth W3146 combined more readily with W112 and its derivatives than with the Y87 stocks, enabling one to distinguish between them. Newton, however, has shown that W3146 should recombine with any Lac_1^-. The supposed reations may again be due to the difference in auxotrophic markers.

Cross-brushes on M-lac+meth of F⁻ prototrophs x Hfr M⁻ to test allelism agreed with Newton's data. The available Hfr Lac_1 strains have been tested, but with no better luck. I am now in the process of trying to derive a prototroph Hfr with a suitable lac marker. Meanwhile, the only promising approach seems to be to do everything in duplicate or triplicate on B-lac and one or more minimal media.

The key-sort cards were just where you said. Thank you.