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NATIONAL RESEARCH COUNCIL

CANADA

ATOMIC ENERGY PROJECT

Biology Branch

CHALK RIVER. ONT.

June 28th, 1949.

Dr. Joshua Lederberg,
Department of Genetics,
The University of Wisconsin,
Madison 6, Wisconsin.

Dear Joshua,

I am at last getting down to the crossing experiments with the strains you sent. In both crosses however (58-161 x W-677, and Y-40 x Y-53) a proportion of the recombinant colonies contain a mixture of more than one type. In view of your recent work on heterozygote formation this was not unexpected in the case of the first of these (I have assumed that W-677 contains the factor Het) but I am surprised that it should occur in the Y-40 x Y-53 cross.

All crosses are made on minimal agar plus thiamin and the parent strains are spread uniformly over the surface of the agar. Two cc of melted minimal agar is then poured over some of the plates, and other are incubated without this additional layer. Colonies are picked, after 3-5 days, into two cc minimal medium plus thiamin, incubated overnight, and streaked. To determine whether there is appreciable parent type contamination streaking has been done in parallel on EMB-Lactose and EMS-Lactose. The results indicate that this is negligible. However, about ten percent of the cultures contain an approximately 50:50 mixture of Lac^+ and Lac^- (% Lac^+ = 35-80).

I would be inclined to suspect bad technique on my part were it not that I get these mixed cultures even when the greatest care is exercised. (well spaced colonies, 2 cc layer of agar, picking small inocula from the portions of those colonies which have grown onto the surface).

If you have run into the same thing and have the answer, I should like to let it drop and get on with the crosses between streptomycin resistant forms. However, if no one has done anything with it, it ought to be followed up. I have been wondering if it represents the recovery of two single crossover strands such as one would expect if two chiasmata were formed in the four strand stage. This interpretation would, of course, only explain mixtures of two types, and I am at the moment testing to see if we get mixtures of three.

Sincerely,

Howard

Howard B. Newcombe

HBN:ar

29 June

SR crosses

(surface plating on EMB agar + thiamin)

Recombinant Step	Lac	V.	cross A Y-40 × Y 53/SR	Cross B Y-40/SR × Y-53	Cross C Y-40/SR × Y-53/SR
R	+	R	24	0	38
R	−	R	25	1	30
R	−	S	14	0	13
S	+	R	7	25	0
S	−	R	8	30	0
S	−	S	5	10	0
R	+	S	3	0	1
S	+	S	0	1	0
Total recombinants			86	67	82
No of colonies			79	63	79
No of duplex colonies obs.			7	4	3

Types of duplex colony observed

Cross A	Cross B	Cross C
(S/R) − R = 3	S(±) R = 3	R(±) R = 3
(S/R) − S = 2	S(± R) = 1	
(S/R) + R = 1		
R(±) R = 1		

Cross Y-40 × Y-53, 60 colonies picked into minimal medium + thiamin, 15 June

Colonies nos.:

Min agar + thiamin:
- 1 − 10 ; spread plates, 0.001 cc inoculum (5-10 prototroph colonies/plate)
- 11 − 20 , spread + 2 cc agar, .001 cc inoculum
- 21 − 30 , spread plates, .01 cc inoculum
- 31 − 40 , spread + 2 cc agar, .01 cc inoculum

EMS-Lac + thiamin:
- 41 − 50 , spread plates, .01 cc inoculum
- 51 − 60 , spread + 2 cc agar, .01 cc inoculum

Typed prototroph colonies observed:

		Ratio +/− on EMB (plating 0.1 cc of 10^{-5} dil)	on EMS
Colony (8)	+ R, − R	146 : 125	130 : 146
(9)	+ R, − R	179 : 247	small colonies
(11)	+ R, − R	112 : 138	" "
(19)	+ R, − R	214 : 84	185 : 59
(26)	− S, − R	0 : 45	
(30)	+ S, − R	75 : 113	43 : 102
* (35)	+ S, − R (+R?)	291 : 8 1	239 : 60
(52)	+ R, − S, (−R?)	28 : 53	3 : 4

* Note: Further testing of +/− on EMB, 36:10; EMB+T1, 4:6; EMS, 39:1; EMS+T1, 7:2 gave following ratios confirming, +S, −R, +R mixture