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Cistron analysis of Lac loci using Lac$_2$-F$_{13}$.

29/VII 1959 REF: W3112F$_{13}$

~~Plate 1~~ ~~Mal$^-$ MH$^-$ Gal~~

Method: Spot method : on MLac. × various Lac$^-$ mutants. (λ^+ Lac$_x^-$)
cultural age : overnight culture in Pen. 5 ml. at 37°C.

W3443 F$^+$ Lac$_2$ λ^+ —, Cu rc.

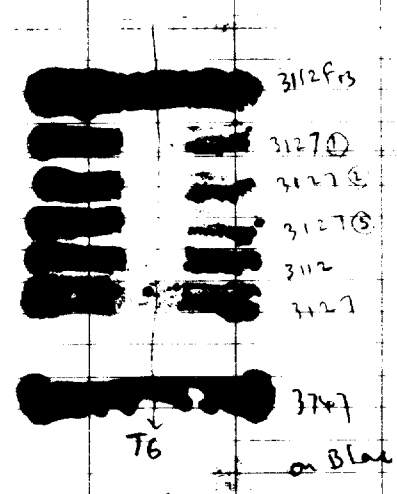
Result:

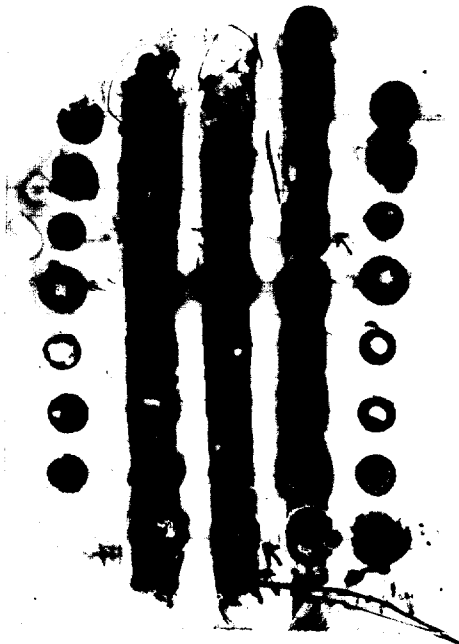
Lac$^-$		× 3112 F$_{13}$ (Lac$_2^-$) nr	× 3112 (Lac$_2^-$)	× 3747 (Lac$^+$ m-F$_{13}$)	blank	Lac Hfr.
DNPG	3112 2	— DNPG-	—	++++	—	✓
unstable	3127 4	++++	+	++++	++ unstable	Lac$_2$ 3164 nr.
	2247 7	—	—	— ?	—	non-transducible Lac.
+ m unstable	3230 11 1a	++	+	++++	++ many unstable	
+ m	3089 12 1b	++ ?	—	++ ?	—	Reaction is very slow.
+ m	4112 52	++++	—	++++	+,	
- f	4121 61	++++	—	++++	—	✓ Lac$_{61}$ 4021
- m	4147 87	++++	—	++++	—	
- m	4148 88	++++	—	++++	—	
- m	4149 89	++++	—	++++	—	
- m	4150 90	++++	—	++++	—	
- m	4151 91	++++	—	++++	—	
- m	4153 93	++++	—	++++	—	
	2243 Lac$_3$	++++	++++ syntrophy	++++	++ unstable	
	2245 5	++++	++++	++++	++++ "	
	2247 Lac$_7$	—	—	—	+$_4$	
	also Gal-			non-transducible Lac		

Test segregants.

This black spot gives many Lac$^-$ on BLac after streaking.

λ_2^S non-transducible Gal





	Lac
3112	2
3127	4
2247	7
3230	1a
3089	1b
4412	52
4121	61
2243	3

3112 F_{13} 3112 3747

F^- Lac^+ F_{13}

M^-

Synthesis between two different Lac^-.

4147	
4148	
4149	
4150	
4153	
2295	
2247	7

3112 F_{13} 3112 3747

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127b

Stability of Lac+. of the treated and untreated heterozygous diploid with AO.

28/11 ; 1959

REF:

AO treated Lac+	1 Lac stability growing	2 T6		3	T6	4 Lac Untreated	T6	5	6 Lac	T6	7	8	9	10
~~1~~	~~U~~ 2594 Lac+/Lac-			51	U m	1	U	m	51	U	m			
2	U	m	control	52	U m	2	S	r	52	U	m			
3	U	m	2594 Lac+/Lac-	~~53~~	~~U m~~	3	U	m	53	U	m			
4	U	m	F-	54	U m	4	U	m	54	U	m			
5	U	m		55	S S r	5	U	m	55	U	m			
6	F- S S	s		56	U m	6	U	m	56	U	m			
7	U	m		57	S S m	7	U	m	57	U	m			
8	U	m		58	S S S	8	U	m	58	U	m			
9	U	m		59	S S r	9	U	m	59	U	m			
10	F- S S	s	F-	60	S S	10	U	m	60	U	m			
11	U	m		61	U m	11	U	m	61	U	m			
12	U	m		62	U m	12	U	m	62	U	m			
13	U	m		63	U m	13	U	m	63	U	m			
14	U	m		64	U m	14	U	m	64	U	m			
15	U	m		65	U m	15	U	m	65	U	m			
16	U	m		66	U m	16	U	r	66	S S	r			
17	F- ~~S~~ U	m		67	U m	~~17~~	~~S~~ control 3747	r	67	U	m			
18	~~S~~ U	m ~~control~~		68	U m	18	U	m	68	U	m			
~~19~~	~~U~~	~~2594 Lac+/Lac-~~ m	F-	69	S S S	19	U	m	~~69~~					
20	U	m		70	U m	20	U	m	~~70~~					
			F-	71	S S r									
21	U	m				21	U	m						
22	U	m				22	S S	r						
23	U	m				23	U	m						
24	F- S	s				24	U	m						
25	F- S S	s				25	U	m						
26	S S	r				26	U	m						
27	U	m				27	U	m						
28	U	m				28	U	m						
29	F- S S	s				29	U	m						
30	S S	r				30	U	m						
31	U	m				31	U	m						
32	U	m				32	S	r						
33	F13 S S	r				33	U	m						
34	U	r				34	U	m						
35	F13 S S	r				35	U	m						
36	U	m				36	U	m						
37	U	m				37	U	m						
38	U	m				38	U	m						
39	U	m				39	U	m						
40	U	m				40	U	m						
41	U	m				41	U	m						
42	U	m				42	U	m						
43	U	m				43	U	m						
44	U	m				44	U	m						
45	F- S S	s				45	U	m						
46	F- S S	s				46	U	m						
47	F- S S	s				47	U	m						
48	U	m				48	U	m						
49	S S	s				49	U	m						
50	S S	s				50	U	m						

Lac+ (stable or unstable)
S: 21
U: 48

Lac+ (stable, or unstable)
S: 54
U: 63

T6-resistance

%		
11.6	V_6^R:	8/69
18.8	V_6^S:	13/69
84.2	V_6^{mix}:	58/69

T6 resistance (Lac+ V_6^R)

	%
V_6^R: 5/67	7.46
V_6^S: 0/67	0
V_6^{mix}: 62/67	92.7

If Lac- (colonies) found it is noted as Unstable. { U: Unstable; S: stable.

Method ①: pick Lac+ from streaked plate, inoculated into Parasy (1 ml), and incubated then for overnight.

② Streak on Blac, and see is there Lac+ only or Lac+ and Lac- colonies on it.

Lac+

1. AO-treatment: AO 30 γ/ml.
   - NSB - pH. 7.6. Inoculate one small loop of Ca 10^7 cells/ml / 5 ml NSB AO.
   - The treated culture was streaked on Blac after 24 hr incubation at 37°C.

m : mixture of V_6^R and V_6^S, segregated from V_6 R/S.

S : V_6^S

r : V_6^R

U : Unstable for Lac+, accounted for Lac diploidy.

S : Stable for Lac+

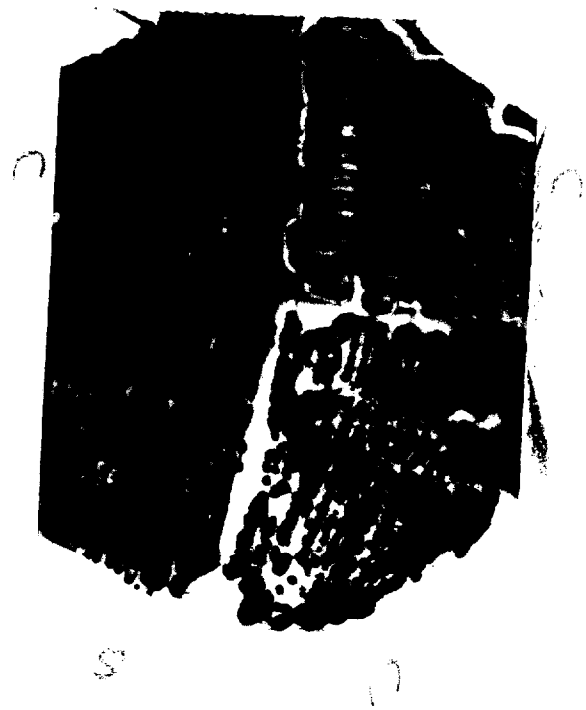
Conclusion

1. Almost of the F- treated with AO Lac-stable, V_6^R or V_6^S, and Lac+-stable.
   11/12: F- Lac+st V_6^R or V_6^S.
   #17 1/12: F- Lac mix V_6^m.

2. Lac st. is T_6^R or T_6^S, $T_6^{r/s}$.

This means, AO remove full segment of FLacU at a time.

U : unstable
S : stable



Elimination of Lac-F13 segment with AO-treatment

23/VII ; 1959 REF: Cf. P118

Strain 2594 Lac⁻/ex Lac⁺

Experimental conditions.

Inoculum size : ca. 10^2 cells /ml. AO : 30γ /ml. Med: NSB for AO-T.

Time : overnight at 37°C. Strain treated w2594 F13 (M S^R V1^R Lac1^+/- V6^R/s)

Method ① Suspend a single colony into 1.0 ml of H_2O, and ~~dilute to~~ use the 10^{-6} ml. Add 0.1 ml to 5 ml NSB with AO 30γ and without AO.

② Seed ~~a~~ 10^{-6} ml on to B Lac. 5×10^{-7} ml of untreated control.

Result I. Elimination of Lac⁺ segment from Lac⁺/Lac⁻ heterozygotes.

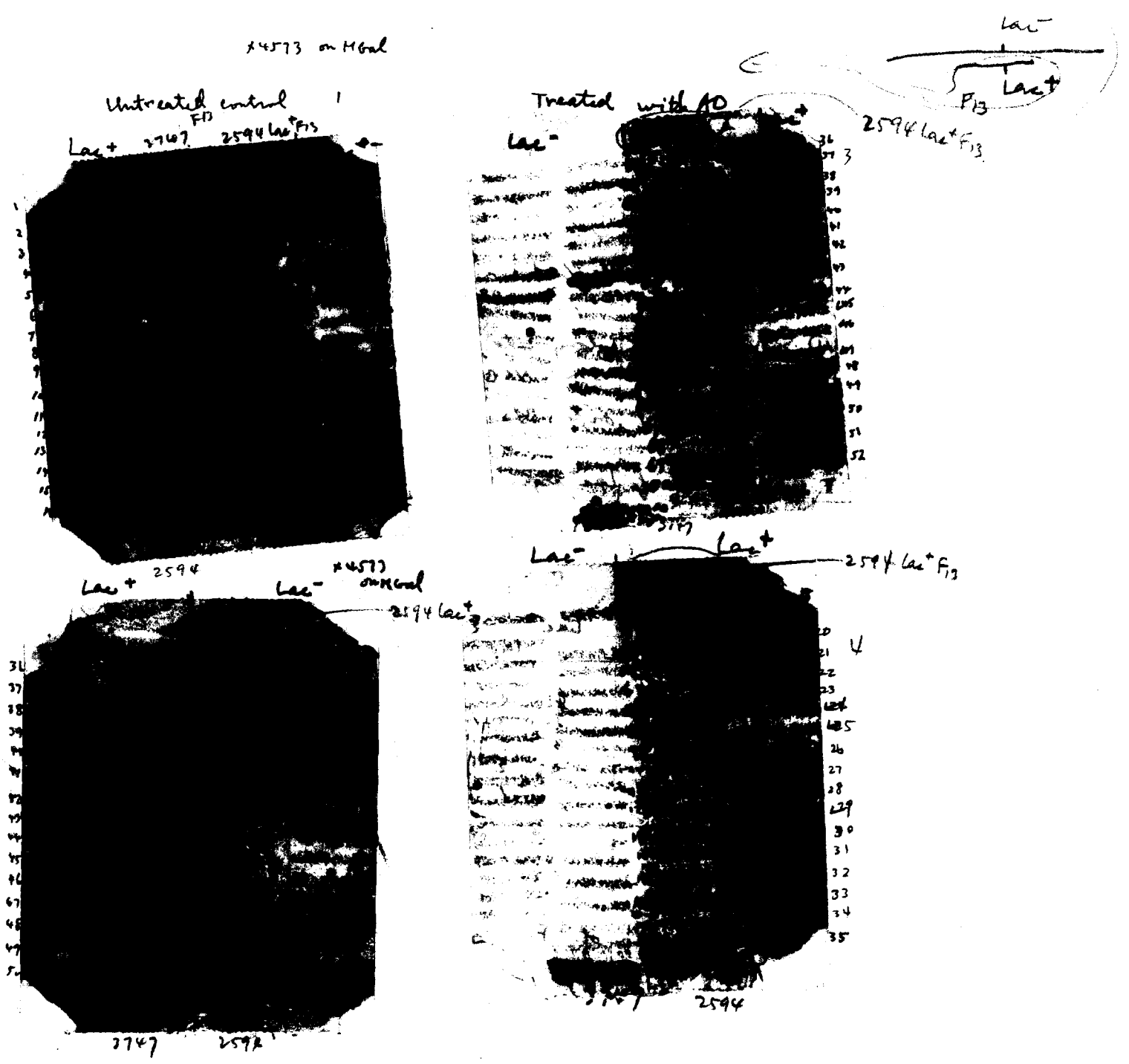
Plate #	AO-treated (30γ/ml) I Lac⁺⁻	I Lac⁺	I Lac⁺ Lac⁻	I Σ (%)	II Lac⁺⁻	II Lac⁺	II Lac⁺ Lac⁻	II Σ (%)	Untreated control C Lac⁺⁻	C Lac⁺	C Lac⁺ Lac⁻	C Σ (%)
1	310	131	4		439	42	2		103	311	2	
2	275	100	2		383	34	3		86	246	2	
3	284	120	3		398	35	2		109	298	3	
4	274	95	2		453	43	2		119	297	3	
5	283	138	2		422	33	1		84	322	2	
Σ %												

Results II. Elimination of F13 from Lac⁺/Lac⁻ hetrozygotes.

	AO-treated (from #II) Lac⁺ F13 HI	Lac⁺ F13 Lo	Lac⁺ F⁻	Lac⁻ F13	Lac⁻ F⁻	Untreated control Lac⁺ F13 HI	Lac⁺ F⁻	Lac⁻ F13 HI	Lac⁻ F13 Lo	Lac⁻ F⁻
Σ %	59	0	13	0	64	67	0	22	50	0

(Tested by Gal-transfer. × 4573)

(see back page)



4421 (Lac61 F⁻)

3112 F_{13} [redacted]

3112 F⁻

on Mac

24 hrs.

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128a

Multiplication of Gal-F_8 segment.

W4520
W6F8 → W4573.

30/11 ~ 1959 REF: p. 123

overnight culture
0.2 ml / 10 ml pln.

Incubate it at 37°C on rotator. for 2 hrs.

Mix { 1 ml W6F8 + 10 ml W4573.

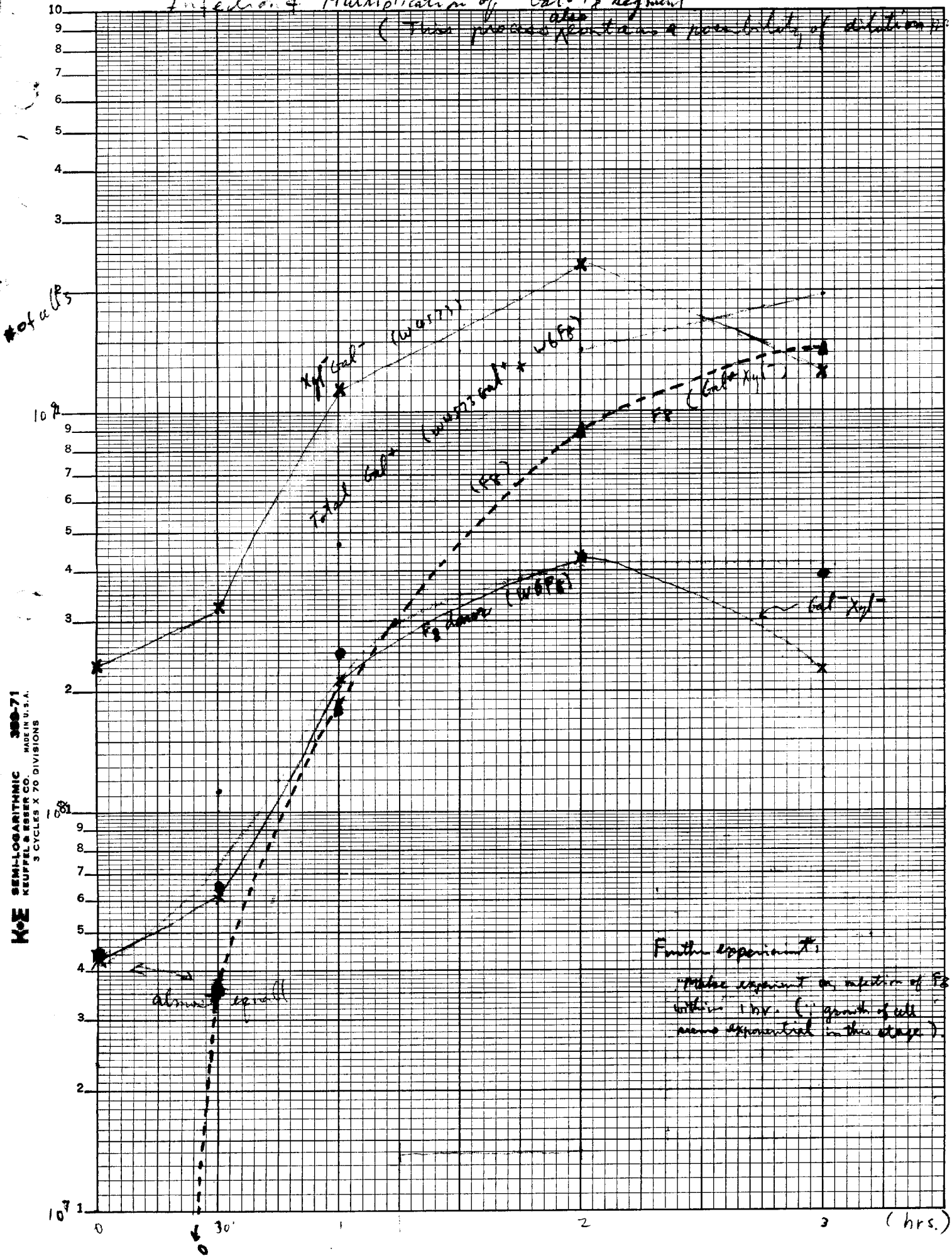
		AM				PM							
		12:00		12:30		1:00		2:00		3:00			
Inoculum size (ml.)		10^{-6} ml		10^{-6} ml		10^{-6} ml		2×10^{-7}		2×10^{-7}		10^{-7}	10^{-7}
Time	Gal	0		30'		1 hr		2 hr		3 hr		4 hr	5 hr
		+	− V	+	− V	+	− V	+	− V	+	− V	+	−
on B2Gal		0	0	0	0 0	0	0 0	0	0 0	0	0 0		
	1	8	54	27	92(1)	84	224(7)	101	167(10)	61	62(6)		
	2	6	36	[illegible]	88(1)	90	238(6)	108	157(9)	69	88(5)		
	3	10	49	19	101(6)	78	211(12)	113	224(10)	68	76(6)		
	4	4	48	29	72(2)	84	258(4)	99	196(11)	103	74(8)		
	5	16	46	12	59(1)	104	229(3)	105	181(4)	60	59(3)		
		+V	−	+V	−	+V	−	+V	−	+V	−		
	C	44	233	112	322	472	1160	570	925	389	359		
		(44) 277		(101,11) 334		(440,32) 1632		(526,44) 1420 1495 ×2.5	2310	(361,28) 1945 748	1290		
		0		0		0		0		0			
on B2Xgl. growth of W6F8	Xgl	+	−	+	−	+	−	+	−	+	−		
		8	54	18	[illegible]	53		35		8			
		6	36	13	[illegible]	51		39		14			
		10	49	11		43		35		16			
		4	48	18		43		31		14			
		16	46	5		60		29		26			
	C	44	233	65		250		169	422	78	390		
% of Lac+	%	15.9		19.5		15.3		12.1		10.4			
multiplication	Gal	+		+		+		+		+			
		0	[illegible]	9		29		66		53			
		0	[illegible]	1		39		69		55			
		0	[illegible]	8		35		78		52			
		0	[illegible]	11		41		68		89			
		0	[illegible]	7		44		76		34			
	C	0	233	36		188		357	892	283	1414		
% of Lac+	%	0		10.8		11.5		23.8		37.8			

0 : actually counted. number

57

Infection & Multiplication of Gal$^+$ F_8 segment

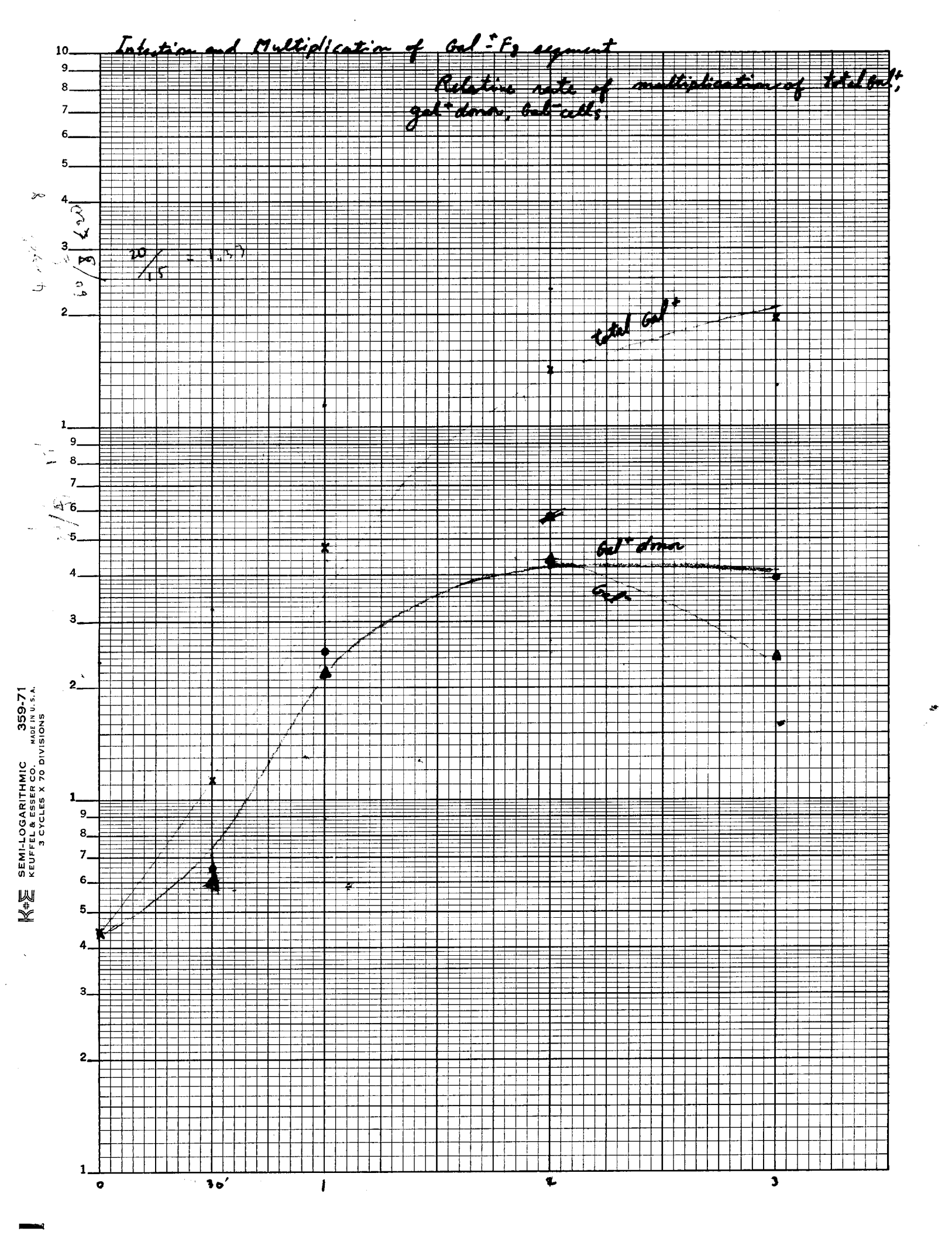
(This process also contains a possibility of dilution process)



Further experiment:

Make experiment on infection of F8 within 1 hr. (∵ growth of cell seems exponential in this stage).

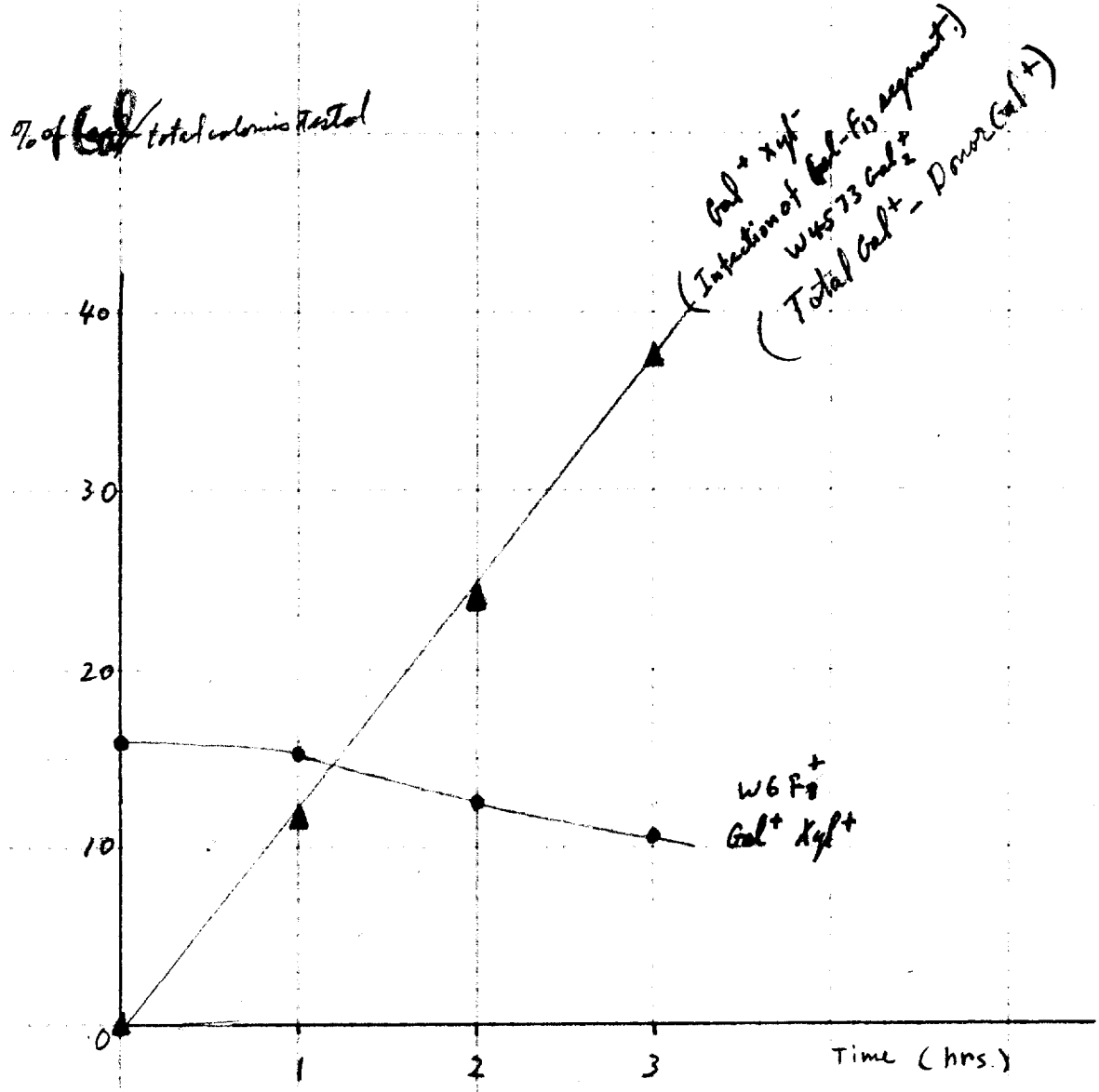
K+E SEMI-LOGARITHMIC 359-71 KEUFFEL & ESSER CO. MADE IN U.S.A. 3 CYCLES X 70 DIVISIONS

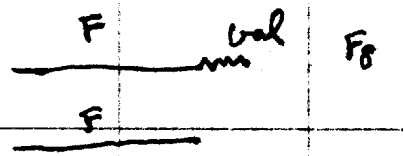

Intestine and Multiplication of Gal⁻ F₂ segment
Relative rate of multiplication of total Gal⁺, Gal⁺ donor, Gal⁻ cells;
total Gal⁺
Gal⁺ donor
Gal⁻
SEMI-LOGARITHMIC 359-71
KEUFFEL & ESSER CO. MADE IN U.S.A.
3 CYCLES X 70 DIVISIONS
0
30'
1
2
3

Rate of multiplication of Gal-F_8 segment. 128L

$W6\,F_8$ — × W4573

37°C, on rotator,
, in penassay broth,
, exponentially growing cultures




F
Wal
F_B
F

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Multiplication of F8 Gal loci.

129

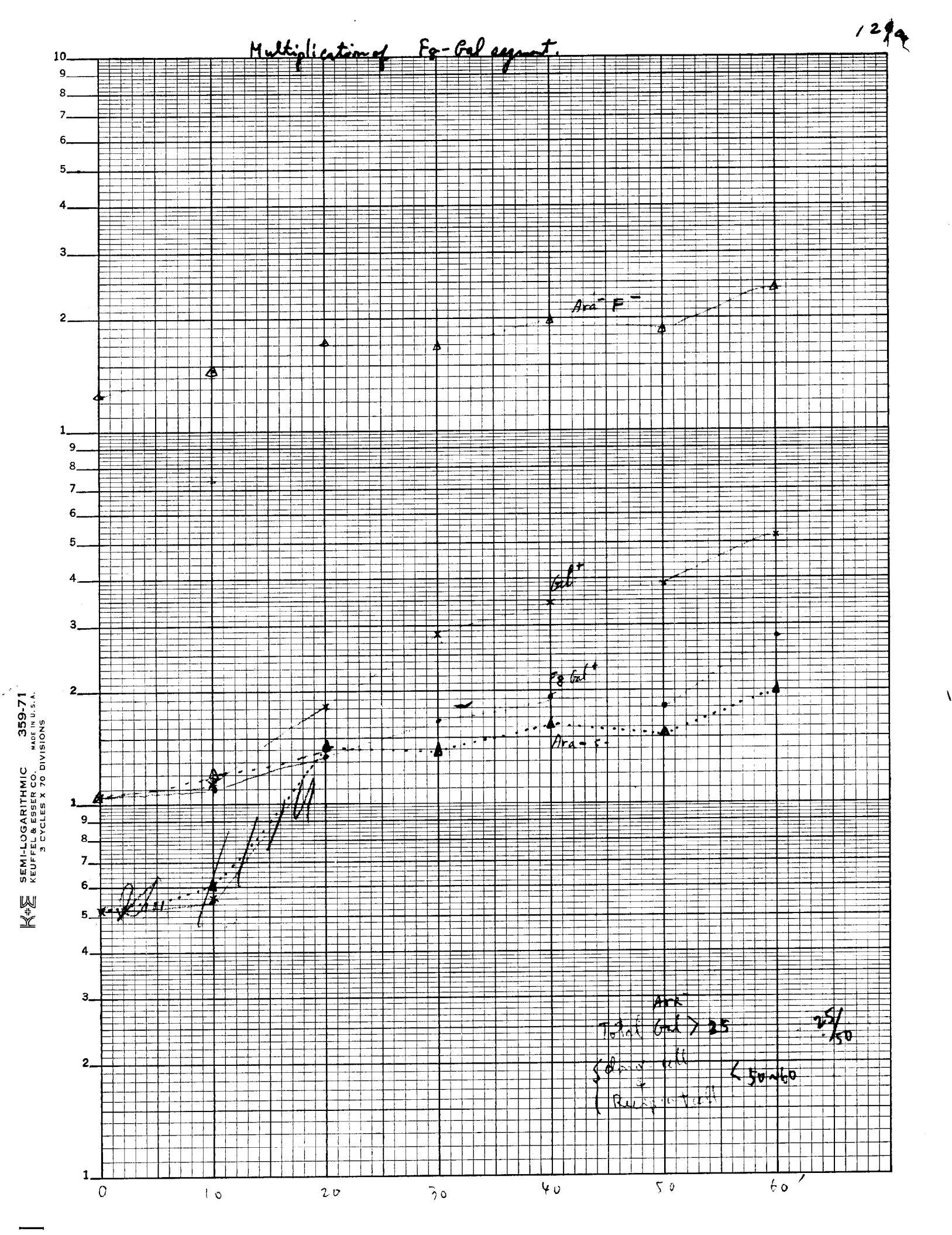
14/VIII 1959 REF:

Principle: W6 F8 → × 4573. Gal_2 Xyl_2 Mtl Ara_2 Mal, S^R.

Method: 0.2 ml overnight culture / 10 ml Pen. → 3 hr at 37°C on rotator 12:00 ~ 2:55. → 5 ml ♀ / 10 ml. + 0.5 ml ♂

time (min)		0		10		20		30		40		50		60	
		2:55		3:05		3:15		3:25		3:35		3:45		3:55	
Dilution		2×10^{-6} ml		1×10^{-6}		2×10^{-6}		..		"		2×10^{-6} ml		1×10^{-6}	
spread		$10^{-2} \times (10^{-2} \times 10^{-1} : 2)$ ml													
		+	−	+	−	+	−	+	−	+	−	+	−	+	−
B Gal	1	39	441	22	201	62	570	82	526	96	615	135	657	74	367
	2	31	408	15	285	65	578	114	598	132	704	147	572	88	389
	3	35	396	19	255	54	577	91	573	115	675	106	637	99	445
	Σ	105	1245	56	741	181	1725	287	1695	343	1994	388	1866	261	1201
														522	2402 (×2)
		+		+		+		+		+		+		+	
B Ara.	1	39		22		48		54		52		56		41	
	2	31		14		47		64		71		69		51	
	3	35		19		40		51		66		55		48	
	Σ	105		55		135		169		190		180		140	
				110										280	(×2)
Net infection.	Δ	0		1		46		118		153		208		242	

Multiplication of Eg-Col segment.



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Stock No, 1~27

130

Segregation of Lac⁻ from Heterogenote for Lac segment.

30/VII ; 1959

REF:

(Lac 61)

W4121 × W3112 F_{13}^{+} (Lac 61 / Lac 2) (Took from spot test (see P. 126))

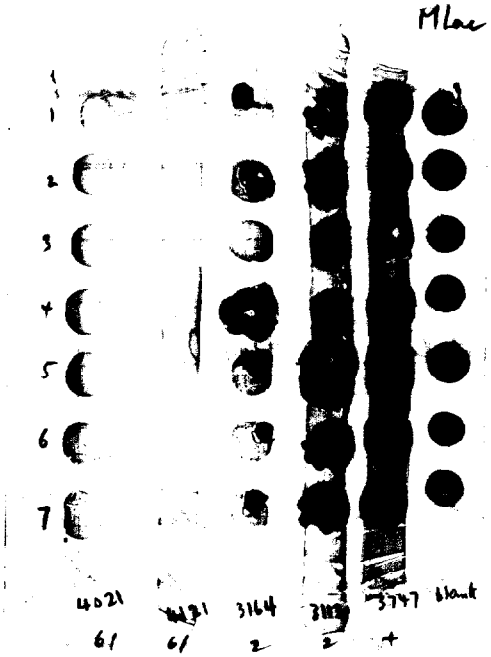
Method: Streak

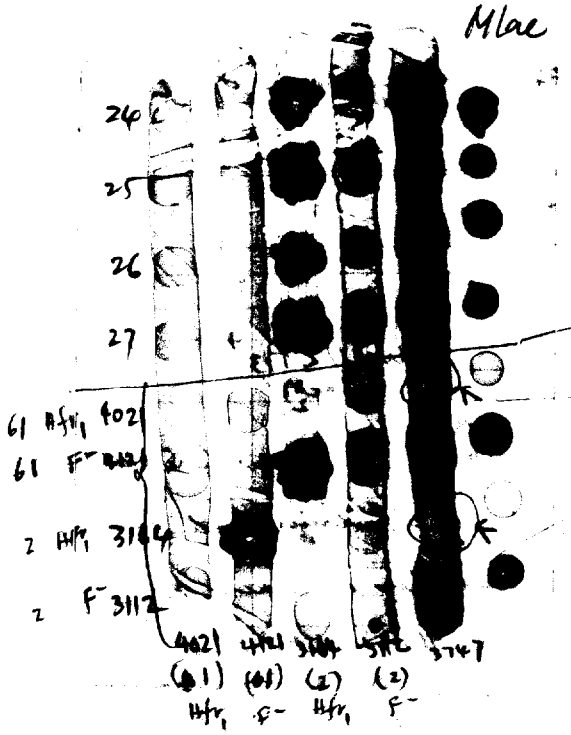
1. purify it on BLac. (5 plates)
2. Pick Lac⁺ colonies and suspend them into Lac⁻ Hfr. tubes.
3. Restreak them on BLac, and pick Lac⁻ segregant (pick one Lac⁻ from each Lac⁺ streaks).
4. streak the isolated Lac⁻ on BLac. (ca. 48 colonies are isolated. Some of them maybe Lac⁺. 29 were Lac⁻.)
5. Test Lac⁻ by cross streaking on MLac. (and sex-compatibility)

	V_6	Lac⁻ mutation end. ex	F	× 3112 (Lac 2) F⁻	× 3164 (Lac 2) = Hfr	× W4121 (Lac 61) F⁻	× W4021 (Lac 61) Hfr	× 3747 (Lac⁺)
1	S	61	♂	+11	+1	●	–	+
2	S	61	♂	+21	+3	–	–	+
3	S	61	♂	+14	–	–	–	+
4	S	61	♀	–	+32	–	–	+
5	S	61 61	♂	+	+2	–	–	+
6	S	61	♂	+21	+1	–	–	+
7	S	61	♂	+14	+1	–	–	+
8	S	61	♂	+16	–	–	–	+
9	S	61	♂	+18	+1	–	–	+
10	S	61	♂	+	+15	–	–	+
11	r/s	61	♂	+	+4	–	–	+
12	S	61	♂	+14	+3	–	–	+
13	S	61 61	♀	–	+26	–	–	+
14	S	61	♂	+18	–	–	–	+
15	S	61 61	♀	–	+24	–	–	+
16	S	61 61	♀	–	+18	–	–	+
17	S	61	♂	+2	–	–	–	+
18	S	61	♂	+13	+1	–	–	+
19	S	61	♂	+13	+3	–	–	+
20	S	61 61	♂	+	+2	–	–	+
21	S	61	♂	+14	–	–	–	+
22	S	61	♂	+10	+1	–	–	+
23	S	61	♀	–	+29	–	–	+
24	S	61 61	♂	–	+23	–	–	+
25	S	61 61	♂	–	+43	–	–	+
26	S	61 61	♂	–	+23	–	–	+
27	S	61 61	♂	–	+34	–	–	+
Hfr 1	4021	Lac 61		(–)	–	–	+	
	4121	Lac 61	61	–	+	–	+	
Hfr 1	3164	Lac 2	2	–	–	+	–	
	3112	Lac 2	2	–	+	–	–	

Conclusion: 27/27 : Lac 61 (100%)

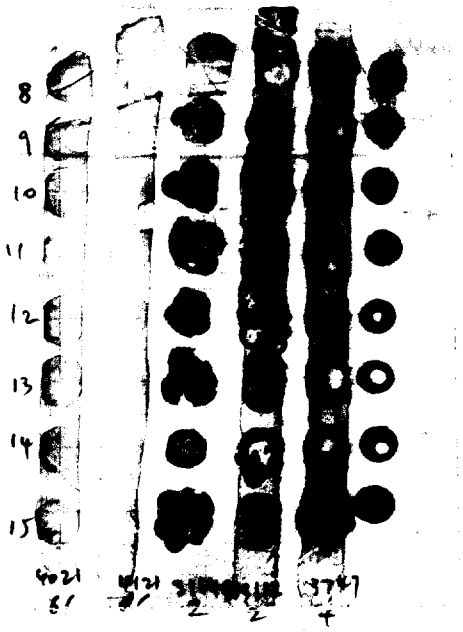
Non-transductants of Hfr 1 and Hfr 3





control.

is Hfr1 x F' steril!

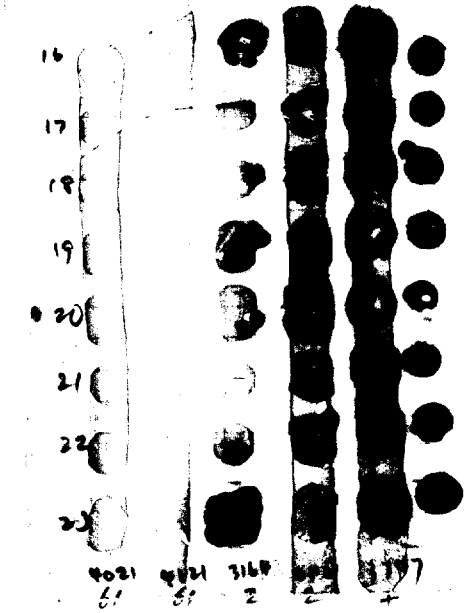


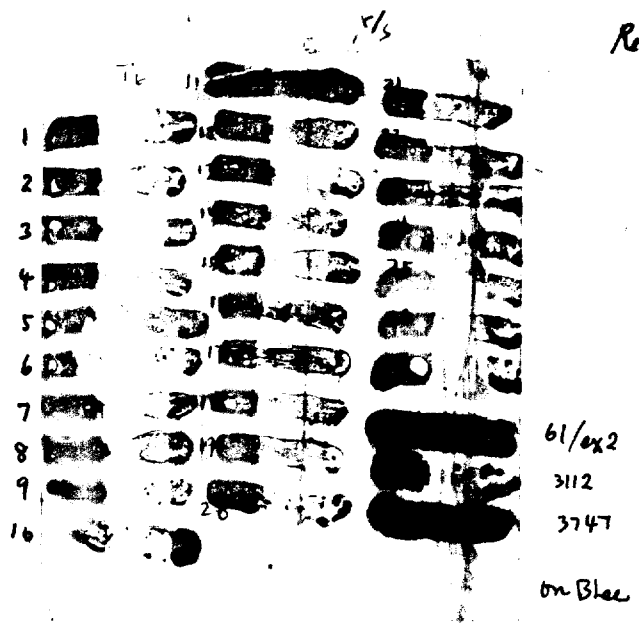
4021 Looks wrong! It may [illegible] (Lac61.

∵ W4021 x Lac2 F- is steril.

Tests for Vi-loci.

Retest #11.





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Stock No 28 ~ 40

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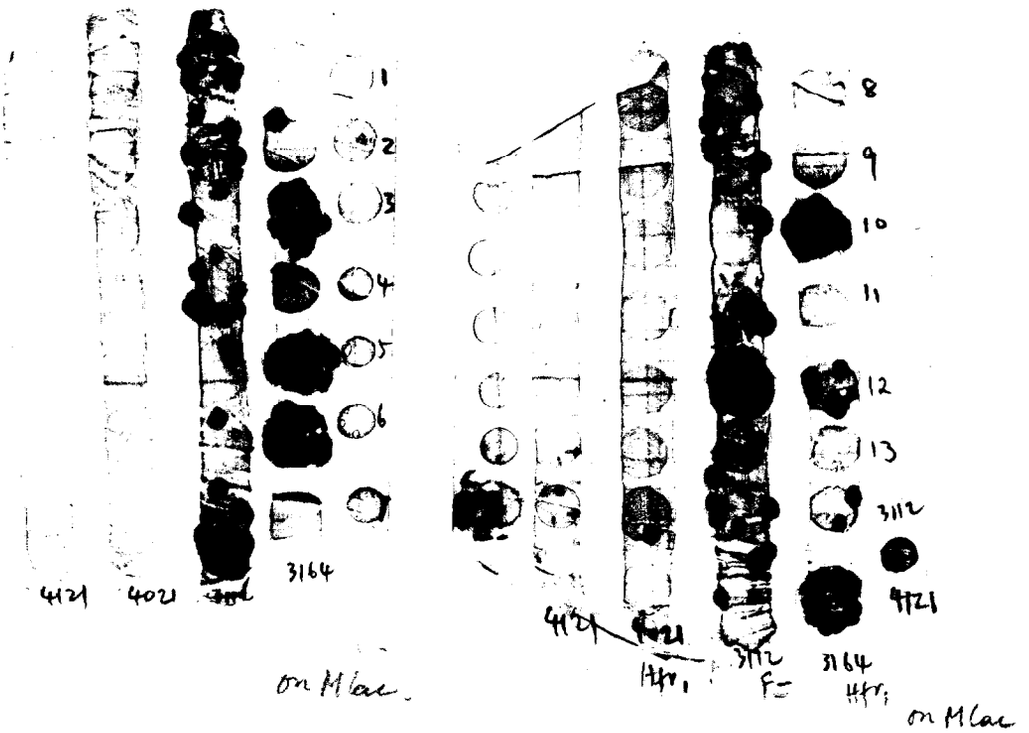
Segregants Analysis of Lac_2^{ex} / Lac_{61} F_{13}

7/xxx ; 1959

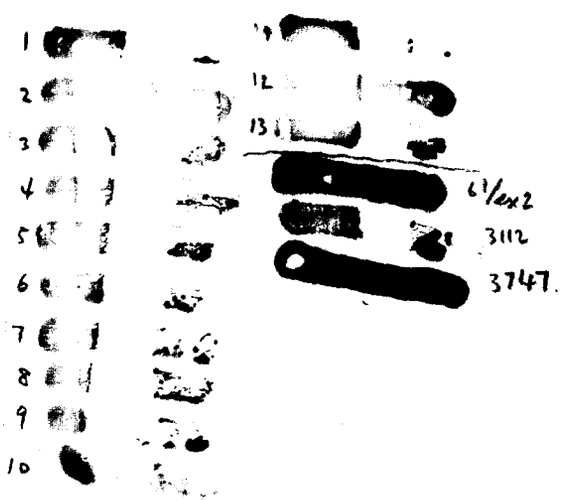
REF:

	1	2	3	4	5	6	7	8	9	10
			W4121 × W3112 F_{13}							
			Lac_{61}	Lac_2						
		Lac	F	× 3112	× 3164	× 4121	× 4021			
	V_L	and$_{61}$ ex$_2$		(Lac_2)	(Lac_2)	Lac_{61}	Lac_{61}			
				F⁻	Hfr$_1$	F⁻	Hfr$_1$			
1										
2										
3										
4										
5										
6										
7										
8										
9	1	S 61	♂	+20	−	−	−			
0	2	r 61	♂	+60	+1	−	−			
	3	S 61	♀	+2	+33	−	−			
1	4	S 61	♂	+9	+1	−	−			
2	5	S 61	♂	+2	+20	−	−			
3	6	S 61	♂	+1	+32	−	−			
4	7	S 61	♂	+23	−	−	−			
5	8	S 61	♂	+11	−	−	−			
6	9	S 61	♂ ♀	+5	−	−	−			
7	10	S 61	♀	+2	+28	−	−			
8	11	S 61	♂	+8	−	−	−			
9 →	12	r/s 61	♂	+61	+9	−	−			
0	13	S 61	♂	+4	−	−	−			
1	[redacted]			[redacted]	[redacted]			See back page.		
2	Lac_{61}/Lac_{ex_2}	r/s								
3	3112	S								
4	3747	R								
5										
6										
7		conclusion:		13/13 : Lac_{61} (100%)						
8										
9										
0										
1										
2										
3										
4										25
5										13
6										27
7										65
8										
9										
0										
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										



Test of V_6 loci

Retest # 12.
This strain looks F_{13}^{+} $Lac_{61}\frac{V_6^S}{Lac_{61}V_6^R}$.



on B lac

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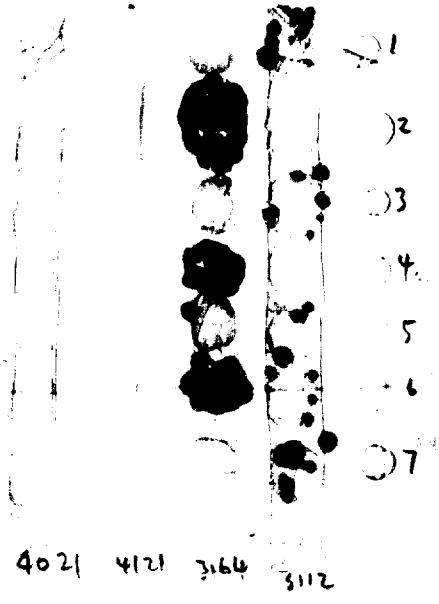
Segregant analysis of Lac61/ex 2. F13.

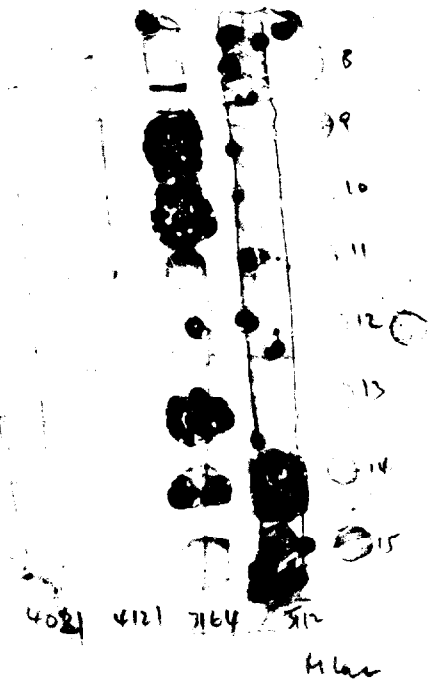
132

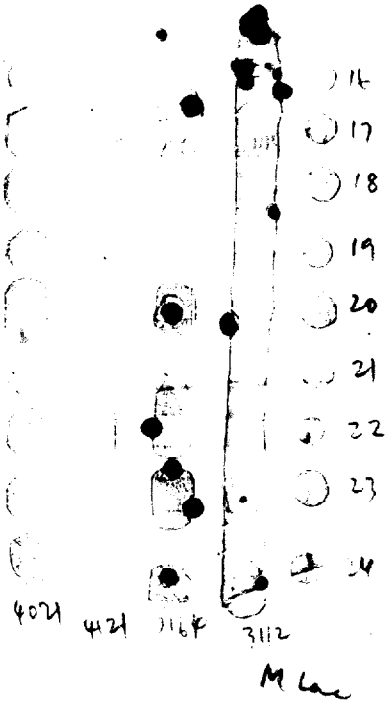
13/VIII :19 59

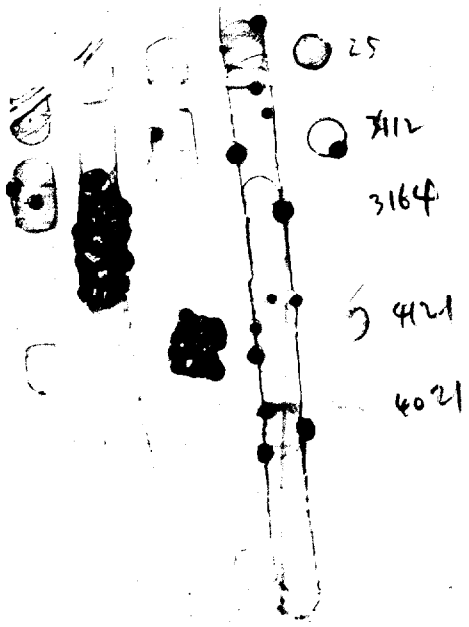
REF:

	1	2	3	4	5	6	7	8	9	10
						× 3112	× 3164	× 4121	× 4021	
		V6	Lac end61 ex2		F	Lac+ F−	Lac+ Hfr1	Lac61 F−	Lac61 Hfr1	
1	1	S			♂	+15	−	−	−	
2	2	S			♀	−	+60	−	−	
3	3	S			♂	+7	−	−	−	
4	4	S			♀	−	+30	−	−	
5	5	S			♀	+2	+2	−	−	
6	6	S			♀?	±2	+36	−	−	
7	7	S			♂	+15	−	−	−	
8	8	S			♂	+6	−	−	−	
9	9	S			♀	±1	+44	−	−	
10	10	S			♀	±	+68	−	−	
11	11	S			♂?	+4	−	−	−	
12	12	S			♂	+7	+1	−	−	
13	13	S			♀	±1	+25	−	−	
14	14	S			♂	±	+10	−	−	
15	15	S			♂	+33	−	−	−	
16	16	S			♂	+15	±1	−	−	
17	17	S			?	−	±1	−	−	
	18	S			?	−	−	−	−	
19	19	S			?	−	−	−	−	
20	20	S			?	±1	±1	−	−	
21	21	S			?	±1	−	−	−	
22	22	S			?	±	±1	−	−	
23	23	S			?	±1	+2	−	−	
24	24	S			?	±1	±1	−	−	
25	25	S			♂?	+2	−	−	−	
					♀					
	61/ex2	r/s								
	3112	S								
	3747	r.								
										??
	3112				F−	±2	±1	−	−	
	3164				F−	−	−	+2	+	
	4121				F−	−	+	−	−	
	4021					+3	−	−	−	

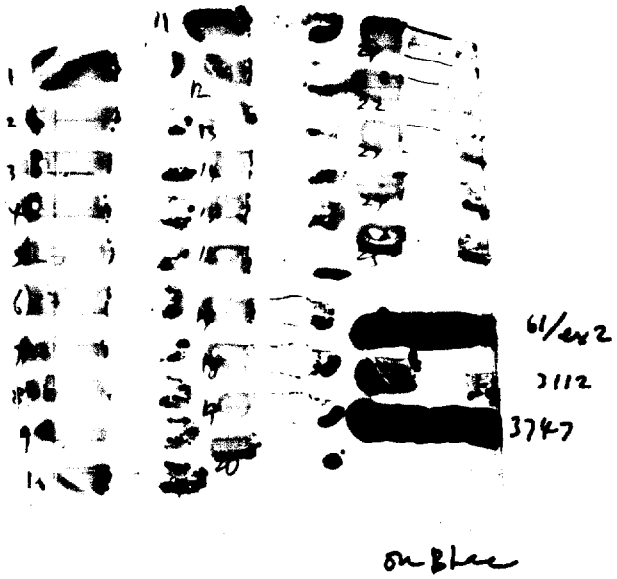








Test of V_6 loci



Retest # 14

This maybe V_6^3 Lac 61/ex 61 V_6^5.

Unsuccessful : use M Lac Sm in next time, (Lac^{61}/Lac_2^- does grow on MLac)

Comparison of the [redacted] frequencies of the transduction of exogenote and endogenote from heterozygous diploid (Lac^{-}_{61}/Lac^{-}_{2}).

4/4/4 ; 1959 REF: cf. 167, 166

Principle

F_{13}. Lac^-_{61} / Lac^-_{2} ex (3112 F_{13} + 4121)

→ × F^- Lac^-_{61} W4121

→ × F^- Lac^-_2 W3112

cultural age : 4 hrs. on rotator.

strain : all these strains used as donor are purified on Blac and Pick single colony and suspended in [illegible] (ca 10^7) 0.1ml is inoculated into [illegible] and kept there on rotator for 4hrs.

Method : 1) Mix Lac^{61-}/Lac^-_2 ex and Lac^-_{61} or Lac^-_2 use excess F^- , Ratio. F_{13} 1 (0.1ml) : F^- 10 (1.0ml)

2) Incubate ~~them~~ for 1/2 hrs. ~~[redacted]~~ at 37°C. 3:25 pm

3.) Seed them on MLac agar after optimal dilutions.

use : 10^{-2}ml, 10^{-4}ml, 10^{-5}ml per plate for each crosses.

4) Count all numbers of each experiment.

Results:

Serival count

	① F_{13} 61^- ½ ex → Lac_2^- W3112		① F_{13} 61^- ½ ex → 4121 Lac^-_{61}		② F_{13} 61^- ½ ex → [redacted] Lac^-_2 W3112		F_{13} 61^- ½ ex 2 → × 4121 61^-		3. F_{13}^+ W3112 → × 2^-	F_{13}^+ W4121 → × 61^-
Serival count	Lac^-	Lac^+	Lac^-	Lac^+	Lac^-	Lac^+	Lac^-	Lac^+	Lac^- Lac^+	Lac^- Lac^+
10^{-6}ml/plate	1057	89	1084	81	944	51	868	85		

Fertility on ml/plate

10^{-1} [redacted]

10^{-3} [redacted]

[redacted] too much, if cannot count them. (Use 1/100 of these experiments)

[redacted] 1084 81 [redacted] 944 51 [redacted] 868 85

[redacted] /ml

ml/plate

* Qualitative experiment.

3112 F_{13} × 4121

3747 1 2

×3112 (2) [redacted]

on MLac

3112 F_{13} × W421.

3747 1 2

4121 (61)

on MLac

Successful.

U.W. MICROBIAL GENETICS

Transduction of Lac⁻ segment from heterozygote, Lac61/ex Lac2.

134.

11/VIII 1959 REF: cf. 145, 146

Principle:

Lac 61 / ex Lac2 S_s → Lac61 F^- S^R W4121 S^R (W4629)

W4121 (Lac61/ex Lac2) → Lac2 F^- S^R W 3112

compare the fertility in both crosses.

Check, Sm-sensitivity of these strains. on B Lac Sm.

Result. → on BlacSm

Qualitative test.

Preliminally test by spotting method 4121 3112 3112F13 4121 (Lac61/ex Lac2)

Spot 4121 (61/ex 2) on W4121 S^R and W 3112 streaked on MLacSm.

Result: see back page.

	(Lac2⁻) 3112	4121 S^R (Lac61⁻)	blank
4121 61/ex 2	+	+++	−
3112 2/ex 2	+ reversion	+++	+ reversion.
3747 +/ex +	+++	+++	−

12/VIII **Quantitative test.**

· cultural age: 0.2 ml: overnight culture / 5 ml pensacy → shake on rotator at 37°C 10:30 ~ 3:30. AM

· Ratio of mix: ~~2 hrs culture~~.
1 ml 3112 + 4121 Lac61/ex 2 0.1 ml
1 ml 4121 S^R (W4629) + 4121 Lac61/ex 2 0.1 ml.

· Time of incubation: ~~1h 2h 3:30 ~ 5:30~~.

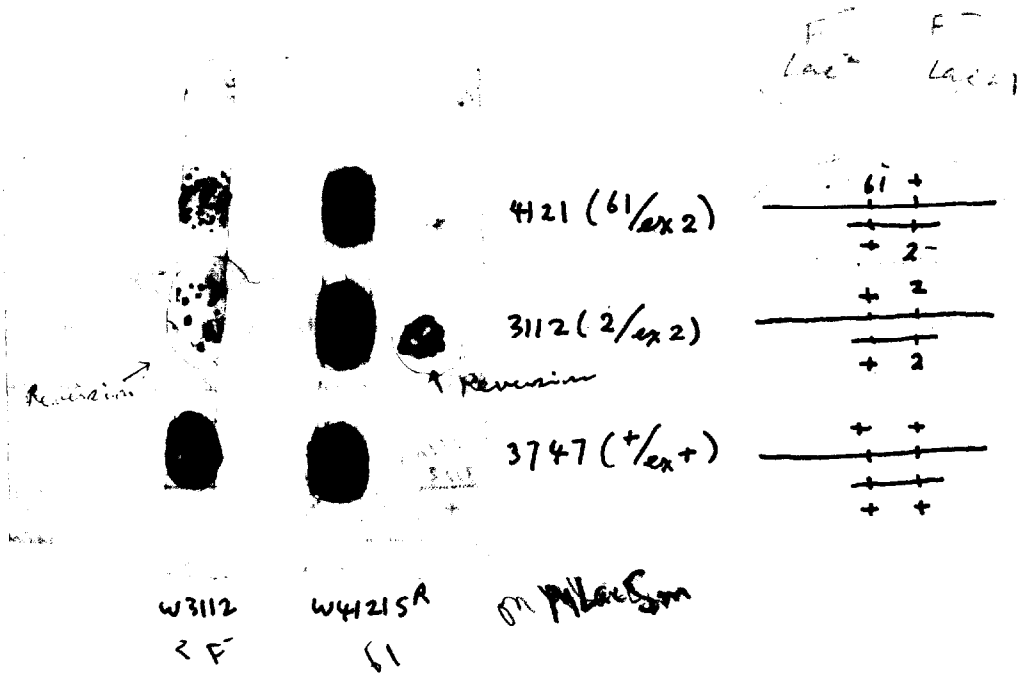
· survival count: inoculum size 10^{-6} ml. of original culture. / plate (4121 61/2 × 3112 Lac⁻ 751 : Lac⁺ .10 4121 61/2 × 4121 S^R Lac⁻ 0 : Lac⁺ 13)

· Inoculum size for counting recombinations. 10^{-3} ml./plate on MLac Sm.

/1x **Result**:

		# of Recombinants. plate 1	2	# of Colonies 10^{-3} ml/plate. %	+	−	+	−	% of Rec.
3747 +/+	× 3112	too much ca 10^3	10^3	%	0	24	2	38	
3747	× 4629	ca 10^3	10^3	%	22	193	38	214	
4121 61/2	× 3112	0	0	%	0	65 ;	1	68	ca $\geq 10^{-3}$
				%	0	112 ;	4	269	
4121 61/2	× 4629	2	5	%	0	66 ;	1	53	ca 10^{-3}
				%	2	136 ;	4	100	

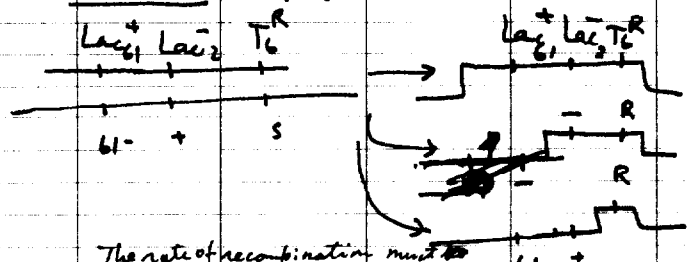
Conclusion: Exogenote is transferred with very high frequency, but not very high for endogenote. This implifies all F13 Lac HI close are diploid in that (Lac) segment. And also possible to speculate that linkage between F13 and endogenote, and exchange of end-exo fragment are relatively low.


F⁻ Lac²
F⁻ Lac²
4121 (61/ex 2)
61 +
+ 2⁻
3112 (2/ex 2)
+ 2
+ 2
3747 (+/ex +)
+ +
+ +
Reversion
Reversion
W3112
2 F⁻
W4121 5R
61

Linkage of $T6^R$ and Lac^-_2 in exogenote of ($Lac_2\ T6^R \times Lac_{61}\ T6^S$) $3112F_{13} \rightarrow 4121$

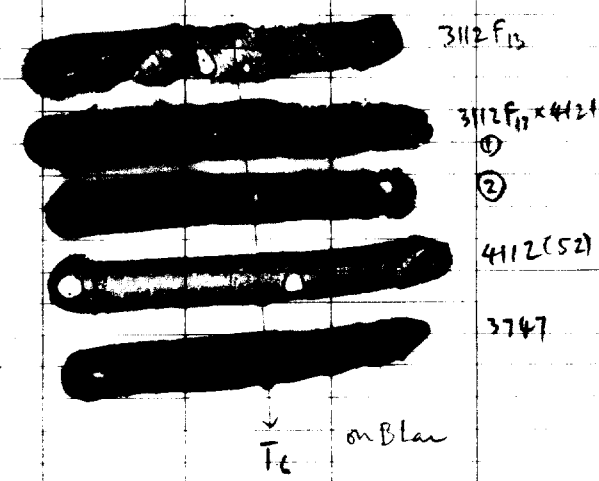
6/VII 1959 REF:

Principle. Segregant (non-recombined) is Lac^-



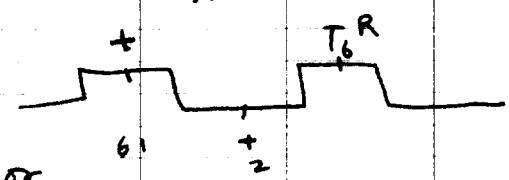
The rate of recombination must express ratio of length of $V_6 - Lac_2 - Lac_{61}$.

If Lac is closely linked with V_6, Lac^- can be found at the lyzed parts of $Lac_{61}\ V_6^S$ / $Lac_2\ V_6^R$ by T_6.



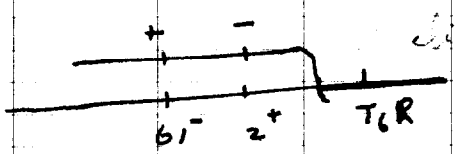
Result: actually the lyzed part is also Lac^+.

explanation: There are enough number of segregants which arized by recombination between two different Lac^- mutations. Their fermentation of lactose may cover the presence of Lac^- colonies.



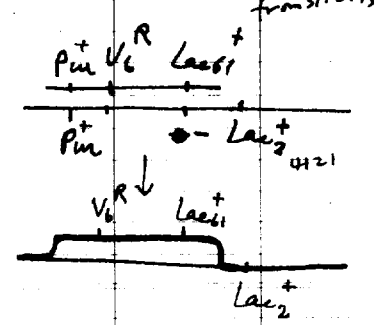
T_6^R Lac^+.

or



elimination of T_6^S segment

or $Lac_2\ F_{13}$ is hemizygote for Lac_2.



Comparisons on the stability of maleness of F^+ and F' to AO treatment.

8/VII ; 1959 REF:

Make oposit combination

Strain W3086 F_8 , ~~W3747~~ , W6 . { W6 F_8 ; 3086 F^+ .

F_8 ~~F_{13}~~ F^+ .

- cultural age : overnight culture in Pheasy broth.
- Inoculumsize : Ca. 10 cells/ml. $10^{-2} \times 10^{-2} \times 10^{-1}$ (0.1 ml)/5 ml
- AO-treatment : AO-medium : 30γ/ml ; NSB pH. 7.6.

Result :

A). Survival count on B Mal. 10^{-6} ml/plate (before treatment.)

Exp II

A).

Mal+ W6 F_8	Mal− 3086 F^+
234	89
327	152
227	107

Exp II compatibility

total W6 F_8 tested	F^-	total 3086 F^+ tested	F^-
44		43	
%			

B). ~~Survival count~~ After treatment. (After treatment)

Treated with AO.

Exp ① 3086 F_8 + W6

W3086 F_8				W6 F^+		
Σ	F^+	F_8	F^-	Σ	F^+	F^-
35		4	31	35	5	30
21	1	2	19	24	1	23
Σ						
56		6	50	59	6	53
%	89.3 F^-			89.9 % F^-		

Replicated on MGal (x 4573.)

$\boxed{\text{Conclusion}}$: Looks no difference in accessibility of F. to acridine method.

Untreated control.

3086 F_8			W6 F^+		
Σ	F_8	F^-	Σ	F^+	F^-
55	55	0	55	55	0
%	100	0	55	100	0

Exp ② W6 F_8 + 3086 F^+.

Untreated control

W6 F_8			W3086 F^+	
Σ	F_8	F^-	F^+	F^-
32	32	0	32	0

x 4573 on MGal.

Treated experiment (with AO: 30γ/ml)

W6 F_8			W3086 F^+	
Σ	F_8	F^-	F^+	F^-
	0	59	0	42
%	0	100	0	100

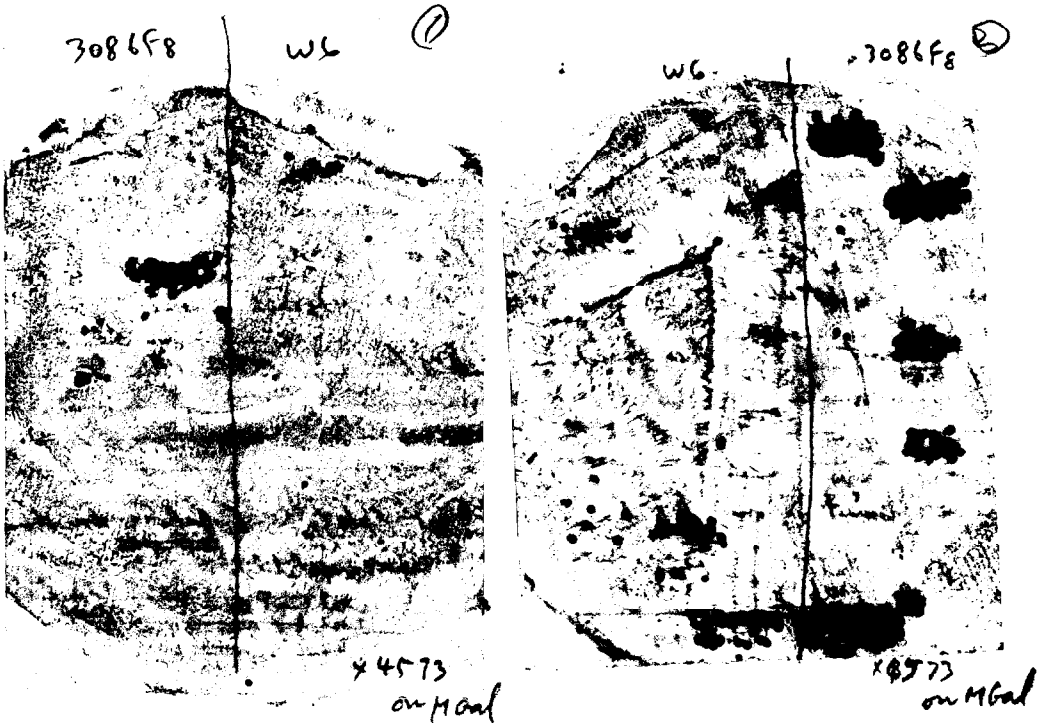
x 4573 on MGal.

Exp (I).

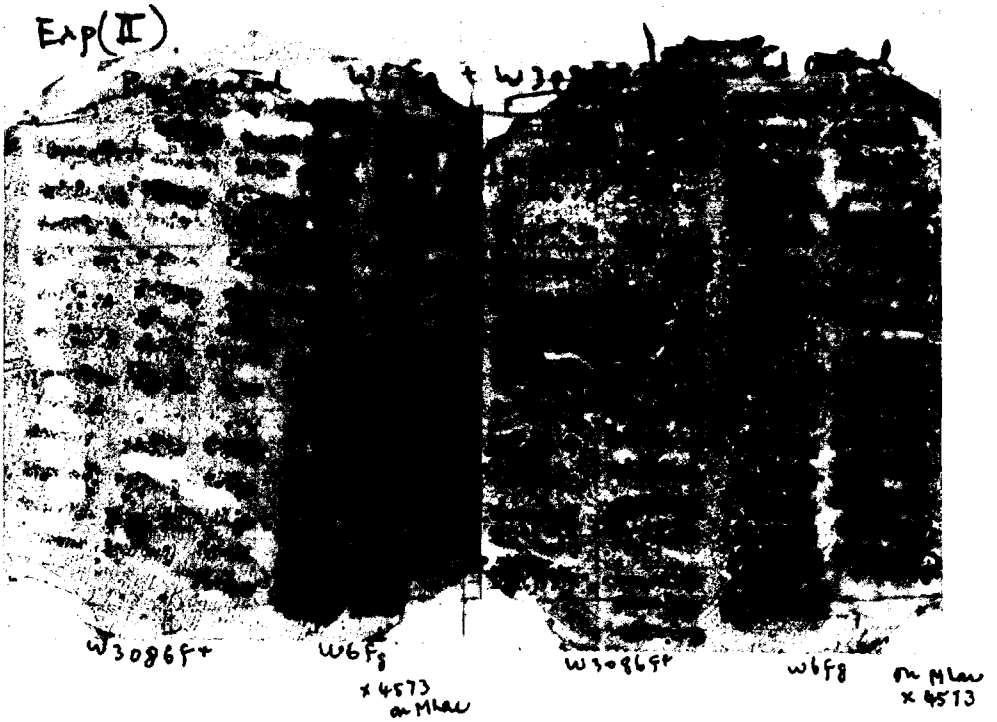
Inoculum ca. 10^2 cells/ml

AO 30 g/ml

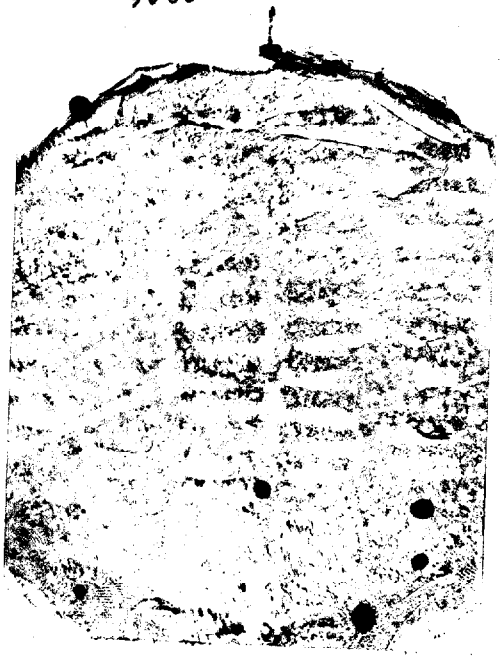
AO 3086Fg + W6 6 min light



Exp (II).

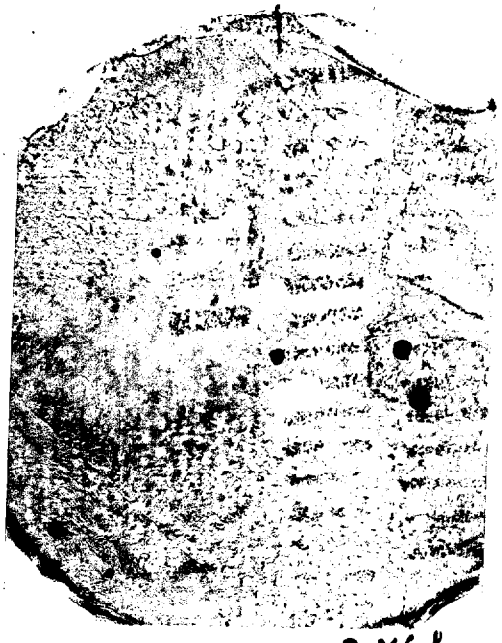


Ao treated
3086 F+ W6F8.



x 4573
on MGel

Ao treated.
W3086F+ W6F8



on D MGel
x 4573

Size of Gal - F_{8D} segment.

12/VIII ; 1959

REF:

Strain used : ♀ Pur⁻ Try⁻ Gal_2^- Xyl 2 Mtl Mal, Ara_2 S^R F^-.

♂ F_8 M^- (W6 F_8)

Cultural age : 0.2 ml : overnight culture / 5 ml Penassay broth.

↓ 2 hr on rotator at 37°C.

10:45 AM ~ 12:45 AM. ca 10^8 cells/ml

Ratio of mix : 1 ♂ F_8 : 0.1 ml F^- → Incubate it overnight at 37°C

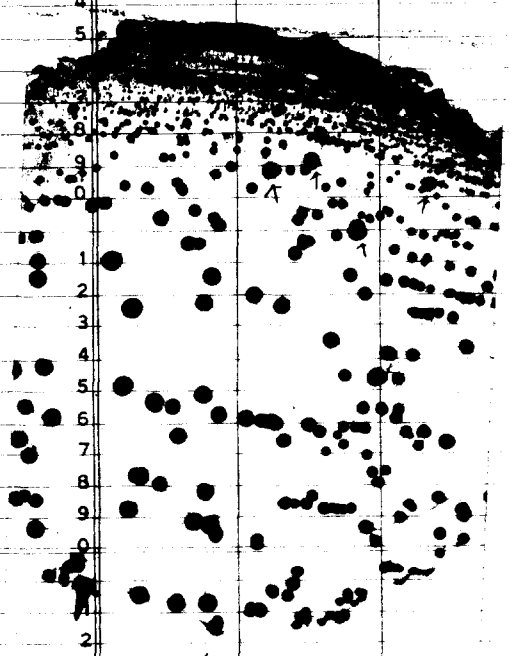
Principle :

W6 F_8 → × F^- Pur⁻ Try⁻ Gal_2^- S^R

Select on B Gal Sm.

Pick Gal^+ and test the other markers.

Result. Very few colonies become Gal^+. It may come from pur⁻ try⁻ of the recipient strain.



On B Gal Sm.

5 days

Gal^- becomes black

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Unsuccessful

Infection of F_3 and Remove of F_1 from it.

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Principle : $F_3 - F_1 = Rfd_3$

20/VM 1959

REF: W4296 shows strong syntrophy with M⁻ see back page

Principle : W4616 (Lac⁻ F_3) ⟶ W4295 $F^- L^- S^R$

[⟶ W4296 $F^- T^- S^R$]

↑ Too much syntrophy.

W4616 ⟶ W4295.

F_3	Total Colonies tested
20	350
15	379

- cultural age : 2 hrs. 0.2 ml / 5 ml.
- Excess F_3^+: 10 : 1. F^-
- Ratio : (2 ml 0.2 ml)
- Purified on B Lac Sm.
- Replicated on M Lac Seeded W3086.

Result: The first step.

3133 F_3 ⟶ 4295

on M Lac

~~X 4573~~

W3086

Retest of newly isolated F_3^+ by spot test. X 3086 on M Lac.

F^-

F^-

F^-

F^+ not F_3

F^-

on M Lac

X 3086

Try it again

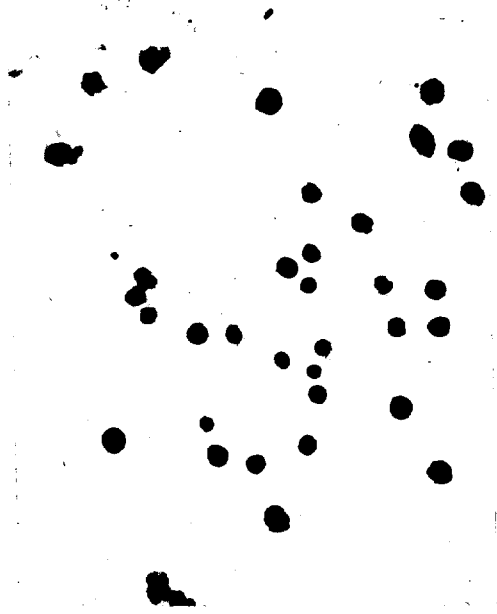
Syntrophy of M and T.

3137 F_3 → W4296

on Mlac

W808

Master plate



on Blac

Unsuccessful.

MICROBIAL GENETICS

Isolation of Lac61⁻ F_{13} by ~~Simple~~ infection of F_{13}

3747 ——— × 4121 S^R

21/VIII 1959 M V_6 F_{13} (Lac61 F^- S^R) REF: cf P120 & P120a P.55

Purpose: Still it is very hard to isolate F_{13} Lac^-_x strain from segregants. This experiment is carried out to find out more easy method.

Principle: 1. F_{13} may split off from Lac loci and infect to Lac^-. (This possibility is very low)

2. Lac^+ F_{13} consist heterozygous diploid state in first step, then recombination occurs, and endogenote Lac^- becomes exogenote. This Lac^- F_{13} becomes infective, and multiplies rapidly.

In any way, if it does occure, it may be possible to isolate Lac^- F_{13} using this method.

Result:

1. Rate of infection of Lac^- F_{13} into 4121 S^R.

Lac^-	91	17.2 %
Lac^+	438	82.8 %

2. Rate of F_{13} · Lac61.

Lac^- isolated	F^-	F_{13}
108	108	0

~~method — The~~ see bellow.

Conclusion: F_{13} itself does not infect into F^- in this experiment. This ratio may be much less than 1 %.

Method:

1. Make 2hrs culture (shaked on rotator in plenary at 37°c.)
2. Mix 10 : 1 : 10
   F_{13} : F^- : fresh broth.
   2 ml, 0.2 ml, 2 ml of fresh broth.
3. Incubate it for overnight.
4. Purify it on Olac 3m.
5. Pick Lac^- colony on Olac 3m.
6. Replicate on MLac, on which Lac^- F^- are seeded.

5/12.

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Isolation of Lac61/ex. Lac61. F_{13}.

24/VIII, 1959.

REF: Lac_x must be Sm^S

Principle:

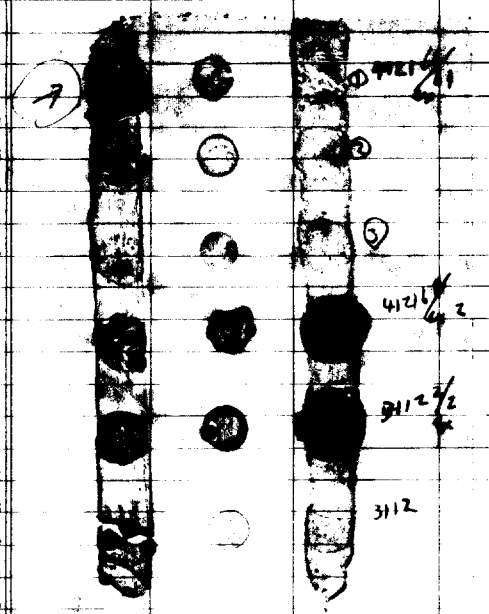
1st step: $Lac_2/_{ex}Lac_2\ F_{13} \rightarrow Lac_{61}\ F^- \rightarrow Lac_{61}/_{ex}Lac_2\ F_{13}$

2nd step: $Lac_{61}/ex\ Lac_2\ F_{13} \longrightarrow Lac_2/ex\ Lac_{61}\ F_{13}$ on MLac^S 3112.
(exchanging Promes of exogenote and endogenote.) (Detect it by replica plating method) see back page.

3rd step: $Lac_2/ex\ Lac_{61}\ F_{13} \rightarrow Lac_{61} \rightarrow Lac_{61}/_{ex}Lac_{61}.F_{13}$

Principle is the indirect selection of a clone which contains many cells of $Lac_2/ex\ Lac_{61}$ arised by exchange of Lac locus from $Lac_{61}/_{ex}Lac_2$.

Retest

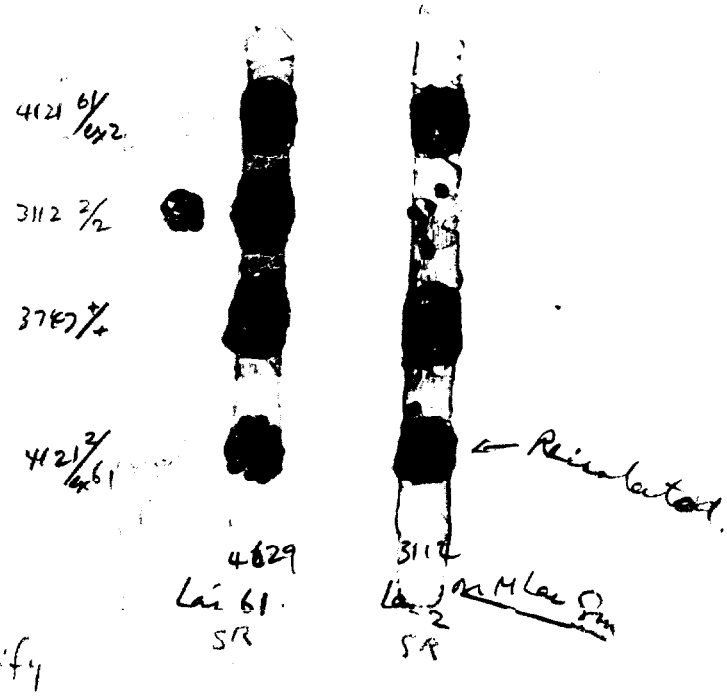


Replicated from BLac.

1st selection.

Pick.

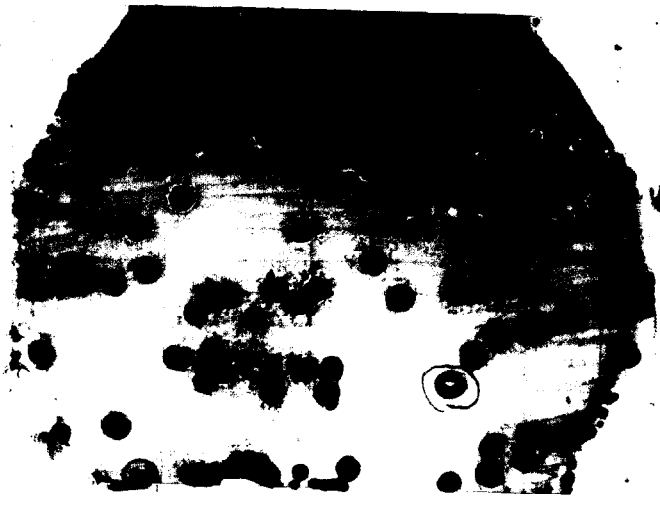
on MLac
× 3112.



Repurify

2nd selection.

on BLac



on MLac

×3112.

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6/40 ; 1959

REF:

	1	2	3	4	5	6	7	8	9	10
1		Isolation of		HI Gal	from W3747.					
2										
3										
4										
5										
6										
7										
8										
9										
0										
1										
2										
3										
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7										
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9										
0										
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										

MGal
×2979.

on Blac

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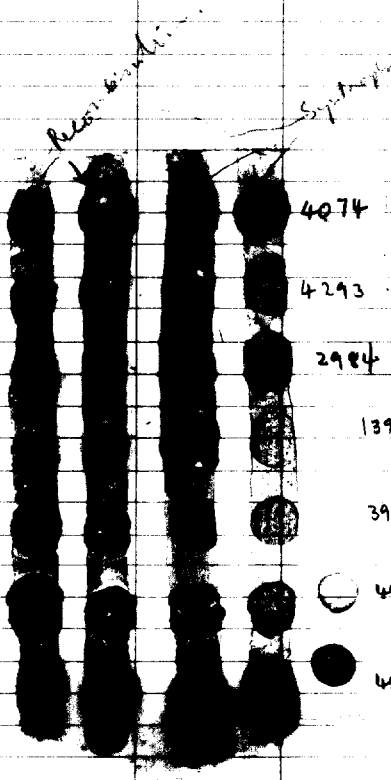
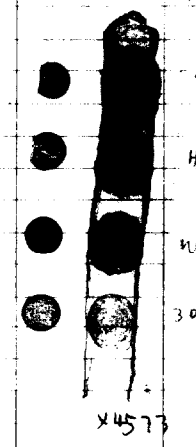
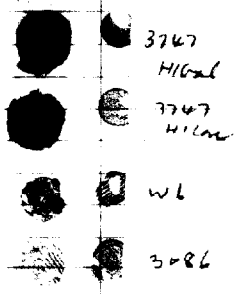
Comparison of the fertility of W3747, HI Gal and HI Lac.
I II.

1959. REF:

Purpose : W3747 shows two kinds of colonies, one is high fertile to







X4573 on MXyl

HI Lac 3747 HIGal 3747 W6 W3086-

on MLac

Conclusion

HI gal TLB1 A1 Try

HI Lac Pur

Lac Pur

gal TLB1 A1 Try

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Order of integration of chromosome via ~~cross~~ $F_2 \times F^-$

26/VIII ; 1959 REF:

W4540

Strains : W6 F_2 , W4573,
H^- F_2 $Ara_2 Lac_{85} Gal_2 S^R Mal_1 Xyl_2 Mtl_1$

Blending : Gage : 60 , 1 min.

overnight culture 0.1 ml/5 ml pen →

Cultural age : 1:30 ~ 4:00 2½ hrs. at 37°C on rotator

W6 F_2 : 2 ml
W4573 : 0.2 ml

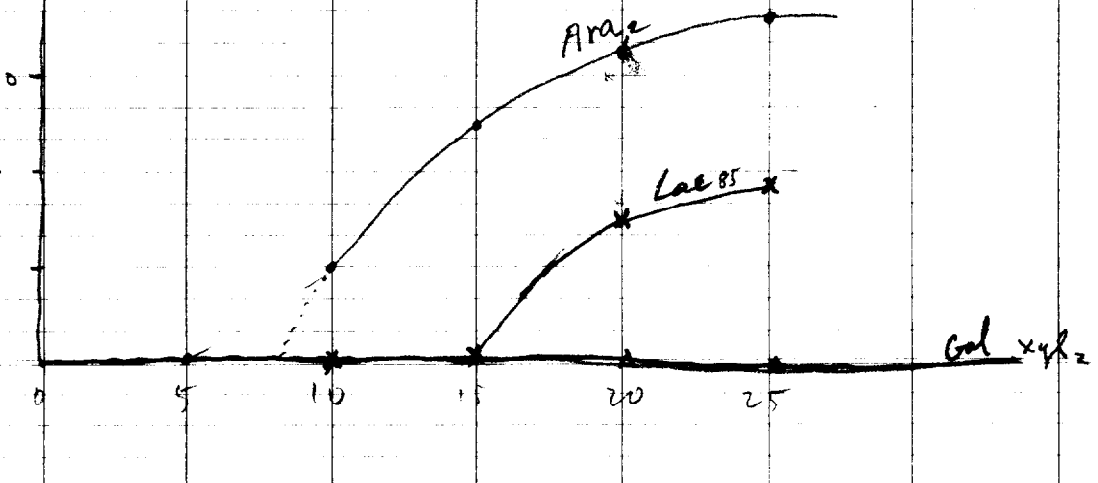
# of Recombinants/plate

Time	0	5	10	15	20	25
	Ara_2 Lac Gal_2 Xyl_2	A, L, G, X	A L G X	A L G X	A L G X	A L G X
plate# 1	0 0 0 0	0 0 0 0	1 0 0 0	9 0 0 0	45 1 0 0	51 3 0 0
10^{-3} ml 2	0 0 0 0	0 0 0 0	0 0 0 0	12 0 0 0	33 1 0 0	57 5 0 0
Σ	0 0 0 0	0 0 0 0	1	21 0	78 2	108 8

Survival count	Lac											
10^{-6} ml	−	+	−	+	−	+	−	+	−	+	−	+
	4	54	6	64	8	51	3	73	6	71	7	102
	●3	29	5	67	6	76	7	63	9	93	9	137

fertility % ca. 0.1 % ca. 1 % ca. 0.5 % ca. 1 %

%



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Comparisons of the fertility of exogenic and endogenic segment.
(Quantitative test.)

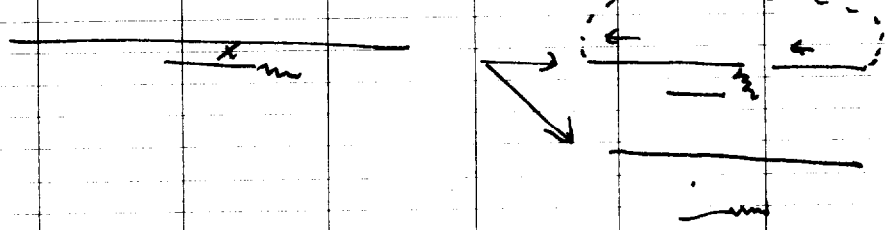
16/IV ; 1959 REF:

	1	2	3	4	5	6	7	8	9	10
		Cultural age:		Overnight culture in Penassay broth.						
1										
2		Cross	:	10:30 AM. ~ 12:30 AM			at 37°C.			
3				· ratio of mix			of 0.1 ml : 8 ml.			
4				· mixed well.						
5										
6										
7					fertility		survival count		10^{-6} ml.	
8					10^{-2} ml	10^{-3} ml	Lac+	Lac-		
9		W 4121	61/ex 2	x 4629		11	4	457		
0							4	382		
			Pre incubation.							
1										
2			Post incubation.							
3										
4										
5		W 4121	61/ex 2	x 3112.		[redacted]				
6						1148	3	364		
7			Pre				4	547		
8										
9			Post.							
0										
1					10^{-3}	5×10^{-4}	93	713		
2		W 3747	x	4629		985	106	754		
3										
4			Pre							
5										
6			Post.							
7										
8										
9		W 3747	x	7112		1065				
0										
			Pre							
1										
2										
3			Post.							
4										
5										
6										
7										
8										
9										
0										
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										

Comparisons of the fertility of exogenetic and endogenetic segment.

1959 REF:

	1	2	3	4	5	6	7	8	9	10
		Cultural age : Overnight cultures.								
1										
2										
3					(F⁻ Lac⁻ 615ˢ)	fertility 10^{-3} ml	fertility 10^{-2} ml	Survival (10^6 ml) Lac⁺	Survival (10^6 ml) Lac⁻	Rec. / Lac⁺
4									+ −	
5		W4121 61/ex 2	→	× W4629		79	1214	8	38 | 8 71	
6						180	1097	5	45 | 8 75	
7										Ca. 10^{-2}
8						Ca. 100	Ca. 1000	Ca. 5×10^8	4×10^7	
9										
0										
1										
2		W4121 61/ex 2	→	× W 3112	(F⁻ Lac₂ 3ˢ)	0	12	1	27 | 18 15	
3						0	20	3	32 | 23 155	
4										
5						0	Ca. ~~10~~ 20	Ca. 2×10^8	Ca 3×10^7	< Ca. 10^{-5}
6										
7										
8										
9						10^{-3} ml	10^{-2} ml	+	− | + −	
0								248	35 | 174 14	
1		W3747 +/+	→	W4629		1231	68	~~262~~ → 79	138 20	
2							101	~~120~~ 46		
3								262	41	
4						Ca. 1000	Ca. 100			Ca. 10^{-2}
5								Ca 2×10^8	4×10^7	
6										
7										
8										
9								+	− | + −	
0		W3747 +/ex+	→	× W 3112		126	52	170	46 | 240 70	
1							113	262	79 | 291 68	Ca. 10^{-2}
2						Ca 1000	Ca 100	Ca. 2×10^8 ; 5×10^7		
3		Conclusion:								
4		Fertility of								
5		exogenetic ≅ endogenetic × 100.								
6										
7										
8										
9		This may be explained by crossing over between F' and host-								
0		chromosome at about 1%.								



Transformation of Gal in K-12.

20/IX. 1959 REF:

Experimental condition:

1 Conc. of Duponol (Na-lauryl sulphate) : 10%.

2 Time of Treatment : 3 hrs at 37°C. in H_2O.
1:45 ~ 4:45.

3 Bacterial culture : overnight; Ca 10^8 ~ 10^9 cells/ml. washed once with H_2O.
in penassy grown. suspended into H_2O.

32 m/s of W6F8 culture was used. (4 tubes of 8 ml pen.)
↓
concentrated into 2 m/s.

1 ml → treated with duponol. final 10%. 1 ml → untreated

20% duponol Soln 1 ml + Bact. suspension 1 ml

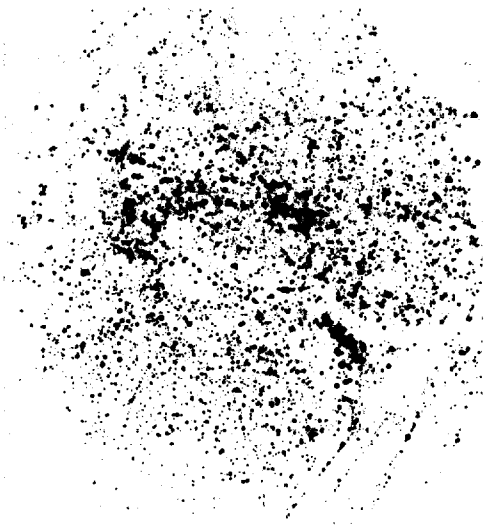
H_2O 1 ml + Bact. susp. 1 ml.

4. Method of transformation.

DNA	dilution of Original culture	10^{-1}	10^{-2}	10^{-3}	
∘ # of Recombinants.		~~[illegible]~~	521	43	
∘ survival cells in 0.5 ml		—	—	2,5 ↑	
Bacteria		10^{-1}	10^{-2}	10^{-3}	10^{-6}
∘ # of Recombinants					41
∘ survival cells in 0.1 ml		—	—	—	435 585 ↑

Next step: Filter this DNA sample.

DNA 10^{-1} dil



DNA 10^{-2} dil

DNA 10^{-3} dil.

Intact.

10^{-2} dil.

10^{-5} dil.

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Transformation of F8 Gal. Negative result.
(filtered material).

23/IX ; 1959 REF:

	1	2	3	4	5	6	7	8	9	10

Isolation of Sample.

1. W6 F8. M⁻ F8 ; W4573 $Lac_{95}Gal_2$ .....
4 tubes, 8 ml. → suspend in 4 ml of H_2O → add 4 ml of dupanol 20 %.
2. ~~Treatment~~ treatment ~~with~~ dupanol : Na-lauryl-sulfate.
(final conc. in dist. W. 10 %.)
37°C 4 hr.
3. Filtered through millipore filter.
4. Mix : dupanolated sample.
0.2 ml of W4573 + 0.2 ml of broth + 1 ml of DNA.
: old culture (2 days)
and incubate it for 30'.
5. Seed it on M Gal.

Result :

1) survival count : 0.2 ml of the extract was seeded onto EMBlac.
No colonies are observed.

2) Recombinating activity.

Extract :	1 ml	(1/10 diluted) 0.1 ml	(1/100 diluted) 0.01 ml
# of Recombinations.	0	0	0

Conclusion :
Mating activity of W6 F8 is in insoluble part.

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Action of propamidine isethionate to F and F' (I)
(P.I.)
4:4'-diamidino-diphenoxypropane-di (β-hydroxyethane sulphonate) 5 gr.

16/IX. 1959 May & Baker Ltd. Dagenham England.

Joshua gave me. on 11/sept. '59.

Stock solution: 100 mg / 1 ml soluble in water.
(Weigh 100 mg of P.I. and dissolved into steril H_2O in vial.)

1. Test survival & bacteriostatic concentrations of P.I.

Conc. of P.I. (cc. of Stock soln.)	0.5 mg/ml (0.25 ml)	1 mg 0.05	100 γ (x1/10) 0.05	10 γ x1/100 0.05	1 γ x1/1000 0.05	Control 0 γ
W6 F8	−	−	−	+	+	+
W6.	−	−	−	+	+	+

· make ~~survival~~ sterility-test of P.I. soln. 100 γ & 10 γ.
− −

" Media. 5 ml Penassy broth, pH. 7.0.

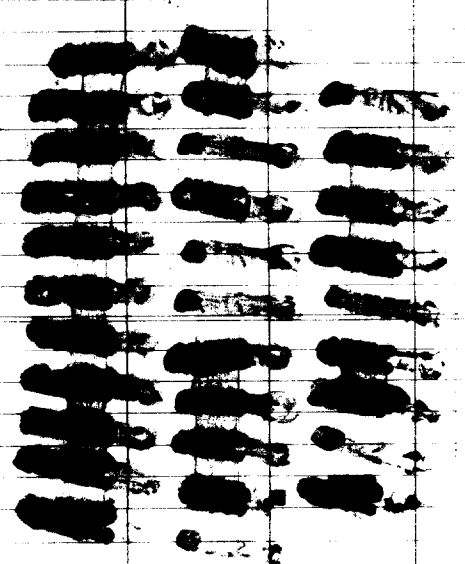
2. Streak these treated cells on Blue agar.
100 γ. P.I.

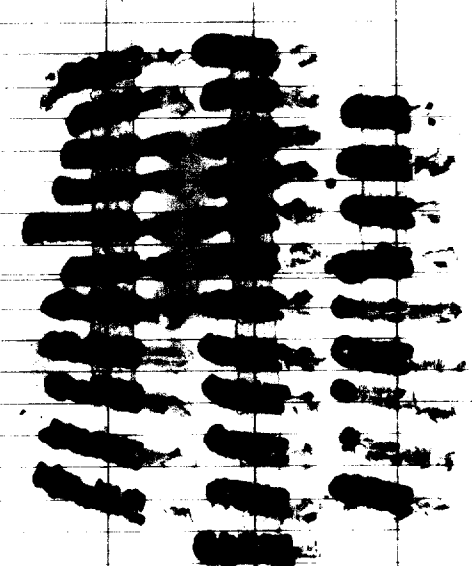
3. Test their compatibility by cross-brushing method.

Result: P.I.-treated 10 γ/ml Rn. Untreated control.

F⁻	F⁺	% F⁻	F⁻	F⁺	% F⁻
7	24		3	29	

W6 P.I. W6 Cont.





MLac N4573

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Action of Propamidine isethionate. on F. II.

150

24/4 ; 1959

REF:

1	2	3	4	5	6	7	8	9	10
	Med. Penassay.	inoculum size: ca. 10^5 cells/ml.			Strain: W6.			(×1/100) Conc. of P.I. Soln used: 1 mg/ml.	
Conc. of P.I.		0 γ / 0 ml	10 γ / 0.05 ml		20 γ / 0.1 ml		50 γ / 0.25 ml	(5 ml plm.)	
Ratio of conversions F⁻/total tested		0/30	0/28		0/28		4/29		
(%).		0	0		0		14.0		
effect		−	−		−		+		

purpose: Is PI active for removal of F? (P.I. can remove chloroplast of Euglena.) In former experiment, 10 γ/ml of PI looks active. Increase the concentration of P.I and see the increase of that activity.

conclusion. P.I. seems effective at 50 γ. in penassay, inoculum size, 10^5 cell/ml.

cont.

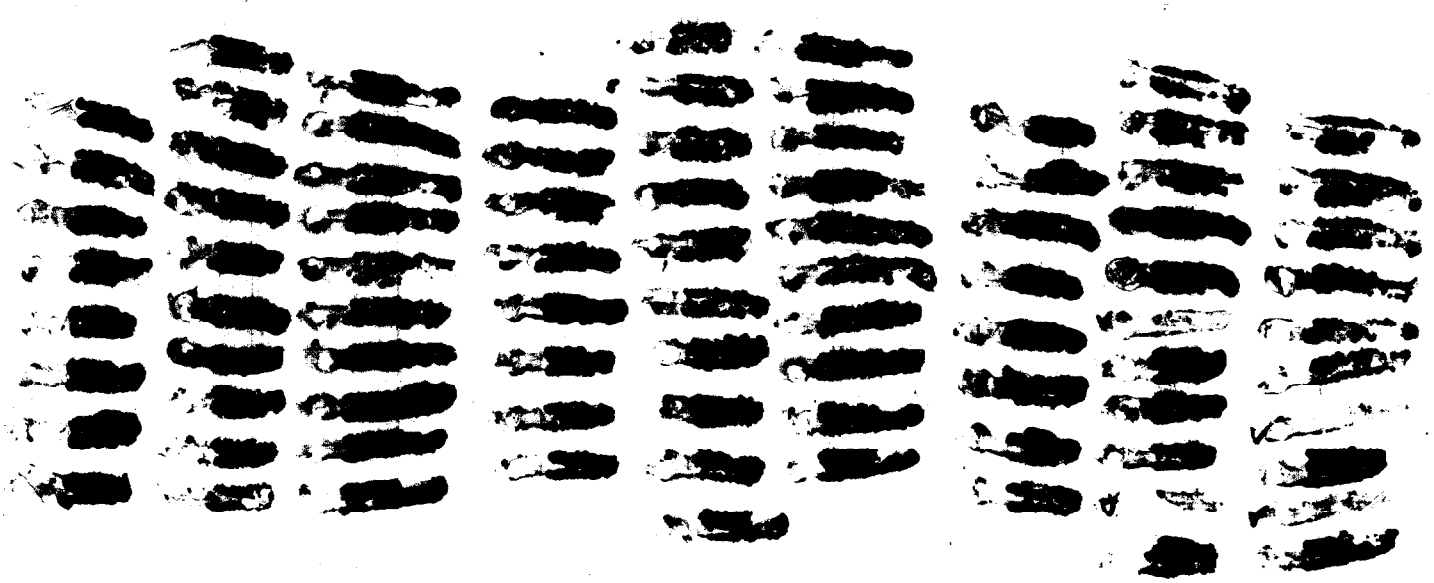
W6 P.I. 0σ.

MLac
X4573

P.I 10σ

P.I. 20σ

P.I 50σ



MLac X4573