U.W.
MICROBIAL
GENETICS

Cistron analysis of Lac loci using Lac₂-F13.

29/VII 1959 REF: W3112F13.

~~Photo +~~
~~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.

W3 F13 F⁺ Lac2 λ⁺ ——> cure.

Result:

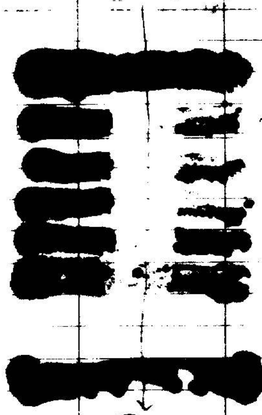
Lac⁻			× 3112 F13 (Lac₂) ONPG	× 3112 (Lac₂)	× 3747 (Lac⁺ M⁻ F13)	blank		
ONPG	3112	2	—	—	###	—	✓	Lac₂ 3164 nr.
unstable ×	3127	4	###	+	###	# unstable		
	2247	7	— (permease and β-galactosidase)	—	— ? check	—		non-transducible Lac.
+ m unstable ×	3230	11a	##	+	###	# many unstable		Recombinant
+ m	3089	11b	+ ? (permease)	—	+ ?			Reaction is very slow.
+ m	4112	52	###	—	###	+,		
− f	4121	61	###	—	###	—	✓	Lac61 4021
− m	4147	87	##	—	##	—		
− m	4148	88	##	—	##	—		
− m	4149	89	#	—	#	-		
− m	4150	90	##	—	##	-		
− m	4151	91	##	—	##	—		
− m	4153	93	##	—	##	-		
	2243	Lac₃	###	(###) syntrophy	###	++ unstable		
	2245	5	###	###	###	### "		
	2247 also Gal⁻	Lac₇	—	—	(—)	+₄		

↑ Test segregants.

This black spot gives many lac⁻ on BLac after streaking.

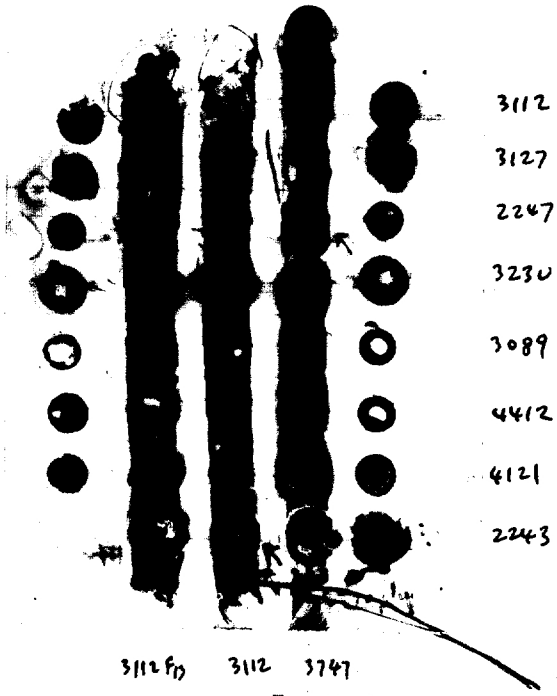
non-transducible Lac

λ_2^s
non-transducible Gal



V6 #s
S
S
S

on BLac



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

3112 F⁻ 3112 3747

F⁻ Lac F⁻

M⁻

Segregating between two different Lac⁻.

4147	
4148	
4149	
4150	
4153	
2295	
2247	7

3112 F⁻ 3112 3747

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

28/xx ; 1959

REF:

AO treated Lac⁺

No.	F	Lac stability	T6	Note
~~1~~				25% Lac⁻
2		U	m	control 2596 Lac⁺/Lac⁻
3		U	m	
4		U	m	
5		U	m	
6	F⁻	S	s	
7		U	m	
8		U	m	
9		U	m	
10	F⁻	S	s	
11		U	m	
12		U	m	
13		U	m	
14		U	m	
15		U	m	
16		U	m	
17	F⁻	U	m	
18		U	~~control~~	
~~19~~		U	m	
20		U	m	
21		U	m	
22		U	m	
23		U	m	
24	F⁻	S	s	
25	F⁻	S	s	
26		S	r	
27		U	m	
28		U	m	
29	F⁻	S	s	
30		S	r	
31		U	m	
32		U	m	
33	F₁₃	S	r	
34		U	r	
35	F₁₃	S	r	
36		U	m	
37		U	m	
38		U	m	
39		U	m	
40		U	m	
41		U	m	
42		U	m	
43		U	m	
44		U	m	
45	F⁻	S	s	
46	F⁻	S	s	
47	F⁻	S	s	
48		U	m	
49		S	s	
50		S	s	

No.	F	Lac stability	T6
51		U	m
52		U	m
~~53~~		~~U~~	~~m~~
54	F⁻	U	m
55		S	r
56		U	m
57		S	m?
58		S	s
59		S	r
60	F⁻	S	s
61		U	m
62		U	m
63		U	m
64		U	m
65		U	m
66		U	m
67		U	m
68		U	m
69	F⁻	S	s
70		U	m
71	F⁻	S	r

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

T6-resistance

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

Untreated

No.	Lac	T6
1	U	m
2	S	r
3	U	m
4	U	m
5	U	m
6	U	m
7	U	m
8	U	m
9	U	m
10	U	m
11	U	m
12	U	m
13	U	m
14	U	m
15	U	m
16	U	r
~~17~~	control 3747	r
18	U	m
19	U	m
20	U	m
21	U	m
22	S	r
23	U	m
24	U	m
25	U	m
26	U	m
27	U	m
28	U	m
29	U	m
30	U	m
31	U	m
32	S	r
33	U	m
34	U	m
35	U	m
36	U	m
37	U	m
38	U	m
39	U	m
40	U	m
41	U	m
42	U	m
43	U	m
44	U	m
45	U	m
46	U	m
47	U	m
48	U	m
49	U	m
50	U	m

No.	Lac	T6
51	U	m
52	U	m
53	U	m
54	U	m
55	U	m
56	U	m
57	U	m
58	U	m
59	U	m
60	U	m
61	U	m
62	U	m
63	U	m
64	U	m
65	U	m
66	S	r
67	U	m
68	U	m
69		

Lac⁺ (stable or unstable)
S : 4
U : 63

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 a Lac⁻ colonies found it is noted as Unstable. { U: Unstable, S: Stable.

Method ①: pick Lac⁺ from streaked plate, inoculated into Penassay (1 ml), and incubated them for overnight.
② Streak on BLac, and see is there Lac⁺ only or Lac⁺ and Lac⁻ colonies on it.

1. AO treatment: AO 30γ/ml.
- NSB - pH 7.6.
~~Inoculate~~ one small loop of ca 10⁷ cells/ml / 5 ml NSB AO.
- The treated culture was streaked on BLac after 24hr incubation at 37°C.

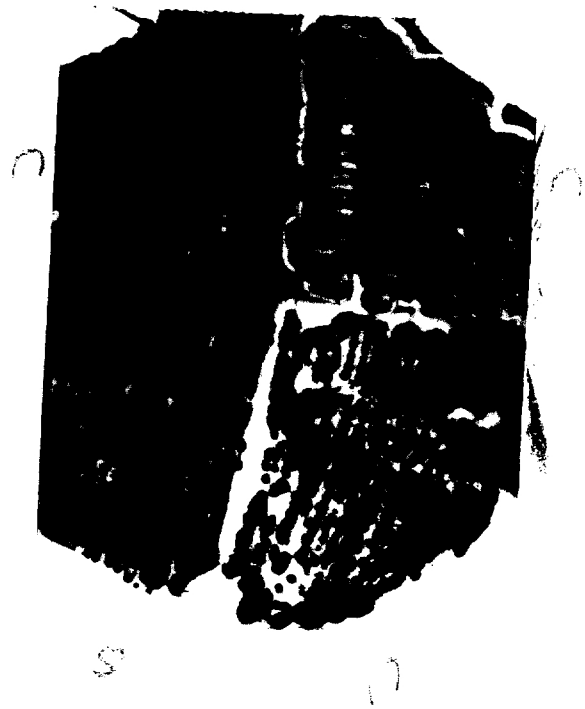
m : mixture of V_6^R and V_6^S, segregated from $V_6^{R/S}$.
S : V_6^S
r : V_6^R
U : Unstable for Lac⁺ accounted for Lac diploidy.
S : Stable for Lac⁺

Conclusion
1. All of the F⁻ treated with AO are 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, not $T_6^{r/s}$.

This means, AO remove full segment of F Lac V at a time.

U : unstable
S : stable



Elimination of Lac - F13 segment with AO-treated F+

23/III ; 1959

REF: Cf. P 118

Experimental conditions.

Inoculum size : ca. 10^8 cells /ml. AO : 30γ /ml. Med: NSB for AO-T.
Time : overnight at 37°C. Strain treated W2594 F13 (M S^R V_S^R Lac_+^+ V_S^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 ca 10^8 /ml on to B Lac. 5×10^{-7} ml of untreated control.

Result I. Elimination of Lac^+ segment from Lac^+/Lac^- heterozygotes.

	AO-treated (30γ/ml) I			AO-treated (30γ/ml) II			Untreated control C		
Plate #	Lac^{+-}	Lac^+ Lacv	Σ (%)	Lac^{+-}	Lac^+ Lacv	Σ (%)	Lac^{+-}	Lac^+ Lacv	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^- heterozygotes.

	AO-treated (from #II)				untreated control			
	Lac^+		Lac^-		Lac^+		Lac^-	
	F_{13} Hi	Lo	F^-	F_{13}	F^-	F_{13} Hi	F^-	F_{13} Hi	Lo	F^-
	59	0	13	0	64	67	0	22	50	0
Σ										
%										

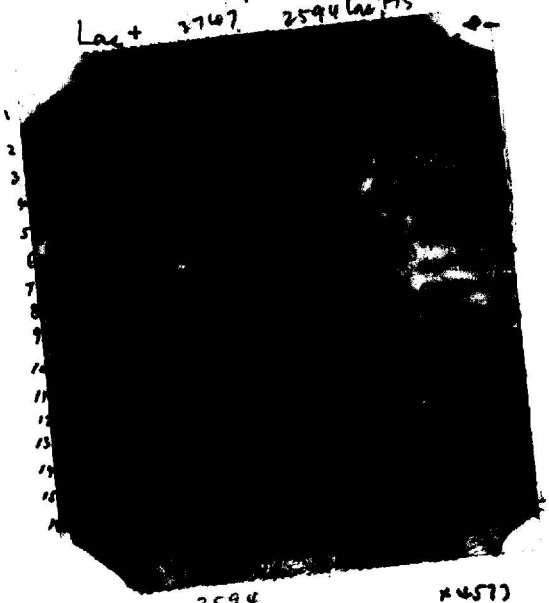
(Tested by bal-transfer. × 45 73)

(see back page)

x4573 on MGal

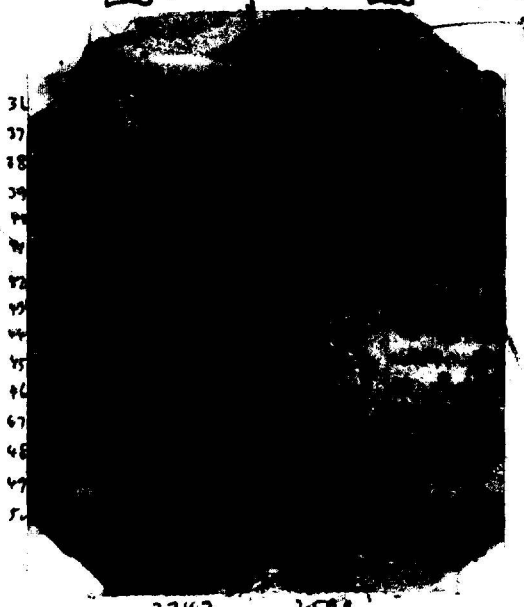
Untreated control

Lac+ 3747 2594 Lac+ F13



Lac+ 2594 x4573 on MGal

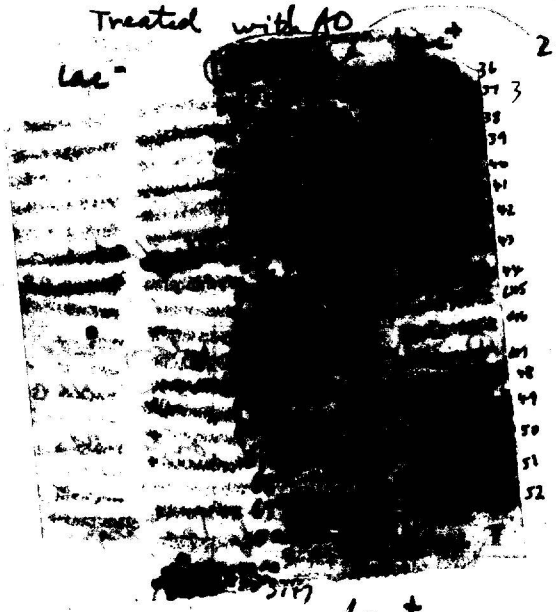
Lac- 2594 Lac+



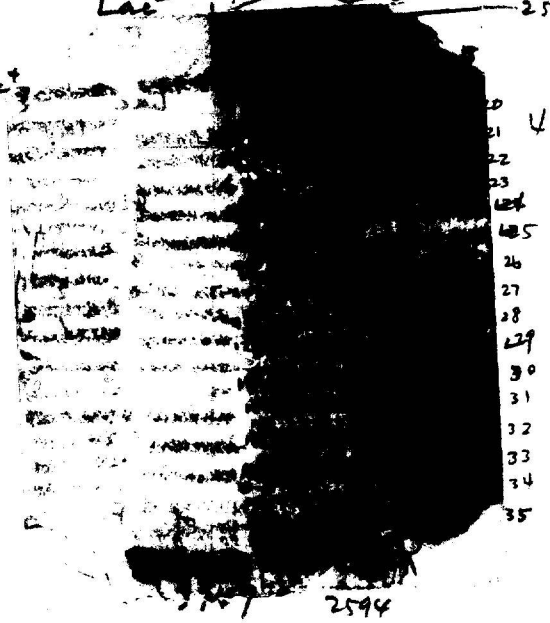
3747 2594

Treated with AO

Lac- Lac+ 2594 Lac+ F13



Lac- Lac+ 2594 Lac+ F13



3747 2594

4121 (Lac61 F-)

3112 F13 [redacted]

3112 F-

on MLac

24 hrs.

Multiplication of Gal-F_8 segment.

W4520
W6F8 ⨯ W4573.

30/VII ~ 1959

REF: p. 123

overnight culture
0.2 ml / 10 ml pen. → Incubate it at 37°C on rotator. for 2 hrs.

Mix { 1 ml W6F8 + 10 ml W4573.

		AM				PM							
		19:00		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		0		30'		1 hr		2 hr.		3 hr		4 hr	5 hr
	Col	+	−V	+	−V	+	−V	+	−V	+	−V	+	−
on B2 Gal		⓪	o	o	o o	o	o o	o	o o	o	o o		
	1	8	54	27	92(1)	84	224(7)	101	167(10)	61	62(6)		
	2	6	36	24	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(4)	104	229(3)	105	181(4)	60	59(3)		
		+V	−	+V	−	+V	−	+V	−	+V	−		
	Σ	44	233	112	322	472	1160	570	925	389	359		
		(44)	277	(101, 11)	334	(440, 32)	1632	(526, 44) 1420	1495 2310 ×2.5	(361, 28) 1945	748 1290 ×		
		o		o		o		o		o			

o : actually counted number

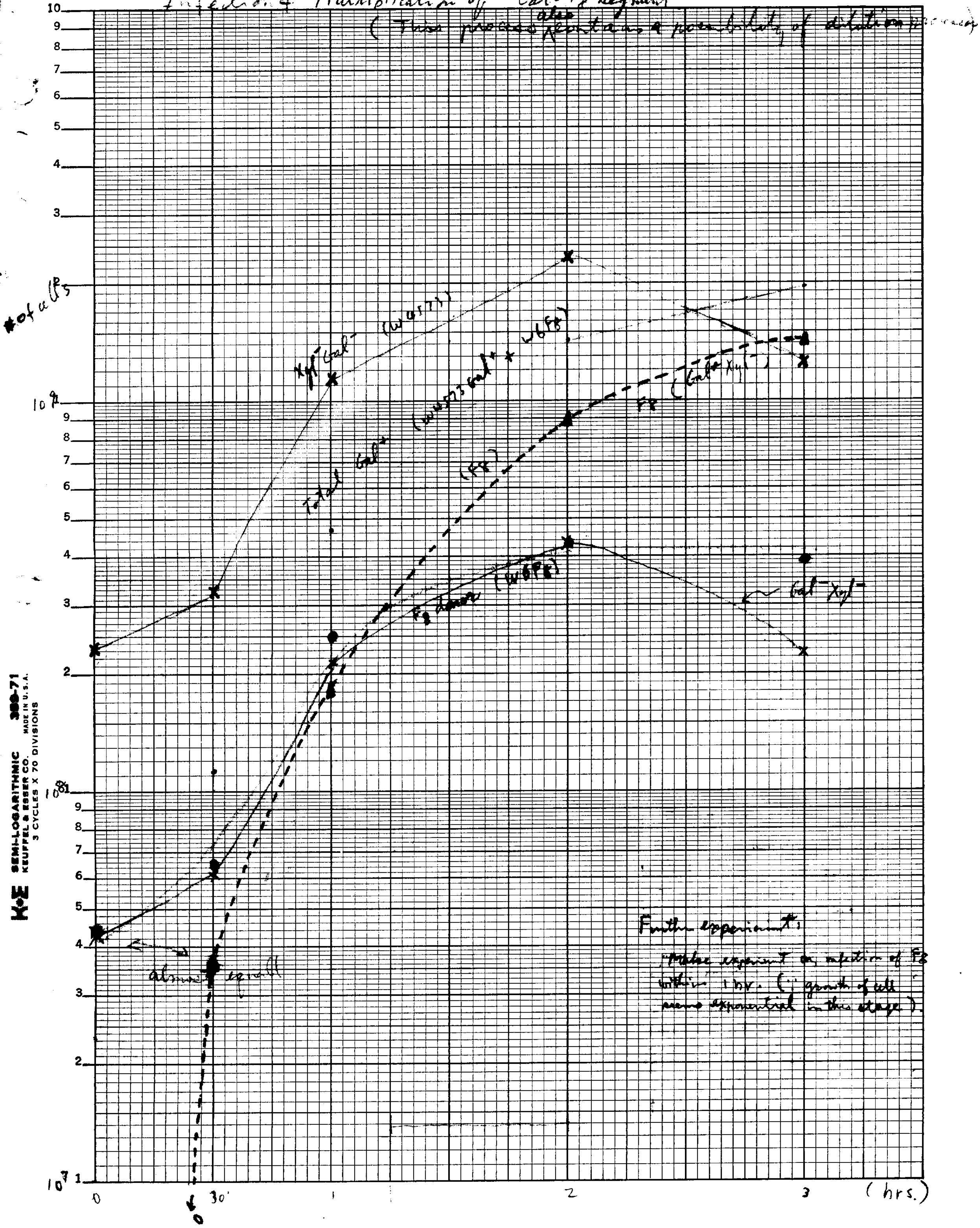
on B Xyl. growth of W6F8	Xyl	+	−	+	−	+	−	+	−	+	−
		8	54	18	100	53		35		8	
		6	36	13	88	51		39		14	
		10	49	11		43		35		16	
		4	48	18		43		31		14	
		16	46	5		60		29	×2.5	26	×5
Xyl+ Lac+	Σ	44	233	65		250		169	422	78	390
% of Lac+	?	15.9		19.5		15.3		12.1		10.4	
Δ multiplication	Gal	+		+		+		+		+	
		0	[illegible]	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	
	Σ	0	~~233~~	36		188		357	892	283	1414
% of Lac+		0		10.8		11.5		23.8		37.8	

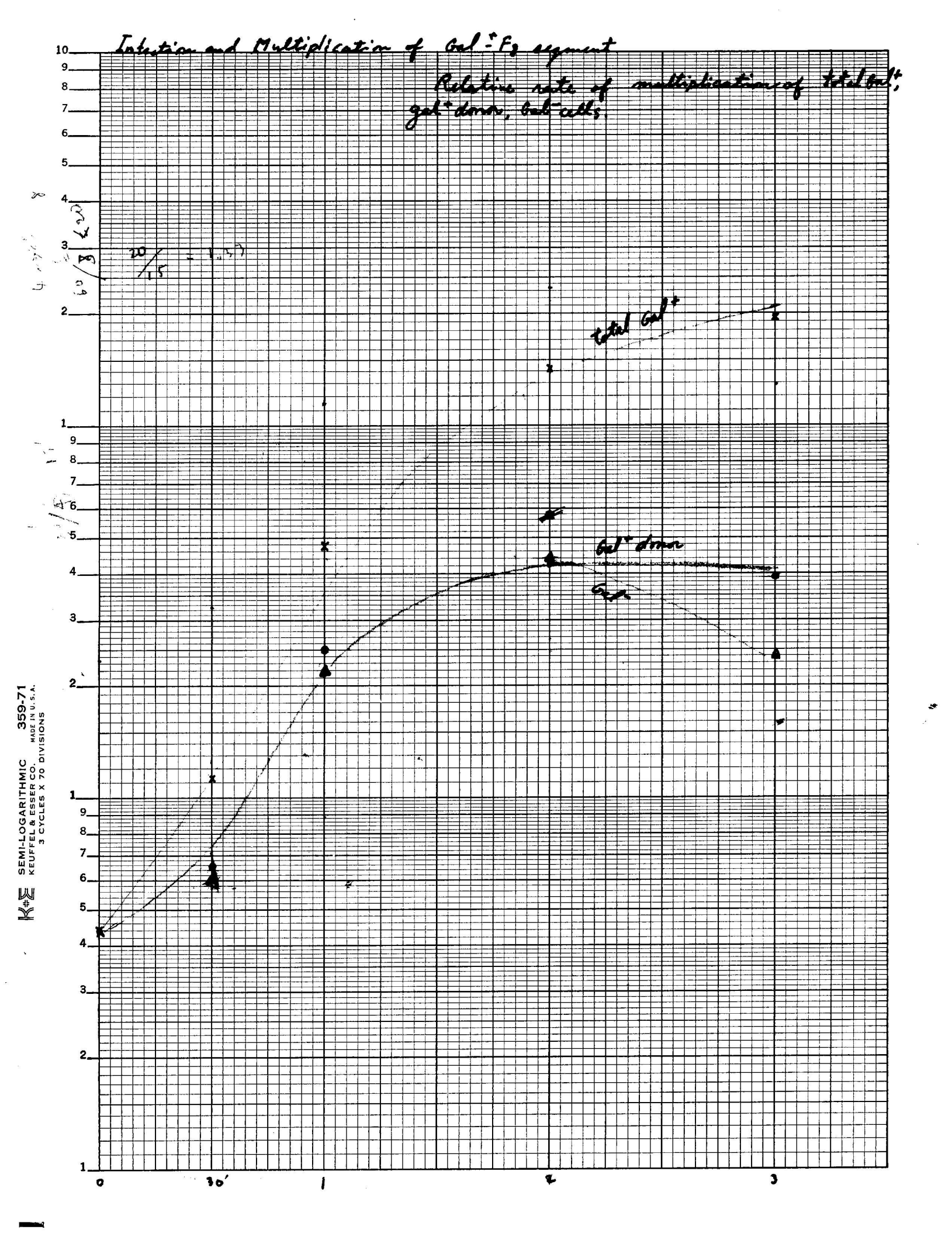
10/4   5/2 = 2.5

57

Infection & Multiplication of Gal+ F8 segment

(This process also contains a possibility of dilution process)

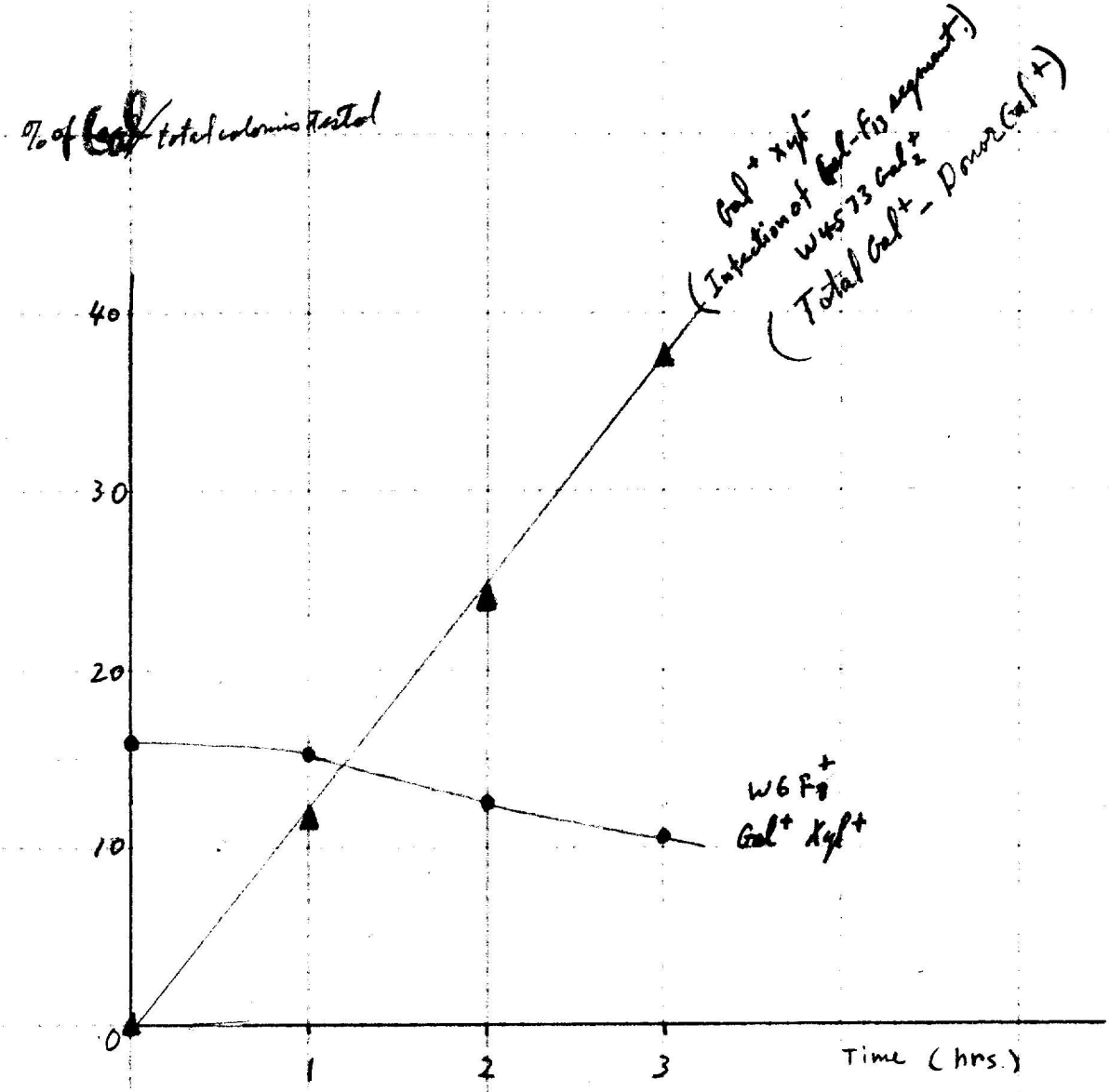


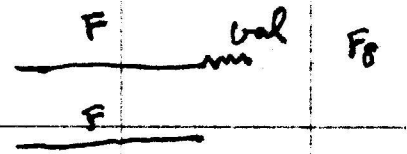

Induction and Multiplication of Gal⁻ F₂ segment
Relative rate of multiplication of total Gal⁺, gal⁺ donor, Gal⁻ cells
20/15 = 1.33
total Gal⁺
Gal⁺ donor
Gal⁻
10
9
8
7
6
5
4
3
2
1
0
30'
1
2
3
SEMI-LOGARITHMIC
KEUFFEL & ESSER CO.
359-71
MADE IN U.S.A.
3 CYCLES X 70 DIVISIONS

Rate of multiplication of Gal-F_8 segment. 128L

$W6\,F_8 \times W4573$

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




F
wall
F_s
F

Multipliation of F_8 Gal loci.

14/VIII 1959

REF:

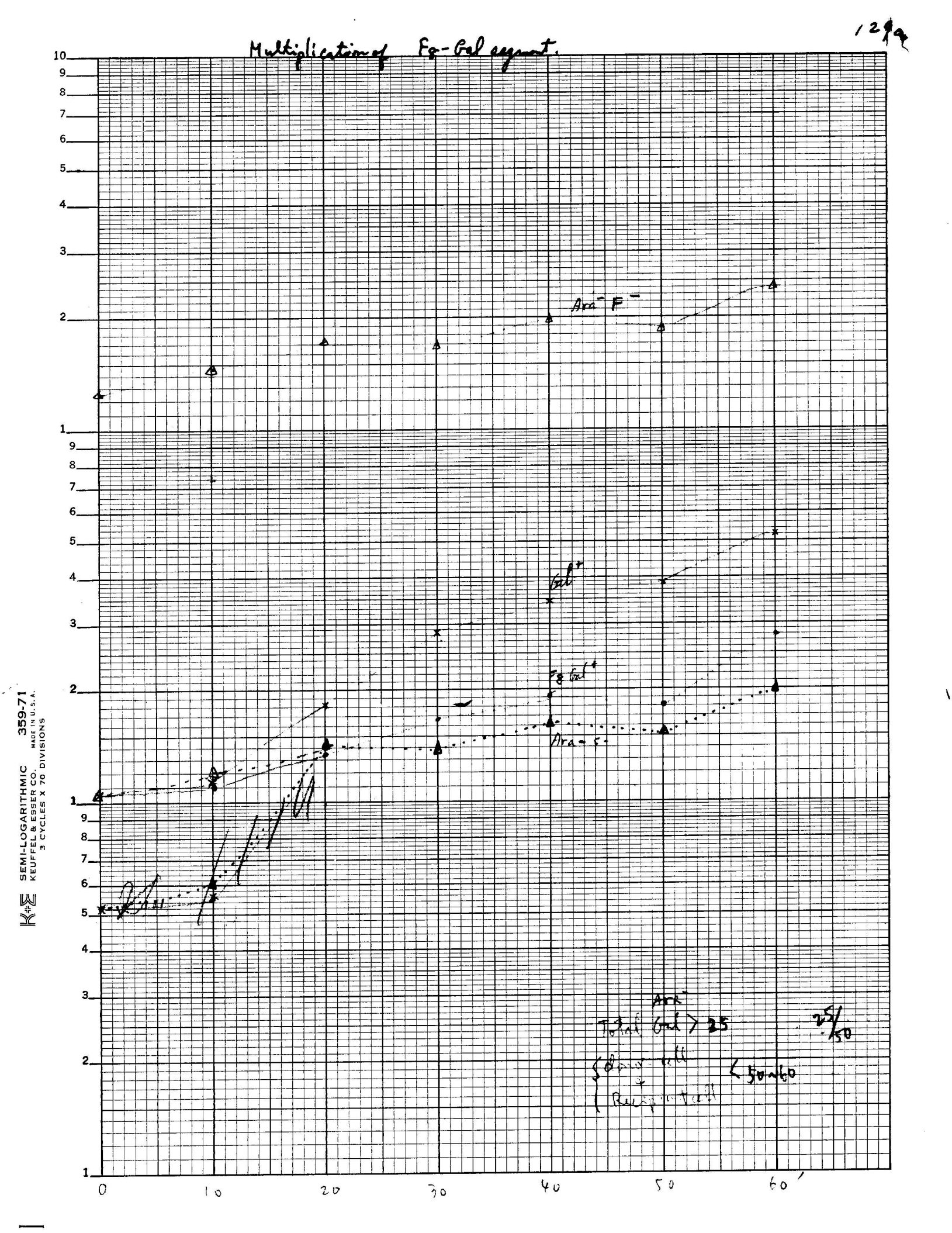
Principle: W6 F_8 ⟶ × 4573. Gal_2 Xyl_2 Mtl Ara_2 Mal_1 S^R.

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

time (min)		0		10		20		30		40		50		60	
		2:55		3:05		3:15		3:25		3:35		3:45		3:55	
1 Innoculum size		2 × 10⁻⁶ ml; $10^2 \times (10^{-2} \times 10^{-1}$: 0.2ml)		1 × 10⁻⁶		2 × 10⁻⁶		..		"		2 × 10⁻⁶ ml.		1 × 10⁻⁶	
		+	−	+	−	+	−	+	−	+	−	+	−	+	−
B Gal	1	39	441	22	201	62	570	82	524	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	1195	343	1994	388	1866	261	1201
														522	2402 (× 2)

		0	10	20	30	40	50	60	
		+	+	+	+	+	+	+	
B Ara.	1	39	22	48	54	52	56	41	
			14	47	64	71	69	51	
	2	31							
	3	35	19	40	51	66	55	48	
	Σ	105	55	135	169	190	180	140	
			110					280	(× 2)
Net infection.	▲	0	1	46	118	153	208	242	

Multiplication of Fg-Gal segment.



U.W.
MICROBIAL
GENETICS

Stock No, 1 ~ 27

Segregation of Lac⁻ from Heterogenote for Lac segment.

30/VII ; 1959 REF:

(Lac61)

W4121 × W3112 F13+ (Took from spot test (~~Lac~~ P.126))

Lac61 (Lac2)

Method: Streak

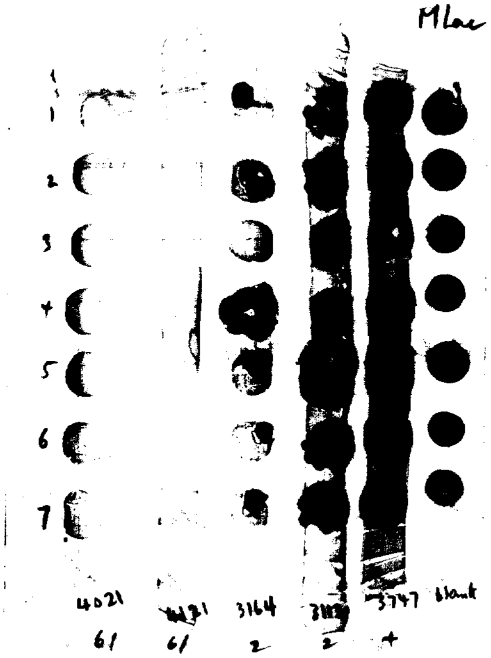
1. purify it on BLac. (5 plates)
2. Pick Lac+ colonies and suspend them into 1ml H₂O-tubes.
3. Restreak them on BLac, and pick Lac⁻ segregant (pick one Lac⁻ from each Lac+ streaks.)
4. streak the isolated Lac⁻ on BLac. (ca. 48 colonies are isolated) (Some of them maybe Lac+. 27 were Lac⁻.)
5. Test Lac x.∧ (and sex-compatibility) by crossbrushing on MLac.

	V6	Lac -mutation end. ex	F	× 3112 (Lac2) F-	× 3164 (Lac20) Hfr1	× W4121 (Lac61) F-	× W4021 (Lac61) Hfr1 ?	× 3747 (Lac+)
1	S	61	♂	+11	+1	−	−	#
2	S	61	♂	+21	+3	−	−	#
3	S	61	♂	+14	−	−	−	#
4	S	61	(♀)	−	+32	−	−	#
5	S	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	(♀)	−	#26	−	−	#
14	S	61	♂	#18	−	−	−	#
15	S	61	(♀)	−	#24	−	−	#
16	S	61	(♀)	−	+18	−	−	#
17	S	61	♂	#2	−	−	−	#
18	S	61	♂	+13	+1	−	−	#
19	S	61	♂	+13	+3	−	−	#
20	S	61	♂	#	+2	−	−	#
21	S	61	♂	+14	−	−	−	#
22	S	61	♂	+10	+1	−	−	#
23	S	61	(♀)	−	#29	−	−	#
24	S	61	♂	−	#23	−	−	#
25	S	61	♂	−	#43	−	−	#
26	S	61	♂	−	#23	−	−	#
27	S	61	♂	−	#34	−	−	#
Hfr1 4021	Lac61			(−)	−	−	−	−
4121	Lac61	61		−	#	−	−	−
Hfr1 3164	Lac2	2		−	−	#	−	−
3112	Lac2	2		−	+	−	−	#

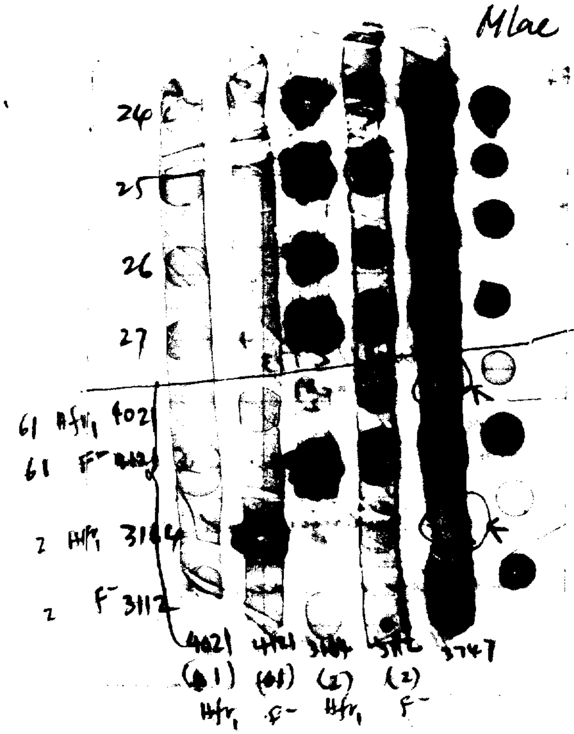
non-transferable 7. Hfr. [illegible] to F13.

Conclusion: 27/27 : Lac61 (100 %)

Mlac

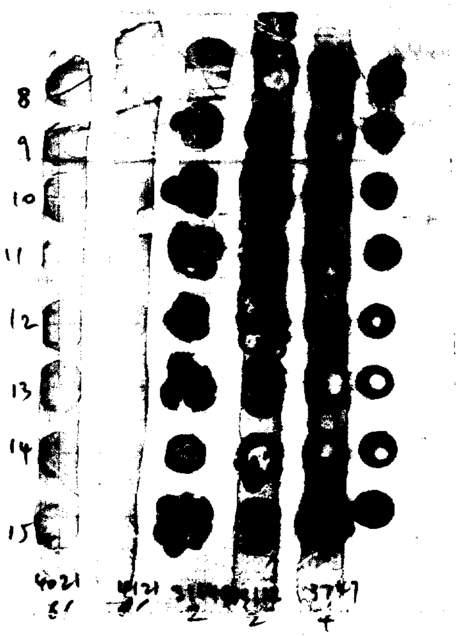


Mlac

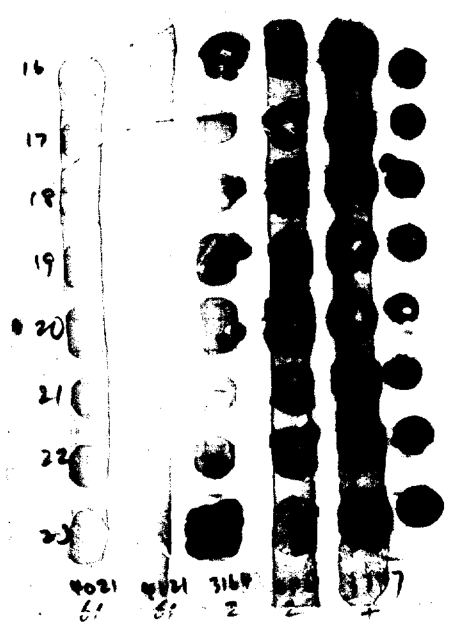


control.

∴ Hfr1 x F' sterile!

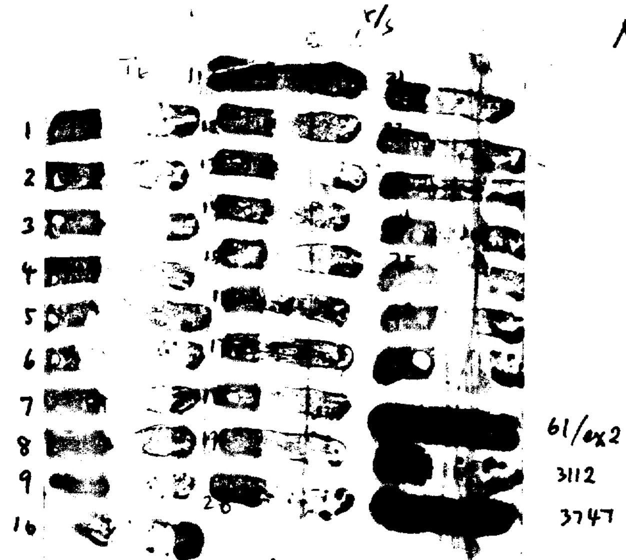


4021 Looks wrong! It may be [illegible] lac61.
∴ W4021 x lac2 F- is sterile.



Tests for T1-loci.

Retest #11.



on Blac

U.W.
MICROBIAL
GENETICS

Stock No 28 ~ 40

131

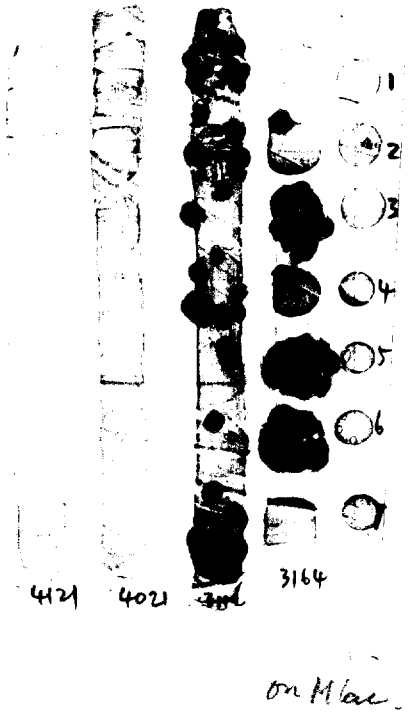
Segregants Analysis of Lac_2^{oc} / Lac_{61} F_{13}

7/xxx ; 1959

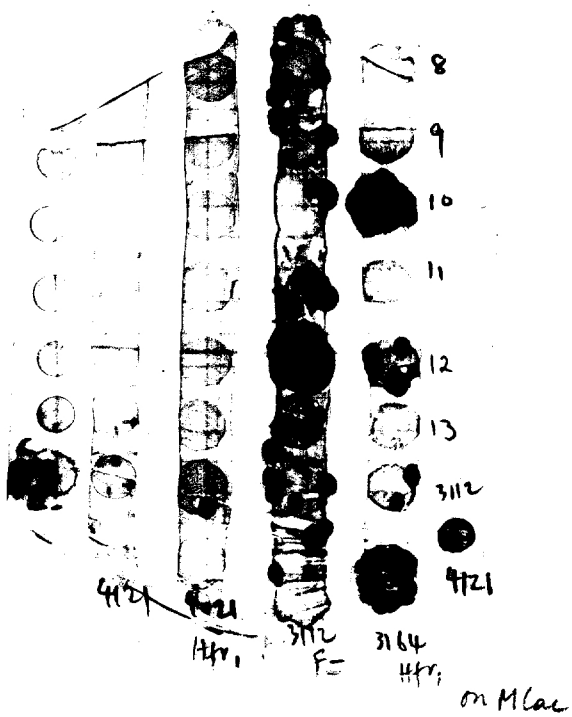
REF:

	1	2	3	4	5	6	7	8	9	10
			W4121 × W3112 F_{13}							
			Lac_{61}	Lac_2						
		Lac	F	× 3112	× 3164	× 4121	× 4021			
	VL	and $_{61}$ lac_2		(Lac_2)	(Lac_2)	Lac_{61}	Lac_{61}			
				F⁻	Hfr	F⁻	Hfr			
1	1 S	61	♂	+20	−	−	−			
2	2 r	61	♂	+60	+1	−	−			
3	3 S	61	♀	+2	+33	−	−			
4	4 S	61	♂	+9	+1	−	−			
5	5 S	61	♂	+2	+20	−	−			
6	6 S	61	♂	+1	+32	−	−			
7	7 S	61	♂	+23	−	−	−			
8	8 S	61	♂	+11	−	−	−			
9	9 S	61	♂	+5	−	−	−			
10	10 S	61	♀	+2	+28	−	−			
11	11 S	61	♂	+8	−	−	−			
12	12 r/s	61	♂	+61	+9	−	−			
13	13 S	61	♂	+4	−	−	−			
14	[redacted]			[redacted]	[redacted]					
15	[redacted]							See back page.		
16	Lac_{61}/lac_2	r/s								
17	3112	S								
18	3747	R								
19										
20										
21										
22		conclusion:		13/13 : Lac_{61} (100%)						
23										
24										
25										
26										
27										
28										
29										
30										
31										
32										
33										
34										
35										
36										
37										
38										
39										
40										
41										
42										
43										
44										
45										
46										
47										
48										
49										
50										

25
13
27
65

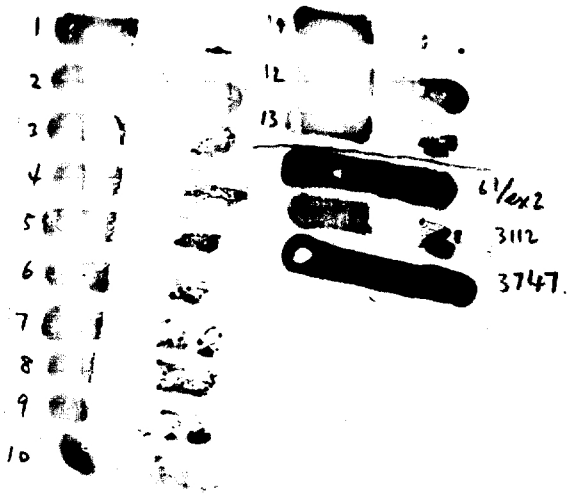


on M Lac



on M Lac

Test of V_6 loci



Retest # 12.
This strain looks $F13^{+}$ $Lac_{61}V_6^S$ / $Lac_{61}V_6^R$.

on B Lac

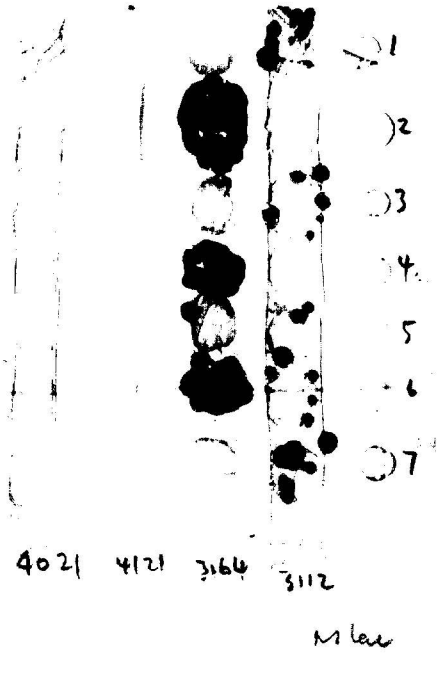
U.W.
MICROBIAL
GENETICS

Segregant analysis of Lac61/ex 2. F13.

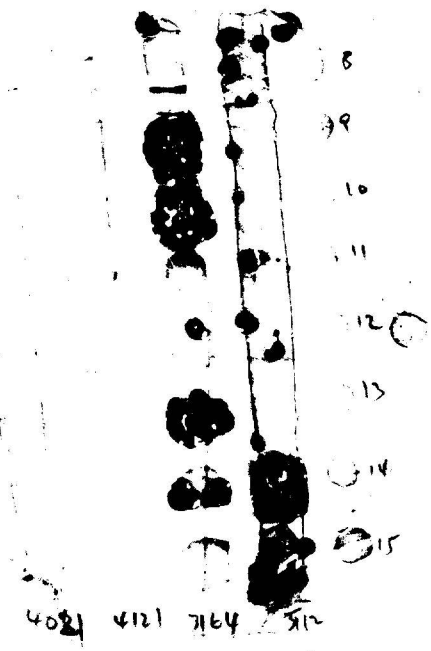
132

13/VIII :1959 REF:

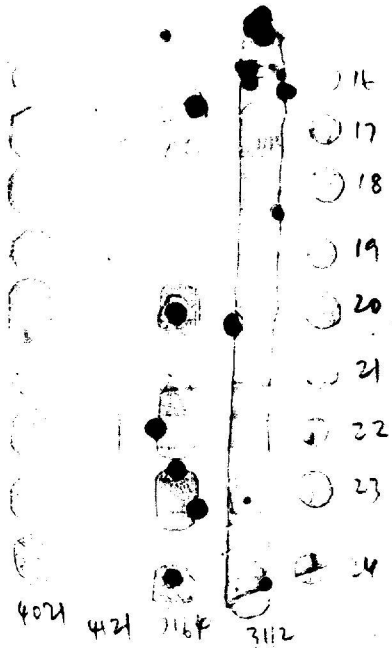
	1	2	3	4	5	6	7	8	9	10
		V_6	Lac end$_{61}$ ex$_2$	F		x 3112 Lac$_2$ F$^-$	x 3164 Lac$_2$ Hfr$_1$	x 4121 Lac$_{61}$ F$^-$	x 4021 Lac$_{61}$ Hfr$_1$	
1										
2										
3										
4										
5	1	S		♂		+15	−	−	−	
6	2	S		♀		−	+60	−	−	
7	3	S		♂		+7	−	−	−	
8	4	S		♀		−	+30	−	−	
9	5	S		♀		+2	+2	−	−	
10	6	S		♀?		±2	+36	−	−	
	7	S		♂		+15	−	−	−	
11	8	S		♂		+6	−	−	−	
12	9	S		♀		±1	+44	−	−	
13	10	S		♀		±	+68	−	−	
14	11	S		♂?		+4	−	−	−	
15	12	S		♂		+7	+1	−	−	
16	13	S		♀		±1	+25	−	−	
17	14	S		♂		±	+10	−	−	
18	15	S		♂		+33	−	−	−	
19	16	S		♂		+15	±1	−	−	
20	17	S		?		−	±1	−	−	
21	→ 18	S		?		−	−	−	−	
22	19	S		?		−	−	−	−	
23	20	S		?		±1	±1	−	−	
24	21	S		?		±1	−	−	−	
25	22	S		?		±	±1	−	−	
26	23	S		?		±1	+2	−	−	
27	24	S		?		±1	±1	−	−	
28	25	S		♂?		+2	−	−	−	
29				♀						
30	61/ex2	r/s								
31										
32	3112	S								
33	3747	r								
34										
35										♂?
36	3112			F−		±2	±1	−	−	
37	3164			F−		−	−	+2	+	
38	4121			F−		−	±	● −	− ●	
39	4021					+3	−	−	−	
40										
41										
42										
43										
44										
45										
46										
47										
48										
49										
50										



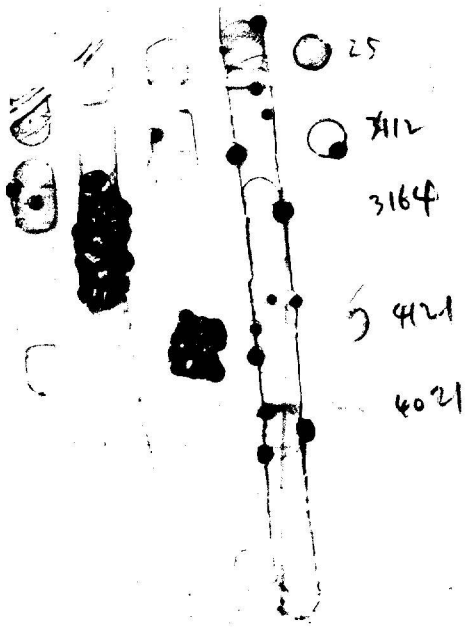
M Lee



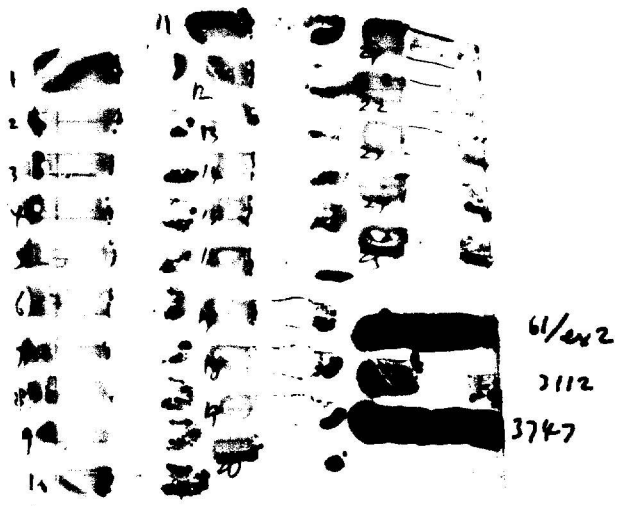
M Lee



M Lee



Test of V_6 loci



On Blue

Retest # 14

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

U.W.
MICROBIAL
GENETICS

Unsuccessful : use M Lac Sm in next time, (Lac61/Lac2 does grow on MLac)

Comparison of the [redacted] frequencies of the transduction of exogenote and endogenote from heterozygous diploid (Lac^-_{61} / ~~Lac^-_2~~ Lac^-_2).

4/VII ; 1959

REF: cf. 105, 146

Principle

F_{13} Lac^-_{61} / Lac^-_2 (ex) (3112 F_{13} × 4121) → × F^- Lac^-_{61} (W4121)
→ × F^- Lac^-_2 (W[redacted]3112)

cultural age : 4 hrs. on rotator.

strain : all these strains used as are purified on BLac and Pick single colony and suspended into B (Ca 10^7) 0.1 ml is inoculated into 5 ml Penassay broth and kept them on rotator for 4 hrs.

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

2) Incubate them for 1/2 hrs. ~~in incubator~~ at 37°C.
3:25 pm

3.) Seed them on M Lac agar after optimal dilutions.
use : 10^{-3} ml, 10^{-4} ml, 10^{-5} ml per plate for each crosses.

4) Count cell numbers of each experiment.

Results :

Servival count

	① F_{13} 61/2 (ex) → × Lac^-_2 (W3112)		① F_{13} 61/2 (ex) → × Lac^-_{61} (4121)		② F_{13} 61/2 (ex) → × Lac^-_2 (W3112)		F_{13} 61/2 (ex) → × 61^- (4121)		3. F_{13}^+ → × 2^- (W3112)		F_{13}^+ → × 61^- (W4121)	
Servival 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				

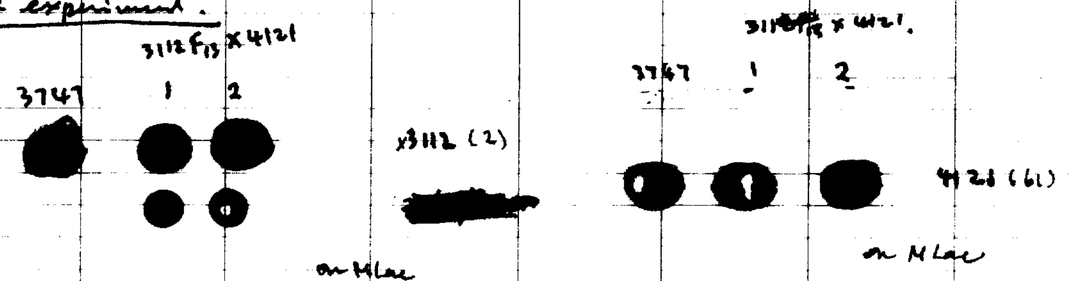
fertility on ml/plate

10^{-1}			~~fertility~~ too much, it canot [redacted] count them. (use 1/100 of these experiments)					
10^{-3}			~~1084~~	~~81~~	~~944~~	~~51~~	~~868~~	~~85~~

~~cells/ml~~

ml/plate

• Qualitative experiment.



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 Lac 2 Ss. → Lac61 F⁻ S^R W 4121 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.

Preliminary test by spotting method

Spot 4121 (61/ex 2) on W4121S^R and W3112 streaked on MLacSm.

Result : see back page.

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

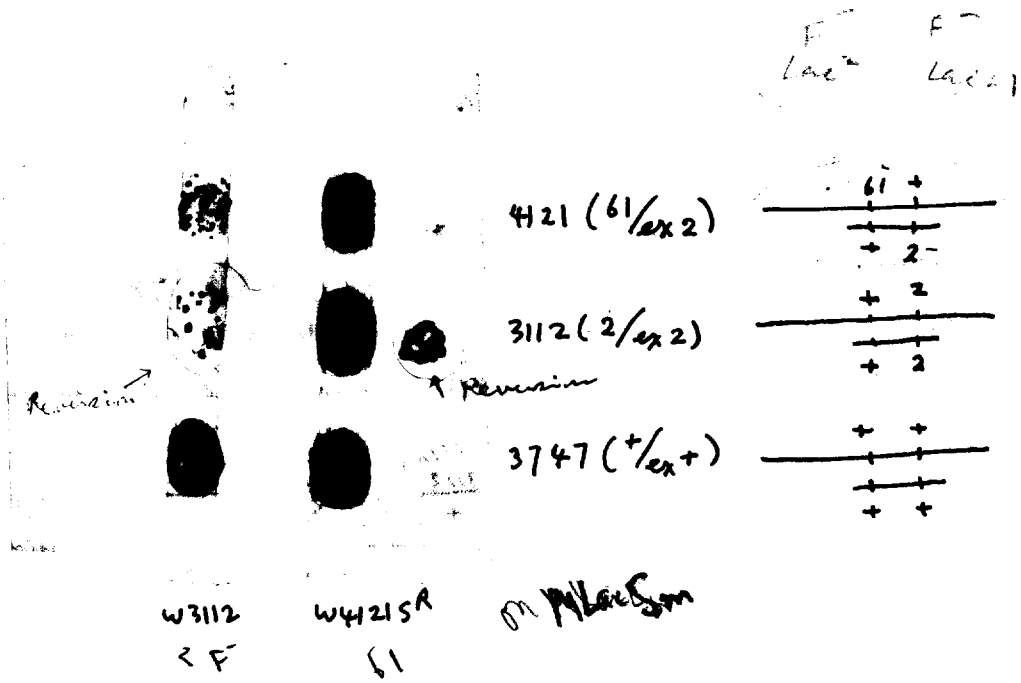
12/VIII **Quantitative test.**

- cultural age : 0.2 ml : overnight culture / 5 ml penassay, at 37°C, shake on rotator, 10:30 AM ~ 3:30.
- Ratio of mix : 1 ml 3112 + 4121 Lac61/ex 2 0.1 ml; 1 ml 4121S^R (W4629) + 4121 Lac61/ex 2 0.1 ml.
- Time of incubation: 1hr ~~3:30 ~ 5:30~~.
- survival count : inoculum size, of original culture. 10^{-6} ml / plate (4121 61/ex 2 × 3112: Lac⁻ 751 : Lac⁺. 10; 4121 61/2 × 4121S: Lac⁻ 0 : Lac⁺ 13)
- Inoculum size for counting recombinations. 10^{-3} ml / plate on MLac Sm.

Result : 1X

		# of Recombinants. plate 1	2	# of colonies 10^{-8} 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 ≥ 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 H1 clone 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.

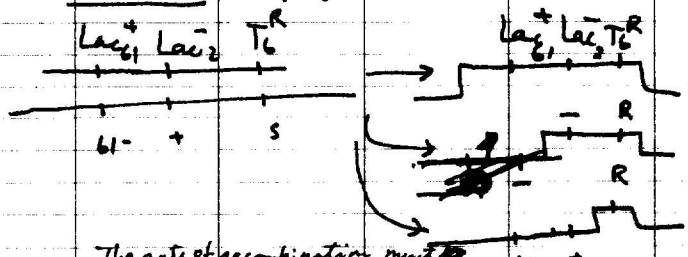

F⁻ Lac⁺
F⁻ Lac⁻
4121 (61/ex 2)
3112 (2/ex 2)
3747 (+/ex +)
Reversion
Reversion
61 +
+ +
+ 2-
+ 2
+ 2
+ +
+ +
+ +
W3112
< F⁻
W4215R
61

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

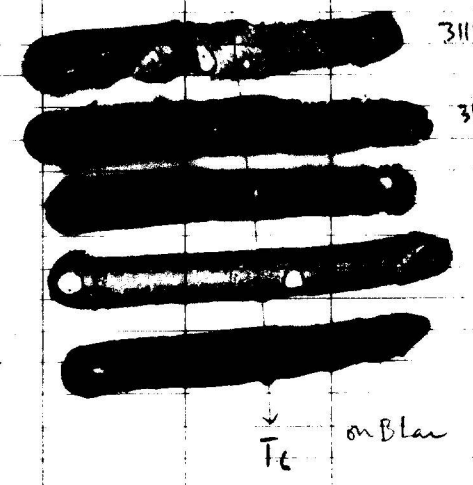
6/VII 1957 REF:

1 2 3 4 5 6 7 8 9 10

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



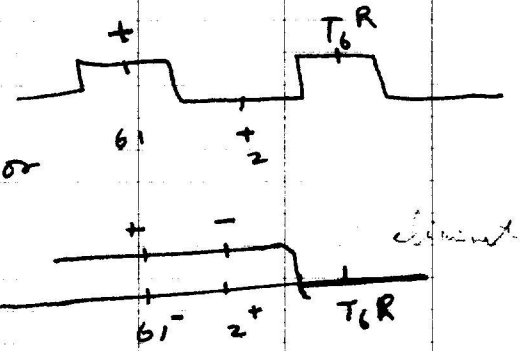
The rate of recombination must be express ratio of length of V_6 – Lac_2 – Lac_{61}.



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

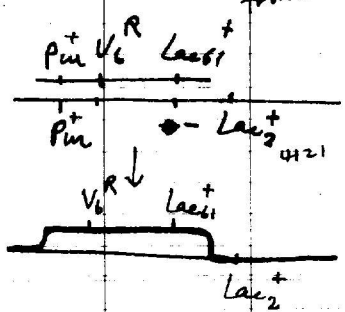
Result: actually the lysed part is also Lac^+.

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



elimination of $T6^S$ segment

or Lac_2 F_{13} is hemizygote for Lac_2.



U.W.
MICROBIAL
GENETICS

136

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

8/VII, 1969 REF:

Make opposit combination
{ W6 F8
{ 3086 F⁺

Strain W3086 F8, W3747 F13 (F8), W6 F⁺.

- cultural age : overnight culture in Penassay broth.
- Inoculum size : ca. 10^4 cells/ml. $10^{-2} \times 10^{-2} \times 10^{-1}$ (0.1ml)/5ml
- 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 F8	Mal⁻ 3086 F⁺
234	89
327	152
227	107

Exp II compatibility

total tested	W6 F8	F⁻	total tested	3086 F⁺	F⁻
44			43		
%					

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

Exp ① 3086 F8 + W6

Treated with AO.

W3086 F8 Σ	F8	F⁻	W6 F⁺ Σ	F⁺	F⁻
35	4	31	35	5	30
21	2	19	24	1	23
Σ 56	6	50	59	6	53
%	89.3 F⁻			89.9 % F⁻	

Replicated on MGal (x4573.)

Conclusion : Looks no difference in accessibility of F. to acridine method.

Untreated control.

3086 F8 Σ	F8	F⁻	W6 F⁺ Σ	F⁺	F⁻
55	55	0	55	55	0
%	100	0	55	100	0

Exp ② W6 F8 + 3086 F⁺

Untreated control

W6 F8 Σ	F8	F⁻	W3086 F⁺ F⁺	F⁻
32	32	0	32	0

x4573 on MGal.

Treated experiment (with AO: 30γ/ml)

W6 F8 Σ	F8	F⁻	W3086 F⁺ F⁺	F⁻
	0	59	0	42
%	0	100	0	100

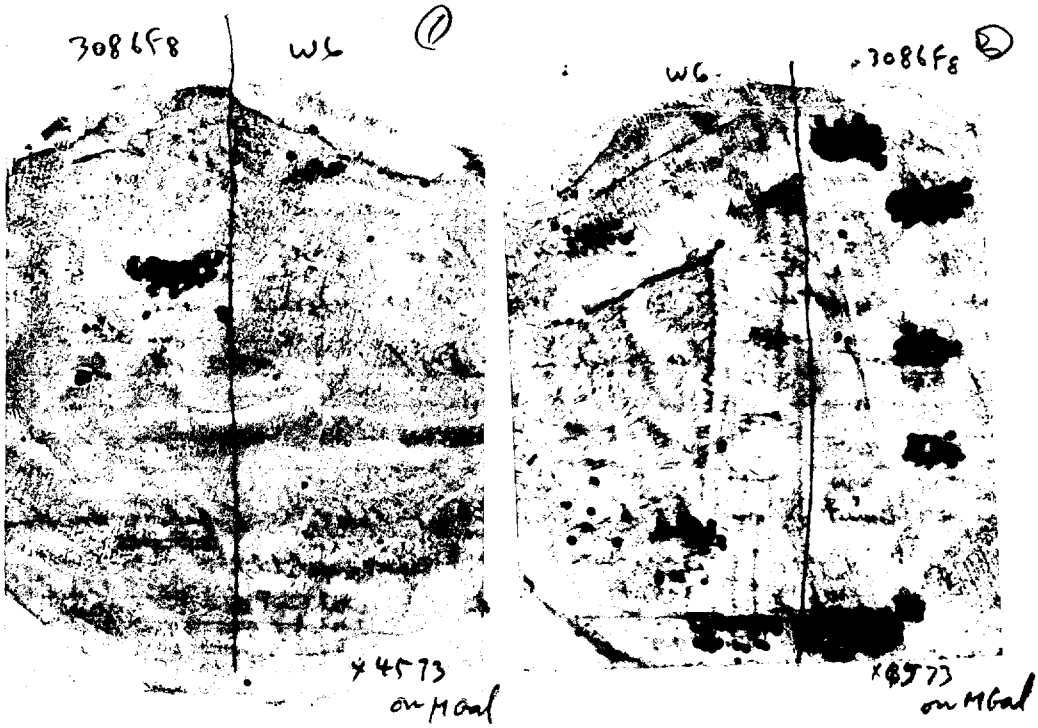
x 4573 on MGal.

Exp (I).

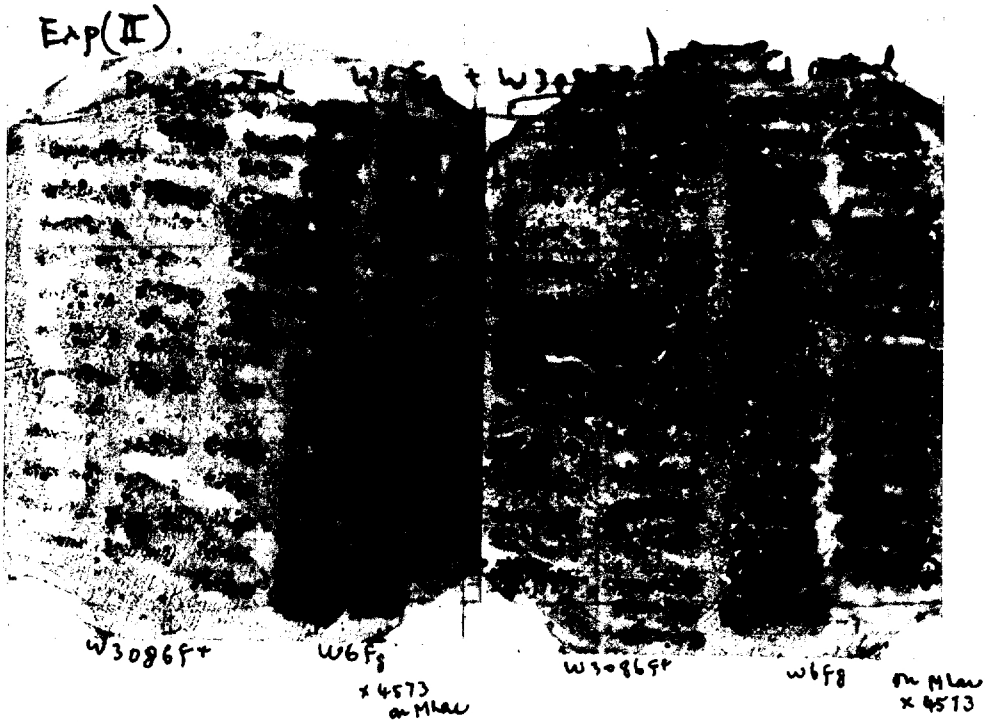
Inoculum ca. 10^3 cells/ml

AO 30 g/ml

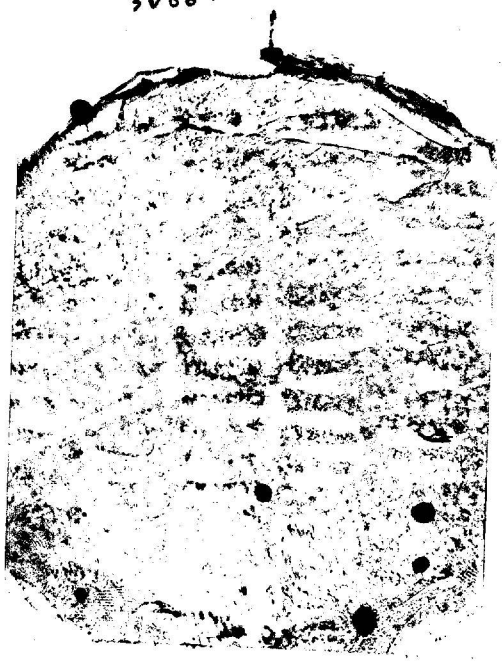
AO 3086F8 + W6 6 min light



Exp (II).



Ao treated
3086 Ft W6 F8.



x 4573
on MGel

Ao treated.
W3086Ft W6 F8

on D MGel
x 4573

Size of Gal - $F_{8\,0}$ segment.

12/VIII ; 1959

REF:

Strain used : ♀ Pur⁻ Try⁻ Gal_2^- Xyl_2 Mtl Mal_1 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

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

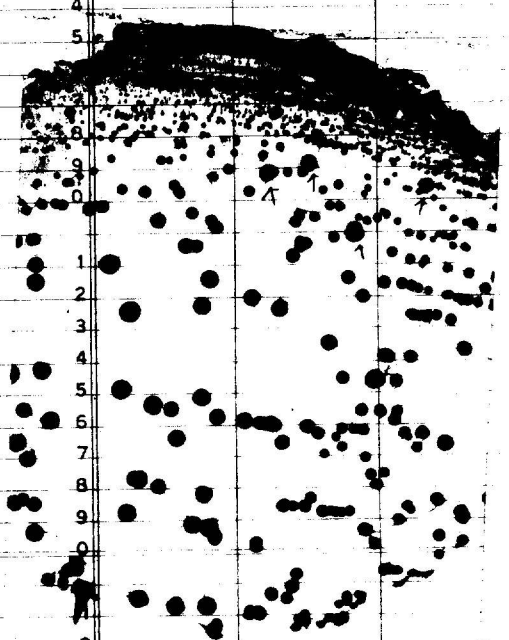
Principle :

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 be came from pur⁻ Try⁻ of the recipient strain.



On B Gal Sm.

5 days

Gal⁻ become black

U.W. MICROBIAL GENETICS

Unsuccessful

Infection of F_3 and Remove of F_1 from it.

138

Principle : $F_3 - F_1 = Rfd_3$

20/VIII 1959

REF: [W4296 shows strong agglutophy with M; see back page 10

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

[→ W4296 $F^-T^-S^R$]

↑ Too much agglutophy.

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

~~4573~~

W3086

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

on M Lac

X 3086

Try it again

Syntrophy of M and T.

3133 F_3 → W4296

on Mlac

W808 W4296

Master plate

on Blac

Unsuccessful.

MICROBIAL GENETICS

Isolation of Lac^{61-} F_{13} by ~~Simple~~ infection of F_{13}

3747 ——— × 4121 S^R

21/VIII 1959 MV$_6$ F_{13} (Lac^{61} 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 the most 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} coexist 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} · Lac^{61}.

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

~~method — Fig~~ 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 plenassy 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.

3/12.

U.W.
MICROBIAL
GENETICS

Isolation of Lac61/ex.Lac61. F13.

24/VIII ; 1959. REF: Lac_x must be Sm^s

Principle:

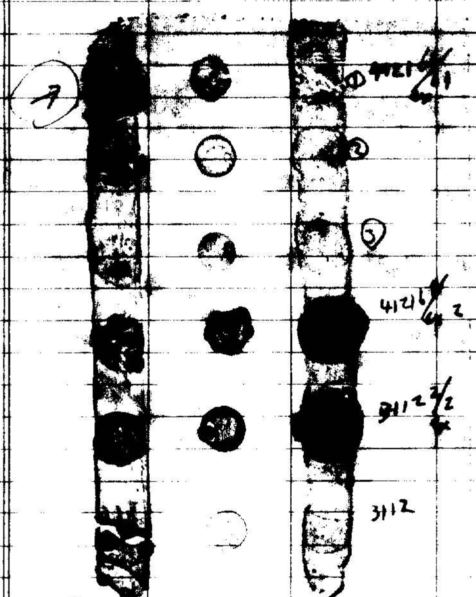
1st step: $Lac_2/_{ex}Lac_2\ F_{13}$ ⟶ × Lac61 F⁻ ⟶ $Lac_{61}/_{ex}Lac_2\ F_{13}$

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

3rd step: $Lac_2/ex\ Lac_{61}\ F_{13}$ ⟶ × Lac61 ⟶ $Lac_{61}/ex\ Lac_{61}.F_{13}$

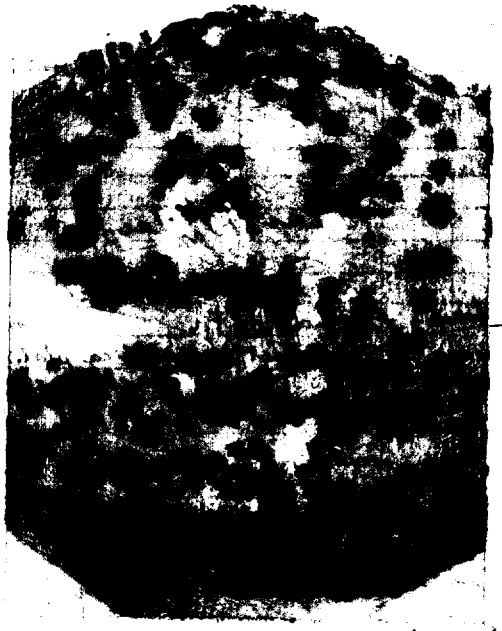
Principle is the indirect selection of a clone which contains many ~~strains~~ cells of Lac2/exLac61 arised by exchange of Lac locus from Lac61/exLac2.

Retest



Replicated from Blac.

1st selection.



Pick.

on MLac
× 3112.

4121 61/4x2.

3112 3/2

3787 1/+

4121 3/4x61

4629
Lac 61.
SR

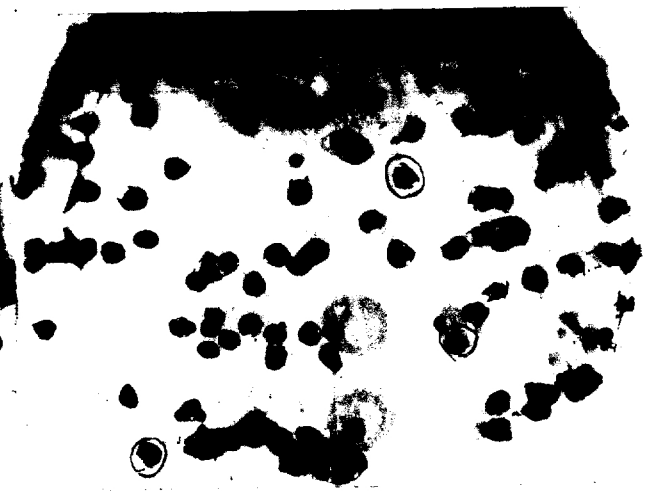
3112
Lac 2
SR

← Replicated on MLac

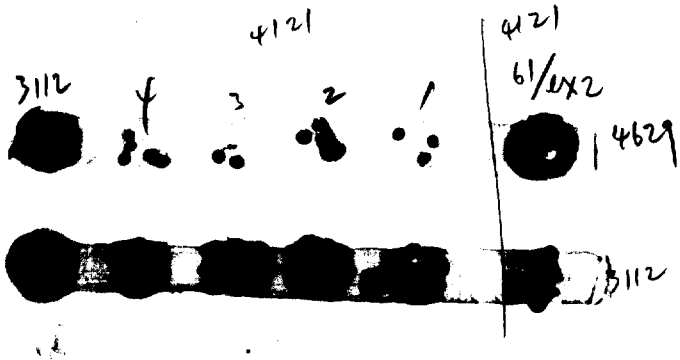
Repunify

2nd selection. on Blac

on MLac



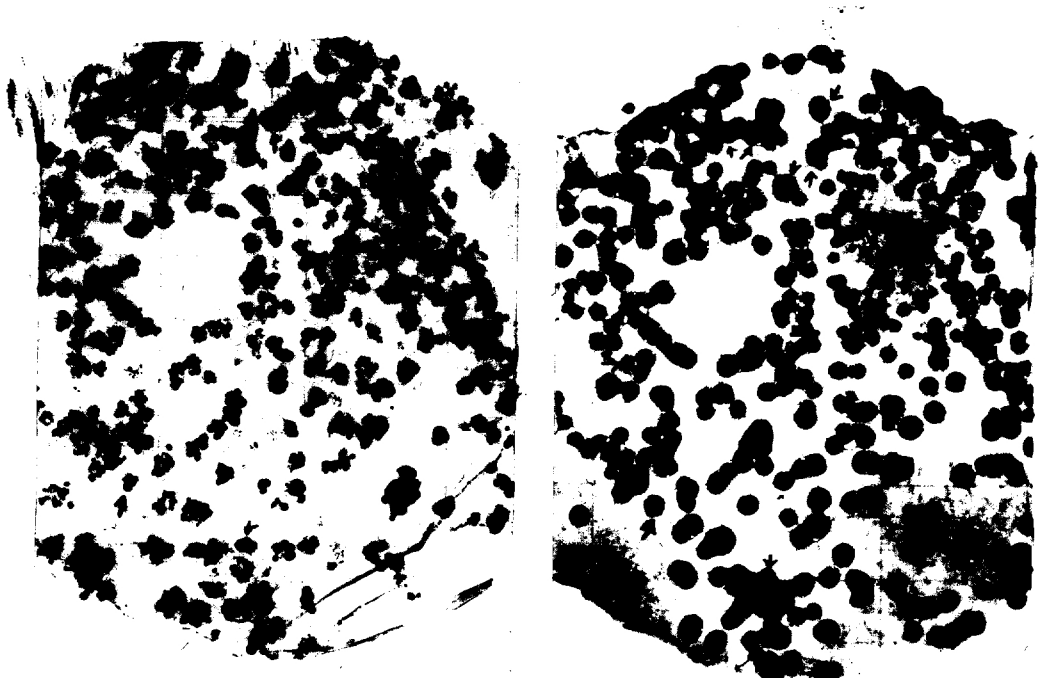
×3112.



U.W.
MICROBIAL
GENETICS

[illegible] ; 1959 REF:

Isolation of H1 Gal from W3747



MGal
×2979.

on Blac

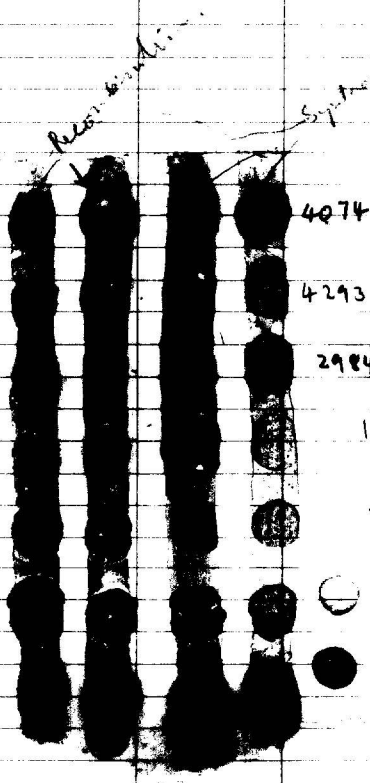
U.W.
MICROBIAL
GENETICS

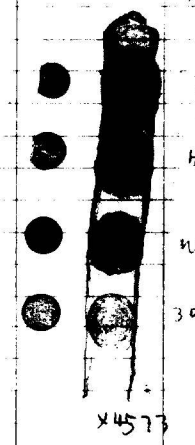
142

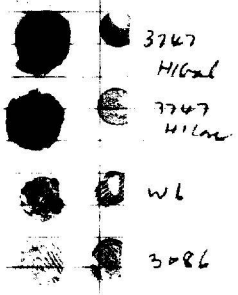
Comparison of the fertility of W3747, HI Gal and HI Lac.
I II.

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

U.W.
MICROBIAL
GENETICS

143

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

26/VIII ; 1959 REF:

W4540

Strains : W6 F_2 , W4573,
H^- F_2 $Ara_2 Lac_{85} Gal_2 S^R Mal, Xyl_2 Mtl$.

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

$W6F_2$: 2 ml
W4573 : 0.2 ml

# of Recombinants/plate

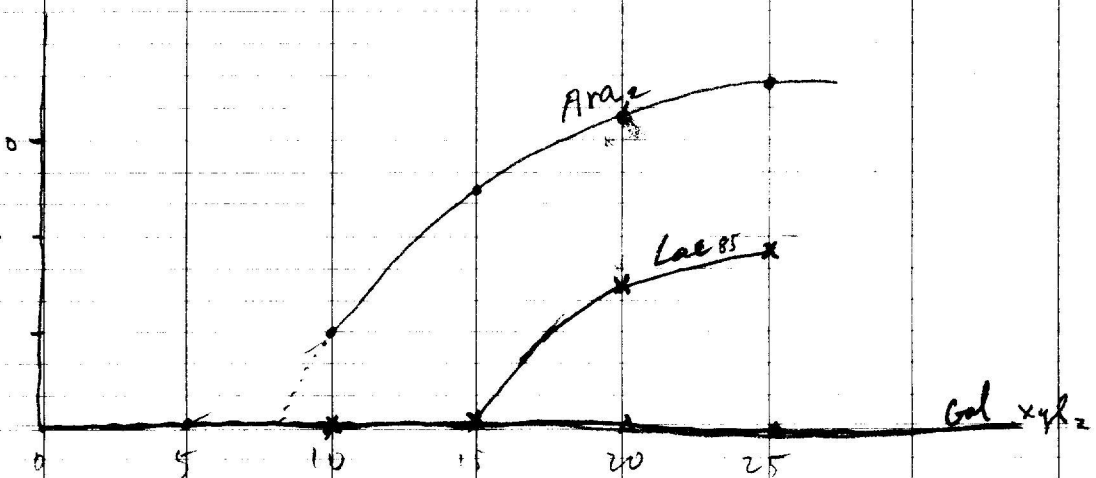
Time	0	5	10	15	20	25
	Ara_2 Lac Gal Xyl	A, L, G, X	A L G X	A L G X	A L G X	A L G X
plate# 1	0 0 0 0	0 0 0 0	1 0 0 0	9 0 0 0	45 1 0 0	51 3 0 0
10^{-3} ml 2	0 0 0 0	0 0 0 0	0 0 0 0	12 0 0 0	33 1 0 0	57 5 0 0
Σ	0 0 0 0	0 0 0 0	1	21 0	78 2	108

Survival count	Lac											
$\times 10^{-6}$ 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 %

%



U.W.
MICROBIAL
GENETICS

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		Cross	:	10:30 AM. ~ 12:30 PM			at 37°C.			
2				. ratio of mix			of 0.1 ml	:	8	1 ml.
3				. mixed well.						
4										
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		
1			Pre incubation.							
2										
3			Post incubation.							
4										
5		W 4121	61/ex 2	x 3112.						
6							3	364		
7						1148	4	547		
8			Pre							
9										
0			Post.							
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:

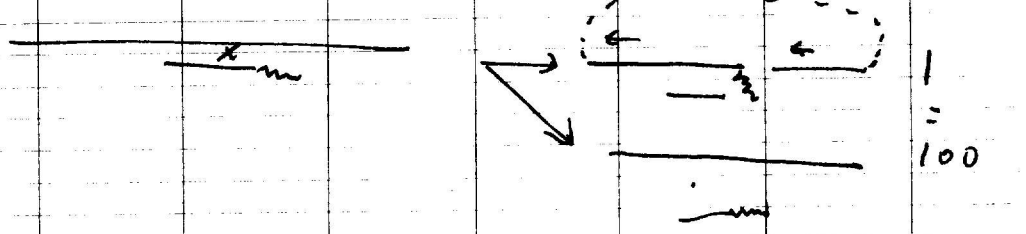
Cultural age : Overnight cultures.

		Fertility		Survival (10^6 ml)			Rec./Lac$^+$
				Lac$^+$	Lac$^-$	+ −	
W4121 61/ex 2 → × W4629 (F$^-$ Lac$_{615}$ S^R)		10^{-3}/ml	10^{-2}/ml				
		79	1214	8	38	8 71	
		180	1097	5	45	8 75	
		Ca. 100	Ca. 1000	Ca. 5×10^8	4×10^7		Ca. 10^{-2}
W4121 61/ex 2 → × W 3112 (F$^-$ Lac$_2$ S^R)		0	12	1	27	18 15	
		0	20	3	32	23 155	
		0	Ca. 10~20	Ca. 2×10^8	Ca 3×10^7		$< Ca. 10^{-5}$
		10^{-3}/ml	10^{-2}/ml	+	−	+ −	
W3747 +/+ → W4629		1231	68	248	35	174 14	
			101	~~262~~ 1~~20~~	~~79~~ ~~46~~	138 20	
				262	41		
		Ca. 1000	Ca. 100	Ca 2×10^8	4×10^7		Ca. 10^{-2}
W3747 +/ex+ → × W3112				+	−	+ −	
		1261	52	170	46	240 70	
			113	262	79	291 68	
		Ca 1000	Ca 100	Ca. 2×10^8	5×10^7		Ca. 10^{-2}

Conclusion:

Fertility of exogenetic ≅ endogenetic ×100.

This may be explained by crossing over between F' and host-chromosome at about 1%.



Transformation of Gal in 4-12.

20/X. 1959

REF:

Experimental condition:

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

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.
the primary grown. suspended into H_2O.
32 m/s of w6fg culture was used. (4 tubes of 8 ml pen.)
↓
concentrated into 2 m/s.

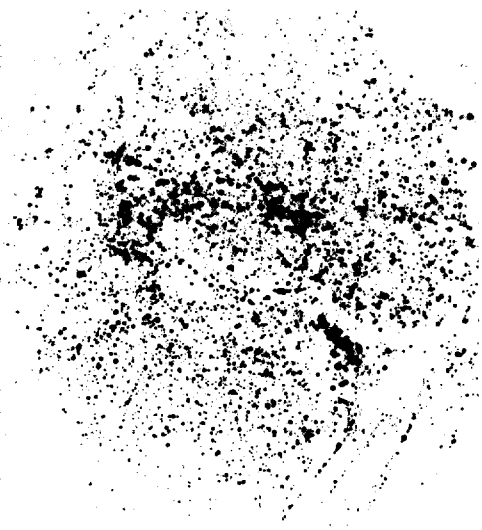
1 ml	1 ml
↓	↘
treated with duponol. final 1%.	untreated
20% duponol Soln 1 ml	H_2O 1 ml
+	+
Bact. Suspension 1 ml	Bact. susp. 1 ml.

4. Method of transformation.

DNA	dilution of Original culture	10^{-1}	10^{-2}	10^{-3}	
# of t_3 Recombinants.		~~numb~~	521	43	
survival cells in 0.1 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.

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

23/IX ; 1959 REF:

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 dupond 20 %.
2. ~~Time of~~ treatment ~~with~~ with dupond : Na-lauryl-sulfate.
(final conc. 10 %. in dist. W.)
37°C 4hr.
3. Filtered through millipore filter.
4. Mix : dupondated sample.
0.2 ml of W4573 + 0.2 ml of broth + 1 ml of DNA.
: old culture (2days)
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.

U.W.
MICROBIAL
GENETICS

Action of propamidine isethionate to F and F' (I)

(P.I.)
4:4'-diamidino-diphenoxy propane-di (β-hydroxyethane sulphonate) 5 gr.

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

Yoshino gave me on 11/Sept.'59.

Stock solution: 100 mg / 1 ml soluble in water.
(Weigh 100 mg of P.I. and dissolved into sterile H_2O — 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	(x1/10) 100γ 0.05	x1/100 10γ 0.05	x1/1000 1γ 0.05	Control 0γ
W6 F8	−	−	−	+	+	+
W6.	−	−	−	+	+	+

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

· Media. 5 ml Penassay broth, pH 7.0.

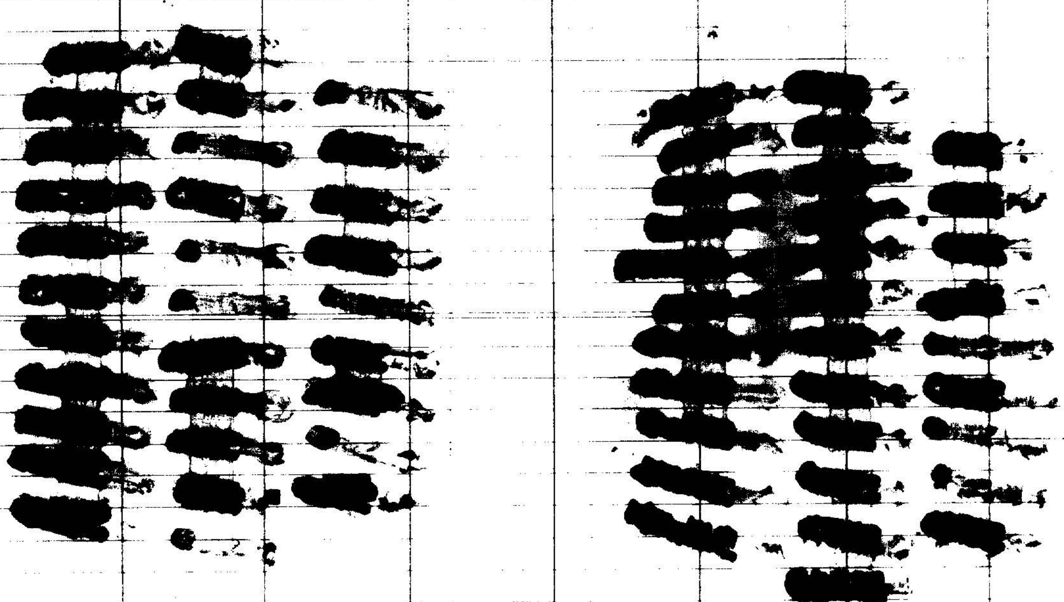
2. Streak these treated cells on Blue agar.
100γ. P.I.

3. Test their compatibility by cross-brushing method.

Result:

P.I.-treated 10γ/ml Rn.			Untreated control		
F−	F+	% F−	F−	F+	% F−
7	24		3	29	

W6 P.I. W6 Cont.



Mlac M573

U.W.
MICROBIAL
GENETICS

Action of Propamidine isethionate on F. II

150

24/IX ; 1959

REF:

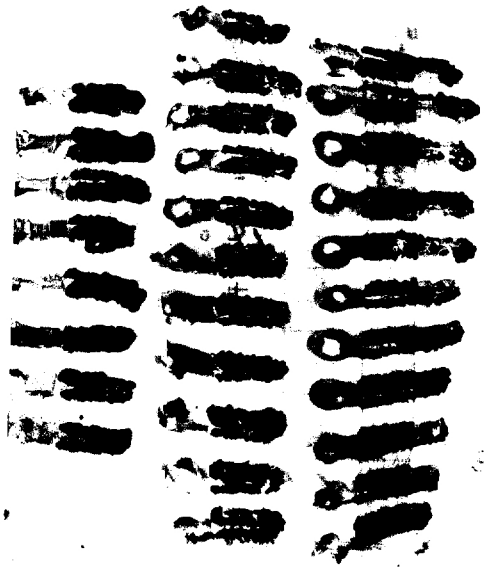
1	2	3	4	5	6	7	8	9	10
	Med. Penassay.	inoculum size: ca. 10^6 cells/ml.			Strain: W6.			(×1/100) Conc. of P.I. Soln used: 1 mg/ml.	
Conc. of P.I.		0γ / 0 ml	10γ / 0.05 ml		20γ / 0.1 ml		50γ / 0.25 ml	(5 ml plm.)	
Ratio of conversions F⁻/total tested		0/30	0/28		0/28		4/29		
(%)		0	0		0		14.0		
effect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

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

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

cont.

W6 R.I. 08.

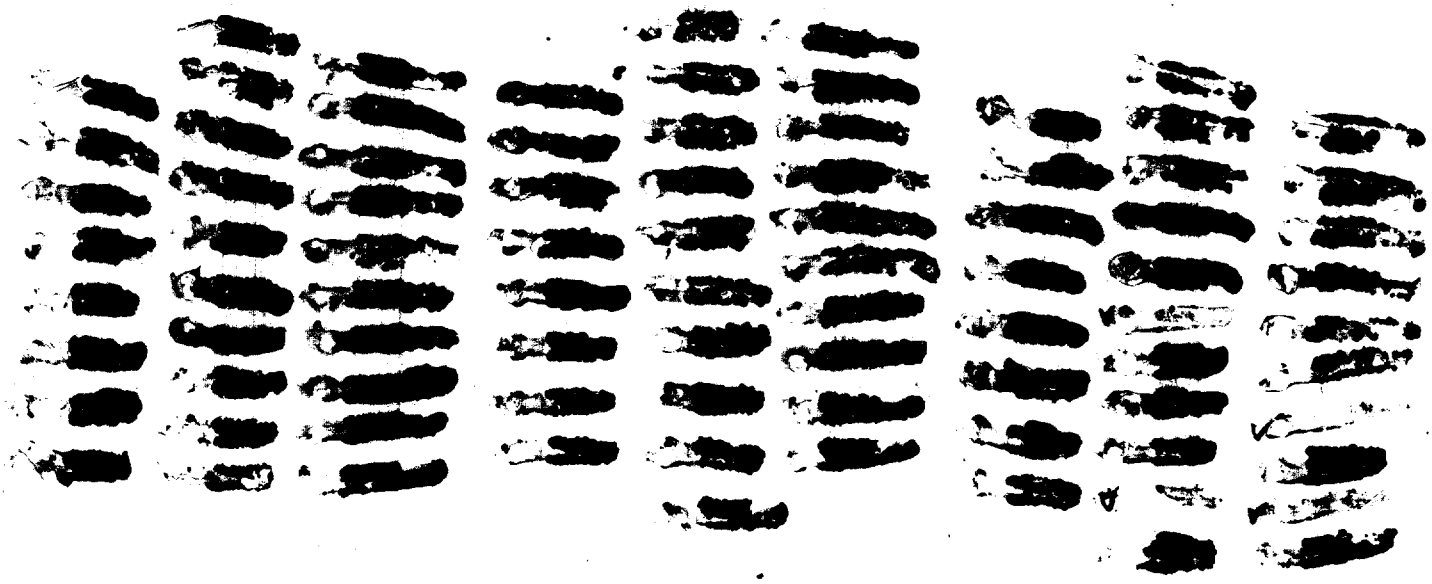


MLac
X 4573

P.I 10g

P.I. 20g

P.I 50g



MLac X4573