March 24, 1950.

Mr. Gordon Allen,
155 Corona Avenue,
Pelham 65, N.Y.

Dear Gordon:

Thanks very much for writing me about your new experiments at such a preliminary stage. They sound very exciting, and I hope you can continue to let me know of the results.

The method should be quite sound, and if you can get a clearcut selection with the markers you have, there ought to be no doubts in the results, with the 5 provisos you tabulated. I have a few suggestions of which you have probably already thought:

Since S^r is "linked" to M, and Az^r to T, I wonder if you don't have a more convenient way to pick principals and complementaries. That is, the cross BM S^r x TL Az^r will give prototrophs which are mostly S^s Az^s. The complements will be S^r Az^r, which you should be able to select with relatively little trouble. This would have the advantage that the principals could, for the most part, be picked out directly, rather than having to make further tests on quasi-prototrophs to see which of them are P-B_1-; rather, I should say that the markers are so arranged that a large proportion of the prototrophs will be S^s Az^s. You undoubtedly have already developed such stocks, but on the offchance you haven't, and that it would save you some time, I'm sending W-1234 (W-677 Az^r). I don't have a 58-161 S^r, unless Norton Zinder has saved some, but you probably will want to use your B-M-P- anyhow. [P.S. He has: W-1302]

As one of the first logical (not necessarily chronological) steps in this type of analysis, I wonder if it would not be most important to establish the following types of statistical complementarity among the population of recombinants at large, namely that $B_1^+P^+$ selections will show a distribution of unselected markers which will be essentially complementary to the M+T-L+ selections. It may be better to use nutritional markers for this purpose, although one could compare Az^s S^s prototrophs with Az^r S^r "non-prototrophs". This may be important to do at an early stage, especially if the Mal locus shows such an aberrant behavior, as I think it does, among prototrophs as among persistent diploids. The expectation might be that, in the crosses above, Mal- might predominate both among the principals and the complementaries.

W-677 has an excellent set of markers [In fact that is its raison-d'etre], which may be more convenient to use than just nutritional. In case you've forgotten, there are Mal-, Xyl-, Mannitol [Mtl]-, and Vl^r, which are easily scored, and also a Gal± and Aralbinose± which are slightly more difficult.

If you need still more markers, you can use W-1272 which differs from W-677 only in carrying a Stl- (sorbitol) and a $V6^p$ (partially resistant to T6; easily scored) However, I don't have a W-1272 S^r, and in fact, if you do develop one to use, would appreciate having one. I'm sending W-1272 just on the off chance as above.

Of the criteria you mentioned in your letter, No. 3 (Correlation of crossing-over) should be the most feasible to test, especially if you use the variety of markers available. Your criterion 5, that "two complementaries should not share a trait of the principal" is based on a rather more restrictive hypothesis of the meiotic mechanism than the others, but at any rate, will be rather hard to test exhaustively. (You will always find a few "spurious" complementaries.)

The picture is brightening just a bit, not much, in my own work. As you clearly saw, I am convinced that Mal duplex prototrophs are the result of segregation from a cell pure for Lac, etc., and heterozygous for Mal. While this might mean a "two-step" reduction, by analogy with the persistent "diploids" which are 2n for Lac but 1n for Mal, another mechanism is also possible now. H-226 is a diploid which is (exceptionally) heterozygous for Lac and for Mal, [obtained not with Het stocks but by Lac_1- x Lac_4-.] Usually it segregates to give Lac_1-Mal- and Lac_4-Mal+ Rather infrequently, H-226 produces apparant partial segregants, i.e., Lac v Mal- [or Mal v Lac-].However, unlike the Lac v Mal- which one usually obtains as the typical persistent diploids, these "partial segregants" are homozygous for Mal, as Mal+ reversions among them then segregate. H-226 is already homozygous for some other factors, so that it is also at least one "partial segregation" removed from the original zygote. The impression that I get is that some sort of autogamy does occur, almost invariably after the original fusion, and occasionally thereafter, which may result in the loss of heterozygosity for various factors, before segregation to recognizable haploids occurs. I don't quite know how to fit this in with the other phenomenon of hemizygosity (viz. of Mal) which seems to occur quite often, but this might be an accident of non-disjunction. I am about to test,now, to see whether the descendants of H-226, in which the elimination for once did not occur, may continue to show this more orthodox behavior in subsequent generations of crossing.

I don't have time to put down the details, but you can tell Bernie that I have some evidence from irradiating diploids, that UV-killing is partly or even largely nuclear, but not, for the most part, recessive lethal mutations. However, even after large doses of UV, a substantial fraction of the survivors can still segregate. In a sense, we are both right in our contentions as to the haploidizing effects of UV.

Sincerely,

Joshua Lederberg