Mutants of F particle.

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

P26 (F13)
P.16 (F4) P57 (F2)
P15 (F11, F4, F6)
3086F': P.7, (F8, F17, F19)
w6F': P. 8 (F8) P.19 (F17, F19)
P.13 (F8)
P.17 (F6, F11)
P57 (F2) P62 (F2)
P.63 (F5)
P.75 (F8)
P.77 (F3) P83 (F3)
P.85 (F6) 3828
P102 Y10 (F3)
P.120 (F13 Lac) 3133
P.103 (F13, 7086)

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

1. High marker

High marker mutant of F	High marker ←——→ Low marker
F8	
F15	
F19	
F1	

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

from one cell to another

They are transfered by short contact, and gives the same high transfering characters to F- cell. (See P.8, P16) In other words, they retains genetic continuity of the traits during transfers.

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

P44. P.76a, b.
P.25, P45. : Resistant to infection of F4, F8, F1

4. 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 F8 ♀3
(p. 46) states
(p55) (p.70)
States: Sometimes they shows two states. but not high for both. After cure of F8 with AO still shows ♀3 trait.

6 Sensitivity to Acridine-treatment. F8 (P.20) (P.22) F.4 Hfr4 (P33) F8 P(34) F8♀3 P70 ♀3 P82 P95 P122 (3133 F3-)
W1394F8. W6 F8 Sensitive to AO.

7. Recombination of F, between Rfd3. and F1. (3442) → Hfr – x 3135 P77, 828 P110.
Is there some kind of exchange reaction between Host cell (F1) and F?

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

P23 P46 P.70 → cure F8 ♀3 and reinfect F1 and see 2 states
P93, P80

8. States of F'♀ and F'+ ♀3
9. ... of infective F from F'
10. minimal cell number for infection (P.47).

P.35, 36, 37.
P54 (infection of F8)
P49: F8 (Final test for fertility)
P51, P52 F4

11) Double F. P.43. ($F_8 \rightarrow \times F_4^+$) P31 (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. strains. 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, ~~B~~ 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 (P35, 36, 37) (P.58). P98. successful. (P.69) (P97)

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

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

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

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

19) Recombination between defective F' (P65) P78. P73, ($F_1 \times F_8^-$)
Unsuccessful.

20). Test other futility 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'. P76 a, b P81. 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 Sun-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,; 113) Pnr

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

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

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

35) Timing experiment of transduction of (Lac - F_{13}) segment (121, 125a, 125b.) (Test for Lac of Syngenotes) F
F_2^+

36) Stability of Lac^+ marker of the AO-treated strain. (127b) (143)

37) Rate of multiplication of Gal-F_8 segment (128a,b) 129a (cf. 32)

38) Cistron analysis of Lac loci (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 (P133, P134), P145 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 the host cell.

These two observation seems quite different from λ. λ becomes deffective because of crossing over. If. F' is not deffective, 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 separated very easily.

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

47) Study of F_{13} (103, 104, [illegible]; 109
[illegible]
1..; 142
[illegible]

Trials to get lac⁻ F_{13} from.

lac⁻ mutation	strain No.	Result	Ref
Lac 85	4411.	F^- and $F^{x?}$ 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.

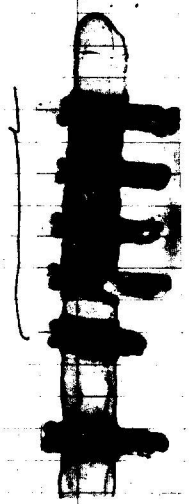
22/10 1959 REF:

Principle: M T L Hfr$_1$ $\xrightarrow{\text{U.V.}}$ M T L F' $\longrightarrow\times$ 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 per c/ml
or mix with W3828 after infection in penicillin. Shake it on rotator for 4 & 8 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 colony. (H1 recombination reaction.) which gives high frequency recombination reactions.



× W3828 on M Lac.

- 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 plate.
- # of W2061 irradiated (inoculated after Ca. 10^8 cells/ml plate. (0.1 ml/8 ml plate)

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) ← add overnight culture of W3086 to it. (0.2 ml/tube)
↓
Shake it overnight on rotator at 37°C.
↓
spread it on BlacSH. → cross brush it× W1394 on MLacB,
↓
Replica plate on MLacB, seeded W1394.

• UV dose : 60 sec. suspended in dist ca 10^9 cells/ml.

Result :

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

U.W.
MICROBIAL
GENETICS

Infection of ~~P~~ Hfr-character to F-

→ W336

17/III ; 1959 REF:

Lo Hi

Principle. Select on B Mal SM:

Gal	Hfr 23	M Lac SM D,	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; Arg; Th	Hfr 5	M φ^s Gal^+	W4097	——→× W3086	v	B Lac SM
	F^+	M	W6	——→× 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/III
2. purify 18/III
3. Test Hi for Gal. (19/III.) by cross brushing method.

1:30 ——→ 3:30

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	−
W6 —→× W3086	3/30	10	+

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

Test for Hfr mutants.

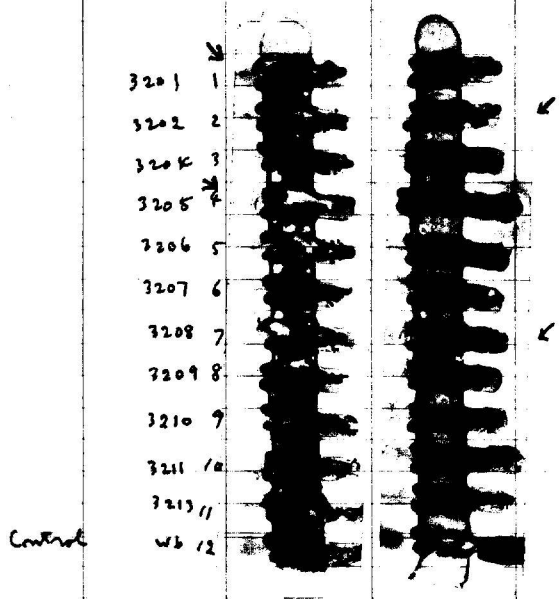
27/III 1959

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

— x 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

Control W6 —× W 3086

in penassay. #. Ca. 10^9 cells/ml.
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-brushing~~ Replica plate it on M Lac and M Gal × W 3828 or × 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.

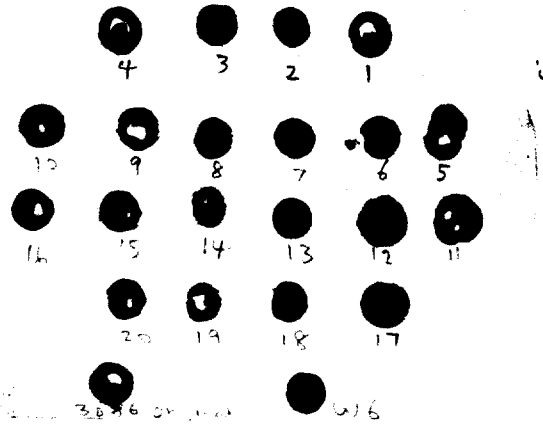
Test for compatibility.

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	+ ++ c	++		—
3208 —× 3086 F8 Lac	7	+++ c	++	F8	—
	8	+++ c	++		—
	9	+++ c	++		—
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		—	—		—

See from the page

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

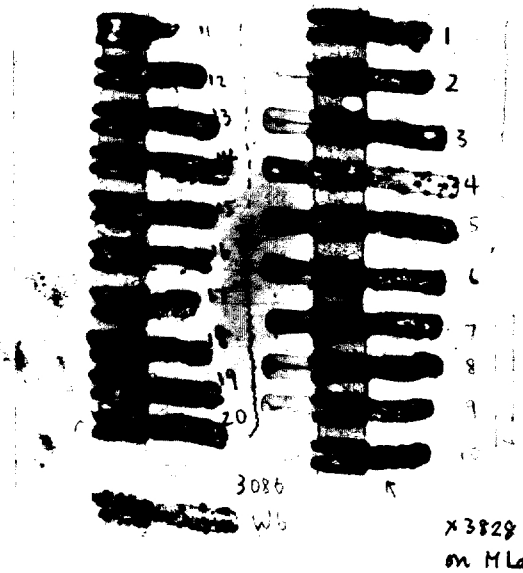
→ X 3086



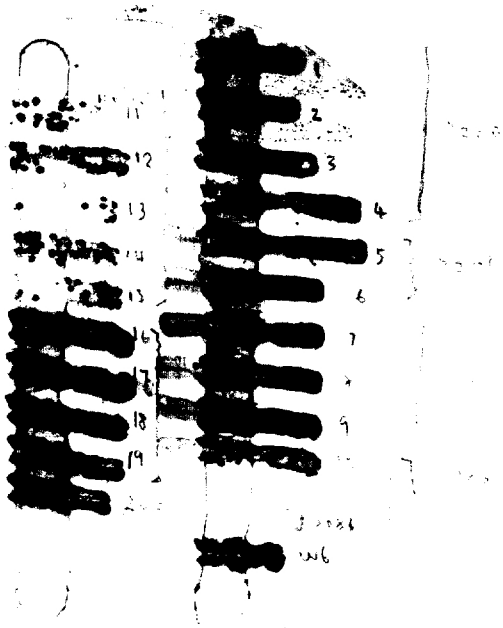
isolation no.

on B Mal.

Hfr → X 3086.



X 3828
on M lac



X 2979
on M Gal

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 Blac SM.

#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 Blac SM agar.
(3086 : SM^R, W4354 SM^S.)

2/30 : 6.67%

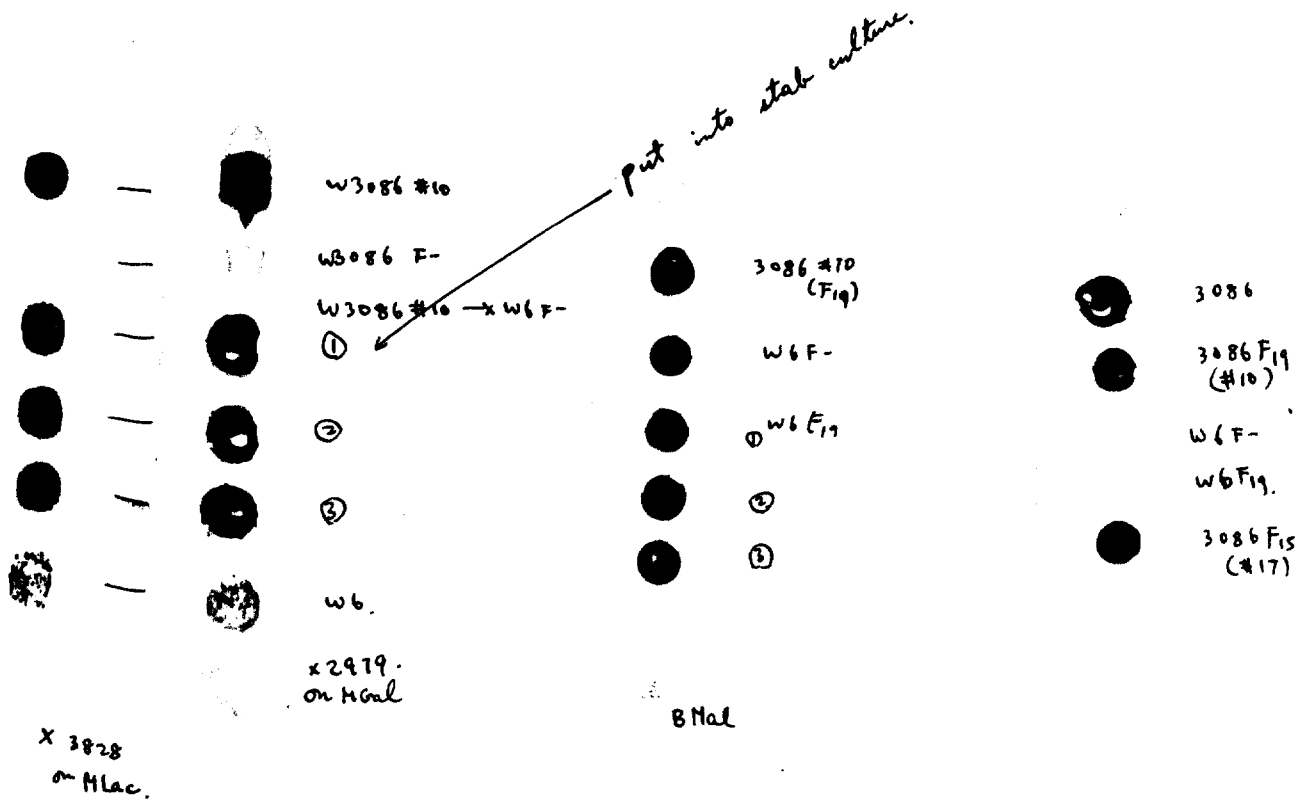
×3828
on MLac
4/33 : 18.2

W4354 W4354

← This is good for standard F8 donor
W6 F8⁺

on Blac Sm

on Blac.


put into stab culture.
W3086 #10
W3086 F-
W3086 #10 → W6 F-
①
②
③
W6
x2979.
on MGal
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)

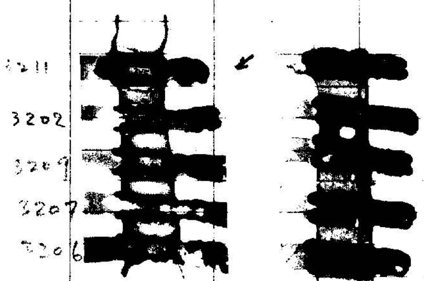
Reisolation of Hfr strains
(from Hfr7 — Hfr16)

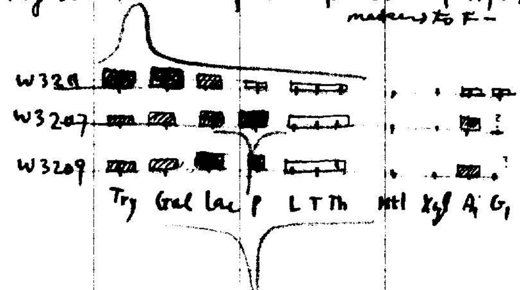
2/14, 1959 REF: See 27/11, 57

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

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

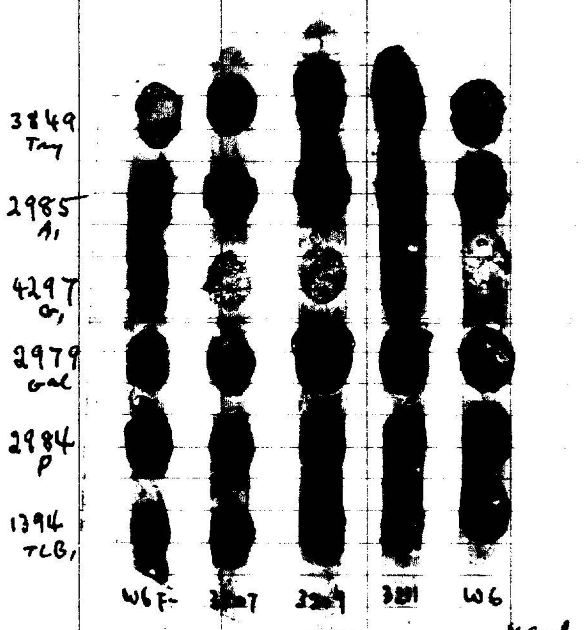
	fertility x 2979 M Gal	x 3828 M Lac	Hfr
W 3211	++++	+	O Hfr11
W 3202	+	+	
W 3209	+	+++	O Hfr9
W 3207 a	+	++	
b	+++	++	O Hfr7
W 3206 a	++	++	
W 6	+	+	

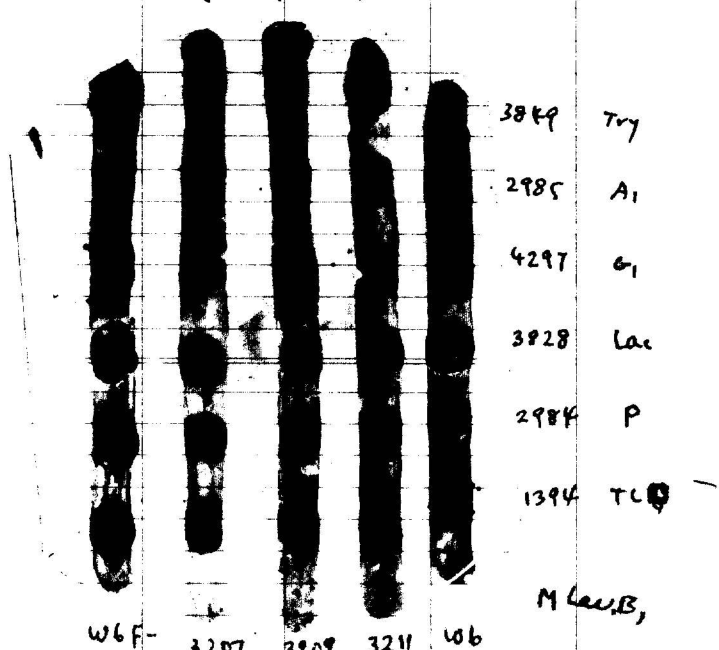




Retest ~~of these Hfr~~ Hi markers of strains. W3211, W3209, W3207.
(Hi : Prolin)

many recombinants appears very late in W3207 + W3209 →





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

3/18 ; 1959 REF:

Experimental conditions.

cultural age: ca 20 hrs.

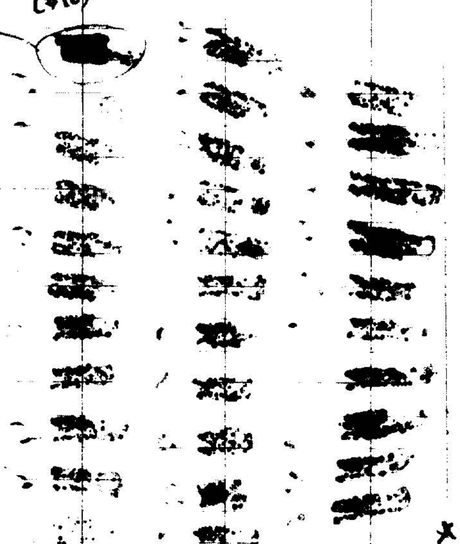
Inoculum size: F8 or F_{15}, or F_{19} 10^9 : 0.1 ml + 3086 : 0.1 ml + 5 ml Pen. (^{5}3876)

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

Retest after growth in penassay broth. (becomes higher)

W3086 F_{19}^+ (#10) → x W6 F⁻

W3086



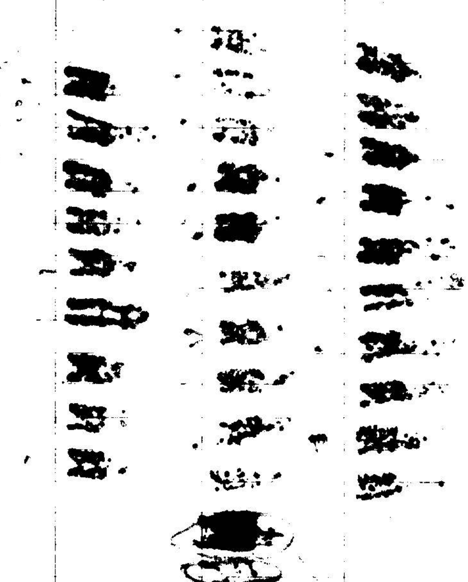
W6F+

*3828 on Hlac.

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

↑ Seems like state II.

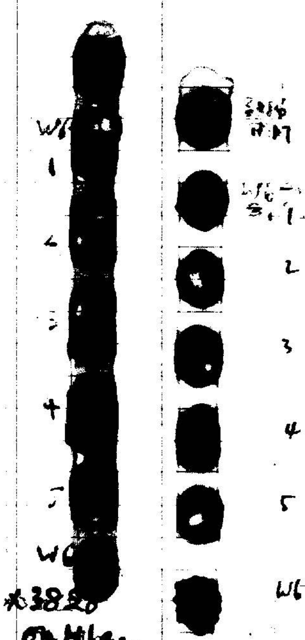
W3086 F_{15}^+ (#17) → x W6 F⁻



W3086 F_{15}^+ (#17)

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

3086 #17



*3828 on Hlac

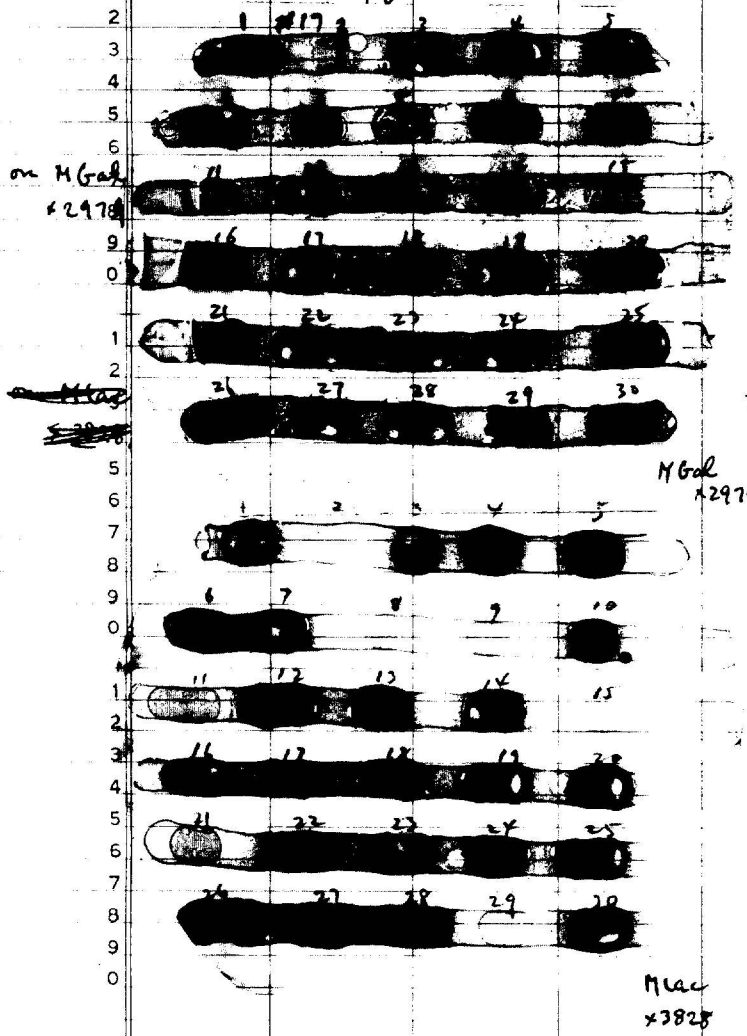
on Hlac

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

State I and state II in F'+ strains.

8/IV ; 1959 REF:

3086 F8



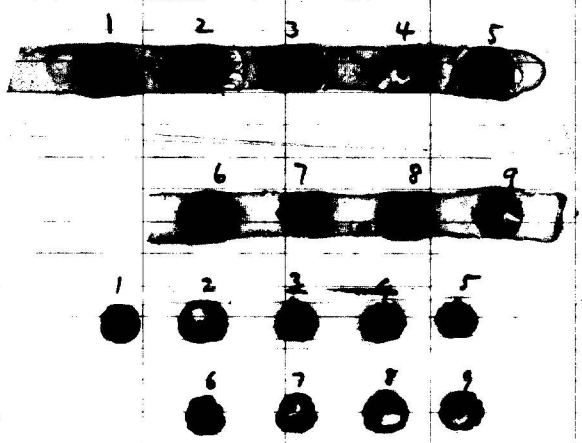
on MGal ×2978

MGal ×2979

7/30 ×100 = 36.3

MLac ×3828

3086 #10 (F19)



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)

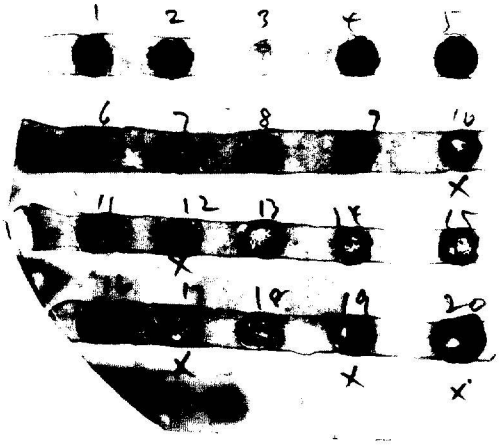
Conclusion : (36.3%)

1) 3086 F8 : (3086 F19) 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 division of #of F8 in cytoplasm is very low.

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

3086 #17

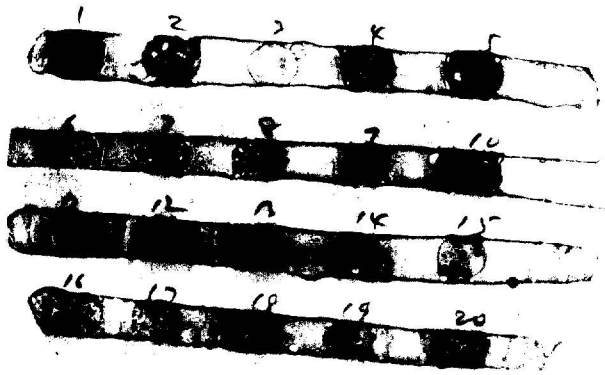
F15



on MGal

3086 #17

F15



on MGal

Treatment of F mutants. by AO.

2/14, 1959 REF:

Purpose : Is F' sensitive to AO-treatment.

Experimental conditions : inoculum size: ca. 10^4 cells/ml , conc. 40γ/ml.
Medium : penassay. 5 ml.
cultural age : overnight culture.
Time of treatment: ... (faint)
strain treated ; W6 (control), 3086-10, 3086-1, 3086-17,

Result

			stat phase II.	F^-/total I	F^-	% of F^-.	effect.
F_1	W6 AO 40γ						
	" control.						
F_8	W-3086-1 AO			11/85	74/85	87	+
	" cont		[struck out]	68/87	19/87	21.8	
F_{19}	W3086-10 AO			8/48	40/48	83.4	+
	~~W3086~~ cont		81/109	28/109	0/109	0	
F_{15}	W3086-17 AO		[struck out]	84/149	65/149	43.6	+
	" Cont.		38/88	50/88	0/88	0	

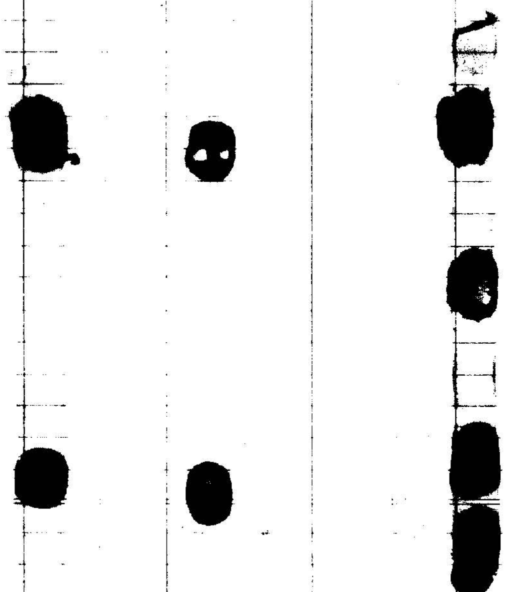
put into stab.

3086 AO #9

C

3086 AO #10

3086 C



3086 #1 AO

3086 #10 C

3086 #10 AO

C

3086 #17 AO

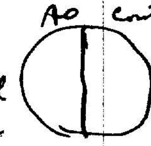
C

W6 control.

MGal Mlac

x 2979 on MGal

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



o Replica plate it on MGal or Mlac
Seeded 2979.

Transmission of F_8 from $W6F_8^+$ to Hfr, and F^-
(W1922) (W1394)

M Hfr$_1$ S^R TLB$_1$ S^R save W1394 F_8^+

5/?/ 1959 REF:

1	2	3	4	5	6	7	8	9	10

Method.

} W6 F_8^+ : 1 ml
Hfr$_1$ or F- : 0.1 ml ($10^{?}$ cell)
5 ml pen.

This data is still not believable
try again using W1895.

1.) Transmission W6 F_8^+ Hfr$_1$ or F-
~~Mix them~~ ~~1000 : 1~~, and ~~shake it~~ stand it overnight.
Select S^R on BLac Sm agar. (ca. 500 colonies per plate).

2) Replica plate it on MGal seeded W2979 on it.

Experimental conditions.
cultural age ;
medium ; Pen. 5 ml. ; Inoculum size and ratio : W6 F_8^+ $10^8 : 10^5$.

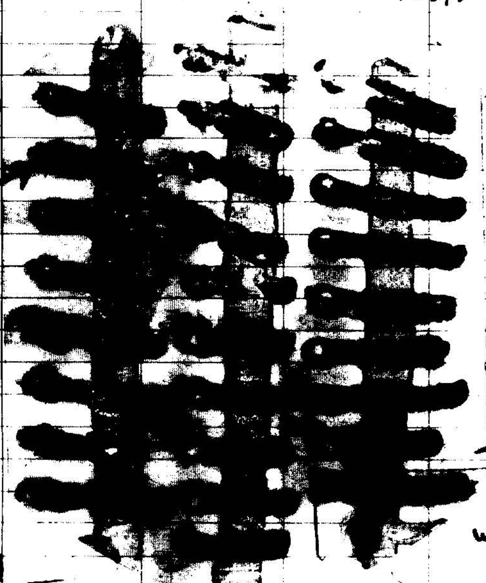
Conclusion : F_8 is transmissible to Hfr$_1$!, and gives Hfr$_1$ character in the sense of chromosome transfer.

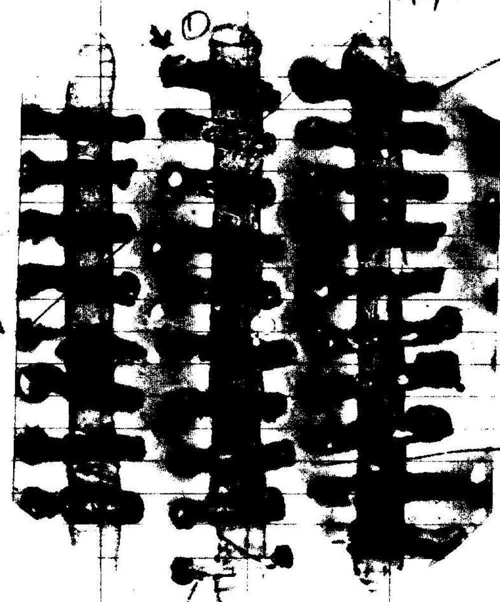
Reselect and put into stab...

Control.

W6 F_8 → x W1394
TLB$_1$ S^R

W6 F_8 → x W1922
Hfr$_1$ M S^R





① ?

②

Put into stab.
W1394 F_8

2/25 x 100 =

W1394 F_8

W6 F_8
W1394

control
W6 F_8
W1922 (Hfr$_1$)

x 3849
x 2985
x 4297
x 2984
x W6-
x 2979
on MGal

12/26 x 100 =

W6 F_8
1922 F_8 1
1922 F_8 2
1922
W6-

x 2979
on MGal

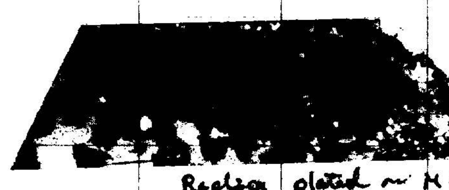
W1394 control

x 2979 on MXyl

x 2979
on MGal.

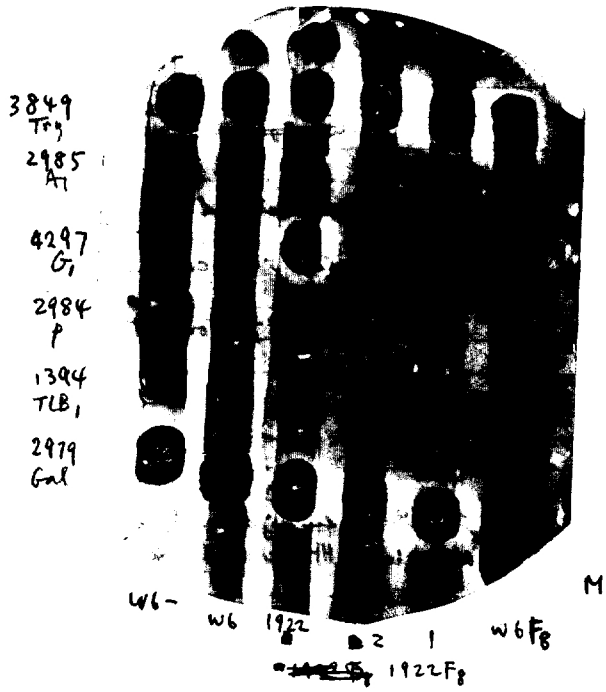
further check.

- Hfr$_1$ Test on MLac B$_1$ x W1394
- F_8 Test on MGal x 2979.

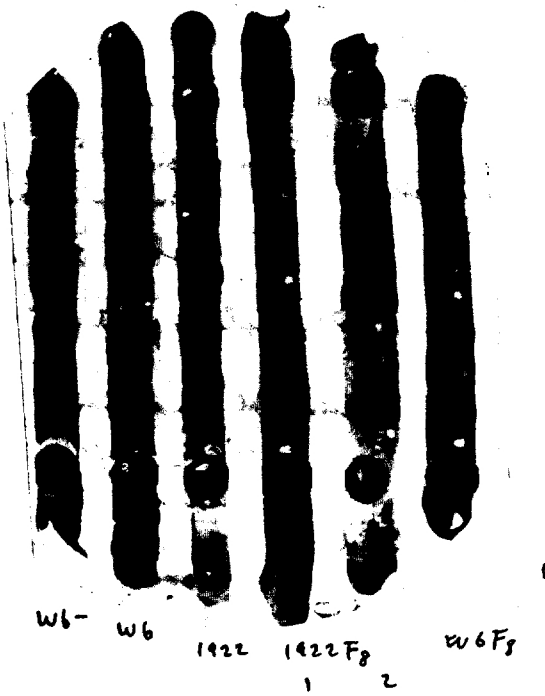


W6 F_8 → x W1922.

W6 F_8 → x W1922 Replica plated on MGal x 2979.

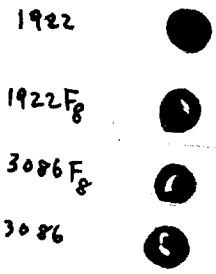


MBal.

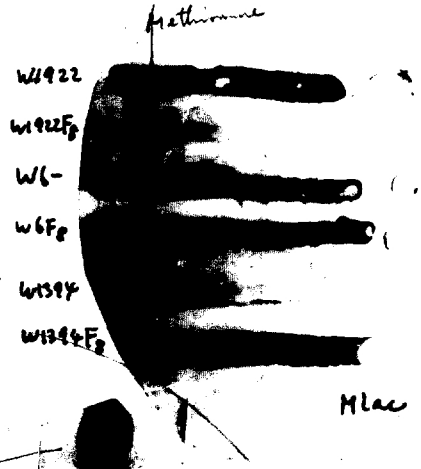


MLac.

1922 Mal test. on B Mal.



nutritional requirment



MLac

Reference

S^R 1922F8 MHtr, F8 S^R

S^R 1922 MHtr, S^R

S^S W6F8 MF8

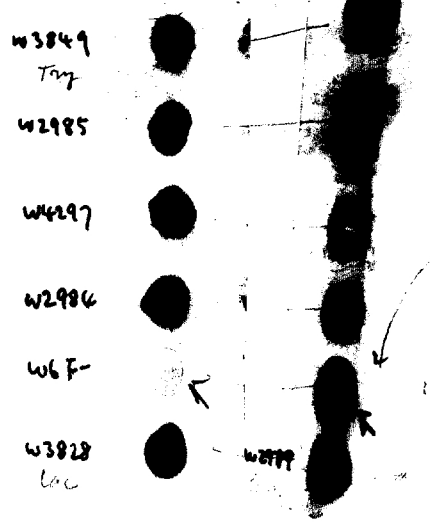
S^R W3086F8 S^R M Mal F8

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

and W6F8

x W1394 TLB, S^R

MLac SM B1



Conclusion

W1394F8 must be M-

W1922F8 must be TLB-

It makes sense that I mixed up with W1394F8 and W1922F8

~~W1394F8~~ on MLac

~~W1394F8~~ W1922F8 on MBal

W6 $\xrightarrow{F_1}$ × 3086 F_8^- (isolated by AO).

W6 $\xrightarrow{F_1}$ × 3086 F_{15}^-

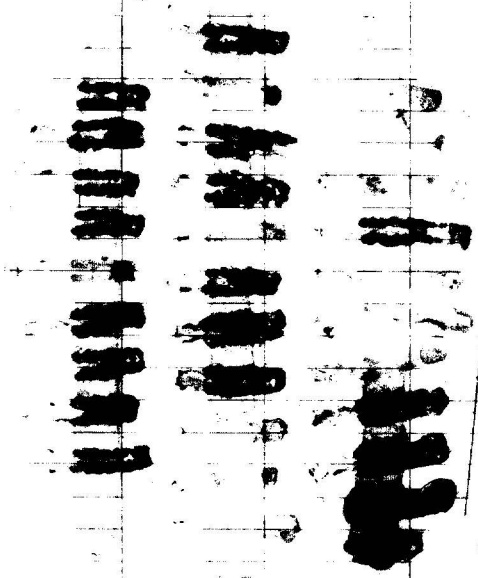
7/14 ; 1959. REF:

Experimental conditions.

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

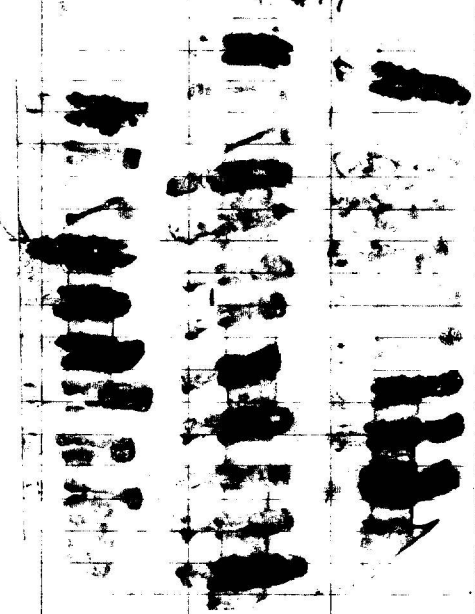
Infection: 37°C overnight. (ca. 20 hrs.)

W6 → × W3086 F_8^- #1



Control W3086 F_8^-
W6
× 2979
on M Gal

W6 → × W3086 F_{15}^- #17



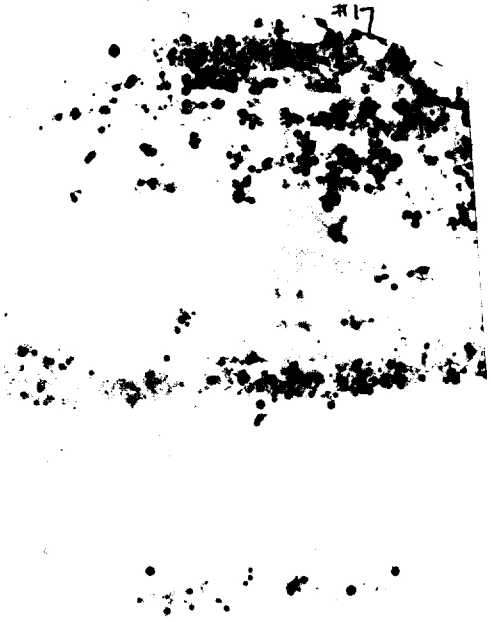
Control W3086 F_8^-
W3086 F_{15}^- #17
× 2979
on M Gal.

Conclusion:

W3086 F_8^- and W3086 F_{15}^- isolated by AO treatment are defective 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 back page: Replica plating method to check their fertility.

W6 ⟶ W3086 F15
#17



W6 ⟶ W3086 F8
#1

ordinary FP

×2979

~ Moal

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 on BMal, and pick Mal+ and test Hfr for Gal (on MGal × 2979)

↓

Test S+ and Mal+, and put into stab.

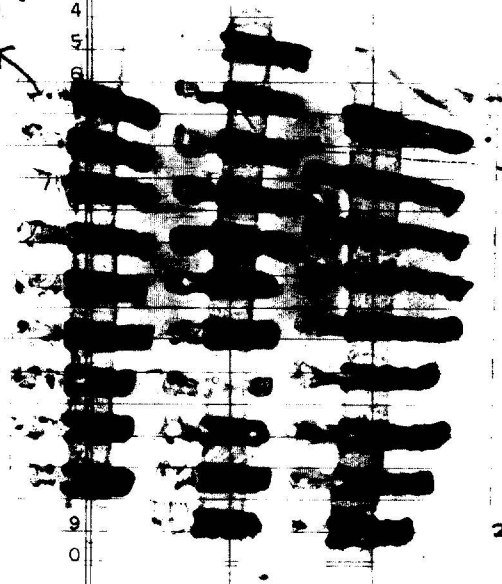
Result:

Rate of transfer. Hfr / total

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

Put into stab. W6 F_{11}.

3086 F_{11} ×W6F−

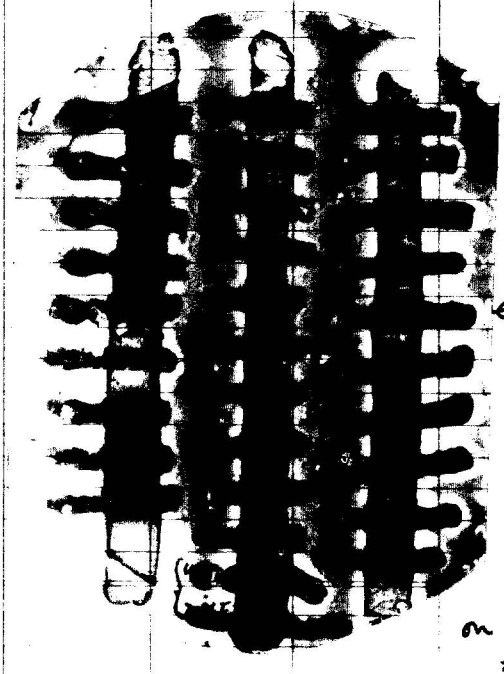


W6F− } Control
3086 F_{11} }

$^{27}/_{28}$

on M Gal ×2979.

3086 F_6 ×W6F−



Put into stab W6 F_6

on M Gal ×2979.

W1394 F_8 TLB, S^R

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

Test for infectivity of F_8 of Hfr, F_8.

12/6/ 1959

REF:

Method :

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

↓

Incubate overnight

↓

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

↓

test Gal and Lac loci in the frequency of transfer.

→ × 2979.

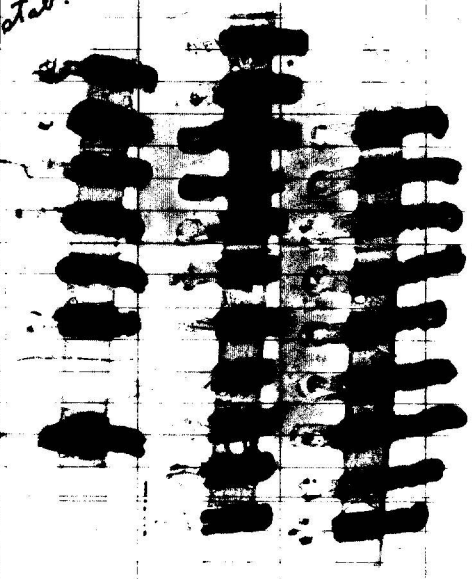
↓

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

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



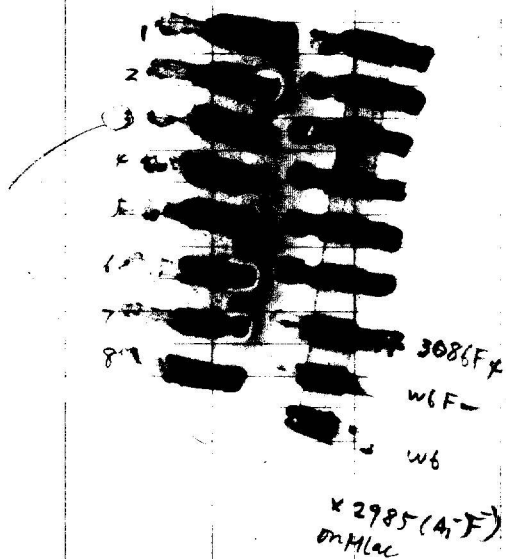
× 2979
on Mal

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

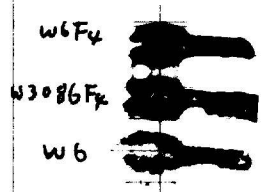
13/IV ; 1959. [redacted] REF:

[redacted] 3086 F_4 1 ml + W6 F− 0.2 ml + 5 ml penassy.
↓
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 F_4.



× 2985 (4, F⁻)
on MLac



× 2979
on MGal.

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

W1394 F8
F8 TLB, SR

12/IV 1959

REF: does F8 be removed by AO treatment?
How about Hfr, mater
Does F8 replaced F1 ?

Method :

1. 10^4 ml of 48hr old culture of ~~Hfr, F8 (W1922 F8)~~ W1394 F8 (penassy) 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 SMB. It does not grow in AO. (It may be too conc.)
Try again.

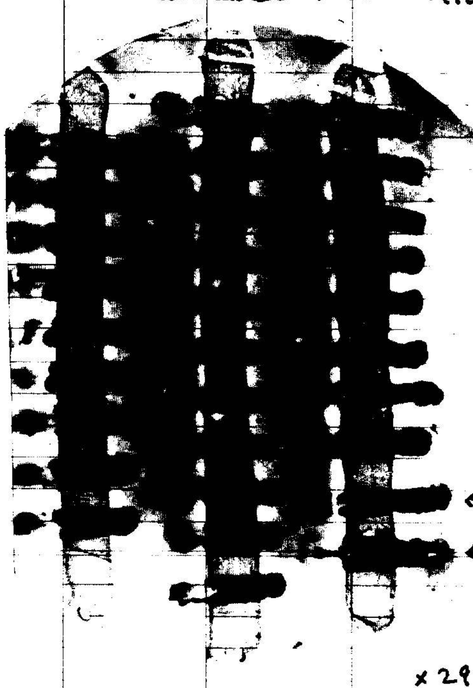
final inoculum size:

13/IV 1) 10^4 cells/ml. young culture (overnight) ; AO conc 30γ, 40γ Penassy; 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 SMB,

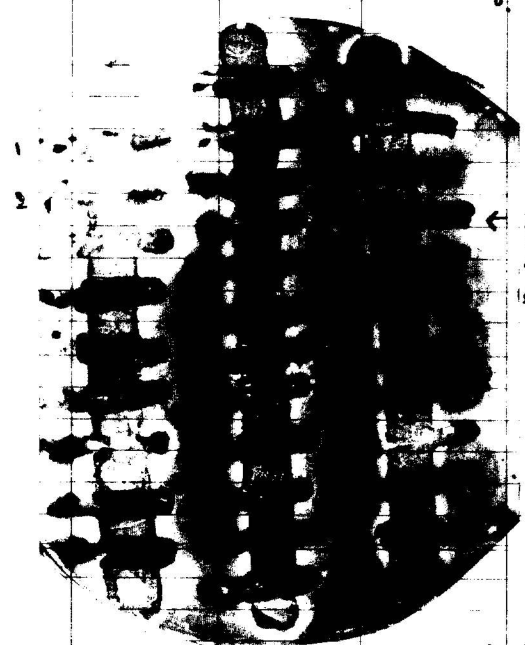
untreated control W1394F8 ~~W1922F8~~



← W1922
← W6F8

x 2979
on MGal

AO treated W1394 F8. ~~W1922F8~~



1
2
← 3? Hfr1? or F8 ...
4 See back page
5

W1922
W6 F8

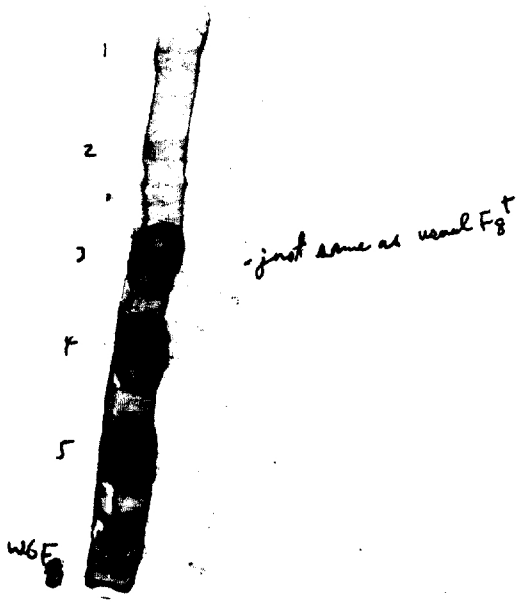
x 2979
on MGal

% of F⁻

0/28 x 100 = 0

6/31 x 100 = 19.4

Retest of fertility
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 like unstable.
How about W6 F_8^+. So it stable or unstable?

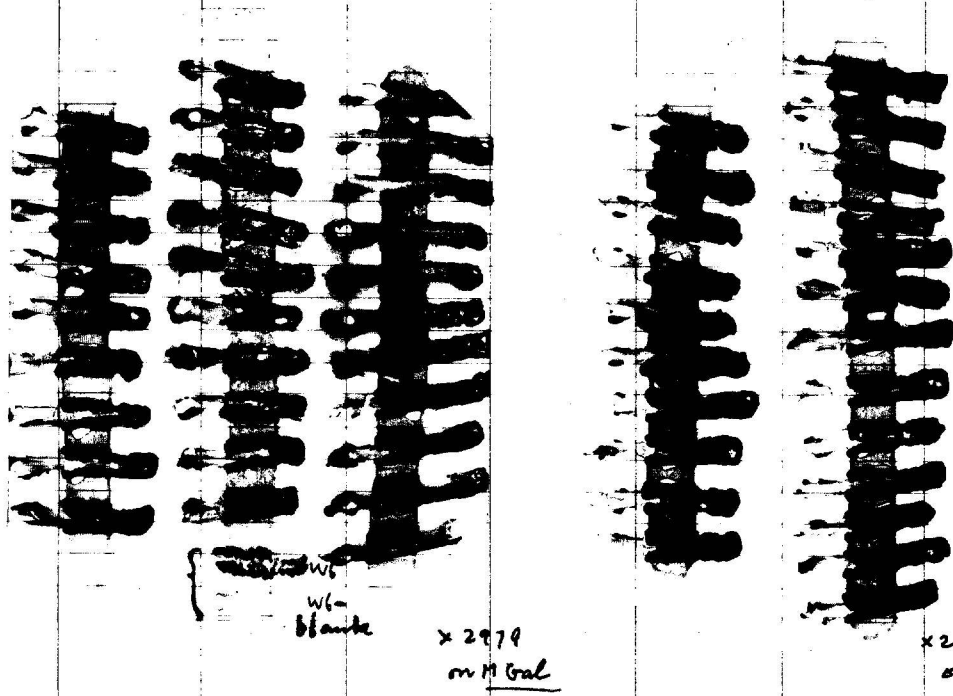
Method :

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



W6F_8/W6F_8.
53/53 × 100 = 100%

Treatment of W6F8 by AO.

15/IV. 1959. REF:

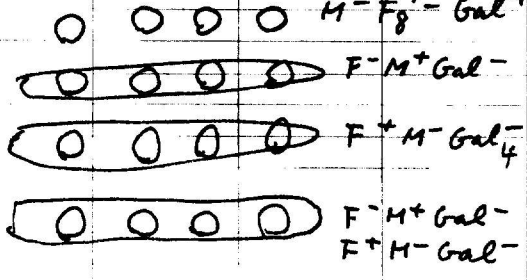
Purpose : Does W6 F8 produce $♀_3$ type F⁻ after treatment of AO?

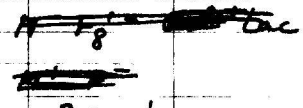
Principle :

M⁻ F_8'⁻ Gal⁺
F⁻ M⁺ Gal⁻
F⁺ M⁻ Gal⁻

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

Method





Principle :

M⁻ ♀ × — Gal⁻ M⁻ F⁺
×
Gal⁻ on MGal.

- Use single colony as a starting W6 F_8.

1. Purify W6 F_8.
2. Treat W6 F_8 by AO. Inoculum size : Ca. 10^8 cells/ml. AO : 30γ/ml 90m.
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⁻ (MGal) according the method described above. Test 50 colonies. (Only 6 ♀s are obtained after treatment.)

Result

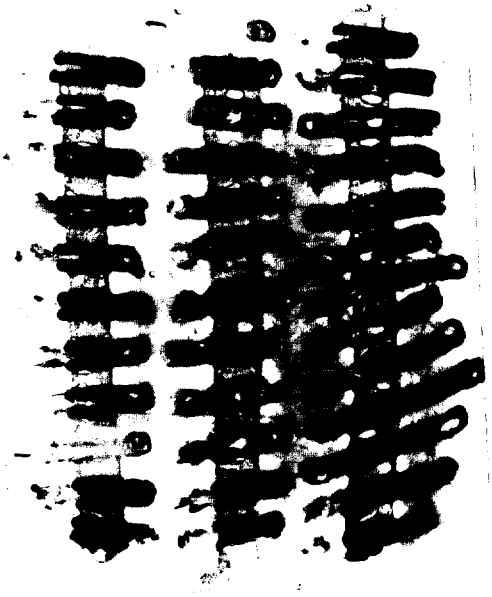
% of ♀ obtained : 8.35 ?

see back page

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) = cytoplasm multiplies independently.

W6 F8 AO treated



W6 F8 AO treated.

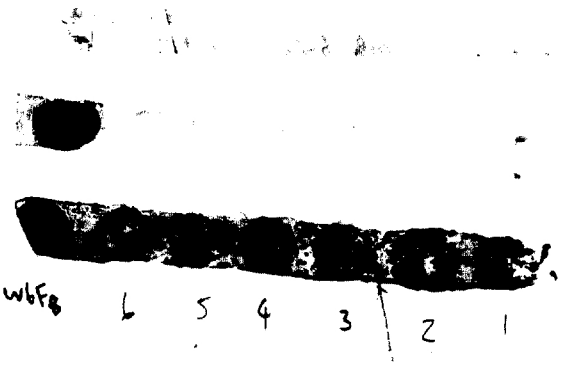
♀ : 6.
♂ : 66.

$^{6}/_{72} \times 100 = 8.35\%$ ♀.

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

×2979
on MGal.

blank.



3046 F⁺ M⁻ Gal⁻₁

2979 F⁻ M⁺ Gal⁻₂

3040+2979

W6F8 6 5 4 3 2 1 – 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, 46.

← W6F8 ← W3644

W6F8 1ml + W3644 0.2ml + Penassy 5ml.
↓
Incubate at room temp overnight. ↓ at 37°C.
↓
Purify on Blac SM.
↓
Test their sero-compatibility (×2979) on MGal.

on BMal on Blac SM

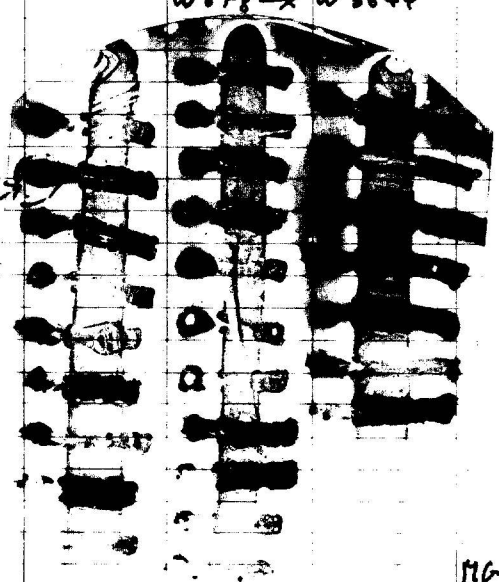
Result.

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

Further experiment:
Compair Hi marker.

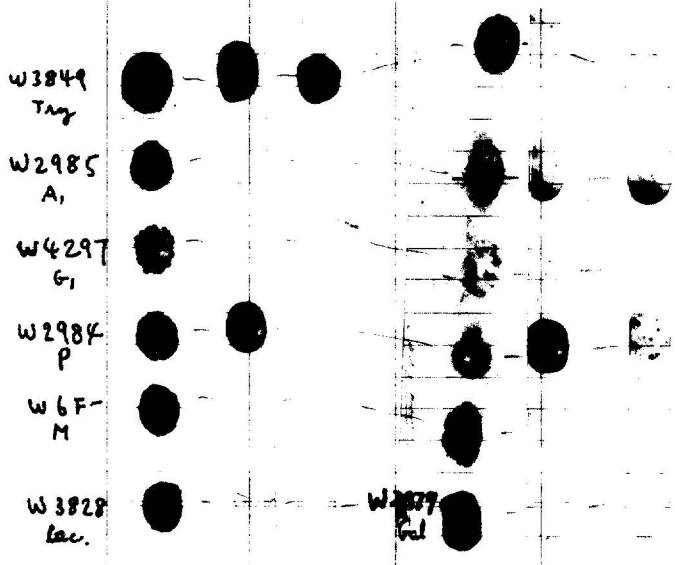
Conclusion: F8 is transmissible to f₃ and gives high frequency recombinations. The rate is about 50% after growth for overnight culture in penassy.

W6F8 → 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, H.

W3644F8 1394 W3644 on MLac.

W3644F8 W1394 W3644 on MGal.

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

↓

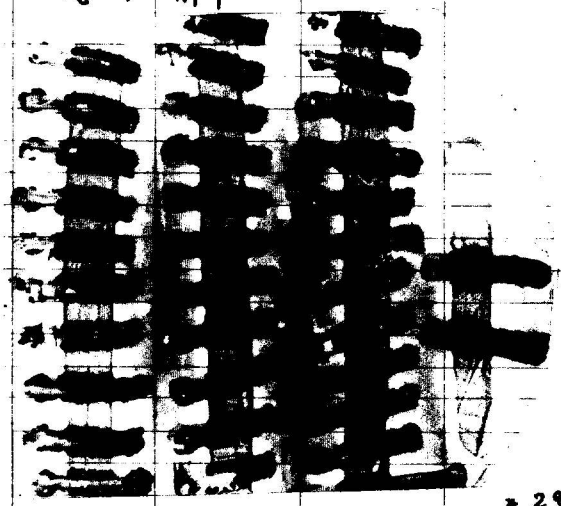
Purify it on B Mal.

↓

Cross brush Mal⁺ colonies on M Gal × 2779

Result : No transfer occured. Seems negative result.

W3086F_8 W1895
F_8 → Hfr,



W1895

W3086F_8

× 2779
on MGal.

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~~ 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 MBGal Sm. → Replica plate it on MGal added F^- Gal⁻ F^- M⁻.