October 13, 1954

Dr. J. B. Clark
Dept. Plant Sciences
University of Oklahoma
Norman, Oklahoma

Dear Dr. Clark:

I am happy to enclose a culture of W-478, which I hope responds to your request of September 30. Diploids are rather troublesome to maintain, and all that had been isolated earlier from the Het crosses published in '49 (PNAS) and '51 (J. Bact. and C.S.H. Sympos.) are gone. From time to time, we have made new diploids by the same procedure, as the need for them arose. While W-478 seems now to give a rather lower yield of diploid progeny than it did earlier, it is still a feasible source.

The most important consideration in maintaining diploid cultures is that they have a strong propensity to segregate and that, on complete medium, the segregants will rapidly outgrow the parent diploid. Therefore, diploids are isolated which are balanced for nutritional requirements and also, say, for lactose utilization, e.g., M- T+L+B_1+ Lac+// M+ T-L-B_1- Lac-, and these are maintained as far as possible on minimal-lactose medium. This discourages the typically auxotrophic or lactose-negative segregants sufficiently to allow one to obtain suspensions with as much as 95% diploid cells and to maintain the cultures in the diploid condition. It will be necessary to repurify the cultures from time to time, preferably by plating for colonies on EMS lactose agar: unfortunately, prototrophic lactose-positive segregants occur by crossing-over from time to time, and thereby displace the diploids even on minimal.

To isolate diploids in the first instance, I would cross W-478 x W-1177 on EMS lactose and proceed as outlined in my '51 CSH paper at p. 421. Lac+ prototrophs (especially those that are <u>slightly</u> delayed) are picked and streaked out in parallel on EMS and EMB lactose agar. When streaks are found that are suspected of variegation (see fig. 2), single colonies on the corresponding EMS plate are saved and tested in the same manner. I need hardly emphasize that a variety of details on diploid behavior are summarized in the papers mentioned. Since the heterozygous compound V_1^p/V_1^r is sensitive to phage T1, while each parent is (more or less) resistant, another expedient would be to make suspensions of lac+ prototrophs from the cross plates and cross-streak them against T1, rechecking any that give a sensitive reaction <u>on EMS</u> (on EMB, resistant segregants and background growth will obscure the result. The ultimate criterion of diploidy is not so much the variegated appearance as the proof of segregation from a single cell, as shown either by repeated single colony isolations or, perhaps more simply by single cell isolation. You should have no trouble, though there will be a bit of work at the beginning.

If you are in any doubts about the characterization of a particular isolate, I will be happy to check it for you. Alternatively, we will doubtless have some occasion during the next several months to prepare some Hfr-cross diploids again ourselves, and I will be happy to send you some of the material then. But I can't promise how long this will be.

I am sorry not to have any appropriate material on hand at the instant, but the loss is not so great. It is hardly less trouble to make and keep one's eyes on a diploid than to isolate it in the first instance, and the latter experience may be worthwhile as such.

You asked whether there would be any conflict of program— probably not. I was interested in the radiobiological behavior of the diploids, as outlined in the '51 CSH paper, but the problem turned out to be unexpectedly complex. In particular, there was no special difference in the kinetics of UV sterilization of diploids vs. haploids, possibly owing to the probably multiplicity of targets (nuclei?) shared by each. Also, there were other, very complex genetic effects for the full interpretation of which, I felt we needed much better and more intimate knowledge of E. coli cytogenetics. Some light is beginning to glimmer through in this. You may be interested that it has recently been possible to discover the conjugal pairs in exceptionally fertile crosses of K-12 with other strains. As in Paramecium, the mates disjoin after an hour or two. Both generally survive to give viable clones, but recombinants have been found only in the progeny of the F- exconjugant. ~~It is the reverse.~~ Thus unlike Paramecium, fertilization is unilateral. At the moment, I am trying to work out pedigrees to study segregation in the exconjugant clones.

I would be obliged if you would keep me current on your work in bacterial genetics through reprints.

Yours sincerely,

Joshua Lederberg
Professor of Genetics

P.S., I probably will get back to the UV/diploid work eventually. Larry Morse came up here as a student to work on this a few years ago, but he got involved in a more interesting problem and is writing his thesis now on a transduction in K-12.