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ENCLOSURE: CULTURES OF ESCHERICHIA COLI
(a harmless bacterium) for scientific investigation

To: Dr. L. L. Cavalli
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Via Darwin 20
Milano, Italia

March 28, 1953

Dear Luca:

I enclose a number of cultures as mentioned in previous correspondence. I apologize for taking so long, but I made a number of attempts to reisolate H-313, which have failed. It would be easier to repeat the cross, and I will do this at an early opportunity. I assume, however, that you are more interested in the variety of segregant types than in this diploid itself. I am therefore sending you a culture which represents the unpurified mixture of segregants from the H-313 stock culture. I was note able to recover the original diploid itself, which is recognized as prototrophic, Mal+ on EMS maltose; but Mal v on EMB maltose agar. Attempts to reisolate H313 from this mess now probably will lead only to new crossings. In addition, as noted, there is a group of isolations previously made from this culture. Their designation as Hfr is tentative, but my records show them to be very active F+ phenotypically, but non-infective. You should have no difficulty in securing prototrophic Hfr recombinants also from the mess.

To avoid confusion, I repeat the correct pedigree:

W-1895 (your Hfr) X W-1177 gave H-310, a Lac v S^r noticed in a cross on EMB Lactose + sm. H-310 appears to be segregating only for Lac, and is pure for the other markers of W-1177 (whether homo- or hemi-zygous I do not yet know). All its segregants so far tested have been F-, but H-310 itself behaves as an Hfr. It is relatively stable, and can be purified easily by picking hazy-mottled colonies on EMB lactose. These rarely throw off typical Lac+ and Lac-.

H-310 x W-1895 on EMS Lac. 1/8 or Mal. 1/12 was Mal v = H313. H313 is pure Lac+ (not surprising as it comes from Lac +/- x Lac+), but segregating for M, TL, S, Mal, Mtl, and Xyl and V_1. [Note, inter alia, that it has a full genotypic contribution from each parent]. Only five segregants have been tested, each behaving like Hfr as mentioned above:

	V_1	Mal.	Xyl.	Mtl	S	TLB_1	M
W-2057	r	-			s	-	+
W-2058	r	-			r	-	+
W-2059	s. = W1895, not included						
W-2060	r	+			s	-	+
W-2061	r	+			s	-	-

These are not a random sample of segregants as I was looking for special types.

The remaining cultures are the partially analysed issue of passages through 2 tubes each of motility agar (formula in Zinder and Lederberg '52).

W-	From	
2206	58-161	This shows very high rate of recombination (not quite as high as W1895, although one does find Lac+S^r recomb. x W-1177) but is still infective F+. It may possibly have a special F+ agent; this needs to be checked, as does its purity (possibility of its being a mixture of Hfr and F+, but doubtful).
2207, 2208	"	seem to be typical F-.
2209	W-1678	(Proline-, glycine(or serine)-). This one is curious. It is very infertile, but does give some prototrophs X W-1177F+. After being grown with W-1177F+, it becomes moderately fertile with W-1177, less with W-1817. This could be explained if

independently of becoming F-, this stock also picked up some modifier that reduced its overall productivity. The original W-1678 is extremely fertile (not quite Hfr) x W1177, much less so x W1177F+.

Jim Watson sent me his Watson-Hayes opus. I have not wanted to polemicize with him, but cannot accept the underlying theory. F+ x F- crosses have given diploids which are deficient for a Mal-S segment from the F- parent, as well as a few which are Mal-S crossovers. This seems to necessitate a post-zygotic elimination, and certainly one which is not absolutely dependent oh F-polarity.
As to the number of linkage groups, an M.A. student (Phyllis Fried-- now working for Ryan) completed an extensive extension of Rothfels' work last June in which S, M, P (proline-), and TL were variously used as elected and unselected markers. We could not confirm the M-Lac linkage, which is based entirely on the segregation ratio of Lac into prototrophs, so the markers seem to fall into the following groups:

$$\underset{[\text{Mal-Xyl-Mtl}]}{\text{S--M---B}_1} \qquad \text{and} \qquad \text{P-Lac-----V}_6\text{V1----TL}$$

The detailed ordering is not entirely worked out. To explain these data, and the unselected Hfr x W-1177, one has to postulate a directed polarized segregation, controlled by F, and directed at two points: one near S, the other near TL.

To counter the possible argument that the diploids mentioned on the 6th line of this page somehow resulted from a cross of inverted polarity, following Ftransduction, I am trying to obtain F- Het stocks (by the semisolid passage technique) so that we can secure diploids from the non-infective Hfr x F-Het cross. But I have almost given up trying to explain this reasoning to Hayes, etc. I would almost rather leave him to make some definitive enough assertion that it will be possible to test it.

Concerning the cultures included, I have of course no objection to your discussing or demonstrating them with anyone, but feel that the same considerations apply to their distribution as to Hfr.

I have word thirdhand that you have recovered a B-M- 58-161. Is this so? I propose we rename our current B+M- culture now W-6 and regard it as a (genetically unanalyzed) reversion from the proper 58-161 type.

I have not forgotten our ms. Thank you for your reprint and microfilms which arrived about the 24th. By the way, I think Umbreit*is all wrong (and not entirely forthright) about the metabolism of S^r mutants: at least as concerns their non-aerobic growth responses. They have had such a culture, but this behavior had nothing to do with S^r: subsequent isolates seem perfectly normal, and they claim to have lost the original S^r. I was once interested (at Stanier's suggestion) to test indirectly selected S^r to determine whether streptomycin had any direct effect on the aerobic metabolism (a la Ephrussi), but could not confirm the premiss. Oginsky sent her strain, with same negative results. But I would not want to bother with this in print.

*preceding pages of that symp.

Sincerely,

Joshua

Joshua Lederberg

P.S.: I have a Pyrex filter on order. When it comes shall I send it direct, which would be much safer, or have my own glassblower make the U-tube, which will be more hazardous to ship?