Mutants of F particle.

I. TRAITS OF F-MUTANTS. F' strains made.

a) Transmission of high-transfer-determinant to various strains.

p26 (F_{13})
p.16 (F_4) p57 (F_2)
p15 (F_{11}, F_4, F_6)
3086F': P.7, (F_8, F_{17}, F_{19})
w6F': P. 8 (F_8) P.19 (F_{17}, F_{19})
P.13 (F_8)
P.17 (F_6, F_{11})
P57 (F_2) P62 (F_2)
P.63 (F_5)
P.75 (F_8)
P.77 (F_3) P83 (F_3)
P.85 (F_6) 3828 Y10
P102 (F_3) 3133
P.120 (F_{13} Lac)
P.103 (F_{13} 3086)

1. High marker mutant of F

High marker mutant of F	High marker ⟵⟶ Low marker
F8	
F15	
F19	
F1	

2. Transmission of High-Transfer-~~Mutant~~ Factor (HTF) to F-. to F'+, Hfr, F^r and F+

They are transfered from one cell to another by short contact, and gives the same high transfering characters to F- cell. (see P.5, P16) In other words, they ~~shows~~ retains genetic continuity of the traits during transfers.

3. Transfer of HTF by cross. (x F-, and xF+ , and xHfr.)

p44. P.76 a, b.
P.25, P45 : Resistant to infection of F_4, F_8, F_1

4. ~~Transfer~~ Infection of HTF to F^r and Hfr, and F+.

(p.24) seems unsuccessful. (p.28) (p.48) clear.
Test again by replica method: use gal.

5. Transfer of HTF to Female-3 and Female-12.

(p 23) trait
w3444 F_8 ♀3
(p.46) states
(P55) (p.70)

States: Sometimes they shows two states, but not high for both. After cure of F_8 with AO still shows ♀3 trait.

6 Sensitivity to Acridine-treatment. (p.20) (p 22) (p33) P(34), P70 P82 P95
w1394 F_8. w6 F_8 F_8 F.4 Hfr4 F8 F8♀3 F3.
Sensitive to AO.
P122 (3133 F_3^-)

7. Recombination ~~of F~~ between Rfd3. and F_1. (3442) > Hfr3 – X 3133 P55 P77, 828 P110.
Is there some kind of exchange reaction between Host cell (♀1) and F_1?

~~8. Host cell and F'. Does host cell retains a factor of F?~~

8. States of F' ♀ and F'++ ♀3W

p23
p.46
P.70, P93, P90 → cure F_8 ♀3 and reinfect F_1 and see 2 states

9. [illegible] of defective F from F'
P, 35, 36, 37.
P49: F8' (final test for fertility)
P54 (infection of F8)

10 minimal cell number for infection (P.47)
P51, P52.
F'4

11) Double F. P.43. ($F_8 \rightarrow \times F_4^+$) P31 ($F^-$ or F^+)
P.42 ($F_4 \rightarrow \times F_8^+$) P42; P.50 (segregation from F_8F_4)
P.53 ($F_8 \rightarrow \times F_1$, $F_4 \rightarrow \times F_1$)

12.) Segregation of different F' from double F. strain. P28, P29, P72, P.73.
P42, P50, P48, ~~P52~~ P53

13) Low markers for F's obtained. (between two himarkers transfered by A_2, A_3, & Hist. P.38 two F's)

14). Reversion of F_8^* and F_4 into F_1 by U.V irradiation. (P.56) (P51) (P52) F_8^- P49. F_8

15). Infection of F' of Killed cell $F'^+ \rightarrow F^+$ F_8 (P 35, 36, 37) (P.58) (P.69) (P97) P98. successful.

16) Test for Immunity of ♀ obtained from F'^+ by U.V. irradiation.

$F' \rightarrow F^R$: 44, 45, 76, 81, 89, 94 $F_8 \rightarrow F_8^-$ P54 (F_8^-); $F_4 \rightarrow F_4^-$ P.59 (F_4^-); $F_1 \rightarrow F_8^-$ P61 (F_8^-)

17) Testing system for F' transfer. (P.64)

18) Recombination between defective (F_8^{ad}) (F_4^{ad}) F' and F_1.
Unsuccessful. P.61 ($F_1 \times F_8^-$) W4544) P68
P78. P.75 ($F_1 \times F_8^-$) W4544 P68, P67 back page.

19) Recombination between defective F' (P65)
Unsuccessful.

20). Test other fertility of W4171 (: W1895 F^+)
P.67. : Same as W6.

22) Recombination between F's. (F_4 and F_8). P.71
Unsuccessful P.79
P.96 (F_4 and F_2)

23) Host range mutant of F'. P 76 a, b P 81. P89, 94.
It was not Hostrange mutant. (F_8) Infective to F^R.

24) Low infectivity of f_3 to F^-. P.84, 83
negative result.

25) 3828 f_3^- isolation: P82

26) Two states of F_8^+ f_3. "W4525" , P86; P90.; P70 a, b.

27) Aggregation of F' P92

28) two states of F_3^+ : P.87. 95

29) Infection of F' by Sm-killed cells : P98, 99, 100, 101, 107, 108

30) Transduction of Lac locus by F_{13}^*. (Test of 3747: P, 103: Segregat. : 104, 105) P106, 109.

31) Size of transduced segment of F_{13}. (P. 112; 112a, b, c, d, ; Pur 113)

32) Elimination of Lac-F_{13} by 40-method. (P. 115; 116; 117, 118a, b, 127,)

33) Rate of multiplication of Lac-F_{13}-segment. (119, 123, 127a graph. 124)

34) Infection of F_{13} to Lac⁻ mutants. (120, 111,)

35) Timing experiment of transduction of (Lac - F_{13} segment) (F_2T) (121, 125a, 125b. (Test for linkage of segments)) (143)

36) Stability of Lac⁺ marker of the 40-treated strain. (127b)

37) Rate of multiplication of Gal-F_9 segment (cf. 32) (128a, b) 129a

38) Cistron analysis of Lac locus (1) (P. 126) (P. 111)

39) Analysis of segregant type from $^{61}/_{ex2}$. (P. 130, 131, 132)

40) Comparisons on the frequencies of transfer of endogenote and exogenote (P 133, P 134), P 145 146

41) Linkage between V_6 and Lac (P. 135) in F_{13} segment.

42) Comparisons on the accessibility of maleness. (P. 136)

43) Recombination using killed ♂. (P. 147, 148) by dupond. 151, 152

44) Action of propamidine isothionate to wild type F (P. 149) P. 150

45) Test on the ~~dual type~~ Compound structure of F'.
Does F'⁺ contain wild type F within the F'⁺ cell. (P. 153 a, b 155

This ~~possibility~~ question came up from two phenomena.
1. F'⁺ cell become F⁺ after UV irradiation
2. F'⁻ segment seems not defective, and can multiply much quicker than host cell.
These two observation seems quite different from 1. F becomes defective because of crossing over. If F' is not defective, how F makes crossing over with bacterial chromosome.

Two hypothesis can be applicable: 1. F' is defective and will co-work with F.
2. F' is not defective, and can be segregated very easily.

46) Reversion test for homozygosity of Lac_2 in 4560 (Lac_2 F_{13}) (P. 154)

47) Study of F_{13} (102, 104, ; 103, 104

142; 142

Trials to get lac⁻ F_{13} . from.

lac⁻ mutation	strain No.	Result	Ref
Lac 85	4411.	F^- and $F\lambda^?$ weak.	P 114
Lac 2	3112	F_{13} & F^-	P 120
Lac 4	3127	No good because of back mutation.	P 120
Lac 52	4112	F_{13} & F^- & F^+?	P 8 (illegible) P 9
Lac 87	4147	F^- , & F^+? , F_{13}.	

Isolation of mutant of F. from Hfr 1.

22/10 1959 REF:

U.V.

Principle:

M T L Hfr$_1$ → M T L F' → × Lac F$^-$ S^R W3828.

W 2061 mutation

T L F$^-$ S^R W1394 (T L Th S^R F$^-$)

M F$^-$ S^R W 3086 (M Hal S^R F$^-$)

Experiment 1. Test Hfr$_1$: H1 for lac transfer

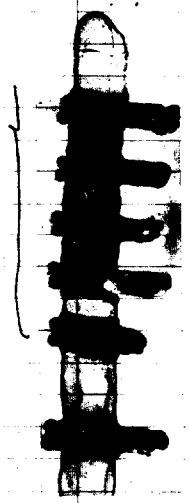
(W2061: 10^9 cells) wash, and

2 Irradiate W2061 by U.V. for 30 sec. → Incubate overnight, 0.1 ml and mix with W3828. in 5 ml pp 0.1 ml

~~or mix with W3828 after infection~~ in penicillin. Shake it on rotator for 48 hrs ~~Incubate overnight~~ at 37°C. (W3828 ca. 10^8 cells/ml)

3. Dilute and spread it on BLac SM. agar and incubate overnight

4. Replica plate it on MLac B, seeded W3086 or W1394 and look for ~~black~~ colony. (H1 recombination reaction.) ~~which~~ which gives high frequency recombination reactions.



× W3828 on MLac.

- U.V dose : @ 30 sec.
- Suspension : 10^9 cells/ml.
- age : Overnight.
- Condition of exposure: ~~=~~ centrifuge resuspend into H_2O.
- Exposed with gently shaking.
- # of W3828 added: Ca. 10^8 cells/ml plm.
- # of W2061 irradiating (inoculated after Ca. 10^8 cells/ml plm. (0.1 ml/8 ml pp)

Isolation of mutant of F II. from Hfr,

21/III, 1959 REF:

Method :

W2061, overnight culture 5 ml.
↓
Suspend into dist. Water (1 ml)
↓
Irradiate it by U.V. for 60 sec.
↓
add 0.2 ml to penassy (5 ml)
↓
Shake it overnight on rotator at 37°C.
↓
spread it on BLac SM.
↓
Replica plate on MLacB, seeded W1394.

add overnight culture of W3086 to it. (0.2 ml/tube)

→ cross brush with W1394 on MLacB,

• UV dose : 60 sec.
suspended in dist
ca 10^8 cells/ml.

Result :

All the colonies tested are not Hfr. : ca. 500 colonies / plate. 20 plates are tested. (∴ ca 10,000 colonies)

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Infection of Hfr-character to F-

→ W536

17/XI ; 1959 REF:

	1	2	3	4	5	6	7	8	9	10

Lo Hi

Principle. select on B Mal SM:

Gal	Hfr 23	M lac SM^r,	W4397	⟶× W3086	v	B Mal SM
Gal	Hfr 6	M φ^s	W3807	⟶× W3086		B Mal SM
Gal	Hfr 13	M φ^+	W3200	⟶× W3086	v	B Mal
L; Axyl Thg	Hfr 5	M φ^s Gal^+	W4097	⟶× W3086	v	B lac SM
	F^+	M	W 6	⟶× W3086		B lac SM

Ratio of Mix 1 : 0.1, Time of mix : ~~overnight~~ 2 hrs at 37°C.

Inoculum size : 10^8 : 10^7 in 5 ml penassay.

tester for Gal : W2979 on M Gal.

Procedure :

1. Mix 17/XI
2. purify 18/XI
3. Test Hi for Gal. (19/XI.) by cross brushing method.

1:30 ⟶ 3:34

Result.

~~W4397 ⟶× W3086~~

Infection	F⁻/Tested	% of Hfr converted	Infectivity
W4397 ⟶× W3086	30/30	0	–
W3200 ⟶× W3086	30/30	0	–
W4097 ⟶× W3086	30/30	0	–
W3807 ⟶× W3086	30/30	0	–
W 6 ⟶× W3086	3/30	10	+

Conclusion : Hfr. ~~character~~ used for ~~a~~ transfer of Hfr-character does not transmit Hfr into F⁻.

Test for Hfr mutants.

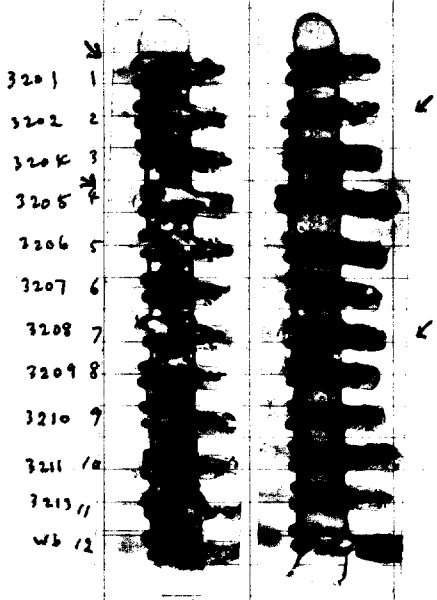
27/III 1959

REF:

M[illegible] Hfr, fertility.

strain #	Lac	Gal	Hfr or F+	# of Hfr
W3201	++	+	Hfr (Lac)	15
W3202	++	++		16
W3204	+	+		18
W3205	++	+	Hfr (Lac)	19
W3206	++	++		20
W3207	++	++		7
W3208	++	++	Hfr (Gal)	8
W3209	+	++		9
W3210	+	+		10
W3211	++	++		11
W3213	+	+		12

Method :
Cross brush on MLac and MGal x W3828 or W2979



Conclusion:
use
- W3201
- W3205
- W3208

— × W3086 for infection.

control W6.

Purify and reisolate by replica plating.

W3202
W3206
W3207
W3209
W3211

Infectivity of Hfr-character.

21/III ; 1959

REF: W 3086 F⁻ M Mal 5ᴿ

W 3201 Hfr M (Hi Lac)
W 3205 Hfr M (Hi Lac)
W 3208 Hfr M (Hi Gal)

Principle.

W 3201 —× W 3086
W 3205 —× W 3086
W 3208 —× W 3086

in penassay. #. Ca. 10^9 cells/ml.

Control W6 —× W 3086 and test 3086
× 3828 H Lac
× 2979 M Gal.

Method

1. Mix them. 5 ml (donor) : 0.1 ml recipient.
2. Shake them for 4 hrs. on rotator at 37°C, 10:00 AM — 2:00 PM.
3. Purify it on B Lac
4. ~~Cross-brush~~ Replica plate it on M Lac and M Gal × W 3828 and × W 2979.
5. Pick Hfr colonies into penassay and test the Hfr-character by cross-brushing method.
6. Put into stock the F′ mutant.

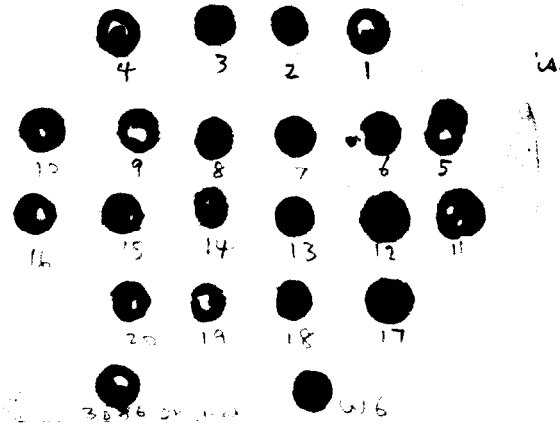
Result.

Test for compatibility.

Transfer	Isolation # from (Replica plate) master plate	Hi for Gal × 2979 (M Gal)	Hi for Lac × 3828 (M Lac)	type	Sugar Marker (check for 3086) Mal
3208 —× 3086 F8 Gal	1	+++ c	+++	F8	—
	2	+++ c	+		—
	3	+++ c	+		—
	4	+	+		—
3201 —× 3086 F15 Gal	5	++	++	F15	—
	6	+ ?	++		—
3208 —× 3086 F8 Lac	7	+++ c	++	F8	—
	8	+++ c	++		—
	9	+++ l	++		—
3205 —× 3086 F19 Lac	10	+	+++ c	F19	—
	11	+	+++		—
	12	+	+++ c		—
	13	+	++		—
	14	+	++		—
	15	+	++		—
3201 —× 3086 F15 Lac	16	++	++	F15	—
	17	++	++		—
	18	++	++		—
	19	++	++		—
3205 —× 3086 F19 Gal.	20	+	++		—
Control W6		+	+		+
3086		—	—		—

conclusion : F Gal and F Lac are isolated.
Further step : Infect these F mutants to W6 F⁻ (W4354)

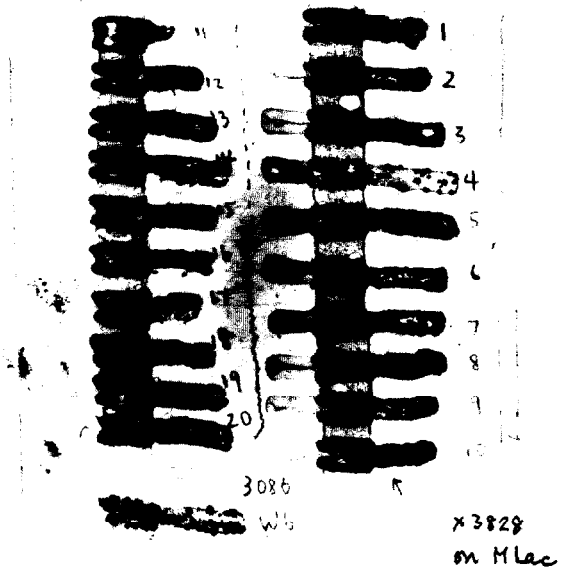
→ x 3086



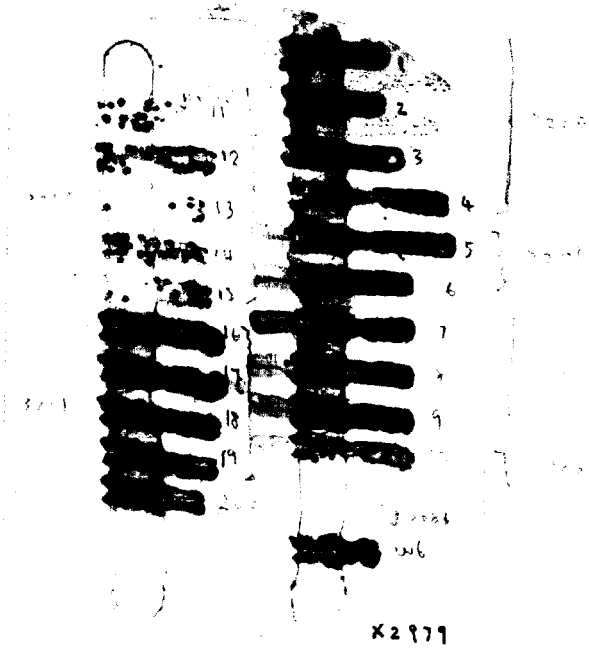
isolation no.

on BMal.

Hfr → x 3086.



x 3828
on MLac



x 2979
on MGal

Infection of F mutants to W6 F⁻ and 3828.

1/IV/ 1959. REF:

Purpose : Make standard Flac and Fgal. (W6 Fgal, W6 Flac etc.)

cultural age : (10:00 —— 3:00.) 5 hrs on rotator (initial inoc. ca. 10^6-10^7)

Principle :

3086 Flac	No 10 —×—	(W6 F-) W4354	Bmal
3086 Fgal	No 1 —×—	(W6 F-) W4354	Bmal.
3086 Flac	No 10 —×—	W3828	Blac SM
3086 Flac	No 1 —×—	W3828.	Blac SM

Method

1). Mix them in 1 : 1. (Inoculum size : 10^8 cells/ml) 1 ml.

2). Shake them on rotator for 3 hrs. (3:00 PM —— 6:00 PM) at 37°C.

3) purify it on Bmal agar.

4) Test mal⁺ colonies in sex-compatibility by cross streaking method. on MLac (× 3828, × 2979), and confirm S^S on BlacSM.

#1 3086 Fgal —× W4354

#10 3086 Flac —× W4354

Hi-marker does not transfered.
→ Why? Is it reverted? or It may be state II.
Try again.

Retest the futility of these clones. ×3828 on MLac
×2979 on MGal.

Retested result.
Hi-marker is transfered to F⁻.
see. back page.
It was state II.

×2979.
on MGal

SM-Resistance are tested on BlacSM agar.
(3086 : SM^R ; W4354 SM^S.)

2/30 : 6.67%

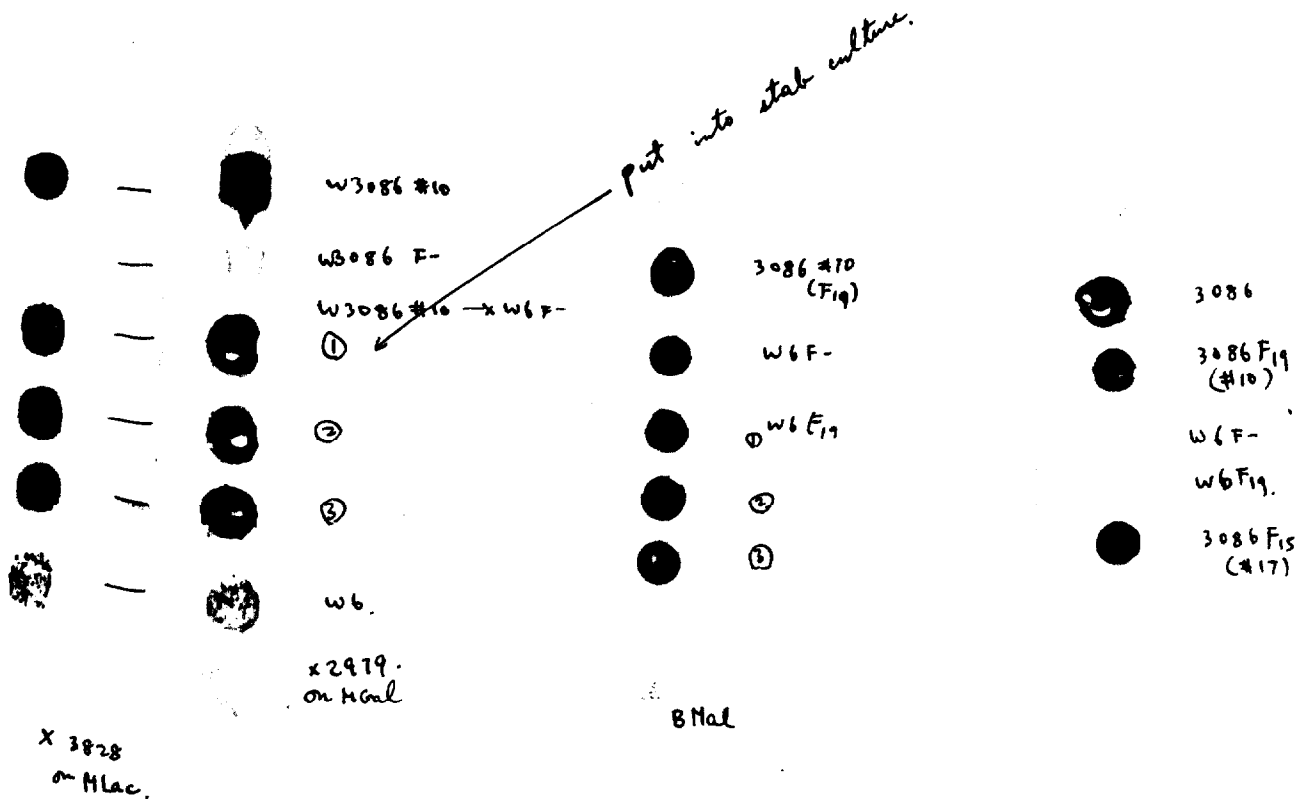
×3828
on MLac
4/33 : 18.2

W4354

← This is good for standard F_8 donor
W6 F_8^+

on BlacSm

on Blac.


put into stab culture.
W3086 #10
W3086 F-
W3086 #10 → x W6 F-
①
②
③
W6.
x2979.
on Mmal
X 3828
on Mlac.
3086 #10
(F19)
W6 F-
① W6 F19
②
③
B Mal
3086
3086 F19
(#10)
W6 F-
W6 F19.
3086 F15
(#17)

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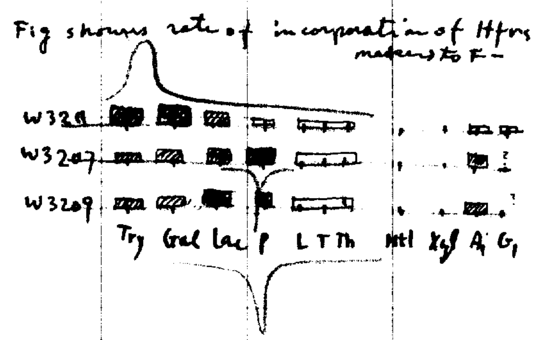
Reisolation of Hfr strains
(from Hfr7 — Hfr16)

2/14, 1959 REF: See 27/11, '57

Purpose : Almost of these Hfr strains are reverted into F⁻.
Reisolation of these Hfr are done by replica method.

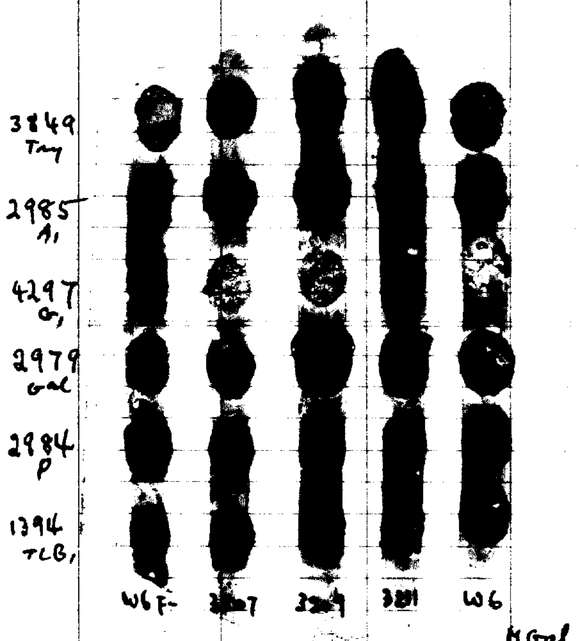
Selective medium : M Gal (x2979)
: M Lac (x3828)

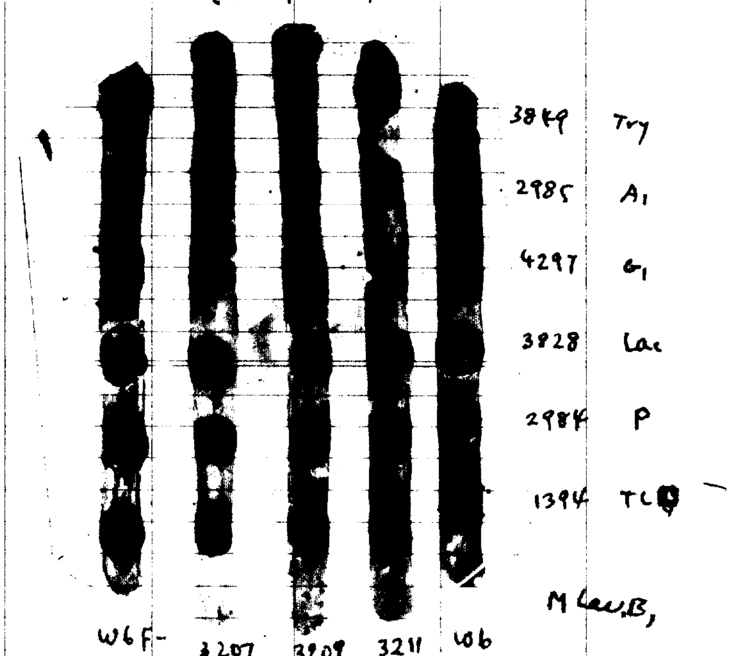
	fertility × 2979 M Gal	fertility × 3828 M Lac	Hfr
W3211	+++	+	O Hfr11
W3202	+	+	
W3209	+	+++	O Hfr9
W3207 a	+	++	
W3207 b	+++	++	O Hfr7
W3206 a	++	++	
W6	+	+	



Retest Hi markers of these Hfr strains. W3211, W3209, W3207.
(Hi : Prolin)

many recombinants appears very late in W3207 + W3209 →





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Infection of ~~F8~~, F_{15}, F_{19} to ~~3086~~ W4354 (W6F−) ~~and W3876~~

3/18 , 1959

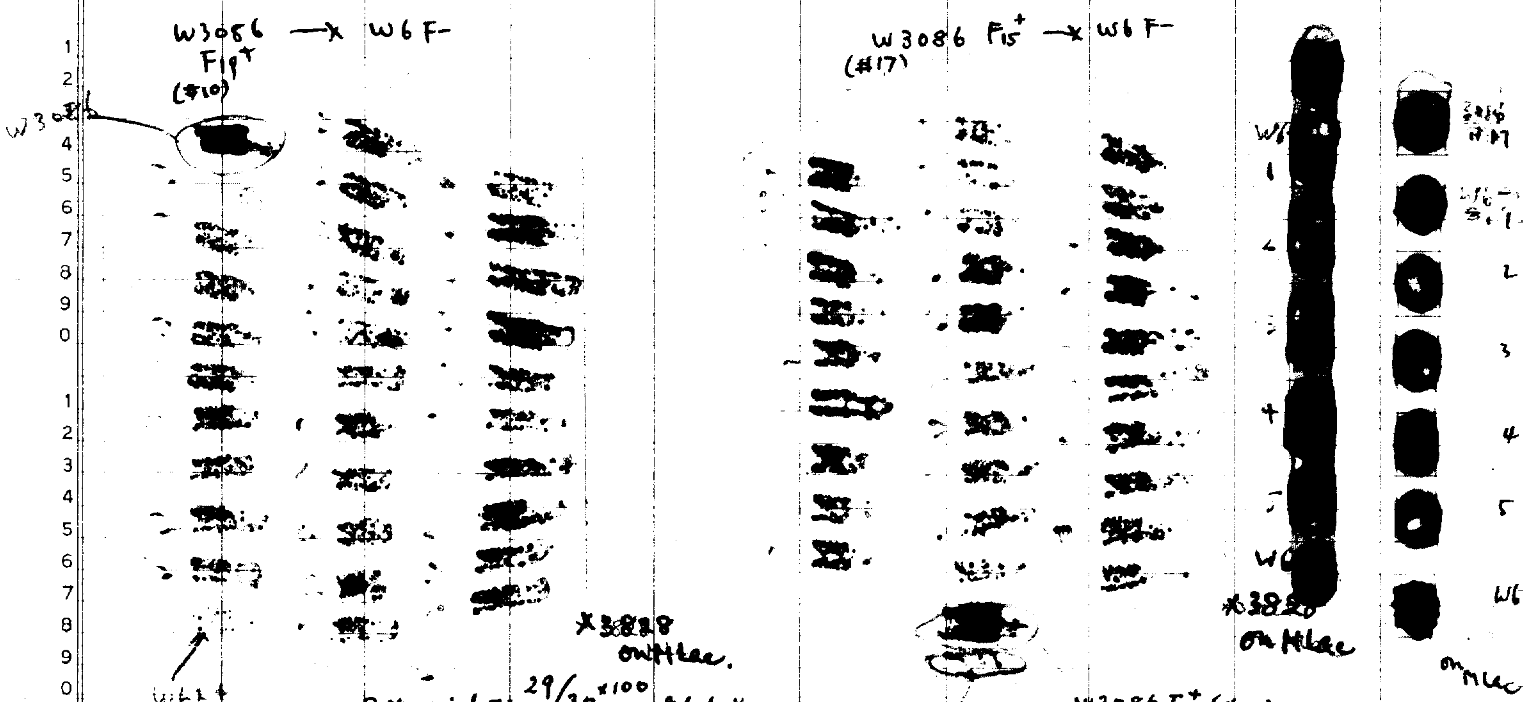
REF:

Experimental conditions.

cultural age: ca 20 hrs.

Inoculum size: F8 or F_{15}, or F_{19} 10^9: 0.1 ml + 3086: 0.1 ml + 5 ml Pen.
(^{5r}3876)

condition of infection: Overnight, at 37°C, standing.



Rate of infection: $29/30 \times 100 = 96.6\%$.

Rate of infection: $29/29 \times 100 = 100\%$

↑ Seems like state II.

Conclusion: Infection of F15 or F19 to F− gives state II (Low frequency male).

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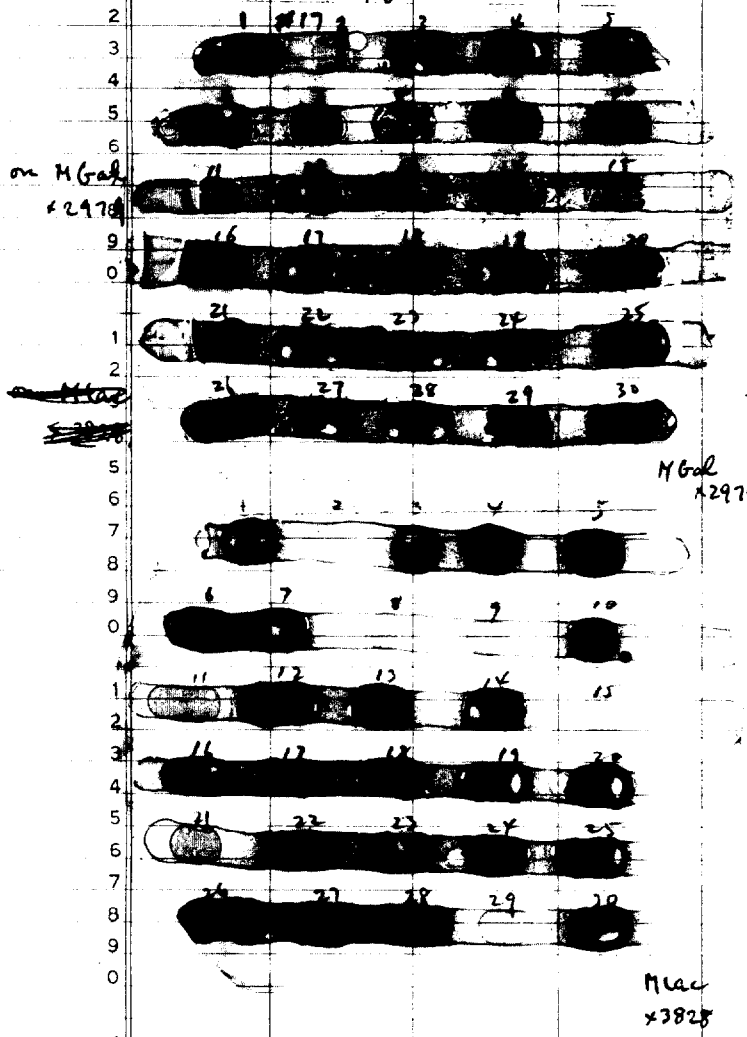
State I and state II in F'+ strains.

8/IV ; 1959

REF:

3086 F8



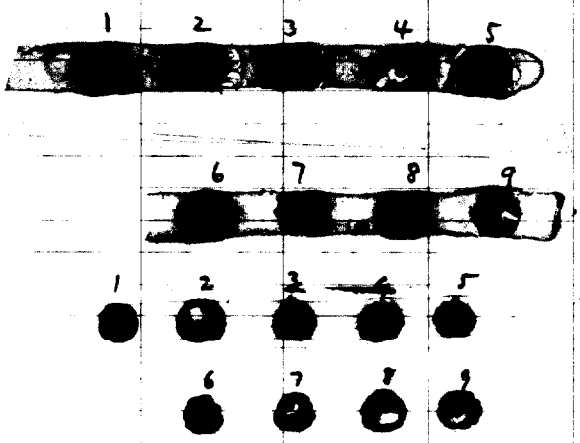
on MGal ×2978

MGal ×2979

7/30 ×100 = 36.3

MLac ×3828

3086 #10 (F1?)



MGal ×2979.

MLac ×3828

Method : 1. Spread on BLac and let it form colonies on it.
2. Pick each colonies and suspend into pipassay.
3. Spot them on MGal (streaked W2979 and W3828. and MLac)

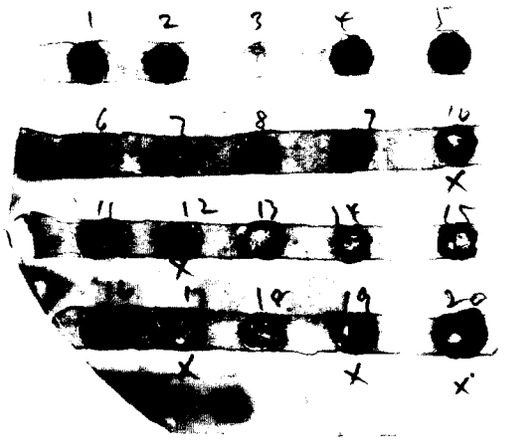
Conclusion : (36.3%)

1) 3086 F8 : (3086 F13) frequently produce F⁻ and seems like single phase.
This high reversion to F⁻ was interpreted by split off of F8 from chromosome and the rate of fission or # of F8 in cytoplasm is very low.

2) 3086 F19 : ( ) gives two state, Hi and Lo.

3086 #17

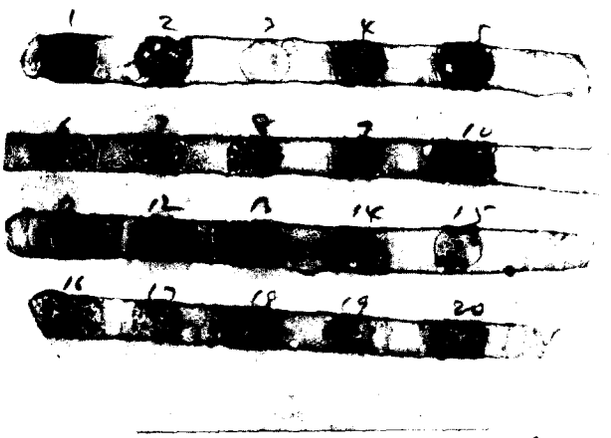
F15



on MCar

3086 #17

F15



on Mcal

Treatment of F mutants by AO.

2/14, 1959 REF:

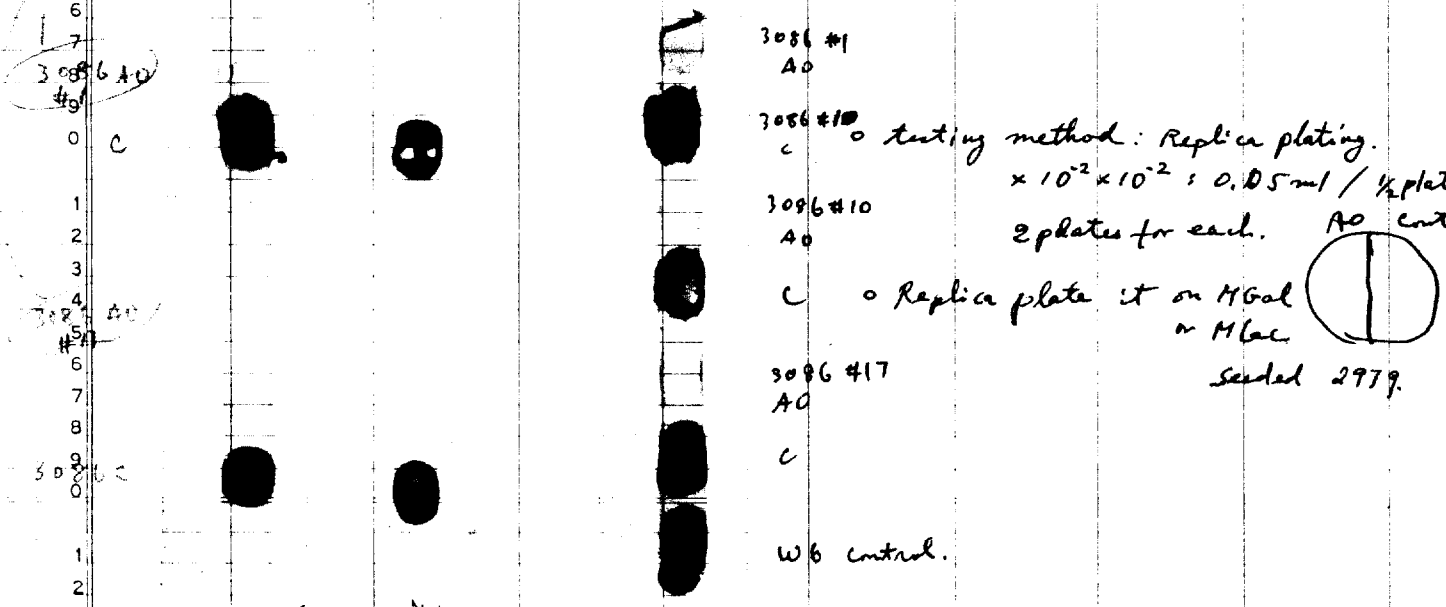
Purpose: Is F' sensitive to AO-treatment.

Experimental conditions: inoculum size: ca. 10^8 cells/ml, Conc. 40 γ/ml.
Medium: penassay. 5 ml.
Cultural age: overnight culture.
Time of Treatment: 10, 20, 30 and 40 min.
Strain treated; W6 (control), 3086-10, 3086-1 3086-17,

Result

		Stationary F⁻/total	Log F⁻/total	% of F⁻	effect.
F_1	W6 AO 40				
	" Control.				
F_8	W-3086-1 AO		11/85	74/85	87	+
	" Cont	~~[illegible]~~	68/87	19/87	21.8	+
F_{19}	W3086-10 AO		8/48	49/48	83.4	+
	~~W3086-10~~ Cont		81/109	28/109	0/109	0	
F_{15}	W3086-17 AO	[illegible]	84/149	65/149	43.6	+
	" Cont.		38/88	50/88	0/88	0

put into stab.



testing method: Replica plating.
$\times 10^{-2} \times 10^{-2}$: 0.05 ml / ½ plate
2 plates for each.

Replica plate it on Mgal or Mlac
Seeded 2979.

U.W. MICROBIAL GENETICS

Transmission of F_8 from W6 F_8^+ to Hfr, and F^-

(W1922) (W1394) 13

M Hfr, S^R TLB, S^R save W1394 F_8^+

5/11 ; 1959 REF:

Method.

1) Transmission W6 F_8^+ Hfr, or F^- mix them 1000 : 1, and ~~shake it~~ stand it overnight.

W6 F_8^+ : 1 ml
Hfr, or F^- : 0.1 ml (10^8/ml)
5 ml pen.

Select S^R on BLac Sm agar. (ca. 500 colonies per plate).

2) Replica plate it on M Gal seeded W2979 on it.

This data is still not believable trying again using W1895.

Experimental conditions.

culture age ;

medium ; Pen. 5 ml. ; growth age and ratio : 10^8 : 10^5. W6 F_8^+

Conclusion : F_8 is transmissible to Hfr! and gives Hfr character in the absence of chromosome transfer.

Control.

W6 F_8 → W1394 TLB, S^R

W6 F_8 → W1922 Hfr, M S^R

Put to stab. W1394 F_8

W1394 F_8

W6 F_8 W1394

W6 F_8 W1922 (Hfr)

control W6 F_8

2/25 ×100 =

× 3949

× 2985

× 4297

× 2984

× W6-

× 2979

12/26 ×100 =

W6 F_8 × 2979 on M Gal

1922 F_8 1

1922 F_8 2

1922

W6-

× 2979 on M Xyl

W1394 Control on M Gal

× 2979 on M Gal.

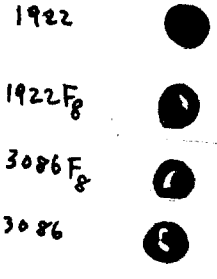
further check.

· Hfr, Test on M Lac B, × W1394

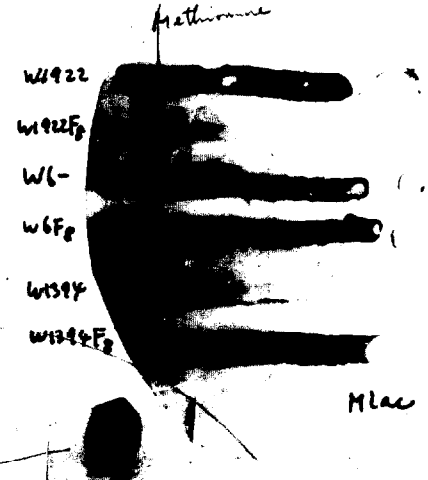
· F_8 Test on M Gal × 2979

W6 F_8 × W1922 Replica plated on M Gal × 2979.

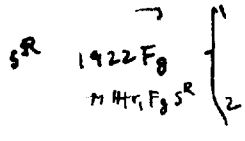
1922 Mal test. on B Mal.



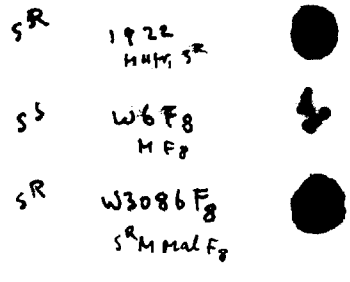
nutritional requirment



Reference



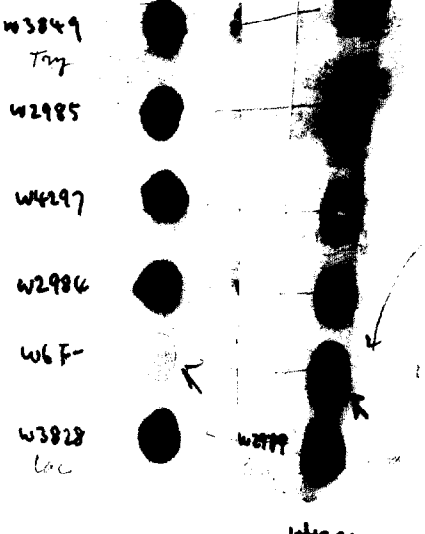
? test nutritional requirement of 1922 F8
did I mixed up with W1394F8 and W1922F8 ?
These culture is made in same time.



and W6F8

X W1394
TLB, SR

MLac SM B1



Conclusion
W1394F8 must be M-
W1922F8 must be TLB-
It is clear that I mixed up with W1394F8 and W1922F8

W6 —F_1→ × 3086 F_8^- (isolated by AO)

W6 —F_1→ × 3086 F_{15}^-

7/IV ; 1959.

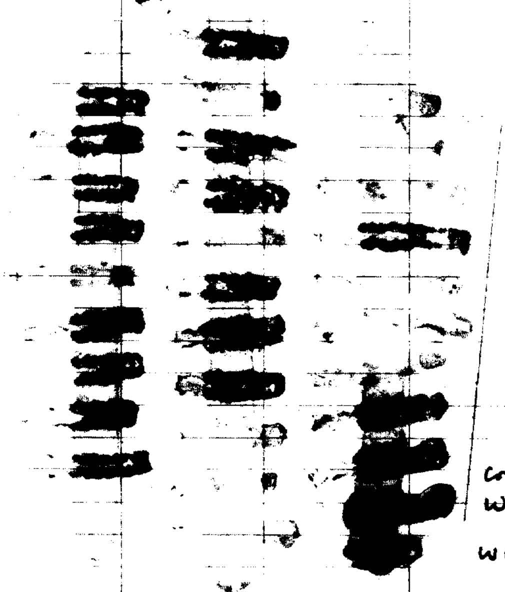
REF:

Experimental conditions.

Ratio: W6 1 ml + 0.1 ml 3086 F_8^- + 5 ml pen.
" + 3086 F_{15}^- + 5 ml pen.

Infection: 37°C overnight. (ca. 20 hrs.)

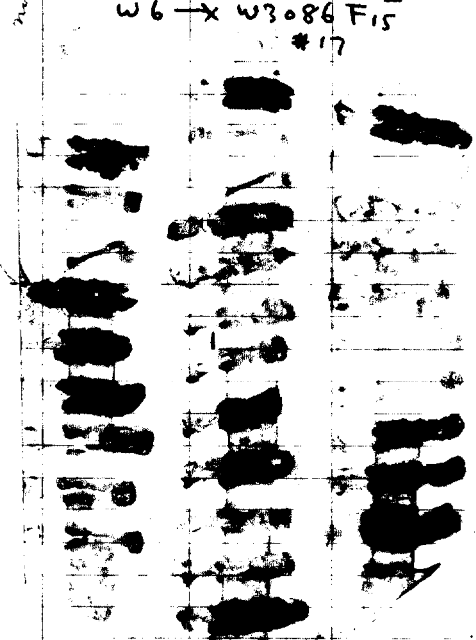
W6 → × W3086 F_8^- #1



control W3086 F_8
W6
× 2979
on M Gal

This is formed due to the [illegible] in impression technique, not spontaneous mutation.

W6 → × W3086 F_{15}^- #17



control W3086 F_8
W3086 F_{15}^- #17
× 2979
on M Gal.

Conclusion:

W3086 F_8^- and W3086 F_{15}^- isolated by AO treatment are ~~ple~~ F^-.
They gives standard F^+ after infection of F_1 to them.
This means change in transfering character is determined by F particle itself.

See pack page : Replica plating method to check their fertility.

W6 × W3086 F_{16}^{-} #17

W6 × W3086 F_{8}^{-} #1

ordinary FP

×2979

~ Mod

3086 F_{11} ——× W6 F−

3086 F_6 ——× W6 F−

12/iv . 1959

REF: Cf p 15

1 2 3 4 5 6 7 8 9 10

Method

1.0 ml of 3086 F_{11} or 3086 F_6 (24 hr old) + 0.1 ml of W6 F− (20 hr.) + 5 ml pen.

↓

Incubate overnight

↓

Plate at 4 on BMal, and pick Mal⁺ and test Hfr on Gal (on MGal × 2979)

↓

Test S⁵ and Mal⁺, and put into stab.

Result:

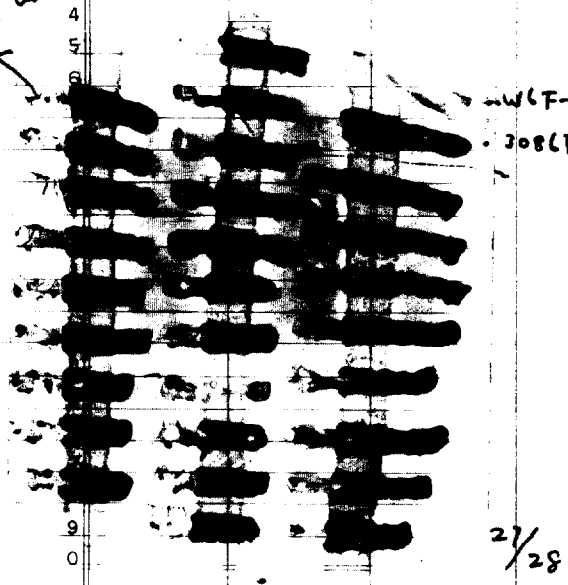
Rate of transfer. Hfr / total

3086 F_{11} —× W6F− : 27/28 × 100 = 96.5 %

Put into stab. W6 F_{11}.

3086 F_{11} —× W6F−

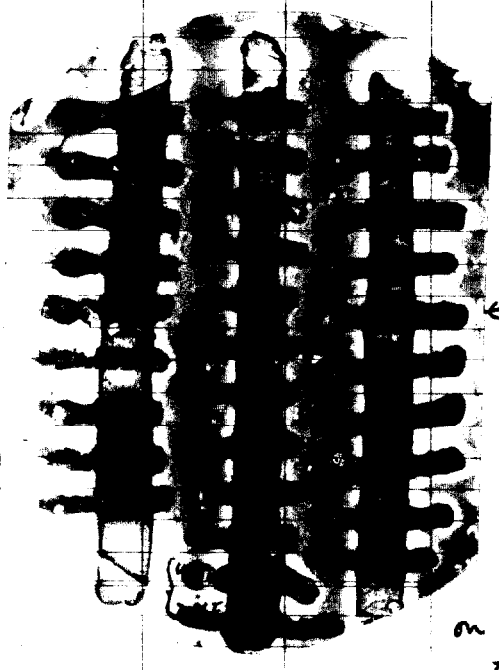
3086 F_6 —× W6F−



W6F− } Control
3086 F_{11} }

27/28

on M Gal × 2979.



Put into stab W6 F_6

on M Gal × 2979.

W1394 F_8 TLB, S^R

~~(Hfr, F_8)~~ —— × (F^-)
~~W1922 F_8~~ 3086.
~~S^R Hfr,~~

Test for infectivity of F_8 of Hfr, F_8.

12/6, 1959

REF:

Method :

1 ml : W1394 F_8 ~~W1922 F_8~~ (20 hr.s old) + 0.1 ml 3086 + 5 ml phrasy

↓ Incubate overnight

↓ Purify it on EMB Mal and pick Mal⁻ (3086)

↓ test Gal and Lac loci in the frequency of transfer.

→ × 2979.

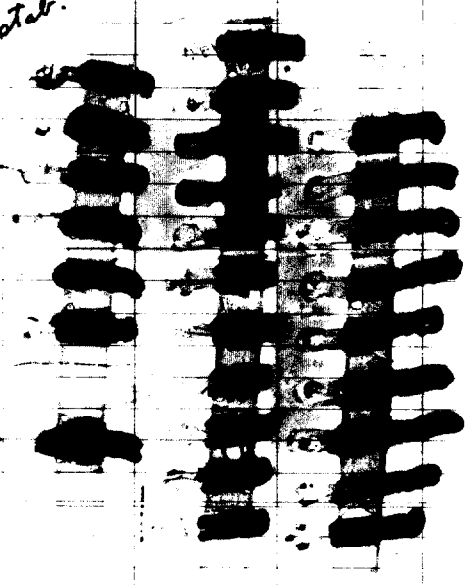
↓ Test Gal Try TLB, C, A, etc. on the strains.

Check for this 3086 F_8^+ is same as original F_8^+. Hfr, F_8^+ modify F_8.

W1394 F_8
~~W1922 F_8~~
(~~Hfr, F_8~~) × 3086.

Conclusion :
F_8 in W1922 F_8 has infectivity to F^-.
It is in cytoplasm of W1922.

put into stab.



Control
3086
3086 F_8

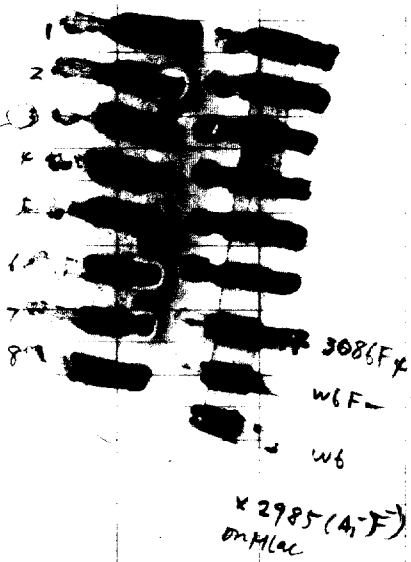
× 2979
on MGal

[redacted] 3086 F4 ⟶ W6 F−
M S^R Mal F_4^+

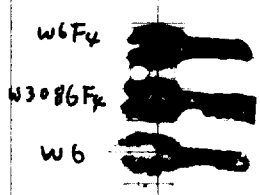
13/IV ; 1959. [redacted] REF:

[redacted] 3086 F4 1 ml + W6 F− 0.2 ml + 5 ml penassay.
↓
Incubate it for overnight.
↓
Purify on B Mal.
↓
Test sex of Mal+ colonies on MGal agar (× 2979)
↓
Put into stab. test Mal. S^S Hi tor #1

Put into stab.
W6 F4.



× 2985 (4, F−)
on MLac



× 2979
on MGal.

Treatment of (~~Hfr, F8~~) W1394 F_8 by AO.

F_8 TLB1 S^R

12/11 1959 REF:

does F_8 be removed by AO treatment?
How about Hfr, make does F_8 replaced F_1'?

Method:

1. 10^4 ml of 48hr old culture of W1394F_8 ~~Hfr, F8 (W1922 F8)~~ (penassay) is inoculated into penassy-AO 40γ/ml medium.

Inoculum size ca 10^5/ml
Time of treatment. ca 20hr.

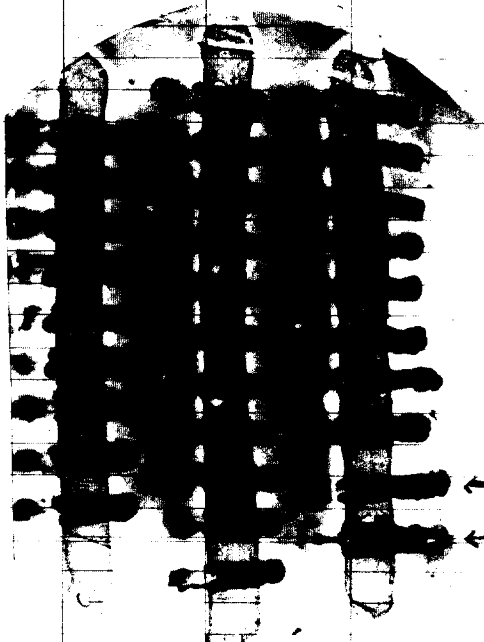
2. Purify it on Blac.
3. test fertility of gal and TL (x2979) on MGal ; (x1922) on MLac SMB1. It does not grow in AO. (It may be too conc.) Try again.

final inoculum size:

13/11 1). 10^4 cells/ml. young culture (overnight) ; AO conc 30γ, 40γ Penassay; 5ml
Incubate overnight. (2:00 pm)
purify it on Blac after treatment.

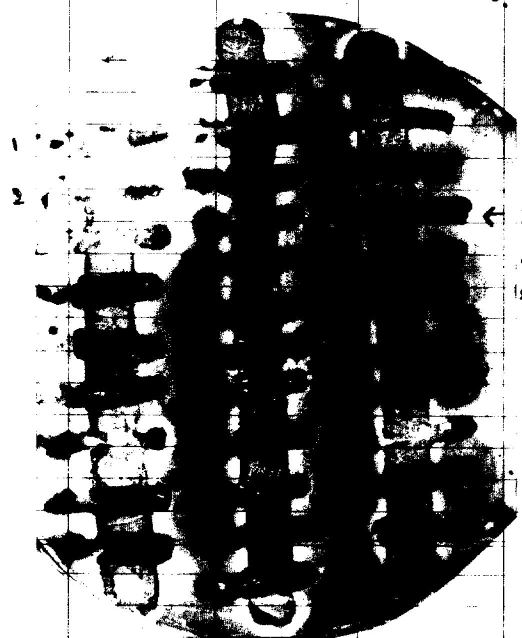
2) test their fertility on MGal and TL (x 2979) on MGal (x 1922) on MLac SMB1

untreated control W1394F_8 ~~W1922F_8~~



← W1922
← W6F_8
x 2979 on MGal

AO treated W1394 F_8 ~~W1922F_8~~



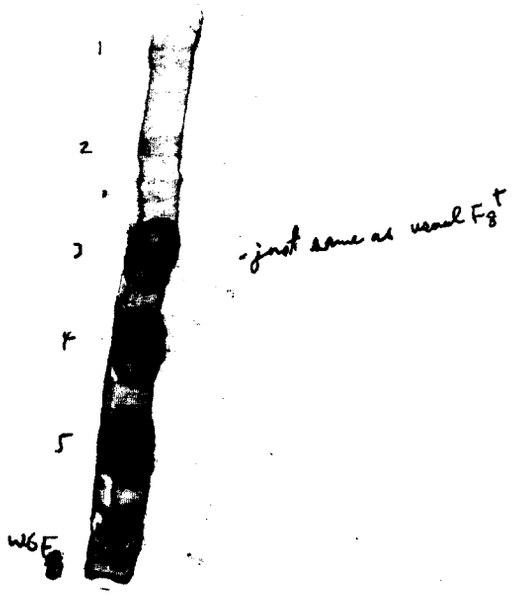
1
2
3 ← ? Hfr1?
See back page
4
5
W1922
W6F_8
x 2979 on MGal

% of F^-

0/28 x 100 = 0

6/31 x 100 = 19.4

Retest of turbidity of SO-treated F_8^+.



- just same as usual F_8^+

*2979
on Hgal.

Stability of W6 F_8^+

13/IV ; 1959. REF:

From 8/IV's experiment 3086 F_8^+ seems liker unstable.
How about W6 F_8^+. Is it stable or unstable?

Method :

1. Purify W6 F_8^+ on Blac. and suspend single colonies into water.
2. Purify it on Blac and test compatibility of each colonies. (on MGal x 2979.)

Conclusion:

W6 F_8^+ is reasonably stable as F^+. The experiment done on 8/IV, 1959 is wrong. or ~~Stability of W6 F_8~~ W6 F_8 is more stable than W3086 F_8.

Reference : AO treatment was done using the same purified W6 F_8 (see P.21).

W6 F_8 / W6 F_8.
$53/53 \times 100 = 100\%$

Treatment of W6F8 by AO.

15/10. 1959. REF:

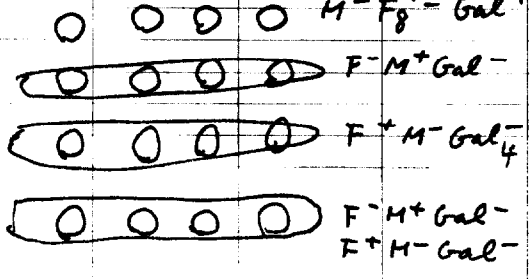
Purpose : Does W6 F8 produce $♀_3$ type F⁻ after treatment of AO?

Principle :

M⁻ F_8'⁻ Gal⁺
F⁻ M⁺ Gal⁻
F⁺ M⁻ Gal⁻

If $♀_3$ type F⁻ arises, it will gives black spot after Mix.

Method



(W6 F_8⁻)
(2979)
(3046.6)

Principle :

M⁻ ♀ × Gal⁻ M⁻ F⁺
×
Gal⁻ on M Gal.

on M Gal

- Use single colony as a starting W6F8.

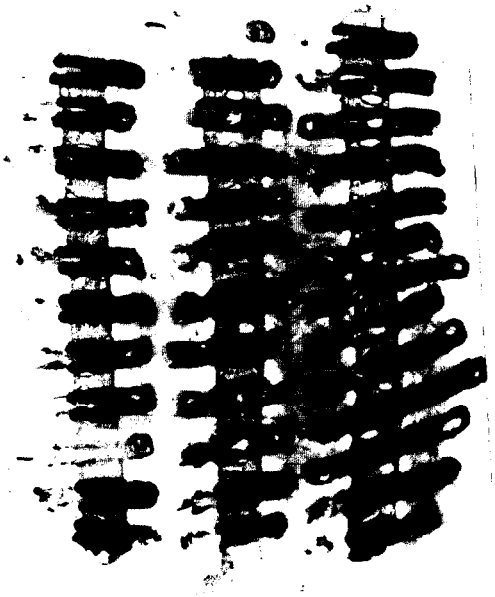
Result

1. Purify W6 F_8.
2. Treat W6 F_8 by AO. Inoculum size : Ca 10^8 cells/ml. AO : 30γ/ml per. % of ♀ obtained : 8.35
3. Test their sex-compatibility by cross-streaking method.
4. Inoculate F⁻ colonies into penney.
5. Spot them on the mixture of F⁻ M⁺ Gal⁻ and F⁺ M⁻ Gal⁻ (M Gal) according the method described above. Test 50 colonies. (only 6 ♀s are obtained after treatment.)

Result : 6 ♀s tested are plain F⁻. (see back page.) They does not gives Hfr after infection of F.

If it does occurs (6 obtained $♀_3$ type F⁻), it is possible to say that (2 parts of F) = episome multiplies independently.

W6 F8 AO treated



W6 F8 AO treated.

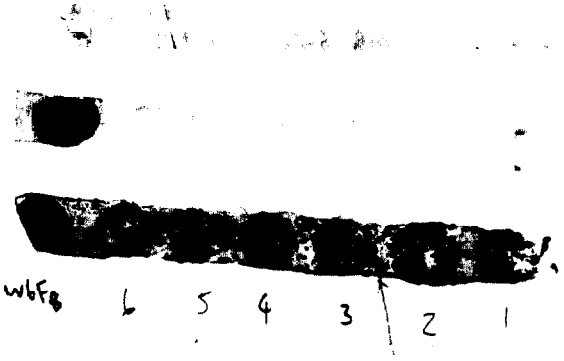
♀ : 6.
♂ : 66.

$^{6}/_{72} \times 100 = 8.35\ \%$ ♀.

untreated control is in P.21
(see page 21.)

x2979
on MGal.

blank.



3046 F+ M- Gal-4

2979 F- M+ Gal-2

3040+2979

on MGal.

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W6526
F⁸ TLB, Mal₅ S^R f₃.

23

W6 F8 ——× W3644
F⁻ TLB, Mal₅ S^R f₃.

12/IV. 1959 REF: cf. 55, 70, 86.

← W6 F8

← W3644

W6 F8 1ml + W3644 0.2ml + Penassy 5ml.
↓
Incubate at room temp. overnight. at 37°C.
↓
purify on Blac SM.
↓
Test their sex-compatibility (× 2979) on M Gal.

on BMal on Blac SM

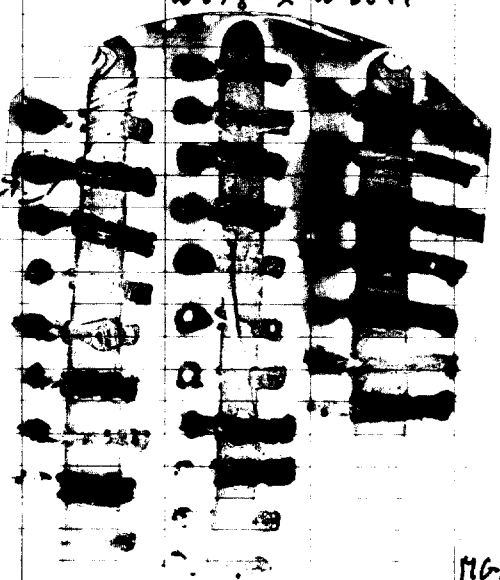
Result.

F8 is transfered to f₃ (W3644). W3644 F8 shows Hi-trait after infection.

Further experiment:
compair Hi markers.

Conclusion: F8 is transmissible to f₃ and gives high frequency recombinations.
The rate is about 50 % after growth for overnight culture in penassy.

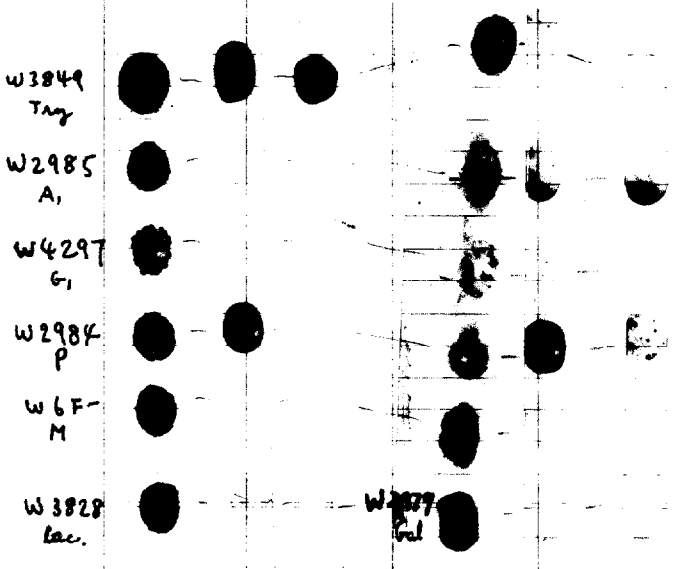
W6 F8 → × W3644



Put into stab.
3644 F8.

MGal
× 2979

13/27 × 100 = 48.2 %



Conclusion:
W3644F8 is not so hi — Meth (but higher than TLTh).
Hi: Tryptophane, Hi: for Gal, Moderate: Pro, M.

W3644F8 1394 W3644 on MLac.

W3644F8 W1394 W3644 on M Gal.

Further experiment: See is there 2 state or not.

Conformation of transfer of F_8' to Hfr,

3086 F_8 → 1895.

16/IV, 1959

REF: unsuccessful result.

Method :

3086 F_8 1ml + W1895 0.2ml + Penassay 5ml.

↓

Incubate 4 overnight

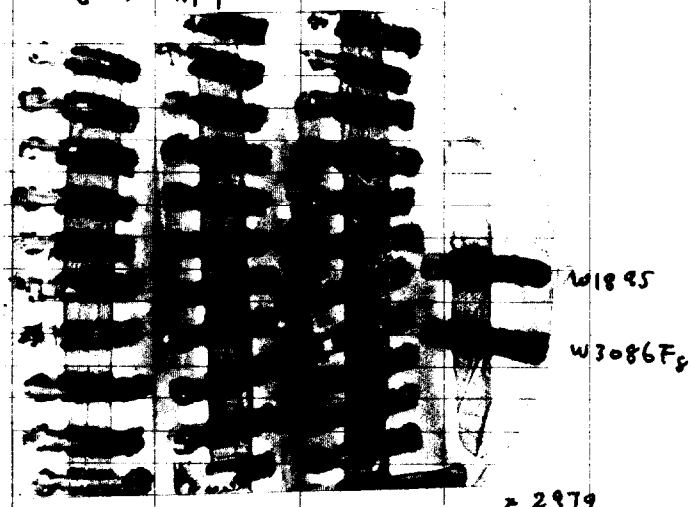
↓

Purify it on B Mal.

↓

Cross brush Mal^+ colonies on M Gal × 2779

Result : No transfer occured. Seems negative result.



× 2779
on M Gal.

0/33. all 1895.

Further experiment : 1. Use replica method to detect the infection of F_8 to 1895.S^R.

2. check the possibility ~~of this~~ that F_8 is infected into Hfr, but does not excert the action.

Design ; Use Gal⁻ M⁻ Hfr, S^R.

W6F_8 → S^R Gal⁻ M⁻ Hfr ; seed M B Gal Sm. → Replica plate it on M Gal added F^- Gal⁻ F^- M⁻.

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Hfr
3200 F13 × W3086 W

26

12/IV : 1959 REF:

Method

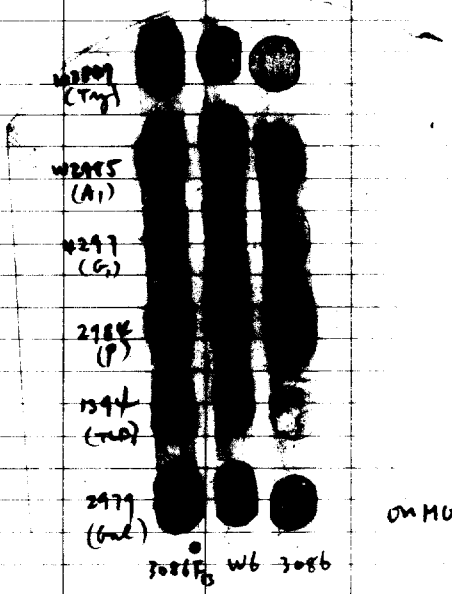
Overnight culture of W3200 5 ml/ pen + 0.1 ml W3086

↓ Incubate overnight (20 hrs)

↓ purify it on Blacsm-agar.

↓ Replica plate it on MGal seeded W2979 on it.

Result & conclusion : F'_{13} is obtained from Hfr_{13} with the method described above. F' gives Hi for Gal; Medium for Try + A_1; Low for G_1 + TLB_1



on MGal

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Comparisons on infectivity of F'+ and corresponding Hfr.

16/VI . 1959.

REF: cf. p. 33. infectivity.

· cultural age : Overnight culture (Penassay)

· Strains: Hfr is isolated by purification of stab culture.
W4321 (Hfr$_4$ M$^-$) W3208 (Hfr$_8$ M$^-$)

· Experimental method:

Hfr or F'+ 1 ml + 3086 0.2 ml + Penassay 5 ml.

↓ Incubate it overnight.

↓ Purify it on MMal Sm agar.

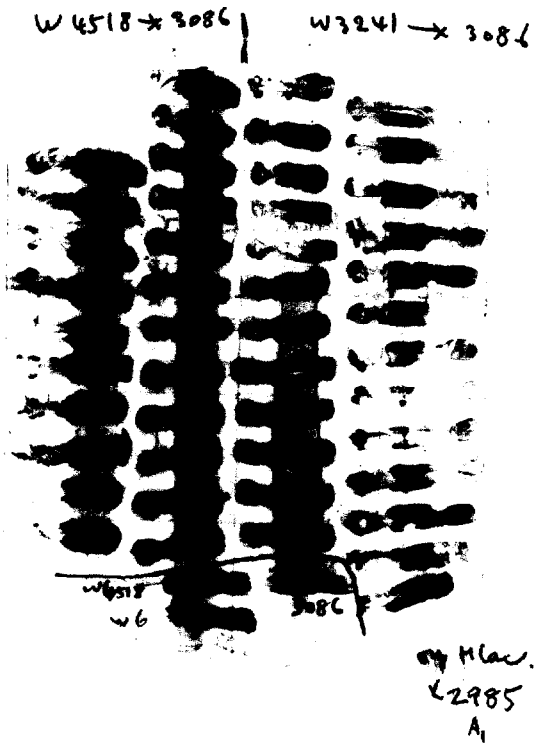
↓ Test sex-compatibility

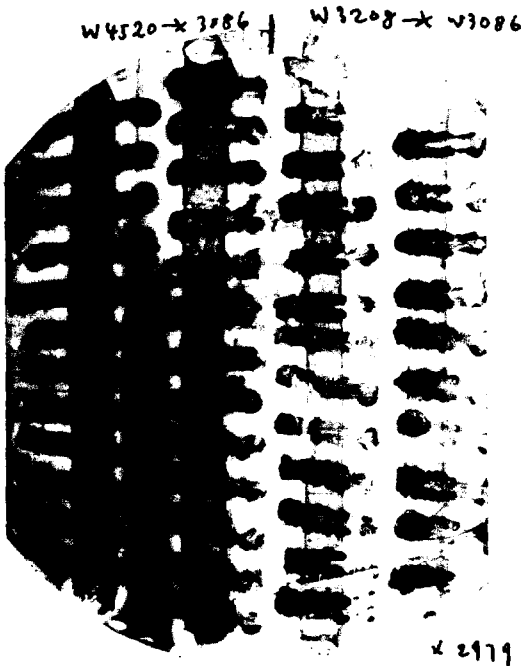
Result:

	Hfr / total.	% converted into F'	infectivity
(Hfr$_4$) W4321 × 3086	0/24	0	−
W4518 × 3086 (W6 F$_4$)	22/22	100.	+
W3208 Hfr$_8$ × 3086	18/22 (not Hfr: F+?)	82 (usual F+?)	see back page.
W4520 × 3086	20/25	80	+

This was not Hfr of W4321

Conclusion : Original stab culture contains 2 kinds of Hfr strain, infective and non-infective.
Infective Hfr may occurred by mutation of non-infective Hfr × F' is simply splited from chromosome.





Mac.
K2985
A1

K 2979
m Mal.

I should use Gal marker, because no asymtrophy.
try again.

3086 F_8 —× W6 ●

F_8 —× F_1 Is it co-exist or exclud ? P.29
[with each others. P. 48
REF:

16/4 ; 19[illegible].

3086 F_8 : 1ml : F_1 0.1ml : 5ml penassay.
W6
↓
Incubate it for 24 hrs at 37°C.
↓
Purify it on D Hal
↓
Test Mal+ colonies on sex. in cross times 2979. on MGal.

3086 F_8 —× W6

test infectivity of F, and F_8 to F- at ~~7~~ 8 (P29)
Does this clone still contain F_1 ?

3086 F_8

×2979 on MGal

sft/total testing : 1/32 ×100 = 3.12

Conclusion : F_8 is infective to W6 F^+.

objection : W6 —spontaneous loss of F→ W6 F^- → W6 F_8
3086 F_8

W6(F_1 F_8) —× 3086

23/I, 1959. REF:

Purpose of this experiment: Does W6F_1F_8 still retains F_1 or not? Cf. P.28.

W6F_1F_8
control 1. or W6F_1 —× W3086
control 2. or W6F_8

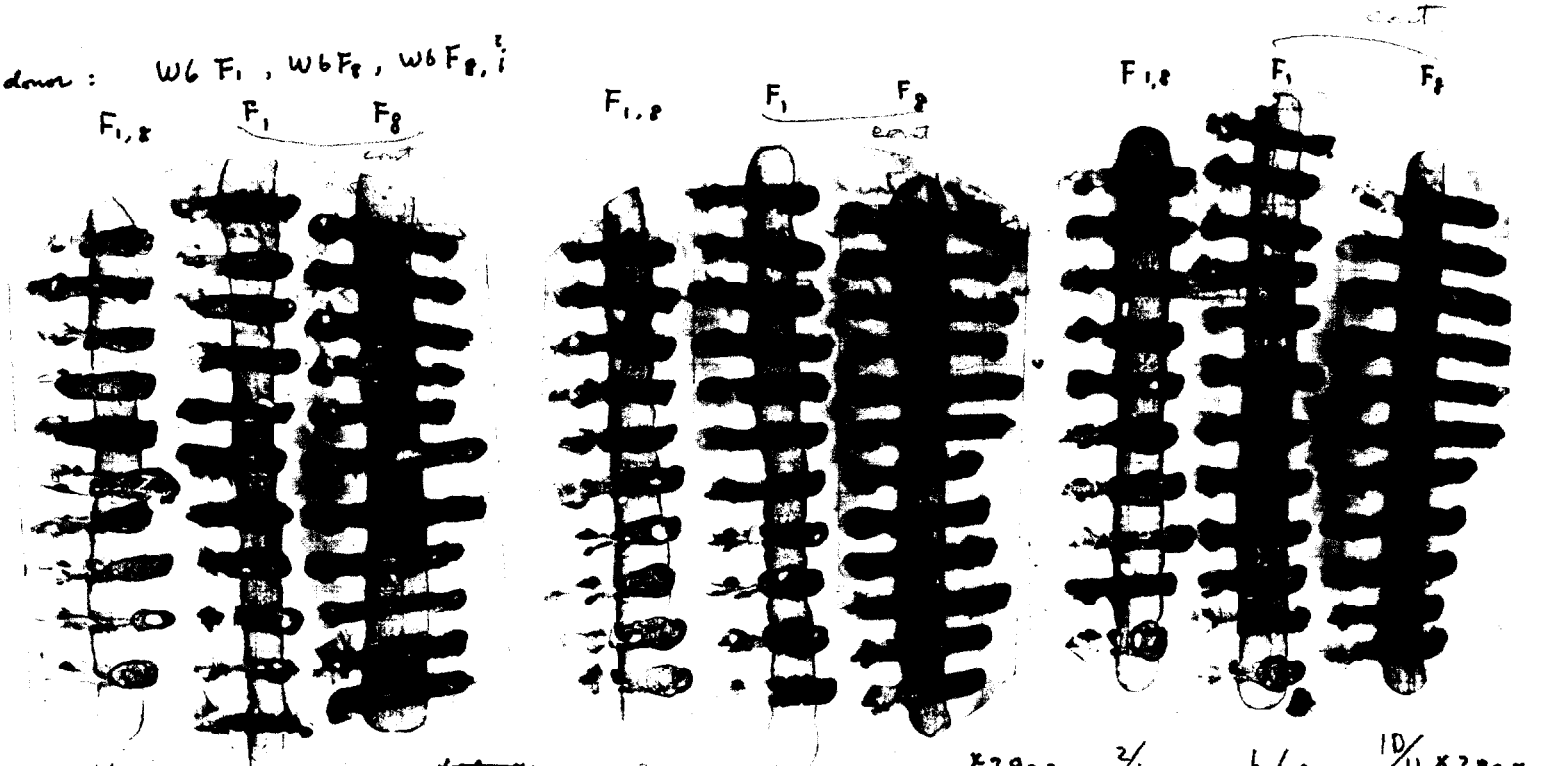
1 ml + 0.1 ml + 5 ml penassay
↓
incubate overnight.
↓
Purify it on Blac Sm.
↓
Cross-brush it in mass × 2979 on M Gal.

		F^8/total (%)	F_1/total (%)
Experiment:	W6F_1 F_8 —× 3086	6/30 (20)	0/30 (0)
Control:	W6F_1 (W6) —× 3086	0/34 (0)	17/34 (50)
	W6F_8 —× 3086	30/34 (88)	0/34 (0)

Conclusion: W6$F_{1,8}$ (obtained by infection of F_8 to F_1^+: W3086F_8 —× W6) does not contain F_1, or not segregate in the time of infection.

~~F_1 and~~ F_8 may exclude F_1 after infection. F_8 > F_1 stronger than.

F donor : W6 F_1 , W6 F_8 , W6 $F_{8,i}$



Y/10 5/11 10/11 ~~[illegible]~~ ×3828 on Mlec

3/10 6/11 10/12 ×3828 on Mlec

2/10 6/12 10/11 ×3828 on Mlec.

F recipient : 3086.

		%
$F_{1,8}$	6/30	20
F_1	17/34	50
F_8	30/34	88.

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Direct demonstration of true recombination.
Not syntrophy!

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Check syntrophy between Gal⁻ and M.⁻
A and M.

20/IV ; 1959

REF:

1. Mix W6 F_8 1 ml + W2979 1 ml + 5 ml pen. Ca. 10^8 cells/ml.
↓
incubate 1 hours at 37°C.
↓
dilute it into 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}.
and spread it on MGal and BGal.
↓
Incubate 40 hrs and see what happens.

See : back page.

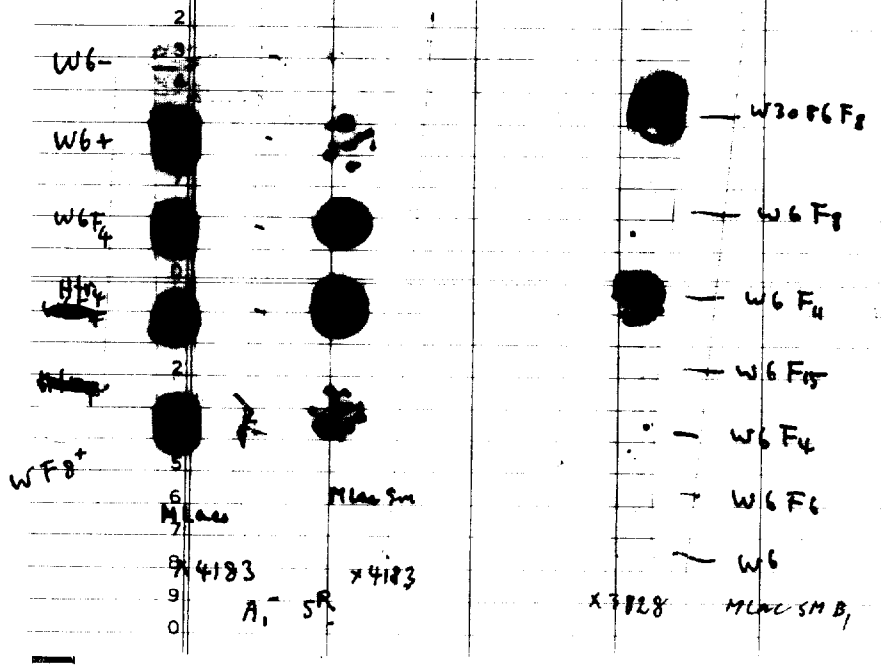
Degree of dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	survivor cont. 10^{-6}
# of colonies	too many	too many	too many	150	32	6	17

2. pick cells from blackspot and ~~streak~~ streak on BGal ~~is it Gal+ or Gal-~~, and test nutritional requirement on ~~MGal (Replica plate from BGal to MGal)~~ replica plate aux/prot.

	isolated on	Test nutritional requirement:	% of x⁺
W6 F_8 x 2979 on MGal	BGal	almost Gal⁺ tested Gal⁺	0/22	0.0
W6 F_8 x 2985 A^- on MLac	Bgal.	All gal⁺	4/21	16.0
W1394 F_8 x 2979 on MGal	BGal.	8/3 gal⁺. tested Gal⁺	2/23	8.0

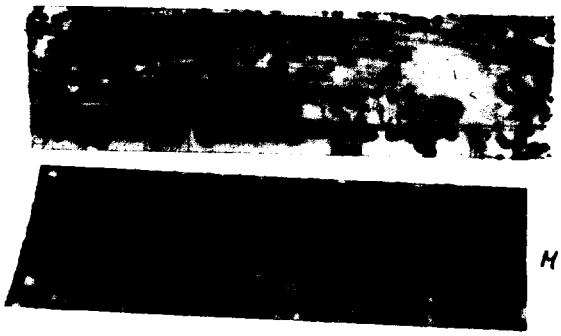
young culture. 4 Spot it on MLac SMB, seeded 3828
old culture

SM destruct male capacity of $^{S^S}_{\lambda}F'$ male but not S^r F' male.

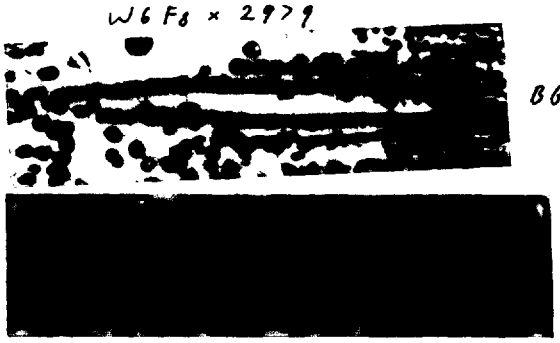


Conclusion : ① This phenomenon is not syntrophy but true recombination.
② F8 x F⁻ S^R (The recombination process is inhibitted by SM-treatment. (It may be interpreted by incorporation of S^S loci to the F-S^R)
③ F4 x F⁻ S^R is not inhibitted by SM.
This phenomenon may be interpreted by incorporation of S^S to F^{S^R}

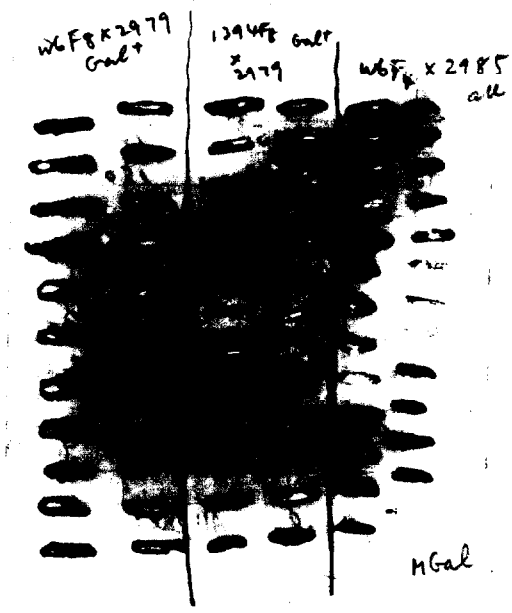
W6 F_4 x W2985



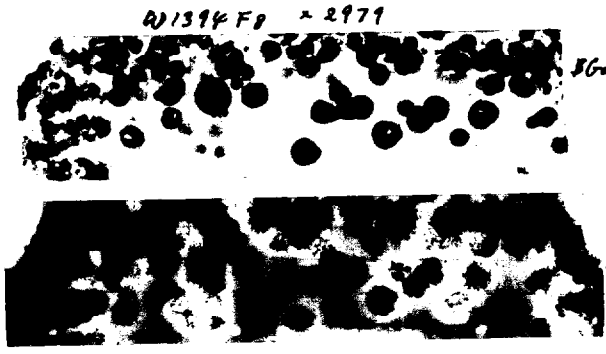
W6 F_8 x 2979



Test of the syntrophy.



W1394 F_8 x 2979



Conclusion:

almost of three Gal+ colonies are

X^+.

Therefore, this phenomena are not syntrophy but recombination.

Genetics.

Preliminary experiment for exclusion of F particles.

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W6 F_4 $\xrightarrow{F_8}$ × 3086 F_8

W6 F_8 $\xrightarrow{F_8}$ × 3086 F_4

16/IV. 1959 REF: cf. p 42 ~ p 50.

Ratio of mix.

Donor (W6 F') 1 ml + Recipient 0.1 ml + Penassay. 5 ml.

↓

Incubation overnight at 37°C

↓

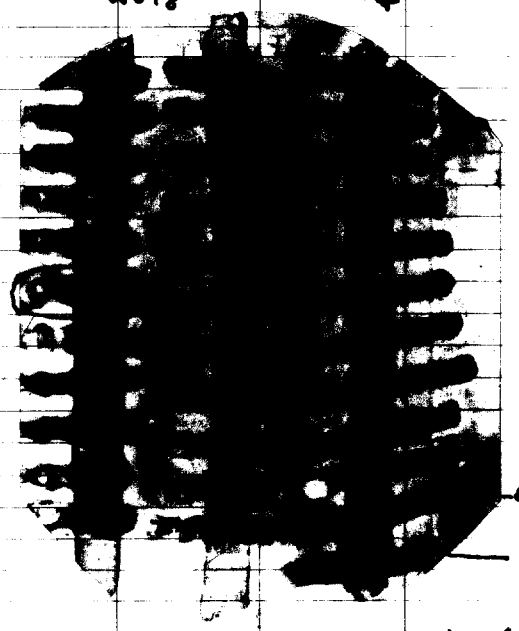
Purify it on B Hard SM.

↓

Test Mal⁻ colonies for sex-compatibility.

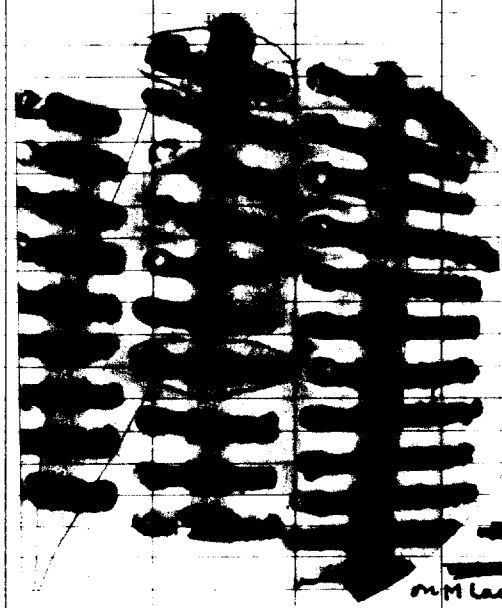
The method used in this experiment is not suitable to know the difference between two different F'+. Use spot method for this purpose. (See back page.)

W6 F_8 × W3086 F_4



on H Gal
× 2979
Gal⁻

W6 F_4 × W3086 F_8



on M Lac
× 2985
A_1^-

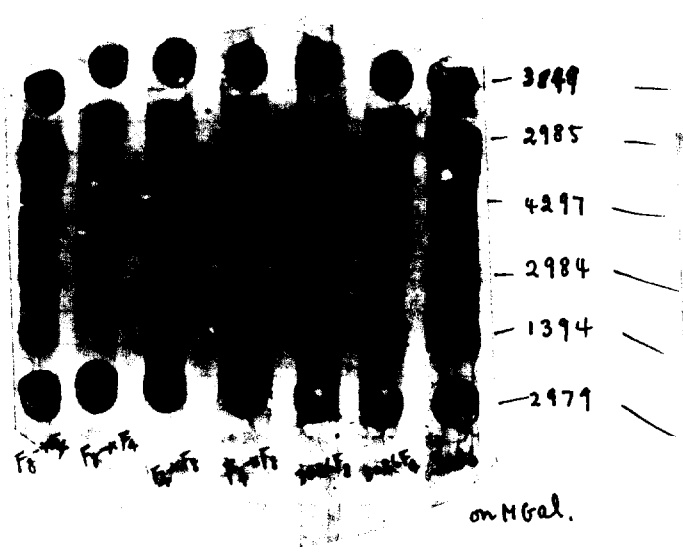
test each H1 markers. Retest H1 markers: See back page

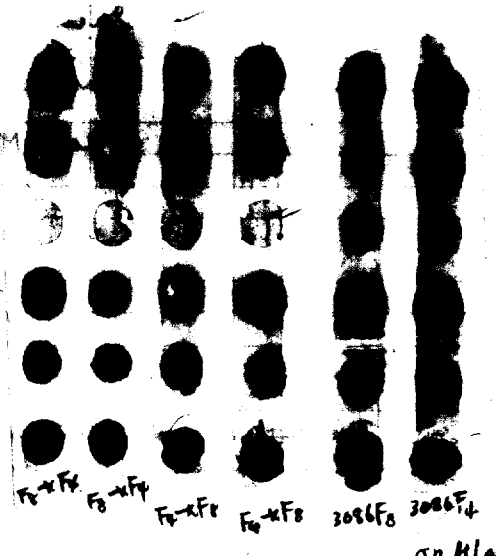
conclusion : F_4 is stronger than F_8. F_4 seems expel F_8 after infection.

But, is it chromosomal level or cellular level? Also phenotypic F_8 (F_8) still contains F_8 or not? (See Back page.)

Next step : $F_{4,8}$ × F^- and see segregation of F_4 and F_8.

Fertility? patterns of F_4, F_8, and $F_4 \times F_8$, $F_8 \times F_4$ obtained by infection of F' to other F^+





F_4 F_4 F_4 F_4 F_8 F_4 F^-

$F_4 \times F_8$ control.

$F_8^{\sigma} \times F_4$

Phenotype F_4 F_4 F_4 F_4 F_8 F_4 phenotype

$F_4 \rightarrow F_8$ control

$F_8 \rightarrow F_4$.

Isolation of F_2 from Hfr$_2$.

4068 Hfr$_2$ ⟶ × 3086. F⁻ M Mal 5^R

16/IV. 1959. REF:

4068 : From Gah. (15/IV) Exponentially growing. it is grown into pm. for overnight

3086 : old broth

Ratio of mix.

4068 /ml : 3088 0.1 ml : Penassy 5 ml.

↓

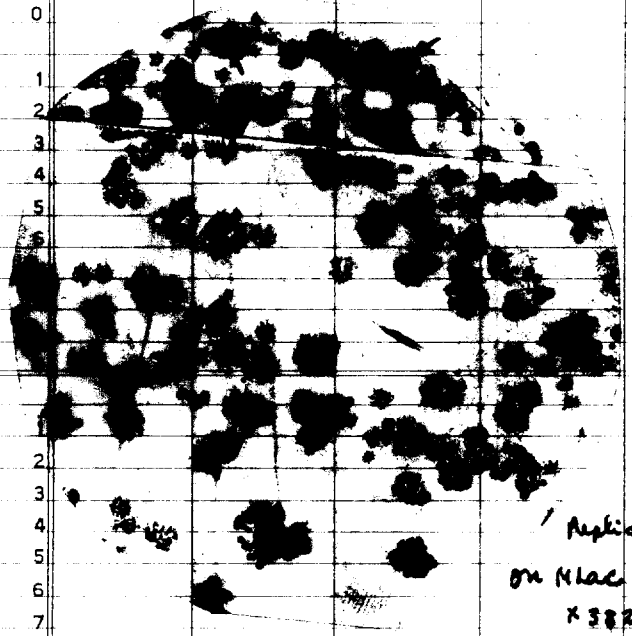
Incubate then at 37°C. for overnight.

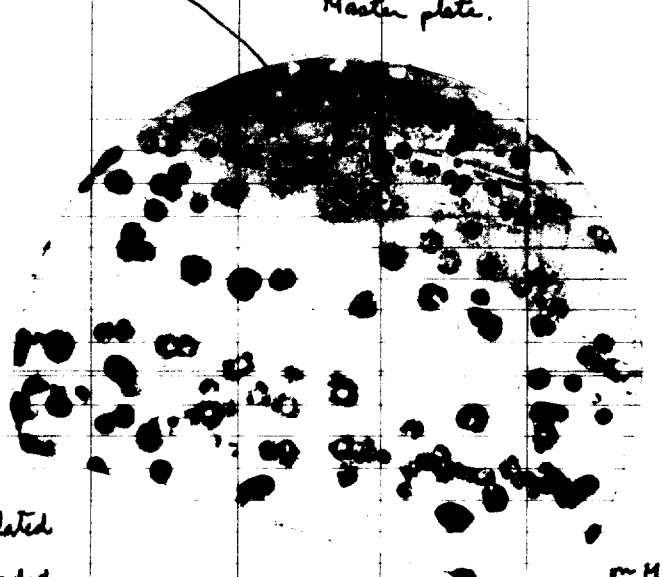
↓

Replica plated on MLac (×3828) or MLac B; SM. (1394).

↓

Pick High recombination coloring and test Hfr quality (×1394) on MLac B; SM





/ Replica plated on MLac seeded ×3828

on MLac Sm.

Treatment of W1922 F8 by AO.

22/IV. ; 1959 REF:

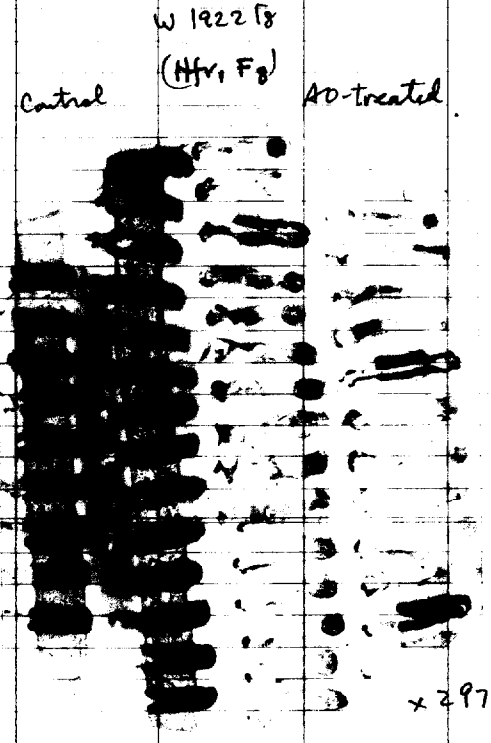
Time of treatment : Ca. 40 hrs. at 37°C. Conc. of AO 30 γ/ml.
5 ml passage.

Purpose : W1922 is Hfr. Has W1922 F8 Hfr, or not. If Hfr, is in W1922 F8
it will gives Hfr, after treatment. Tester : W2979 or MGal.

Result:

	AO treated W1922 F8	Untreated W1922 F8.
+/total	27/27	0/23
% of +	90	0

Conclusion :
W1922 F8 does not give arised Hfr, after AO-treatment. Only gives F⁻.



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FR. from F8 3086.

28/N — 1959

REF: cf. p35
P37 51 49

	1	2	3	4	5	6	7	8	9	10
1		U.V.	10 sec.		Initial number of cell /plate : ca. 10^7~10^8 cells/pl					
2					cultural age : overnight. seeded on B Gal.					
3					Strain : W2979					
4					Med : on M Gal.					
5										
6										
7	plate #	# of colonies/plate		# of Rec+ colonies.			% colony appeared.			
8										
9	1									
10	~~2~~									
	3									
1	4									
2	5									
3	6	19		18			1 ✓	pick and retest the clones of		
4	7	26		26			0	fertility.		
5	8	36		36			0	and test immunity.		
6	9	31		31			0			
7	10	90		90			0			
8	11	35		35			0			
9	12	53		53			0			
10	13	37		37			0			
	14	37		37			0			
1	15	49		49			0			
2	16	63		63			0			
3	17	53		53			0			
4	18	29		29			0			
5	19	25		25			0			
6	20	28		28			0			
7										
8										
9	Σ.			610			1			
10										
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										

Interrupted mating experiment of $W6F_8$.

18/IV ; 1959. REF: H is low mosaic line

F⁻ : W4352 F⁻ Try P M Gal$_6$ Lac$_{eq}$ U_6^R Lp$^+$ Make S^R mutant.
Hfr : W4520 Fg H.
(W6F8)

Selective marker:	Medium	Purpose
Try	M Glucose + Meth + Pro	Try incorporation.
Try & Pro	M Gal + Meth.	2 Which is earlier Try or Pro.
Try Lac.	M Lac + Meth + Pro	Which is earlier Try or Lac.
"	" + Meth + Pro + Trp	1 Lac incorporation
Try & Gal	M Gal + Meth + Pro	which is top. Try or Gal.
Gal	" " " + Trp	Gal.

Method:
1. Purity W4352 on Blac agar. (It was very small colonies).
2. Inoculate purified colony into peptone and incubate it at 37°C. for one night.
3. Transfer it into peptone broth (10ml) and inoculate W 4520 into 10ml peptone and shake it for 4hrs. (11:00 — 2:45)
4. Mix them (1:1) take a sample, and dilute it into chilled H_2O. ($\times 10^{-3}$, & 10^{-5} dil: ca 10^6 & 10^8 0time 0time culture/ml)
5. Spread over each selective medium at each time.

Selective Medium	Time / Selective marker	2:45 0 (# of cells ×100. after mix.)	2:50 5	2:55 10	3:00 15	3:05 20	3:10 25	actual time. min.
Gal-M-P	Try	0	0	0	0	0	0	
Gal-M-P	Try-Gal	0	0	0	0	0	1	
Lac-M-P-Try	Lac	0	0	0	0	0	0	
Gal-M-P	Try-Lac	0	0	0	0	0	0	
Gal-M-P	Gal.	3 ($\times 10^2$)	1	?	5	9	0	
BLac	Survival Count							# of cells used in Exp one ($\times 10^2$).
F_8	# of 4520 a	0	0	0	0	0	0	
	# b	0	0	2	0	1	0	
F⁻	# of 4352 a	7	12	33	15	12	7	
	# b	12	24	38	18	27	11	

Method of dilution.

0.1/10 × 0.1/10 : 0.1 /plate : 5 min —— 25 min. (selective agar)

0.1/10 : 0.1 /plate : 0 min. only. (selective agar)

0.1/10 × 0.1/10ml × 0.1/10ml : 0.1ml : 0 min —— 25 min (EMB Lac)

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Crude timing experiment of for chromosome transfers from F'+ to F−
markers observed : Lac.

24/10 ; 1954.

REF:

Cultural age : ca. 5 hr. on rotator.

cell number : Ca. 10^8 cells/ml.

Phenotype.

selective media : M Lac Sm.

Strain : W6 F_8 ♀ W3828.

dilution		
10^{-3}	10^5 : 0.1ml/10ml	: 0.1ml/plate
10^{-4}	10^8 : 0.1ml/10ml	: 1ml/10ml : 0.1

Time (min) / dilution	0	0	10	20	30	40	50		120
Ca. 10^5/plate 10^{-3}	13	1	10	70	56	81	80		172.
Ca. 10^3/plate 10^{-4}	0	0	0	1	4	5	7		30

0.01 % # of recombinants.
of recombinations.

9
8
7
6
5
4
3
0.02
1

0 10 20 30 40 50 min

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W6 F8 × W3828.

Is there 2 states in W6 F8 ?

23/10. 1959 REF:

W6 F8 × W3828. on MLac Sm, & MLac.
M⁻ F8 Lac⁻ S^R.

Hypothesis: Interpretation for low fertility of W6F8 on Sm-media.

: Almost of the F8 cells are in state I (cytoplasmic state), and not state (II).
Only state II can donate chromosome even in the presence of Sm.

: If it is true, all of W6F8 colonies show Hi on MLac, and only (clone) four W6F8 shows Hi fertility in the cross on MLac Sm. (W6F8 only shows the state II phenotype on (MLac) Sm-media) and almost of the W6F8 colonies are low fertile.

Method:

1) Make W6 F8 colonies on BLac agar. (10 plates).

2) Replica plate it on MLac and MLac Sm seeded W3828 on it,

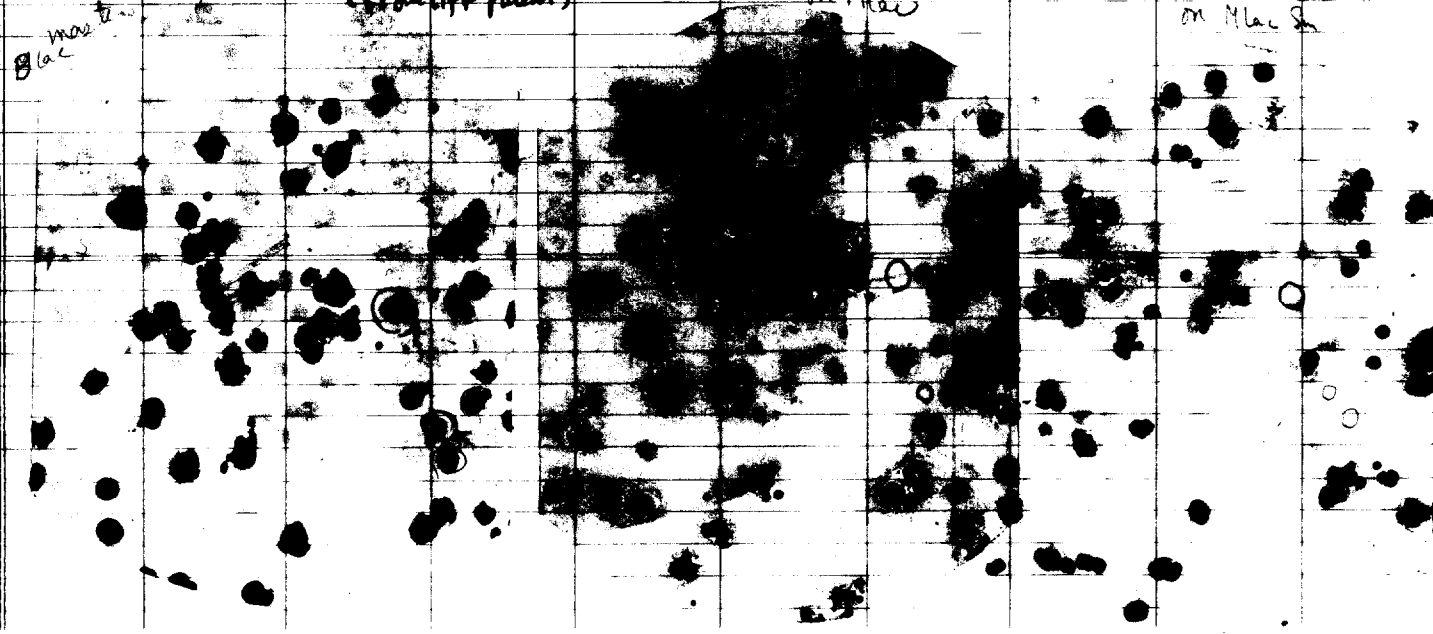
3) Incubate it for 30 hrs.

plate #	# of colonies	Hi # of Fertile colonies on MLac	on MLac Sm.
1	135	135	135
2	112	112	112
3	130	130	~~129~~ 129(1)
4	127	127	127
5	58	~~57~~ 58(1)	58(1)

Result.
apparently, fertility of F8 W6 (on MLac Sm) is much low than MLac. But there is almost no fluctuation among replicated colonies.
all state I ?

F⁻ or F^R =

Conclusion:
This phenomenon (inhibition of recombination (formation) by Sm.) may be interpreted by the incorporation of S^S segment to S^R F⁻.
(from Hfr parent)



one example.

2nd trial of infection of F_4 — × F_8.

23/4, 1959. REF: Cf. p 31. Cf. 50

• Method : (W6) (W3086)

Mix F_4 1ml : F_8 of 1ml : 5ml phaseay. Control F_8 only.

(W6 F_4 W3086 F_8 is purified on Olac. before using.)

↓ Incubate it overnight.

↓ Purify it on BMal Sm.

↓ ~~Inoculate~~ Mal⁻ Sm^R colonies into 1ml phaseay tubes. and let it grow for 5 hrs at 37°C

↓ Spot them on (~~W3086~~, W2985, W2979.) m. Gal.
(~~Sup~~) (A_1) (Gal)

and compair Hi recombination pattern.

• Strain : W6 F_4, W6 F_8, W3086 F_4, W3086 F_8 all of them are purified. before using in this experiment.

• Result :

Experiment	HI for A_1 only (F_4)	HI for Gal only (3086 F_8)	HI for both (double)
W6 F_4 × W3086 F_8	1 # 28 (F_8 may be expelled by F_4)	38	5 1 2 15 31 34
Control W3086 F_8 only	0	40	0

Double F strains. (F_4 + F_8 in W3486)

• Conclusion :

1. F_4 is infectable to F_8^+ cell, and sometimes expel F_8 from the host cell and sometimes make double F strain.

2. These fertility ▶ may depends on the segregation of two types F^+. (∵ If A_1 becomes higher than parent Gal will become lower or vice versa, but not both.) See segregation of F characters by spot test and replica plating method.

[redacted]

efficient method : application of Replica plating method.

W6 F_4 × 3086 F_8. Use Xyl to detect F_4

Seed on BMal Sm.

Replica plate on MXyl seeded W2979.

(F_4 is Hi for Xyl but F_8 is very low for Xyl, therefore it is easily differentiate between F_4 and F_8.)

F_4

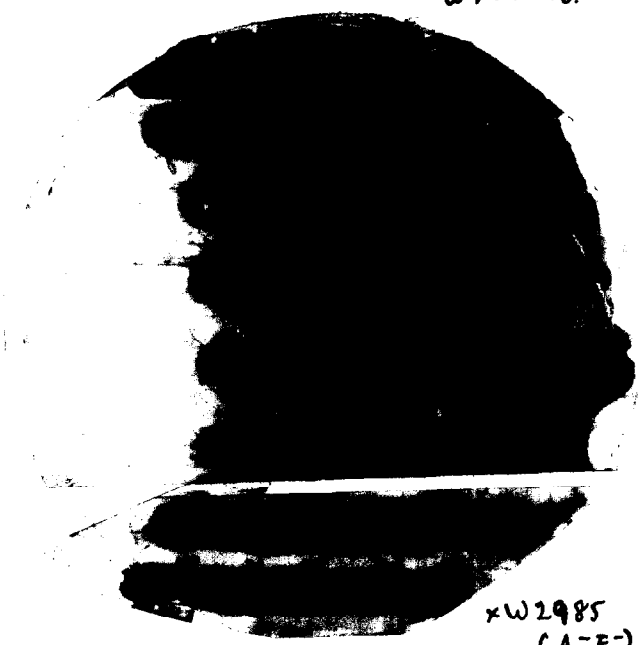
3026

×2979 (Gal⁻) 2985 (A_1) on MGal

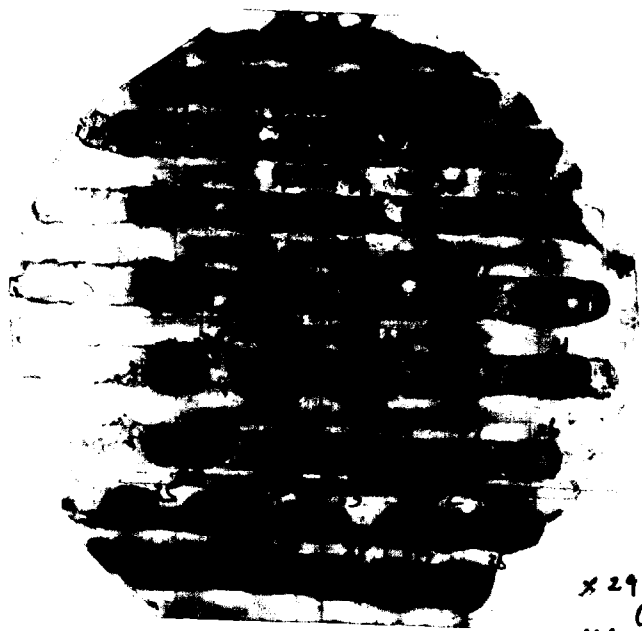
on MXyl W2979

Take HI colony, ~~on Xyl~~ transfer and see segregation of the marker by replica plating in same way.

Control W3086 F_8.



×W2985 ($A_1^- F^-$) on MGal

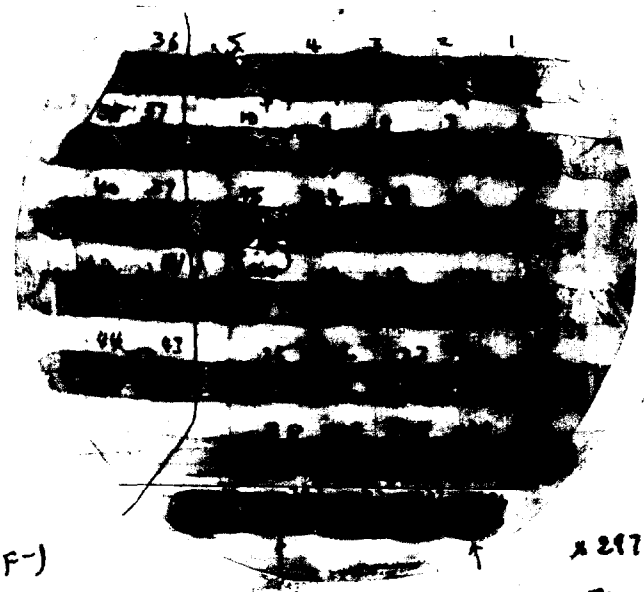


×2979 ($Gal^- X^+$) on MGal.

infection. Look for double F.

W6 F_4 — × W3086 F_8

×W2985 ($A_1^- F^-$) on MGal



×2979 ($Gal_2^- X^+$) on MGal

Test No. 28, ●. No. 29. and No 31. No 1.
on. Segregation, or [redacted] H1 for both.

2nd trial of infection of F_8 to F_4^+

W6 F_8 —× W3086 F_4^+.

23/IV 1959

REF: (cf. p 31.) $F_4 \rightarrow F_8$

Method: is completely same as W6 F_4 —× W3086 F_8.

Purpose: Is W3086 F_4^+ accessible to the infection of F_8?

Result:

			H_1 for A_1 only F_4 type	H_1 for Bal_2 only F_8 type	H_1 for both (double F)
	W6 F_8 —× 3086 F_4		35/35 (100)	0/35	0/35
Control	3086 F_4 only.		35/35 (100)	0/35	0/35

All the colonies of 5 tested

Conclusion: [3086 F_4] does not express F_8 character after mixed culture with W6 F_8.

This may be explained as ① lack of infection from F_8 to F_4.

or ② it is infectable to F_4 but recessive for phenotypic expression.

or ③ it is infectable to F_4 but is expelled after infection by exclusion between F_4 and F_8. F_8 is weak to the competition than that of F_4.

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Test for 2 states of fertility in $F_8^+ f_3$

W4525 (W3644 F_8^+)

1/14 ; 1959.

REF: ~~FLB, Hel, SR f_3.~~ Cfp 55, p 70, p 23

W4525.

1. Purify $F_8^+ f_3$: (W3644 F_8). on B Lac.
2. Inoculate it into penassay and let it grow. for overnight.
3. Make colonies (single colony) on Blac. and replica plate it on M Gal plates ~~W3644 F M Gal~~ W2979 ~~+~~ or W6 F−.

Purpose :

Does $F_8^+ f_3$ shows two states on Gal or M Lac or not ?
(only M or both or only Gal ?)

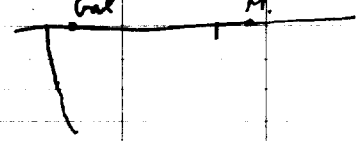
Result :

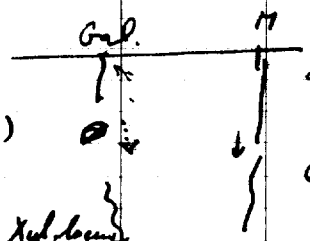
Only one state of fertility was observed. (see below) in both cross. (× M−, × Gal−)

Conclusion : F8 does not fixed on Rfd3 locus. Only attach at the Xyl locus

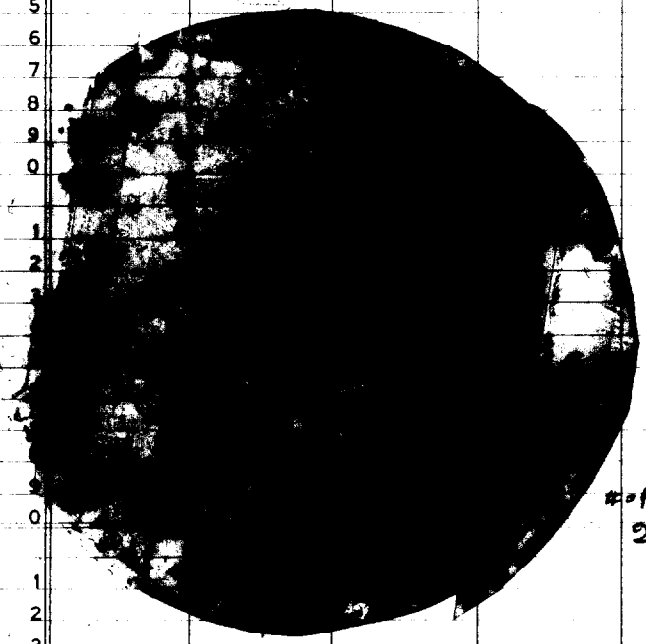
(∵ Lo. for Meth (Rfd make hi). ∴ Hi for Gal. and also only one state was observed.)

This means F_8 does not split off as easily as F_1.



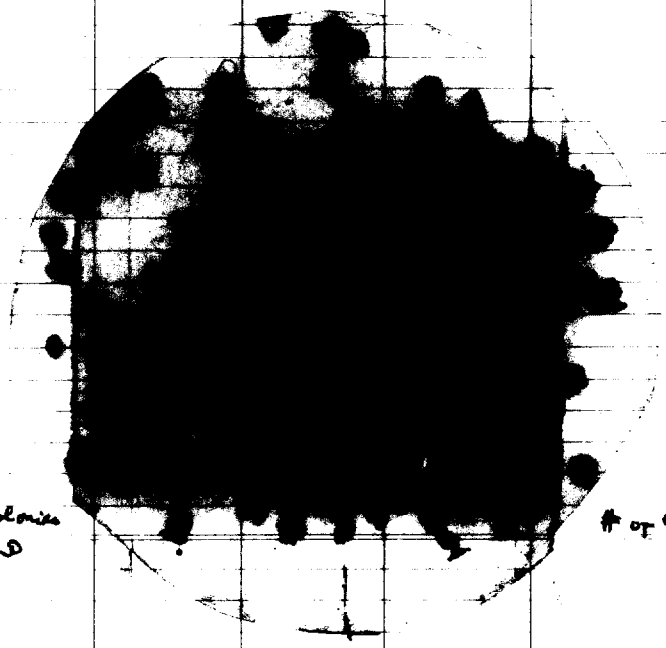


W4525



# of colonies 220

× 2979 on M Gal



# of colonies 220

× W6 F− on M Lac

Minimal cell number for infection of F' to F⁻

5/V ; 1959 REF:

1. Overnight culture (penassay culture) of W6 F_8 and W3086 are inoculated into penassay medium. (/ml culture/5ml penassay).
2. Incubate it for 2 hrs. at 37°C. (To make new culture.)
4. Mix them in the ratio of 1 : 10^6 cells/ml

No of tube	1	2	3	4	control
W6 F^8 :	$\times 10^{-2}$ (2×10^6) ↓ 0.1ml (2×10^5) +	10^{-2} (2×10^4) ↓ 0.1ml (2×10^3) +	$\times 10^{-2}$ (2×10^2) ↓ 0.1ml (2×10^1) +	$\times 10^{-1}$ (2×10^1) ↓ 0.1ml (2×10^0) +	⇐ 0
W3086 Ca. 10^8	1 ml	1 ml	1 ml	1 ml	1 ml.

5. Add 5m into the mixture. at the final concentration of 1,000 unit /ml. to kill F_8 donor. ~~after~~ 2 hrs after mixing, and incubate it overnight. 4:00 pm. 5/V/59.

2g/5ml H_2O $\times 10^{-1}$: 0.2ml / 1ml pen. final : 0.8 mg /ml.

6. Survival count of W6 F^8. & W3086.

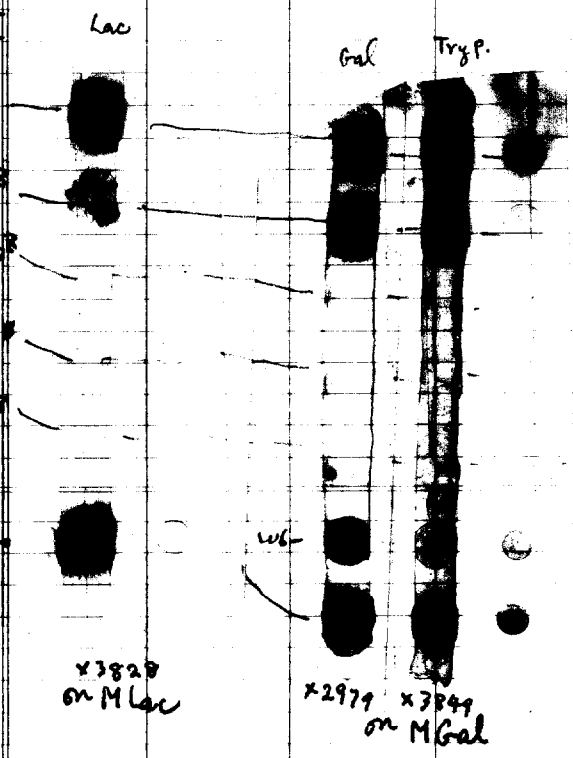
W6 F^8: $\times 10^{-2}\times10^{-2}\times10^{-1}\times10^{-1}$ (0.1ml/plate) — 230, 189

W3086: $\times 10^{-2}\times10^{-2}\times10^{-1}\times10^{-1}$ 0.1ml. — 206, 185

Cell Numbers used : Original cell #. — 2 x 10^8 cells/ml : 2 x 10^8 cells/ml.

number of F_8 donor

W6 F_8	3086
# of F^1	# of F^1
2×10^5	10^8
2×10^3	10^8
2×10^1	10^8
2×10^0	10^8
~~0~~	10^8
W6 F_8	—



Summary of experimental conditions.

young culture : ⇐ 2 hrs. culture in pen. at 37°C.

Inoculum size :

Minimal cell number for infection of F' (within 2 hrs.)

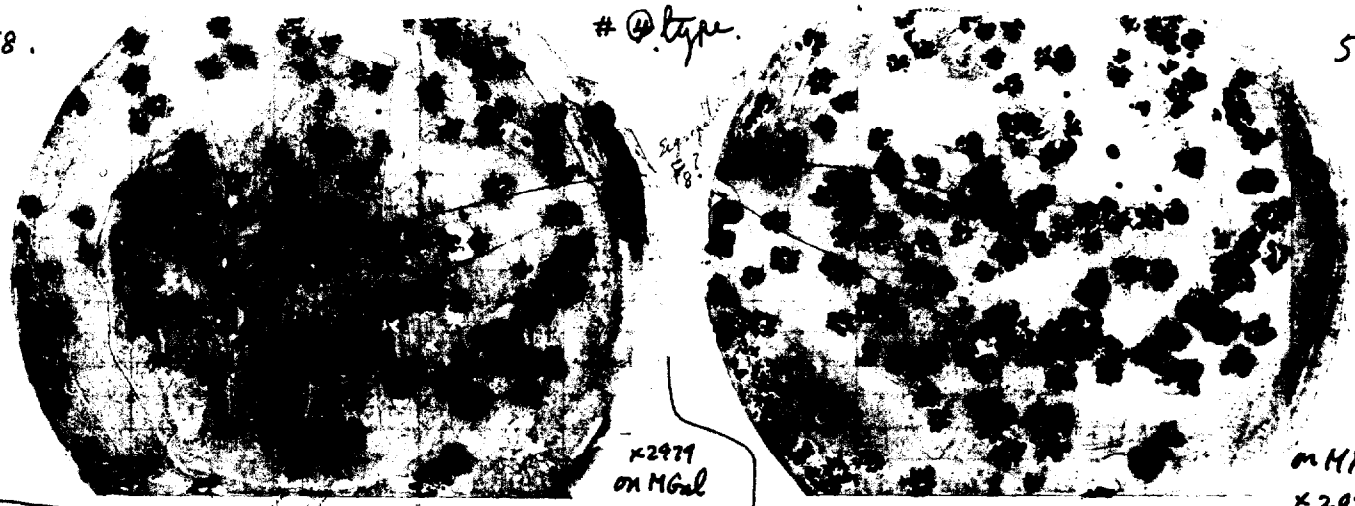
$2\times10^3 \sim 2\times10^1$

2000 ~ 20

Ca. 100^2.

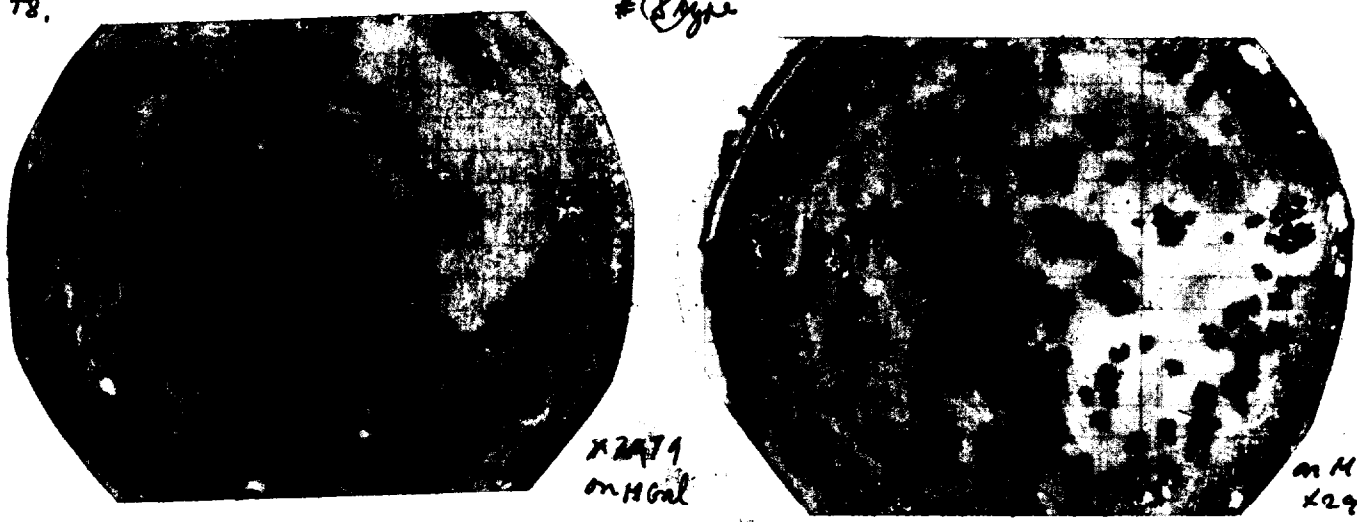
F4 → F8.

④ type.



F4 → F8.

⑧ type



Segregation pattern of F_8 and F_4 from double F. ($F_{4,8}$). judged by Replica plating method

Cf. ♀42

Compair these two segregant. above 4 type ; bellow 8 type.
Xyl plate is ok : F4 type is more futile on MXyl, than F8 type.
But MGal plate is not suitable for distination between F8 and F4.

Use # 2 and # 31. to detect recombinats between F'.

Segregation of F'+ from double F'+

30/IV : 1959 REF: cf. P. 42

1 2 3 4 5 6 7 8 9 10 — Isolating of various infected strains.

① Purify double F clone on Blac. (See: P 42. 23/IV ; exp.)

② Test individual colonies on fertility × A_1^- and Gal_2^- by spot test and replica plating method (Xyl : 2979 on MXyl.

③ See segregation of F.

Purpose :

① Is Replica plating on MXyl enough to tell whether F_4^+ or F_8^+ or not?

② Is F" segregate or not. If yes, how about the rate.

Results.

① By replica plating on MXyl seeded W2979 on it.

	# of colonies counted	F_4 (+++)	F_8 (+)
WLF4 × W3086 F8			
①	46	33	13
②	89	66	23
③1	67	17	50
③4	48	47	① stmil. ← (spontaneous loss? or after [redacted] between two different F's)
②8	36	36	0

② By Spot test

Pick typical segregants for expression of F_4 type and F_8 type and test complementality in the fertility on Gal_2 and Xyl_2. (namely Hi for Gal_2 and low for Xyl_2, or Hi for Xyl_2 and Lo for Gal_2.)

See back page.

: Segregation was not observed; ① gives all F_8 type
② gives all F_4 type.

This results can be explained by mutual exclusion during the growth in peptone medium for overnight.

Conclusion : These results seems parallele to the spot test done on 23/IV See P.

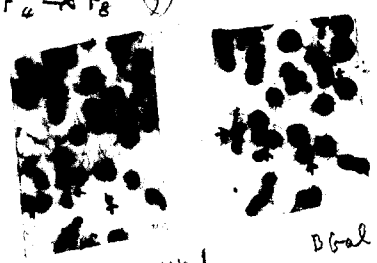
Further experiment : Does segregant segregate the other F'+ after multiplications? How about F_4 & F_8.

In this kind of experiment, I must also replica plate on MGal seeded 2979. To show the reverse relationship for fertility of Gal and Xyl.

Replica

master

$F_6 \rightarrow F_8$ (1)



mMXyl

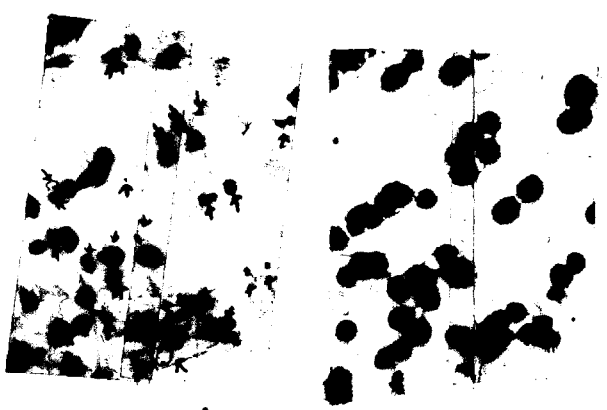
BGal

$F_7 \rightarrow F_9$

(31)

Replica master

F_8 F_4



MXyl

BGal

Further test for

(33)

F2 type

see front page; compare with the fertility.

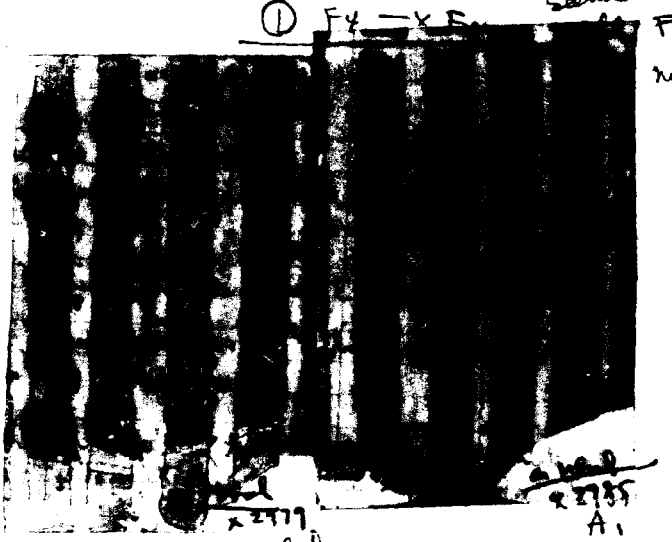
sel: Gal_2

sel: A_1

(1) $F_4 \rightarrow$ F same

F_3 type.

not segregate



Gal2

A_1

F_4^-

Isolation of (F_4^R) from 7086 F_4

5/IV ; 1959 REF:

Method :

1). Inoculate 7086 F_4 to penassay 5 m/.

→ 2). Shake it overnight on rotator at 37°C. (This makes more efficiently for isolating F^-)

20 plates → 3). Seed 0.2 m/ / plate (on BGal)

4) Irradiate it by U.V. for 10 sec. to each ~~colony~~ plates.

● 5). Incubate it overnight

Ca. 200 colonies per plates → 6). Replica plate it on MGal seeded w 2985 (A_1^-) on it.

of F^- gal: Ca. 2 ~ 3 colonies/plate 7). Pick F^- colony and inoculate it into broth.

8) Retest the fertility cross times w 2985 on MGal.

9). Test infectability to $F_1^{\bullet}$ or F_4 or F_8.

Result :

1. # of colonies tested : Ca. 4000
2. # of F^- colonies obtained : 72 (~~abortive~~ only 3 were F^- the other are F^{+}) recombination-
3. # of F^- colonies obtained : after testing by cross brushing method

see p. 52. and the back page.

4. Rate of F^- obtained

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Isolation of more-infective F' from F4.

Mutant "Rapid infection"

7/14 1959 REF:

• Experimental schedule:

W6
~~3086~~ F4 : 10^3 cells/ml F4 + ~~3086~~ F- 10^8 cells/ml : 1 ml
W6 F4

↓ incubate overnight

W6
~~3086~~ F4 : 10^3 cells/ml F4 + ~~3086~~ F- 10^8 cells/ml : 1 ml
W6 F4

↓

Repeat 10 times.

• Method: ① 1st step.

W6 F4 $\times 10^{-2}$ $\times 10^{-2}$: 0.1 ml /5 ml. : Ca. 10^3/ml.

W6 F- 1 ml + /5 ml. : Ca 10^8/ml.

+

5 ml fresh penassay.

* tubes for each.
1 tube for W6 F4
1 tube for W6 F-

1st : 5:30 p.m.

↓

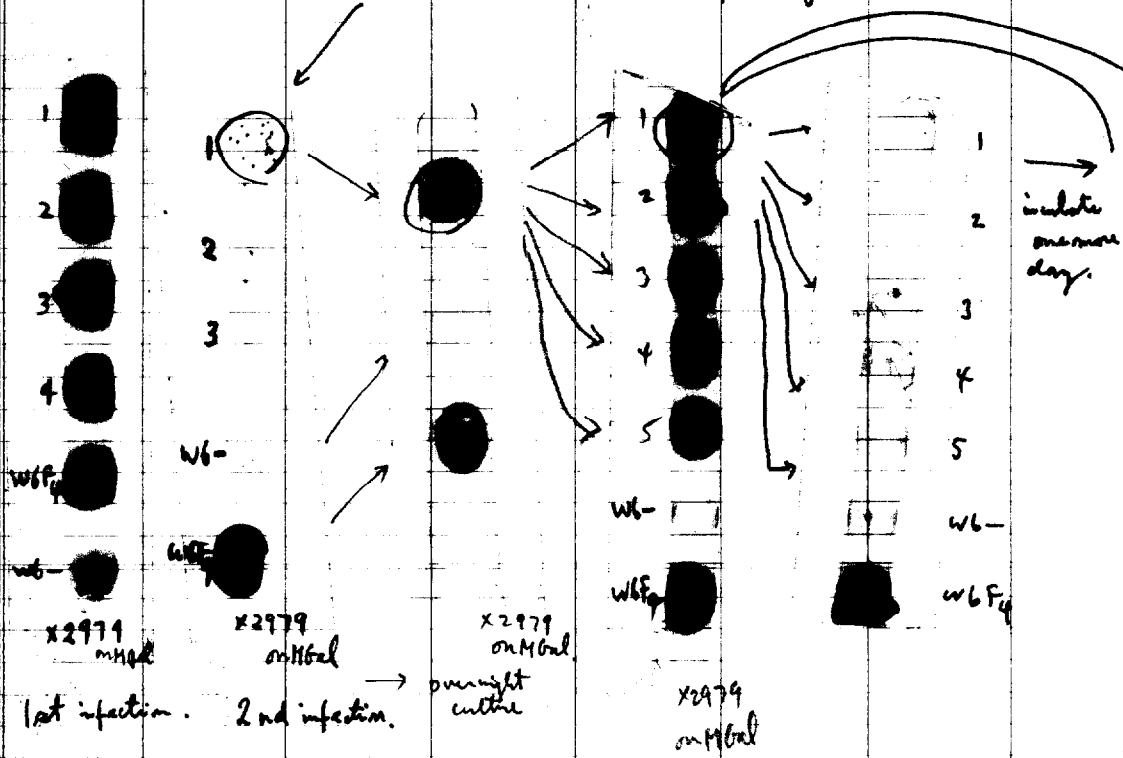
Incubate overnight. — Test fertility by spot test. Save it in cold room.

↓

Use most fertile tube: Repeat again.

(2nd) W6 F4 $\times 10^{-2}$ $\times 10^{-2}$ $\times 10^{-2}$: 0.1 ml /5 ml Ca. 10^1/ml 3 tubes each.
control. W6 F4 ① W6 F- ②.

(3rd) Inoculate No 1 into penassay. 5 ml. and incubate it overnight.



Test for immunity to the infection of F_8 (of F_8 F^- strains)

9/4 ; 1959

REF: See p 49.

Principle: F_8

$M\ Gal_4^- \longrightarrow \times$ ⑤ $M\ Gal_4^+\ F_8^{R?}$

(W3220 F_8)

×

$M^+\ Gal_2^-\ F^-$

(W2979)

Method

W3086 F_8 ; W3086 F_8 + W2979

W3220 F_8

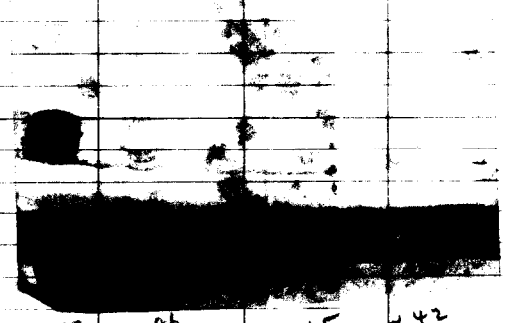
W2979

W3220 F_8 + W2979.

on M Gal

o Difficulty is Gal_4 and Gal_2 are not allelic with each others. Therfor, background gives many a black spot. But it may be possible to differenciate between infectable spot and noninfectale spot.

Unsuccessful result



might be 3220 F_8 × 2979 → M Gal

9/4: Other method testing F_8^R strain. by replica method.

1). ~~W3220~~ W6 F_8 + 3086 F_8^R → on 5m B Gal → Replica plate on M Gal Streaked W2979 on it.

Ratio of mix. 1 : 1. in pen. → incubate it overnight → seed it on B Gal →
→ Replica plate it on M Gal. (& 2979).
Inoculum size : 10^8/ml.

2	5	#2	W3086
↑	↑	↑	↑
W6 F_8	W6 F_8	W6 F_8	W6 F_8.

control.

Results : All the clones, # 2, # 5, #42, are infected by F_8, in otherward, these F^- strains are plain F^- in the sense of immunity. But looks more resistant to the infection of F_8. See back page.
(Compair with control)
5 is most promissing.

control

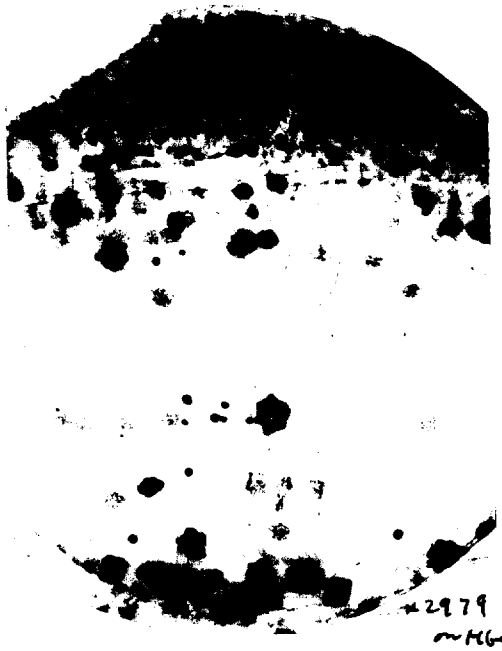
$F_8 \rightarrow$ 3086

×2979 on MGal.

$F_8 \rightarrow$ #5

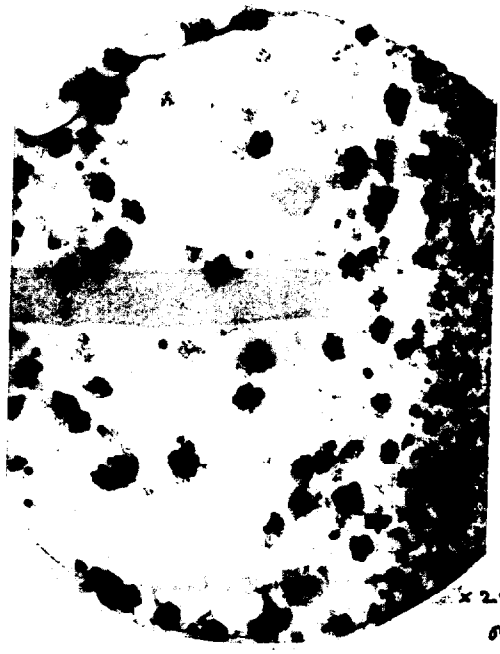
×2979 on MGal.

$F_8 \rightarrow$ #2



×2979 on MGal.

$F_8 \rightarrow$ #42



×2979 on MGal.

next step : Infect F_1 to these & strains and see F_2 coming up or not.

(See p. 61)

Test for transfer of Hfr$_3$ to F$^-$.

W3234 (Lp$^+$ ~~Hfr$_3$~~ Mal$^-$ MOal$_2$ Lac$_1$)

W1394 (TLB$_1$ F$^-$ S^R) × W6 F$^-$ on Mlac

Test this possibility.

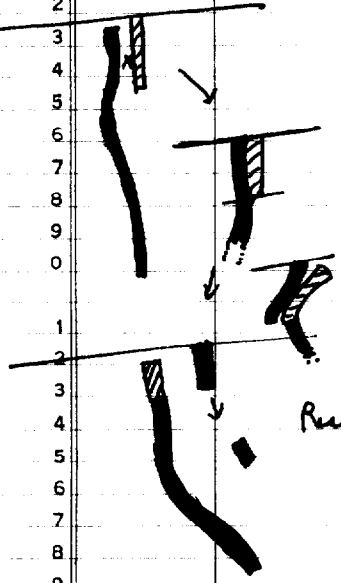
Procedures.

① Purify W3234 on Blac checks S^s before use.

② old culture of W3234 1ml + W1394 ~~culture~~ 0.1ml + 5ml phosp. and incubate it overnight.

③ Streak it on Blac Sm.

④ Replica plate it on Mlac needed W6 – on it.



Result: negative result See bellow.

Isolation of F_2 from (3000 (Hfr_2 Th) for Adelberg) W4531

13 NOV. 1959

REF: Cf. p 62.

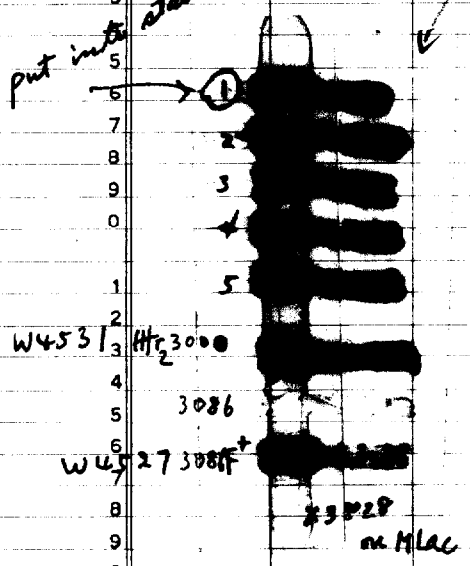
Method:

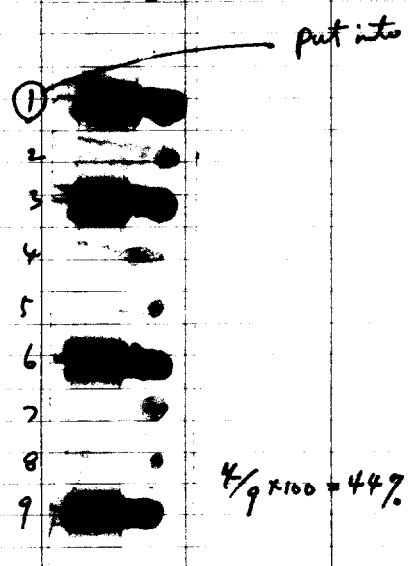
1. 3000 5 ml overnight culture + 0.1 ml phage W3086 •
   ↓
   Incubate it overnight at 37°C.
   ↓
   Seed on B Lac Sm.
   ↓
   Replica plate on M Lac seeded W3828 mixt.
   ↓
   Look for Recombination + colonies from it.

2. Test Hi-markers with 3000 as a control and infect F_2 to W6−.
   Isolated F_2: 3086 F_2 1, 2, 3, 4, 5; 3000 control 3086 3086 F^+
   × 3828 on M Lac.

   See ~~back page~~.

3. Infect F_2 to W6−, ~~and check~~ and compair W6 F_2 and W3086 F_2 in the pattern of chromosome transfer to F^-.





Infection of F' after killing of F'+ by U.V.

12/4, ; 1959 REF:

1. Wash W6 F4 once by H_2O, and suspend into 1 ml of Water.
   cultural age: Overnight culture.
2. Irradiate it various times, and add to W6−, and incubate them overnight

	U.V. W6 F4	+ W6−	+ 5 ml pheasey broth.
~~F+~~ :	0.1 ml.	1 ml	

- Time of irradiation:

  0, 10 sec, 15, 20, 25,

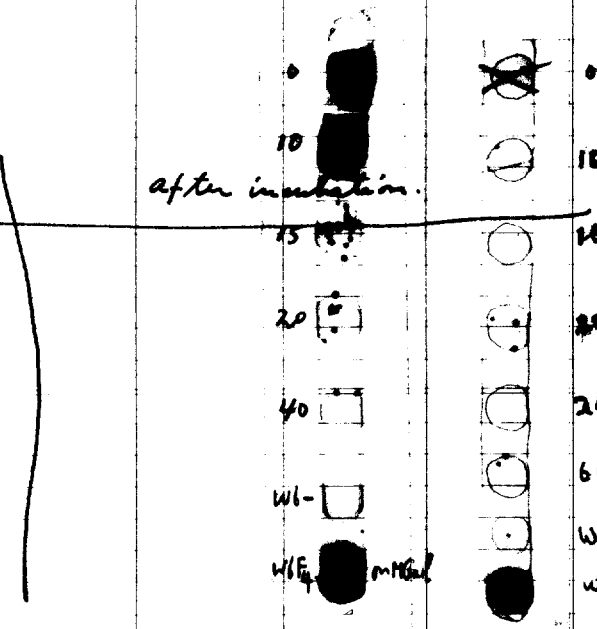
- Spread 0.1 ml of $\times 10^{-2}$-diluted suspension (over EMB.): survival test.

Result:

Time of U.V.-irradiation	0	10	Time of exposure (seconds). 15	20	25	60
Infectivity of F4.	−	±	−	+	−	+
survival count: # of survival cells	152×10^6 / 129×10^6	0 / 0	1 / 3	2 / 2	0 / 0	11 / 19

(Same number as much as used in infection.) 0.1 ml.

(11/19: ~~area~~ thickness of layer irradiated are ca. 2 mm.)



before incubation — after incubation.

Repeat again.: Use thin layer and mix well during and after irradiations.
applay DNase to the sample. Test lysophil. or temp sensitivity

Test of the infectability of ♀ strains obtained from F4 by U.V. irradiation

11/4 ; 1959.

REF: cf. ♀52

Infectability of ♀ #39, 69, 78 were tested by mixed culture with W6 F4.

W6F4 1ml + ♀ strain 0.2ml + 1ml phosphate

↓ Incubate it overnight at 37°c.

↓ Purify it on Blac Sm.

↓ Replica plate them on Hbal seeded W2988 F⁻A₁⁻ on them

Results:

all of these ♀ strains are infectable by F4. (See back page.)

Not immune to the infection of F4.

These ♀ strains are same as F⁻ in the sence of ~~Resist~~ not immune to the infection of F4.

78 may be more resistant than plain F⁻. (3+6)

control.

W6 Fq → x 3086

×2979
on MGal.

W6Fq → #39.

×2979
on MGal.

W6Fq → #69.

×2977
on MGal.

W6 Fq → # 78

×2979
on MGal.

↑
78 maybe promising.

Mix #78 and #5, or #2, or #42 comes from F8, and test the fertility.
(F10) See p.54 Try all the combinations.

Segregation of F_1 and F_8 from double F. ($F_{1,8}$ / $F_{4,8}$)
(2nd segregation.)

14/V ; 1959 REF: Cf. P48, P53.

Test of 1 2nd segregants 3 4 5 6 7 8 (from master plate / replica plate) 9 10

1. These strain used are obtained from P. 53 - experiment (Replica plate.) of 2nd segregations. # express isolation numbers of p.53. (see p53 front page bellow). Replica plated on MGal seeded W2979 on it.

Results:

From $F_{8,8}$.

1. F_8 type segregants only gives F_8 type only. (Sometimes gives few F^-). This may be reversion.
2. F^- type segregant which comes from $F_{8,1}$ always gives sexual F^+ type.

From $F_{1,4}$.

3. F_4 type segregant gives F_4 type with very few exceptions.
4. F_1 type segregate few F_4 and many F_1. But some colonies show higher futility (than F^+). They may still have F_4 in it.

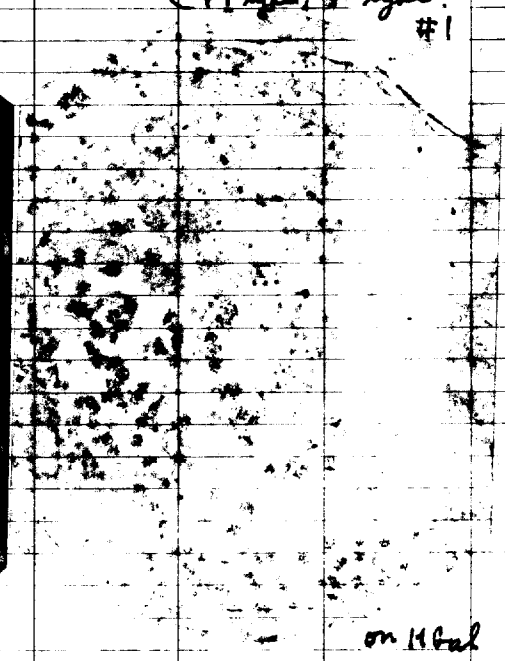
[See back page.]

Segregant from $F_{8,1}$. (Example.)

F_8 type #1

(F_1 type) F^- type #1

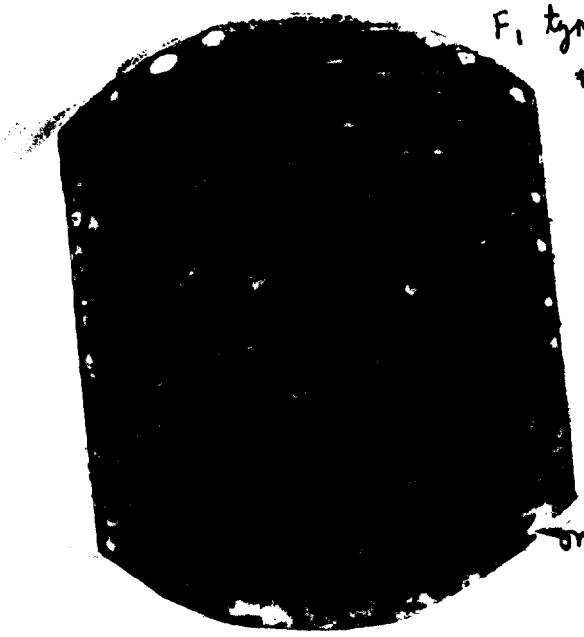
on MGal ×2979



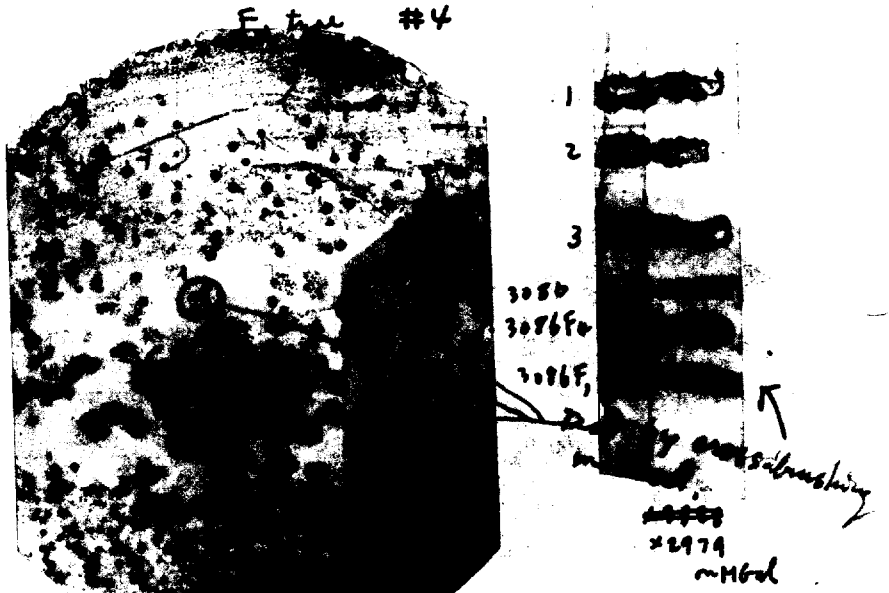
on MGal ×2979.

○ Check if (F^-) arised after infection of F_8 to F^+. It might be the result obtained from exclusion between F^+ and F^1 as reported in the exclusion between virulent phage and temperate phage.

○ Check. F_4, or F_8 segregated are still gives F^+ or not.



Segregation of F_1 and F_4 from $F_{4,1}$. F_1 type segregant gives only F_1 type after division.



Probably, F_4 may can grow with F_1 without interference. Both F_4 and F_1 can grow within one cell without competition.

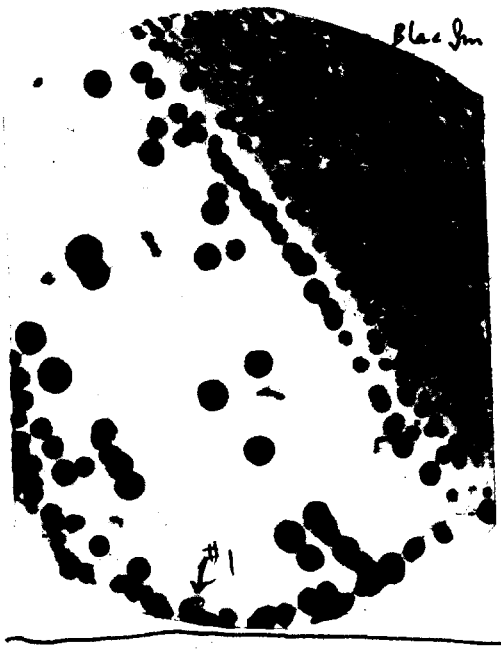
F_1 and F_8 are not miscible, but F_1 and F_4 are missible.

F_1 and F_8 exclude with each others. But F_1 and F_4 is compatible in one cell.

(Some colonies ~~assemble~~ assemble to F_4 and some colonies assemble to F_1)

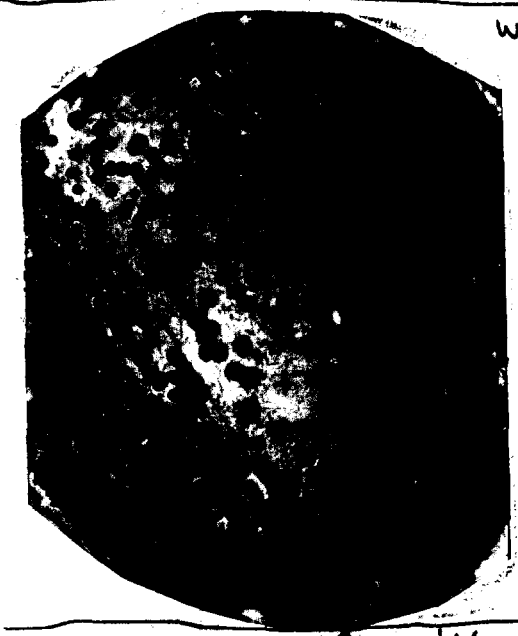
Interpretation: Both kinds of Fs are spread into the bacterial populations, and they are distributed with different proportion of particles.

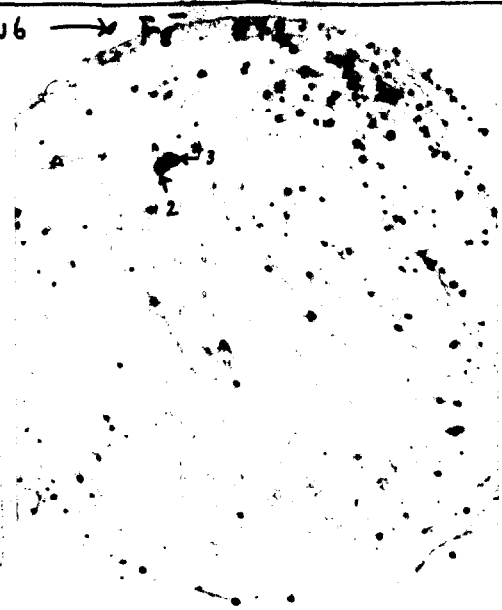
Master plate W6 → F_8^- # 2. continued.



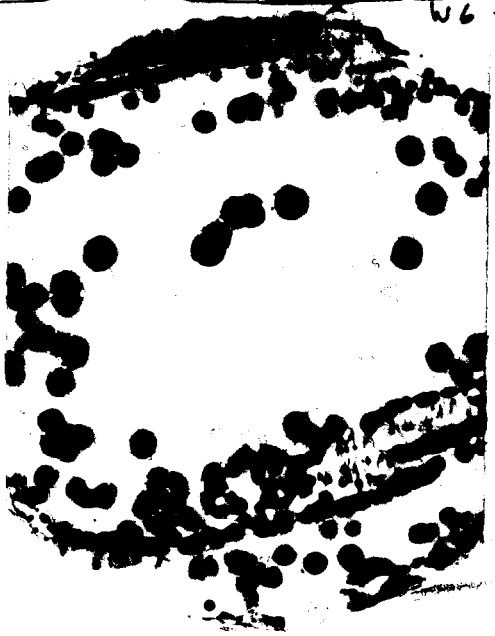
×2979 in Kbal

W6 → F_8^-





W6 → F_8^- #2.



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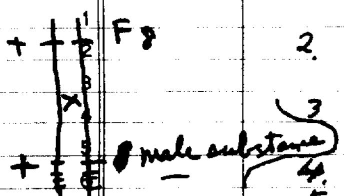
Infection of F_1 to F_8^- W6 $\xrightarrow{F_1}$ × F_8^- 3086.
2, 5, 42.

14/v ; 1959. REF: cf. p.58. see P.68

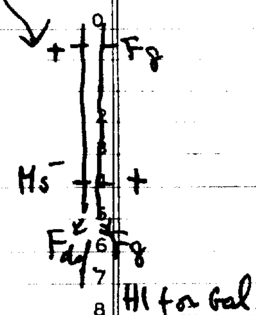
\#2,[6] #5, #[7]42.

Principle

1. Mix W6 1ml + F_8^- (M 5[?] Mal.) 1/ml + 5 ml [illegible]
2. Incubate it overnight.
3. Purify it on BLac Sm.
4. Replica plate it on M Gal seeded 2979 on it.
5. Pick Hfr colonies and retest by cross brushing, or spotting method.



Result : Seems promissible! Ratio is quite low, but gives Hfr colonies by infection of F_1 to F_8^-.

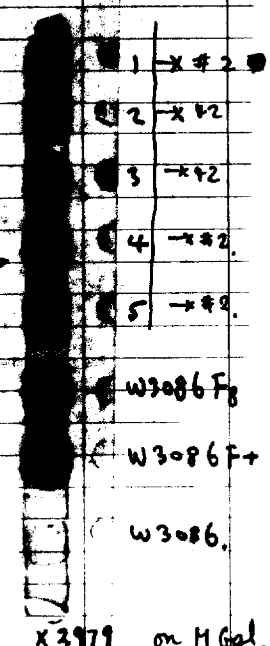


1. 3086F- is more infectable by F_1 than 3086 F_8^-. But both of strains are infectable by F_1. Susceptible to infection of F_1.
2. Infected F_8^- by F_1 gives Hfr colonies. This may be a recombinant of F.

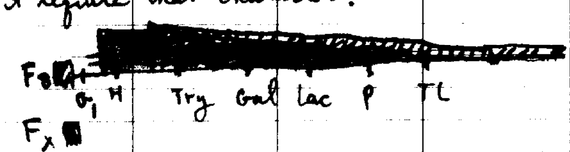
Further experiment:

1. Pick Hfr colonies and test by (cross brushing method) spot test (see bellow)
2. Recombine ~~[illegible]~~ these defective P'.
3. Retest using more larger scale.
4. check it if type male or Standard type or F' type

put into stab.
Test infectivity of this F'?
and test pattern of fertility on various markers.



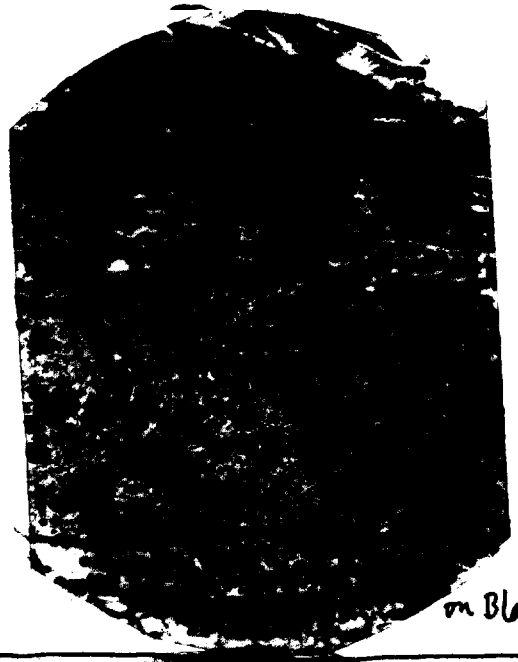
H1 for Histidine : This Hfr type mutant seems differ from F_8, however, it is quite useful because H1 transfer[illegible] character for histidine, it is linked to antigenic characters.
How does it require that character? H1-marker seems shift a little to Histidine side.



W6 → × 3086
control.

Master plate

Replica plate.

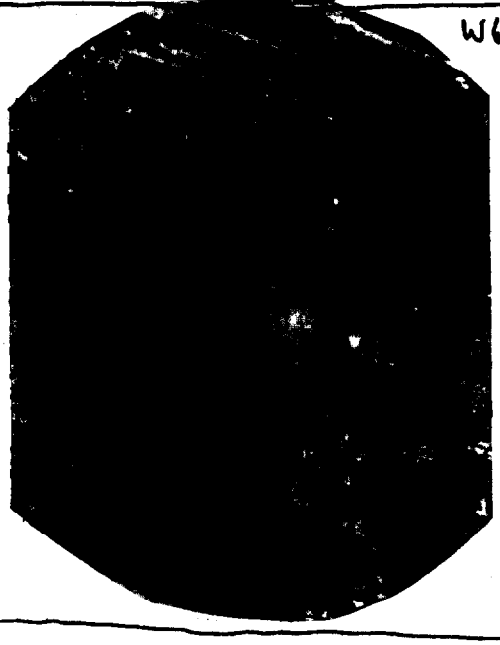


× 2979
on MG al

on Blac 9m.

compare ratio of inactivation of F_1

W6 — × F_2 #5.



× 2979-
Mgal

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Comparison of Hfr_2 and F_2 strains.

17/4, 1959 REF: Cf 857

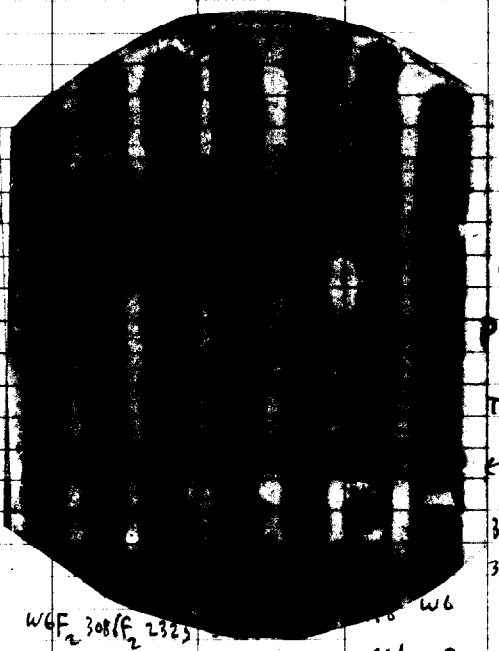
1 conclusive 2 3 4 5 6 7 8 9 10

	B_1	TL	Ara_2	P	Lac	Gal	Try
		?					
			H1	H1	H1	H1	H1.

F_2 shows quite same quality as Hfr_2. in transfer of chromosome.

Hfr_2 F+ F+ F- T-

on Mgal.



WGF_2 308 (F_2 2323) Hfr_2

on M lac B_1

Try Syntrophy.

A_1

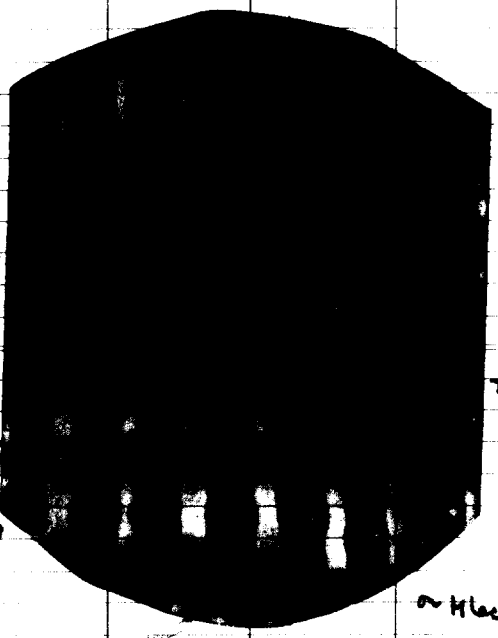
G_1

TL

← This is mistake: 2979 was spotted on M lac B_1

3824 Lac

3950 A_2



← Syntrophy

TLB_1

← mistake W2979 was spotted by mistake.

on M lac

W6 —

3086 —

W6

3086+

Test Ara_2 2383

$3086F_2$

$W6F_2$

Selected H+ Ara_2^+

on M Ara_2 ×2979

Isolation of F_5 from Hfr_5 (W4537: Hfr_5 [redacted]) (W6-, 3086)

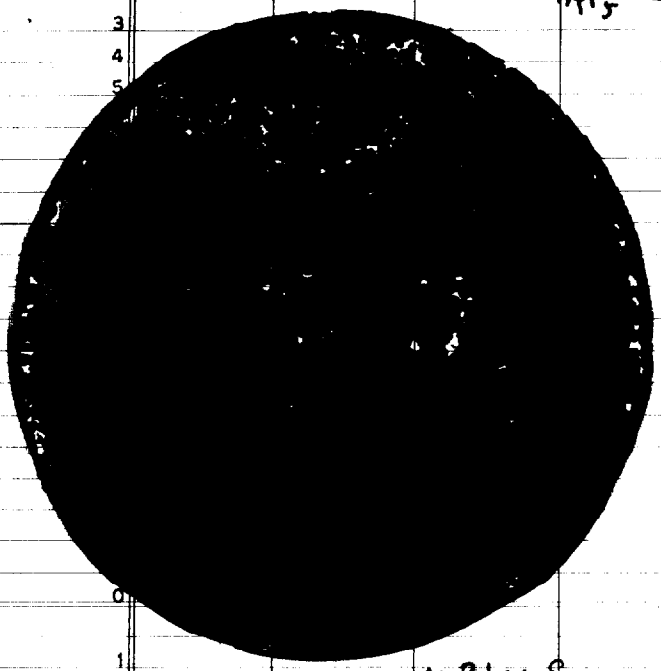
16/4 ; 1959

REF:

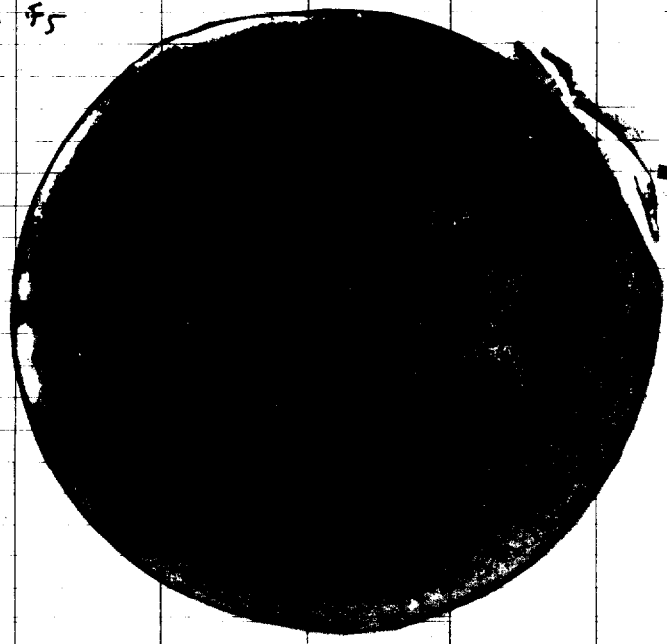
Method : Standard method for isolating F' from Hfr strain was used.

1. Replica plated on MXyl seeded 2979 on it, and looked for Hfr colony formit.
2. Test infected F'_5 by cross streaking method. (check H1 for Xyl)
3. Input F_5 to W6-. see back page :
4. Compair fertility-pattern to original Hfr_5 (Hfr_5 & H1 for Th, Xyl, A,)

W4537
Hfr_5 → F_5



on B Lac Sm



3086 F_5

1

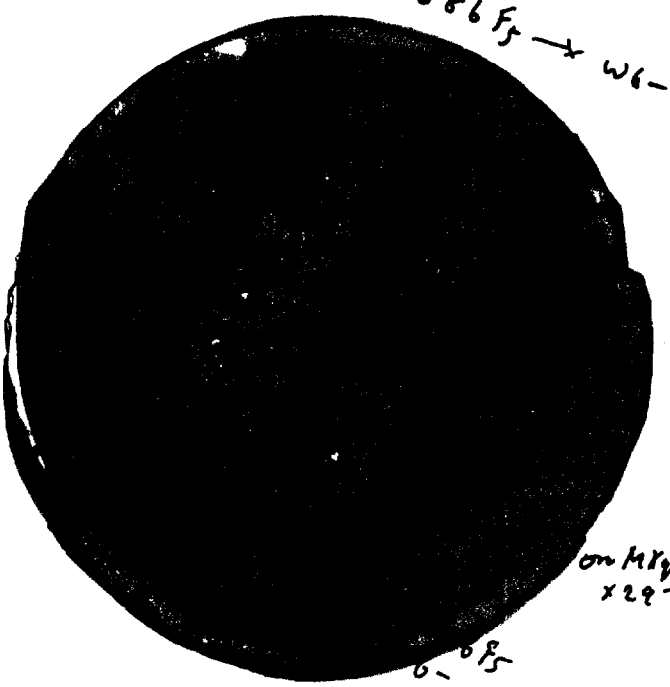
2

3086+

3086-

×2979
on MXyl.

on MXyl.
×2979.



Ratio.
F^+ F^- F'
17/26 = 65.4% 9/26 = 34.6% 0/26 = 0%

This infection does not give arised ~~(F5)~~ Hfr type. Then, what factor was left behind? If more isolates were tested, it may be obtained W6 F_5^-. (Test at least 100).

3086 F_5 —cure by AO.→ 3086 F_5^- ← F_1

↓

? If a factor which controls high fertility was left ~~out~~ back, infection of F_1 to 3086 F_5^- may ~~give~~ results Hfr 5 type cell.

Testing for infectivity of $(F_8)F'$. system

17/V ; 1959 REF:

Principle : M⁻ Gal⁻ ● F_8
M⁻ + F⁻
↓
+ Gal⁻ F⁻

(W3220 F_8) W4534
(W6 F⁻) W4354
W3994.

Select on MGal.

Purpose : Does this system work well ?

Result : ok.

Method :
overnight culture : Penassay 5 ml
at 37°C.
↓
spot test,

F⁻ M⁻ Gal⁺ W3220 — M⁻ Gal⁺ F_8 W4534 F_8 lysis

W3994 (● Gal F⁻)

W6 − (M F⁻)

W3994 + W6 −

● ↖

Use young culture.

MGal

Developed method to look for colony of F.

1. Dilute W4534 into optimum concentration.
2. Spot it on MGal seeded W3994 + W6 −, and incubate it — 40 hrs at 37°C.
3. Pick — colony from the spot, and inoculate it ● into penassay. (5 ml)
4. Infect the F to 3994 F⁻ (mix culture ● it with 3994 ● and purify it)
5. Test the infectivity by spot test.

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Unsuccessful result.

Recombination between F_8 defective mutants (polysaccharide defect.)

12/V ; 1959.

REF:

P_8^- : Polysaccharide deficient : P_8^+ : wild type.

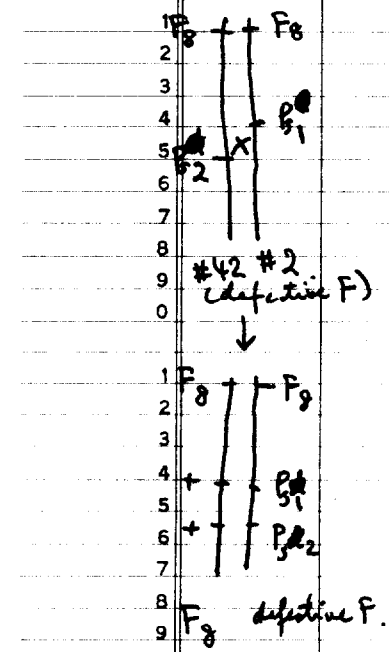
~~Escherichia~~ Strain used ; # 2, 5, K 2, (W3086 F) -.

Combination.

1 2, 5, K2. ; 2 2, 5 ; 3 2, K2 ; K 5, K2 ;

5 2, ; 6 5, ; 7 K2, ;

9 2, - ; 10 5, - ; 11 K2, - ; 8 - only



• Experimental conditions.

Age ; Overnight culture of W3086 F_8^- (W3086 F_8^- 2,5, W3086

Ratio : 0.4 ml F_8^- + 1 ml penassay broth.

↓

Incubated overnight at 37°C

↓

Spotted on 2979 & 3994 on MGal. (Control : W3086 W3086 F

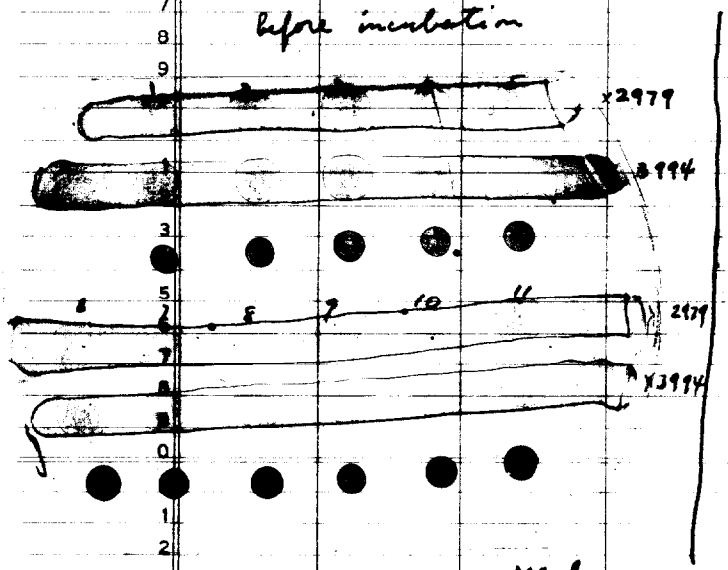
Sel: M^+ Gal_2^- or Gal_2^+

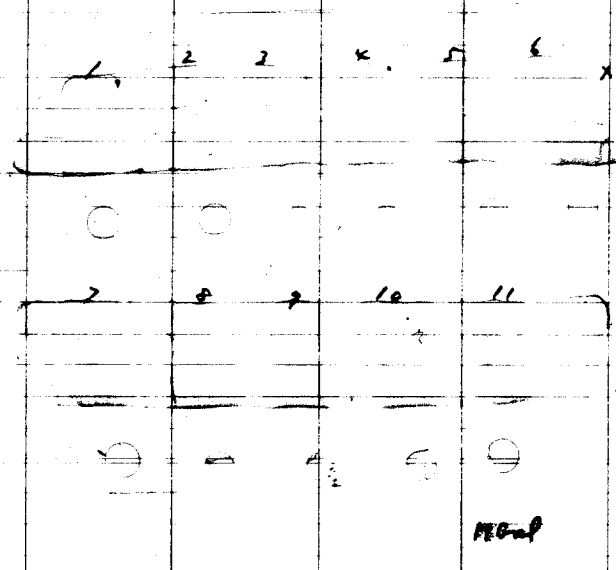
• Interpretation for this negative result.

Cell contacts or conjugation does not occur between those defective F^+ (just like F^-)

After incubation.

Before incubation





Test W4171 (1895 F+ : Novick's F+). W4171 (M) reverted from W1895. sent by Novick.
(Test H1 marker).

21/V., 1959 REF:

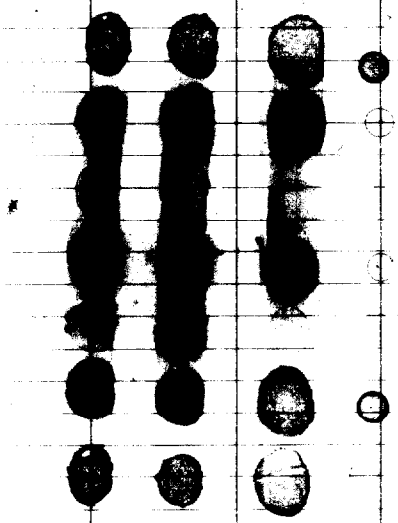
purpose: Is it F+ or F'+? Test H1 marker.

method: 1. purify W4171 on B bac. and pick single colony from it.
2. Incubate it overnight.
3. Spot test. on M Gal. Control W6, W6F-.

Result: W4171 shows same fertility pattern as F+ (W6).

Conclusion: W4171 seems not F'.

Summary of W4171
① It carries "Infective F."
② curable by AO treatment.
③ fertility is low as same as F+ on every markers.



on M Gal.

Test (on the infectivity of) F of W4544

20/V 1959 REF: cf. P.61.

Purpose : Does F of W4544 infective? Is it Removable by AO treatment?

Principle : 3086 $F'_{Rec.}$ ⟶ × W6−

(I) Infectivity.

method : 1ml 3086 $F'_{Rec.}$ + 0.1 ml W6− + 5 ml phosay.

↓ Incubate overnight

↓ purify on BMal.

↓ Pick Mal+ and test their fertility in cross × 2979. on MGal.

This strain carries F_1 or F', and is not standard Hfr. Is O3 type Hfr? What was left behind F_0, then?

(II) Treat W4544 by AO. F in W4544 is sensitive to acridine treatment. (see back page)

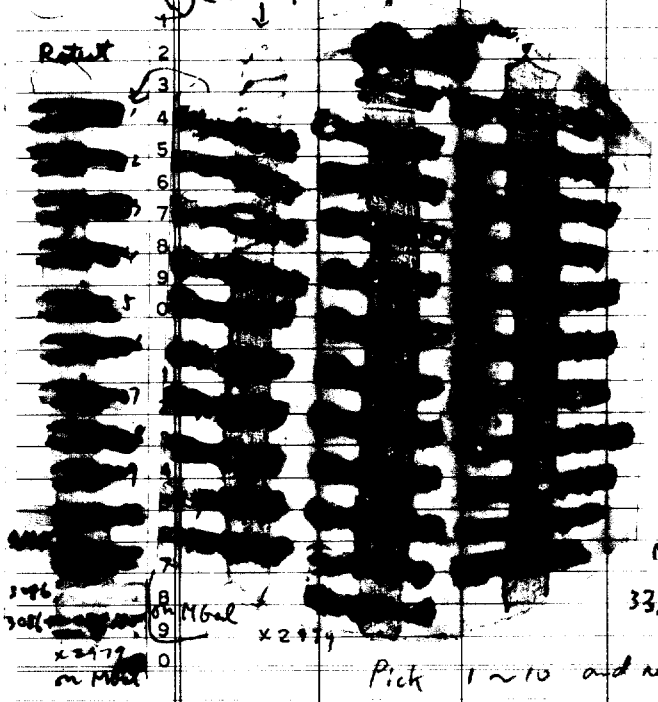
Result: AO F^-/F^+ 13/13 ; Control F^-/F^+ 24/24

Result.

(I) Infectivity W4544 $\xrightarrow{F_1^2}$ × W6−

↓

Result



Rate of infection

33/33 × 100 = 100 %.

Pick 1 ~ 10 and retest

Result

1. W4544 infect F to F^-.

Effect of UV to infectivity of F'.

22/V ; 1959 REF:

Experimental conditions.

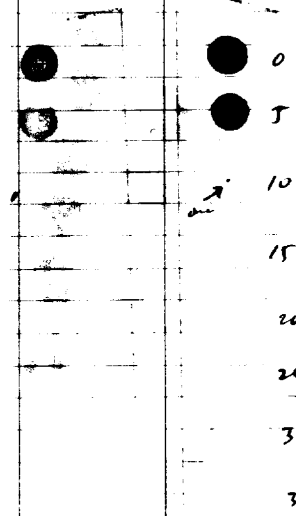
overnight culture. W4534 (M⁻ Gal₄⁻ F₈ p^s)
W6⁻ (F⁻ M⁻)
W3994 (F⁻ Gal₄⁻)

U.V. 6.5 cm.

1ml (*Penassay culture) of W4534 → Spread it onto petri-dish. diameter 8cm.

Take one drop of sample after each UV irradiations.
Spot it on BGal and on MGal streaked a mixture of W3994 + W6⁻ (1:1).

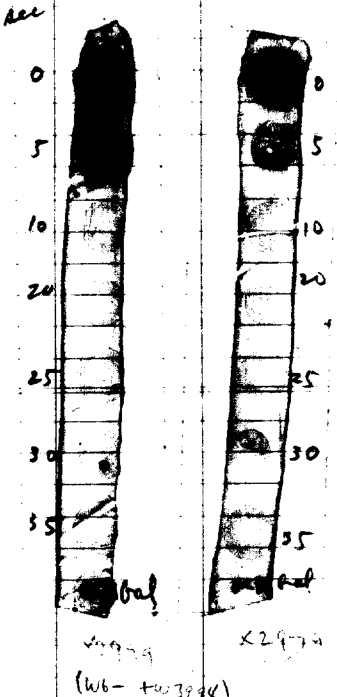
Survival test.



Conclusion:
F' itself or mechanism for infection of F' are sensitive to UV as well as host cell.

on BGal. stained. Fast green 5% Ac. Acid EtOH. Petri's method

Time of UV irradiation sec



W3994 (W6⁻ + W3994) X2994

Replica plated on M Lac B, from Blac Sm

Untreated Control.

AO treated

Looks two states.

×3825 on M Lac B,

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F8 × F4. Ref. P42. P50

Recombination of F itself: selection of Hi for (G1 or A2.) [Histidine] or Hi for both markers.
22/V ; 1959
REF: (F⁻ A1 Xyl Mal1 Gal Try Pur, Lac P.)

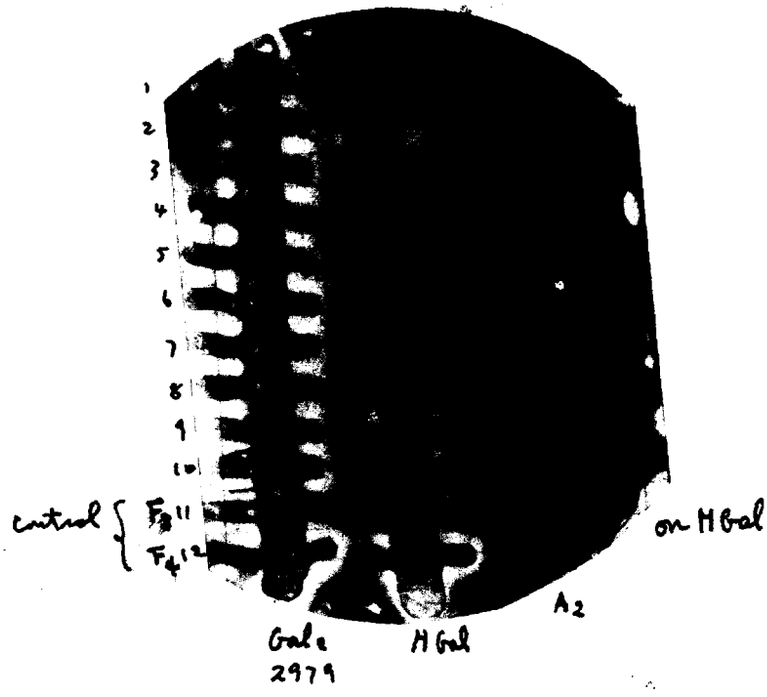
1. Take doubly infected compound male. (# 1, 2, 31) see p. 42
2. Make colony on B Gal
3. Replica plate it on ~~Hi~~ M Gal. seeded ~~W4529.~~ W3861 F⁻ A2⁻
4. ~~Look~~ for HI colonies to ~~Histidine~~ A2.
5. Pick the colonies HI for ~~histidine~~ A2. Infect the F' to the other F⁻.
6. Confirm stability and uniformity of the progeny. (non-segregational.) (Heritable.)

Replica | Cross brushing

Ca 500 colonies per plate.

		plate No.	# of Hfr mating	Cross brushing
F48	1.	1	–	
		2	–	
		3	1.	
		4	–	
		5	–	
		6	2	
		7	–	
F48	2	8	–	
		9	2	
		10	–	
F48	31	11	–	
		12	1	
		13	–	
		14	–	
		15	1	
F48	C	16	–	
		17	1	
		18	1	
		19	1	
		20	–	

A2 : F4
Xyl : F8.



#10 obtained from control !!
([redacted] of F_8.)
spontaneous mutation ?

3rd Conformation of the Infection of F' to F+.

F_4 —x F_1^+

20/V ;1959 (W6 F_4) (W3086 F^+). REF: cf. P53.

W4518 W4527

1. purify both strains before using.
   Purified on Blac. and inculated into 5ml plusay.
2. Ratio of mix: 1:1 (1ml + 1ml) overnight culture.
   1ml W527 (control)
3. Incubate then overnight.
4. Streak on B Lac Mal Sm.
5. Replica plate it on M Gal seeded W2985 on it.
6. Pick Single colony of $F_{4.1}$ and suspend it into plusay. Streak it on Blac Sm. $R^- A_1^-$
7. Replica plate it on M Gal seeded W2985 ($A_1^- F^-$).

Results and conclusions.

1. $F_{4.1}^+$ is infectable by F_4. and gives $F_{4.1}$ and F_0 probably $\pm F_r$.
2. The percentage of infectivity of F4 to F_1^+ is ca. 1 %.

on Mgal.
x2979

6/669 x 100 = 0.90 %

ca. 1 %

Scheme. $F_4 \times F_1^+$

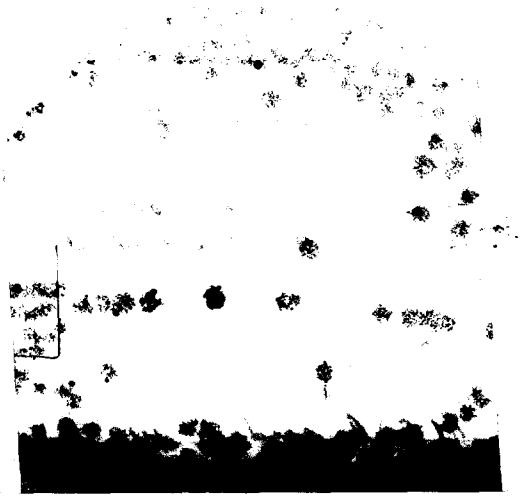
$\downarrow$

F_{41}^+

$\swarrow \quad \searrow$

$F_4^+ \quad F_1^+$

Segregant F_1 type.



Double F type F_{41}. # 1

W6 $\xrightarrow{F_1}$ × 3086 F_4^d Control $\frac{F_1}{W6}$ × 3086.

W6 $\xrightarrow{F_1}$ × 3086 F_8^d

20/4 ; 1959 REF: Cf. P.61

method : Same as P.61.

Strains : 3086 F_4^d #78 (see p.59), 3086 F_8^d #2.

W6 : F_1 donor cultural age : overnight culture.

Control W6 ⟶ 3086.

Replica plate on MGal Seeded W2979 (Pat F⁻) (Select M Gal⁻.)

Replica plate. Retest the Hfr colonies by Cross-lining method

	No of colonies tested	Hfr colonies (Hi for Gal.)	Hfr clones	
F_1 W6 × F⁻ 3086	286	1	1	1
	255	0		
	334	0		
	269	0		
	256	0		
	232	0		
	Σ 1632	1		
F_1 W6 × F_8^-	88	0		
	74	0		
	91	0		
	103	0		
	94	0		
	450	0		
F_1 W6 × F_4^-	185	0		
	206	0		
	240	2	1	3
	158	0		
	168	2	1	
	162	1	1	
	Σ 1119	5		

Retest by cross-lining method

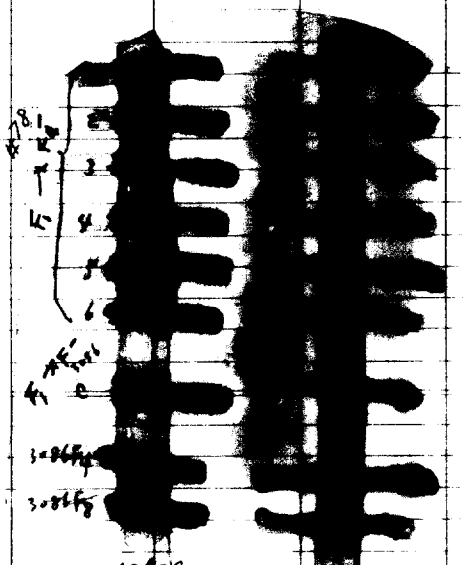
1. Test infectivity and accessibility to UV-treatment.
2. Remove F and reinfect F_1 to it. See what happens.

Conclusion :

1. Even plain F⁻ gives Hfr colony by infection of F_1. This is unexpected result.

But, this suggest that F⁻ can mutate into F_3 type female by itself, or F_1 can mutate into F' by spontaneous mutations, and become F'⁺ or Hfr.

2. All these Hfrs are not F4 type. This means 3086 F_4^d has no F4 character in there.



×2979 ×2985

Retest on MGal 2, 4, 5, 6, Hi marker by spot test (see back page)

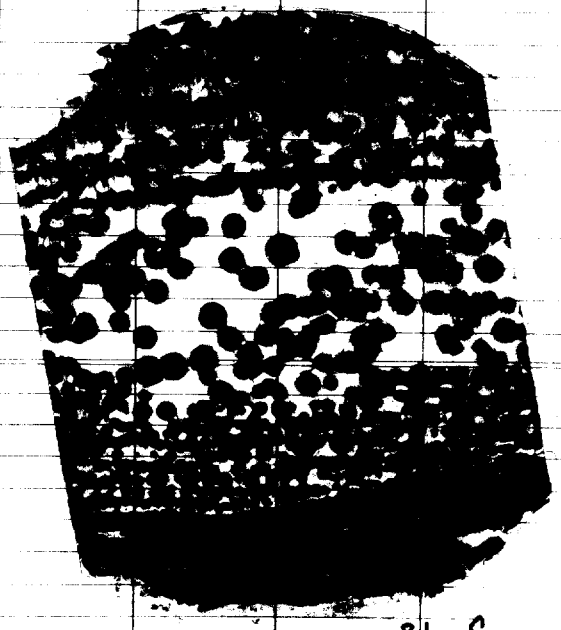
~~W3898~~ → W3086
W3924

23/4 : 1959. REF:

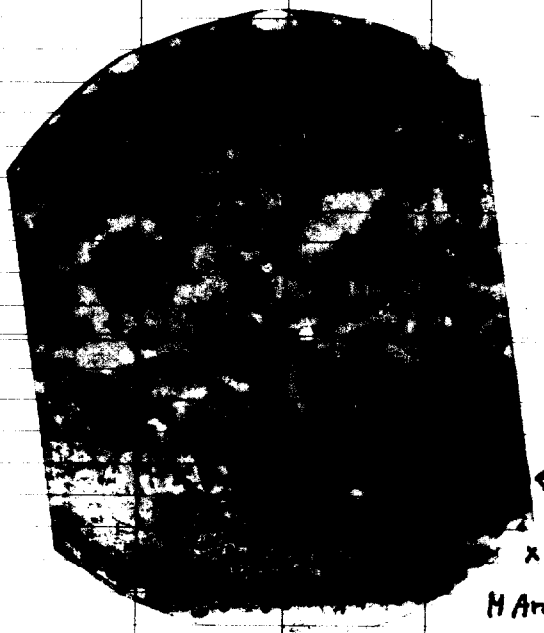
Hfr x Lys⁺ Thr⁺ Hist⁺ Lac⁺ V₁ P100.
Jacob's Hfr.

Method: Just same as standard method.

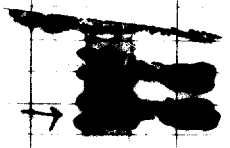
Replica plated on MAra plates seeded W2979 on them.
[Meth - Ara₂ selection.]



BLac Sm.



x 2979.
MAra



MAra
x 2979

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W3703 → W6- Ref: F of W64 does not
(W64 Hfr.) REF: infect to K-12 F.

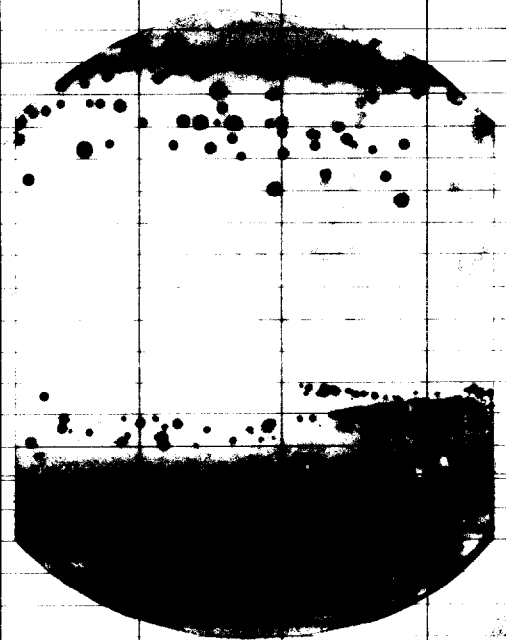
2/4/59 ; 1959

L Try Lac Mal Str.

1 2 3 4 5 6 7 8 9 10

Method : W3703 1ml + W6- 0.1 ml + 5ml phage.
↓
Incubate 24hrs. at 37°c.
↓
Spread on MLac + M
↓
Replica plate it on MGal seeded 2979 on it.

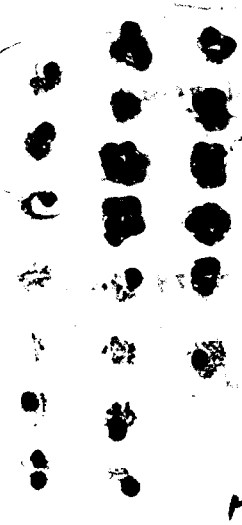
Result : No F+, and no Hfr was observed.



MLac + Meth.

MGal
x2979

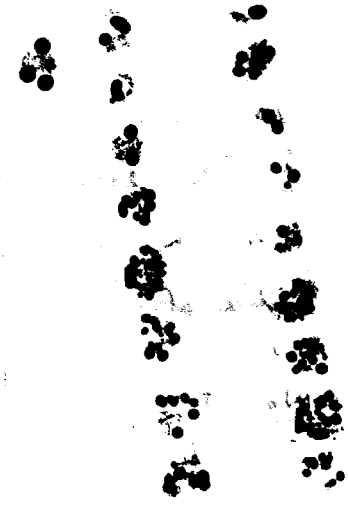
5 – x WB –



MLacB,

x3828

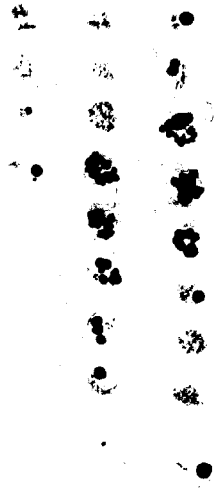
1 – x WB –



MLacB,

x3828

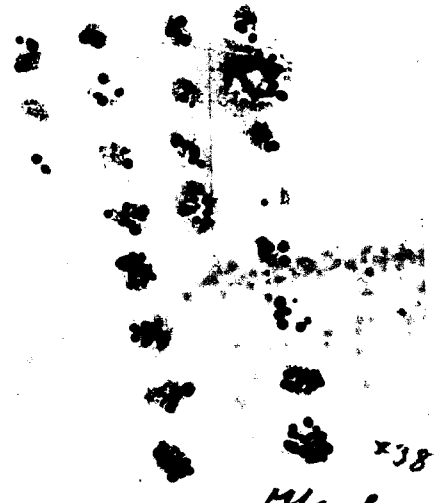
2 – x WB –



x3828

MLacB,

4 – x WB –



x3828

MLacB,

~~Infectivity~~ Infectability of F^R to F_8. purpose: Does F^R keep F_8 for a while? Temporally F_8^+

28/4 ; 1959

REF: P.44 P.45 P25.

Method : (Overnight culture (aeration) (passage 5 ml.)
Ratio of mix : 1 : 1.
(Spot them : Untreated.
Add Sm. 400γ/5m ml.
Keep them at 37°C for ½ hr. : Treated by Sm.
spot them.

Experimental condition for F_8 infection:
Shaked : 3 hrs. at 37°C on rotator.

Principle:
$M\,Gal_4\,F_{85}\,S^S$ W4534 × $M\,F^R\,S^R$ × $Gal_4^-\,S^R$.

	1	2	3	4	5	6	7	8	9	10
			1	2	3	8	4	5	6	7
			3220 F^- MGal$_4$	4534 F_8 MGal$_4$	4526 F^R M S^R	~~3086 F- His^R~~	4534 + 4526 F^R	4534 + 3086 F^- (Shaked 3 hrs. 24°)	3220 + 4526	3220 + 3086
recombination	3994 (F^- Gal$_4$ S^R)		−	−	−		++	++	−	−
	3086 (F^- M$^-$ S^R)		−	−	−		−	−	−	−
Infectivity	3086 + 3994		−	++	−		++	++	−	−

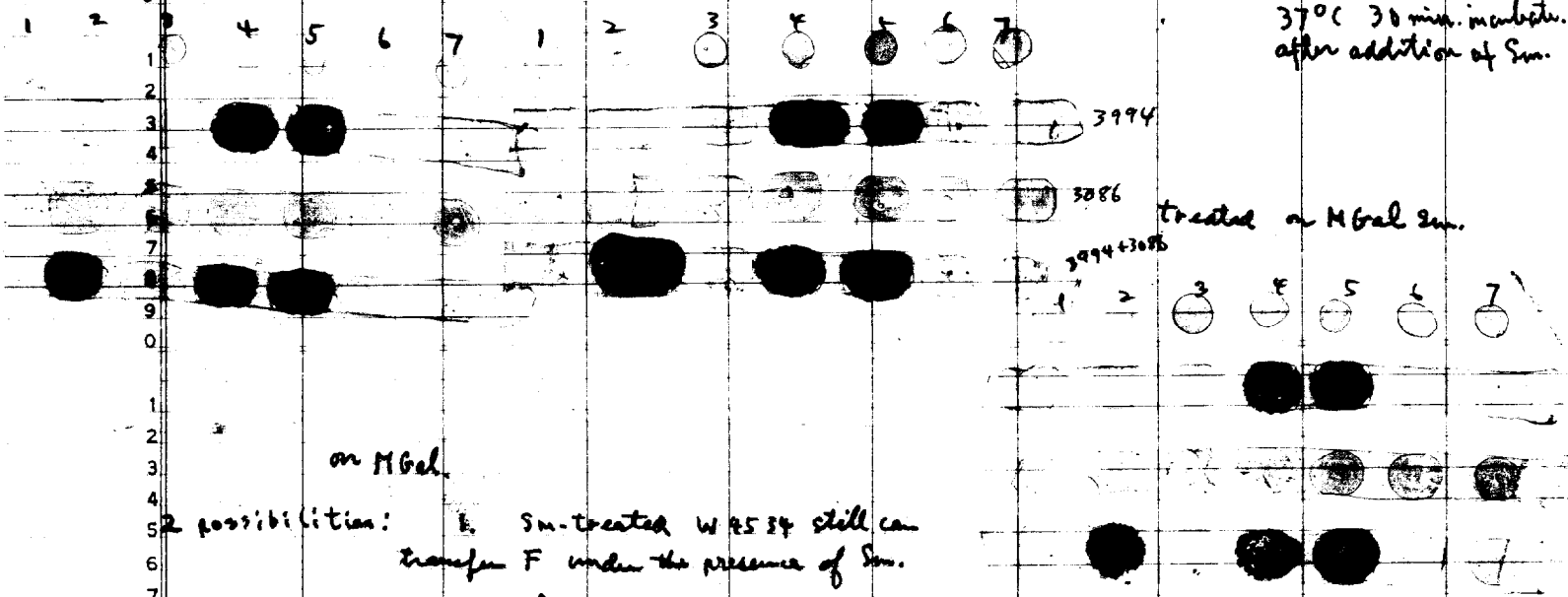
Next step ① purify 4534 → 4526 & 4536 → 3086 on B Lac Sm. and test accompatibility by replica-method. (see next page. P76 b)

on MGal and on MGal Sm.

After treated by Sm. on MGal on MGal Sm

Untreated. untreated on MGal Sm

Sm. 37°C 30 min. incubation after addition of Sm.



treated on MGal Sm.

on MGal.

2 possibilities: 1. Sm-treated W4534 still can transfer F under the presence of Sm.

2. F^R can carries F_8 for a while. and possesses F_8^+ character for a while. But it can't continues those traits.

Continued from former page

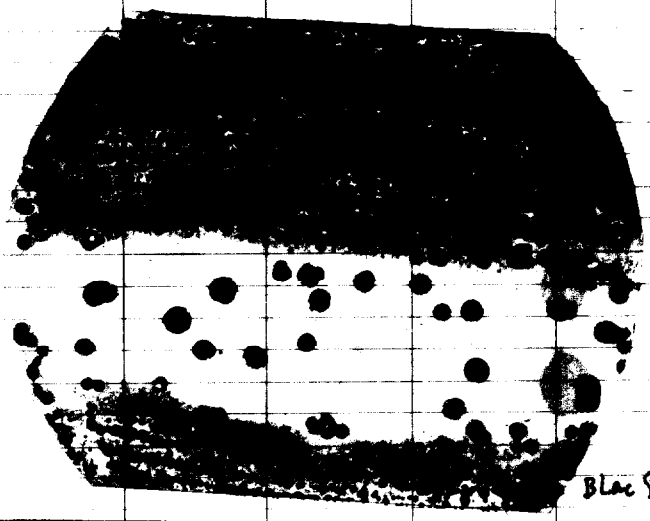
3D/4 ; 1959.

REF: cf. p76a : (Next step)

Method : ① # 4 and # 5 (4534 × 3086) were streaked on Blac Sm.
(4534 × 4526)
② Replica plate on MLac B_1 seeded W3828, and see futile colony is there or not.

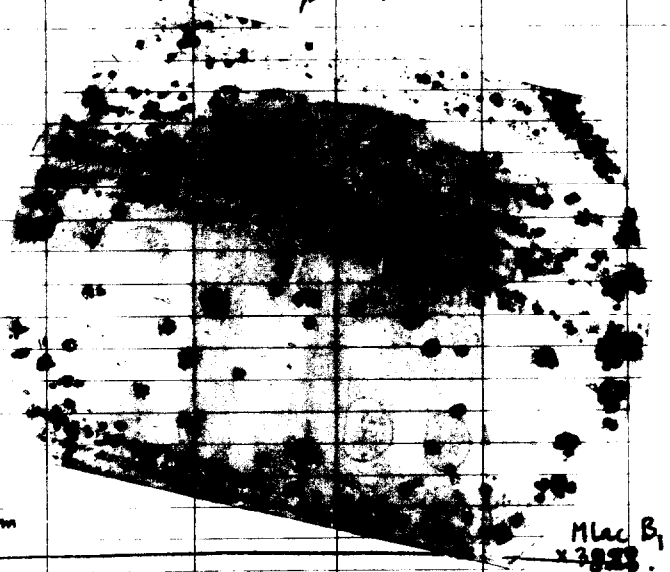
Control. 4534 × 3086

Master plate



Blac Sm

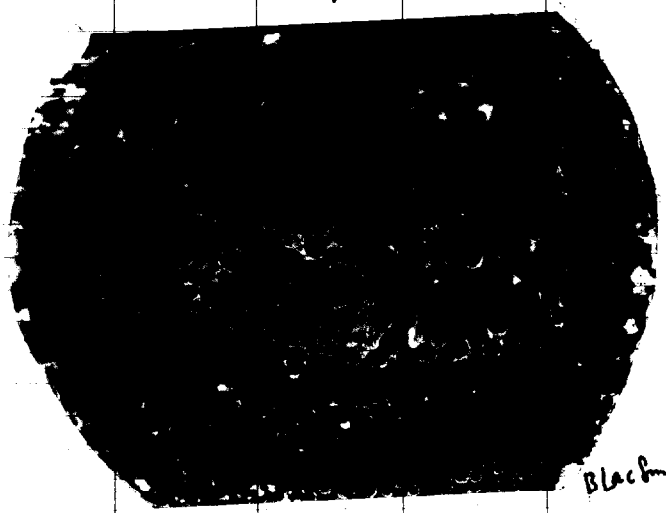
Replica plate.



MLac B_1 × 3828.

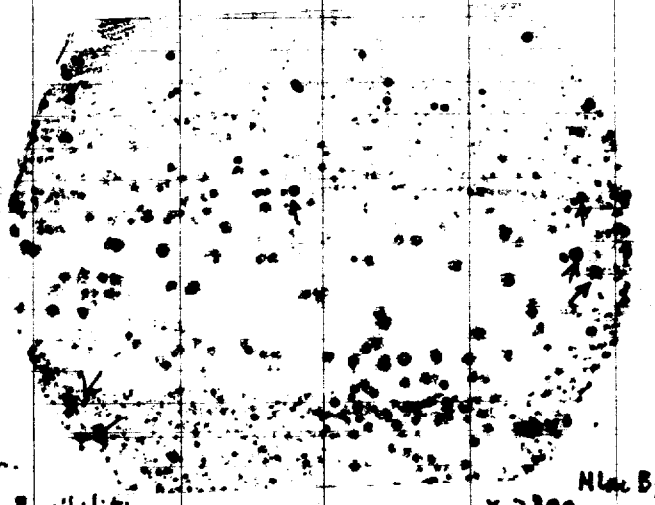
4534 × 4526

Master plate



Blac Sm.

Replica plate.



MLac B_1 × 3828

Possibilities :

① Is it mutant of F_8 (Host range mutant)

② Spontaneous (reversion) mutant of F^R to Hfr_1.
(This seems unlike, because there is no recombination in 40,3 ; see p76a)

③ Recombination between F^R and F_8 and get Hfr_1 after recombination.

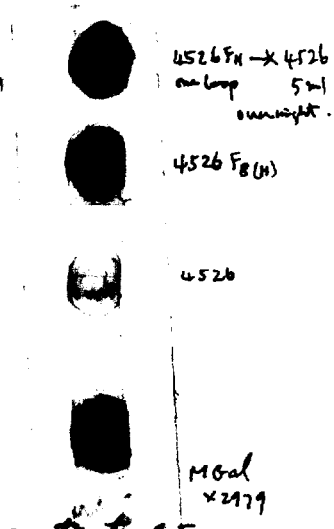
F_8 + + +
+ + F^R

Next step :

① Repeat this experiment with control.

② If it is host range mutant of F_8, it must be ~~insensitive~~ sensitive to F^- and F^R. And also H1 for Gal ~~unlike~~ like Hfr_1. (See back page). This is H1 for Gal.

Control to F^R



Rough estimation of Host range hypothesis.

① Mix W4526 F_{8H}. one loopful genera. broth culture → × W4526 5ml plenary. overnight grown culture.

② Make spot test. on MGal. ×2979.

If. black spot was obtained, it may be Host-range mutant of F_8.

Result and conclusion: 4526 F_H → × 4526 does not become black spot. Infectivity of $F_{(H)}$ is still very low to 4526 or not sufficient to FR.

#2 —× W6 −
#4 —× W6 −
#5 —× W6 −
C —× W6 −

AO #2
AO #4
AO #5
AO #5. REF: cf. 74.
AO C

27/4 ;1959

Experimental conditions: 0.1 : 0.1 : 5ml 0.1ml, AO 30γ/ml, 9 p.m.
grown overnight at 37°C.

Result: F of
1. A #2, #4, #5, C, are infective, and gives low fertile, probably F+ (See back page).
F of
2. (#2, #4, #5, C, are sensitive to acridine treatment.

#5

C

AO

MLac B, ×3826

F' —× 3026

C

AO

MLac B, ×3828

unsuccessful result.

Detection of recombinants from double F^+ strain.

$F_4 \times F_8$

1/VI ; 1959 #2 was used. cf REF. p42, p50.

Principle.

F_8 F_4

\+ × + F_4

F_8 + +

↓

F_4 + +

F_8 + +

F_{48} F^+

↑ detcd F^+

point : Look for F^+ carefully.

assumption : F_8 and F_4 is not allelic.

Method 1. Seed #2 on B Lac Sm. ($\times 10^{-2} \times 10^{-2} \cdot 0.1 \to 1$) + ($10^{-2} \times 10^{-2} \times 10^{-1} \cdot$ of $\times 2$.

2. Replica plate on M Gal Seaded 2979. on it.

3. Look for Lo colonies. 2985

	# of colonies tested	# of Y colonies steril (on M Gal *2979	Hfr colonies
1	451	1	450
2	437	0	437
3	415	0	415
4	395	0	395
5	510	0	510
6	372	0	372
7	376	0	376
8	333	0	333
9	422	0	422
0	533	(1) F^+ or F^-	532
1	Σ 4044	2	4042

check Gal marker

There two colonies are F^- (See bellow).

Possibility : 1. spontaneous reversion of F_8^+ or F_4^+ into F^+ (or F^-)

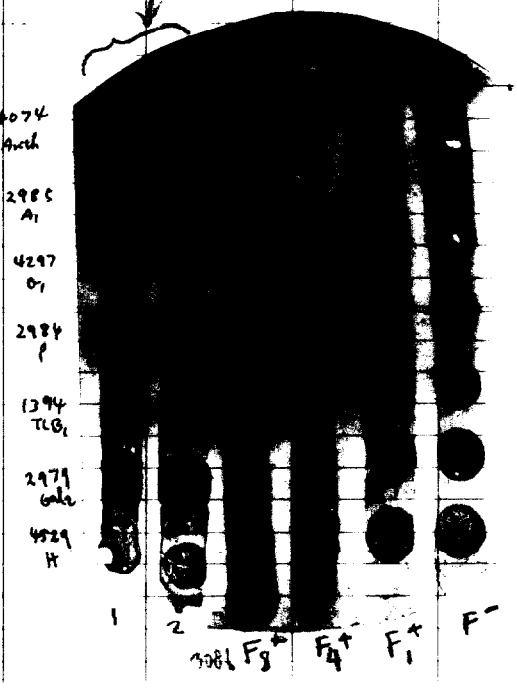
Result : F^+ was not observed (negative result was obtained)

Conclusion : ① F_4 and F_8 locus are not allelic, and cannot recombine, or ② they are very close with each others or ③ does not make synapsis.

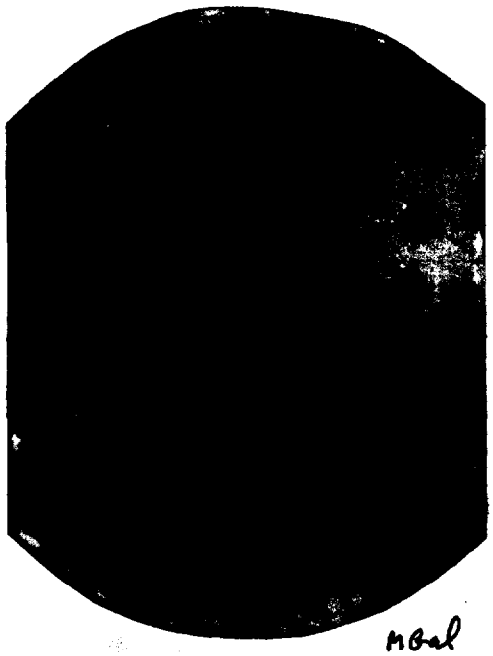
① F_8 + + F_4

② F_8 + + F_4

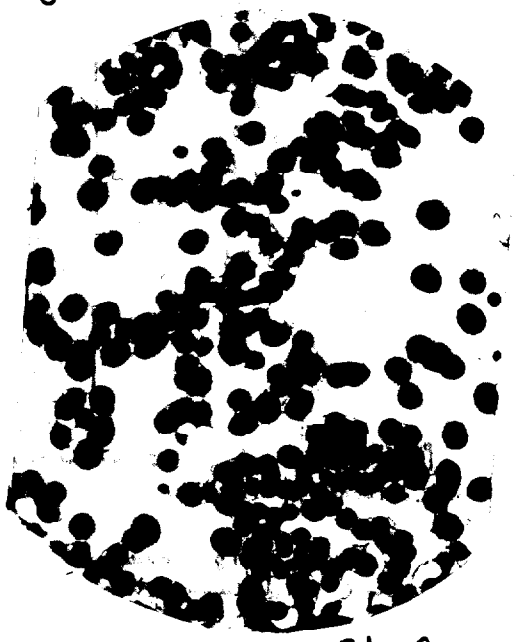
③ F_8^+ F_4^+



An example: Replica plating method gives clear spots in this case.



MBal
KE979



n BlacSm

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Isolate Hfr$_8$ from W3208. unsuccessful result.

1/VI ~ ; 1959

REF:

Purpose : It is necessary to get Hfr$_8$.

Method : 1. Take W3208 from stab culture, and purify it on Blac.

2. Cross-brush the 10 colonies. (×2979 on MGal)

3. pick Hfr and treat by AO. and see it is resistant to AO or not. infect F to 3086. and see is it infective or not. Use F$_8$ control.

Method for infection:

① W to F$_8$, or W3208 Hfr$_8$ 1 ml + 3086 0.1 ml : Pouring with 1 ml.

② incubate it overnight at 37°C.

③ purify on Blac Pm.

④ cross on MGal ×2979.

1/10

×2979 on MGal.

Result : This strain is infective to F$^-$ ∴ F$'_8$ (not Hfr$_8$)

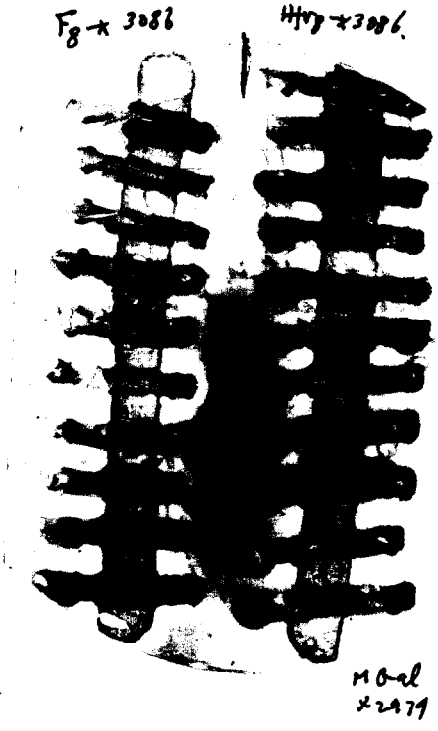
Ratio of infection : Hfr$_8$ + F$^-$ 3086 $15/23 \times 100 = 65.2$ (%) ← (see back page)

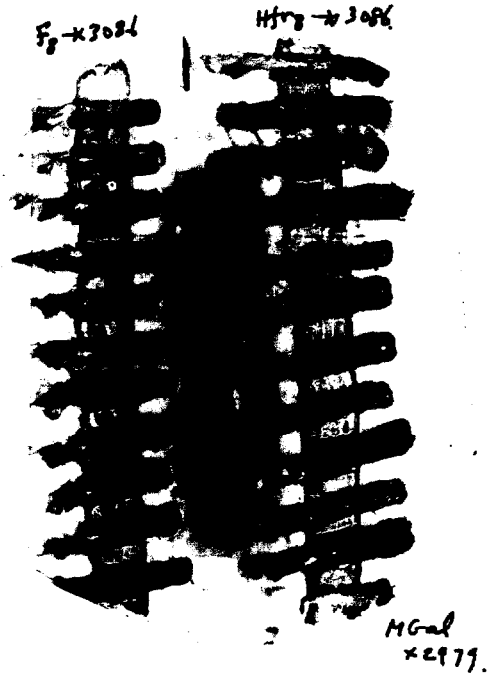
control. F$_8$ + 3086 $17/21 \times 100 = 81.0$ (%)

Next step :

Treat W3208 with AO, and look for Hfr or use lyophilized culture for reisolation (no mutation) of Hfr$_8$.

Comparison of infectivity of F_8 to F-





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GENETICS

W4568

81

Characterization of Host range mutant of P_8.

4526 $F_{8.H.}$

4/4/1 ; 1959

REF: cf. P76a, b.

Purpose : Confirm this is "Host range mutant" of F_8, and this trait is heritable character determined by (itself)

1. Infect $F_{8H.}$ to W3735 ($4^5 F^R$ M), and isolate W3735 $F_{8H.}$

· Mix 1 : 1 in passage (1ml : 1ml : 1ml)
4526 $F_{8H.}$ 3735 Ra.

· Purify it on BGal and replica plate it onto Blac Sm. and see which colony is W3735. or W4526.

· Pick W3735 (S^5) and cross-streak with W2979. on MGa

Purpose : put more markers into F^R mutant to differentiate between F^R mutants.

2. Isolate Gal^6 from ~~W3735~~ W4526 ~~F8H~~

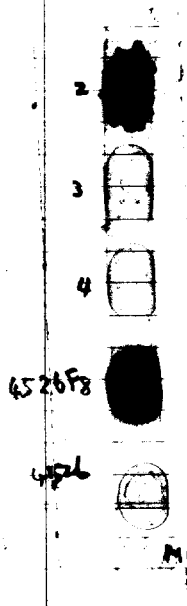
① HFT-6 ⟶ × W4526
0.2ml 1ml overnight culture. Keep this one hour.

② Spread on B-D. and incubate it overnight.

③

3. Infectivity to F^-. 3735 $F^+_{8H.}$ ⟶ 3006. ; Method : standard method was

Result: Infective (very efficient) to F^- 100 %. (21/21)



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3828 F_3 → Y10.

4/6 ,1959 REF:

1 2 3 4 5 6 7 8 9 10

1st trial.

Method : 1. Mix them 1 : 1.

2. Incubate it overnight.

3. Streak it on Blac, and pick Lac+. Test 1gl. transfer. ×2979. on Mlgl+B_1

Result : Strong competition was observed. 3828 has more selective advantage over Y10. Unsuccessful all F^-. try again.

2nd trial:

Method : 1. Mix them 3828 Y10 2 : 1 (1ml 0.5ml), incubate it overnight. Ca. 24hrs.

2. purify it on Blac.

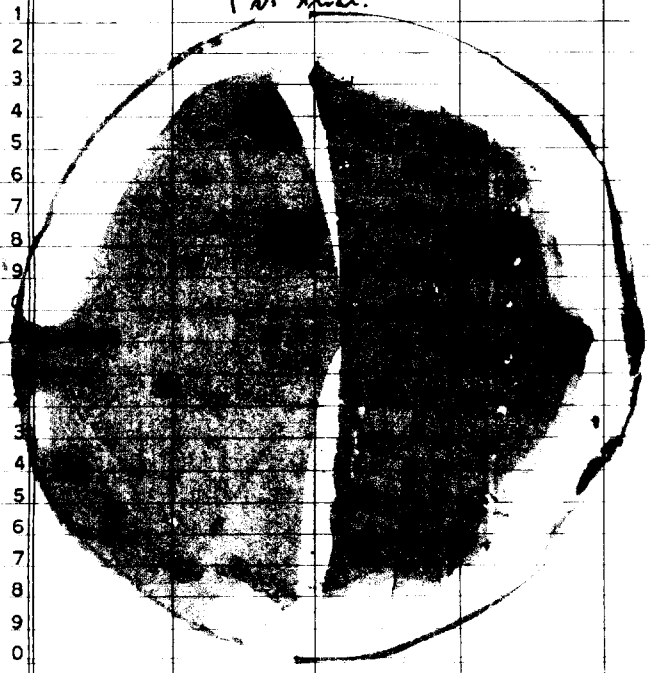
3. Cross brush Lac+ (Y10) against 3086 on MLac B_1.
test at least 100 colonies!

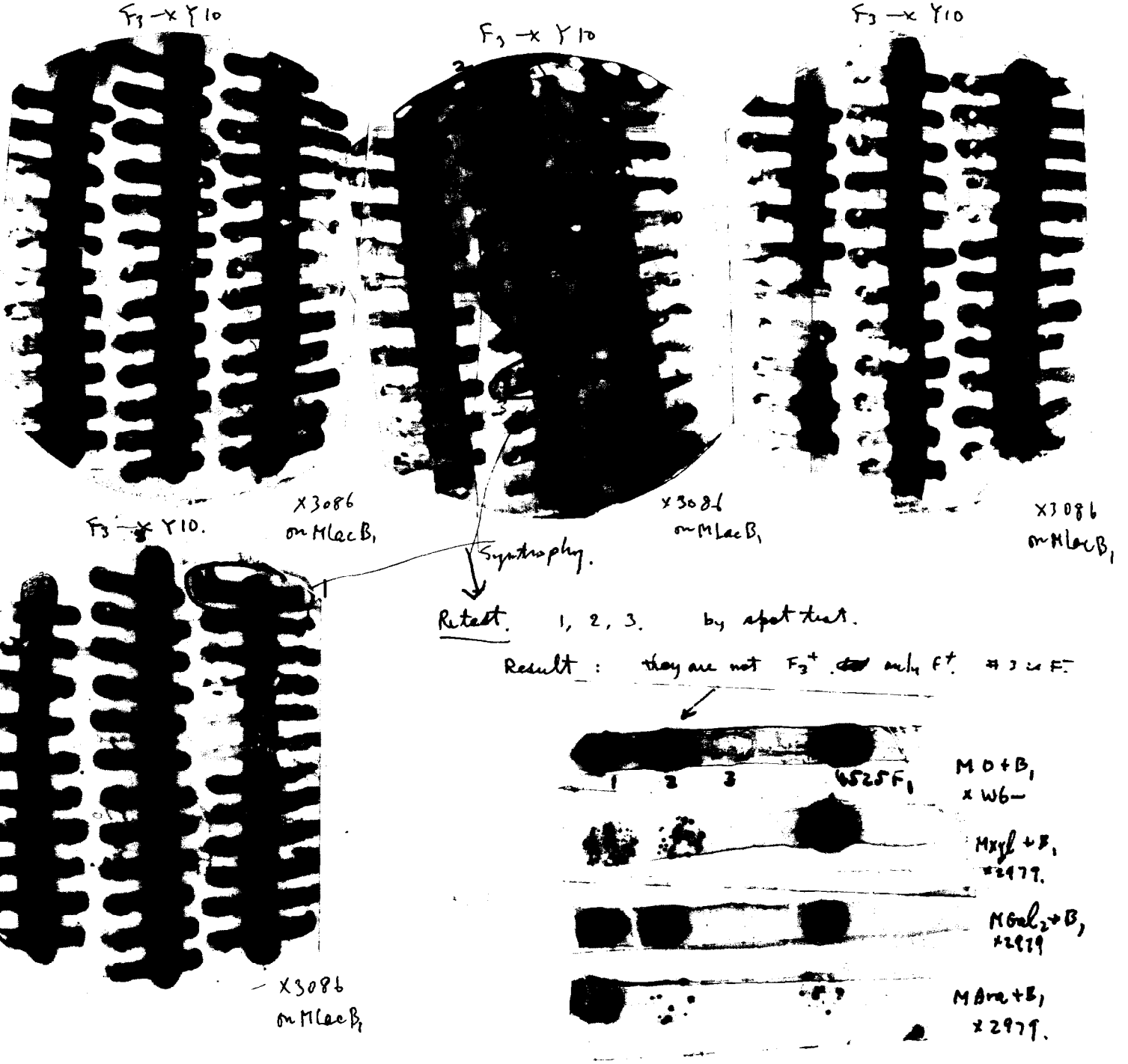
Ratio : (See back page).
Hfr/$\bar{\Sigma}$. 0/139.

No F_3 were transfered to Y10.

3828 F_3 only gave F_1 not F_3.

1st trial.





Retest 1, 2, 3. by spot test.

Result : they are not F_3^+ only F^+ # 3 is F^-

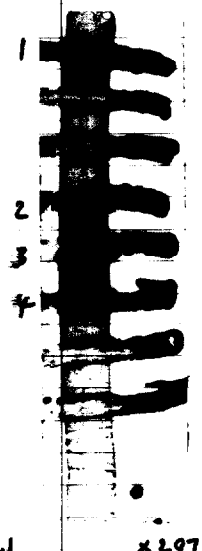
3828 F_3 → × W6−

6/11 ; 1959. REF:

Method 1. Mix them 1 : 1
2. Incubate + on mipt. at 37°C
3. purify it on Blac and test Lac+ on sel-compatibility, ×2979 on MXgl.

Result.
W6↗ was obtained after mixed culture with 3828 F_3 and W6−.
Is it O_3 or F^+?

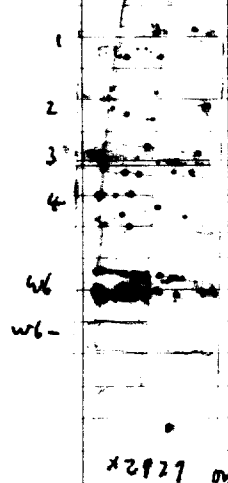
3828 F_3 → × W6−



×2979 on MXgl.

4/8 = 50%

Retest



×2979 on MXgl

W4576 (F_4 A_1^- Gal_6^-)

Infect F_4' and F_4'' to W4528.

(F_4'' (selected 10 times for infectivity)) F^- A_1^- Gal_6^-

8/VI ;1959 REF:

Purpose: Comparison of the infectivity of F_4 mutants.

1st comparison: Result.

	F_4^+/total (%)	
W6 F_4' → W4528	0/34 (0)	Select on BGal.
W6 F_4'' → W4528	4/35 (11.4)	Compare rate of infection.

Test on M1 for Gal. (on MacConkey agar)

(See back page)

2nd trials of infection of F_4 (non-selected F_4) to W4576 was not successful.
0/32 : 0% rate of infection: all F^-.

2nd Comparison

Spot W4528 F_4' and W4528 F_4'' on W4293 $F^-A_1^-$ and W3996 $F^-Gal_6S^R$

7/IV. 1st trials.

Method for selection of more infective F_4.

W6 F_4 one loopful (overnight culture) + W6 F^- 5 ml ca. 10^8 cells/ml.

↓ incubate overnight.

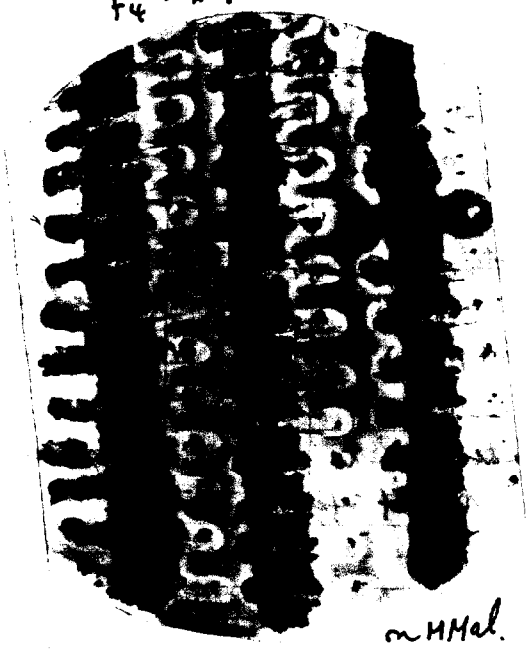
one loopful : + W6 F^- 5 ml ca. 10^8 cells/ml.

↓ Repeat this process.

This selection was done for 10 times: W6 F_4''.

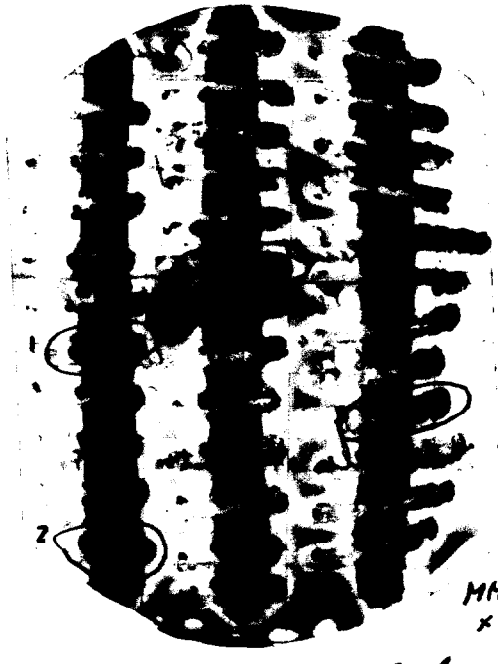
2nd trial: From 8/VI's experiment, the infectivity of F_4'' is still not enough. Try selection again using W4528.

F_4 — × 4528



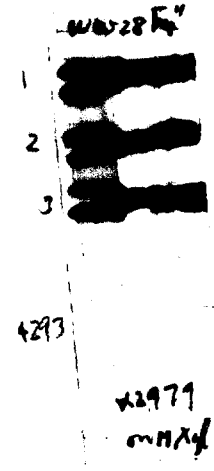
on MMal.
×2979.

F_4'' — × 4528



MMal
×2979.

Retest of infected F_4''



×2979
on MMal

Mal, marker is reversible.
Even though, F_4'' can be told from highly probability

2nd trials of infection of F_4 to ω4528
: unsuccessful :
May be some reason

Treat W4552 (3086 F_5^-)
with AO, and reinfect F_1 to it.

1959, Test the possibility of σ_3 type F'. REF:

Purpose and Principle:

3086 F_5^- ~~and 3086 F_5~~ transfer their F to F^-. But the infected cell shows low fertility after infection. Then, what factor was left behind at the time of F transfer. If a factor which determines Hfr character cooperated with F, was left behind after treatment of those F'^+ strains, reinfection of F_1 to F^- obtained after treatment of the F'^+ organism arise Hfr or F' mutant with high frequency.

Hfr

Method of treatment: Usual method. conc of AO. 3 σ, Pen. 5 m/. overnight at 37°C.

Rfd

F_1

Curve from F_5^- with AO.

Result: Rate of F^-/11 = Ca. 1 % (See back page.)

Plot off.

Infect F_1 to 3086 F_5^-: W6 × 3086 F_5^- Use usual method for infection.

1st run was not successful. × 2979 on MXyl.

Result: Rate of infection: (see back page)
0/66 = 0 %

Why?

2nd run was successful, and get F^+. Rate of infection:
try again. 23/58 = 39.7 % (See bellow)
Expt. Condition: 5 ml pen. + 1 ml W6 + 0.1 ml 3086 F_5^-.

F'^+

Conclusion:
F_1 × 3086 F_5^- gives plain F^+, not F_5^+.

σ

F_3^-

removable by AO.

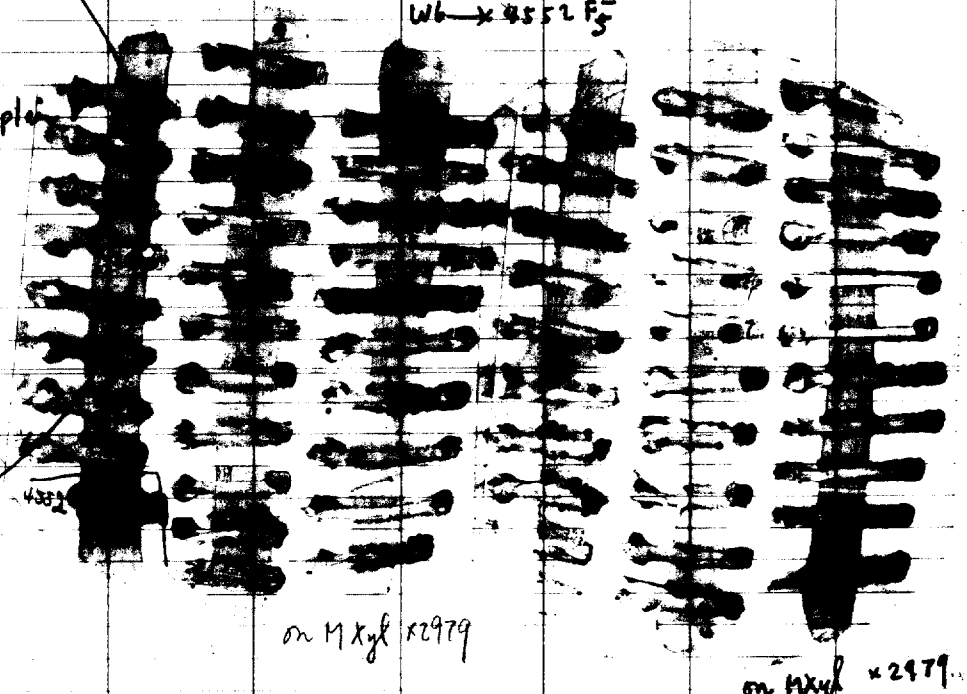
This Hypothesis looks incompatible to the result. (3086 F_5^- × W6) gives F^+.

W6 × 4552 F_5^-

plate

F_3 type F^- not simple F^-

Reinfection of F_1



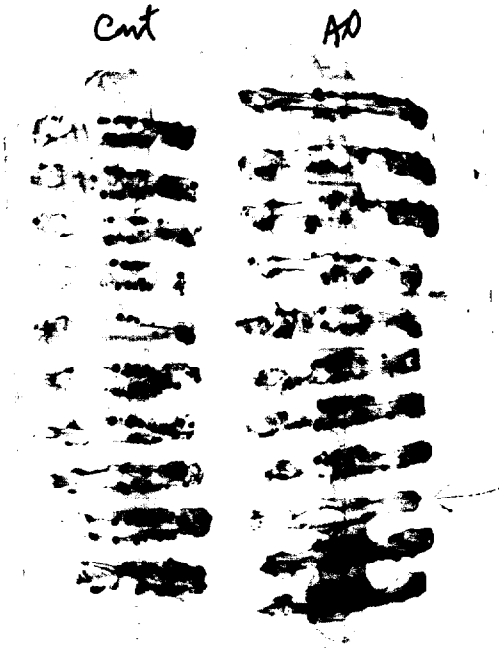
on MXyl ×2979

on MXyl ×2979

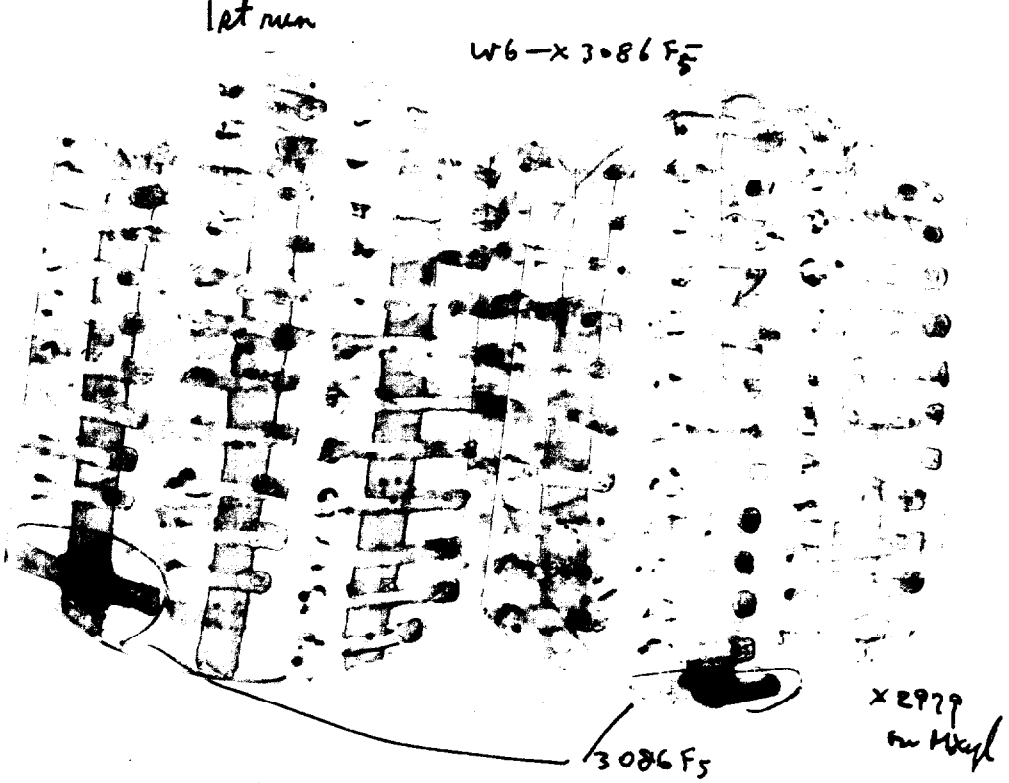
① cure from F_5.

3086 F_5

on B Mal.



② Infect F_1 to 3086 F_5^-



Unsuccessful. compair infectivity of F_3' of W3642 F_1^+ to F^-

Take control (W6) F^+ M Mal$_5$ Gal$_2$ Lac$_1$

15/VI ; 1959. REF:

Exp. design. : W3642 F_1 → × 3086

W6 (control) → × 3086

Select on BGal Sm.

Replica plate also on MXyl. × 2979.

Method:

Result:

	Exp. W3642 F_1 × 3086 # of colonies	# of Hfr_3 colonies (%) (select on MXyl.) × 2979	Control. W6 × 3086 # of colonies	# of Hfr_3 colonies (%) (select on MXyl × 2979)
1	618	0 (0)	4 249	0 (0)
2	661	0 (0)	5 208	0 (0)
3	700	0 (0)	6 245	0 (0)
*	$\Sigma_{3642 \times 3086}$	0 (0)	$\Sigma_{W6 \times 3086}$	

Further experiment:

Use 3833 for F receptor.

Agglutination of F' strains.

18/4 ; 1959

REF: Peter Hayes also observed this phenomena.

1	2	3	4	5	6	7	8	9	10

Starting point: W4293 F_4 showed agglutination. but F^- was not
(A; Gal$^-$) (λ^+ Sugar$^-$ S^R)

young culture : 2hrs. showed no distinct difference between them.

Experiment:

1. Inoculate

Hfr_2 F_2 , Hfr_4 , F_4 , Hfr_5 , F_5 ; F^- ; F^+

4321 , ~~W4321~~ $W6F_2$, W4321 , $W6F_4$, 4536 , $3086F_5$, W6 ; 3086 ; W6 , W3086F^+

and incubate them for overnight. All culture was obtained from stock collection in cold room.

In overnight culture, agglutination was not observed.

2. Inoculate 0.2 ml of the overnight culture into 5 ml. phrasey broth. and shake it on rotator at 37°c for 2hrs. 10:00 AM — 12:AM.

Result: Agglutination was not observed in Hfr, F', F^+ and F^-. after 2 hrs, 4hrs. incubation.

Conclusion: Agglutination of F^+ cells are sometimes observed, but not always does it. Presumably, it is influenced by unknown experimental conditions sensitively.

W4526 F_8^- (Infectability to F_1)

19 REF: cf. P 89

	1	2	3	4	5	6	7	8	9	10

Principle :

Control { W6 → × W4526 (F^R) — 0/26
W6 → × W3086 — 22/28

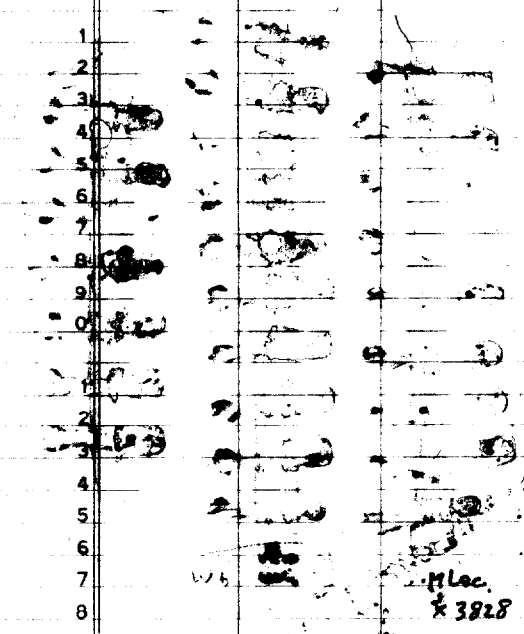
W6 → × W4526 F_8^- (1) — 10/31
W6 → × W4526 F_8^- (2) — 10/29
W6 → × W4526 F_8^- (3) — 11/29

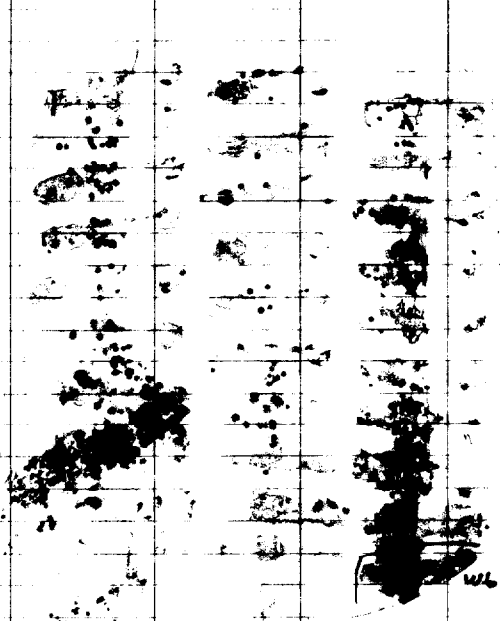
Ratio.

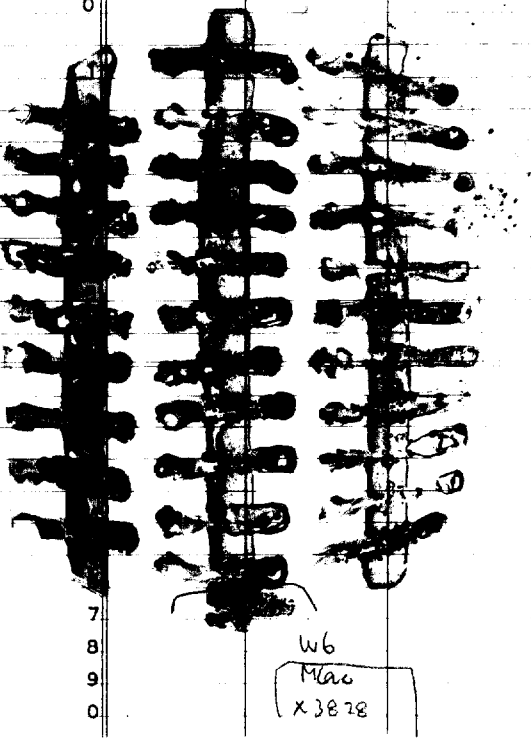
Mixed culture.
Overnight:
W6 1 ml, & o.1 ml.
Plate 37°C.
Purify on BLac Sm.

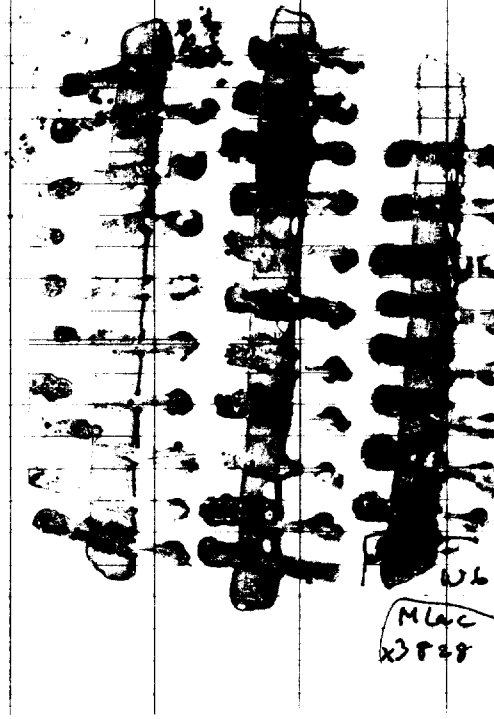
W6 → × F^R control.

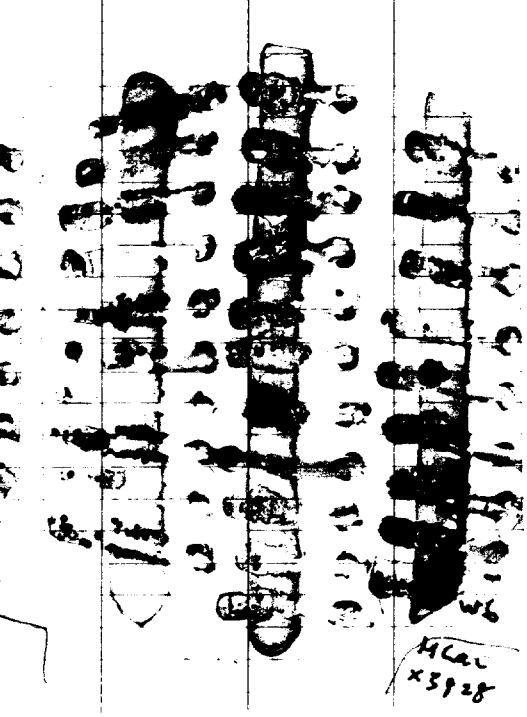
W6 → × 3086











2nd trial on : Take all F_3 apart. from (W4583 3828 F_3^+)

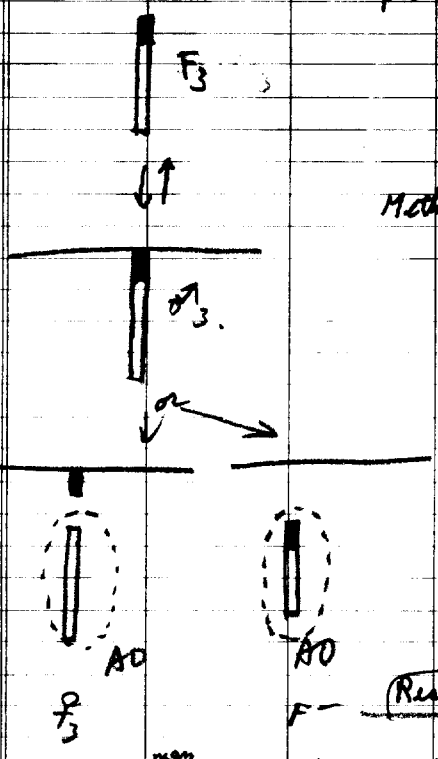
" Isolation of $\mathcal{F}_3$ from F_3^+.

5/4 ; 1959

REF:

If it does work, this may be apply to other F^+

Apossibility.



most of them may become plain F− but some of them may become $\mathcal{F}_3$ type

Method for detection of $\mathcal{F}_3$ among many F− colonies.

Principle : F^+ M^- Lac⁻ : W1816.

F_1 × $\mathcal{F}_3$ M^+ Lac⁻ : W3828 F_3^- (arised from W3828 F_3 by AO treatment)

$M^+ \times$ F⁻ M^- : W3086

Method :

① treat W3828 F_3 with AO (30γ/ml penassay broth; 5 ml, treated for 48 hrs)

② Seed it on BLac. ~~Same Blac~~.

③ ~~Replica plate it on Mlac Seeded with W3086~~

Cross-brush the treated W3828 F_3 against W3086 on Mlac. ~~and Mlac seeded with W3086~~

Results: 2 : 51 / total 92 : 55.4% See : back page.

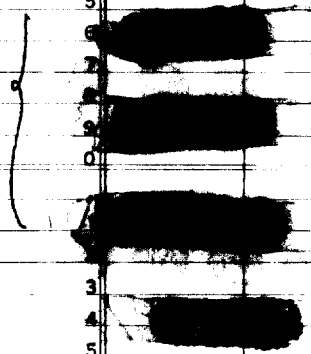
④ Inoculate those ~~male~~ female clones into penassay.

⑤ Make spot test as P.82. using W3086, W1816. on Mlac. Use control of $\mathcal{F}_3$: W3876 ; F⁻ W3828.

Result: All of the females are all $\mathcal{F}_3$, not F⁻! See back page.

not very clear cut, but still higher than F− (control)

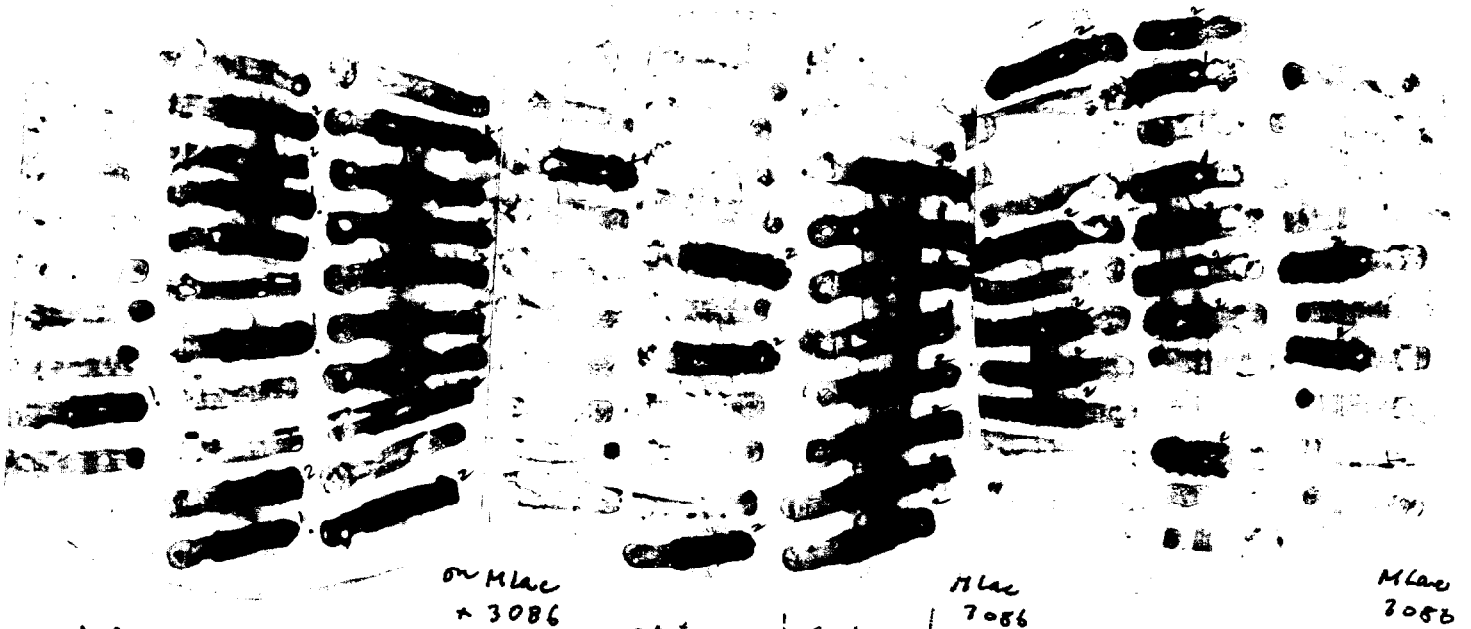
W6 W6−



W3828 F_3^- #1	H1	+ 3086
W3828 F_3^- #10		+ 3086
W3876 : $\mathcal{F}_3$ control		+ 3086
W3833 : $\mathcal{F}^-$ control.		+ 3086

on M Lac

AO-treated (W3828 F_3) W4083



on Mlac × 3086

State I (Hi for Lac) : 10

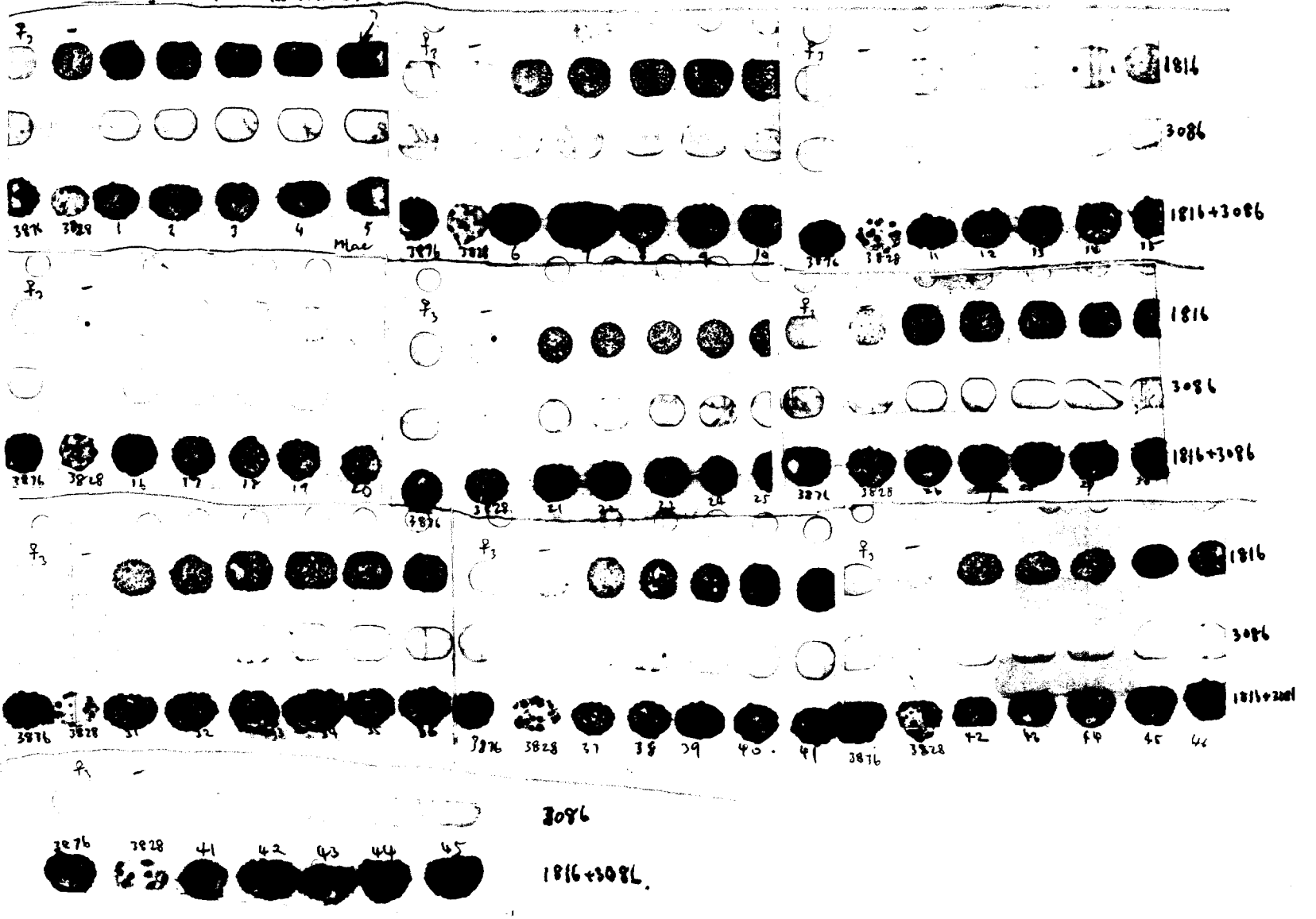
State II (Lo for Lac) : 32

? : 51

Test on F_2 or F^- : a Mlac.

State I: 9	State II: 32	Mlac 7086 F^-: 50

Mlac 3086



Cross $F_2 \times F_4$

with F_4 Hi for F_2

select Ara_1 and Ara_2 on M Ara.

20/4 – 23/4 1959.

REF:

1. Purify W6 F_2, W6 F_4, W3086. on BLac.
2. Make overnight culture of them.
3. Add 0.2 ml of the culture into pen (5 ml) and incubate 4 hrs.
4. Mix W6 F_2, W6 F_4, W3086. and shake it for 2 hrs. at 37°C.

	W6 F_2	W6 F_4	W3086.
	1 ml	1 ml	0.1 ml.

4:30 ~ 6:30 PM.

① W6 F_2 × W6 F_4 → × 3086

② W6 F_2 → × 3086

③ W6 F_4 → × 3086

5. Seed it on BLac Sm. and incubate them.
   dilution: $10^{-2} \times 10^{-2}$ 0.1 ml/plate.
6. Replica on the M Ara seeded W2979 and WK5-50 ($Ara_2 F^-$) [10^8 cells 0.3 ml/plate] ($Ara_1 F^-$)

Result: Fertility of W6 F_4 on transfer of Ara_1 is very low as Peter said; therefore it is very hard to tell is it F^+ or not, however, rate of infection under this condition was confirmed.

Control F_2 × W3086

total no. of colonies.	F_2^+	(%)
341	230	(67.5)
325	198	(61.0)
394	258	(65.5)
346	208	(60.2)
322	194	(60.3)
Σ		

Control F_4 × W3086

269	152.	(56.5)
155	89	(57.5)
260	132	(50.4)
323	182	(56.4)
304	189	(62.2)
Σ		

F_4. F_2 → W3086

444	41	Lo	
304	77 (F_2)(111)54	54	(12.2)
346	72 (F_2)(111)37	F_4	(12.3)
239	66	(19.4)37	(11.1)
305	45	(19.8)36	(15.0)
	48	(15.7)37	(12.1)
Σ			

Effect of U.V. to infectivity of F'

23/VII — 25/VII ; 1959 REF:

Experimental conditions: 1. Purify all mutants on B Gal. before use.
2. Use young culture: 4hr at 37°C in water bath. (overnight culture 0.2ml / 5ml per. → incubate for 4hrs)

Principle:

W4534 (M⁻ Gal⁻ Sᵗ Fᵦ)
W3637 (M⁻ Sᴿ Sᵗ F'⁻)
X
W3104 (Gal⁺ Sᵗ F⁻)

Exp 1.

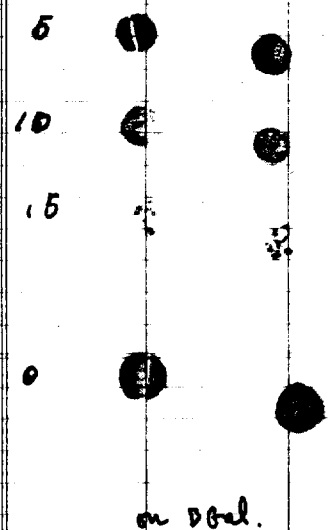
Time of irradiation:

	F_β infection	0.1ml. Colony count. /plate
0	+++	—
5		—
10	+++	too much
15	+	ca. 10^4 /plate

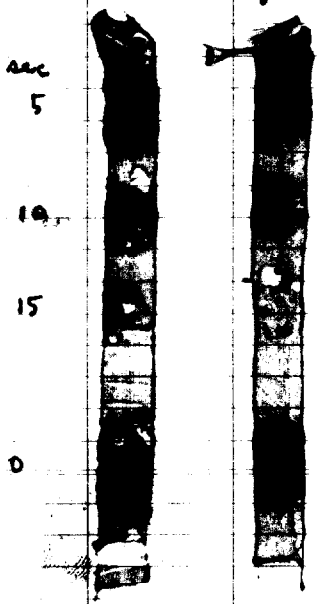
Method:
Survival of Fβ. : 0.1ml W4534 UV + (1ml:W3637 / 1ml:W3104) → incubate it overnight. → spot on MGal.
survival of all: 0.1ml /plate.

Exp 2. Qualitative test

Survival test.



Test for Infection.



Method: ① 1ml : W4534. : broth culture : ca. 5m diameter :
② U.V.-irradiated additionally.
③ spotted on BGal., MGal.

4534 → (UV.) × W3637 / W3104

Infection of F' by Killed cell.

Sm. EtoH, $CHCl_3$, CCl_4.

28/11 ; 1959 REF:

Principle :

F_8 : 4534 F_8 → F^- M Gal_4 $4p^s$
3637 F^- M $4p^s$
3104 F^- Gal_4 $4p^s$

F_4'' ~~4528~~ F_4'' F_4'' A_1 Gal_4 $4p^s$
3106 F^- Gal_4 $4p^s$
2985 F^- A_1^- $4p^+$

Method : ① Treat w 4534, and w 4528 F_4'' with various agents.

1 ml of F'-donor possess overnight grown culture

Agent used :
- one drop $CHCl_3$
- CCl_4
- 0.1 ml Sm (x100) (usually use 1 mg/ml : Sm 0.1 ml (100 ml)) — keep it for 15 min. at room temp.
- 1 ml of Et·OH (95%) — 1 hr.

② Expel the treated agent by centrifugation or bubbling air.

$CHCl_3$ & CCl_4 is adsorbed into plastic Petri dish, therefore it is easily removable by this procedure.

Et·OH is removed by centrifugation (once).

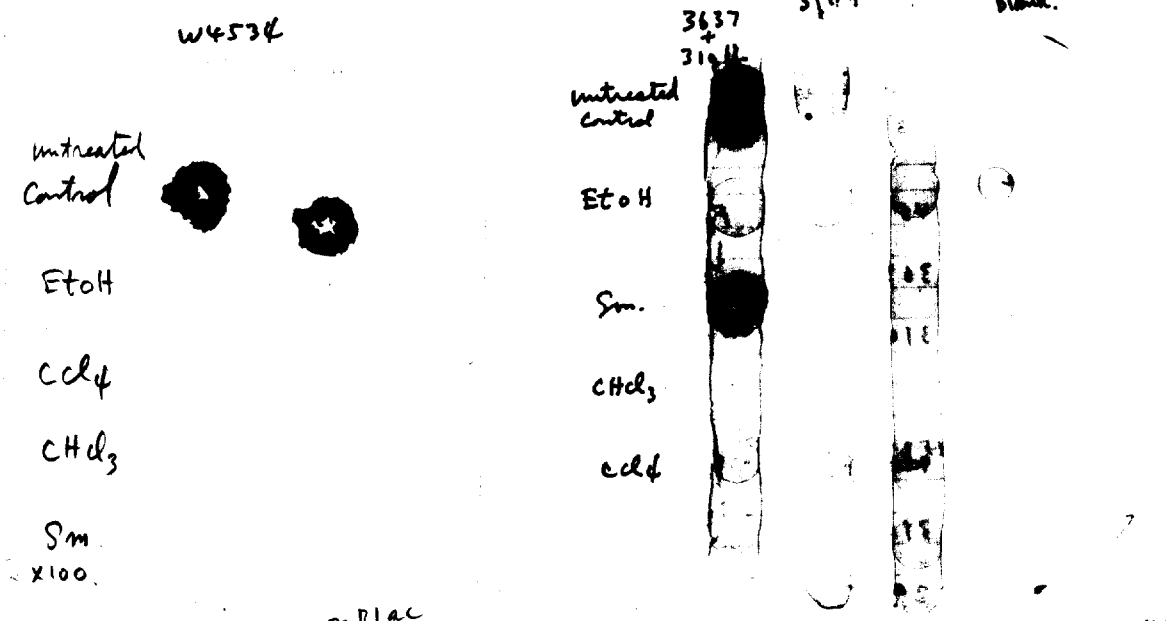
Sm is removed by centrifugation (twice).

Centrifuge → discard the supernate.
Add 5 ml pen.
↓
Centrifuge → discard the supernate
Add 1 ml pen. and suspend it.

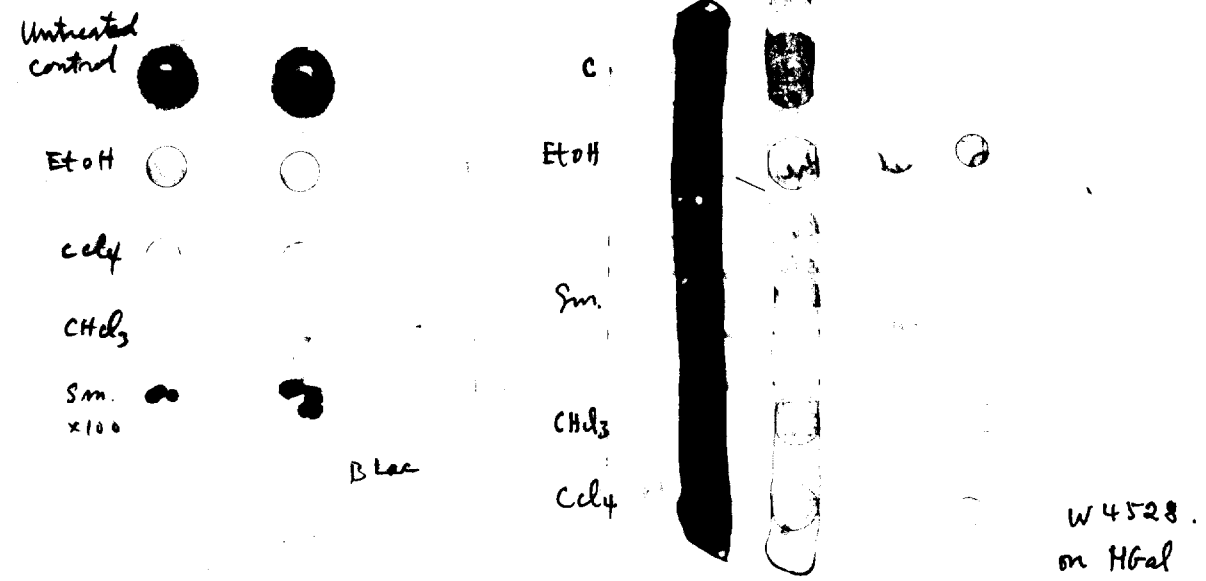
Result : Streptomycin looks good. Almost no decrease.

Next step: Check various concentration of Sm, and various time of treatment.

W4534 ($MGal_4Gp^5F_8$)



W4528 ($A_1Gal_6F_4''$)



Conformation : Infection of F_8 to F^- by Streptomycin-killed cell.
(24 hrs treatment) at 37°C.

28/11 ;1959 REF: 1mg/ml Sm.

Principle :

W4534 M Gal$^+$ F_8 → F^+ ^{SR}Mal$^+$
W3086 M F^+
W3994 Gal$^+$ F^- ^{SR}

Method : ① Add 0.2ml of Sm to overnight culture of W4534 (ca 10^8 cells/ml), 2ml. Plenary. 10 times conc.
Control : non-addition of Sm. 2ml.

② Incubate it overnight at 37°C.

③ Wash twice with broth. (2ml) 5min : centrifugation.

④ Spot it on MGal streaked W3086 + W3994 on it.

5ml overnight culture → 2ml → 2ml
Sm 0.2ml
Incubate them for overnight at 37°C.
Test their infectivity by spot-test.

Survival test by spotting on Blac.

W4534

treated by Sm (overnight) ×10 : 24hr

untreated

W6- {

on Blac.

Infectivity of F_8

Control. 3220 →

4534 untreated control →

4534 Treated with Sm. ×10 for usual time. 24hrs.

3086 M | 3994 Gal$^+$ | 3086 M × 3994 Gal$^+$ F$^+$

on MGal

Conclusion : Sm-killed cells still have some F^+ infecting ability to F^-, but it decreases into about < 1 ~ 0.1%.

Transferring ability of F'^{+} to infect F' to F^{-} after killing ~~by~~ by Sm.
(Short time treatment:)

28/VI ~ 30/VI ; 1959 REF:

Method:

Conc. of Sm:
200U x 200 : 0.2 ml Sm sol'n. : ~~1000 U/ml~~ 2 mg / 1 ml
100U. x 100 : 0.1 ml Sm. soln. : " 2 mg / 1 ml.

Time : ½ hr. & 2 hr.
½ ; 3:15 ~ 3:30
2 : 3:15 ~ 5:15

System :

W3994 F^{-} Gal_6 S^R) x F_8 W4534 F
$W3^{+}086$ F^{-} M S^R

W2985 F^{-} A_1^{-}) x F W4576
$W3^{+}106$ F^{-} Gal_6

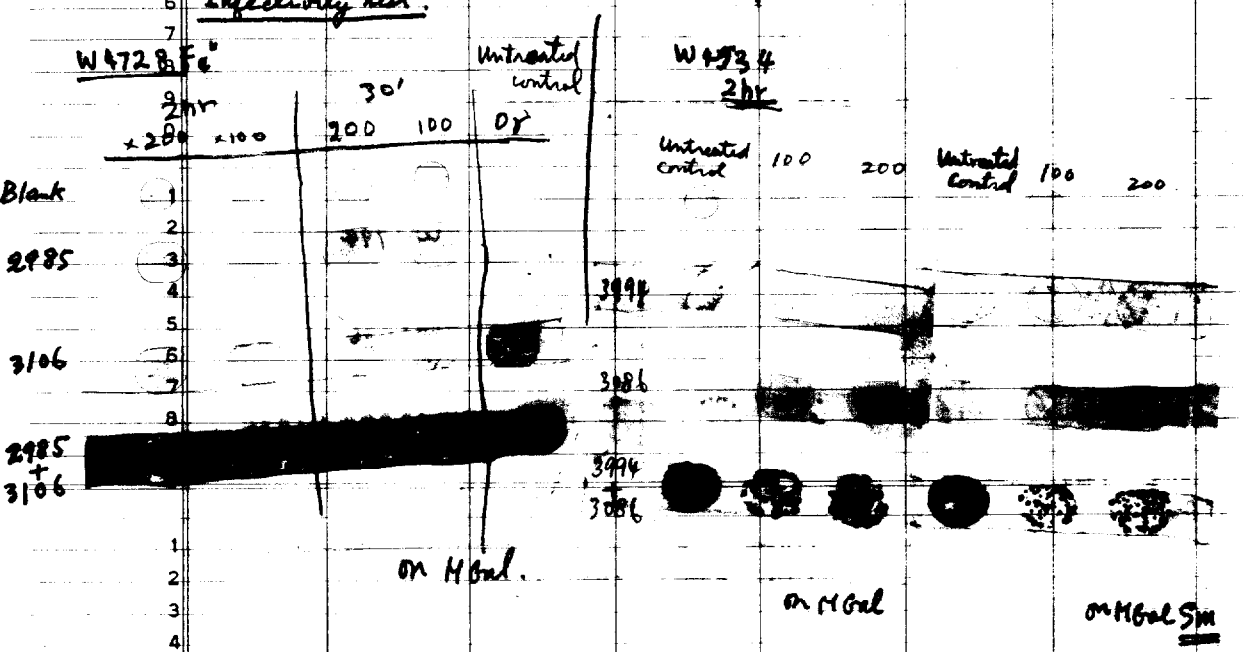
Method :
1. Add Sm 200U & 100U /ml. separately.
2. Incubate them for ½ hr. & 2 hr.

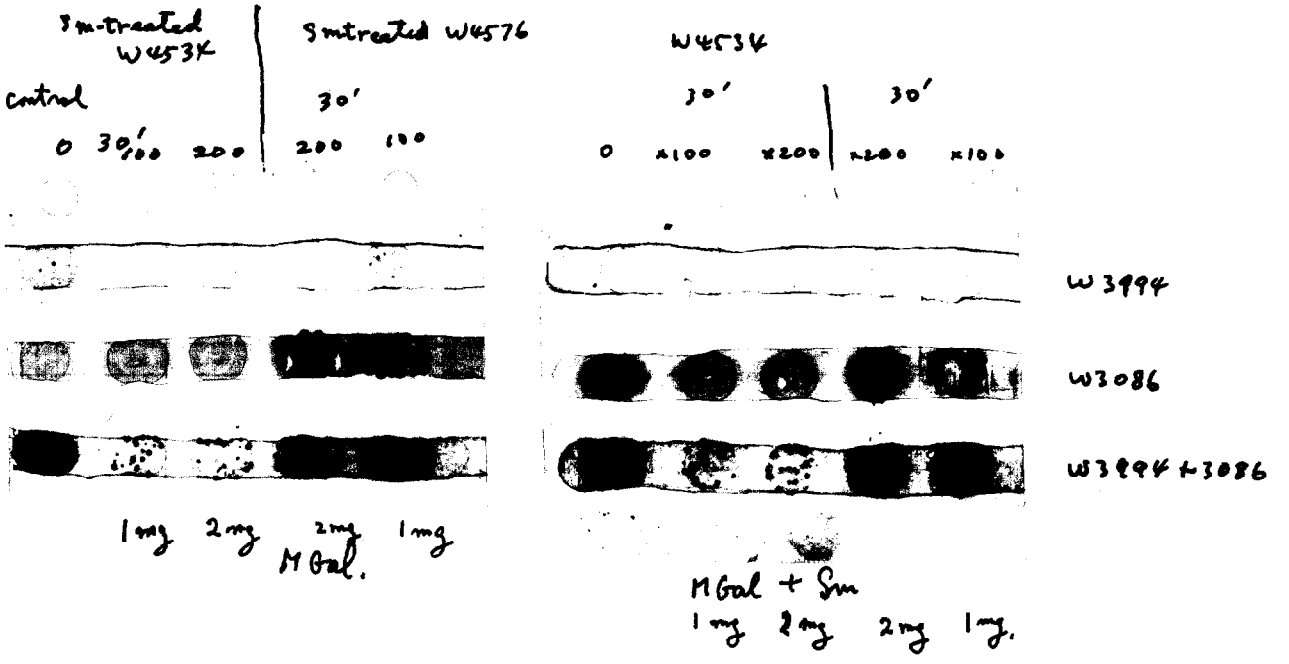
Survival test : 0.1 ml / plate : after washing : Same as sample as used as F donor.

W4728(F_6^{+})

	100U-30 min,	200U—30 min.	100U ···· 2hr.	200U ··· 2hr.
1	3	0	0	0
2	0	0	0	0
W4534				
4	0	0	1	0
5	0	0	0	0

Infectivity test.




Sm-treated W4534
Sm treated W4576
W4534
control
30'
30'
30'
0 30' 100 200
200 100
0 ×100 ×200
×200 ×100
W3994
W3086
W3994+3086
1mg 2mg 2mg 1mg
MGal.
MGal + Sm
1mg 2mg 2mg 1mg.

Quantitative measurements of the transfer of F' to F- by Sm-killed F^+ cells.

30/11 : 1959 REF:

Principle: W4534 (F_8) × 3086 × W4573 (MGal# F_8)

Method: 1. Treat W4534 with Sm. ×10 conc. than usual Sm. (1 mg/ml)
×10 diluted Sm. 0.2 ml / 2 ml culture
Cultural age: 24 hrs.; penassy grown; Wash twice with broth:
Time of treatment 9:00 pM → 1: pM. (next day)
at 37°C

2. Mix → Incubate it overnight.

2' Test survivors.

- 0.1 ml/plate Sm-killed cell
- 10^{-6} ml/plate untreated cell

Experiment: Sm treated W4534 + 3086 (ca. 10^6 cells/ml)
control: Untreated W4534 + 3086 (ca. 10^6 cells/ml.)
and incubate them for overnight.

Check: survival: 2 hr. culture & overnight grown culture → 0.5 ml/5 ml pa. → Incubate it for 2 hrs more

3. Seed it on BLac Sm, and select W3086. ×10^{-5} ml & ×10^{-6} ml.

4. Replica plate on MGal needed W4573 on it.

Result:

1. # of survivors (10' overnight) : colony forming activity.

0.1 ml/plate Sm-treated	control: Sm-Untreated (×10⁻⁶) 10^{-6} ml/plate.
0	460
0	516
0	540 ?
0	520
0	575
Σ 0	

2. # of (F_8) infections.

Sm treated		control Sm-Untreated.	
F_8^+ / # of colonies tested (10^{-6} ml/plate.)	(%)	F_8^+ / # of colonies tested. (10^{-5} ml/plate)	(%)
1 709 : / 2104	(33.8)	1 59 / 68	(87)
2 584 : / 2390	(24.4)	2 65 / 54	(88)
3		3 60 / 69	(87)
4		4	
5		5	
Σ	(%)	Σ	(%)

Sm untreated control, F_8^+ is survivor. Therefore, it shows strong competition with 3086? But Sm-treated group is not. It is clear to see their difference.

% of infections per survivors:

W4534 : overnight culture in Penassay broth. + Sm 10 μg/ml
5 ml. < 2 ml : Sm-treated
2 ml : untreated control. (x10 much than usual use.)

↓

Incubate it overnight at 37°C.

↓

Wash twice by Centrifugation. (with penassay). (10 min. for each centrifugation)

↓

Suspend it into penassay. 1.5 ml.

↓

Test: 0.1 ml/plate Sm treated; No addition: 0.1 ml. × 10^{-6}/plate untreated.

Add. young culture. (2 hr : ① overnight culture 0.5 ml + 5 ml/plate incubate it 2 hrs more ② dilute. × 10^{1} ; add 0.1 ml to 1 ml of W3086. (F' recipient). Add: 10^{6} cells into the 1 ml of W534

↓

Incubate it for overnight at 37°C.

↓

Seed W3086 on Blac Sm. and incubate them.

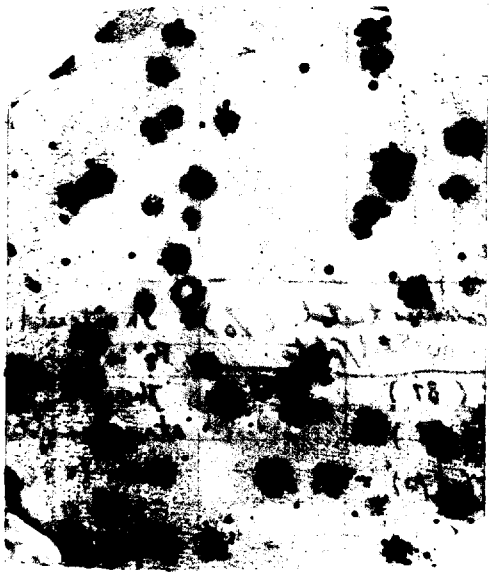
↓

Replica plate them on MGal seeded W4573 on it.

↓

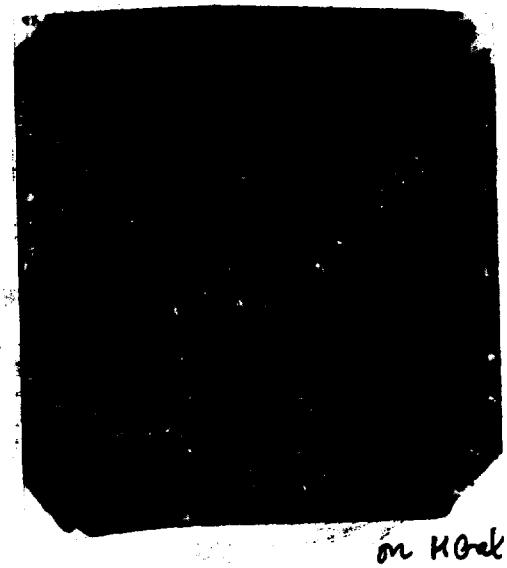
count the percentage of $F8^{+}$ infected.

untreated control.
W4534 + W3086



untreated control.
X W4573
on MGal

Sm killed W4534 + X 3086
Treated control.



on MGal
seeded W4573

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MICROBIAL
GENETICS

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2nd trial for isolation of F_3^+.

(W3642F_1): F^+ H Mal_S Gal_2 Lac_1.

2/11 ~ ;1959 W4554 REF: see p 122

Principle.

① W3642 F_1 × 3133.

W6 × 3133

② select on DO.

③ Replica plate on MLac seeded W3086. and look for H1 for M.

Results.

W3642 F_1 × 3133			W6 × 3133		
# of colonies	Hfr	%		Hfr	%
627	1		586	0	
613	2		673	0	
676	3		517	1 ?	
693	10		684	1 ?	
1742.	2		509	0	

④ Test the fertility again. (× 3086, on MLac) by cross-streaking method

⑤ purify #2 and #6 on Blac and reisolate 3133 F_3.

3642F^+ × 3133

#2 #6. 55 colonies of 3133F_3 are reisolated and tested on the fertility in transfer of Mt. Best non of them are Hfr. (see backpage)

∴ It means the 3133F_3^+ is very unstable after infection of the F' to F⁻. It segregate many F^+

1 → 2 3 → 4 5 → 6 7

3133

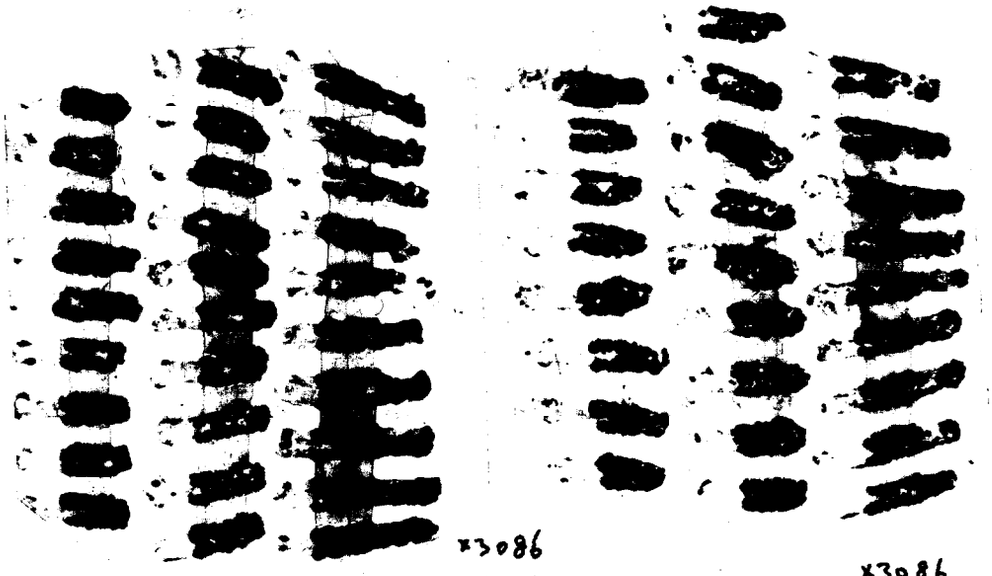
× 3086

⑥ Try Replica plating method to for reisolation of 3133F_3.

Purification of 3133 F_3.

#2

#6



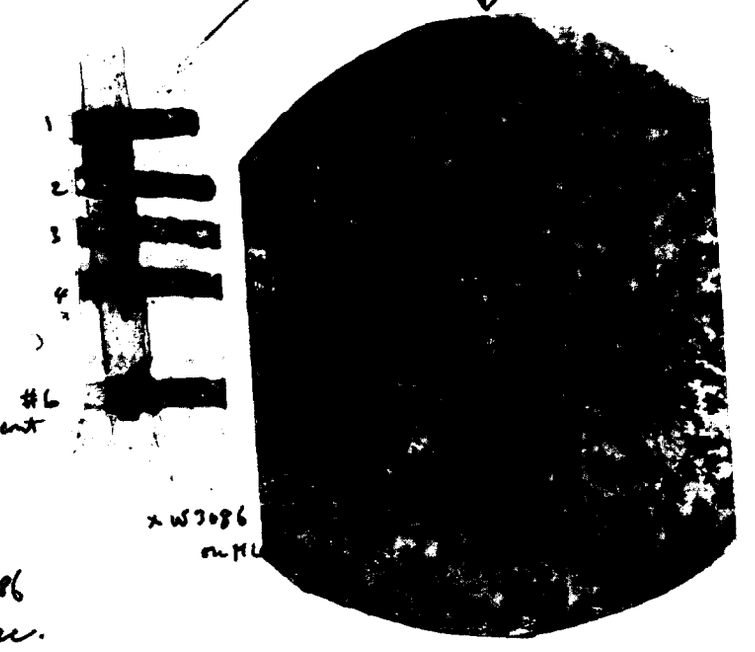
×3086 on Mlac.

×3086 on Mlac

Reisolation of 3133 F_3^+ by cross-streaking method was not successful use replica-plating method for 3133 F_3^+ isolation.

these reisolated F_3 looks reasonably stable.

Seeded W3086 on Mlac.



#6 cut

×W3086 on Ml

Test W3747. (derived strain from W3213)

17/11 ;1959 $Lp_4^R M^- V_6^R Hfr_{13}$. REF: Hfr_{13}.

J.L says: Alan found that it is HI for Lac (x 3133 on MLac) but the many of these recombinants to show Lac+. This seems quite peculiar phenomenon, it may (linked transfer of Lac and F' ~~dependent~~ on Hfr marker. (Hfr may linked with Lac.) (a strange Hfr)

1. Purify W3747 on B Lac and test the fertility of each picked colonies from the Bla (x W3133 on MLac)
2. Look for Hfr_{13} and pick recombinant from cross-streaked lesion. Purify it on B Lac.
   Result: All the colonies are Hfr. (Fig 1)
3. See variegated colonies on the B Lac plate.
   Result: It segregate Lac+, Lac-, and Lacv. (See bellow Fig. 2) several colonies/plate are variLac.
4. Pick 12 Lacv, suspend it into water, and streak it on B Lac, Gal, Mal, Mtl, Xyl, to know the size of incorporated chromosomal segment.
5. Pick Lac+ and Lac- and cross x F-, and see the relation between F and Lac marker.
6. Treat #19 with A0. and conform the infectivity of Hfr-character.
   A0 20', and A0 30'. W3747 → x W3086.
   Result: This strain W3747, transfers the Hfr-character into F- (3086). Therefore, it is not Hfr but F' (Hfor Lac.) (see back page).
   Hfr 16/28 total x100 = 57 (%)
2. A0 does not work in this experiment. (25/25 were Hfr. untreated control: 27/27 Hfr. after treatment) tester 3028

W3747

Fig 1

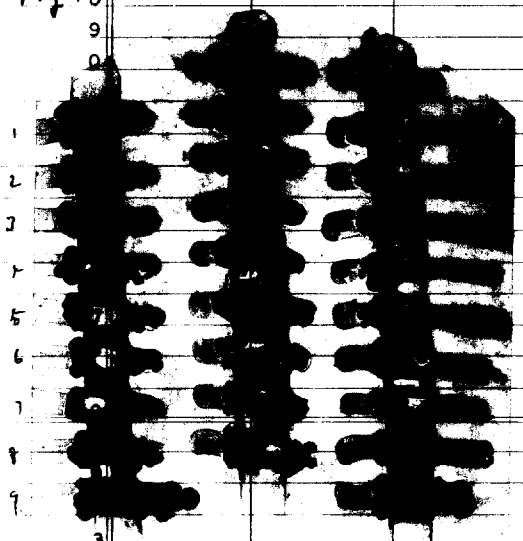
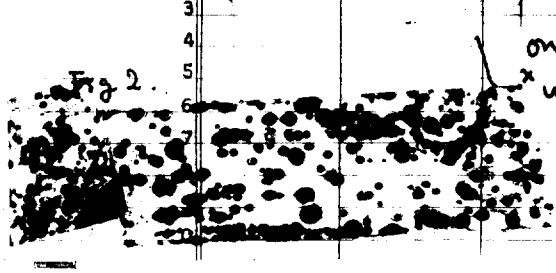


Fig 2.

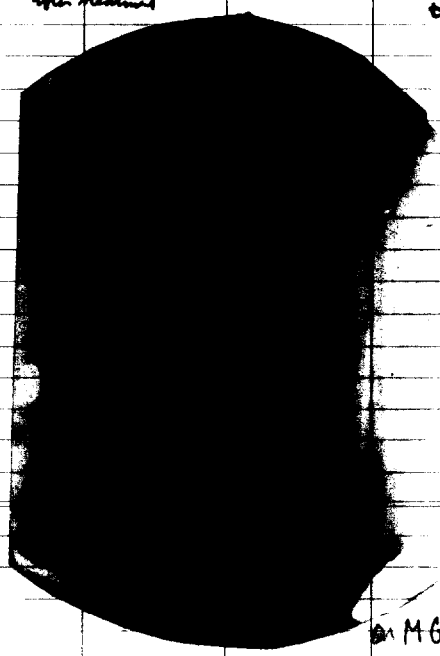


Lac v.

Blac

on MLac x W4573

on BLac cont.

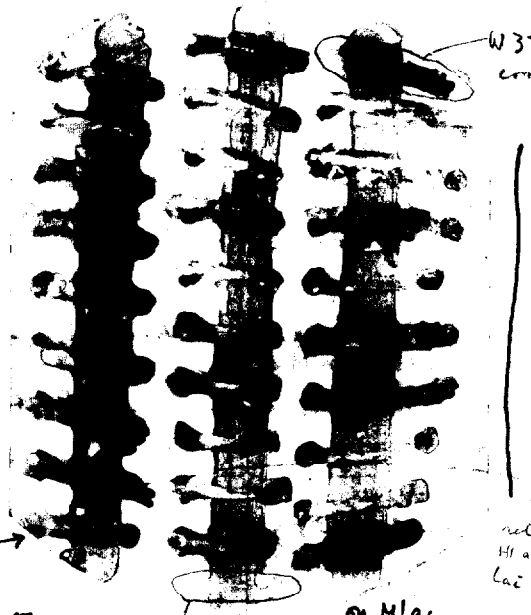


on M Glucose

Tests on infectivity of F_{13} to 3086

$Hfr_{13}\ M^-V_6^R\ Lp_4^R$ $\quad F^-M^-Mal_1\ S^R$

W3747 → × W3086



This colony is hi for Gal, instead of Lac!
This means there are two states.
Hi for Lac or Hi for Gal.
See below.

Data from Cross-bunching method

	control	W3086	Rate of conversion
Hi : Lac	—	16/28	(57 %)
Lo : Lac	—	1/28	(3.7 %)
total		17/28	(60.8 %)

on Mlac
×3828

Data from Replicaplating

Hi : Lac 101/576 (17.6%)
Lo : Lac 34/576 (5.9%)

F_{13}

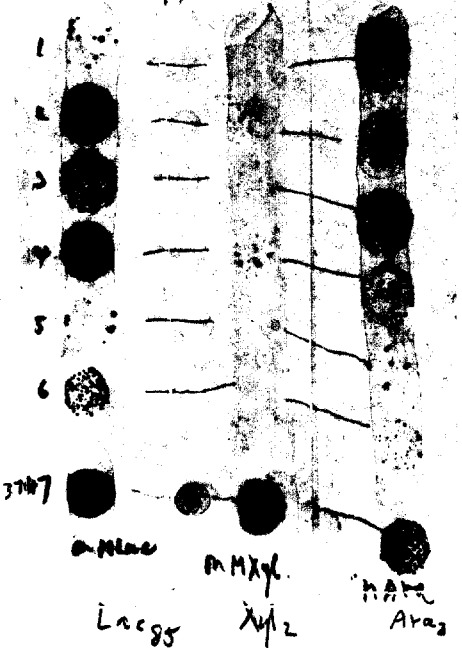
W3747 → × W3086 :

on Blac Sm → replicated on Mlac seeded W3828.

Test of Hi and Lo colonies of W3086 F_{13} on the transfer of various markers.

3086 F_{13} → × W4573.

2, 4 : Hi Lac 1, 3, 5, 6 Lo : Lac.



Hi : Lac is low for all markers, but Lo : Lac is Hi Gal, relatively Hi for Ara_2.

It is not so strict. Hi for both

Futility of infected colonies by F_{13}.

Save one as W3086 F_{13}.

ity on the futility of Lac and Gal transfer, However, It may be interpreted by the mixture of both types of clones.

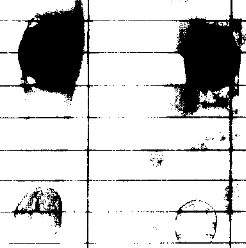
Fertility of the segregants ~~from~~ of a sectored-colony arised from the cross W3747 x 3828.

30/10 1959 REF:

3743x

Method: 1. ~~Spot W3743 x 3828~~. Cross-brush W3743 x 3828. on HLac.
2. Purify the black area on BLac.
3. Pick sectored colony and suspend it into water (1ml) and streak it on BLac again.
4. Pick (Lac+ and) Lac− colonies, respectively, and test their fertility by cross x 3086. (M− F−) on HLac.
5. Result.

#7 19



control. W—

Hl for M: Hfr 1/33

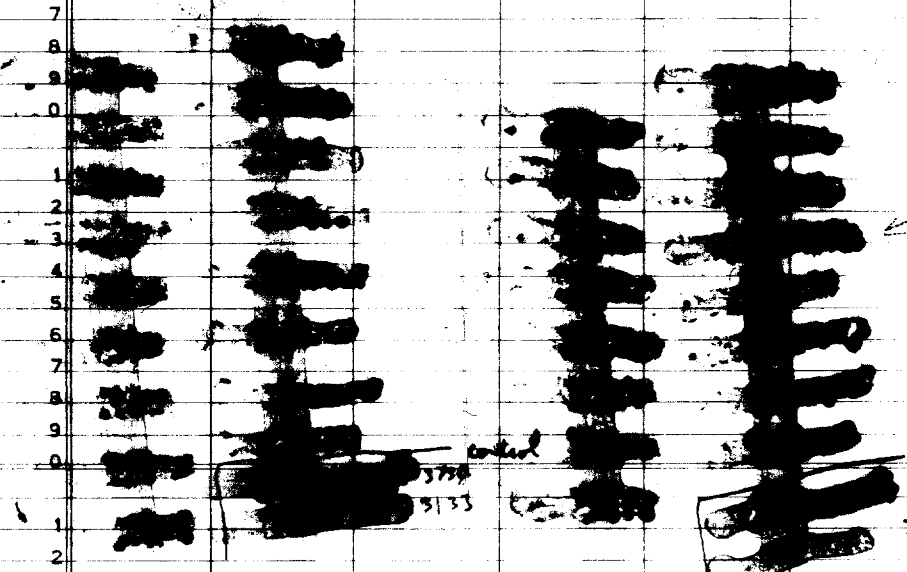
Lo for M: 32/33

Conclusion: Lac− Segregants from diploid colony ~~are~~ are all ~~or~~ not ♀ unlike as usual Hfr.

x3133 x4573 on HLac

all the sugar markers are checked by replica plating method
All, Lac−, Mal+, Mtl+ Gal+ (see back page)

#1 #2



on MLac x3086

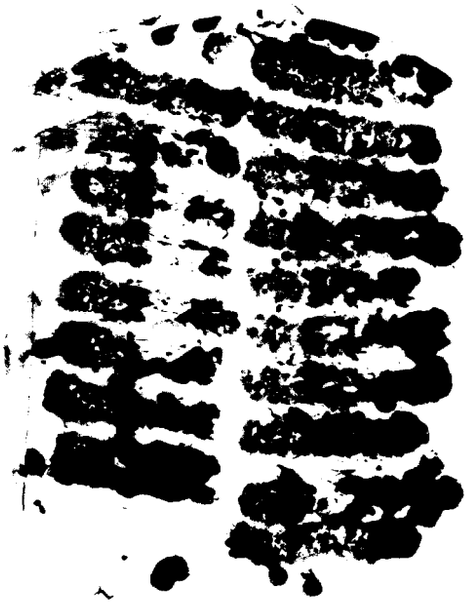
on MLac x3086

Hfr? Save this it may be F_{13}^{+} Lac S^R

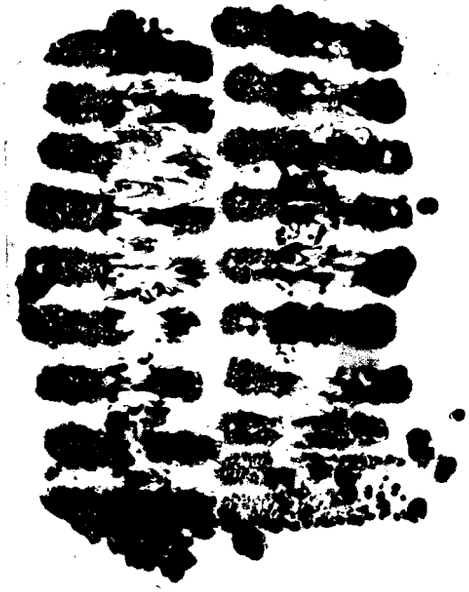
These 2 kinds of colonies, Hl for M Lo for M may be interpreted by two states of colonies.
Hl Lac is Lo the others, but Lo Lac is Hl the others. (esp. Gal)

Hypothesis:
1) Transduction of Lac result Hl Lac but it will mutate to Lo Lac.

#2.



#1



Sex-compatibility of Lac⁻ segregants from Lac+ colonies obtained from the cross W3747 × W3133.

30/VI ; 1959

REF:

Method : 1. Cross W3747 × W3133 on MLac agar. by spot test.
2. Purify it on Blac agar.
3. Pick Lac+ and repurify on Blac.
4. Pick Lac⁻ segregants and cross-brush it with W4503 (Pur⁻ F⁻) on MLac agar.

Result.
all of the Lac⁻ segregants were F⁻. (see bellow.)

Lac+ : #1. 0/26 all 26 were F⁻ (0 %)
Lac+ : #2. 0/27 " 27 " " (0 %)

Conclusion: This result seems contradict from the former result.
Try again.

#1

3747
3133
× 4506 (Pur⁻ F⁻)
on MLac

#2

3747
3133
× 4506 (Pur⁻ F⁻)
on MLac.

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L06

Transduction of Lac-loci by F_{13} (W3747)

$S^S F_{13} M 4^S V_6^R$

29/VI ; 1959 REF:

Method : 1. Mix W3747, W4573. 1 ml + 1 ml + 1 ml fresh broth.

2. Incubate it overnight.

3. Dilute and seed onto EMBLac Sm.

4. Count the ratio of numbers of Lac^+ and Lac^- colonies on it.

5. Test compatibility of those colonies

Marker selected: Sm^R

Results:

1. Rate of infection of Lac^+ loades into Lac^-_{85} strains.

plate No.	Lac^-	Lac^+	% of Lac^+
1	20	9	31.2
2	28	2	6.67
3	13	2	13.3
4	14	5	26.3
Σ	75	18	19.4 %

on BLac Sm.

(: rate of infection of Lac^+ to Lac^-)

Conclusion : This rate (19.4 % : totals colonies tested) is very high.
Other kind of transfer of Lac^+ marker occured. (mechanism of not recombination) to F^-

2. Test the Compatibility of the Lac^- and Lac^+ colonies isolated from above experiment.
Look for Lac^- F_{13} comes from splitting of F_{13} from Lac by X

Method : Replica plate on MXyl seeded W4506 on it. (save it)

Result: All Lac^+ is compatible with F^- (x F^-Pur^- : W 4506), but fertility is lower than control : Parent. (Test fertility of Lac^+ transfer).
All Lac^- are F^- (Steril in cross x W4506) on MXyl.
41 (Lac^+) all σ : 88 (Lac^-) all φ. (see back page)

3. length of the transduced-segment by F_{13}.

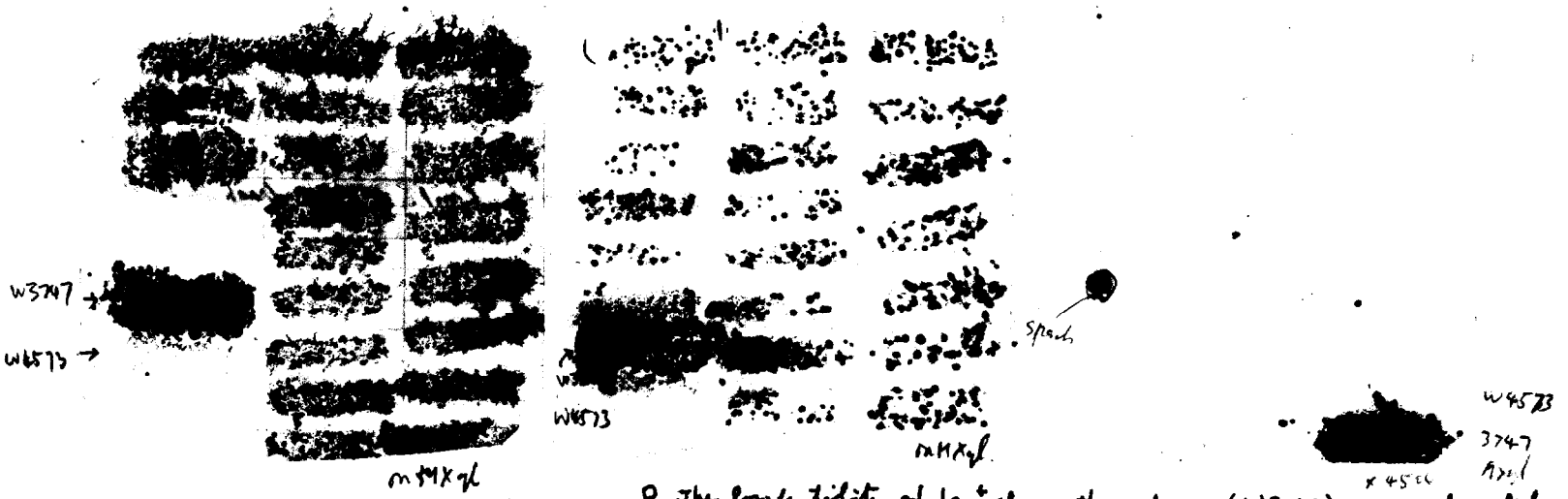
V_6	Lac_{85}	Gal_2	Ara_2	Xyl$_c$	Mtl.	●	Mal_1	M.	# of colonies tested.	
S	+	-	-	-	-		-	+	41	Lac. transduced.
S	-	-	-	-	-		-	+	88	F^- Parental type.

No other combinations of these markers were found.

Conclusion : Size of genetic materials transferred is very small.
It looks only Lac is transferred into F^-, even no selective marker was used for near Lac-region.

Next step : Use reverse transduction of Lac^+_{85} to Lac^- other origin M^- F^-
Use W6 F_{13} and W4541 ($F^- S^R$ Lac Xyl$_2$ Mtl Mal_1 Gal_2 V_6 P)

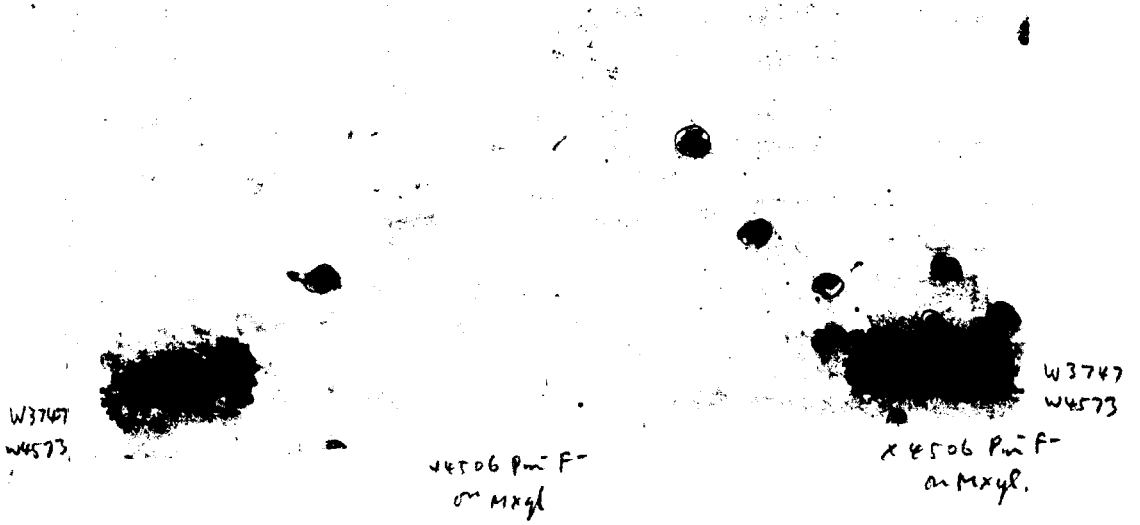
1 (Lac$^+$) 2 (Lac$^+$) 3 (Lac$^-$)



○ The low fertility of Lac$^+$ clones when donor (W3747) may be interpreted by two states. (Lo for Lac is Hi for the other marker, but Hi for Lac is Low for other marker.)

Test ● Lac-transfer. of Lac$^+$.

4 (Lac$^-$) 5 (Lac$^-$)



Transfer of F' of $^{V}F_8^+$ (W4534 Sm-killed) to F⁻; 1 mg/ml Sm.

1/VII ; 1959 REF:

1	2	3	4	5	6	7	8	9	10

• Principle:

W4534 (Sm-killed cells.) $\xrightarrow{F_8}$ ~~W4534~~ W4573 + W3994 (↑↓ Gal_4^+ and Gal_2^+)

W4573: F^- Ara_9 $X^s_{1/2}$ MCI Gal_2 Lac_{85} Mal_1 (Lp^+)

W3994: F^- Gal_4 $(Lp^+)S^R$

Lp^s M Gal_4 on BGal + BGal Sm

• Further experimental design.

① (Use S^R Lp^s for all strain)
W3104 (S^R) Lp^s Gal_4 F^-
W3102 (S^R) Lp^s Gal_2 F^-
No S^R.

② If Gal_2^- Gal_4^- S^s F_8 was used for F_8-donor, it is better.

• Possibility:

These infective F' of Sm-killed W4534 may be streptomycin-resistant F_8'.

(there is a sign (use: 0.1 ml to 100 ml) of Sm

• Experiment:

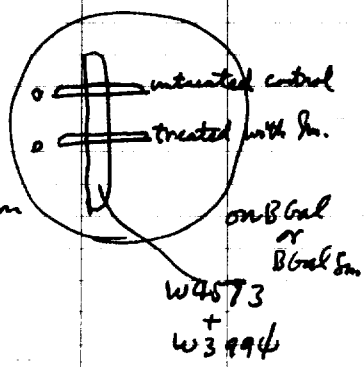
Method: ① add 0.1 ml to 1 ml of overnight culture of W4534.

② Incubate it at 37°C for 2 hrs.

③ Wash it with 5 ml of Pensasy once.

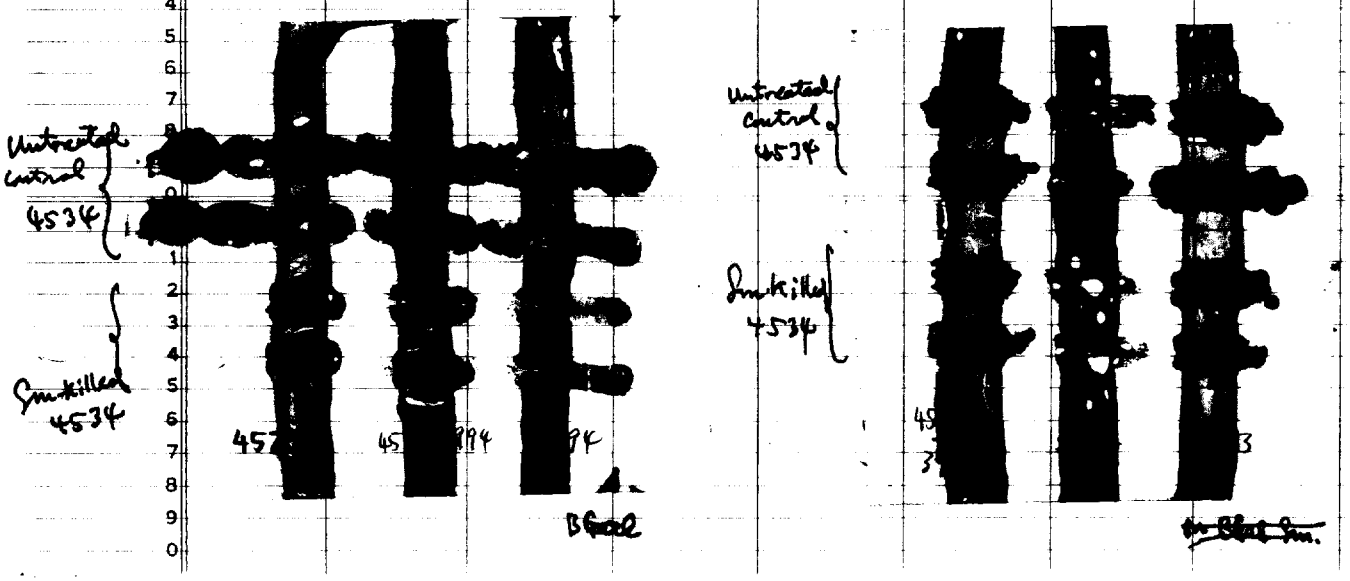
④ Cross-brush it with the mixture of W4573 and W3994 on BGal and BGal Sm.

⑤ Incubate it overnight.



• Expectation:

If Sm-treated W4534 cells are completely killed, and still have some infectivity of F_8, it may gives gal^+ recombinant at the contact area by infection and chromosome transfer from each others.



(Treat W3747 with Sm.)
Transduction of Lac-marker to F⁻ by Sm-killed F13.
(and isolation of Sm-resistant F13 from it.)

2/4 :1959

Method (Add)

1. (Treat) W3747 with Sm. (×10 conc.; overnight-treated in phenasay-Sm) overnight grown culture in penassay broth.
2. Incubate it for overnight at 37°C. (Sm-treated cell ● shows no growth but untreated control is 4.10 times turbid)
3. Wash it once with 2 ml of Penassay broth and resuspend it into 1 ml of Penassay broth, and test the fertility ~~infectivity~~, by cross-streaking method. and by standard recombination method.

Result: See back page

● Cross-streaking method: Cross-brush it with ~~[redacted]~~ 3828, and W4573. on MLac, Blac, and BLac Sm. use untreated W3747 as a control.

● Semi-plating method:

1. Spread W4573 on MLac, and add 0.1 ml W3747-Sm-killed cell to ● half of the plate, other half is control. Also try to make control.
2. Incubate it 40 hrs.

Result:	+ Sm killed W3747	control
377	0	see back page

● Standard method for testing fertility of Sm-killed cells.

	# of Recombinants / 10^{-4} ml
Untreated control:	542
	: 579
	: 609
Sm treated	: 63 / 10^{-1} ml
	109
	62

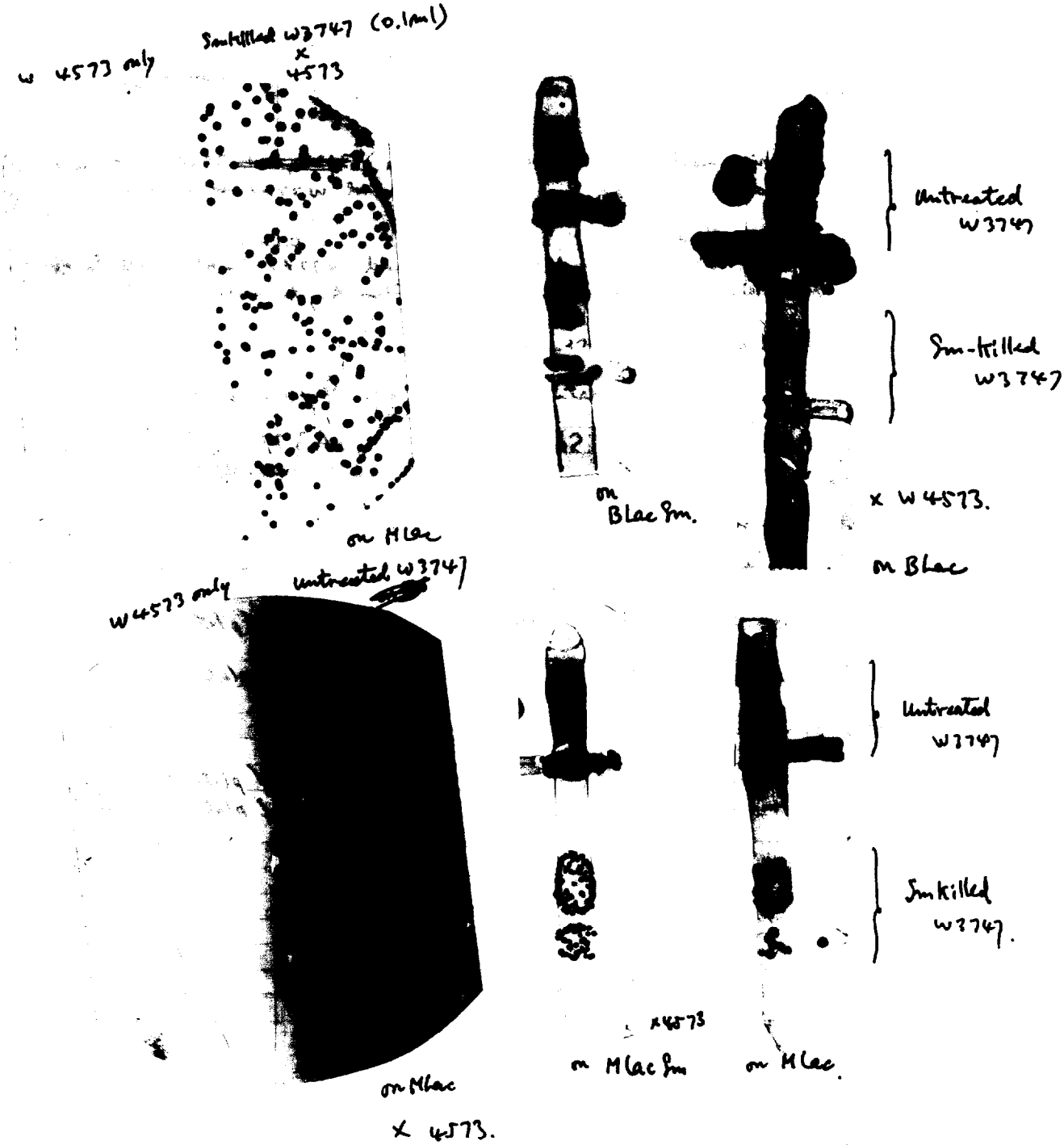
1. Add 0.2 ml of Sm-killed cells to W4573: 1 ml. Use untreated W 3747 as a control. Add 1 ml of fresh-broth to it.
2. Incubate it for 2 hrs. at 37°C. (11:45 AM ~ 1:45 PM.)
3. Dilute them in adequate concentration, and seed it on MLac.

Untreated control: ~~[redacted]~~ Use 10^{-4} ml. / plate.

Sm-killed cell: " 10^{-1} ml / plate.

● ~~[redacted]~~ Survival test

		colonies / plate
Untreated control:	Use 10^{-7} ml / plate.	248 ; 303 (3×10^9)
Sm-killed cell:	" 10^{-1} ml / plate.	0 ; 1 (0 or 10)

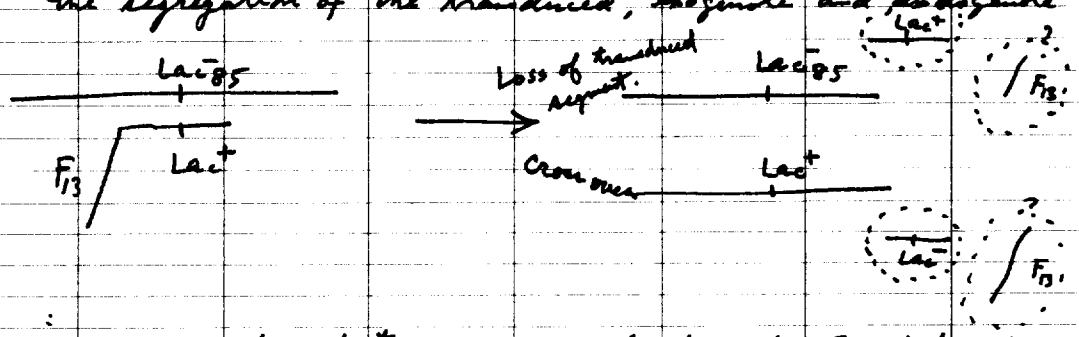

W 4573 only
Sm-killed W3747 (0.1ml)
x
4573
on MLac
W4573 only
Untreated W3747
on Mlac
x 4573.
on Blac Sm.
Untreated
W3747
Sm-killed
W3747
x W 4573.
on Blac
Untreated
W3747
Sm killed
W3747.
x4573
on MLac Sm
on MLac.

Test stability of transduced marker "Lac+"

7/VIII ; 1959 REF:

Principle : Lac+ is dominant marker, therefore, diploid strain exhibits Lac+ phenotype as well as haploid Lac+.

If it is ~~[redacted]~~ hemizygous diploid at Lac loci, it must show the segregation of the transduced, exogenote and endogenote marker.



Method :

Test unstability of Lac+ of newly isolated W4411 F_{13} Lac+. (see 15_3 6/VIII)
Use 5 strains

1. Dilute it in adequate ~~[redacted]~~ cell number. and plate it on Olac, incubate it overnight.
   use 10^{-7} ml / plate.

2. Count total number of colonies and Lac⁻ colonies

Ref : These culture is independently isolated ~~[redacted]~~ transduction of Lac+ to 4411 from W3747. Lac+ colonies are inoculated into penassay broth and incubated ca. 7 hrs at 37°C. ~~[redacted]~~ These cultures were used for this experiment.

Result :

Esolation number :		# of total colonies obtained	# of Lac⁻ colonies	% of Lac⁻
2	2	0	0	
		0	0	
3	3	186	1	
		134	0	0
		173	0	0
4	4	377	3	0.7.9.6
		384	2	0.5.2.1
		394	2	0.5.0.8
5	5	668	2	0.3.0.0
		714	1	0.1.4.0
		683	2	0.2.9.3
1	1	10	0	
		4	0	
		0	0	

1 , 2 was too few colonies

Further work : Test sex-compatibility of Lac⁻ colonies which are segregated from the transduced Lac+ probably Diploid for Lac.

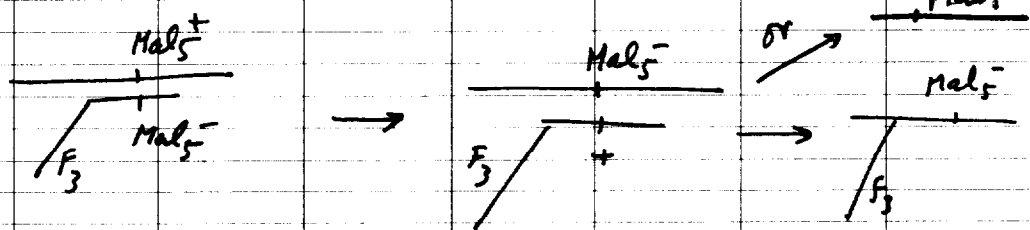
Result : #3 of 1/7 F⁻ #5 of 1/5 F⁻

Recombination between Mal_5^- and F_3.

7/4/11 ; 1959 REF:

Principle: F_3 may transfer Mal_5^- in the transduction, but Mal_5^- is recessive. Look for segregation of Mal_5^- from 3133 F_3 (Mal_5^-/Mal_5^+)

Scheme:



Method: ① Dilute 3133 F_3^+ : Use 10^{-6} ml /plate ; and seed it on B Mal.

② Incubate it overnight at 37°C.

③ Look for Mal^-.

Control 3133		Experiment. 3133 F_3	
Mal+	Mal-	Mal+	Mal-
244	0	841	0
224	0	920	0
239	0	856	0
222	0		
287	0		

allelism test using 3828 F_{13}. (Lac_{12})→(Lac^{3229})
Called $11D_3$.

6/VII/1959 REF:

1	2	3	4	F⁻ 5 x⁺	6	7 F⁻ x⁺	8	9 History of 10 $11D_3$.
very unstable:	Lac_1 : Permease			W 3238		Lac_{61} : W4121		Lac_1 T87
	Lac_3 : Lac^- gal⁻ mal⁻ Gal⁻			W 2243				Lac_1 W518
	Lac_5 : Lac^- Mal⁻			W 2245 (S^R)				" W1578
	$Lac_{2.4}$: β-galactosidase			W 3112 (S^R)				" W1321
	4 : β-galactosidase			W4287, (W3127)				Lac_1 W1687
	Lac_7 : Permease and β-galactosidase			W 2247				Lac_{12} W3120
	Lac_{11} (11)			W 3230				Lac^{3229} W3229
	16 (12)			W 3089 (Mal S)				Lac^{3229} W3133
								Lac_{11D_3} W3828

Principle : W3828 F_{13} × F⁻ Lac⁻ on MLac.

Method :

1.) Purify all Lac⁻ mutants on Blac agar.

2.) Inoculate purified colony into 5 ml/ passage broth.

3.) ~~Hatch~~ Spot test on each strains. for transfer of Lac from W3828 F_{13}.

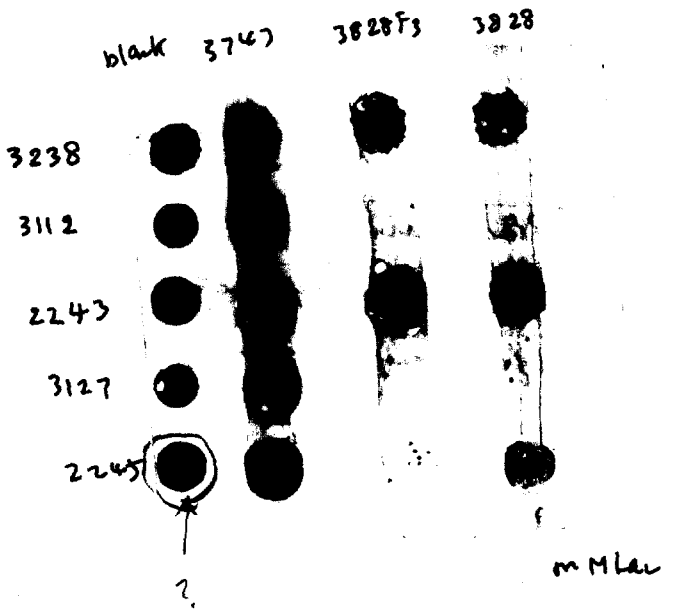
Result :

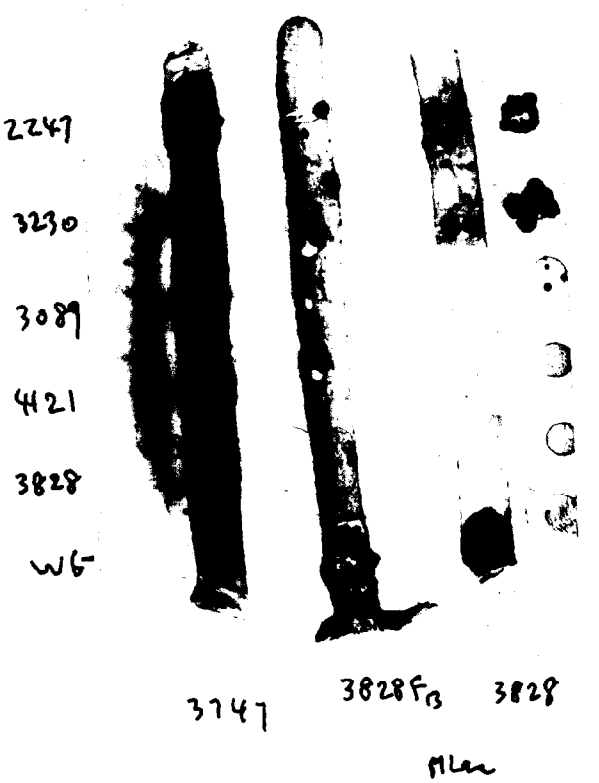
Lac	W-	3828	3828 F_{13}	3747	blank.
1	3238	(+) −	(+) −	+++	(+)
2	3112	−	−	+++	−
3	2243	? +++	? +++	? +++	+++? remain
4	3127	−	−	+++	−
4	2245	+	+	+++	+++ ?
7	2247	−	−	+++	−
11 (12)	3230	−	−	+++	−
12 (16)	3089	−	−	+++	−
61	4121	−	−	+++	−

on MLac 4 on MLa Sm.

see back page :

Conclusion : W3828 F_{13} may be wrong, not F_{13}^- but F⁻


blank
3747
3828F3
3828
3238
3112
2243
3127
2245
?
mMLac


2247
3230
3089
4121
3828
WG-
3747
3828F3
3828
MLac

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Transduction of Lac-loci by F_{13}.

[W6 F_{13} → × W4541
W4511 F_{13} — × W2594]

3863 Pa_c^- F^-

7/VII 1954 REF:

1	2	3	4	5	6	7	8	9	10

Principle :

One way : W6 F_{13} (transducible) → × W 45[illegible]. ($F^- S^R Lac_{BS} Xyl_2 Mtl Mal_1 Gal_2 V_6$ P)
M F_{13}.

back : Lac^+ [illegible] W4411 F_{13} → × W 2594 ($F^+ M Lac\ S^R\ U_1^R$)
P Lac^+ P Xyl_2 Mtl Mal_1 Gal_2 V_6

Method :

1. Mix 1 : 1 : 1 (fresh broth) and incubate it overnight at 37°C.
2. Purify them on Blac 9m. count the ratio of Lac^+ and Lac^-. Use 10^{-3} ml/plate
3. Test on all markers of Lac-transduced W4541. and W2594.

W6 F_{13} × W4541

① Ratio of Lac^+ and Lac^- (+/−)

; 239/249 (51.1) ; 224/267 (45.6) ; 255/266 (49.0)
; 285/213 (52.4) ; 209/242 (46.4)

F	S	Lac	Xyl	Mtl	Mal_1	Gal_2	V_6	P	M
r	+	−	−	−	−	r	−	+	
		..		+		..			

Plate	1.	2	3	4	5	6	7	8	Σ	%
	40	40	35	38	37	38	38	40	306	97.2
	1	0	2	1	3	0	0	2	9	2.86

W4411 F_{13} → × W2594

① % Ratio of Lac^+ / Lac^- Partial[R] ; $\frac{+}{178}/\frac{-}{260}$ (40.6) ; $\frac{+}{152}/\frac{-}{237}$ (39.1) ; $\frac{+}{125}/\frac{-}{229}$ (35.3)
; $\frac{+}{135}/\frac{-}{206}$ (39.6) ; $\frac{+}{201}/\frac{-}{258}$ (43.8) :

② Pick Lac^+ and test other markers.

Plate No.	F	S	Lac	Xyl_2	Mtl	Mal_1	Gal_2	V_6	P	M	T_1	No of colonies	%
2	r	+	+	+	+	+	+	S^r (r:1) (s:1)	+	−	r	53	
4	r	+	+	+	+	+	+	S^r (r:4)	+	−	r	48	
8	r	+	+	+	+	+	+	S^r (r:2)	+	−	r	39	
2	r	+	+	+	+	+	+ (−:1)	S^r (r:2)	+	−	r	39	
	r	+	+	+	+	+	+	$S^{r:3}_{s:2}$	+	−	r	53	
1	r	+	+	+	+	+	+	$S^{r:}_{s:}$	+	−	r	40	
6	r	+	+	+	+	+	+ (−:1)	$S^{r:3}_{s:1}$	+	−	r	39	
7	r	+	+	+	+	+	+	$S^{r:1}_{s:}$	+	−	r	38	
5	r	+	+	+	+	+	+	S^r (r:2)	+	−	r	44	

Total Tested 393

V_6^s 24
Gal_2^- 2

	S^r	Lac^+	Xyl_2^+	Mtl^+	Mal_1^+	Gal_2^+	$V_6^{s'}$	P	M	T_1^+	: 367	93.4	
	r	+	+	+	+	Gal_2^-	$V_6 S^r$	+	M^-	T_1^r	: 2.	0.508	Lac^- 0.508
	r	+	+	+	+	+	V_6^r	+	M^-	T_1^r	: 19	4.83	Lac^+ 99.492 %
	r	+	+	+	+	+	V_6^s	+	M^-	r	: 5	1.27	

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REF:

	plate 7	1	2	3	plate 8	5	6	plate 9	8	9	10
		V6	Lac	Gal	V6	Lac	Gal	V6	Lac	Gal	
1		I	H1	H1	I	H1				H1	
2		I	I								
3		r	H1								
4		I									
5								r	(H1)	ex ap tr.	
6											
7											
8											
9											
0											
11											
2						Endomix?					
3					r	H1	Except				
4					I						
5											
6											
7											
8											
9											
20											
21											
2											
3											
4											
5											
6											
7											
8											
9											
30											
31					r	Lo					
2					I	H1					
3	Gal-										
4											
5											
6											
7											
8											
9											
40											
41											
2											
3											
4											
5											
6											
7											
8											
9											
50											

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REF:

	Plate 4 1	2	3	Plate 5	5	6	Plate 6	8	9	10
	V6	Lac	Gal	V6	Lac	Gal	V6	Lac	Gal	
1	I	HI	HI	I	HI	HI				
2										
3										
4										
5										
6										
7										
8										Gal –
9										
0										
11										
2	I									
3	r	Lo								
4	I	HI								
5										
6				R	Lo					
7										
8										
9										
20							R	Lo		
21							R	(HI)		exceptional
2							R	Lo		
3										
4										
5										
6										
7										
8										
9										
30										
	I									
31	r	Lo								
2	I	HI								
3										
4	I									exceptional
5	r	Lo								
6	I	HI					(S)	Lo		
7										
8										
9										
X0										
X1										
2										
3										
4										
5										
6										
7	I									
8	r									
9										
0										

Crossover?

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19 REF:

Plate 3	1	2	3	4	5	6	7	8	9	10
9	V_6	Lac	Gal		V_6	Lac	Gal			
1	I	H	H	51						
2				52						
3				53						
4										
5										
6										
7										
8										
9										
10										
11										
12										
13	r	Lo								
14										
15										
16										
17										
18										
19										
20										
21	S	Lo								
22										
23										
24										
25										
26										
27										
28										
29										
30										
31										
32	r	Hi								
33										
34	r	Lo								
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39										
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41										
42										
43										
44										
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47										
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49										
50										

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Resistant: Sensitive;
V_6 : r. S. I. Intermediate
Lac : H (HI) ; L (Lo)
Gal :

REF:

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Plate #① / Clone #	1 V_6	fertility Lac	3 Gal	Plate-2.	5 V_6	MLac ×5573 Lac	MLac. ×5573 Gal	8	9 V_6	10 Lac	Gal
1	I	H	H		I	H	H	51			
2								52			
3								53			
4											
5	r	Lo									
6											
7					S	Lo					
8											
9											
0											
11											
12											
13	r	Lo									
14											
15											
16											
17											
18											
19											
20											
21											
22											
23											
24											
25											
26											
27											
28											
29											
30											
31											
32											
33											
34											
35											
36											
37					r	Lo					
38											
39											
40											
41											
42											
43											
44											
45											
46											
47											
48											
49											
50											

Transduction of Lac-segment to F⁻ pur⁻ by F_{13}.

4411 F_{13} → 93:85. (pur V_6^R S^R F⁻ Lac85 [illegible] p^+

REF:

7/VII ;1959

1	2	3	4	5	W4637 6	4647 ?(8 101 85)9	10

Purpose: Is pur^+ transduced into F⁻ by F_{13}.

Procedure: 1. Mix 4hrs culture of Parents and incubate it overnight at 37°C.

2. Plate out it on BLac Sm. after optimal dilutions.

3. Count ratio of Lac^+ and Lac^-. and pick Lac^+ (1 $\frac{1}{4}$ p) and streak it onto BLac Sm.

4. Replica plate it on DO, DO + pur. + Prol. Bou

Results

1. Rate of infection.

Lac^+	Lac^-	% of Lac^+
63	720	
45	662	
44	685	
60	684	
50	703	
57	503	
319	3857	Σ4176

7.42 %

2. Transfer of marker by replica. Selected

All colonies showed X^+.

(Hi Gal)	(Hi Lac)	F_{13}	V_6	Gal	Prol	Pur	$Lac^+ S^R$	Number of colonies tested.
Hi	Hi	F_{13}	r	+	+	+	+ r	149

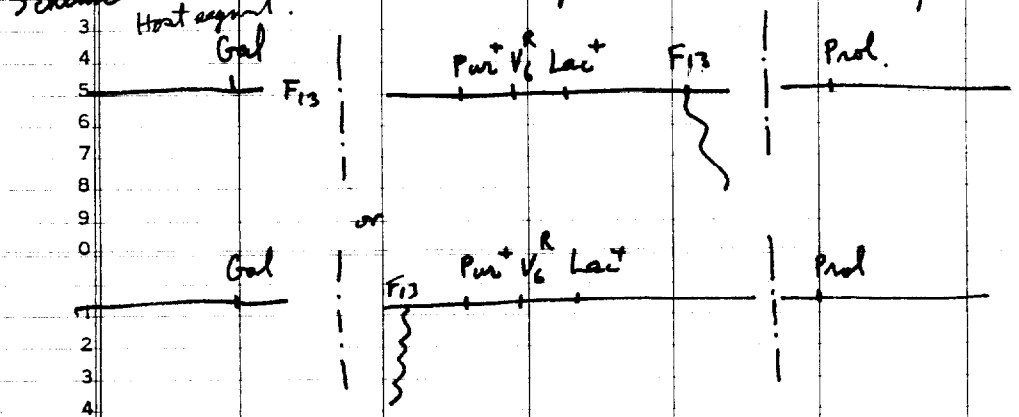
all colonies can grow on DO. no the other kind of colonies were found.

Conclusion:

Pur. is also transferred with F_{13}.

Rate of multiplication of the frequent recombinants is relatively low in pur⁻ stock.

Scheme



Tests of Sex-compatibility and Lac-marker of segregants from "Diploid colonies" which obtained by transduction of Lac+.

8/VII. ,1959 REF:

Ref.
1. Lac loci of W4411 is Lac 85.
2. W4411 was originally F−.

Method
1. Streak all lac− colonies on BLac.
from # 4 ; 7 : # 5 , 5.

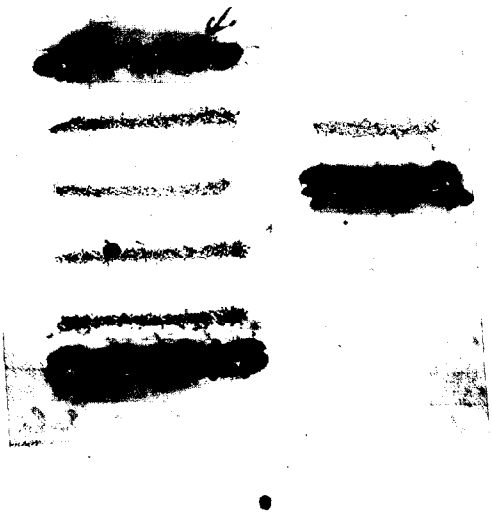
2. Replica plate it on MLac seeded W3086 on it. ● Sel: Lac M.
Replica plate it on MLac seeded W4573 on it.

Result:

Segregants from # 4		Segregants from # 5	
♂ / ♀(F−)	(%)	♂ / ♀(F−)	(%)
1/7	(14.3%)	1/5	(20.0%)

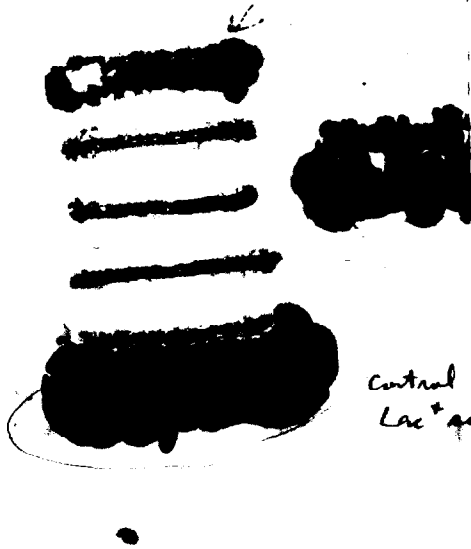
Ref: fertile Lac− grows better than infertile Lac−, on BLac

From 4411 F_{13}
#5



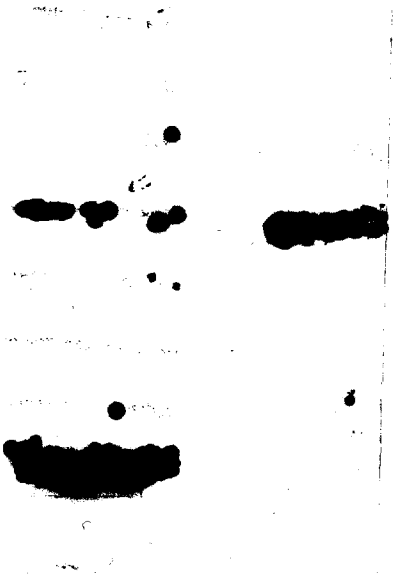
on MXyl
×3086

#5



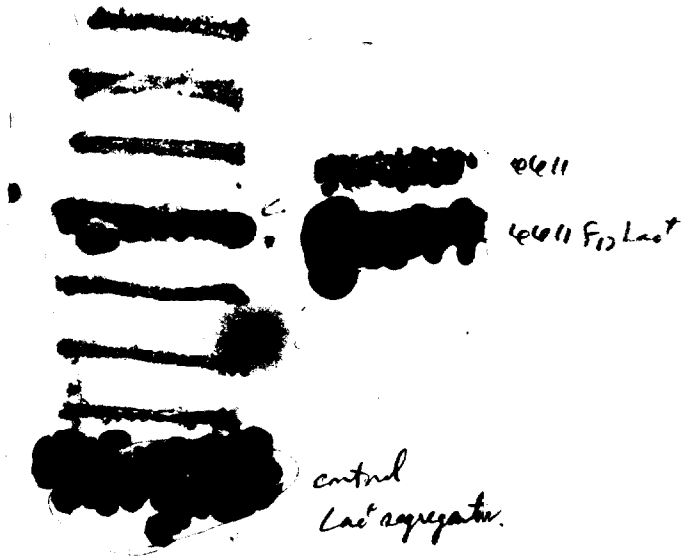
on Blac

#4



on MXyl
×3086

#4.



on Blac

Treatment of Lac+ transduced F_{13} with AO.

8/VII ;1959 REF: Cf. P117, 127, 118

Purpose : Is F_{13} sensitive to AO-treatment?

Strain : W4411 F_{13} Lac+. (This is diploid in Lac loci.) Cf. P114

W6 F_{13}

AO : 50γ/ml Med : Nutrient broth pH. 7.6. : 5 ml.
overnight treated at 37°C. ← 0.1 ml ca. 10^6 cells/ml.

Result :

1. 4411 Lac+ F_{13}

a. Lac marker :

		AO-treated	Control
Lac+/total	1	13/29	6/27
	2	9/25	6/24
	3	15/27	8/28
Σ		37/81	20/79
		% (35.7)	(25.3)

Treated by Lac+ transfer.

b. F_{13}.

		AO-treated		Control	
		×4573 (Mlac)		×4573 (Mlac)	
F−/total	1	0/16		0/21	
	2	0/19		0/12	
	3	0/16		0/18	
Σ					

2. W6 F_{13}

		AO treated		Control	
		on Mgal ×4573	on Mlac ×4573	Mgal ×4573	Mlac ×4573
F−/total	1	2/25	2/25	0/26	0/26
	2	1/27	1/27	0/28	0/28
	3	1/23	1/23	0/23	0/23
Σ F−/total		4/75		0/77	
%		5.34		0.00	

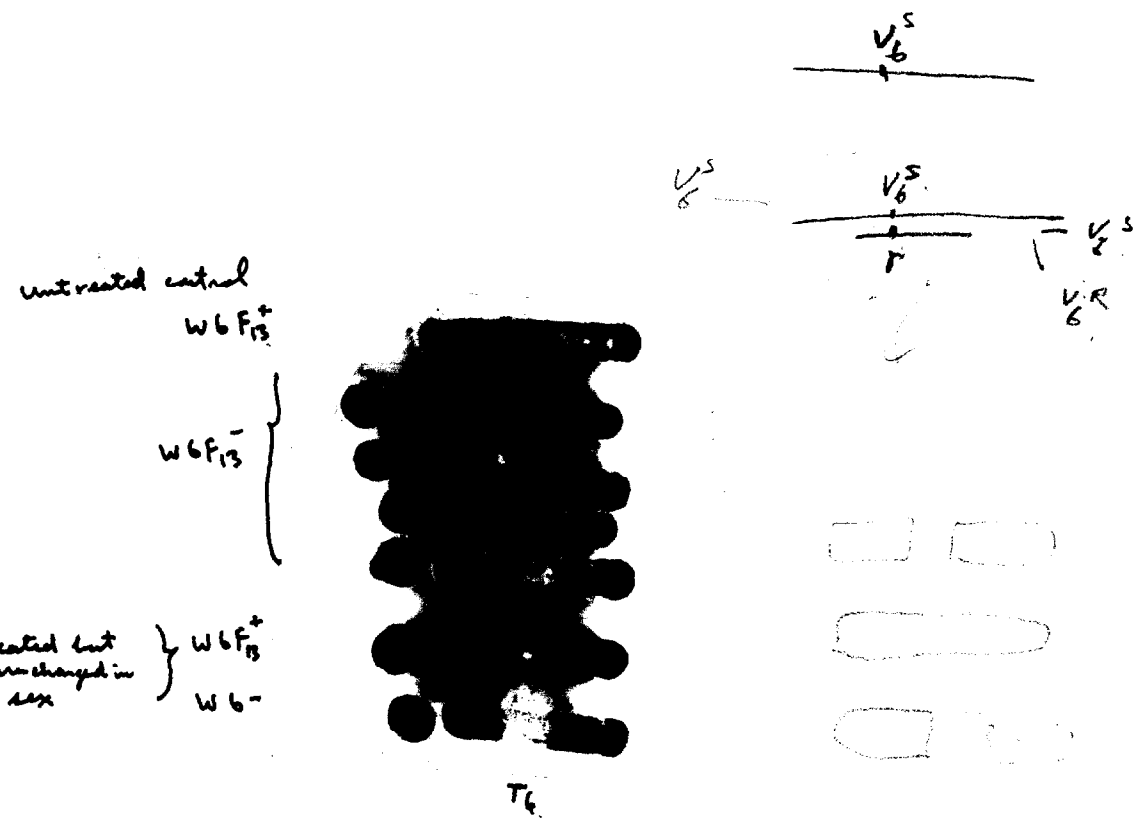
Test T_6-resistance.

3/4 : T_6^S

1/4 : Intermediate.

Conclusion :

1. Acridine does work to Lac-V_6 segment at the same time to attack F_{13}, and result loss of V_6^R segment.
2. But the rate is relatively lower than F^+.


untreated control
W6 F13+
W6 F13−
Treated but unchanged in sex
W6 F13+
W6−
T6
V6S
V6S
V6S
V6R

Treatment of Lac⁺-transduced W4611 with AO.

19 REF:

Purpose: I_3 V_6 Lac^+ F_{13} loci sensitive to AO-Treatment.

strain W4611 F_{13}.: F_{13} Lac^+ / Lac_{85} V_6^S / V_6^R Mal, Ag_2 [redacted] Mtl S^R

Experimental method:

1. Inoculate 0.1 ml of overnight culture of W4611 F_{13} into AO-NSB, pH. 7.6 : AO 5 γ/ml: 6.10^6 cells/ml.
2. Incubate it overnight.
3. Seed it on $BLac_3$ and incubate them overnight at 37°C.
4. Count Lac^+ and Lac^-

	AO-treated W4611 Lac^--F_{13}	Untreated control.
Lac^+ / Lac^- (% of Lac^-)	97/242 (27.8%) 349	267/623 (30.0%) 890
	105/273 (~~27.8~~ ~~30.9~~) 378	249/702 (26.2%) 951
	91/283 (24.3) 374	237/585 (28.9%) 822

Conclusion:

AO-treated group gives slightly lower ratio of Lac^+, but almost same as untreated control.

This result seems not conclusive.

Further Test: Use Lac^+ heterogenote which contains high rate of Het and not segregate progeny.

Elimination of Lac-F_{13} segment with AO-treatment. U)

16/10 ; 1959

REF: 2594 F_{13} (M S^R V_1^R Lac V_6 Lac$^+$ +/ h_2
C.f 118, 127, 115

Purpose: Is Lac-F_{13} segment sensitive to Acridine treatment.

Strain: W2594(F_{13} Lac$^+$) cf. P112.
Purify a Lac$^+$ colony on Blac, pick a Lac$^+$, and suspend it into 1 ml distilled water. (ca. 10^7~10^8 cells/ml)
Use the 10^{-3} ml for AO treatment.

AO treatment: inoculum size: ca. 10^5~10^6 cells/5 ml. ; Medium: NSB-conc. pH7.
AO conc.; 30γ/ml : 37°C, overnight treatment.

Procedure:
Seed 10^{-2} ml of AO-treated and untreated culture onto Blac agar, and count the ratio of Lac$^+$ and Lac$^-$ colonies.

Result:

Tube A		AO-treated (30γ AO)				Untreated control			
		Lac$^+$	Lac$^-$	Lac v.	% of Lac$^-$	Lac$^+$	Lac$^-$	Lac v.	% of Lac$^-$
Plate #	1	52	110	3 165	66.7	250	66	1	317 20.8
	2	65	150	1 216	69.5	289	54	1	344 15.7
	3	65	171	1 237	72.2	246	82	1	329 24.9
	4	71	117	2 190	61.7	243	73	3	319 22.9
	5	38	102	4 144	71.0	220	58	2	280 20.7
	Σ $\bar{0}$ %	291	650	11 952	68.2 %	1248	333	8	1589 21.0 %
	~~% of Lac$^-$~~								
Tube B (30γ AO)	1	12	59	0 71		382	46	3 431	
	2	31	107	1 139		374	42	3 419	
	Σ $\bar{0}$ %	43	166	1 210	79.0 %	756	88	6 850	10.3 %

Further work

1. Test [redacted] V_6 r or s. or intermediate.
2. Test F_{13} by Gal-transfer.
pick Lac$^+$ Lac$^-$ of Untreated and treated
↑ These all Lac$^-$ gives Lac$^+$ papillae on Blac.

Conclusion:
AO-can eliminate exogenote with very high frequency

History of 2594 (Lac$_{II}$)

X87 Lp^S Gal$^-$

↓

W518 M Lac, Gal$^+$ Lp^S V$_1^R$

W'1578 Lp^S F$^-$ M Lac, Gal$^+$ V$_1^R$

↓

W1321 Lp$^+$ F$^-$ M Lac, Gal$^+$ S^R V$_1^R$

↓

W'1607 F$^-$ Lp$^+$ M Lac, Gal$^+$ S^R V$_1^R$

↓

W'2594 F$^-$ Lp^S M Lac, S V$_1$

Untreated control
Lac+ colonies

ATO-Treated Lac+ colonies

on Blac

on MGal
+ 4573.

on M Gal
+ 4573

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Elimination of Lac-F_{13} segment with AO-treatment. (continued.)

22/VII; 1959.

REF: Cf 1274, 115, 117

1	2	3	4	5	6	7	8	9	10

strain treated with AO: W2598 F_{13} ($M S^r V_1^r$ Lac$^{+}/$Lac^{-} V_6^r …)

Continued.

Purpose : Test sex-compatibility : Does AO remove F_{13} with Lac ?

Principle : Pick Lac$^+$ and Lac$^-$ colonies from the plates obtained after seeding treated and untreated cultures with AO.

Method : Replicate the streaked clones on MGal seeded 0.2 ml broth culture of W4573 (Gal_2^0 Lac_{85} Ara_2 Mal, Xyl_2 MtI S^r F^-)

Ref. { This Lac$^-$ is ~~stable~~ unstable. It gives several papillae per one streak. on DLac agar.

Result :

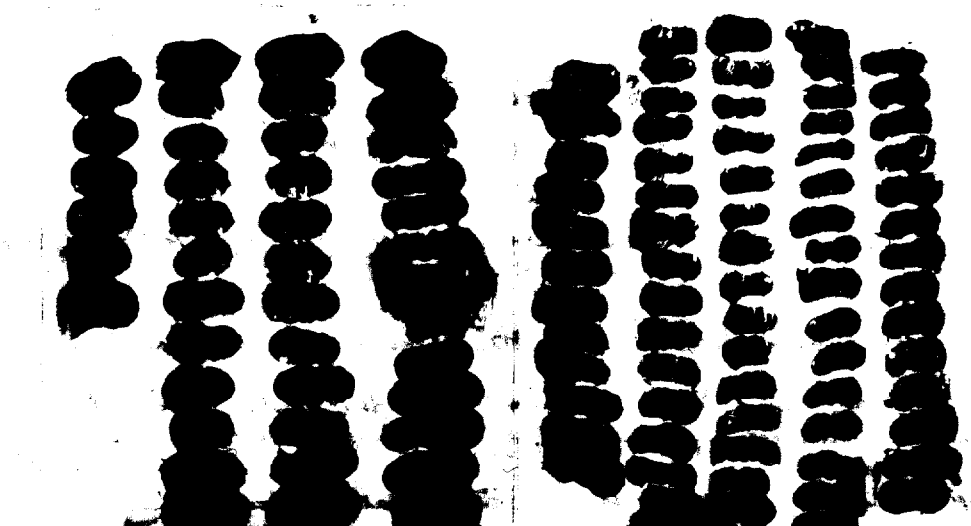
	Isolated from AO treated culture								Isolated from untreated control.							
	Lac$^+$				Lac$^-$				Lac$^+$				Lac$^-$			
	F^+		F^-		F^+		F^-		F^+		F^-		F^+		F^-	
Gal	Hi	Lo		Σ	Gal Hi	Lo		Σ	Gal Hi	Lo		Σ	Gal Hi	Lo		Σ
	148	2	0	149	13	9	116 (116)	138	146	0	0	146	104	12	0	116
%	98.6	1.3	(0)		9.43	6.52	(84.1)		100	0	(0)		89.8	10.3	(0)	

Conclusion : ① Acridine acts on F_{13} as well as Lac. (84.1 % of Lac$^-$ is F^-)

② No F^- is observed in AO treated Lac$^+$. (This may contains Lac$^+/^-$ and Lac$^+$)

③ In control experiment, no F^- was found. (0%) in both Lac$^+$ and Lac$^-$ ~~segregants~~ colonies.

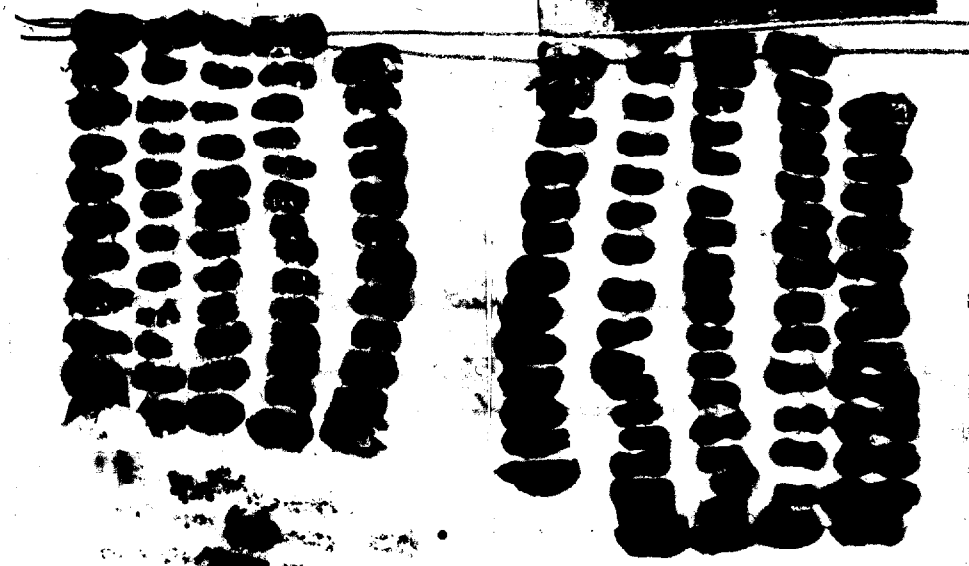
a untreated control Lac⁻ each streaks has papillae. ∴ lac, is unstable.



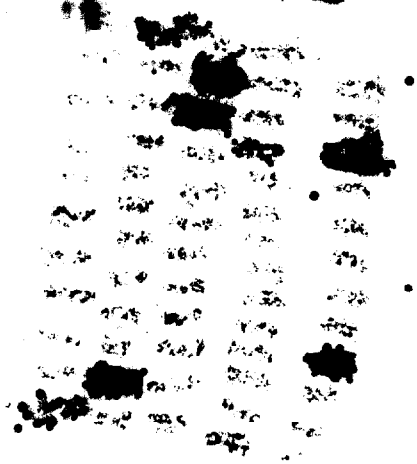
on Blac.

on MGal x4573

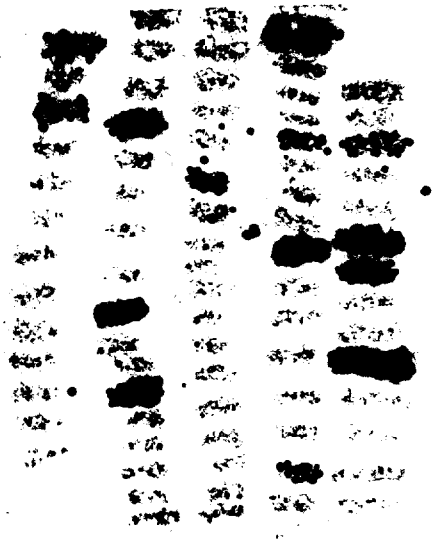
AO-treated, lac⁻
each streaks has papillae.



on Blac



on MGal x 4573



on MGal x4173

Rate of multiplication of F_{13} Lac segment.

(Infection of Lac-F_{13} segment to F^-)

W4573 F^- Gal$_c$ Lac$_{85}$ Mal, Xyl$_c$ MtlA Str$_c$ S^R.
W3747 M^{R5FR}_{6} F_{13}

18/III ; 1959

Purpose ; Is Lac-F_{13} segment multiplies more than cell? host

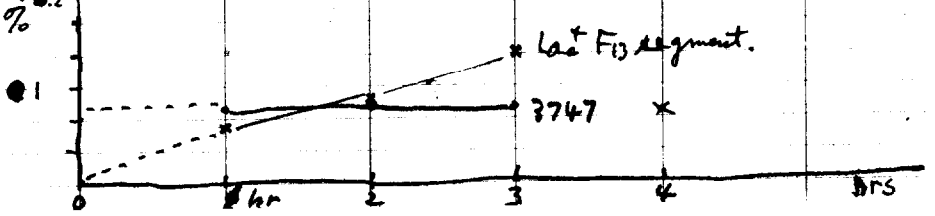
1. Make overnight culture of W4573 and W3747.

2. Make fresh culture of these strain by inoculating the 0.2 ml of the culture into 10 ml penassay broth and shaking on rotator at 37°C.
   P.M. 9:10 ~ 1:00. P.M.

		1:00	2:00	3:00	4:00
	Time	0 hr 10^{-5} ml	1 hr 10^{-6} ml	2 hr 10^{-7} ml	3 hr 10^{-7} ml.
BLac		16/1011	0/125	2/164	~~3/164~~
		8/890	2/151	5/151	~~5/151~~
		15/964	1/123	2/129	~~3/129~~
multiplication of Lac marker :	Σ	39/2865	3/399	9/444	
original Lac$^+$	Lac$^+$+Lac$^-$	2909	402	453	
	%	1.34	0.746	1.99	%
		Ara$^+$/Ara$^-$			
BAra		8/1011	0/125	0/164	
		6/890	0/151	3/151	
		8/964	0/123	0/129	
multiplication of Ara. cell itself.	Σ	22/2865	0/399	3/444	
	Ara$^+$+Ara$^-$	2887	399	447	
	%	0.762	0.	0.671	%
		Xyl$^+$/Xyl$^-$			
BXyl		8/1011(8)	0/125(0)	0/164 (2)	
		6/890(2)	0/151(2)	3/151 (2)	
		8/964(7)	0/123(1)	0/129 (2)	
			17/2865	3/399	6/444
Difference multiplication of Lac$^+$-segment.	Multiplication of Lac$^+$ (Lac$^+$-Ara$^+$)		2882	402	450
	%		0.592	0.746	1.33. %

Result : After 3 hr incubation, F_{13} becomes approximately double.

Further experiment : Use 1/10 of mixture of F_{13} and F^-.



Transfer of F_{13} to W3127, W3112,

5/VA ; 1959 REF:

Purpose : For cistron-analysis.

Method :

1. Spot W3747 on W3127, W3112, and W3230. on MLac.
2. Purify them on BLac and incubate it overnight at 37°C.
3. Look for Lac_v and purify Lac_v on BLac.
4. Pick Lac^- derived from Lac_v, and test the compatibility, streak them on BLac. Replicate it on BLac and make copy.
5. Replica plate on MLac seeded various Lac indicator strains on it.

Indicator strain. Lac_{1a} (11) W3230 ⟷ W3112 : Lac_2

" ⟷ W3127 : Lac_4.

Isolation of F_{13}^+ W3127: Result :

1. All Lac^- colonies which isolated from sectored colonies, purified on BLac are very unstable and gives many papillae on the streaked colonies. After replicating these streaks, they gives various kinds of fertility according to Lac^- mutations. This unstability is still not
Under that Lac^+ appeared by segregation or reversion of Lac^-.

Then, the streaked are purified on BLac again, and picked Lac^- from the and tested the fertility by cross breeding (it gives almost Lac^+!) on MLac with W3230. However, they show very unstable Lac^- character on MLac again. (see back page).

But, 2 of them showed fertile cross times W3230! (Lac_{1a}) on MLac

② Test V_6-resistance. (See back page).
Keep it.

W3112: Result.

① Test 174 of Lac^- segregated from Lac^+ diploids. (picked 2 Lac^- from each Lac_v colonies) by replicating on MLac seeded W3230 (Lac_{1a})

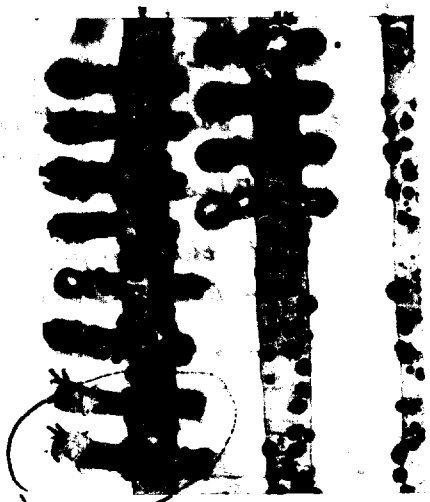
Σ	HI Lac	Lo Lac	F⁻ (probably, because Lac_2 is unstable)
174	88	80	6. (but better than 3127)

② one of the isolated clone shows HI fertility, and shows mixed clone in resistance to T_6. (see p.126)

Result: ③ Test fertility, all Lac^- are tested in their compatibility

marker transferred	Gal	Lac_{85}	Lac_{88}
HI	72	0	58 (80.6 %)
Lo	0	0	14 (19.4 %)
on	MGal (Gal-transfer)	MLac	MLac Lac transfer

Lac_{85} include or accompanied with Lac_2 (W3112)



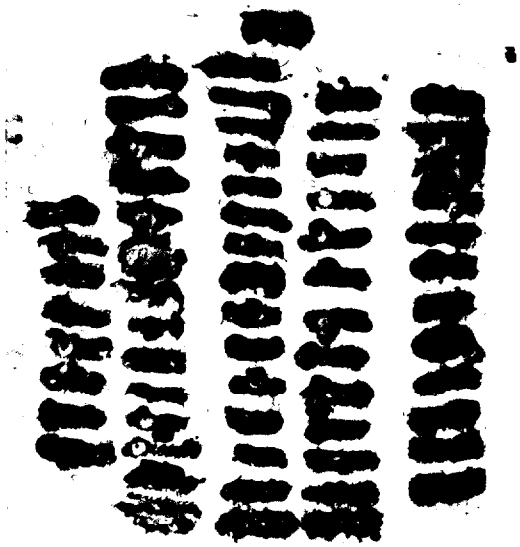
× 3230 on Hlac

Segregants from hac 2 F13/+ F-

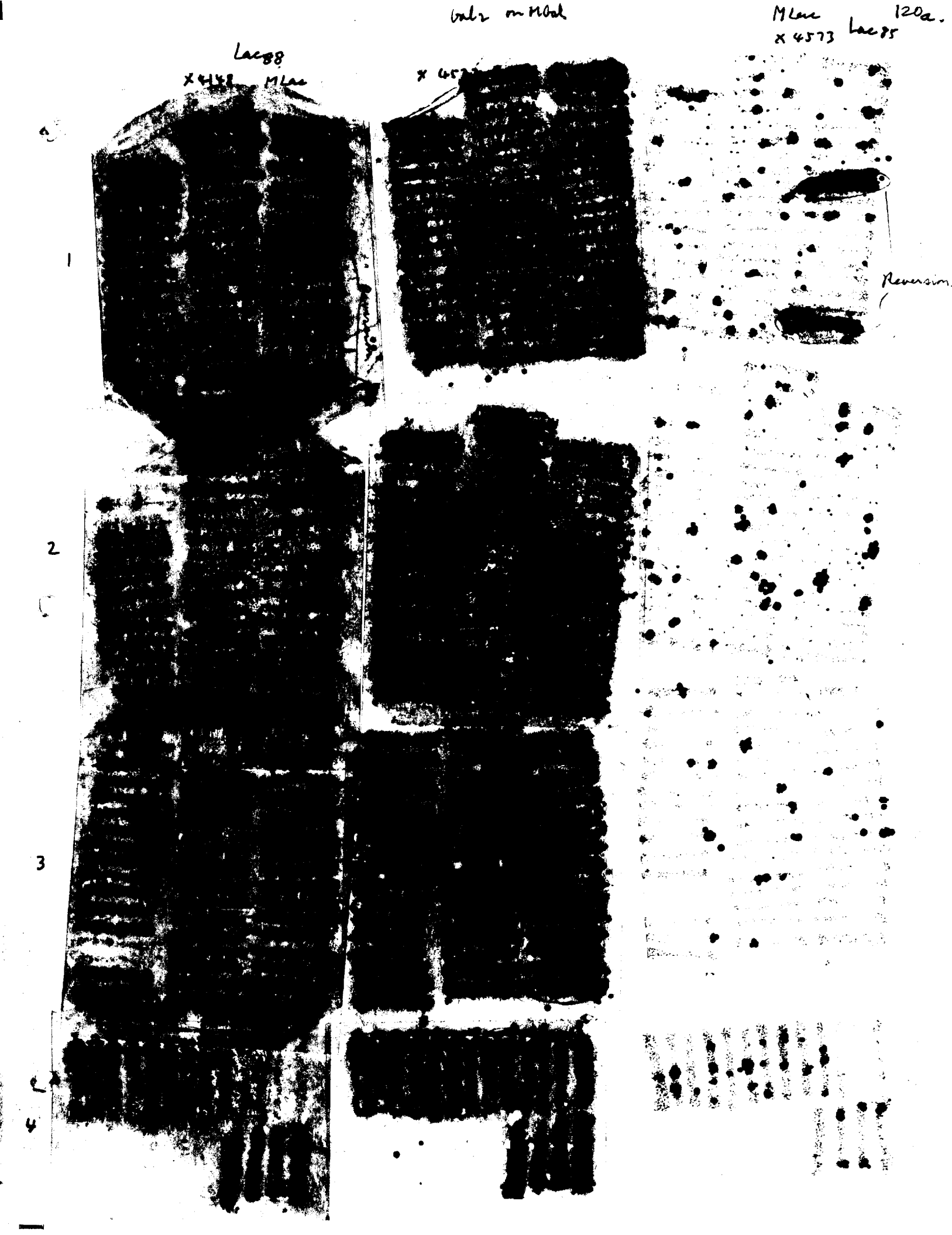
W3112 F13

on Hlac

× recded W 3230



on Blac


Lac88
MLac
Lac88
MLac
Lac85
120a.
Reversion
1
2
3
4

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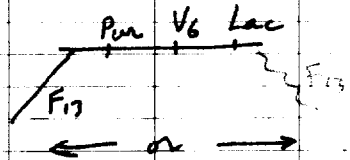
Timing experiment of transduction of Lac F_{13} segment. I

20/VII ; 1959 REF:

Strain : ♀ : AC: 93:85 : Pur⁻ V_6^R S^R F^- Lac 85

♂ : W3747 H- V_6^R F_{13}.

Purpose : Test the direction of Lac-F_{13} transfer from F_{13}^+ to F^-



Experimental design:

- Use 2 hrs old culture of both strains (ca 10^8 cells/ml) overnight culture 0.5 ml / 10 ml pen. incubate it (2 hrs on water bath). AM 9:00 ~ AM 11:00.
- Mix 10 ♂ : 1 ♀.

Result :

- Inoculum size (Recombination plate : 10^{-5} ml /plate.
  (Survival counting plate : 10^{-7} ml./plate

Media \ Time	Marker selected	0	2.5 min	5 min	7.5 min	10 min	15 min	20 min
# of Recombinants. MLac + Sm	Pur, Lac	0	0	0	0	0	0	0
		0	0	0	0	0	0	0
MLac + Pur + Sm (Adenin)	Lac	0	0	0	0	0	0	Lac⁺ → 2
		0	0	0	0	0	0	0
MO + Sm.	Pur.	0	0	0	0	0	0	0
		0	0	0	0	0	0	0
EMB-Lac (10^{-7} ml)		− + 0/13	− + 0/11	− + 1/15	− + 1/19	− + 2/11	− + 2/32	− + 1/23
		0/12	4/12	3/13	1/18	0/16	5/26	1/20

blank (10^{-6} ml /plate) $\bar{1}7 / \overset{+}{1}10$; $\bar{2}6 / \overset{+}{1}37$; $\bar{1}7 / \overset{+}{1}41$:

① # of cells used { ♂ initial : 1×10^8 cells; final : 2×10^8 cells
: ♀ : ca 1×10^7 cells

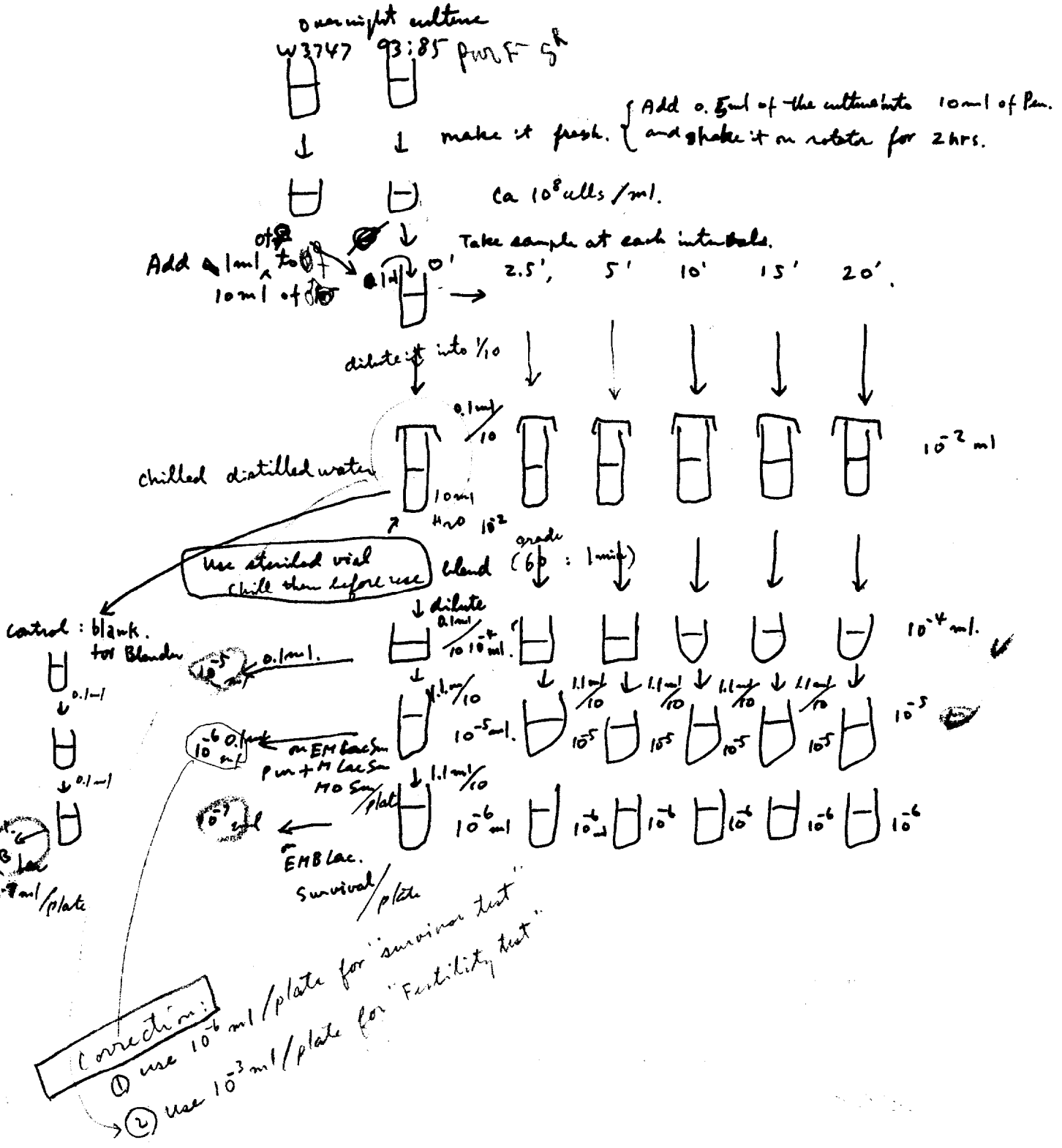
② Rate of Recombination :

Rate of Recombination in this experiment :

Rects / ♀ ca. $\frac{1 \times 10^2 \text{ ♀ cell}}{\text{after 20 mins}}$ (Lac⁺)

1. Test F_{13} by Gal-transfer
2. Test Pur⁻ → both strain is purine⁻.

Experimental method



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Isolation of 3133 F_3^- with AO method.

122

20/VII ; 1959

REF:

AO : 30γ/ml : NSO conc. standard for AO method pH 7.6.
Inoculum size ca 10^5 cells/ml. overnight treatment.

3133 F_3^+ [redacted]

3133 F_3^-

3133 F_{10}^+ [redacted]

3133 F_3^- [redacted]

×40-

on plate

Result.

AO-treated		Untreated control.	
−	+	−	+
1	210	0	359.

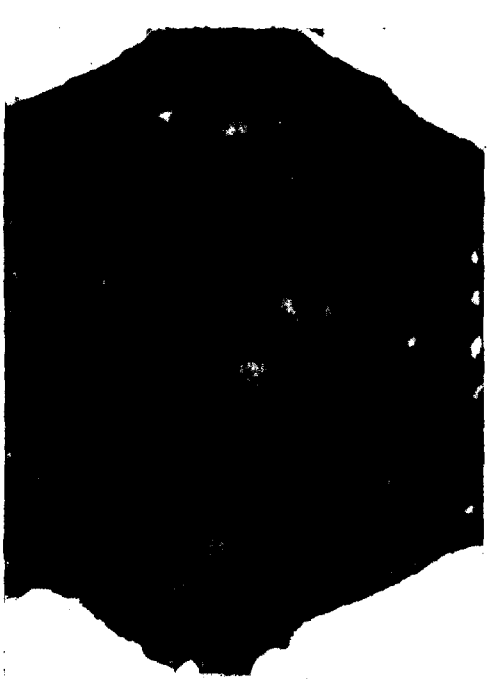
See back page

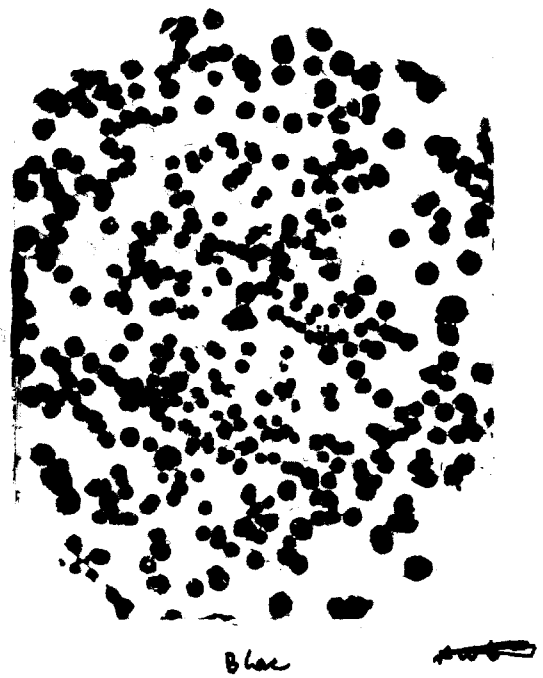
Further work

① Save this.

② Test F_3 type F^- or plain F^-.

untreated control

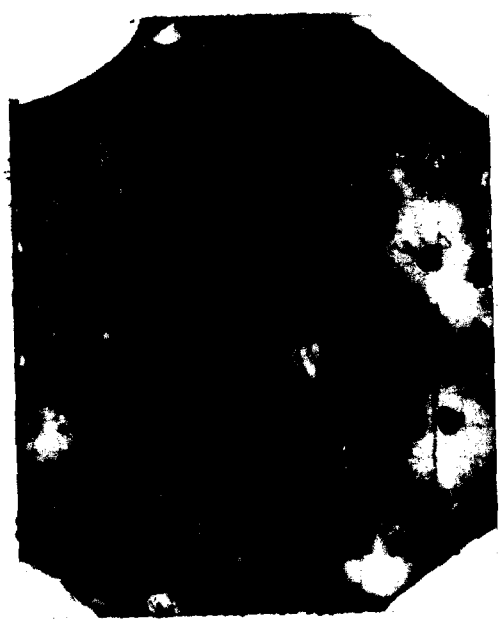


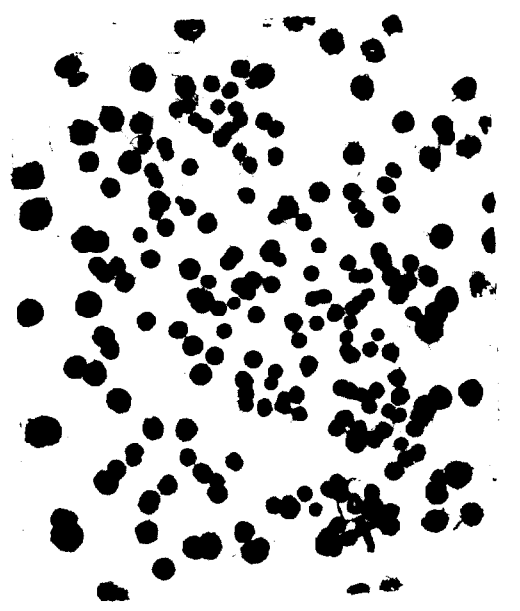


Mlac x W6

Blac

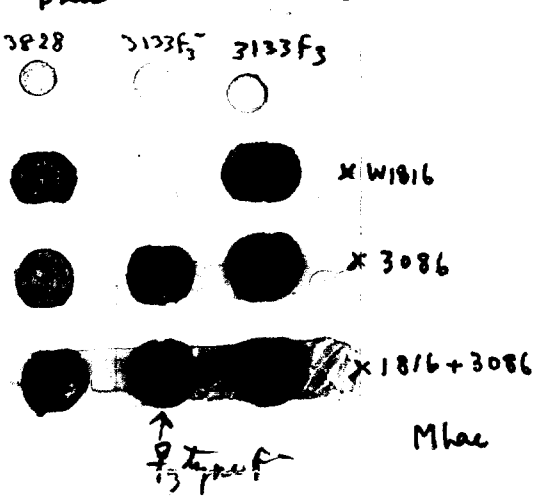
Treated with AO





Mlac x W6

Blac



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Infection of Lac- F_{13} segment. (II).

21/VII 1959 REF:

Principle[2]: W3747[3] —F_{13} Lac+[4]→ W4573[5]

Strain: Log-phase 3747 + 4573 { 1:00 PM. → 3:00 PM. 0.2 ml / 10 ml pbs. Made fresh on rotator

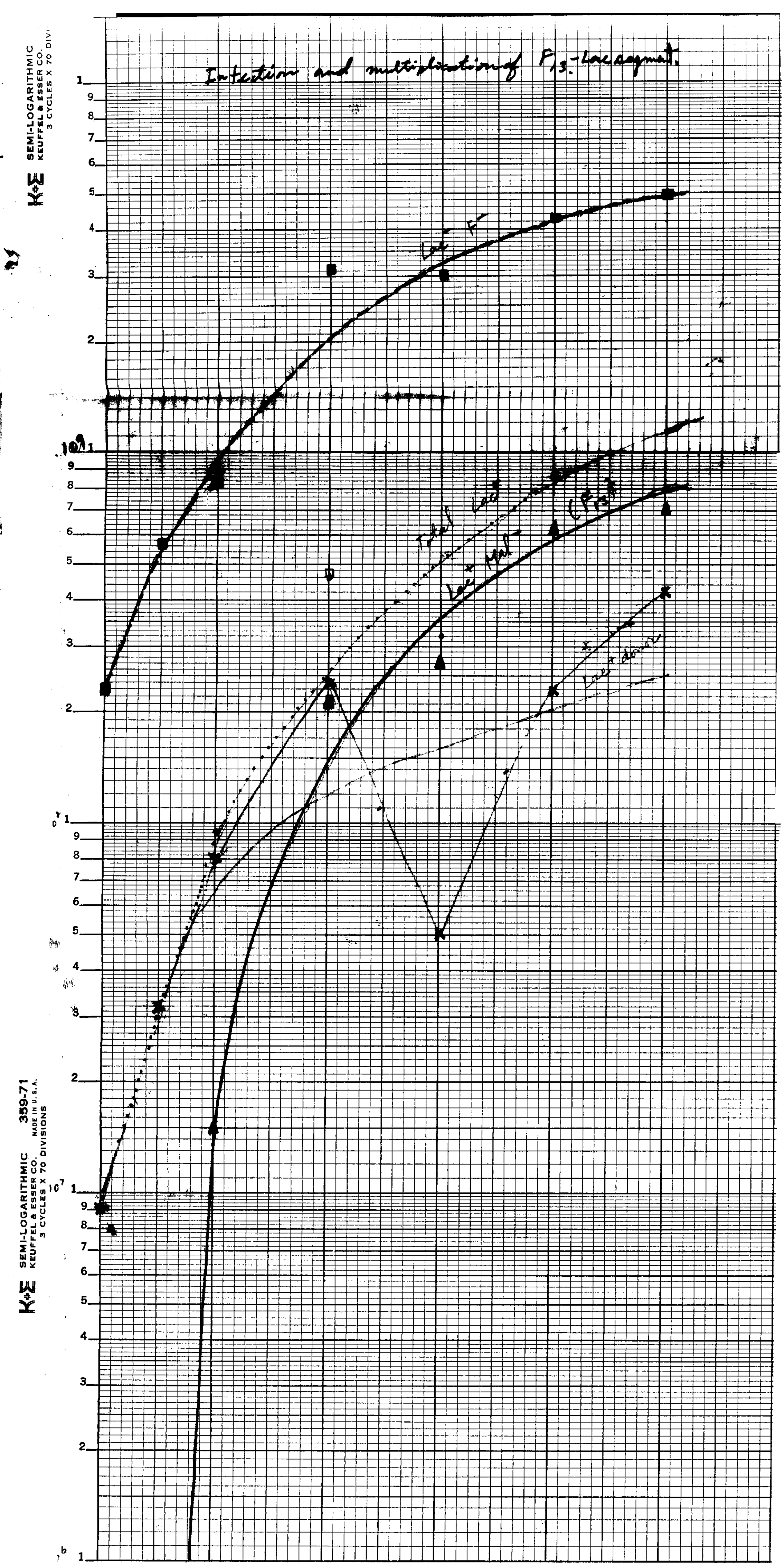
① Mix: Ratio 1.1 ml + 10 ml Ca 10^8 cells/ml.

② Shake on rotator. Take samples at each time from the mixed culture.

③ Dilute it with H_2O into 10^{-6} to ~10^{-7}. Seed on B Lac. Incubate them for overnight at 37°C. Replica plate them on BXyl, BMtl, BAra, and MGal & seeded 93:85 on it.

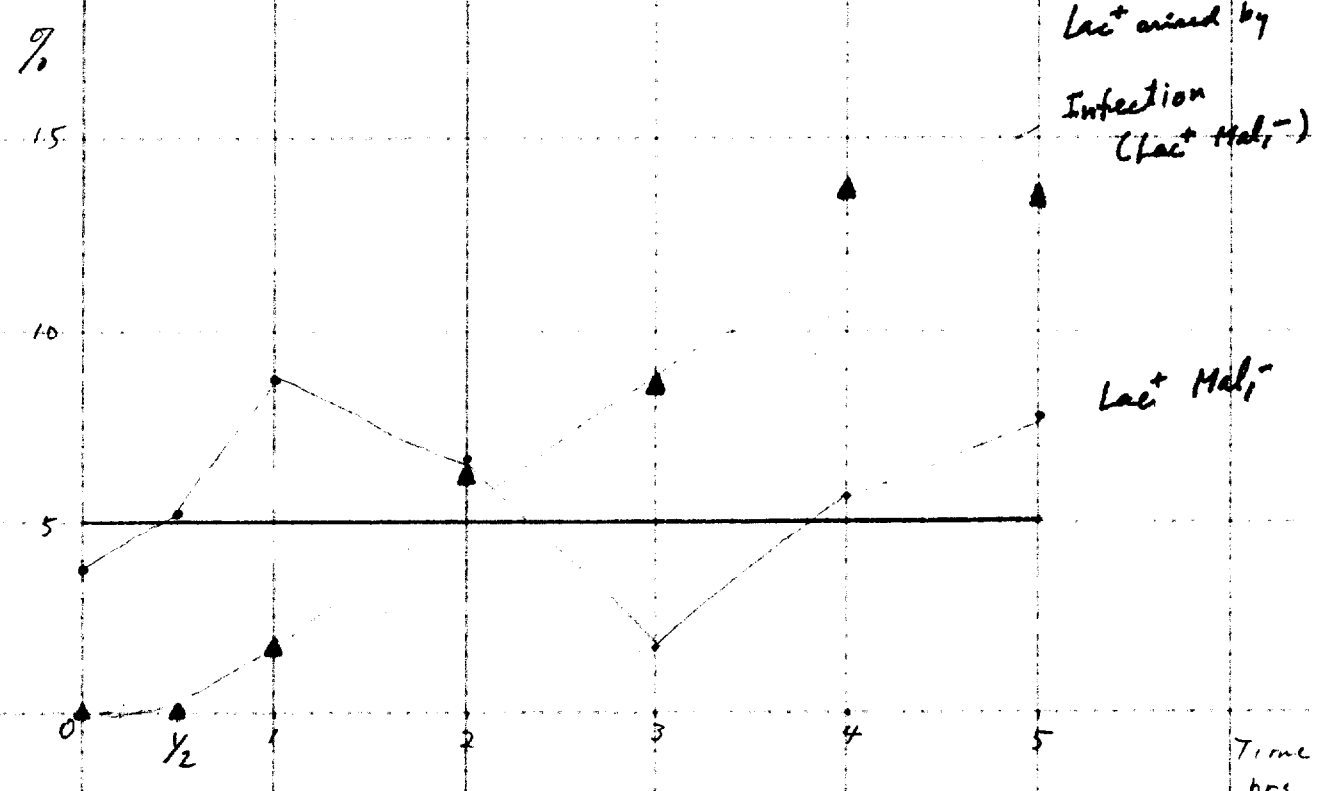
	1	2	3	4	5	6	7	8	9	10
Time		3:05	3:35	4:05	5:05	6:05	7:05	8:05		
Inoculum size		10^{-6} ml/plate	10^{-6} ml	2×10^{-7}	10^{-7}	10^{-7}	10^{-7}	10^{-7}		
Time		0 hr	1/2 hr	1 hr	2 hr	3 hr	4 hr	5 hr		(hrs.)
on B Lac		+ / −	+ / −	+ / −	+ / −	+ / −	+ / −	+ / −		
	1	1/39	5/111	0 3/38	3 13/68	1 3/68	14 20/61	12 24/95		
	2	2/38	6/92	1 4/48	7 9/68	6 7/58	11 17/100	15 21/93		
	3	2/45	3/117	1 7/38	5 8/71	8 9/77	8 14/113	12 19/101		
	4	1/49	10/119	1 5/42	4 8/66	3 5/58	14 17/75	23 30/96		
	5	3/59	8/126	[illegible]	4 8/63	7 8/40	15 18/83	11 17/102		
				+5 95 [illegible]	+10					
	Σ	9/230	32/565	19/166	46/336	32/371	86/432	111/487		
		(239)	(597)	(185)	(382)	(333)	(518)	(598)		
	%	3.77	5.36	10.3	12.0	9.65	16.6	18.5		
on BMal		△	△	△	△	△	△	△		
	1	0 1/39	0 5/111	0, 3/38	3 10/68	3 0/68	15 5/61	12 12/95		
	2	0 2/38	0 6/92	1, 3/47	7 2/68	6 1/58	11 6/100	13 8/93		
	3	0 3/45	0 3/117	1, 6/38	5 3/71	8 1/77	8 6/113	11 8/101		
	4	0 1/49	0 10/119	1, 4/42	4 4/66	3 2/58	14 3/75	23 7/96		
	5	0 3/59	0 8/126		3 5/63	7 1/40	15 3/83	11 6/102		
Δ Infection of Fi Lac		△	△	+5 80 15	△	△	△	△		
		0, 9/230	0, 32/565	3, 16/166	22, 24/336	27, 5/308	63, 23/432	70, 41/482		
		(239)	(597)	(182)	(360)	(308)	(455)	(523)		
	%	0, 3.77	0, 5.37	1.65, 8.80	6.12, 6.67	8.82 1.66 ?	13.8 5.06	13.4 7.83		

Conclusion: Lac-segment multiply about 2 times more than host cell after 5 hrs. at 37°c on rotator.


Infection and multiplication of F13-Lac segment
Lac F⁻
Total Lac
Lac+ Mal⁻ (F13+)
Lac+ donor
SEMI-LOGARITHMIC
KEUFFEL & ESSER CO.
3 CYCLES X 70 DIVISIONS
359-71
MADE IN U.S.A.

Rate of Multiplication of Lac- F_{13} segment.

3747 ⟶ 4573
M V_6 F_{13} Gal$_2$ Lac$_{g5}$ Ara$_2$ X$_{gl2}$ Mtl Mal$_1$ Su.



Multiplication of Lac-F_{13} segment. (continued)

23/VII 1959 REF:

Make cultures fresh : 1. Inoculate 0.2 ml of the overnight broth culture of W3747 and W4573. into 10 ml of Penassay broth. and incubate it for 2hr on rotator at 37°C.

Dilute 2. Dilute W3747 into 10^{-6} : Use 10^{-7} ml of W3747 for F_{13}-Lac donor. count number of cells used.

Mix 3. Mix 1 ml of W4573 and 10^{-6} ml of W3747, and 10 ml penassay broth. (ca 10^3 cells + ca 10^2 cells + 10 ml per)

4. ~~Shake on rotator~~ Incubate it at 37°C for overnight.

Result :

I] Initial ratio

Survival count :

	# of colonies in 10^{-6} ml / plate	Inoculum size :	Ratio (Initial ratio) of Lac^+ and Lac^-
W3747	73	8 cells /ml	ca 10^{-7}
	85		
	84		
W4573	115	1×10^7 /ml.	
	88		
	107		

5. Inoculate 0.1 ml of the mixed culture into 10 ml of Penassay broth.
count the Lac^+ and Lac^-.

6. Incubate it overnight at 37°C.

II] After 24hrs incubation : Seed 10^{-7} ml / plate. (EMB Lac agar).

plate #	Lac^+	/ Lac^-
1	0	485
2	0	504
3	0	496
4	0	485
5	0	471

III] After 48 hrs incubation.

Inoculate 0.1 ml of the mixed culture into 10 ml Penassay-broth, and incubate it overnight at 37°C.

Plate #	Lac^-	/ Lac^-
1	1	95
2	2	106
3	0	101
4	1	137
5	0	106

The segregants are tested for these 4 colonies.

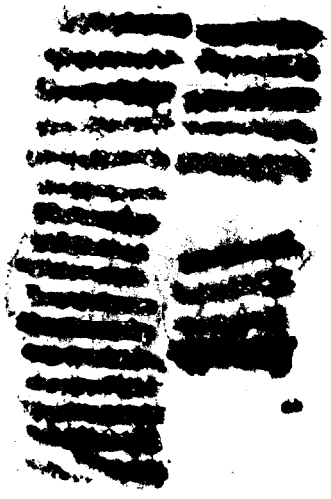
2 Mal_3^+ ? 2 Xyl^+

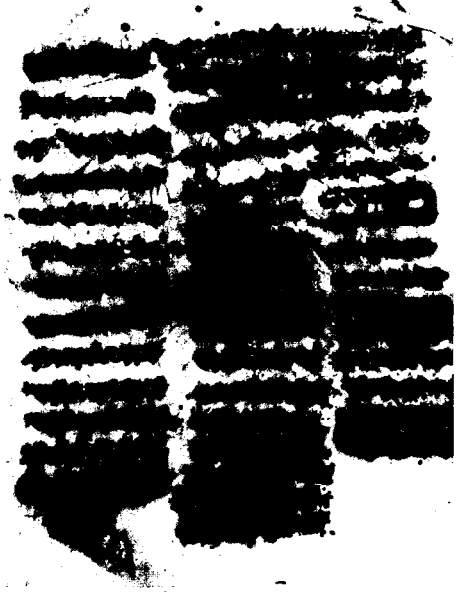
2 Mal_1^- 2 Xyl^-

Selective marker : Leu - Meth.

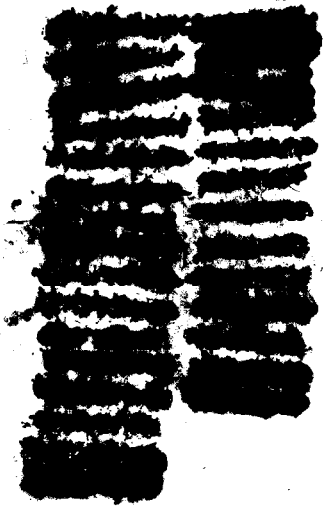
10'

15'

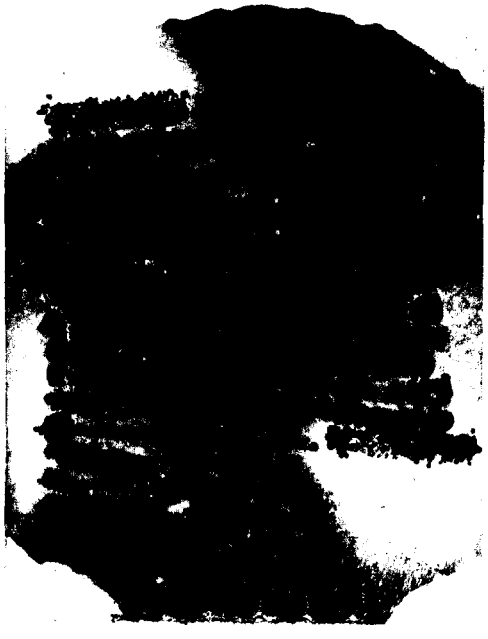




15'



20'



20'

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1205a

Time experiment of Lac – F13 transfer.

25/VII ; 1959 REF: 125b.

1 2 3 4 5 6 7 8 9 10

○ F_{13} ♂ : 3747 ; ♀ 93:85 ; Cultural age : 2hrs aerotator at 37°c

0.5 ml overnight culture Penassay grown / 10 ml Penassay.

: Ratio ♀ 1 ml : ♂ 9 ml.

○ Method : exactly same as former experiment except inoculum size of the mixed culture onto selective medium. (See P.121.)

○ blending ; Gage : 7 ; 1 min. Temp. for mating : 35°C.

Result :

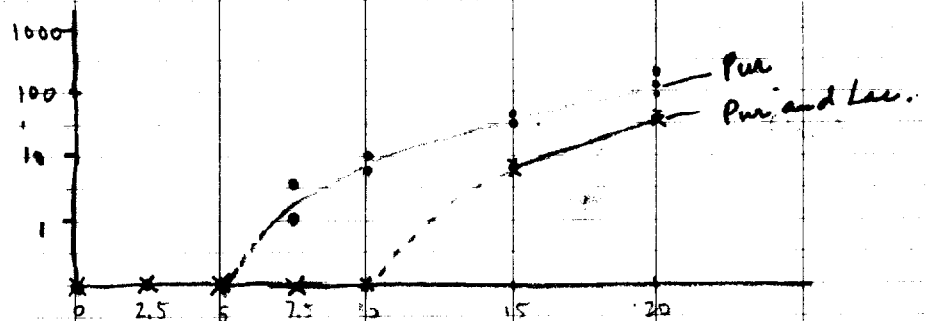
Media \ Time	Marker selected	Time after interruption (min.): 0	2.5	5	7.5	10	15	20
M Lac Sm (10^{-3} ml)	Pur Lac	0	0	0	0	0	8	67
		0	0	0	0	0	7	75
M Lac -Pur- Sm Adenine B_1 (10^{-3} ml)	Lac	0	0	0	0	0	8	93
		0	0	0	0	1	5	41
M Glct Sm. (10^{-3} ml)	Pur.	0	0	0	1	5	43	154
		0	0	0	1	9	40	182
EMB-Lac. ♀ (10^{-6} ml)		149	163	[illegible]124	152	125	127	196
		141	149	[illegible]130	113	121	187	180
Blank (before blending) (10^{-6} ml)		143						
		127						
		121						

# of Recombinants per plate.

① # of cells used ♂ : Ca. [illegible]1.5×10^8
♀ : Ca. 1×10^7

② Frequency of Pur^+ transfer within 20 min. ca. 10^{-2} ♂ and ♀
1 %.

Conclusion : ① Purine looks first, and Lac is next.
② Test [illegible]Lac, Pur^-, and [illegible]B. of these recombinants.
There is no marker to test F_{13} cells.



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markers

~~125~~ 126

Test ~~Pur~~, Lac, ~~Pur~~, F_{13} of the recombinants obtained from timing experiment in cross W3747 × 93:85.

30/VII ,1959 REF:

Purpose : Reconfirm which end is earlier ~~from the markers of these~~ recombinants (cf. p.125).

Method : (1.) Streak them on M-0 + Sm. and incubate it overnight at 37°C.

(2.) Replica plate it on BLac V_6, ~~+Pur~~, DO, MGal seeded with W4573.

Result

1. All colonies grown on M Lac Sm are Lac^+

Time (min)	15'	60'
# of colonies Tested	14	125

2. Recombinants grown on M gal Sm are Lac^+ ~~or~~ Lac^-.

Time (min)	7.5	10	15	20'
Lac^+	0	0	16, 6) 22	49, 34) 83
Lac^-	2	21	29, 23) 52	26, 11) 37

Conclusion from 1. 2. Pur is first, Lac is second.

Pur^- Lac^- Method : (3) Replica plate on M Lac seeded Pur^- F^- on it. (Test purine transfer) W4506.

Pur^+ Lac^+ Principle : If it is F_{13}^+ the Lac^- strain can transfers pur^+ to F^-, but if it is F^-, they can't.

Which side of the fragment is it attaching

Result :

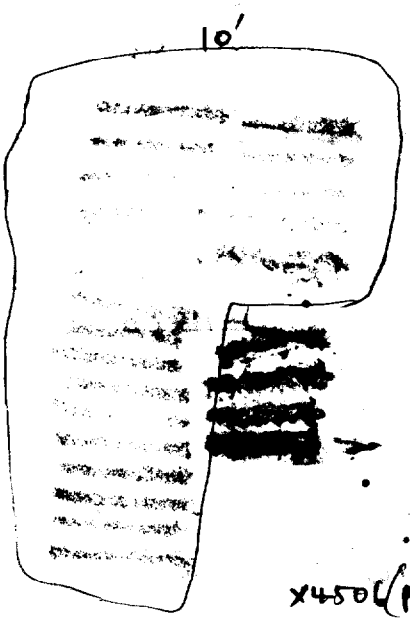
1. purine is first : Pur – Lac – F_{13}
2. F_{13} is latter than Lac.

Conclusion :

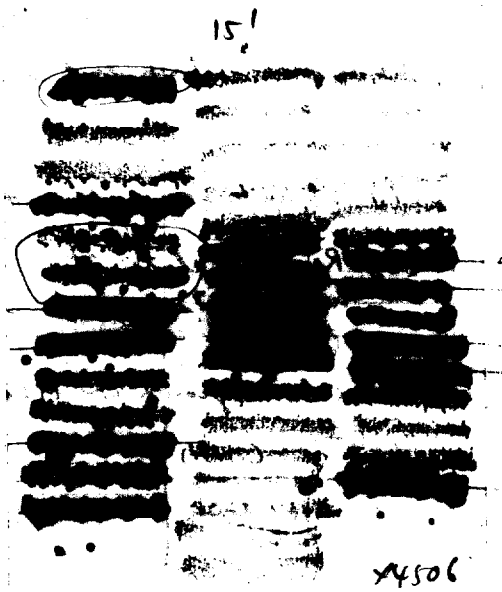
– –

Pur^+ Lac^+ F_{13}

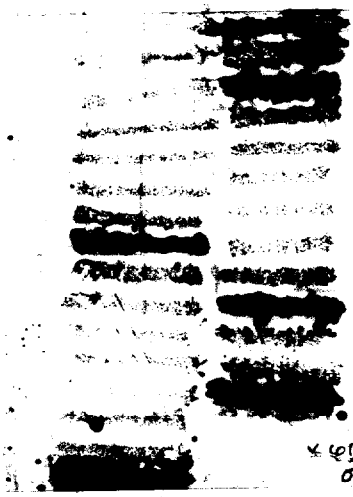
Selected on Lac - Pur transfer



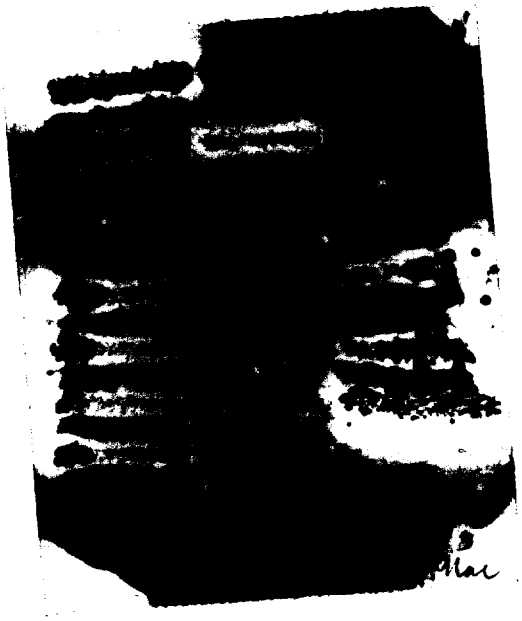
X4506 (pur F⁻)
on M Lac



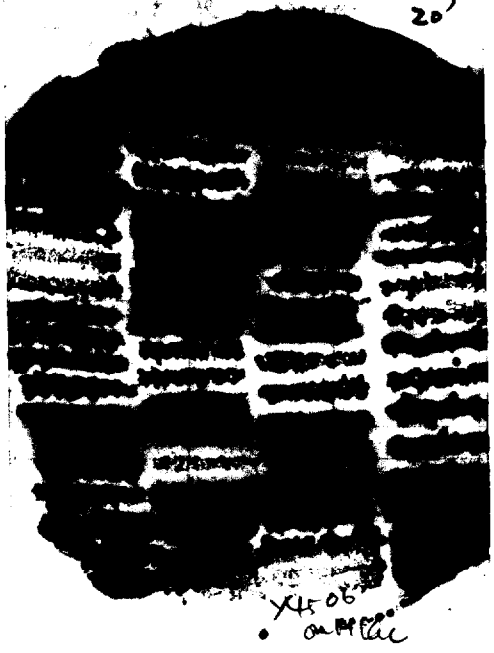
X4506
on M Lac



X4506
on M Lac



M Lac



X4506
on M Lac

Cistron analysis of Lac loci using Lac$_z$-F_{13}.

29/VII 1959 REF: W3112F_{13}.

~~Photo +~~
~~Med. 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(13) $\underline{F^+}$ Lac z $\overline{X^+}$ ——→ cure.

Result:

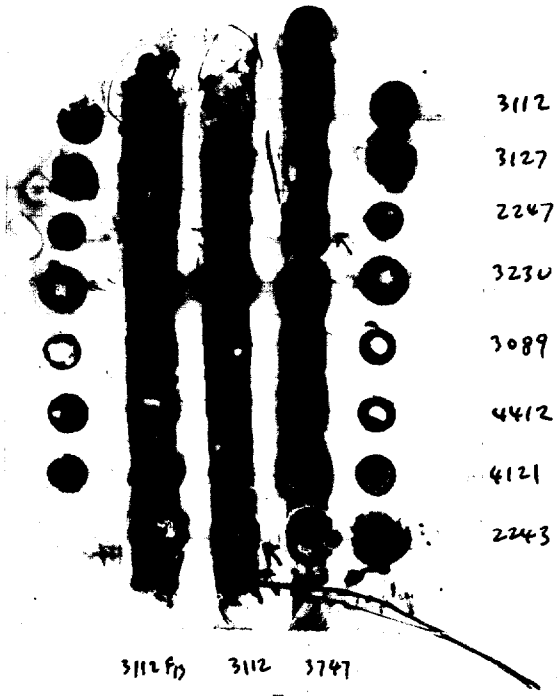
Lac⁻		× 3112 F_{13} (Lac$_z^-$) nr	× 3112 (Lac$_z^-$)	× 3747 (Lac$^+$ M-F_{13})	blank		Lac Hfr.
ONPG	3112 2	(ONPG -)	−	###	−	✓	Lac$_z$ 3164 nr.
unstable X	3127 4	###	+	###	++ unstable		
	2247 7 (permease and β-galactosidase)	●	−	(— ?) check	−		non-transducible Lac.
+ m unstable X	3230 11a	++	+	###	● many unstable	Recombinant …	
+ m	3089 11b	+ ? (permease)	−	++ ?	●	Reaction is very slow.	
+ m	4112 52	###	−	###	+,		
− f	4121 61	###	−	###	−	✓	Lac$_y$ 4021
− m	4147 87	###	−	###	−		
− m	4148 88	##	−	###	−		
− m	4149 89	##	−	##	−		
− m	4150 90	##	−	##	−		
− m	4151 91	##	−	##	−		
− m	4153 93	##	−	##	−		
	2243 Lac$_z$	##	(###) syntrophy	###	++ unstable		
	2245 15	##	###	###	### "		
	2247 Lac$_y$ also Gal-	−	−	(−)	● +,		

↑ Test segregants.

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

non-transducible Lac
λ_2^s
non-transducible Gal

V6 ϕ_3



	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⁻

Segregation between two different Lac⁻.

4147	
4148	
4149	
4150	
4153	
2295	
2247	7

3112 F⁻ 3112 3747

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

28/[illegible] 1959

REF:

AO treated Lac⁺

No.	F	Lac stability	T_6
1		~~[illegible]~~ 25% lac⁻	
2		U	m (control 2598 lac⁺/lac⁻)
3		U	m
4		U	m
5		U	m
6	F⁻	S S	s
7		U	m
8		U	m
9		U	m
10	F⁻	S 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	s
26		S S	r
27		U	m
28		U	m
29	F⁻	S S	s
30		S S	r
31		U	m
32		U	m
33	F_{13}	S S	r
34		U	r
35	F_{13}	S 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	s
46	F⁻	S S	s
47	F⁻	S S	s
48		U	m
49		S	s
50		S S	s
51		U	m
52		U	m
~~53~~		~~U~~	~~m~~
54	F⁻	U	m
55		S S	r
56		U	m
57		S	m
58		S S	s
59		S 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	s
70		U	m
71	F⁻	S S	r

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

T_6-resistance:

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

Untreated Lac

No.	Lac	T_6
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~~	~~S~~	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
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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 S	r
67	U	m
68	U	m

Lac⁺ (stable or unstable): S : [illegible]; U : 63

T_6-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

Notes

If 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.

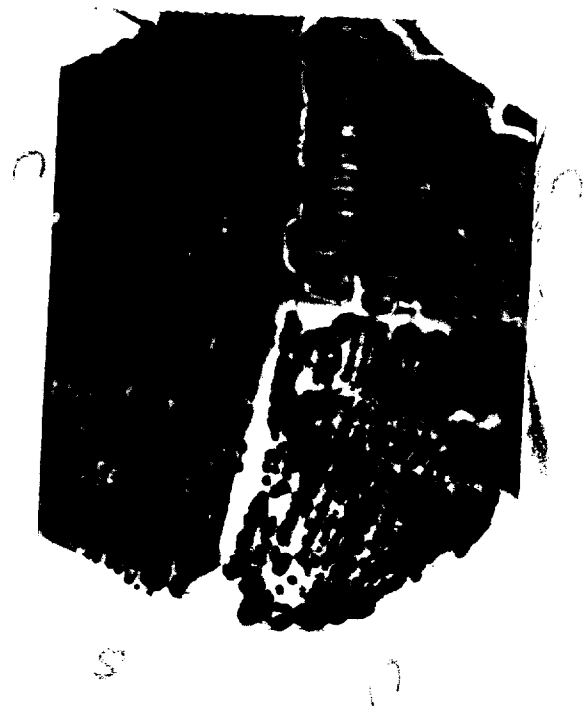
1. AO-treatment: AO 30 γ/ml.
- NSB - pH 7.6.
- ~~Inoculate~~ one small loop of ca 10⁷ cells/ml / 5 ml NSB AO.
- The treated culture was streaked on Blac after 24 hr incubation at 37°C.

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 (treated with AO)
1. All of the F⁻ 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. Lacst is T_6^R or T_6^S, not $T_6^{r/s}$.

This means, AO removes full segment of F Lac U at a time.

U : Unstable
S : stable



Elimination of Lac-F_{13} segment with AO-treated F_{13}

23/IV ; 1959

REF: Cf. P 118

Experimental conditions.

Inoculum size : ca. 10^2 cells/ml. AO : 30γ/ml. Med: NSB for AO-T.
Time : overnight at 37°C. Strain treated w 25% F_{13} ($M\ S^R\ V_1^R\ Lac_1^+/_0\ V_6^R/_S$)

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 $AO_{30\gamma}$ 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.

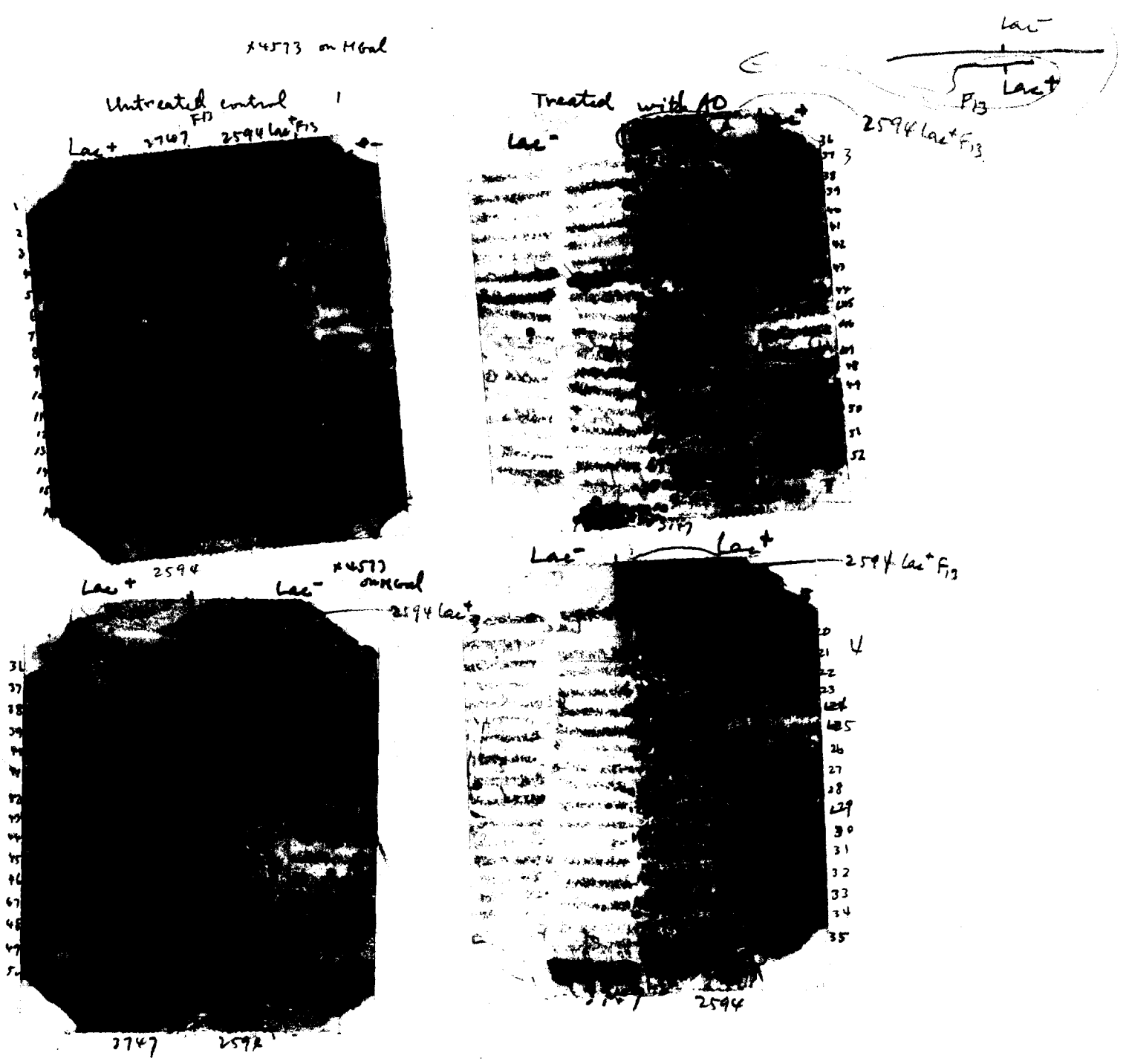
	AO-treated (30γ/ml)						Untreated control			
	I				II		C			
Plate #	$Lac^{+/-}$	Lac^+ Lacv	Σ	(%)	$Lac^{+/-}$	Lac^+ Lacv	Σ	(%)	$Lac^{+/-}$	Lac^+ Lacv	Σ	(%)
1	310	131	4		439	42	2		103	311	2	
2	275	100	2		383	34	3		86	246	2	
3	284	120	3		398	35	2		109	298	3	
4	274	95	2		453	43	2		119	297	3	
5	283	138	2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

Results II. Elimination of F_{13} from Lac^+/Lac^- heterozygotes.

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

(Tested by bal-transfer. × 4573)

(see back page)



4121 (Lac61 F-)

3112 F_{13} [redacted]

3112 F-

on MLac

24 hrs.

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MICROBIAL
GENETICS

128a

Multiplication of Gal- F_8 segment.

W4520
W6F8 × W4573.

30/4 1959 REF: p. 127

1 2 3 4 5 6 7 8 9 10

overnight culture
0.2 ml / 10 ml plm.

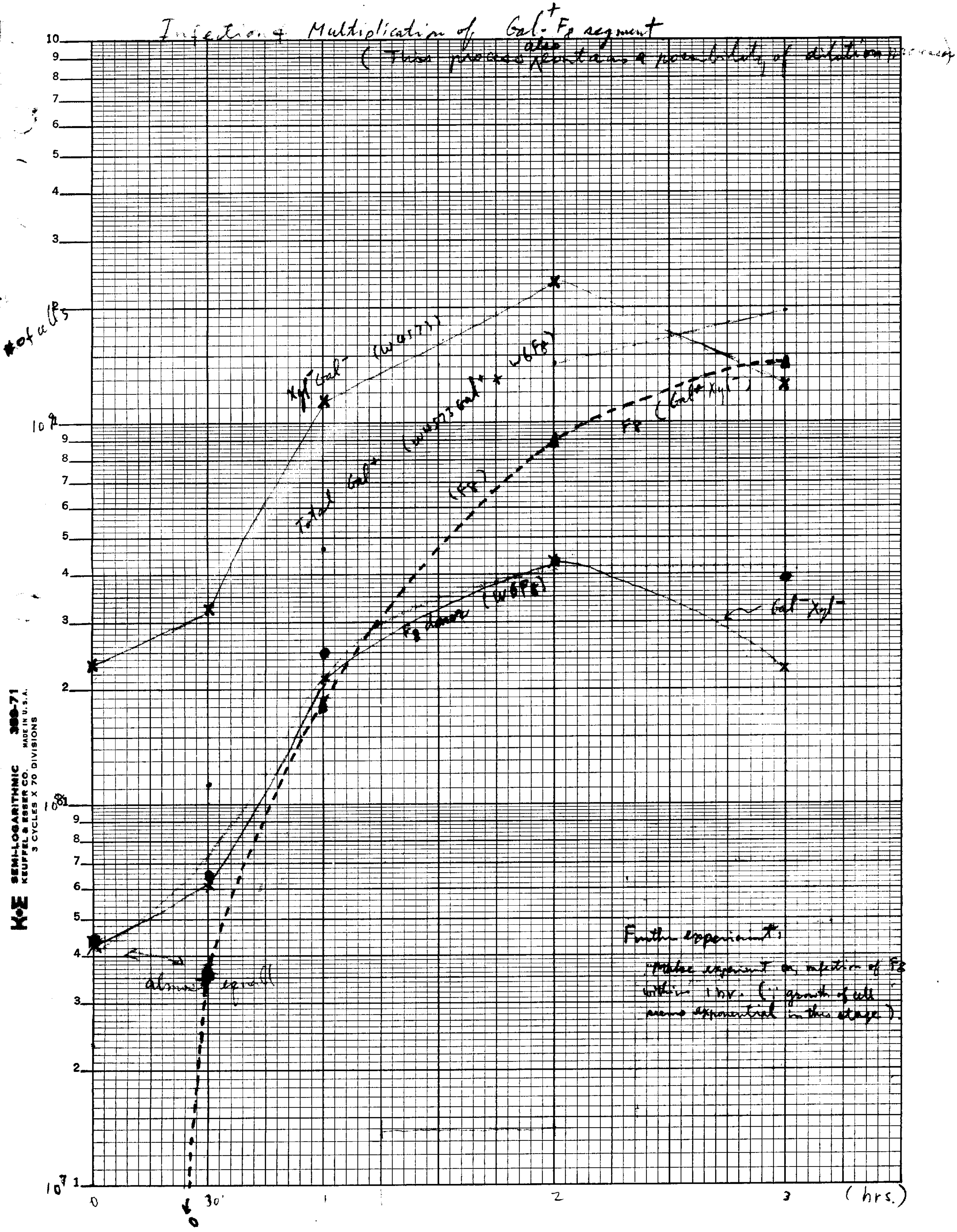
Incubate it at 37°C on rotator. for 2 hrs.

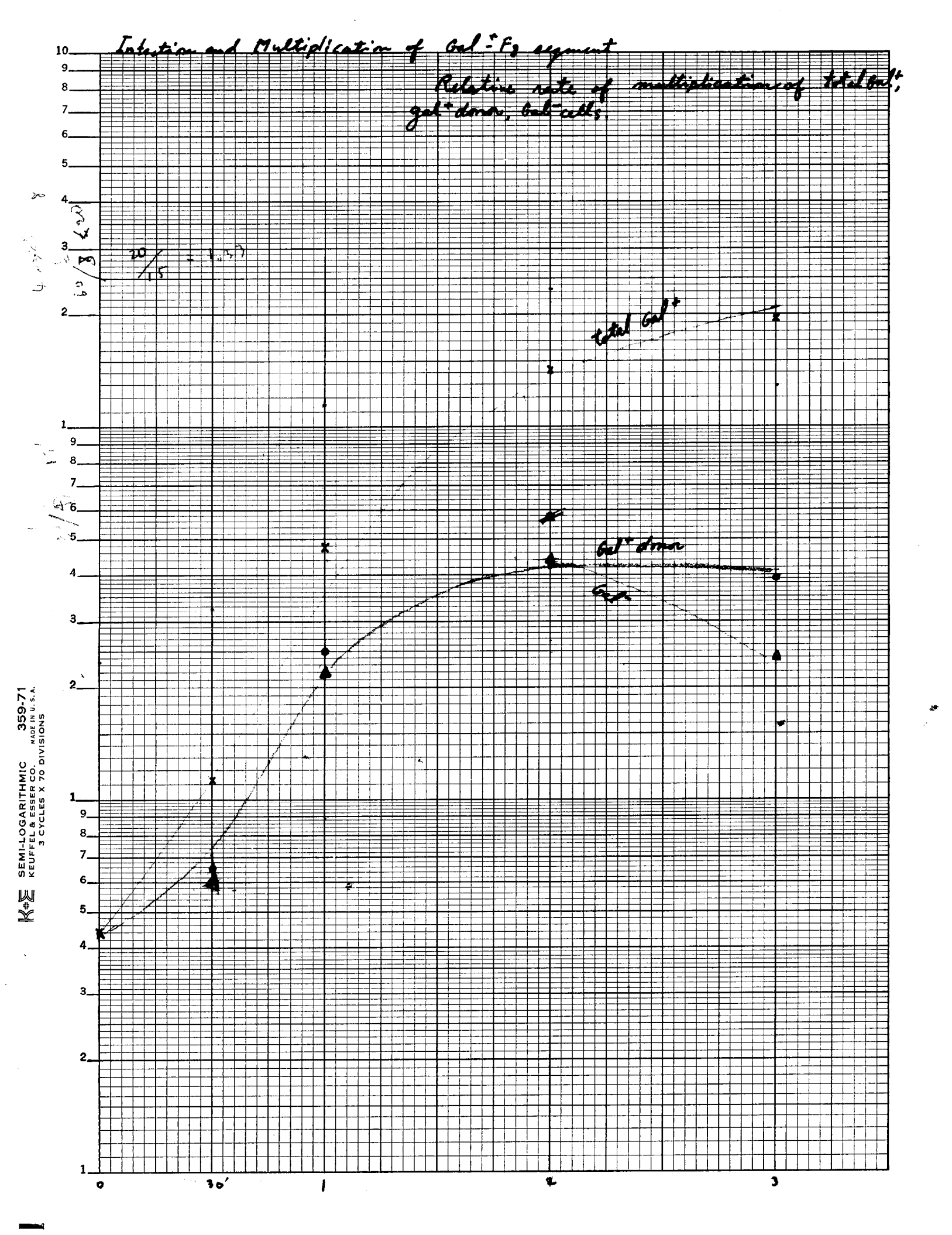
Mix { 1 ml W6F8 + 10 ml W4573.

	AM				PM									
	12:00		12:30		1:00		2:00		3:00					
Inoculum (ml.)	10^{-6} ml		10^{-6} ml		10^{-6} ml		4×10^{-7}		2×10^{-7}		10^{-7}		10^{-7}	
Time	0		30'		1 hr		2 hr.		3 hr		4 hr		5 hr	
vol	+	−	+	−	+	−	+	−	+	−	+	−	+	−
on Bact Gal	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	68	88(4)	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)		(101,11)		(440,32)		(528,44)		(361,28)					
	277		334		1632		1820	1495	1985	748				
	0		0		0		0	×2.5	0	×2.5				
on Bact.	+	−	+	−	+	−	+	−	+	−				
growth of W6F8	8	54	18	108	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				
Σ	44	233	65		250		169	422	78	390				
% of Lac+	15.9		19.5		15.3		12.1		10.4					
multiplication	+		+		+		+		+					
	0	54	9		29		66		53					
	0	36	1		29		69		55					
	0	48	8		35		78		52					
	0	48	11		41		68		89					
	0	46	7		44		76		34					
Σ	0	233	36		188		357	892	283	1414				
% of Lac+	0		10.8		11.5		23.8		37.8					

D : actually counted number

57

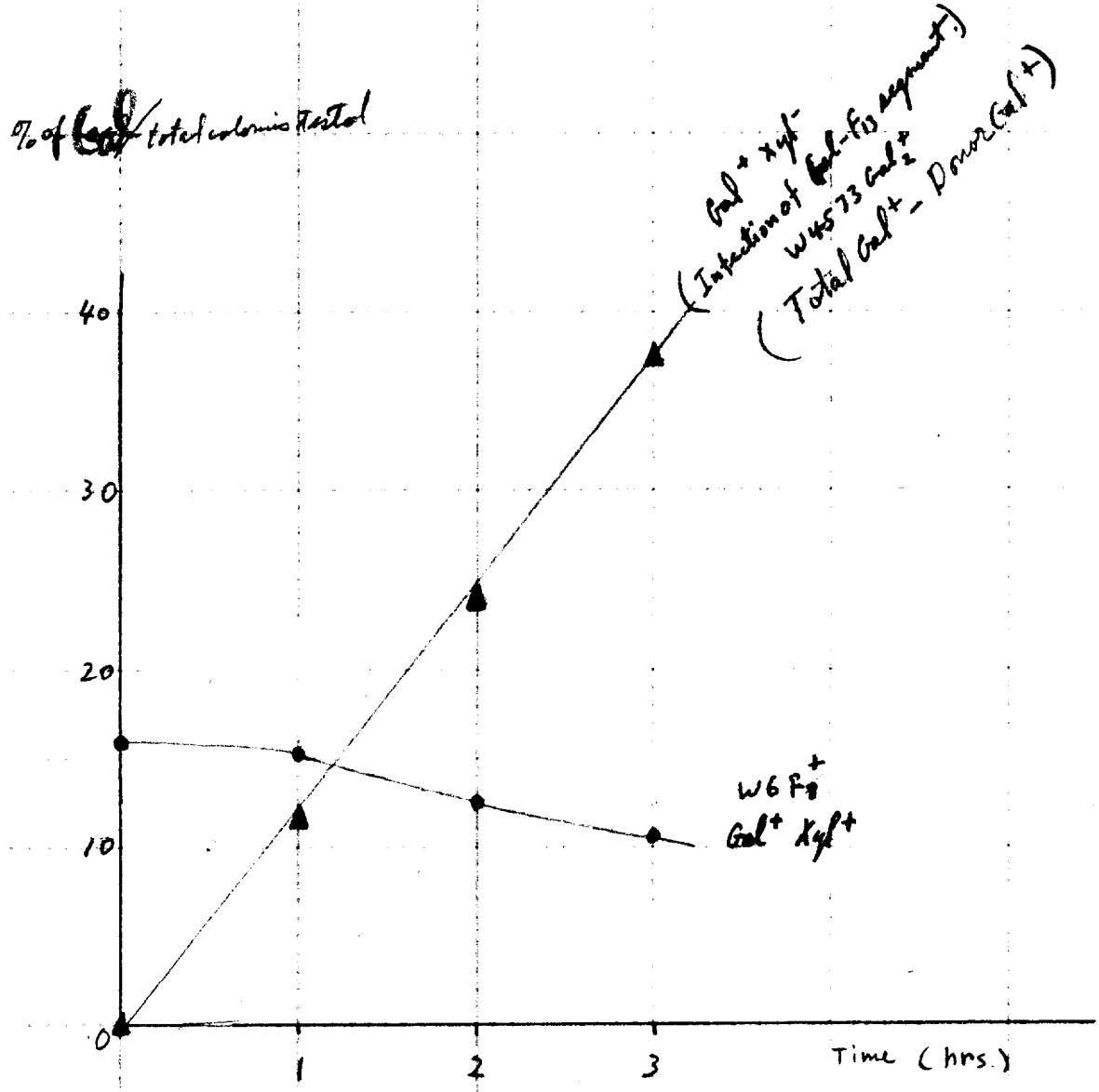

Infection & Multiplication of Gal+ F8 segment
(This process also contains a possibility of dilution process)
# of cells
Xyl- Gal- (W4177)
Total Gal+ (W4177Gal+ × W6F8)
(F8)
F8 (Gal+ Xyl-)
F8 donor (W6F8)
Gal- Xyl-
almost equal
10^9
10^8
10^7
0
30'
1
2
3
(hrs.)
Further experiment:
Make experiment on infection of F8 within 1 hr. (∵ growth of cell seems exponential in this stage).
K+E SEMI-LOGARITHMIC 358-71
KEUFFEL & ESSER CO. MADE IN U.S.A.
3 CYCLES X 70 DIVISIONS

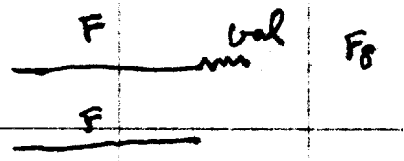

Intestine and Multiplication of Gal⁻ F₂ segment
Relative rate of multiplication of total Gal⁺, Gal⁺ donor, Gal⁻ cells;
20/15 = 1.33
total Gal⁺
Gal⁺ donor
0
30'
1
2
3
SEMI-LOGARITHMIC
KEUFFEL & ESSER CO.
3 CYCLES X 70 DIVISIONS
359-71
MADE IN U.S.A.

Rate of multiplication of Gal-F_8 segment. 128L

$W6\,F_8$ — × W4573

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




F
val
F_B
F

Multiplication of F8 Gal loci.

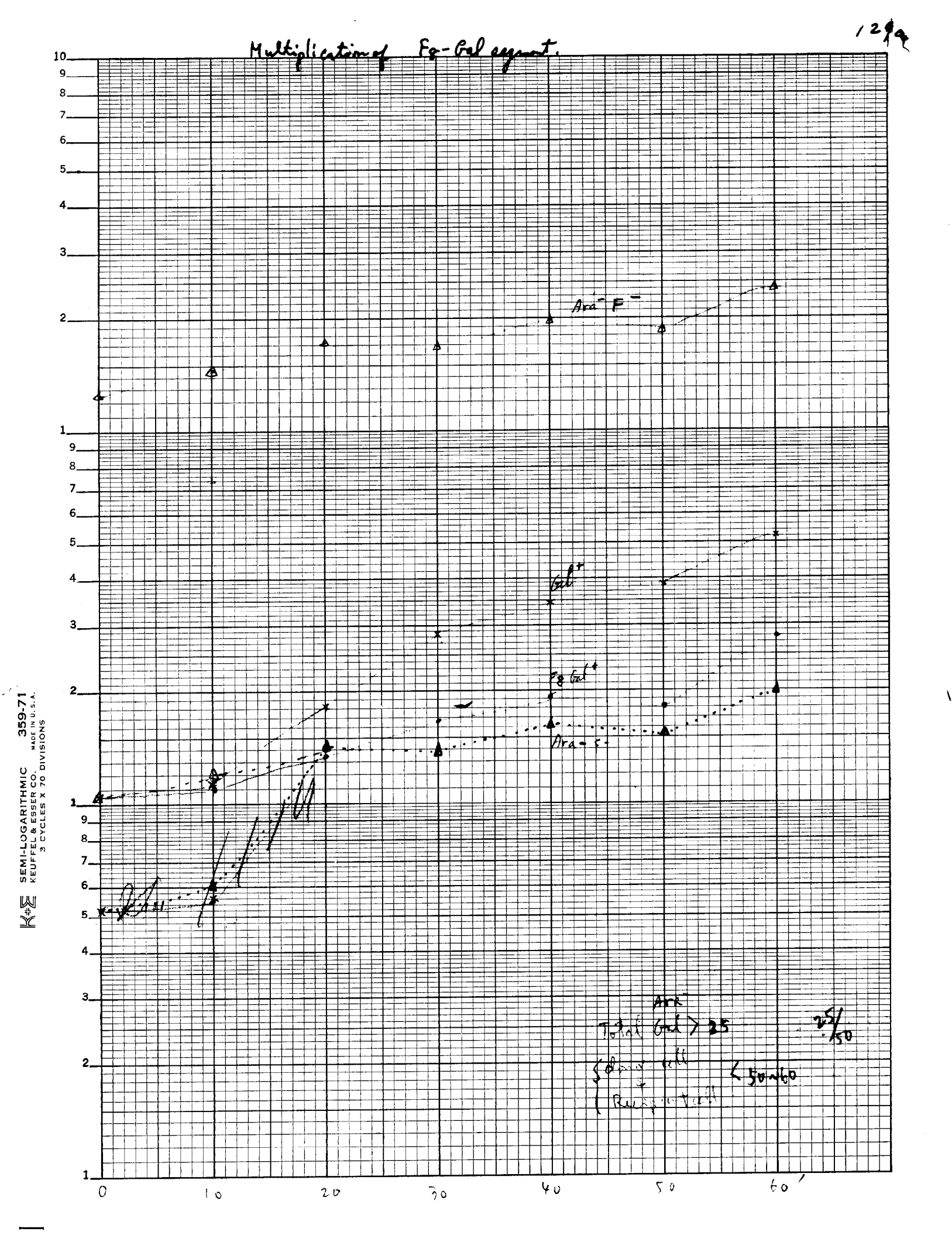
14/VIII 1959 REF:

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. → 0.5 ml ♀ + 0.5 ml ♂ / 10 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	$10^{-2} \times (10^{-2} \times (10^{-1} : .2 ml)$													
			+	−	+	−	+	−	+	−	+	−	+	−	+	−
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)
			+		+		+		+		+		+		+	
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 Fg-Gal 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}^{+} (Took from spot test (~~Lac~~ P126))
Lac61   (Lac2)

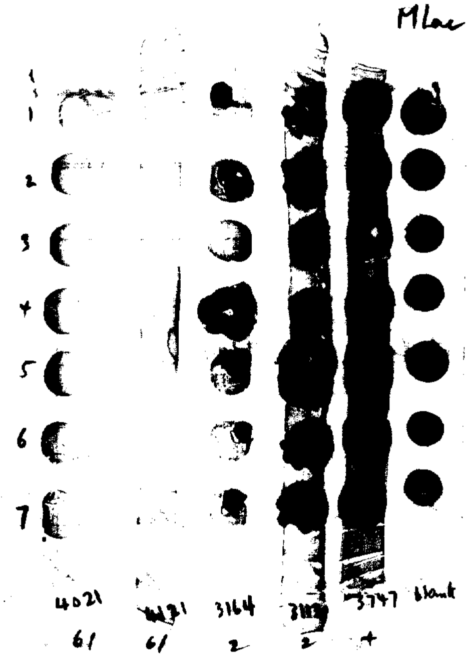
Method:
1. Streak purify it on BLac. (5 plates)
2. Pick Lac⁺ colonies and suspend them into 1 ml H_2O-tubes.
3. Restreak them on BLac, and pick Lac⁻ segregant (pick one Lac⁻ from each Lac⁺ streaks.)
4. streak the isolated Lac⁻ on BLac. (ca. 48 colonies are isolated) (Some of them maybe Lac⁺. 27 were Lac⁻.)
5. Test Lac x. and sex-compatibility by cross brushing on MLac.

	V6	Lac-mutation end. ex	F	× 3112 (Lac2) F-	× 3164 (Lac20) Hfr,	× W4121 (Lac61) F-	× W4021 (Lac61) Hfr, ?		× 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	♂	++23	−	−	−		++
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	−	−		++
Hfr, 4021	Lac61	61		(−) ?	−	−	−		−
4121	Lac61	61		−	++	−	−		−
Hfr, 3164	Lac2	2		−	−	++	−		−
3112	Lac2	2		−	+	−			++

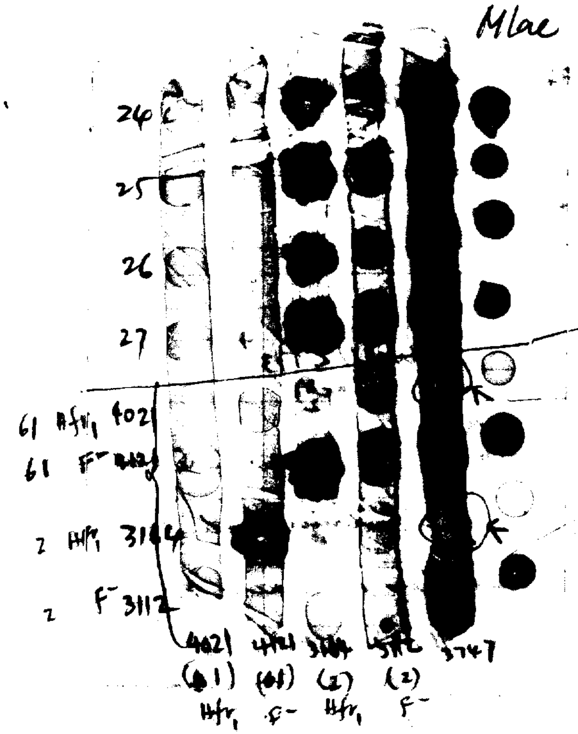
non-transducible ?. Hfr. [illegible]

Conclusion: 27/27 : Lac61 (100 %)

Mlac



Mlac

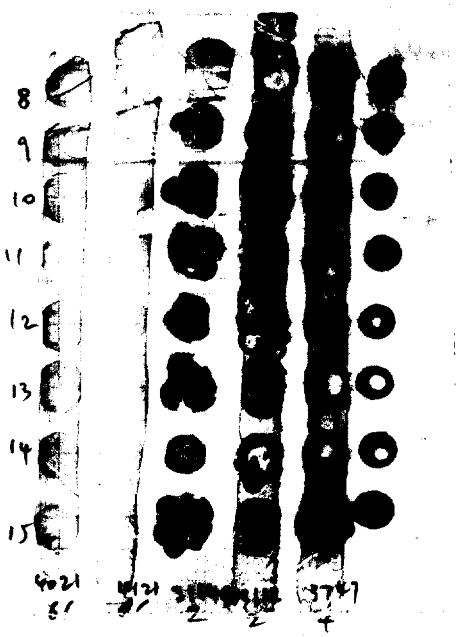


control.

* is Hfr1 x F' steril!

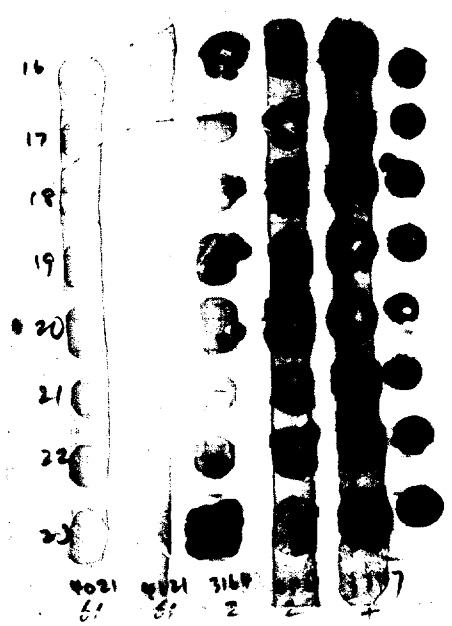
4021 Looks wrong! It may [illegible] (Lac61.

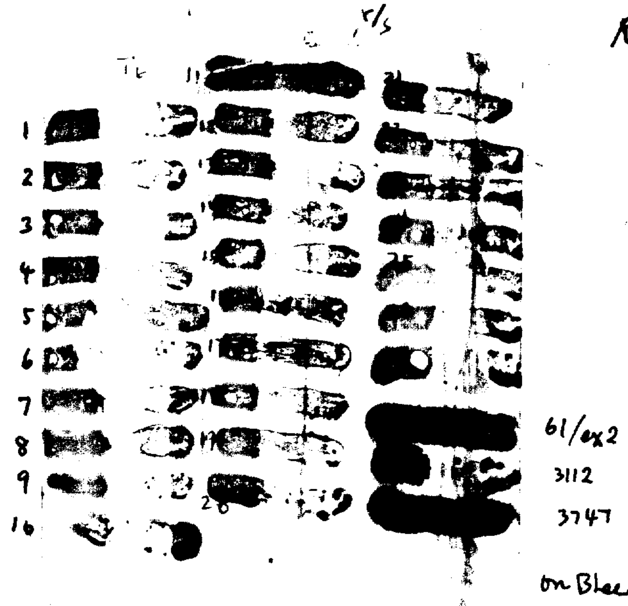
∴ W4021 x Lac2 F- is steril.



Tests for Ti-loci.

Retest #11.





on Blac

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Stock No 28 ~ 40

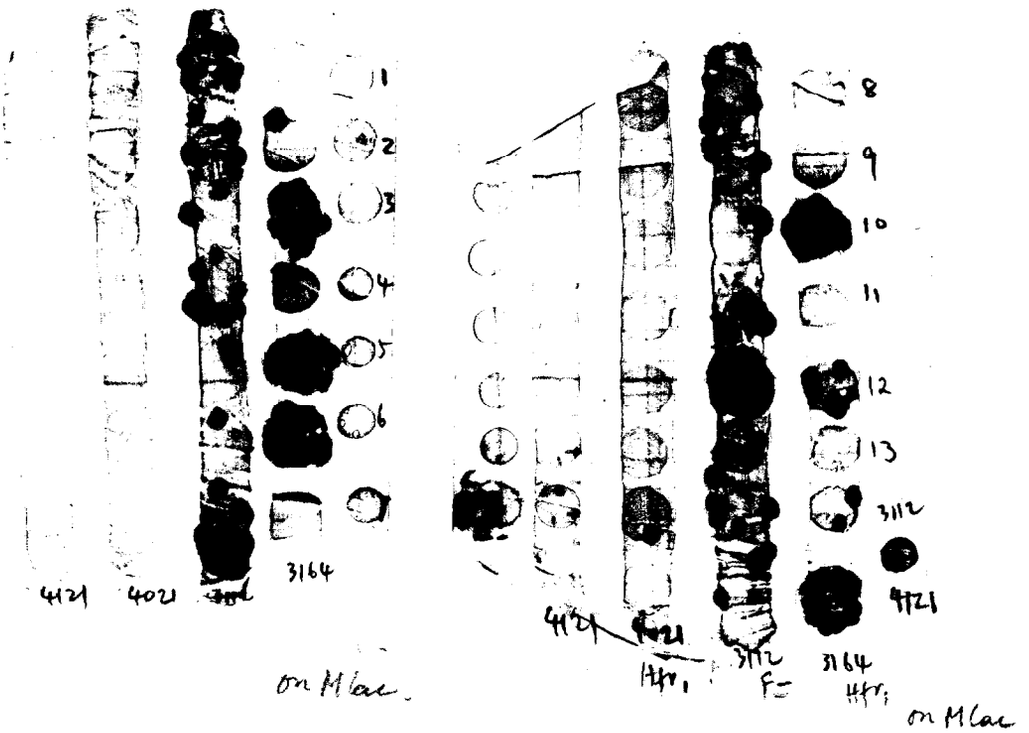
131

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

7/xxx ; 1959

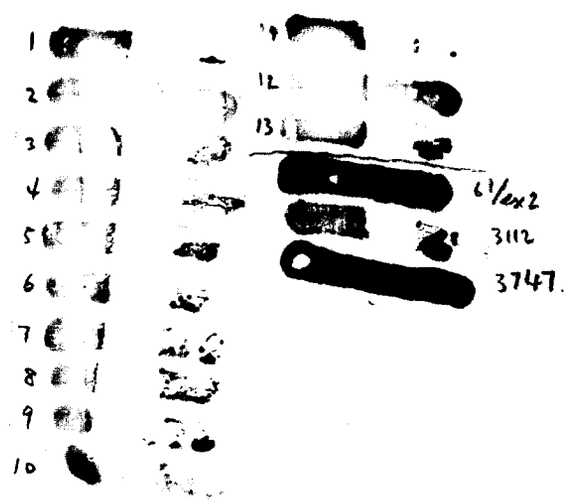
REF:

	1	2	3	4	5	6	7	8	9	10
			W4121 × W3112 F_{13}							
			Lac_{61}	Lac_2						
		Lac and $_{61}$ ex $_2$	F	× 3112 (Lac_2) F⁻	× 3164 (Lac_2) Hfr$_1$	× 4121 Lac_{61} F⁻	× 4021 Lac_{61} Hfr$_1$			
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}/ $_{ex_2}$ r/s									
3	3112 S									
4	3747 R									
5										
6										
7		conclusion :		13/13 : Lac_{61} (100%)						
8										
9										
0										
1										
2										
3										
4										
5									25	
6									13	
7									27/65	
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 Blac

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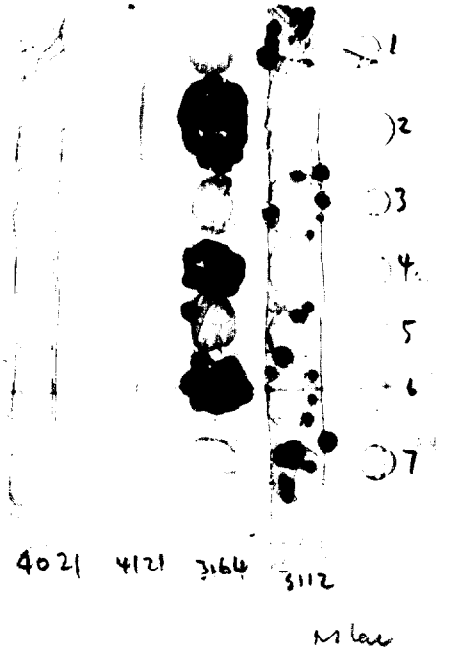
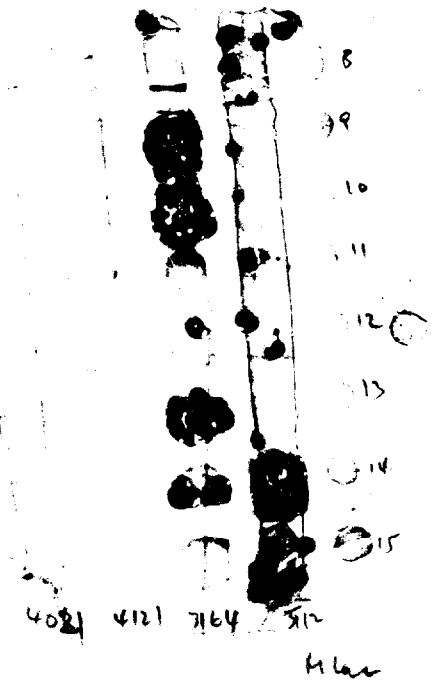
Segregant analysis of Lac61/ex 2. F13.

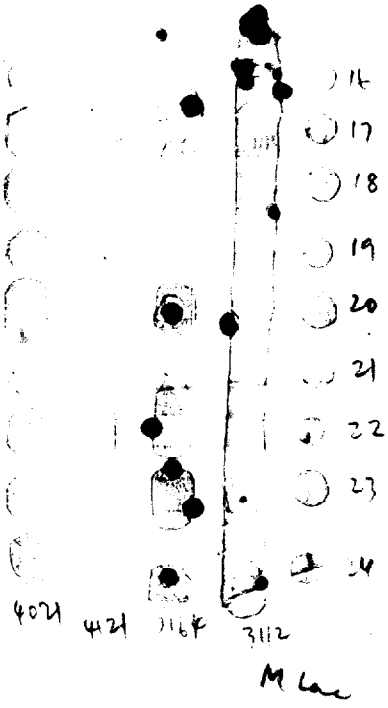
132

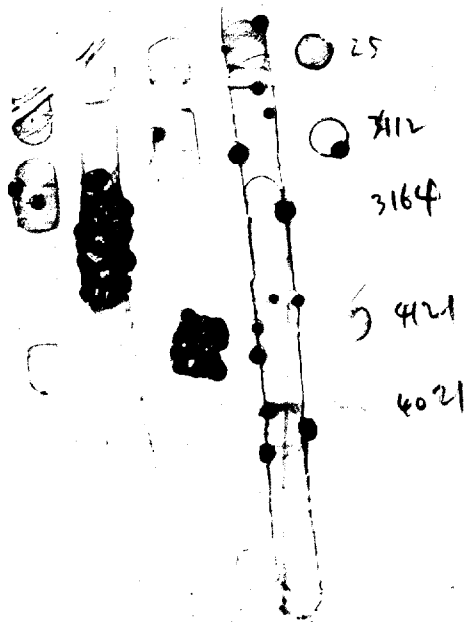
13/VIII :1959 REF:

	1	2	3	4	5	6	7	8	9	10
		V6	Lac ex61 ex2		F	×3112 Lac+ F−	×3164 Lac+ Hfr1	×4121 Lac61 F−	×4021 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	−	−	−	

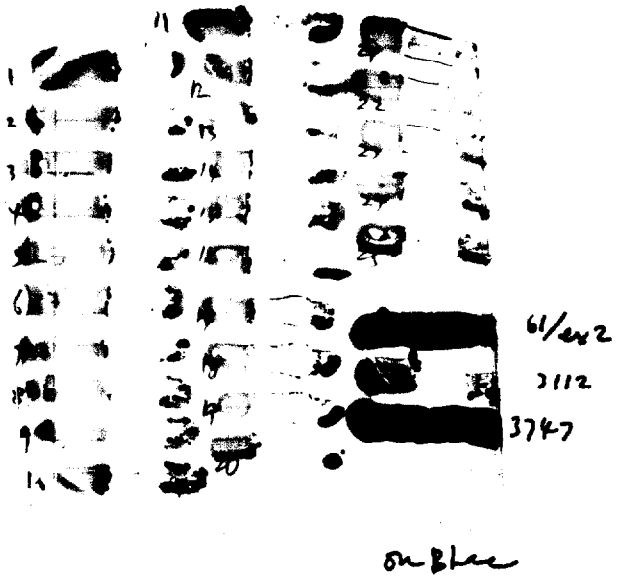








Test of V_6 loci



Retest # 14

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

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

Comparison of the frequency of the transduction of exogenote and endogenote from heterozygous diploid (Lac61̄ / Lac2̄). cf. 105, 146

4/VII ; 1959 REF:

Principle

F_{13} Lac61̄ / Lac2̄ (ex) (3112 F_{13} × 4121) ——→ × F^- Lac61̄ (W4121)
——→ × F^- Lac2̄ (W3112)

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 Penassy broth and kept them on rotator for 4 hrs.

Method : 1) Mix Lac61̄/Lac2̄ (ex) and Lac61̄ or Lac2̄
use excess F^- , Ratio , F_{13} 1 (0.1 ml) : F^- 10 (1.0 ml)

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

3.) Seed them on MLac 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:

Survival count

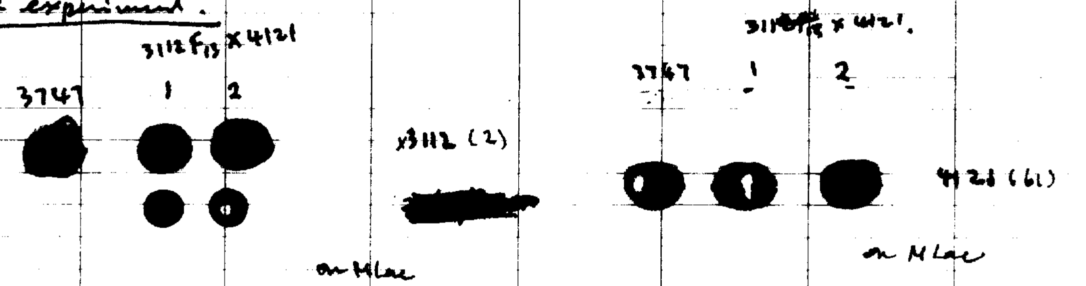
	① F_{13} 61/2 (ex) → × Lac2̄ (W3112)		① F_{13} 61/2 (ex) → × Lac61̄ (4121)		② F_{13} 61/2 (ex) → × Lac2̄ (W3112)		F_{13} 61/2 (ex) → × 61̄ (4121)		3. F_{13}^+ → × 2̄ (W3112)		F_{13}^+ → × 61̄ (W4121)	
	Lac⁻	Lac⁺	Lac⁻	Lac⁺	Lac⁻	Lac⁺	Lac⁻	Lac⁺	Lac⁻	Lac⁺	Lac⁻	Lac⁺
Survival count 10^{-6} ml/plate	1057	89	1084	81	944	51	868	85				

fertility on ml/plate

10^{-1} too much, it canot count them. (use 1/100 of these experiments)

10^{-3} ~~1084 81~~ ~~944 51~~ ~~868 85~~

• Qualitative experiment.



Successful

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Transduction of Lac⁻ segment from heterozygote, Lac^{61}_{ex}/Lac_2.

134.

11/VIII 1959 REF: cf. 145, 146

Principle:

Lac 61 /ex Lac 2 S_S. → Lac 61 F⁻ S^R W 4121 S^R (W4629)

W4121 (Lac 61 /ex Lac 2) → Lac 2 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 3117 F13 4121 ($Lac^{61}/_{ex}Lac_2$)

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

Result: see back page.

	3112 (Lac_2^-)	4121 S^R (Lac^{61-})	blank
4121 61/ex 2	+	++	−
3112 2/ex 2	+ reversion	++	+ reversion.
3747 +/ex +	++	++	−

12/VIII

Quantitative test. at 37°C Shaken on rotator

· cultural age: 0.2 ml : overnight culture / 5 ml/ phase 10:30 a 3:30.

· Ratio of mix: 1 ml | 3112 + 4121 $Lac^{61}/_{ex}2$ 0.1 ml

1 ml | 4121 S^R (W4629) + 4121 $Lac^{61}/_{ex}2$ 0.1 ml.

· Time of incubation: ~~1h 2:30 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

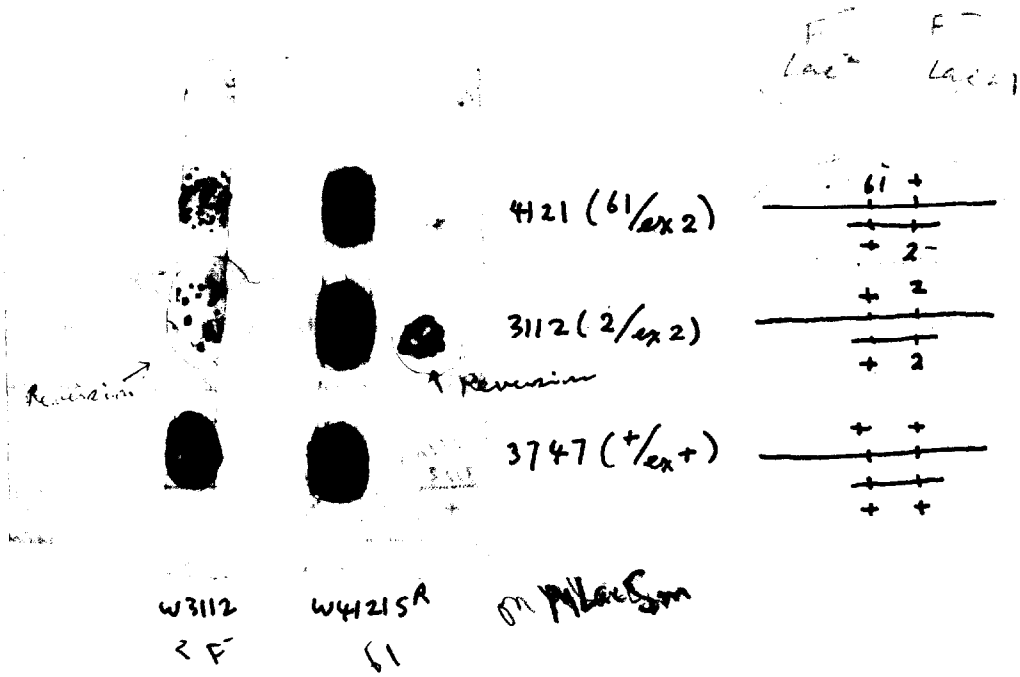
· Inoculum size for counting recombinations. 10^{-3} ml. / plate on MLac Sm.

12/IX

Result:

		# of Recombinants. Plate 1	# of Recombinants. Plate 2	# of Colonies 10^{-3} ml / plate. +	# 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	1/10 22	193	38 212	
4121 $61/_2$	× 3112	0	0	1/2 0	65	1 68	$Q \geq 10^{-3}$
				1/10 0	112	4 269	
4121 $61/_2$	× 4629	2	5	1/2 0	66	1 53	$Q \leq 10^{-3}$
				1/10 2	136	4 100	

Conclusion: Exogenote is transferred with very high frequency, but not very high for endogenote. This implies all F13 Lac HI 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.

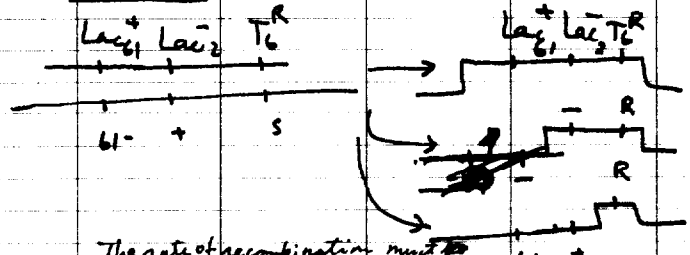

F⁻ lac⁻
F⁻ Lac⁺
4121 (61/ex 2)
3112 (2/ex 2)
3747 (+/ex +)
Reversion
Reversion
W3112
< F⁻
W4121 SR
61

Linkage of $T6^R$ and Lac^-_2 in exogenote of ($Lac_2\ T6^R \times Lac_{61}\ T_6^S$) $3112F_{13} \rightarrow 4121$

6/VII 1957

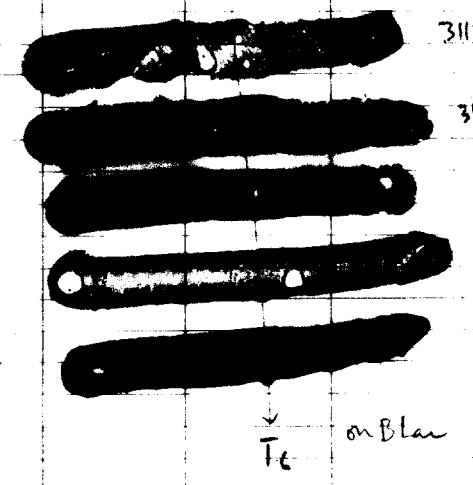
REF:

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



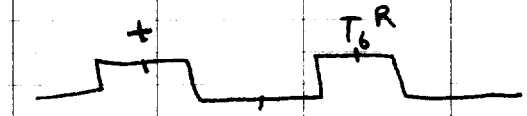
The rate of recombination must be express ratio of length of $V_6 - Lac_2 - Lac_{61}$.

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



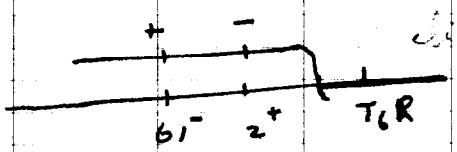
Result:
actually the lysed part is also Lac^+.

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.



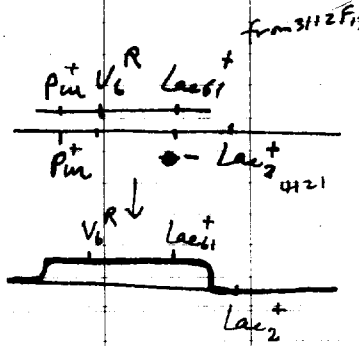
$T_6^R\ Lac^+$.

or



elimination of T_6^S segment

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



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

8/44 , 1969 REF:

1 2 3 4 5 6 7 8 9 10

Strain W3086 F_8 , W3747 F_{13} , W6 F^+ . Make opposit combination { W6 F_8 / 3086 F^+.

- cultural age : overnight culture in Penassay broth.
- Inoculum size : ca. 10^8 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~~ before treatment.)

Exp II A).

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

Exp II ~~[illegible]~~ compatibility

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

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

Exp ① 3086 F_8 + W6

Treated with AO.

W3086 F_8 Σ	F_8	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 (x 4573.)

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

untreated control.

3086 F_8 Σ	F_8	F^-	W6 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 Σ	F_8	F^-	W3086 F^+ F^+	F^-
32	32	0	32	0

Treated experiment (with AO : 30γ/ml)

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

x 4573 on MGal.

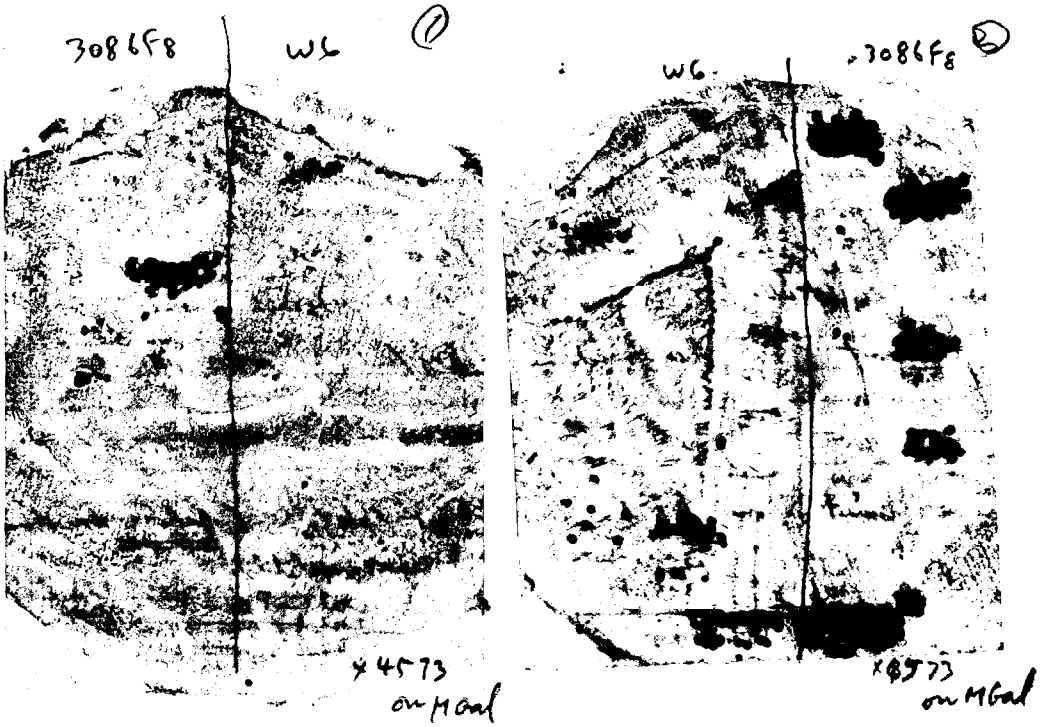
x 4573 on MGal.

Exp (I).

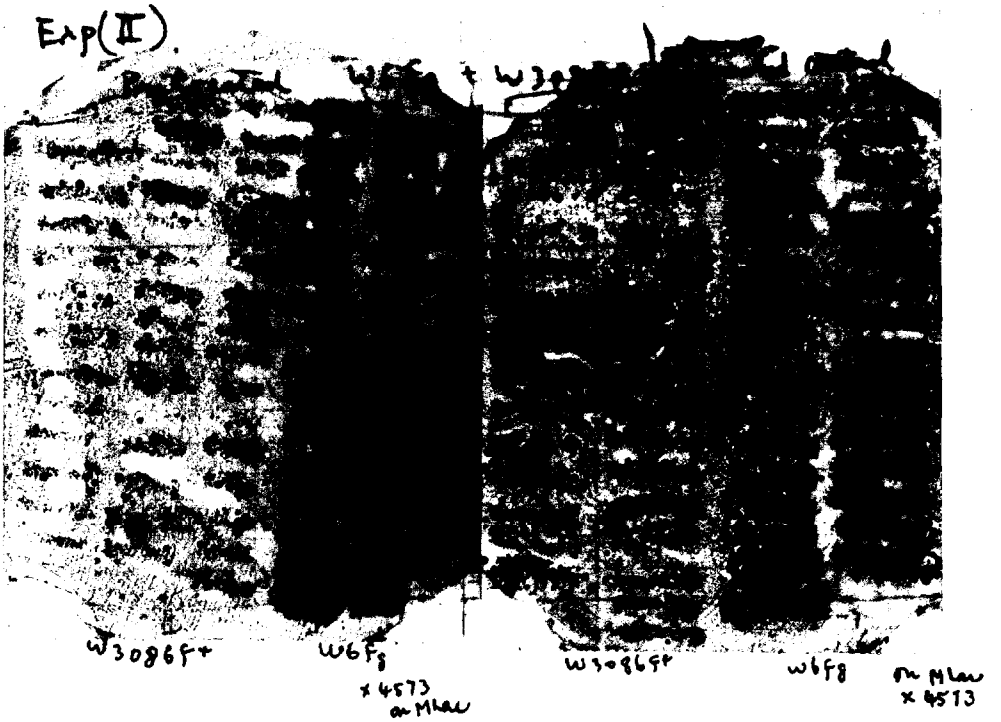
Inoculum ca. 10^2 cells/ml

AO 30 g/ml

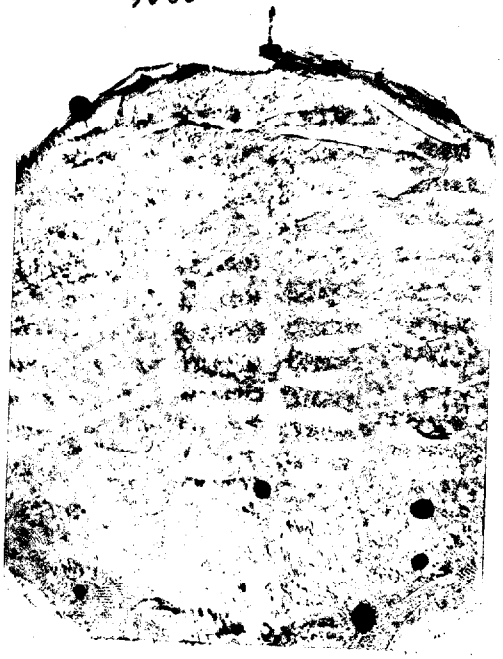
AO 3086Fg + W6 6 min light



Exp (II).

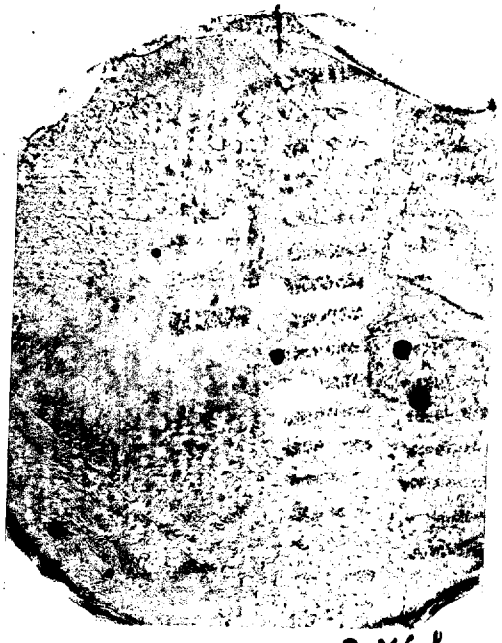


Ao treated
3086 F+ W6F8.



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

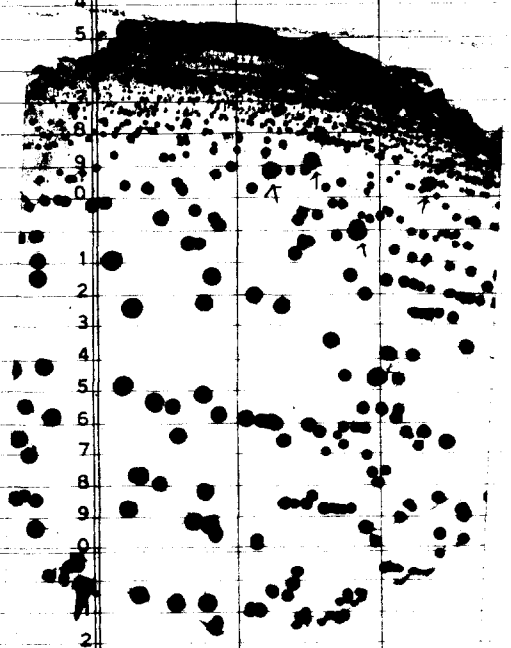
Principle :

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/VIII 1959

REF: [W4296 shows strong agglutophy [with Mt] 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

~~4577~~

W3086

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

on M Lac

× 3086

Try it again

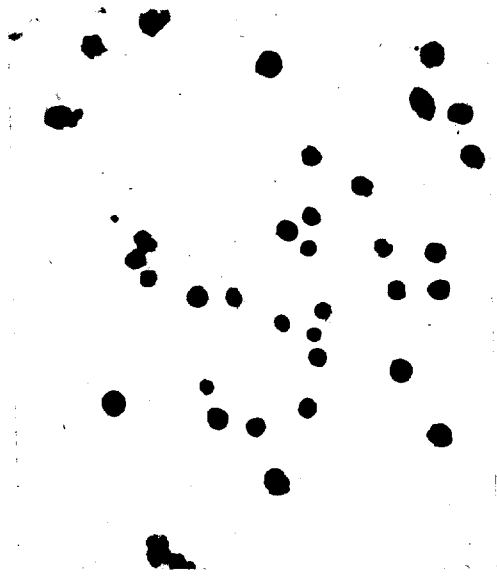
Syntrophy of M and T.

3137 F_3 → W4296

Master plate

on M Lac

W808



on B Lac

Unsuccessful.

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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 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} 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~~ 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+ must be S^s

Principle:

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

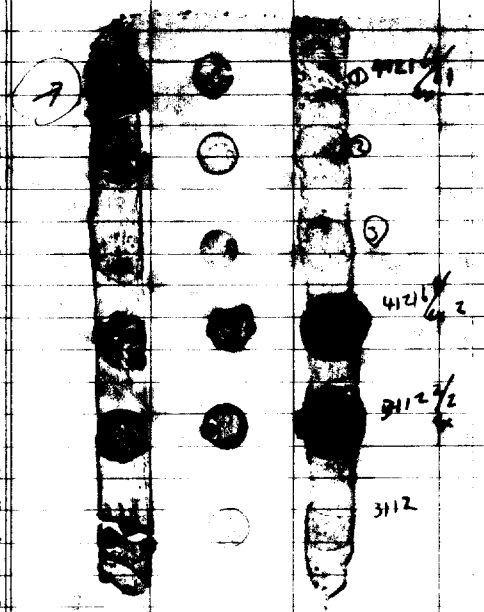
2nd step: $Lac_{61}/ex\ Lac_2\ F_{13} \longrightarrow Lac_2/ex\ Lac_{61}\ F_{13}$ on MLac (X 3112.

{exchanging Proms of exogenote and endogenote.} (Detect it by replica plating method) see back page.

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

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

Retest



Replicated from BLac.

1st selection.

Pick.

on MLac
× 3112.

4121 61/4x2

3112 2/2

3787 1/1

4121 3/4x61

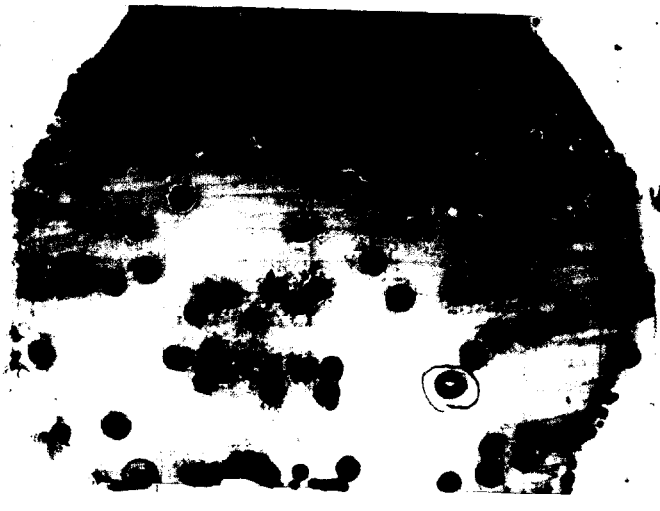
4829
Lac 61.
SR

3112
Lac 2
SA

← Reisolated on MLac

Repunify

2nd selection. on BLac

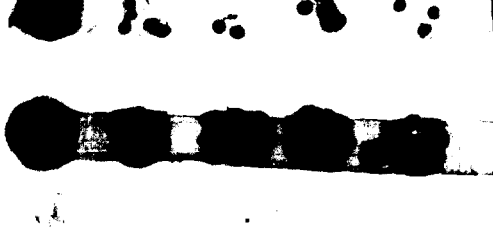


on MLac

×3112.

4121

3112 4 3 2 1



4121
61/4x2

4829

3112

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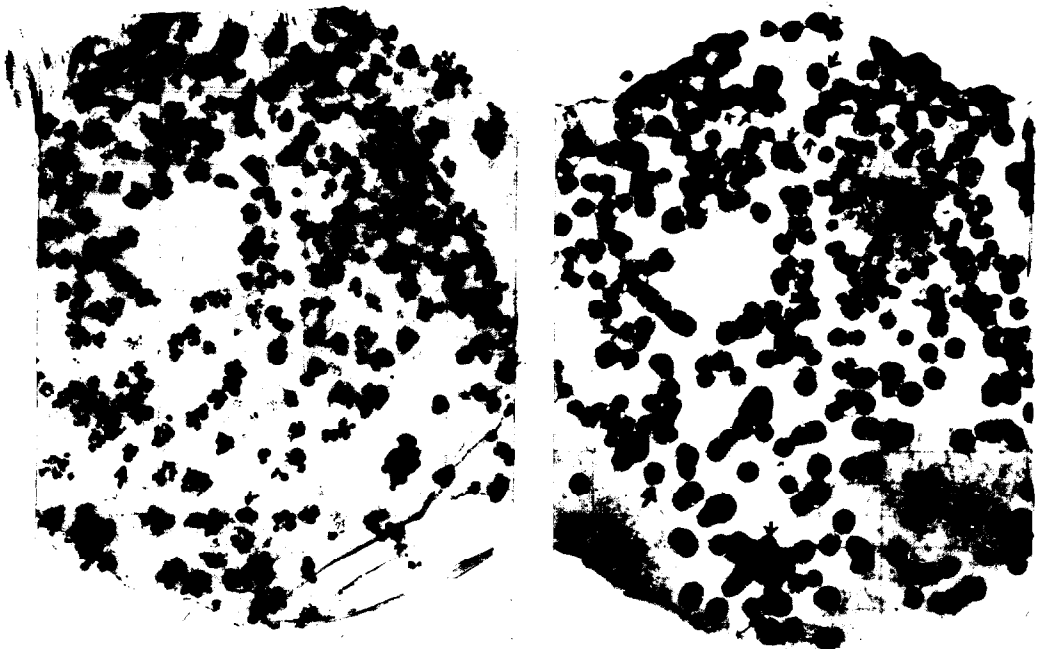
[illegible] ; 1939 — REF:

1	2	3	4	5	6	7	8	9	10

Isolation of HI Gal from W3747



MGal
×2979.

on Blac

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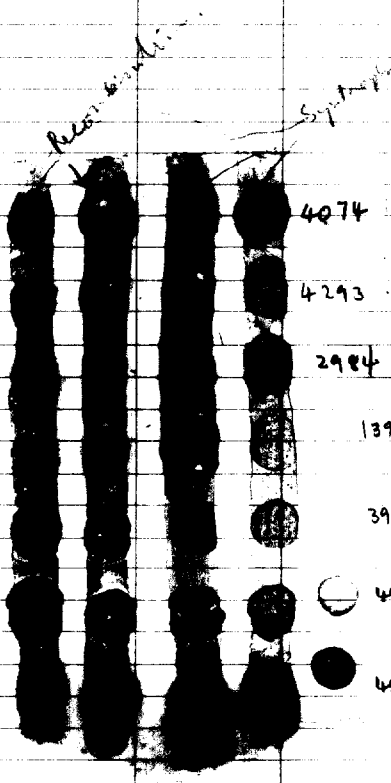
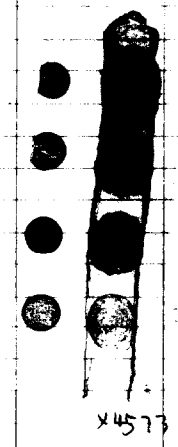
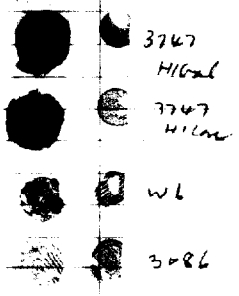
Comparison of the fertility of W3747, HI Gal and HI Lac.
I II.

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 →

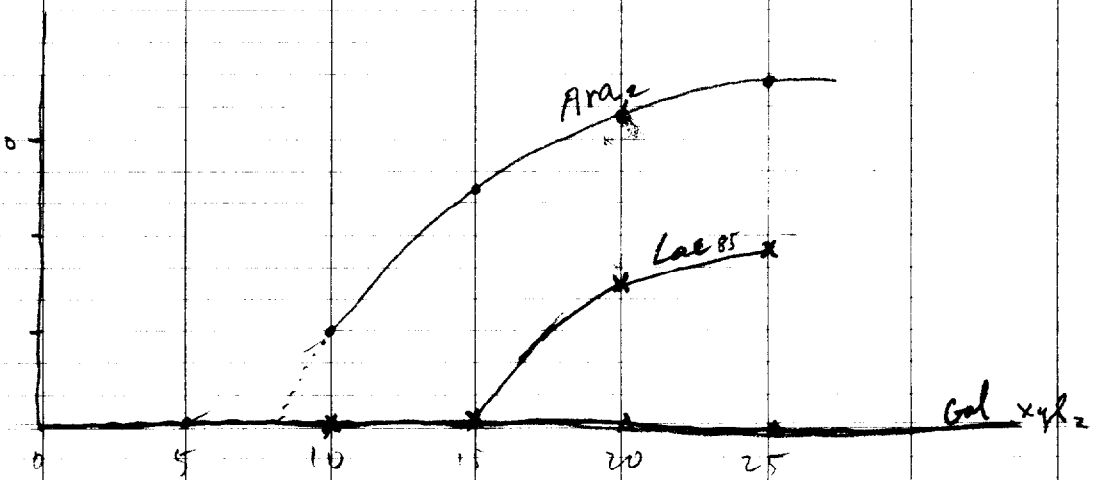
Cultural age : 1:30 ~ 4:00 2½ hrs. at 37°C on rotator

W6 F_2 : 2 ml
W4573 : 0.2 ml

# of Recombinants/plate

Time	0		5		10		15		20		25	
	Ara_2 Lac Gal 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 8	
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 0.1 %		ca 0.5 %		ca. 1 %	

%



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

16/IX ; 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 exogenotic and endogenotic segment.

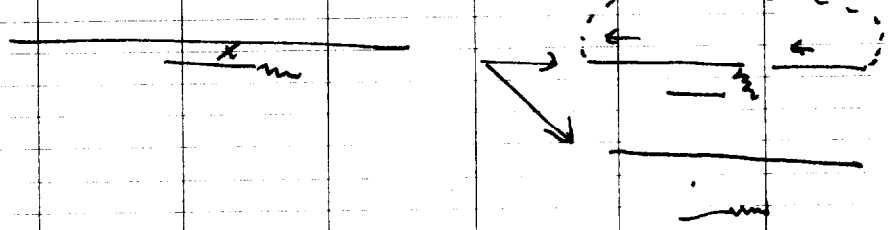
1959 REF:

	1	2	3	4	5	6	7	8	9	10
		Cultural age : Overnight cultures.								
1										Rec. D. / Lac+
2						fertility		Survival (10^{-6} ml)		
3					(F⁻ Lac 61 S^R)	10^{-3} ml	10^{-2} ml	Lac+	Lac⁻	+ −
4		W4121 61/ex 2	→	× W4629		79	1214	8	38	8 71
5						180	1097	5	45	8 75
6										
7						Ca. 100	Ca. 1000	Ca. 5 × 10⁸	4 × 10⁷	Ca. 10^{-2}
8										
9										
0										
1		W4121 61/ex 2	→	× W 3112 (F⁻ Lac 2 S^R)		0	12	1	27	18 15
2						0	20	3	32	23 155
3										
4										
5						0	Ca. ~~10⁻²~~ 10⁻³	Ca. 2 × 10⁸	Ca 3 × 10⁷	< Ca. 10^{-5}
6										
7										
8										
9						10^{-3} ml	10^{-2} ml	+	−	+ −
0		W3747 +/+	→	W4629		1231	68	248	35	174 14
1							101	~~262~~ 220	~~79~~ 46	138 20
2								262	41	-
3						Ca. 1000	Ca. 100			Ca. 10^{-2}
4								Ca 2 × 10⁸	4 × 10⁷	
5										
6										
7										
8										
9								+	−	+ −
0		W3747 +/ex+	→	× W 3112		126	52	170	46	240 70
1							113	262	79	291 68
2										Ca. 10^{-2}
3						Ca 1000	Ca 100	Ca. 2 × 10⁸ ; 5 × 10⁷		

Conclusion:

Fertility of exogenotic ≅ endogenotic × 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.
as primary growth. suspended into H_2O.
32 m/s of WBFB culture was used. (4 tubes of 8 ml per.)
↓
concentrated into 2 m/s.

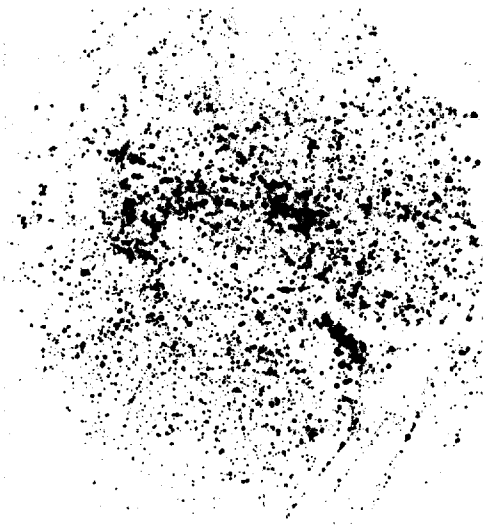
1 ml	1 ml
↓	↘
treated with duponol. final 1%.	untreated
20% duponol Soln 1 ml	H_2O 1 ml
+	+
Bact. Suspension 1 ml	Bact. susp. 1 ml

4. Method of transformation.

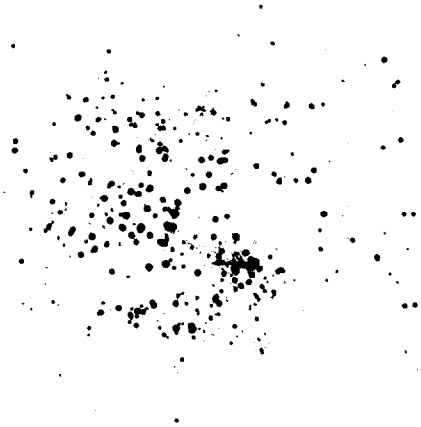
DNA	dilution of Original culture	10^{-1}	10^{-2}	10^{-3}	
# of T_3 Recombinants.		~~much~~	521	43	
survival cells in 0.5 ml		—	—	2,5 ↑	
Bacteria		10^{-1}	10^{-2}	10^{-3}	10^{-6}
# of Recombinants					41
survival cells in 0.1 ml		—	—	—	435 585 ↑

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 dupond 20 %.
2. ~~Time of~~ treatment ~~with~~ dupond : Na-lauryl-sulfate.
(final conc. 10 %. in dist. W.
37°C 4 hr.
3. Filtered through millipore filter.
4. Mix : deproteinated 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 Recombinants.	0	0	0

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

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

189

4:4'-diamidino-diphenoxypropane-di (β-hydroxyethane sulphonate) 5 gr.

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

7 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 sterile H_2O — vial.)

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

Conc. of P.I. (cc. of stock soln.)	.05 mg/ml (0.05 ml)	1 mg 0.05	(x1/10) 100 γ 0.05	x1/100 10 γ 0.05	x1/1000 1 γ 0.05	Control 0 γ
W6 F_8	−	−	−	+	+	+
W6.	−	−	−	+	+	+

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

Media . 5 ml Penassay broth, pH. 7.0.

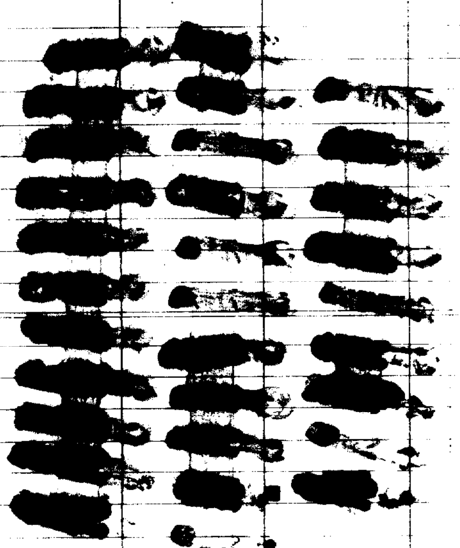
2. Streak these treated cells on Blue agar, 100 γ. P.I.

3. Test their compatibility by cross-brushing method.

Result: P.I.-treated (10^8/ml Rec.) untreated control.

F−	F+	% F−	F−	F+	% F−
7	24		3	29	

W6 P.I.



W6 Cont.

Mlac 573

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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. 10^5 cells/ml.			Strain: W6.			(×1/100) Conc. 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 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.

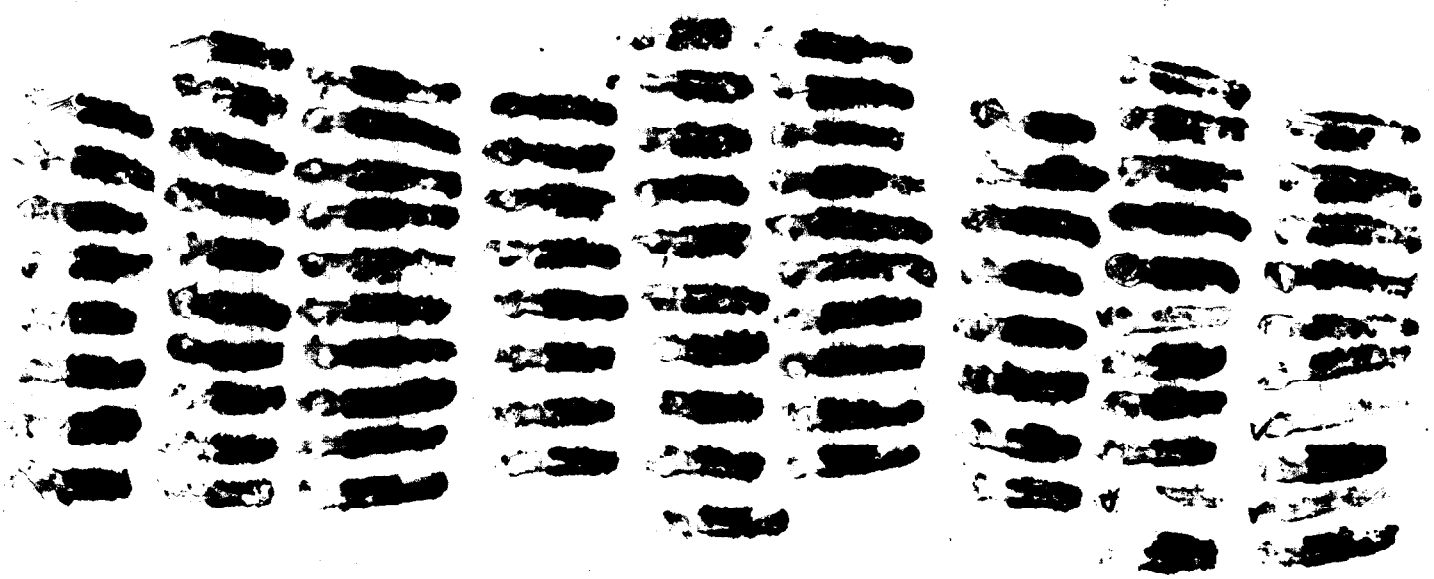
W6 P.I. 0δ.

MLac
X4573

P.I 10δ

P.I. 20δ

P.I 50δ



MLac X4573

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151

Last trial on the killing of cells without loss of mating capacity.

17/xo 1957 REF:

5 ml overnight culture of W6 Fg. in Pen

↓

Centrifuge, discard the supernate, resuspend it's 1 ml of H_2O.

↓

distribute the 0.2 ml of W6 Fg into dupont soln. {sodium lauryl sulphate 1:100 PR

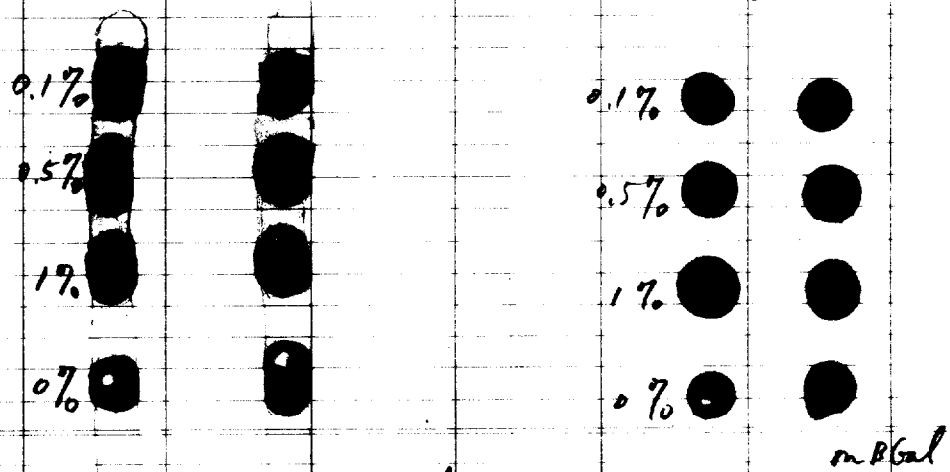
↓

Incubate it for 2 hrs. at 37°C.

↓

Wash it once and spot them on MGal x 4573 & on BGal

Conc. of dupont.	survival	fertility.
1 %	+	+
0.5 %	+	+
0.1 %	+	+
control. 0 %	+	+



Conclusion: Use much conc. of dupont. (2 %)

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2nd trails on the killing cells, without removal of mating capacity.
(duponol)

17/IX ; 1959. REF:

1 2 3 4 5 6 7 8 9 10

20% 1. W6 F_8 overnight culture 8 ml / tube. 4 tubes.

Centrifuge - Wash once - put 4 tubes together - resuspend them into 1 ml Tris.

15% 3. Add 0.2 ml of the Bacterial suspension into duponol soln. 0% 10%
15% 20% and incubate them for 4 hrs. at 37° C.
3:00 PM ~ 7 PM.

10% 4. Test survivals & fertility of these treated and untreated W6 F_8.
on B Gal and B M Gal x 4573.

0 2 days old.

1/19 1 Penicillin grown.

Exp. I. 4573 + duponol-lysed F_8^+ (with Duponol) → incubate at 37°c for 30'
0.2 ml original 0.5 ml
0.2 ml 1/10 diluted 1 ml

→ wash once → ~~spot~~ the 0.1 ml on M Gal. (7:30 PM ~ 8:00 PM,
(centrifuge for 5 min.)

Result.

	survival	Recombinant
10%	2×10^3	$> 2 \times 10^3$
15%	"	"

Transfer of Lac.

Exp II. dilute the duponol-lysed material (10^{-1}) → spot on MLac on which W4573 was streaked.
non-diluted material (10^0) → "

untreated control.

0

10%

15% ~~Centrifuge and resuspend in 1 ml Tris and spot on MLac x 4573~~

20%

Conclusion: ① Killed cells with duponol does not transfer Lac to F^-.

② However, the treated material shows recombinating activity in transfering Gal as same numbers of survival cells.

x 4573

1/19 dil On MLac 1 mail into

Next step: ● Make exact experiment.

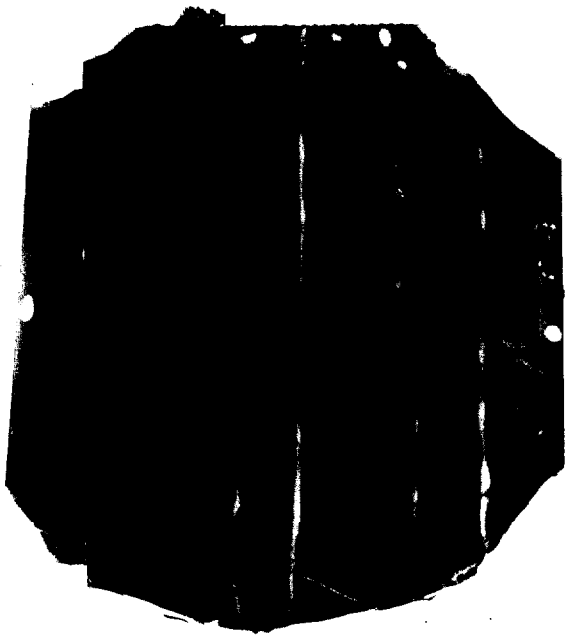
4:30

4297 4296 2985 4539 4627 4354 4295
G T A_1 H Try M L

1
2
3
4
5

blank #295 #354 4627 4539 2985 4296 4297

on MGluc.



4297 4296 2985 4539 4627 4354 4295 blank

on MGluc.

Continued from 153a.

F status of segregants from ♀ 3747 × 4574.

4574 : F^- P Ara_2 Mal_8 Mtl Ara_2 Xyl_2 Gal_2 SR.

primary exconjugants of (cf. P)

24/II ; 1960

REF:

Exp. I This experiment does not work well. The fertility was so low in this time. only one Lac$^+$ was tested.

25' : at 37°C. / recombinants (Lac$^+$) — purified on BLac Sm — Pick Lac$^+$ & Lac$^-$ onto in incubator. BLac Sm — Replicate on DO + 4506 (F^- pur$^-$)

(Lac$^-$ colonies (4574) are so crowded around the Lac$^+$, therefore, Lac$^-$ F^- (4574) can be contaminated with segregants)

#1 Lac$^+$.	# of Lac$^+$ tested	sex F_{13}	# of Lac$^-$ tested	sex
	15	F_{13} : 15 F^- : 0	24	F^- : 21 F_{13} : 1 ← Save this F^+ : 2 (or F_{13}) recheck!

#of Lac$^-$ Lac$^+$
876 : 1

See back page Ia, & Ib.

Exp. II Exp. does work beautifully.

25' : at 37°C : in incubator room : on rotator.

Lac$^+$. tester : 4506 on DO.

	Lac$^+$ tested	sex	Lac$^-$ tested	sex.
#1	8	F_{13} : 8	10	F^+ : 10
#2	10	F_{13} : 10	1	F^+ : 1
#3	4	F_{13} : 4	1	F^+ : 1
#4	8	F_{13} : 8	—	—
#5	6	F_{13} : 6	—	—
#6	7	F_{13} : 7	—	—
#7	8	F_{13} : 8	—	—
#8	6	F_{13} : 6	—	—
#9	5	F_{13} : 5	—	—
#10	6	F_{13} : 6		
Σ	58	: 58	12	12

① Mix 4hrs exponentially growing and culture of 3747 (10) + 4774 (1) Shake it on rotator for 25' at 37°C. Chill & Then blend the mix, dilute it into 10^{-3} : 0.1 ml plate on BLac Sm.

② Pick well separated Lac$^+$.

③ purify them on BLac Sm.

Pick Lac$^+$ segregants & Lac$^-$ segregants. and test sex by cross-brushing method on DO. × 4506 (See back page II.

• F^+ means as follows :
1. Not F^- from the cross × F^-
2. Not F' by low fertility.
3. Same fertile as F^+ in cross × F^-

Fertility of F^+ & Infectivity of F^+ should be checked.

• 3 colonies out of 10 showed segregation of Lac$^-$ # 1, 2.

— : No Lac$^-$ colonies can be picked after streak on BLac Sm.

% of F_{13} of Lac$^+$ segregants : 100. % of F^+ of Lac$^-$ segregate : 100.

Conclusion : 1. Lac$^+$ becomes F_{13}. suggesting close linkage between Lac & F_{13}.

2. Lac$^-$ becomes F^+. (These F^+ (presumably?) should be checked. in its purity. and low fertility & infectivity for many matings) → These are really F^+! see back page.

further experiment : Pick 3 colonies from #1, #2, #3. and test compatibility of each 3. 1, 11, 12.6 (see back page II)

Speculations : 1.) F^+ (cell) carries wild type F, or at least F' which can distribute F into their progeny after mating in separate way from F'. This may be interpreted by 2 alternatives : 1. F^+ carries wild type F ; 2. F' is easily separate from F and the newly formed F will spread over the progeny.

2) Hfr may be defective F^+. It carries defective F by recombination between F and bacterial chromosome.

Ia:

Lac⁻ ⟷ Lac⁺ on DO ×4506

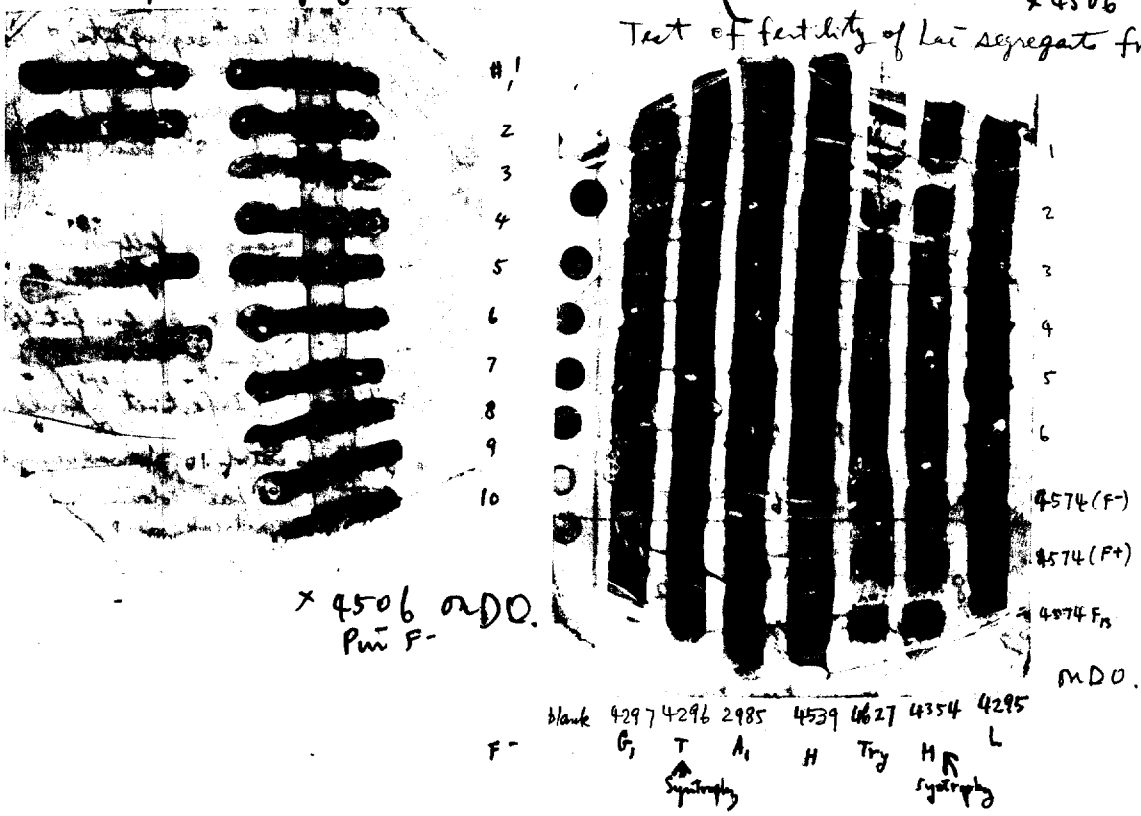
Ib. · Retest fertility of all Lac⁺ and 2 fertile Lac⁻ and sterile Lac⁻.

Make cross-streaking.

on DO ×4506 Lac⁻ / Lac⁺

II. ~~Tested by~~ Sex of Lac⁻ segregants from primary zygote

Test of fertility of Lac⁻ segregate from Primary zygote



×4506 on DO. Pm F⁻

on DO.

Test for dual structure of F'-recs.

(Is F' defective? Usual F'^{+}_{REF} contains standard F.)

10/II ; 1960

1 2 ~~3 hrs~~ 3.5 hrs 4 5 6 7 8 9 10

Cultural age : 11:30 ~ 15:00 Inoculum size : 0.2 ml / 10 ml pen.
Shaken on rotator at 37°C. Overnight.
Pen. grown culture

Principle. : Does F' segregate out F after infection of F_{13} from F_{13}^{+} (3747) to F^{-} (4574)? F^{-} Lac$_{85}$ P Ara$_2$ Gal$_2$ Xyl$_2$ Mtl, Mal, S^R.

Experimental conditions to set it up. : tester { Lac$_{85}$ Pur^{-}. F^{-} Lp^{+} W4642 / S^R W4647 } F_{13} V_6^R M

Use excess F^{+} (10 times more). (Add 1/2 ml F' to 10 ml F^{-}) Blend the mix with mixer. 20':10, 25':10, 30':10

Seed on Blac Sm. pick Lac^{+}. Purify each Lac^{+} on Blac Sm.
Restreak the Lac^{+} on Blac Sm. again.
Pick Lac^{-} and Lac^{+} from the segregants. Test sex selecting X^{+}
10 10
on M glucose. seeded W6- on it.

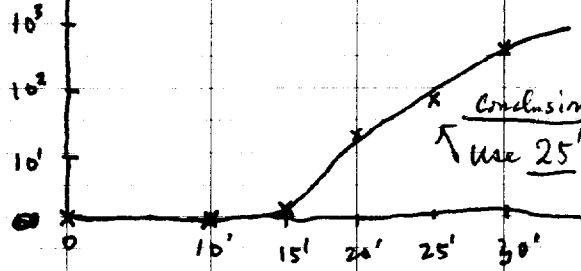
Time of Interruption : 0' 10' 15' 20' 25' 30'

Speed of blending 70 : 1 min. Dilute the mix into 5×10^{-4}

Result :

1). Timing experiment : at 34°C, standing in water bath.

Time of blending after mix	0'	10'	15'	20'	25'	30'
# of Lac^{+} S^R	0, 0, 0	0, 0, 0	0, 1, 0	11, 9, 12	38, 35, 23	155, 129, 121
Σ Lac^{+} S^R	0	0	1	32	96	405



Lac^{+} transfer by F_{13} (3747) in Pen. at 34°C,

Conclusion: ↑ use 25' and try more diluted 1/10 sample. use 10 plates for only 25'.

2). Repeat this experiment at 37°C. in incubator room. in pen. 10 ml. at 37°C on rotator
on rotator.

- Cultural age : 13:00 ~ 16:00
- Inoculum size : 0.5 ml / 10 ml of pen. (overnight grown)
- Mix 10 : 3747 with ~~4574~~. 1 ml : 4574.
- Select on Blac Sm. 10^4 : 0.1 , 0.5

4:20 ~ 4:45

Result :

Lac^{-} / Lac^{+}
428 : 4
967 : 4
419 : 3
429 : 4
454 : 3

Streak Lac^{+} on Blac Sm. and pick Lac^{+} & Lac^{-} colonies & test sex-compatibility.

continue to 1536

This data is in 1536.

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Experiments to show whether W4630 (Lac_2) homogenote or heterogenote?

22/II. 1960

REF: Lac_2 F^+ : 2242
S^R Lac_2 F^- : 3112.

1 2 3 4 5 6 7 8 9 10

Experimental design:

1. Pick independent Lac_2^+ reversions and purify them on BLac. (Pick 20.)
2. Treat those Lac^+ with AO, and see are there any Lac^- segregate or not.

As a control, use Lac^+ revertant from 3112. (This strain should not segregate Lac^- after AO-treatment.) also $3112\ Lac_2^-/Lac^+_{ex}$. (This segregate Lac^- by AO)

3. Check Lac^- segregant for Lac_2-ness.

Points expected for this expts.

1. If it is homozygote for Lac_2, all the Lac^+ does not produce Lac^-_2.
2. If it is homozygote for Lac_2, three possibilities can be expected.
   a. Lac_2^- will be segregated after AO treatment from Lac_2^-/Lac_2^+ (exogenotic revertants).
   b. Lac_2^- will not be segregated after AO-treatment from Lac_2^+/Lac^-_{ex} (endogenotic revertants).
   c. Suppressor mutation in outside of F_{13} will not segregate Lac^-.

Experiments:

1. Purify W4630 on EMB Lac Sm. and pick 20 Lac$^-$ single colonies. Suspend these colonies in 1 ml distilled water and spread on BLac separately. Test all F_{13} Lac_2^- by cross-streaking with 4573 ($Lac_{\Delta 5}$ F^-) on MLac. ~~Inoculate the colony in 8 ml nut. broth.~~ (See back page 1)
2. ~~Inoculate 0.2 ml of the culture on MLac and~~ Incubate them for 24 hrs. at 37°C. Pick Lac^+ from each Lac^- 4630 colonies.
3. Purify the Lac^+ colonies on BLac. 20 colonies.
4. Treat these revertants with AO. exponentially growing cell.

Results:

Medium : Nutrient broth : pH. 7.6 : AO : 20γ/ml : 37°C. overnight @100 rpm-Amco

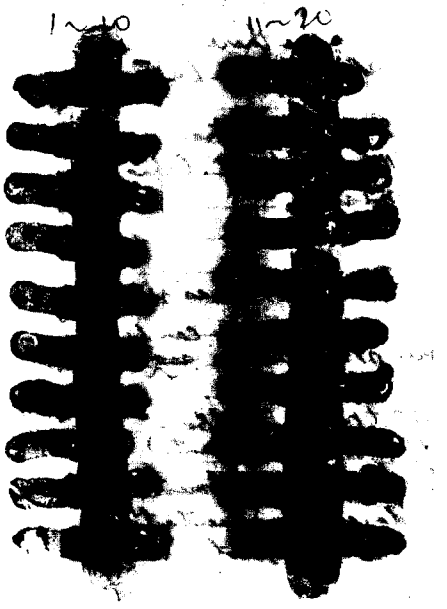
Inoculum size : one loop of 10^7 cells/ml 4630 Lac^+ revertants / 1 ml of medium. 20γ/ml of AO.

Isolation No. of 4630 Lac^+ Revertants.	Lac ν or + before AO	Lac ν or + after AO	Isolation No. of 4630 Lac^+ revertants	Lac ν or + before AO	Lac ν or + after AO
1	No	No	16	No	No
2	No	Segregate	17	No	No
3	No	Segregate	18	No	No
4	No	No	19	No	Segregate
5	No	No	20	No	No
6	No	Segregate	control.		
7	No	No	3112 Lac^+	No	No
8	No	No	3112 $Lac_2/ox\,Lac^+$	Seg	Seg
9	No	No			
10	No	No			
11	No	No		Seg: 2	Seg: 6
12	No	No	Σ Lac ν / total No. tested	No: 18	No: 14
13	Segregate	Segregate			
14	Segregate	Segregate			
15	No	No			

Streak the treated & untreated cells on EMB Lac. and see are there any Lac^- segregation or not.

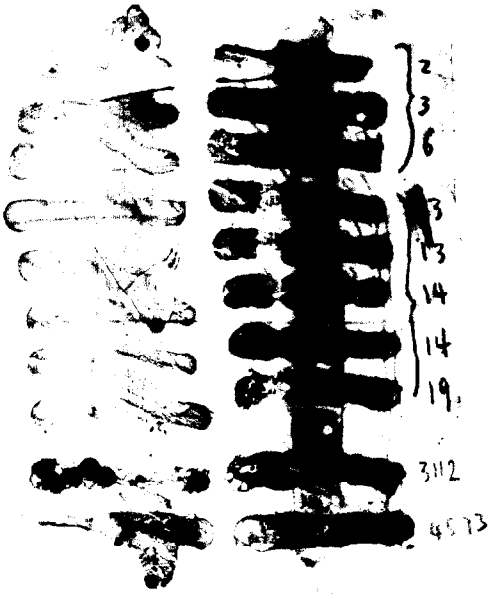
6. Restreak from 12, 13, 14, 15, 18, 19 (Single colonies and make sure.) 5. Pick Lac^- segregate and test the Lac marker by cross | x 3747 x Lac_2F_{13} | on MLac

4630
Isolated from streaking
on Phac Sm

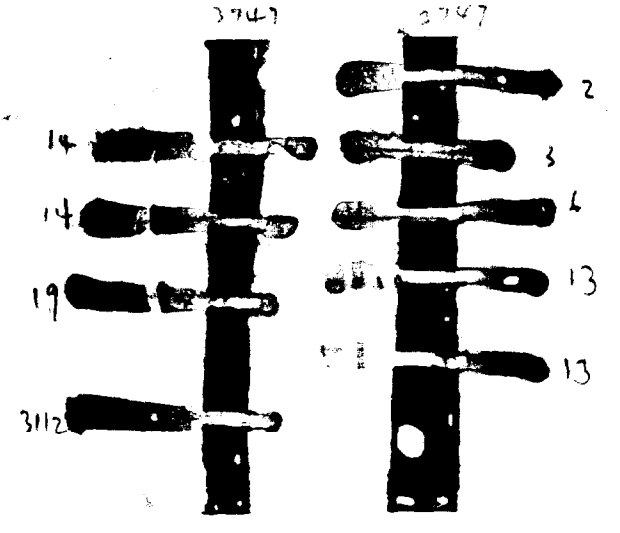


X 4573
m M Lac

segregants from Lac mutants of 4630



V4630 X3747
on M Lac



Compound structure of F' in 3747.
Testing for segregation of F' and F from primary exconjugate.

25/II ; 1960 REF:

Cultural age : exponentially growing culture in 10ml plain broth on rotator. (Inoculum size : 1ml/10ml plain)
4:30 ~ 6:30

Mix them : 3747 : 10ml + 4574 1ml.

↓

Keep them on rotator for 25'

↓

Dilute and chill them in distilled water, then blend the mix.

↓

Dilute it into 10^{-1} : 0.5 : 10^{-2} : $10^{-2} \times 5$: 0.1/plate

↓

Inoculate the 0.1ml on EMB Lac 9m.

Result :

	# of Lac⁻	# of Lac⁺
	367	208
	319	196.

Sex

	Lac⁺		Lac⁻		
	# of Lac⁺	Sex	# of Lac⁻	sex	
# 1	3	F13	7	F⁻ 5 ; F⁺ 2.	Tester 4506
# 2.	10	♂ 10	5	F⁺ 5	Media } MBlue. selected } Spit.
3.	8	" 8	9	F⁻ 9	
4	3	" 3	9	F⁻ 9	
5	5	" 5	9	F⁻ 9	[illegible] colonies which segregate : Lac⁻ P⁺ : 2 ; Lac⁻ P⁻ : 7
6	4	" 4	9	F⁻ 9	
7	2	" 2	8	F⁻ 8	
8	2	" 2	10	F⁻ 10	
9	6	" 6	9	F⁻ 9	

Conclusion : ① F' segregate F to their progenies after mating. But it seems not always occurs. Some F' does but the others are not.
② Fertility of Lac⁺ varies, some shows high the others shows lo. There seems no distinct difference.
Only a General principle is Lac⁺ is always male.

Test Infectivity of F and F'.

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Transformation of Gal with λ helper.
(Dr. Keiser's system)

22/III, 1960 REF:

Helper: 434hy C_I21 10^{12}/ml

dilute I, II in 2M NaCl. ID, IID

Recipient:
W3104 (434hy / T_1) infected with λ:
grown in P med
- .02M KPO_4 pH 7.0 : Conc. of Bact. 4×10^9/ml.
- .001 $MgSO_4$
- .001 $(NH_4)_2SO_4$ OD: 1.78
- 10^{-6}M Fe
- 0.5 mg/ml glucose.

DNA: Extract DNA from W4687 (c.f. 19/III/60). : Dialyzed at 5°C for 3 hr vs. 2 l. vs. .15M NaCl, .01 Tris pH 7.4 ID IID.

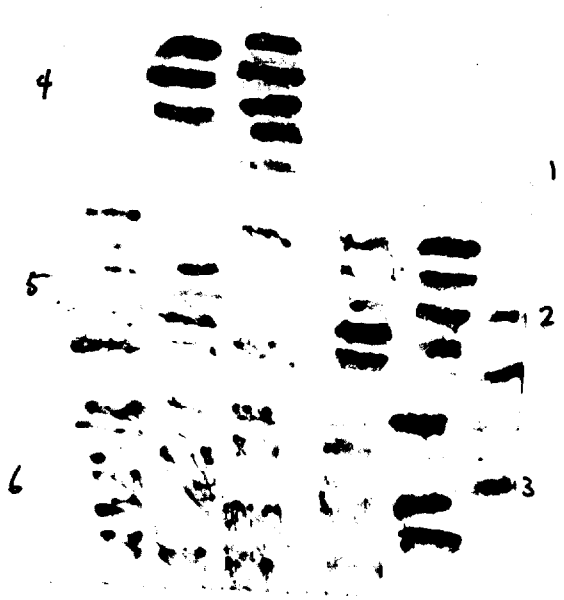
Reaction Mix:-
0.1 ml DNase (control).
0.1 ml DNA (200 µg, 20 µg) W4687
0.1 ml Bacteria

Keep the mix at 30°C for 1 hr.

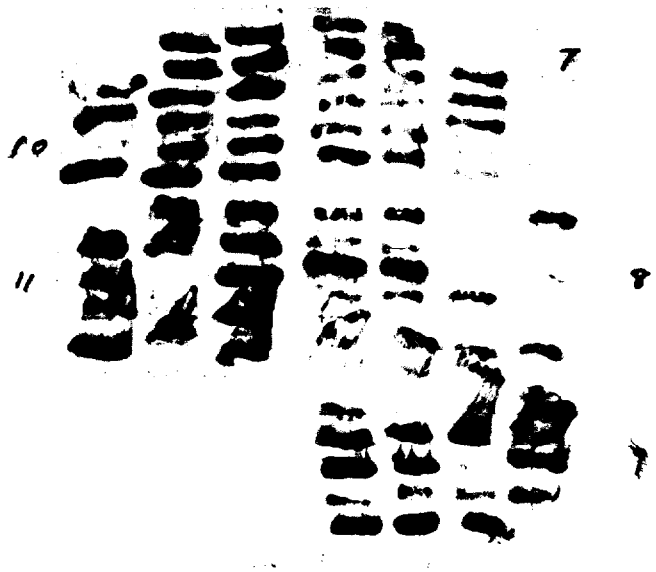
	on TTC Plaque	Gal	(Dil's count)	on B Lac Sm	DNase 30 µg	DNA	# of Gal+/− from replica (on BGal)
1			(1000)	0	—	I 0.1 ml	+48
2	341	198	(500) red	0	—	I 1/10 0.1 ml	+2/66 ±4
3			(10) red	0	—	II 0.1 ml	+0, −6 ±2
4	73	41	(10) red	0	—	II 1/10 0.1 ml	+3, −5
5	(423×4) 1692		(500) blue	0	—	ID 0.1	+0, −10
6	(423×2) 846	211	(500) blue	0	—	I_D 1/10 0.1	+0, −17
7	58		(2000) blue	0	—	IID 0.1	+0, −17
8	44		(1000) blue	0	—	IID 1/10 0.1	+0, −18
9	0	660	(500) red	0	0.1	ID 0.1	+1, −14
10	136		4/500 by blue	0	—	λdg DNA 1/6 10^4 0.1	+15, −0
11	0	73	100 red	0	—	—	reversion control. +0, −14
12	0	0	0	0	—	I 0.1	
13	0	0	0	0	—	II 0.1	
14	0	0	0	0	—	ID 0.1	
15	0	0	1	0	—	IID 0.1	

F8
3/4 on W3104
λdg Gal+ — infective W3104
λdg 0
Gal+ λp+ (3110 ...)

Conclusion:
1. DNA Extracted from 4687 shows plaque forming activity on W3104. (434hy/T_1)
2. Some colonies which form TTC+ shows gal+ quality on BGal.
3. Such activity will be loose by treatment of DNase.



on B Gal.



on B Gal.

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Extraction of DNA from Gal-heterogenote W3094D. (4/ex 2)

23/III 1960 REF:

[redacted]-source : (from W3094 derived strain (4pt Gal+ F⁻)) 4/ex 2. (Made by HFT-Gal$_2$ transduction)

21/III

1. Shake it on shaker (2.5 l in 4 l flask.) at 37°C for overnight.

22/III

2. C.f.g. collect, and wash the cells with 0.14 M NaCl 3 times. 4000 g — 20 min.
3. Store it in refrigerator for overnight at ca. 5°C. (Ca. 20 hrs)
4. Treat with duponol (30 ml : 15 % : at room temp :) for 5 hrs. pH. 7.5 7:10 → 12:10
5. p.p.t with 95 % of Et-OH.
6. Wash the p.p.t with 80 % Et-OH. twice.
7. Suspend the p.p.t in saline-citrate soln with aid of glass-homogenizer 60 ml.
8. C.f.g. 3,000 xg : 20 min and repeat this twice.
9. Sediment saved.
10. Extracted twice in 40 ml of 2 M NaCl on Magi Mix. at 4°C for 30'.
11. C.f.g 20,000 xg for 20 min. at 4°C.

23/III

12. Add it to 100 ml of 95 % Et-OH.

24/III

13. Fibers suspended in 5 ml of 2 M NaCl.
14. Deproteinize with sevag's method : Add equal volume of CHCl$_3$ and mix. Shake it for 5 min. Repeat [redacted] times. No interface.
15. P.p.t with Et-OH [redacted]
16. Dissolve in small 2 M NaCl. soln.
17. Digest RNA with RNase. (50 γ/ml. final. : 1 hr at 37°C.)

1 mg/ml lysozyme 10 min : 37°C

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Mating capacity of dupnonlated 3747.
(15% dupond).

11/III, 1960 REF:

Method :

Use 20 ml culture of 3747 (phenary grown for overnight at 37°C)
(2 tubes of 10 ml)
Recipient 4573.

↓ C.f.g.

↓ resuspend it in 1.5 ml of H_2O.

↓ Add 1 ml of the suspension to 5 ml of 15% dupond.

↓ keep it at 37°C.

Take 1.0 ml of the sample, add 2.0 ml of H_2O
↓ C.f.g. {12000 rpm at each time 0 hr, 1 hr, 3 hr.
20'
wash it once with 5 ml of H_2O.

↓ resuspend it in 1 ml of H_2O.

Use this as a dupnonlated cell.

↓ Use 0.2 ml.

Test mating capacity. for survival, for mating, for DNase digestion.

0.2 ml 3747 dupnonlated + 4573, 1 ml + Pan 8 ml.
Exp. grown.

Result :—

Volume of dupnonlated 3747 susp. used	3:00	4:00 +DNase	5:00	No DNase added.		
	1 hr	2 hr	3 hr	1 hr	2 hr	3 hr
# of survival colonies on EMB Lac plate 10^{-7} / 0.2 ml. of the treated suspension.	17, 20	4, 2	0, 1	14, 12	5, 2	4, 3
# of Recombinants / plate. 0.2 ml / plate. on Mlac Sm.	0, 0	0, 0	0, 0	0, 01	0, 0	0, 1

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Mating capacity of duponolated 3747. (10% duponol).

12/III ; 1960

REF:

	1	2	3	4	5	6	7	8	9	10
		• Cultural age :		overnight culture of 3747 in Penassay broth. (30 ml)						
		Recipient :		4573.						
		• Treatment :		10% duponol : final conc. at 37°C.						
		• Time :		1 hr. (2:30)	2 hr. (3:30)	3 hr. (4:30)				
		• c.f.g. :		aerval ; 12000 rpm. for 20 min.						
				3.5 ml H_2O + 1.5 ml. duponolated 3747						
				Wash it with ↓ 5 ml of water						
				Resuspend it into ↓ 1.5 ml of H_2O.						
		• Result :								

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Mating capacity of duponolated 3747.
(10% duponol)

12/XII ; 1960

REF:

1	2	3	4	5	6	7	8	9	10
	• Cultural age :		Overnight culture of 3747 in Penassay broth. (30 ml)						
	Recipient :		4573.						
	• Treatment :		10% duponol : final conc. at 37°C.						
	• Time :		1 hr. (2:30)	2 hr. (3:30)	3 hr. (4:30)				
	• c.f.g. :	serial	; 12000 rpm. for 20 min.						
			3.5 ml H_2O + 1.5 ml duponolated 3747						
			Wash it with	↓	5 ml of water				
			Resuspend it in	↓	1.5 ml of H_2O.				
	• Result :								

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Transformation in E. coli K-12.
using Helper (Hfr, F+, F−)

14/III ; 1960 REF:

	1	2	3	4	5	6	7	8	9	10

• Strains used:

DNA from 3747 : 1 month old. (Feb. 10. in 2M saline. kept in refrigerator.
Recipient ; 4573 : F− Lac85
Helper : 4574 F+ : F+ Lac85
4574 : F− Lac85
4049 ; Hfr, (M P V_0^R Lac$_{89}$ Hfr,) m.

(These culture was taken from old broth culture). (several weeks)

• Cultural condition:

Inoculum size : 0.2 ml / 8 ml penassay. ; Incubate at 37°c on rotator. 11:00 ~ 2:00

Use exponentially growing cells.

• Experimental condition:

Inoculate 0.1 ml of Recipient
+
0.1 ml of Helper
+
0.1 ml of DNA. (ca. 20γ /ml)
+
1.5 ~~1.8~~ ml of Penassay broth.
(make whol volume into 2 ml.). 2:15~ ca. 10^9~10^{10}.

→ Incubate it at 37°c for overnight on rotator. → Seed it on Hlac Sm.

Use 0.2 ml / plate EMlac Sm.

• Result : After overnight shaking concentration of cells become

Exp. #	Mix: Recipient	Helper	DNA	# of Lac$^+$ X$^+$ on Hlac Sm
1.	4573	+ 4574	+ 3747	0, 0
2.	4573	+ 4574 + 4574F+	+ 3747	0, 0
3.	4573	+ 4574F+	+ 3747	0, 0
4.	4573	+ 4574F− 4049	+ 3747	0, 0
5.	4573	+ 4049	+ 3747	0, 0
6.	4573	+ 4574 4574F+ 4049	+ 3747	0, 0
7.	4573	+ 4574F+ 4049	+ 3747	0, 0
8.	4573	—	+ 3747	0, 0

Test sterility of DNA. on Blac. 0.1 ml / pen tube — OK ; Steril after overnight shaking on rotator which is same as 1 ~8.

order of additions of the DNA cultures: I II II.

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DNase-sensitivity of λ mating capacity of Sm-killed cell. ②

14/III ; 1960 REF:

Experiment : Strain. : 3747 overnight culture at 37°C.
↓
+ Sm 1 mg : final conc.
↓
keep ~~6~~ incubation overnight.
↓
c.f.g + resuspend it in 5 ml of Penassay broth.
↓
0.2 ml bacterial suspension. + 0.1 ml enzyme soln.
+ DNase 200 γ/ml
+ RNase 100 γ/ml
+ lipase.
4:00 ~ 5:00.
Incubate it at 37°C for 1 hr.
↓
+ 4573 1 ml + Penassay 1 ml
↓
Incubate the mix for 1 hr. at 37°c.
5:00 ~ 6:00
↓
plate the mix onto MLacSm. 10^{-1} : 0.1 ml / plate.

Result :

	● No enz.	+ DNase	+ RNase	+ lipase
# of Recombinants / plate EMLacSm 10^{-1} : 0.1 ml				
# of survival cells/ml on EMB lac. 10^{-1} : 0.1.				

Conclusion : seems no difference.

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Transformation of E. coli K-12.
(Hfr, F^+, F^- Helper). without shaking.

15/III. 1960 REF:

$Lac^-_{85} \rightarrow Lac^+$

- without shaking. Idea : DNA may be taking ⊕ at the time of infection of F^+ to F^- with
- Cultural age of strains used.
- 0.2ml / 10ml pen. 10:30 ~ 1:30 at 37°C.
- Conc. of DNA. 200γ/ml : final concentration.
- Experimental condition for transformation of E. coli.

Mix these bacteria and DNA.

0.1 ml	4049	Hfr, helper.
0.1 ml	4574	F^- helper.
0.1 ml	4574	F^+ helper.
0.1 ml.	4573	: Recipient
200γ/ml : 0.2 ml/2ml		: DNA. (kept in refrigerator for 1 month)

final Ca. 10^7 cells/ml for each strain.

Make this mix into 2ml with pen. broth.

↓

- keep incubation for 3 ⊕ hrs / overnight at 37°C without shaking. (just stand the mix.)

↓

- Score $Lac^+ S^R X^+$.

↓

Inoculate 0.2 ml of these cultures on M Lac Sm agar.

- Result :

Vol. of Pen. (ml)	Exp. #	Constitution of the mix: Helper	DNA	Recipient	# of transformants/plate 3 ⊕ hr	24 hr
1.6	1	4574 F^+	3747	4573	0, 0	0, 0
1.5	2	4574 F^-	3747	4573	0, 0	0, 0
1.5	3	4049	3747	4573	0, 0	0, 0
1.4	4	4574 F^+ + 4574 F^-	3747	4573	0, 0	0, 0
1.4	5	4574 F^+ + 4049	3747	4573	0, 0	0, 0
1.4	6	4574 F^- + 4049	3747	4573	0, 0	0, 0
1.3	7	4574 F^+ + 4574 F^- + 4049.	3747	4573	0, 0	0, 0
1.6	8	—	3747	4573	0, 0	0, 0
1.4	9	4574 F^+ + 4574 F^- + 4049	—	4573	0, 0	0, 0
10 Km		3	1	2		

Conclusion : No transformants were observed under this condition.

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Extract DNA from W4687.

(Use this DNA as Gal-transfer.)

18/III. 1960 REF:

EML.

1. Grow up cells in penassay broth (Inoculated from broth given by Esthy)
10 ml. : overnight grown.

2. Inoculate the 10 ml into 2 l of Penassay in 6 l flask.
Shake it for overnight at 37°C. 2:00 ~

3. Collect cells by centrifugation.

· Continuous flow.
refrigerating centrifuge.

· Bactogar.

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Isolation of DNA from 4687.

P_1 → × 4520
→ × 4621 F^- Ind Lp^+ Gal_{27}^{--} S^R
#74 (Gal+/–, F_8; P_1^+? 9 Lp^+?)

19/III, 1960 — REF:

18/III — 1. Strain W4687 grown in pen at 37°C with heavy shaking for 6 hrs. on shaker. (2.5 l. in 6 l flask)

2. Collect cells with Servall centrifuge: 4000 rpm. for 10 min. Wash the cells twice with 0.14 M saline. Ca. 3 ~ 4 grs ?

19/III — 3. Let it suspend in H_2O by addition of 2 ml H_2O.

4. Keep it in refrigerator: 9:30 PM ~ 7:30 AM.

5. Add the bacterial suspension to 30 ml of duponol soln (15% pH 6.8)

6. Keep it at room temp for 5 hrs (7:30 ~ 12:30).

40°C — 7. P.P.T. with 95% Et-OH.

8. Wash the p.p.T. with 80% Et-OH. twice.

9. Suspend the pp.T in Saline-citrate soln. (0.15 M) (0.05 M) with the aid of glass homogenizer (Ten Broeck)

10. C.f.g. & save the ~~sup~~ pellet.

11. Extract DNA with 40 ml of 2M NaCl (~~2 times~~ Twice) on Magi Mix at 4°C for 30'

12. C.f.g at 20,000 xg for 20' at 4°C.

19/III — 13. Add the supernate to 100 ml cold 95% EtOH.

14. Suspend pp.T in 7 ml of 2M saline.

15. Keep the solution at 5°C for overnight.

20/III — 16. Deproteinize with $CHCl_3$–octanol method (5) : (1) { $CHCl_3$ + octanol 1 = DNA soln 1 }

Make 5 × Sevag.

17. ~~Deprotein~~ p.p.T : DNA with 80% Et.OH.

18. Dissolve it in 5 ml 2M NaCl. (Sterilized) → Sample I.

19. Take 2.5 ml of the soln twice and add 50 γ/ml of RNase (Boiled) (final conc.) Keep at 37°C for 60'.

20. P.P.T. DNA with 80% Et-OH and dissolve it in 2M salt. → Sample II.

11/18/59 EML 59.67

P.1 + W4520 HFr

——x W 4621
F^- Ind$^-$ Lp$^+$ Gal_2^- y$^-$ S^R

transductants. 73 + 74

Gal +/–
F'8
P_1+ ?
Lp+ ?
S^R Ind$^-$

re isol. 3/15/60 as W4687
#74
as Gal +/- heterogenote.

(i.e. Segregation Pattern: details later)

♀ Gal_6 S^S 3096
S^R 3996

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DNA-analysis.

20/III ; 1960 REF:

Ref: Burton, K. : Biochem. J., 62, 315~323 (1956)

Reagent:

1. Diphenylamine Reagent.

Dissolve 1.5 gr of Fisher certified diphenylamine in 100 ml of redistilled acetic acid and add 1.5 ml of Conc. H_2SO_4. Store in brown bottle.

On the day it is to be used 0.10 ml of aqueous acetaldehyde (16 mg/ml) is added for each 20 ml of reagent req'd.

Procedure:

The author states that extraction with 0.5 N $HClO_4$ at 70° for 15 min. (2x) will liberate 95% of the nucleic acid from E. coli. This is comparable to extracting at 90° for 15 min. with 5% TCA.

The final conc of $HClO_4$ must be 0.5 N before add'n of diphenylamine reagent.

1.0 ~ 2.0 (usually 1.5 ml) of sample is mixed with 2 volumes (3 ml) of diphenylamine reagent containing acetaldehyde.

Tubes containing known amounts of Standard DNA and a blank containing 0.5 N $HClO_4$ but no DNA are also prepared.

Color is developed by incubating at 25~30°C for 16~20 hr. Provided that all tubes are at the same temp, constancy of the temp. is not essential, and variations at least between 25°C and 35°C do not appreciably affect the readings.

Read at 560 mμ.

Added. read at ~ P.M.

Standard: 5γ, 50γ/ml of DNA.

Use 0.1 ml (5N) P.C.A to 1.5 ml of sample and 3 ml

Volume of Sample used.

Conc. Standard		ml Sample I		ml. Sample II	
5 0γ	5γ	0.1	0.3	0.2	0.3
.85	.075	0.36	1.9	0.24	2.0

0.1 2 0 ml. EN 0.045 1.5 ml

0.015 3 ml.

Exp.:-

0.1 ml + 1.4 ml H_2O + 0.15 ml PCA (5N)

0.3 ml + 1.2 ml H_2O + 0.15 ml P.C.A (5N)

↓

+ 3 ml diphenylamine reagent cont. CH_3CHO.

Standard (deoxyadenylic acid. 5γ, 500γ/ml.)

0.1 ml + 1.4 ml H_2O + 0.15 ml P.C.A (5N)

blank — + 1.5 ml H_2O + 0.15 ml PCA (5N)

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Pentose determination.

29/III ; 1960 REF:

Ref. Colowick & Kaplan "method in Enzymology" Vol III, p. 87.
(Dische modification: J. B. C., 204, 983 (1953))

Reagents:

Acid Reagent – 10% $FeCl_3 \cdot 6H_2O$
0.5 ml of $FeCl_3$ sol'n in to 100 ml of Conc. Hcl.

Orcinole Reagent – 6% soln of orcinol in 95% Et. OH.

Procedure:

To 1.5 ml of unknown is added 3 ml of the $FeCl_3 \cdot 6H_2O$ (acid reagent above).

Add 0.2 ml of freshly prepared Orcinol soln

Heat in Boiling H_2O bath for 20 min.

Read in Klett with #66 filter.

Comments:

Under these condition, both ribose & ribose-5-PO4 are completely developed in 20 min.

No other sugars besides pentoses can be present.

Standard soln of ribose used as control.

Standard: 5 to 50 γ/ml. : final.
(1/10)
ribose. soln is used as standard

Vol. Sample (ml)
0.1 ml

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2nd Extraction of DNA From 4687.

25/III ; 1960 REF:

1. Grow up cells in penassay (2.5ℓ) on rotary-shaker for overnight at 37°C.
2. C.f.g. & wash two times. with 0.14 M saline. Store in refrigerator at 5°C for overnight. 25/III
3. Add 1% (pH. 7.5 ajusted 0.5 ml of 0.01 N HCl) @, 30 ml of Onpond sol. and keep it at room temp for 5 hrs. 7:30 ~ 12:30
4. p.p.t with 95% of Et-OH.
5. Wash the ppt with 80% Et-OH twice.
6. Suspend the ppt in 60 ml of saline-citrate sol. with aid of glass-homogenizer.
7. C.f.g. (30,000 xg : 20 min) Sediment saved. Repeat this three times.
8. Extract twice in 40 ml of 2M NaCl on Magi mix at 4°C for 30'.
9. C.f.g (20,000 xg : 20 min) at 4°C.
10. Add it to 100 ml of 95% Et-OH. 36/III
11. Fibers suspended in 10 ml of 2M NaCl.
12. Deproteinize with Sevag's procedure: $CHCl_3$ 5 : N-octyl alcohol 1 Mix 1 : 1. Repeat 5 times.
After 7& times Sevag, there is no interface.
13. p.p.t with 100 ml, 95% Et-OH.
14. Dissolve in 10 ml of 2M saline. ——4687 I

80% 200 ml = 95% 168 ml + H_2O 32 ml

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Extraction of DNA from 4520. ($H\ \lambda_L^2\ F_8$)

21/I ; 1960 REF:

1. Grow up cells for 6 hrs in 2.5 l pan (in 6 l flask) with shaking at 37°C.
2. Wash cells 3 times with 0.1 M saline. keep it in refrig. at 5°C for overnight
3. Treat with 15% duponol at room temp for 5 hr. 7:30 AM ~ 12:30
   cell suspension was not well homogenized : looks bad.
4. p.p.t. with 95% Et-OH.
5. Wash ppt with 80% Et-OH. twice
6. Suspend p.p.t in Saline-citrate soln. with the aids of glass-homogenizer
7. C.f.g. it (@ 30,000 xg). Saved p.p.t. Repeat 2 times.
8. Extract DNA with 40 ml of 2M saline. (2 times.) (20 min extraction at 5°C with Mag. Stir
9. p.p.t. DNA with 95% Et.OH. 100 ml.
10. Dissolve the p.p.t. in 2 M salt. Store in refrigerated at 5°C.
11. Deproteinize it 4 times by Sevag's procedure. there is very little interface insoluble remain.

    In the course of this procedure, the sample was kept at room temp. For 1 hr.
12. p.p.t with 100 ml of 95% Et.OH.
13. Dissolve it in 5 ml of 2M saline. (steril.)
    • this sample seems more easily dissolved in 2 M salt than the other samples. Also seems very thick.

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Biological activity of DNA extracted from several Strains. (Gal⁻ → Gal⁺ : plaque forming activity).

1/11 ; 1960 REF:

	1	2 Lysogenic for	3	4	5	6	7 Condition:	8	9	10
	Recipient :	434 hy	: Gal+		Helper : 434 hy.		at 30°C. for 1 hr.			
1										
2										
3										
4										
5	plate #.	Strain Extracted DNA	0.1 ml DNA 0.1 ml Bact.	0.1 ml DNA : 1 ml of Media		# of plaques	# of gal⁺ on T.T.C & on MGal (T.T.C : soft agar)			
6										
7	1	4687 "New"		0.1	TTC	0	37 ?			
8										
9	2			0.1	EMGal	0	0			
0										
	3				TTC	Ca. 1000	0			
1										
2	4				EMGal	527	0			
3										
4	5	from 3094.		0.1	TTC	4	1			
5										
6	6			0.1	EMGal	1	2			
7		4^+ 4/4x² (dg)								
8	7				TTC	480	0			
9										
0	8				EMgal	199	0			
1	9	4520		0.1	TTC	4	1			
2										
3	10	4^+ Gal⁺/Gal⁺ F_8		0.1	EMGal	10	0			
4										
5	11	(Through this DNA seems no good must be repeated.			TTC	3	4			
6										
7	12				MGal	0	0			
8										
9	13	old 4687		0.1	TTC	3	6			
0										
	14			0.1	EMgal	5	1			
1										
2	15				TTC.	395	2			
3										
4	16				Mgal.	46	0			
5										
6										

Conclusions:

1. DNA from 4687, & 3094 have plaque-forming activity.
   a. It forms plaques.
   b. Such activity will disappeared by DNase-treatment.
   c. That activity is retained or in preparation during the process of preparation of DNA
2. There is no transforming activity for Gal.

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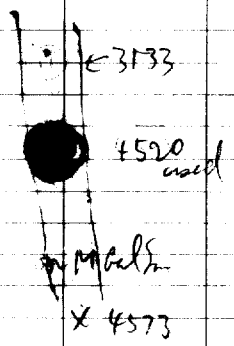
2nd Extraction of DNA from 4520.

2/14 1960 REF:

1. Let 4520 grow for overnight on rotator.
2. Collect cell with 0.14M saline. Wash 3 times with saline.
3. keep it at 5°C for overnight.
4. lyse cells with Duponol (15%) for 5 hrs. at room temp.
5. ppt. & rinse it with 95% Et. OH
6. Wash cells with Saline-citrate mix 60 ml. 3 times.
   0.14M 0.05M.
   Suspend the ppt in Saline-citrate mix. Mix with Mag-mix. for 20 min.
   c.f.g. x 50,000 g for 20'. Save sediment.
7. Extract DNA with 2M saline (40 ml : 2 times) with Mag.mix. twice
   30'. at 4°C. 2:15 ~ 2:45.
8. c.f.g. 20,000 xg for 20'. Sup. saved.
9. Add. it to 100 ml of 95% Et-OH.
10. Dissolve it in ± 10 ml of 2M NaCl.



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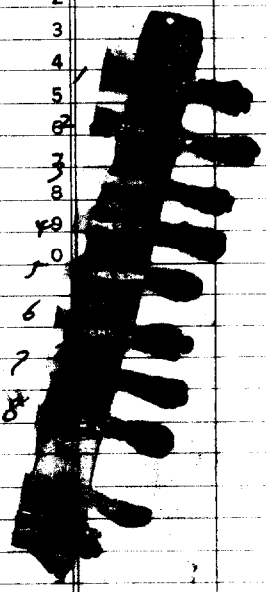
W 3104 F_8

11/14. 1960 Made by 4520 F_8 × 3104 REF:

Test of compatibility.

4520 × 3104



3104

4520

Gal+ F_8

1. W3104 F_8 : grow up cells in 10 ml broth. 4. Add it in ~~25l~~ pm. Shake it for 5 hr. on rotator.
2. C.f.g. & collect cells by small c.f.g. wash it twice with 0.14 M saline.
3. Treat with 15% dupanol at room temp for 5 hrs. 10:30 AM ~~—~~ 3:30 pm.
4. P.P.T. DNA with 95% Et-OH (100 ml) Rinse it twice
5. Wash p.p.t. with citrate - Nacl - Soln. twice, Save sediment. Homogenized by pestle-type homogen.
6. Extract DNA with 40 ml of 2M Nacl twice.
7. P.P.T it with 100 ml of cold Et-OH. Dissolve it in 10 ml of 2M Nacl

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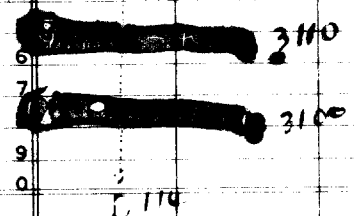
B 114 (W3104 1dg) lyse by U.V. No plaque
Immune to λ infection.

15/11 1960 REF:

1. Grow up cells in 2.5 l pen. for overnight at 37°C.
2. Collect cells keep it in refrigerator for overnight. (5°C).
3. Lyse the cells with 15% chloroform at room temp (11:00 ~ 4:00) for 5 hrs.
assay of plaque forming activity. 10^{-1} : 0.1 / plate on 3110.
4. Wash the cells with ~~95%~~ 70% Et-OH. (100 ml.) CHCl₃-treated. — c.f. 3 - Store in refrig.
5. Wash the precipitate with citrate-saline soln. twice.
6. Extract DNA with 2M saline : 40 ml : twice. on Mag. mix. for 30'. at 5°C.
7. P.P.T the extract with 95% Et-OH. (100 ml), & Dissolve it in 2M saline (10 ml)
8. Deproteinize it with Octanol-CHCl₃ soln. (5 times) until no interface
9. P.P.T DNA with Et-OH and redissolve it in 5 ml of 2M saline.
10. Store in refrigerator at 5°C.

Test for λ production.



No plaque was found on 3110.

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Labeling DNA with P^{32}

1960. REF:

Medium low in phosphate for making P^{32} DNA in E. coli.

Ref. Lehman, Doesman et. al. : J. B. C., 233, 163 (1958)

The medium contains .34 μM of $PO_4^{\equiv}$ / ml.
Coli requires ca. .70 μM/ml for optimal growth.

Preparation of Medium.

Na-lactate 70 m Mol.
to 100 ml of hot lactic acid (Mark 85%), add 40% NaOH (~100 ml) to pH. 7. Boil 3 min. Check pH.
Cool & filter if necessary. Soln is yellow viscus.
concentration ~ 6 M (~100 ml)

glycerol	4g
NaCl	5 gr.
KCl	2 gr
NH_4Cl	1
$MgCl_2$	1 m Mol
$CaCl_2$	0.1 μ Mol
gelatin	0.01 gr
P (as ortho PO_4)	10 mg P
S (as $SO_4^{\equiv}$	10 mg S

To 1,000 ml should be pH. 7.
generation time : ~ 1½ hr.
cells smaller than in most of the synthetic med.

Preparation of Stock Soln.

A.) Na-lactate ~6 M 58.0 ml
glycerol 95% 16.0 ml
NH_4Cl 5.0 g
→ 200 ml pH. 7.2

B)

NaCl	25g		
KCl	10		
$MgCl_2$	0.3 M	16 ml	
$CaCl_2$	1 M	0.5 ml	
gelatin	1%	5 ml	
KH_2PO_4	pH. 7.4	1 M	1.7 ml

to (5 ml) 200 ml. pH. 7.3.

C). Na_2SO_4 - 0.2% After autoclaving may be mixed with B (after B has been autoclaved)

Autoclave all 3 stock Soln 20'

Preparation of complete medium.

92 ml	steril H_2O (+ P^{32})
4 ml	A
4 ml	B
4 ml	C.

● continued to back page

Prep. of P^{32} med. — Want 0.6 ~ 0.8 μM P/ml

Add hot P^{32} + adequate cold KPO_4 1 M.

pH. ~7. to ~~adjust~~ give difference from 0.34 μM/ml for E. coli

for P^{32} studies, the P^{32} should be added to the water, autoclaved, and then neutralized with sterile NaOH.

(Best autoclave ~~with~~ while P^{32} in ~~dilute~~ acid, since any polyphosphates will then be broken down.)

Inoculum — can be grown in Bunsen. Use a small inoculum, and grow with vigorous aeration.

Labelling cells with P^{32}

c.f. Forro, F
13/II ; 1960 Exp. cell. Res. 12, 363~73 (1957)

P^{32} : PO_4 in weak HCl
conc. : 200 mc/ml.
~~Specific activity~~ : 55350 mc/g. of original P^{32}
date : 2-29-60.

purpose : 1. for autoradiography of bacteria.
2. Testing leakage of P^{32} from P^{32}-labelling bacteria after dilution.

Method ① grow up cells in H-medium for overnight at 37°C on rotator.

② ~~Wash the cell~~ Add the 10λ ~~[illegible]~~ of the culture to 9 ml. made by method as follows.

a. To 0.8 ml H_2O cont. (1/40 casa ord.) add 0.01 ml (10λ) 5 stock.
2% CH : 0.1 ml/10 ml H med. ; final conc. : 0.2%.
25% CH : 0.1 ml/2 ml H ; 0.25%

b Evaporate it and redissolve it in 0.8 ml H-medium.
adjust pH. 7.2. Add. 0.1N KOH ca. 0.05 ml.

③ Grow cells for 3 hr on shaker at 37°C.
1:100 ~ ~~1500~~
Inoculum size ca. 10^7
10λ .8 ml.

④ Wash cells 5 times with ~~[illegible]~~ water : 2 ml.

No of Planchet ① before wash (total)

# of	Washing/cell.	counts/min. x1000
2	0 (Sup.)	
3	1	
4	2	4553
5	3	1493
~~8~~ 6	6 (suspended in 2 ml)	12217 x 5
		61085 count/min

50% 120,000
x10 1200,000

10^{-3}

⑤ ~~grow~~ Add 0.25 ml of the suspension to 10 ml of protoplastic broth (c.f. back page) shake it on rotator.

⑥ make high speed c.f.g. (50,000 xg : 20') Save sediment. Add 1 ml of H_2O to both tube. Assay Radioactivity in supernate.

(Keep it 15' after + H_2O) c.f.g. x 3,000 x g

	7 + P	8 − P (x1000)
Count/.1 ml/min.	2284	2248

1200

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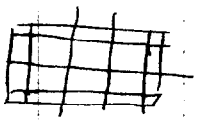
Autoradiography.

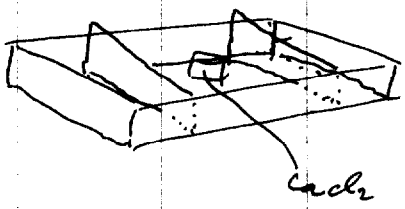
15/IV 1960

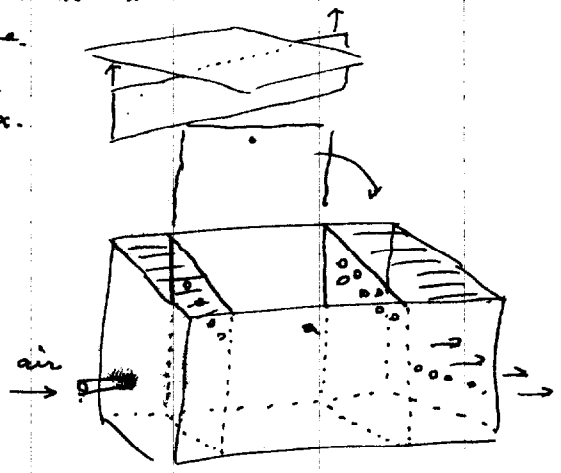
REF:

1. Treat slide-glass with gelatin.
   a. Clean slide-glass with soap, acid, rinse.
   b. Dip slide-glass in 0.5% gelatin solution. for min.
   c. Let it dry for min.

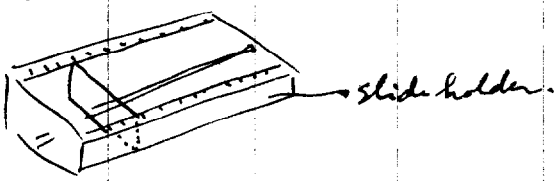
2. Put samples onto the slide-glass. Let it dry.

3. Cover the slide with gelatin film.
   a. cut gelatin-film into 6 pices. →
   
   b. ~~Take and~~ strip each pieces off from the plate.
   c. Let it place on surface of the water. (20 ~ 22°C)
      keep moisture ~~of~~ from
   d. Pick the film from water with slide. (film will attach very well on slide)
   e Dry it with air for 30 min. in Dark box.
      Pinch with clamp & hang it from wire.
   f. Take the slide out. and keep it in refrigerator with $CaCl_2$ in small box.





4. ~~8~~. develop the slide.
   Kodak Developer D-19.



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4520 ⟶ × 3104
18/11 ,1960 H F8 Lp+ Lp^s Gal- F-

REF:

Purpose : ① DNA-Donor for transformation experiment.
② Test a posibility of location of Lp-locus on F8 Gal segment.

Experiment 1. ~~Mix~~ Mix 4520 1ml & 3104 1ml, and incubate it overnight at 37°C.

2. Streak on ~~M~~ M Gal. purify each isolates on B Gal.

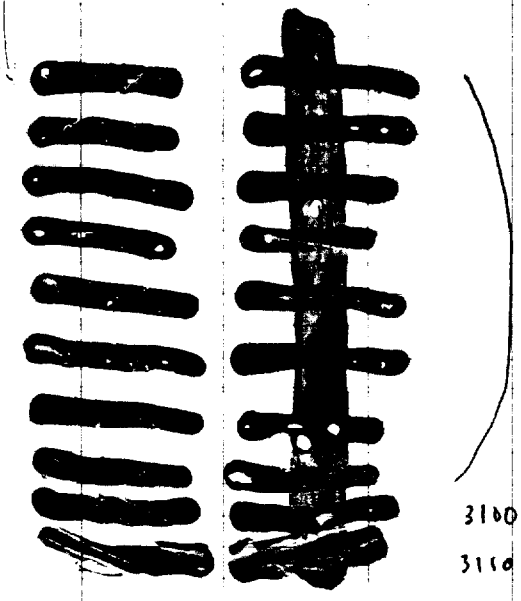
3. Test production of λ and sensitivity to λ. by cross-brushing on B-O.

Result :

all isolates (8) shows Lp^s character.

Save ~~m~~.

λ ~~3100~~ 3110



3100
3110

on B-O

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So called Shigella. from Dr yanofsky.

10/IV/ 1960 REF:

This strain was obtained on 3/2/60 & 4/9/60 : 5h/5.
as broth culture maintained in refrigerator.

1. purpose of this strain was for typing of Phage P_1.

2. Strange things happen. when tested phage-sensitivity. The phenomenon is as follows:

This strain is sensitive to 1_2 434 hy (Same as 1 except C loci).
But immune to 1.

Hypothesis given by Julian & Kaiser was that this shigella carries 1.

3. Cross-brushing exp. was done on B-O. vs. 3110 & 3100
cp^s cp^+
also UV & Un irradiated control.

Result shows Shigella produce phage (plaque forming-center) on 3110 and lyse the 3110. But lysis was not observed on 3100. This lysis is enhanced after U.V. irradiation. (5 second/plate)

4. Sugar fermentations and nutritional marker were checked.

Result is as follows. When Shigella is streaked on Blac, all (the colonies shows Lac.

X	Lac	Mal	Mtl	Ara	Gal
−	+	+	+	+	+

This marker shows strong syntrophy on Mglucose agar.

5. This strain can grow on SS-agar.

6. This strain is rod. Almost of the cells (were) Fla⁻ seems

Conclusion: ① This strain produce phage which has same host range as 1.
② and it can ferment sugars.
③ This seems rod-unflagellated bacteria. (not quite sure yet.)

If this is shigella, it should be untypical strain from above data.

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Extraction of DNA from B120.

3110 4p+ (carries mutant marker)

12/11/ ; 1960 REF:

1. Grow up cells in 2.5 l ~~flask~~ pan for several hrs. ~~in pan~~ at 37°C.
2. Collect cells and store it in refrigerator at 5°C for overnight.
3. Treat it with 15% duponol for 5 hrs at room temp.
4. ppt it with 95%, 100 ml of Et-OH, and suspend it in citrate-NaCl Soln. with the acid of glass-homogenizer. and wash it ~~twice~~ twice.
5. ~~Extract~~ Extract DNA with 5x 2M saline (40 ml) on Mag. mix. for 30'. twice.
6. ~~Dissolve it~~ ppt. with 100 ml of Et-OH, and redissolve it in 10 ml of 2M saline.

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Test of biological activity of DNA extracted from various strains.

20/IV / ; 1960

REF:

1	2 plate No.	3 DNA	4 DNase 30 μg/ml	5 # of Gal+ / # of Plaques
	1	A	—	0 / 526
	2	A	—	0 / 623
	3	A	0.1	2 / 1
	4	B	—	0 / 347
	5	B	—	0 / 325
	6	B	0.1	0 / 3
°	7	C	—	60 / 60
°	8	C	—	108 / 124
	9	C	0.1	4 / 2
	10	D	—	0 / 2
	11	D	—	0 / 2
	12	D	0.1	0 / 7
✓	13	E	—	1 / 3
✓	14	E	—	7 / 4
	15	E	0.1	0 / 3
	16	F	—	3 / 14
	17	F	—	~~+~~ 2 / 56
	18	F	0.1	contaminants / 14
	19	G	—	3 / 377
	20	G	—	1 / 323
	21	G	0.1	1 / 5
	22	H	—	0 / (993×2) 1986
	23	H	—	0 / (1207×2) 2414
	24	H	0.1	2 / 5
150 '3000 °	25	I	—	contaminant / 196
18000g	26	I	—	98 / 168

150 ← 3hrs. | 15 ... H1. 正 沢

6 plate No.	7 DNA	8 DNase	9 # of Gal+ / # of Plaques	10
27	I	0.1	2 / 2	
28	—	—	2 / 5	
29	A	—	—	
30	B	—	many contaminants. ca. 1000	
31	C	—	—	
32	D	—	—	
33	E	—	—	
34	F	—	—	
35	G	—	—	
36	H	—	—	
37	I	—	—	

Recipient : B 7/1 infected with 434hy.

DNA : 1. Duponol, 2M NaCl extracts of following strains.

A	W3100	: 3110 Lp+	+
B	W4520	: F_8 M⁻ Gal+ (λ)	+
C	B16	: Gal_4^- (λdg, λ) / $λ_2$	+
D	W[illegible] F_8	: Gal_4^- (F_8 Gal+)	0
E	B114	: Gal_4^- (λdg)	0
F	B120	: W3110 (λref)	0
G	W3094 ex 2	: Gal_4^- (λdggal_2^-, λ)	+
H	W4687	: $Gal_{2,7}$, P_1 Gal_{27}^+ F_8 λ	+
I	III × dg dilute	.01/.25 .1/5 .1/5 TCM ●	+

Summary : See back page.

Possibility : ① Only free phage DNA is active.

② Transduced DNA cannot make complementation : c.f. 7, 8, 13, 14, 20.

Strain number	genotype	plaque	gal-transduction.
3100	(φ^+)	+	−
B120	Gal_4 (φ ref)	+	−
B16	Gal_4 (1dg 1)/1$_2$	+	+
B114	Gal_4 (1dg)	−	− or ±
W4520	F_8 M$^-$ Gal$^+$ (1)	+	−
W3104 F_8	Gal_4 (F_8 Gal_4^+) φ	−	−
W3094ex2	Gal_4 (1dg 2$^-$ 1).	+	−
W4687	φ^+ $gal_{2\,7}$ (I_{1++} F_8)	+	−

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DNA - Assay of DNA. samples. isolated from various strains. and RNA

21/IV 1960 REF:

Ball- 5581
(remove filter) core.

	DNA			RNA.	
Sample of DNA		Volume	Reading.	Volume	Reading
3100	(0.1 ml, 0.3 ml	0.1	0.105		
B120	(0.1, 0.3	0.1	.036		
B16	{0.1, 0.3	0.1	.070		
B114	(0.1, 0.3	0.1	.056		
W4520	(0.1, 0.3	0.1	.034		
W3104 F8	(0.1, 0.3	0.1	.025		
W3094 ex2	(0.1, 0.3	0.1	.075		
W4687	(0.1, 0.3	0.1	.086		
50γ per ml Standard	500γ, 50γ, 5γ	0.1	.85, .00.		

4/28/60

Pentose determination

·1 & ·3 ml of unknowns

Standard curve. 5 → 50 γ

Stnd. 10 mg/ml. ·1 → 10 ml = 100 γ/ml A B C D A. B 10 γ 20 γ

Tube #		Sample	H_2O	Acid reagent	K/H reading
1	·1		1·4	1 ml	# 66
2	·3		1·2		
3	·1	G 3094	1·4		135
4	·3	4 koc 2	1·2		151
5	·1	B 4520 ✓	1·4		404
6	·3		1·2		> 900 F5
7	·1	F B 120 ✓	1·4		79
8	·3		1·2		124
9	·1	H 4657 ✓	1·4		400
10	·3		1·2		568
11	·1	C B 16 ✓	1·4		165
12	·3		1·2		600
13	·1	A 3100 ✓	1·4		73
14	·3		1·2		249
15	·1	D 3104 F 5 ✓	1·4		520
16	·3		1·2		> 900 F5
17	·1	E B114 ✓	1·4		300
18	·3		1·2		540
19	0		1·5		
20	0		1·5		
A	·1 ml std.		1·4		190
B	·2 ml std.		1·3		271

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Test hypothesis of compound structure of F.

Lac91 : ONPG (−)

24/IV. 1960

REF: 4354 4520. Purify it on BGal. before use.

Time

Procedure : W4351 F⁻ Lac₉₁ Ara₂ V_6^s Mal₁ Xyl₂ Mtl Gal₂

1:07 1. 4520 M F_8 → × Gal₂ F⁻ S^S select on M gal.

Dilute it in H₂O 10^{-2} to stop further infection of F_8)

1:37 2. (Purify it on BGal pick Gal⁺. Make sure their purity. Incubate them in Pen (37 tube) Let it grow at for overnight.

ratio 1 : 5 ml pen. 3. Mix it with F⁻ S^R Gal_2^- ^4573 and incubate it overnight at 37°C

×2 90⁴/plate Streak it on BGal. ~~Sm~~ Count ratio of Gal⁺ & Gal⁻ on the EMB Sm agar.

~~get gal⁺ in spent ? infection.~~

~~4. Save to little which has not give few gal⁺ infections.~~

Ratio : F_8 1 : F⁻ 100

In Pen.: Incubate the mix for 40' at 37°C on rotator.

Gal⁺ 1 ml
F⁻ gal⁻ 1 ml
Pen : 5 ml.

~~4~~ Test Infectivity of Gal⁺ and sex-compatibility of Gal⁺-transductants.

4351 F_8 → × 4573.

Expected character of defective F'

1. Not infective, a (maybe steril like as F⁻ in × F⁻ but segregate Lac⁻)
b. (or may be fertile in case for × F⁻ but cannot multiplice as F.)
If it comes from revertants, it must be F⁻ and does not segregate Lac⁻.

2. It will become F' after infection of F to the hypothetical defective F_8^+.
To detect this, just mix defective F' with F⁺ ~~and~~ as exp. and F def with F⁻ as control, and see spreading of gal⁺ character in gal⁻ population.

Result :

Isolation No	Infectivity.	fertility
→ 1	+	++
2	+	++
3	+	++
4	+	++
5	+	++
6	+	++
7	+	++
8	+	++
9	+	++
10	+	++
11	+	++
12	+	++
13	+	++
14	+	++
15	+	++
16	+	++
17	+	++
18	+	++
19	+	++
20	+	++

Isolation No	Infectivity	fertility
21	+	+
22	+	+
23	+	+
24	+	+
25	+	+
26	+	+
27	+	+
28	+	+
29	+	++
30	+	++
31	+	++
32	+	++
33	+	++
34	+	++
35	+	++
36	+	+
37	+	++
Control 4520	+	++

Infectivity & fertility.
+ ++ : gives more than 50% of gal⁺ colonies (by infection of F_8. ~~counted after infection of F_8~~)
from 4351 F_8 → × 4573.

1 : 1 : 5 ml penassay : 37°C overnight : Standing culture.

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Effect of RNase, omission of helper (434 hy) for DNA-transduction.

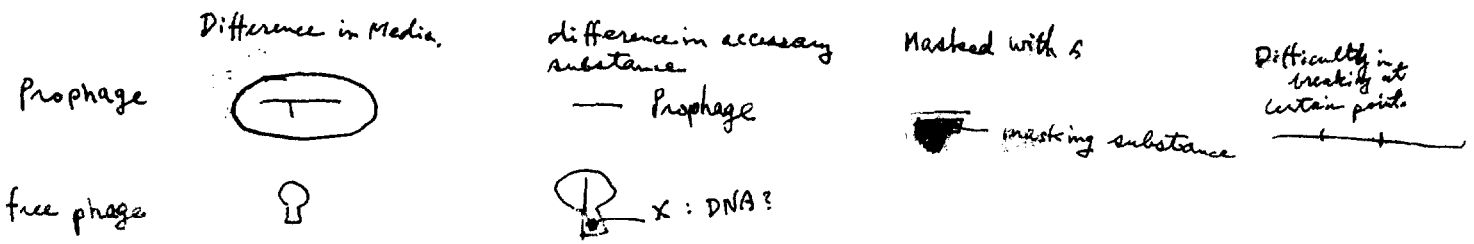
; 1960.

REF:

	1		2 30 µg/ml + DNase	3 Boiled 20 µg/ml + RNase	4 + helper	5 # of Plaques	6 # of gal+	7	8 Strain No.	9 Genotype	10
1	1	A	—	0.1	—	0	0	A	B16	gal4 /ex dg+ λ/λ2	
2	2	B	—	0.1	—	0	0	B	B114	gal4 /ex dg	
3	3	C	—	0.1	—	0	0	C	3100	gal + (λ)	
4	4	D	—	0.1	—	0	0	D	3104 F8	gal4/ex gal+F8	
5	5	A	[redacted] -	[redacted] -	—	0	0				
6			[redacted] -	—							
7	6	B	[redacted] -	—	—	0	0				
8	7	C	[redacted] -	—	—	0	0				
9	8	D	[redacted] -	—	—	0	0				
0	9	A	0.1	—	+	0	1				
	10	B	0.1	—	+	0	0				
1											
2	11	C	0.1	—	+	0	0				
3	12	D	0.1	—	+	1	0				
4	13	A	—	0.1	+	4	6 ←				
5	14	B	—	0.1	+	0	0				
6	15	C	—	0.1	+	28	0				
7											
8	16	D	—	0.1	+	0	0				
9	17	A	—	—	+	6	6 ←				
0	18	B	—	—	+	0	0				
[redacted]	19	C	—	—	+	42	0				
1	20	D	—	—	+	0	0				

B 7/1 c.f.g, resuspend in P med 0.125 + helper glucose.

+	OD	269	0.09	$MgSO_4$ + salt →	4 ml + 0.0[redacted] λ434 hy	37°C 15' c.f.g.
3	.26					
127	1.32					
177	1.50					
203	1.66					


Difference in Media.
difference in accessary substance
Masked with S
Difficulty in breaking at certain points
Prophage
Prophage
masking substance
free phage
X : DNA?

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Effect of UV-irradiation on transformability of Recipient cell.

W3350

5/14 1960 REF:

Idea. : U.V might block the immunity of recipient cell for transformation.
As in the case of recombination of prophage and exogenotic phage.
or in the case of transplantation of homograft-tissue.

Experimental design :

Recipient : 3350  1^- 2^- Lp^s F^- : from Weigle.

DNA Donor : DNA from 4520 H^- Gal^+ F_8
4687 Lp^+ Try Cal_2 Gal_7 $/ex^+$ P_1 F_8
3094D. Lp^+ $^x/_{ex}$ 2. (dg).

Method : Irradiate the recipient with various doses of U.V. (different time)
↓
Add DNA (3350) 0.1ml to 0.1ml of bacterial culture. + or - DNase
↓
Incubate it at 30°C for 1 ~ 2 hrs.
↓
Seed the mix on BGal. or on MGal.

Media : for transformation reaction.
a. penassay broth. b. Spiz + Y.E. c. DO.

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Transformation of 3350 irradiated with UV.

1⁻ 2⁻

19 REF:

	1	2	3 Bacteria 3350 grown in Spiz.	4	5 DNA Ext.	6 DNase	7 # of Gal⁺ BGal(A)	8 BGal(B)	9	10
1		1	0.1		0.1 from 4520	0.1				
2							0	0		
3		2	0.1	Gal^r F_8	0.1 4520	—	0	0		
4										
5		3 5′	0.1		0.1 4687	0.1				
6				2⁻ T⁻/ ex⁺⁺ + F_8			0	0		
7		4	0.1	P, λ 4⁺	0.1 4687	—	0	0		
8										
9		5	0.1		0.1 3094	0.1	0	0		
0				4/ex 2.						
		6	0.1		0.1 3094	—	0	0		
1										
2		7	0.1		—	0.1	0	0		
3										
4		8	0.1		4520 : 0.1	—	0	0		
5				+ F_8						
6		9	0.1		4520 : 0.1	0.1	0	0		
7										
8		10	0.1	2⁻ T⁻/ ex⁺⁺	4687 : 0.1	—	0	0		
9		[redacted]								
0		11 [redacted]	0.1	P, λ 4⁺	4687 : 0.1	0.1	0	0		
1		[redacted]								
2		12	[redacted]	4/ex 2.	3094 : 0.1	—	0	0		
3		13	0.1		3094 : 0.1	0.1	0	0		
4										
5		14	0.1		—	0.1	0	0		
6										
7										
8										
9										
0										

Cultural condition ① 3350 grown in Pen. for 5 hrs. → c.f.g. resuspend in 5 ml. of Spiz + C.H. (5% / 0.1 ml) & Pen.

② 5% [redacted] : 0.5 ml / 5 ml (ca. 2^4 T) 2 tubes Spiz. glucose. for 2 hr.

③ 2:45 —— [redacted] 3:45. c.f.g. resusp. it in 1 ml of Spiz + C.H. [redacted]

④ UV [redacted] : [redacted]. 1 ml of bacterial suspension in Spiz + C.H.

⑤ Contact cell with DNA for 1 hr. at 30°C.
5:45 ~ [redacted] 8:00

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Isolation of DNA from 3100 ($4p^+$ 3110)

7/ , 1960 $4p^+ x^+$ REF:

1. Grow up cells in 2.5 l of flu. for overnight on shaker.
2. Collect cells & store in refrigerator at 5°C for overnight.
3. Add it into 15 % duponol soln. (pH. 7.0) and keep it at room temp. for 5 hr.
4. p.p.t. with 95 % Et-OH (10:30 ~ 3:30). Wash it with 80 % Et-OH.
5. Wash it with citrate-NaCl soln. twice. Sediment saved.
6. Extract DNA with 2M NaCl. on Mag. mix for 30'. twice.
7. p.p.t. DNA with 95% Et-OH. Dissolve it in 2M NaCl. (10 ml)
8. Keep it in refrigerator.
9. Deproteinize with $CHCl_3$-octanol mix for 5 times.
10. p.p.t with 95 % Et OH. (100 ml.) (No interface).
11. Dissolve it in 5 ml of 2M saline (steril).

Use this as DNA.

Extraction of DNA from B16

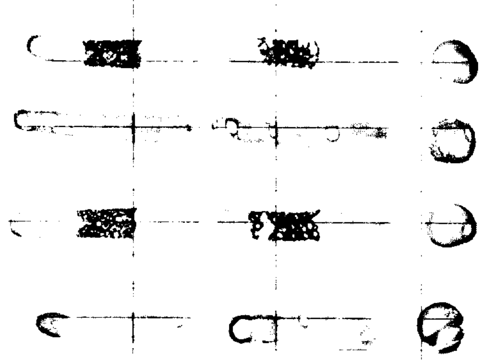
9~11/10, 1960

gal₄⁻/ex ldg λ_2^R from Kaiser. REF. λ.

1. grow up cells in 2.5l pen A shake it overnight at 37°C.
2. Collect cells wash it with 0.15M saline for 3 times. (cold room.)
3. Suspend cells in 10ml saline. (0.15M): Final vol. 16ml.
   Add the 1ml to 10ml H_2O + $CHCl_3$, c.f.g. ~~final 2d at 10⁻¹ ... 10⁻² ... / plate.~~ on 3110.
4. Add the 15ml to 30ml of 1.5% duponol soln. (pH. 7.0.)
   Keep it at room temp (20°C.) for 5hrs
   1:30 ~ 6:30.
5. P.P.T. DNA with 95% cold Et-OH.: 100ml in cold room.
6. Wash it with 80% Et-OH. twice.
7. Wash it with 100ml of citrate-NaCl soln twice. Sediment saved.
8. Extract DNA from it with 40ml of 2M saline twice. on Mag mix. (30')
9. p.p.T it with 95% EtOH. (100ml).
10. Dissolve it in 10ml 2M saline, Store in refrig. 2 days.
11. Deproteinize it with $CHCl_3$ - Octanol mix. 5 times.
12. p.p.T. it with 95% Et-OH (100ml).
13. Dissolve it in 10ml of steril saline. Store in refrigerator at 5°C.

λ B16

3110
3100
3110
3100



on B0.

# of Free phage-λ (on 3110) in 1ml of original susp.

	# of plaques/plate.
10⁻¹ : 0.1	194, 213.
10⁰ : 0.1	×10² (1ml of original susp.)
# of Free phage/~~ml~~ per ml. (×10)	2 × 10⁴ /ml of original bacterial suspension.

total number of λ-particles.

15ml × 2 × 10⁴ ; 3 × 10⁵ (# of λ in starting material.)

plaques volume used of DNA
Ca. 100 : 0.1ml ;
Total volume was dissolved in 5ml of

100 × 10 × 10 = 10⁴

% recovery $\frac{10^4}{3 \times 10^5} = 3\%$.

U.W.
MICROBIAL
GENETICS

Isolation of DNA from B114.

2/17 : 1960 REF:

A : + 0.9 ml of 1 ref.
B : No 1 ref.

1. B114 was grown in 2.5l [redacted] on [redacted] shaker for overnight at 37°c.
2. Collect cells, ~~[redacted]~~ wash twice, and divide it into 2 parts. (A, B).
   Add 1 ref to A, without + 1 to B.
3. Add 14 ml of bacterial suspension to 30 ml of deponal (5%) Soln.
   Keep it at room temp for 5hrs. (1:15 ~ 6:15 pH).
4. P.P.T & wash it with 95% Et-OH. Homogenize with potter-type glass-homogen
5. ~~Extract [redacted]~~ Wash it with Nacl-citrate Soln. 2 twice.
6. Extract DNA with 2M saline. (40 ml.) twice. P.P.T it with 95% Et-OH.
   on Mag. mix for 30'. at 5°c.