April 28, 1948.

Dr. Leo Szilard,
Institute of Radiobiology & Biophysics,
University of Chicago,
Chicago, Illinois.

Dear Szilard,

I'm sorry our phone connection was so bad yesterday; however, I think that I heard what you said, despite the clatter of office machines in our office. It is not usually so bad. Nevertheless, I think it wise to send this amplification by fast mail.

1. As to the time of recombination: the data are not really conclusive on this point. However, the speed with which the prototrophs develop suggests that there can not be a very extended delay- possibly a few hours. For the preliminary experiments, I should think that it would be convenient merely to spread the mixed cells on agar, and spray phage at varying lengths of time.

2. The trace element solution is referred to in my papers to the publication of Gray and Tatum, 1944, Proc. Nat. Acad. Sci. 30, 404. It is the same as used for Neurospora, see: Beadle & Tatum, 1945, Amer. J. Bot. 32:678-686. However, by mistake, I find that it is superfluous even in liquid media. It is the experience of workers in the field that the requirements of bacteria for the trace metals are so low that special procedures must be used to purify the best qualities of reagents. Probably a solution of USP Ferric Chloride, 1 ppm, would be the best thing that you could use, if you wished to insist on adding these materials. Even the most drastic washing of agar probably will not rid it of trace metals unless one displaces the positive ions with something like Calcium or Hydrogen in the washing process. This can be done, but is rather more laborious than it would be worth.

3. If you are looking specifically for the multiple requiring recombinants, some attention must be paid to the linkage relationships. In the first place, it will be difficult to secure B_1- together with B-M- due to the close linkage (about 10 units) between them. Secondly, if you cross B-M-V_1^r x T-L-B_1-V_6^r only a small fraction of the $V_1^r V_6^r$ recombinants will be B-M-T-L-; rather, they will be mostly prototrophs and it would require a triple crossover to give the multiple deficient type. On the other hand, the cross B-M-V_6^r x T-L-B_1 V_1^r should give you the desired class much more frequently (by a factor of 50 or more). All this on the assumption that you have mutations at the same loci that I worked with. V_1 is the /1,5 type.

4. I was not entirely clear on what had been found re K/1,6.... Do you find such a multiple resistant with appreciable frequency? If they are somewhat rarer than K/1 and K/6 respectively, I should like to have a few of them for a problem I discussed with Aaron.

With best regards,

Joshua Lederberg