Mutants of F particle.

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

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

1. High marker

mutant of F	High marker ⟷ Low marker
F8	
F15	
F19	
F1	

P26 (F13)
P16 (F4) P57 (F2)
P15 (F11, F4, F6)
3086F': P.7, (F8, F17, F19) F1
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 (F4) 3828 Y10 P102 (F3) 3133
P.120 (F13 Lac)
P.103 (F13, 3086)

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

They are transfered ~~to F-~~ from one cell to another by short contact, and gives the same high transfering characters to F- cell. (see P.8, 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 F4, F8, F1

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 gel.

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

(p23) 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 F.4 Hfr4 F8
(P.20) (P22) (P33) P(34). P70 F8 ♀3 P82 F3.
W1394F8. W6F8 Sensitive to AO. P.95
P122 (3133 F3-)

7. Recombination ~~of F1~~ between Rfd3. and F1. (3442) > Hfr3 - X3133 P55 P77, 82, 8
Is there some kind of exchange reaction between Host cell (♀3) and F1? P110.

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

P23
P.46
P.70, P93, P90 → cure F8 ♀3 and reinfect F1 and see 2 states

8. States of F'+ and F'+ ♀3

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

9. Isolation of Infective F. from F'

10 minimal cell number for infection (P.47).

11) Double F. P.43. ($F_8 \to F_4^+$) P31
P.42 ($F_4 \to F_8^+$) P.42; P.50 (segregation from F_8F_4) one F^-
P.53 ($F_8 \to F_1$, $F_4 \to F_1$)
P28, P29, P72, P.73.

12.) Segregation of different F' from double F. strain.
P42; P50, P48, ~~P52.~~ P53

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

14). Reversion of F_8 and F_4 into F_1 by U.V irradiation. (P.56) (P.51) (P.52) F_4^-
P49. F_8

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

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

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

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 ($F_1 \to F_8^-$) W 4544 P.68
P78. P.73, ($F_1 \to F_4^-$)

19) Recombination between defective F' (P65.)
W 4544 P68, P67 back page.
Unsuccessful.

20). Test the 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'. P76 a, b. P81. P89, 94.
It was not Host range 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" , P46; P90.; P70 a, b.

27) Aggregation of F' P.92

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) 106, 109.

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

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 graph, 124)

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

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

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

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

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 hyp~~ 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 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]; Seg 104

[illegible]

[illegible]

[illegible]; 142

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}.	

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1

Isolation of mutant of F. from Hfr 1.

22/11 1959 REF:

Principle:

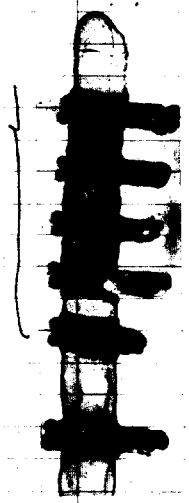
MTL Hfr1 (U.V.) → MTL F' → × Lac F⁻ S^R W3828.
W2061 mutation
× TL F⁻ S^R W1394 (TLTh S^R F⁻)
M F⁻ S^R W3086 (MHal S^R F⁻)

Experiment 1. Test Hfr1 : H1 for Lac transfer × 2979 on MLac.
(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 0.1 ml) or mix with W3828 (after irradiation) in penassy. Shake it on rotator for 48 hrs at 37°C. (W3828 ca. 10^5 cells/ml)

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

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

W2061



W6

× W3828 on MLac.

- U.V dose : 30 sec.
- Suspension : 10^9 cells/ml.
- age : Overnight.
- Condition of exposure: centrifuge reanspend into H_2O.
- Exposed with gently shaking.
- # of W3828 added: ca. 10^5 cells/ml. pen.
- # of W2061 irradiated (inoculated after) ca. 10^8 cells/ml pen. (0.1 ml/5 ml pen)

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 BLac SM. → cross brush it× W1394 on MLac B,
↓
Replica plate on MLac B, seeded W1394.

• 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)

Infection of Hfr-character to F-

17/11 ; 1959 REF:

→ 4536

Principle. Select on B Mal SM:

Gal	Hfr 22	M Lac SM D,	W4397	—x W3086	v	B Mal SM
Gal	Hfr 6	M Cp⁺	W3807	—x W3086		B Mal SM
Gal	Hfr 33	M Cp⁺	W3200	—x W3086	v	B Mal
L ; Axyl Th	Hfr 5	M Cp⁺ Gal⁺	W4097	—x W3086	v	B Lac SM
	F⁺	M	W 6	—x W3086		B Lac SM

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

Inoculum size : 10^8 : 10^7 in 5 ml ~~[illegible]~~.

Tester for Gal : W2979 on M Gal.

Procedure :

1. Mix 17/11 1:30 —→ 3:30
2. purify 18/11
3. Test Hi for Gal. (19/11.) by cross brushing method.

Result.

~~W4397 —x W3086~~

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

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

Test for Hfr mutants.

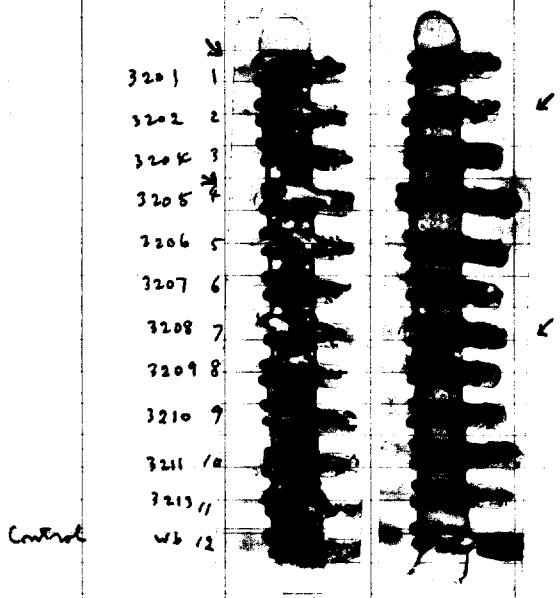
27/III 1959

REF:

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

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5

Infectivity of Hfr-character.

21/III ; 1959

REF: W 3086 F⁻ M Mal S^R
W 3201 Hfr M (Hi Lac)
W 3205 Hfr M (Hi Lac)
W 3208 Hfr M (Hi Gal)

Principle.

W 3201 —x W 3086
W 3205 —x W 3086
W 3208 —x W 3086

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

Control W6 —x W 3086

and test 3086
x 3828 M Lac
x 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 [illegible]
4. ~~Cross-brush~~ Replica plate it on M Lac and M Gal x W 3828 or x W 2979.
5. Pick Hfr colonies into [illegible] and [illegible] the Hfr-character by cross-brushing method.
6. Put into stab. the F' mutant.

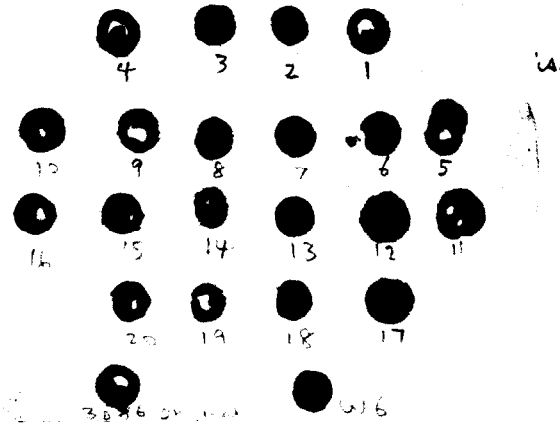
Result.

Test for compatibility.

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

Conclusion : F Gal and F Lac are isolated.
Further step : Infect these F mutants to W6F- (W4354)

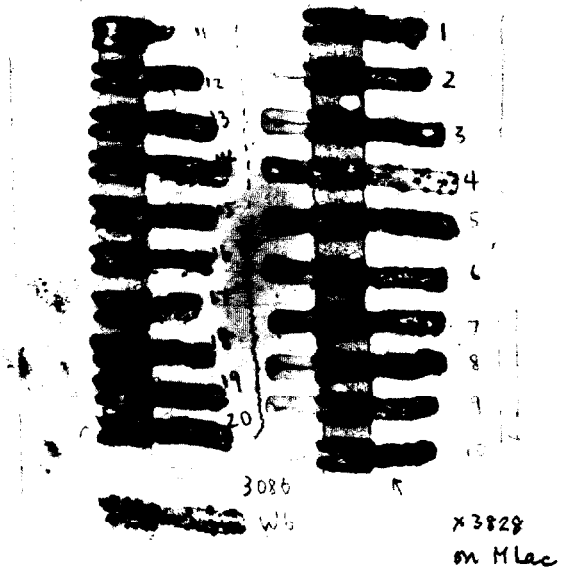
→ x 3086



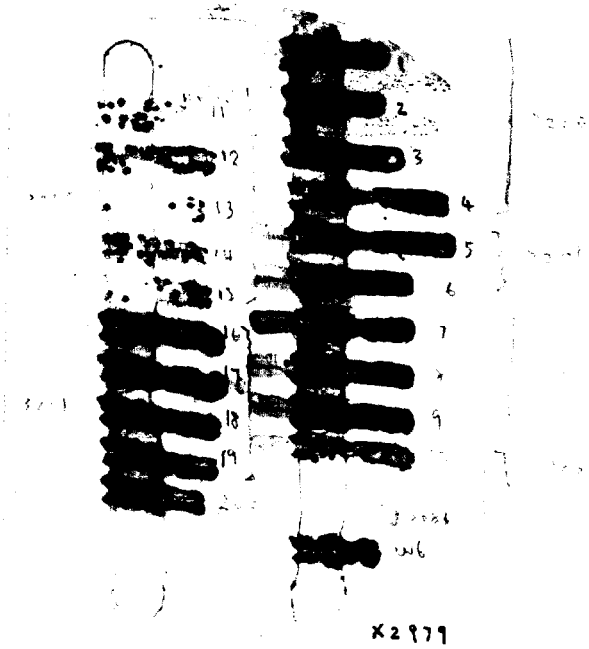
isolation no.

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

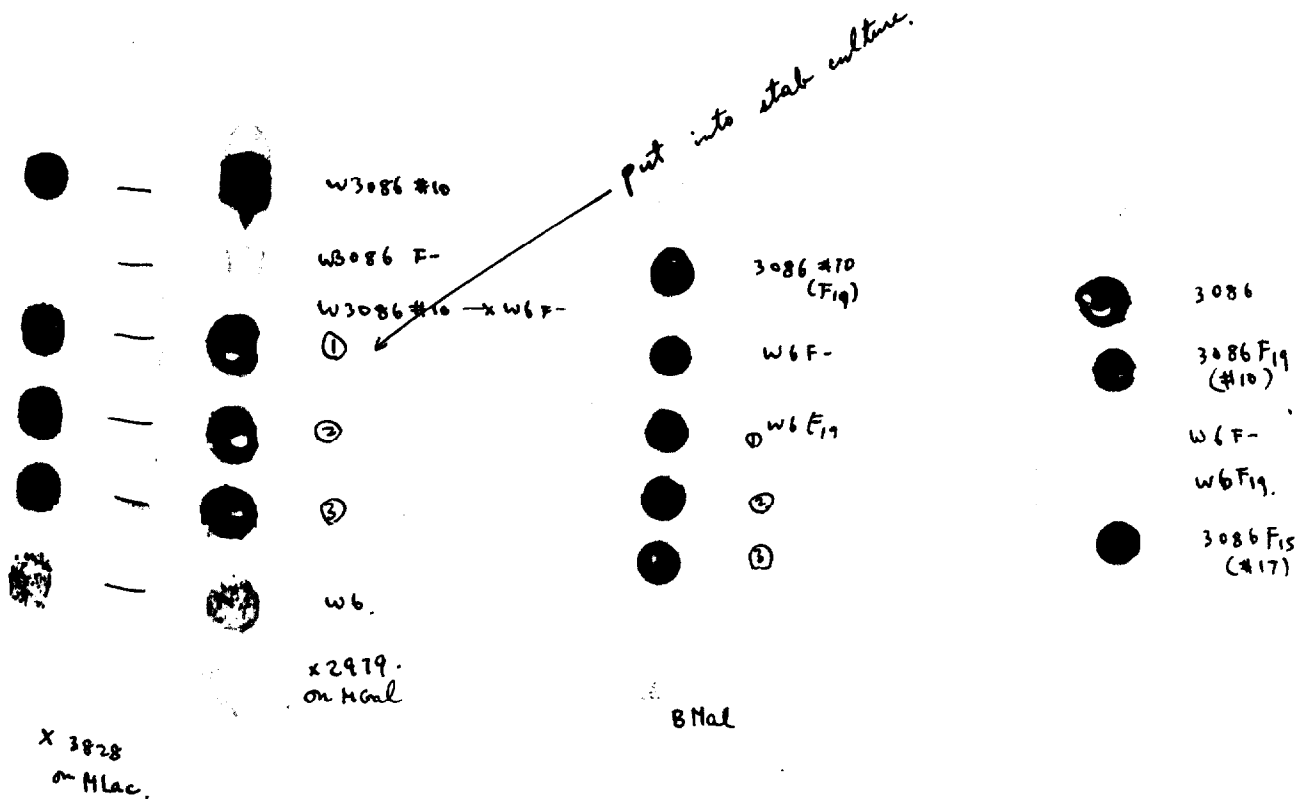
4/33 : 18.2

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 Mal
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)

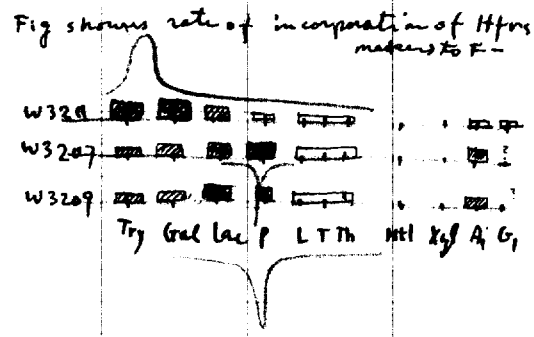
2/16, 1959 REF: See 27/10, '59

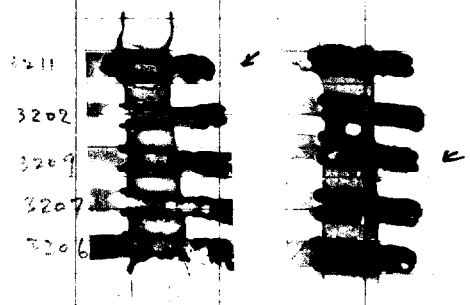
Purpose: Almost of these Hfr strains are reverted into F-.
Reisolation of these Hfr are done by depletion method.

Selective media: M Gal (x2979)
: M Lac (x3...)

	Fertility x 2979 M Gal	Fertility x 3202 M Lac	Hfr
W3211	++++	+	O Hfr11
W3202	+	+	
W3209	+	++	O Hfr9
W3207 a	+	++	
b	+++	++	O Hfr7
W3206 a	+	++	
W6	+	+	

Fig shows rate of incorporation of Hfrs markers to F-

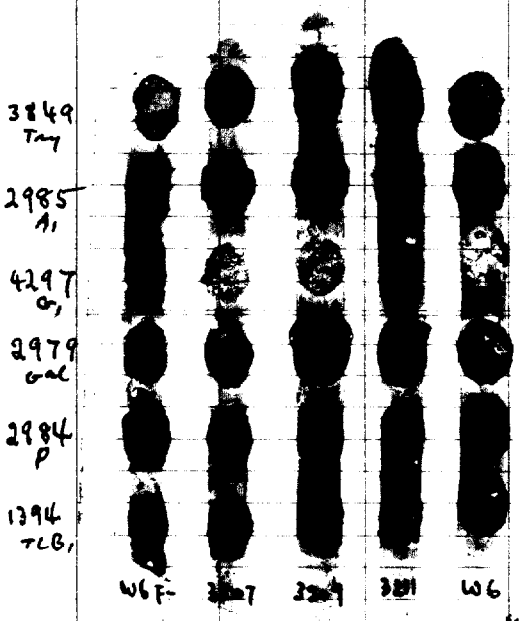


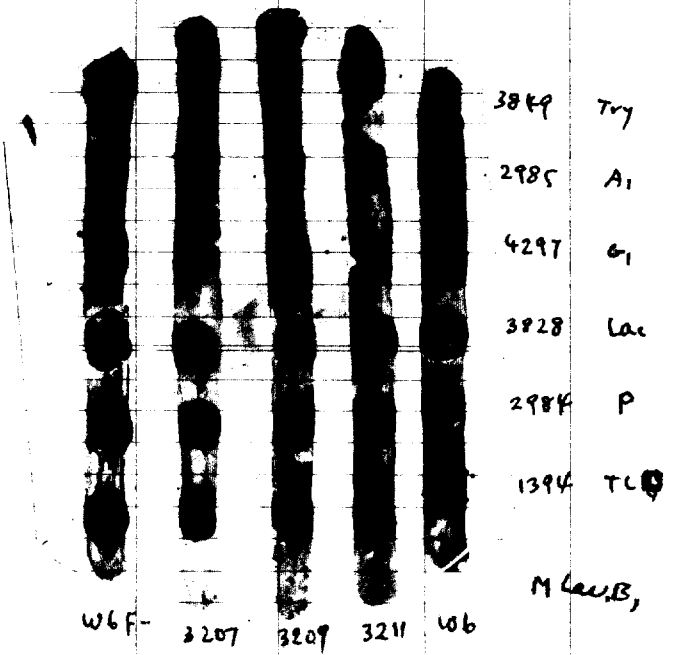


on MGal on Mlac

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

many recombinants appears very late in W3207 + W3209





Infection of [illegible], F_{15}, F_{19} to W4354 ~~3086~~ ~~and W3876~~ (W6F-)

3/14 ; 1959 REF:

Experimental conditions.

cultural age: ca 20 hrs.

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

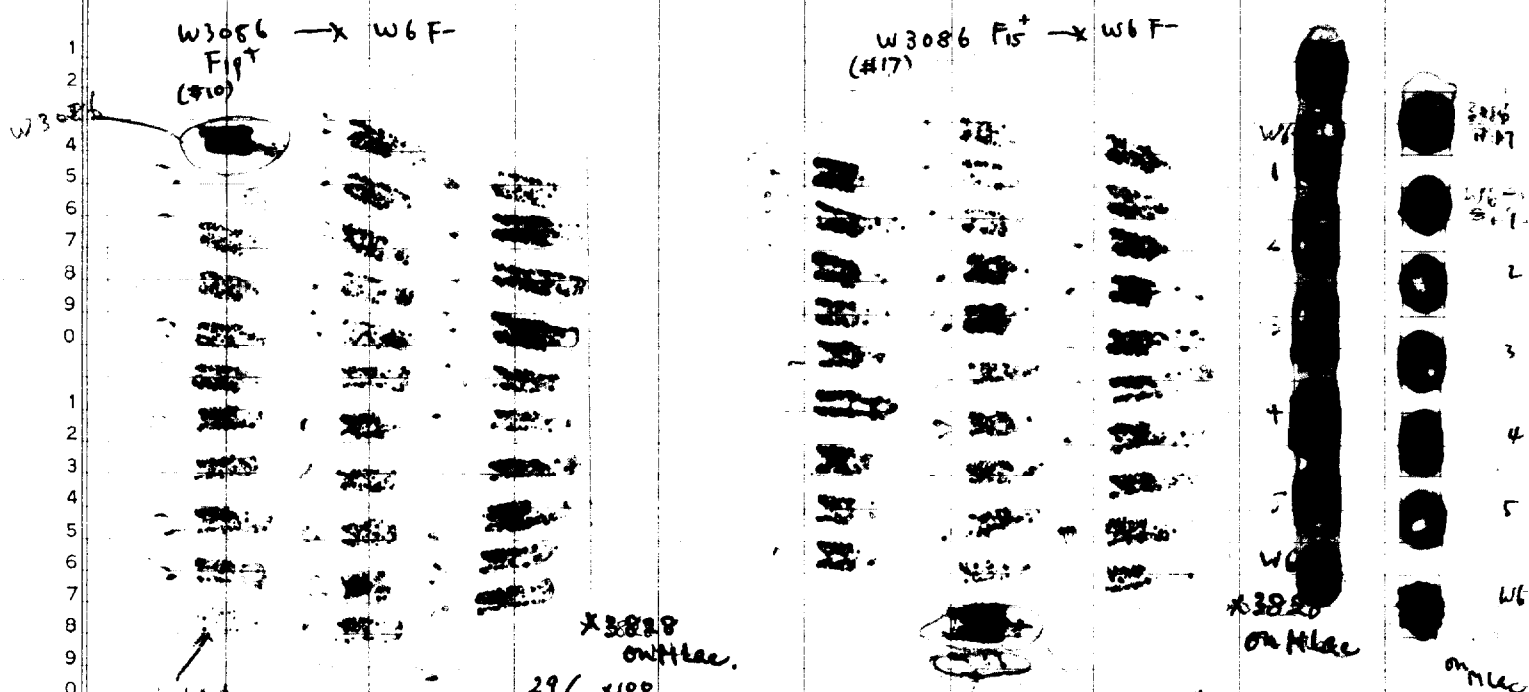
condition of infection: Overnight, at 37°C, standing.

Retest after growth in penassy broth. (becomes higher)

3086 #17

W3086 F_{19}^+ (#10) → W6F-

W3086 F_{15}^+ (#17) → W6F-



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

W3086 F_{15}^+ (#17)

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

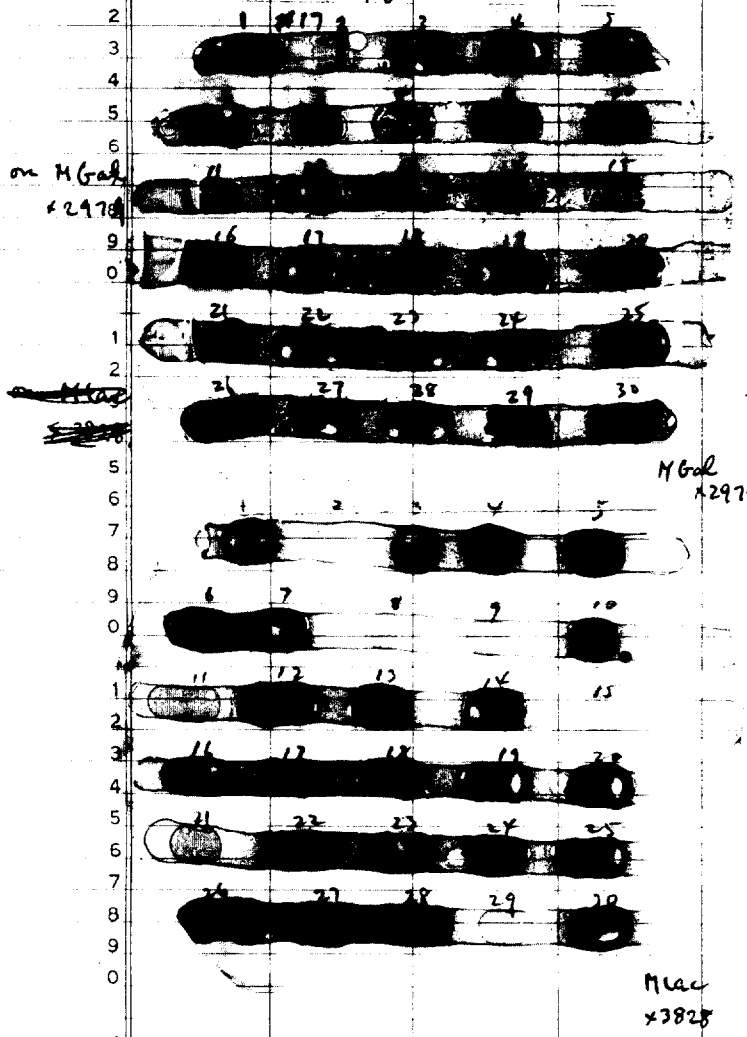
Seems like state II.

Conclusion: Infection of F18 or F19 to F- gives state II (Low frequency male).

State I and state II in F'+ strains.

8/IV ; 1959 REF:

3086 F8



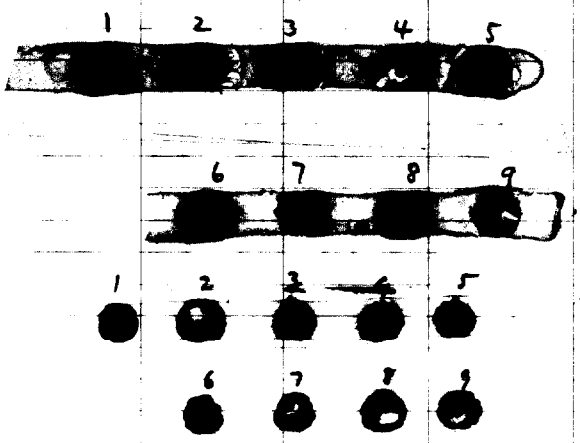
on MGal ×2979

MGal ×2979

7/30 ×100 = 36.3

MLac ×3828

3086 #10 (F19)



MGal ×2979.

MLac ×3828

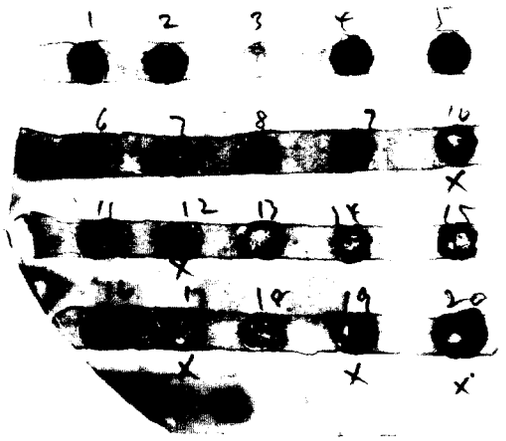
Method : 1. Spread on BLac and let it form colonies on it.
2. Pick each colonies and suspend into pipassay.
3. Spot them on MGal (streaked W2979 and W3828. and MLac)

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 fission or # of F8 in cytoplasm is very low.

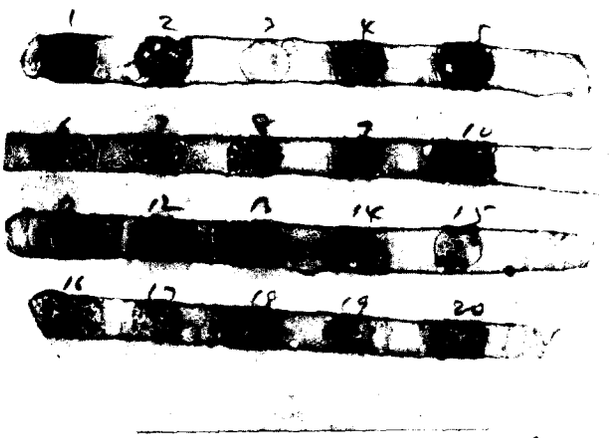
2) 3086 F19 : ( ) gives two state, Hi and Lo.

3086 #17
F15



on MCal

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. 10^4 cells/ml, conc. 40g/ml.
Medium: penassy. 5 ml.
cultural age: overnight culture.
Time of Treatment: [illegible]
Strain treated; W6 (control), 3086-10, 3086-1, 3086-17.

Result

		F⁻/total stat. II.	I	F⁻	% of F⁻.	effect.
F_1	W6 AO 40g					
	" control.					
F_8	W-3086-1 AO		11/85	74/85	87	+
	" cont		68/87	19/87	21.8	
F_{19}	W3086-10 AO		8/48	49/48	83.4	+
	~~W3086~~ cont	81/109	28/109	0/109	0	
F_{15}	W3086-17 AO		84/149	65/149	43.6	+
	" cont.	38/88	50/88	0/88	0	

put into stab.

3086 AO #1

C

3086 AO #17

3086 C

3086 #1 AO

3086 #10 C

3086 #10 AO

C

3086 #17 AO

C

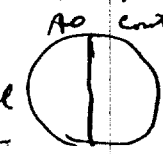
W6 control.

MGal

Mlac

× 2979 on MGal

○ 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.

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Transmission of F_8 from $W6F_8^+$ to Hfr_1 and F^-

(W1922) (W1394)

M Hfr_1 S^R TLB, S^R save $W1394F_8^+$

13

5/12 ; 1959 REF:

Method.

~~$W6F_8^+$~~ { $W6F_8^+$: 1 ml; Hfr_1 or F^- : 0.1 ml (10^{-2} dil) 5 ml pen.

1.) Transmission $W6F_8^+$ Hfr_1 or F^-

Mix them 1000 : 1, and ~~shake it~~ stand it overnight.

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

This data is still not believable trg again using W1895.

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

Experimental conditions.

cultural age ;

medium ; Pen. 5 ml. ; Inoculum size and ratio : 10^8 : 10^5. $W6F_8^+$

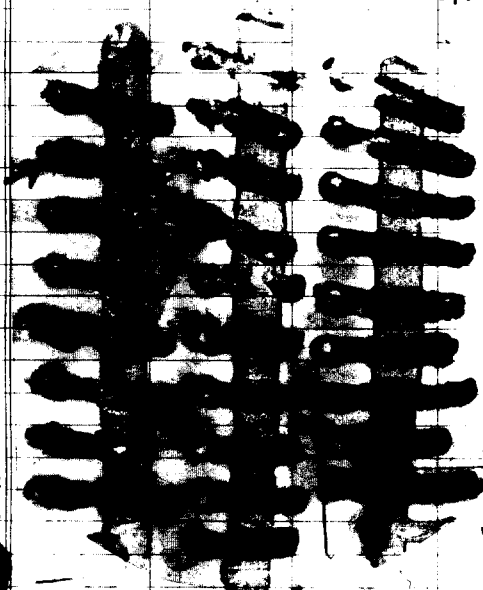
Conclusion : F_8 is transmissible to Hfr_1 !, and gives Hfr_1 character in the sense of chromosome transfer.

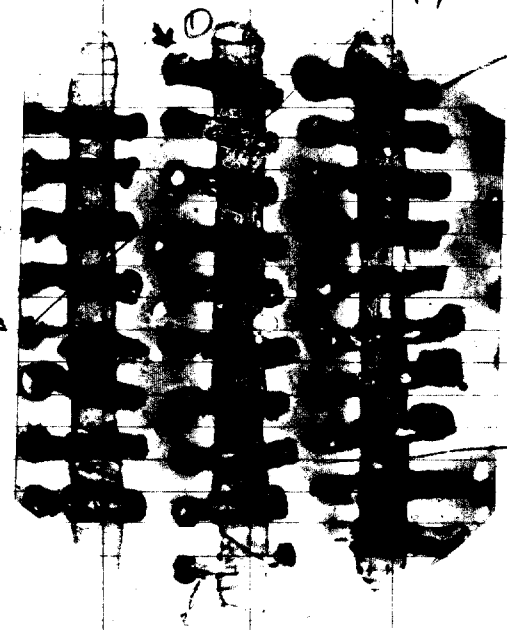
Control.

Select and put into stab.

$W6F_8$ → x W1394 TLB, S^R

$W6F_8$ → x W1922 Hfr_1 M S^R





Put into stab. W1394F_8

W1394F_8

$W6F_8$ W1394

2/25 x100 =

control $W6F_8$ W1922 (Hfr_1)

x 3849

x 2985

x 4297

x 2984

x W6-

x 2979

12/26 x100 =

$W6F_8$ x 2979 on MGal

1922 F_8 1

1922 F_8 2

1922

W6-

x 2979 on MXyl

x 2979 on MGal.

further check.

· Hfr_1 Test on MLac B_1 x W1394

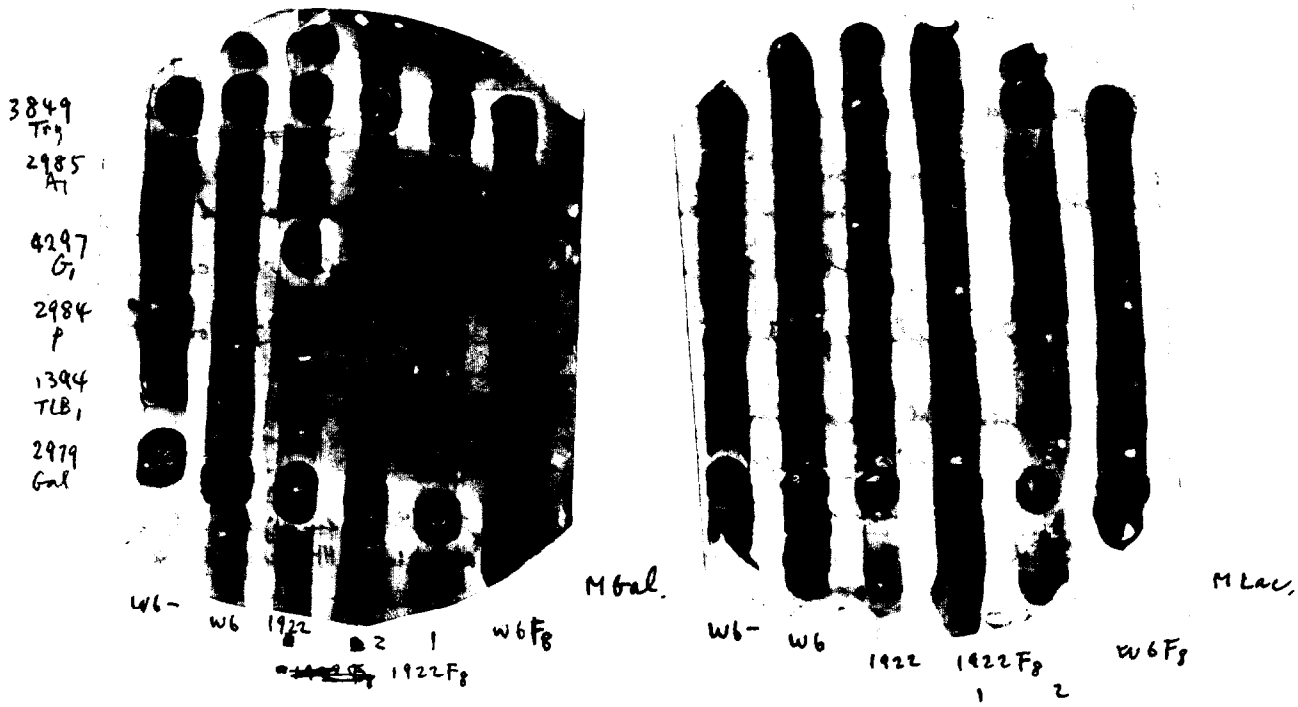
· F_8 Test on MGal x 2979

$W6F_8$ → x W1922

$W6F_8$ → x W1922 Replica plated on MGal x 2979.

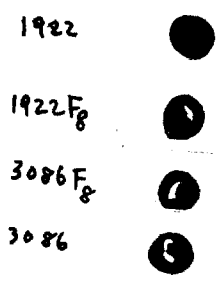
on MGal

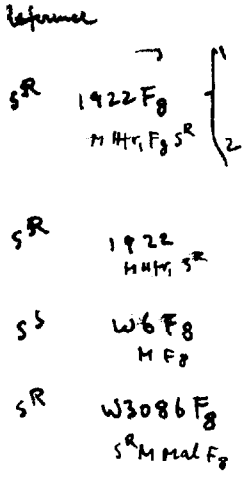
W1394 control



1922 Mal test. on B Mal.

nutritional requirement



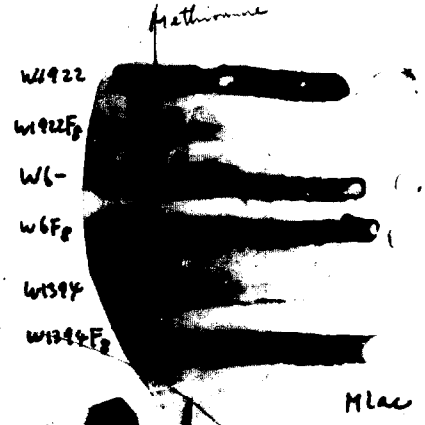


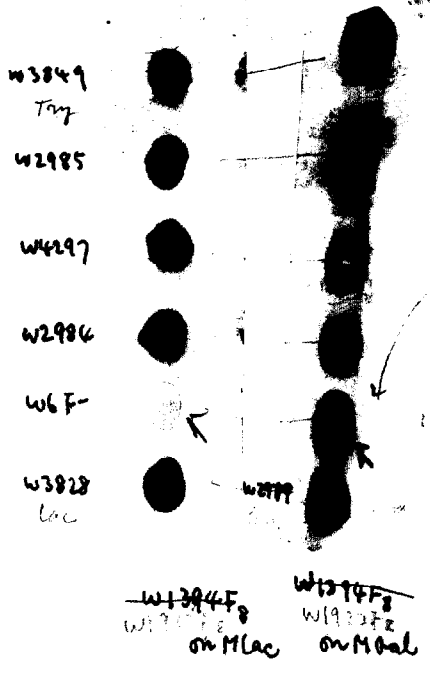
? test nutritional markers of 1922F8
did I mix cal up with W1394F8 and W1922F8?
These culture is made in same time.

and W6F8

X W1394
TLB1 SR

MLac SMB1





Conclusion [illegible]

W1394F8 must be M-

W1922F8 must be TLB1-

It is clear that I mixed up with W1394F8 and W1922F8

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

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

7/IV ; 1959. REF:

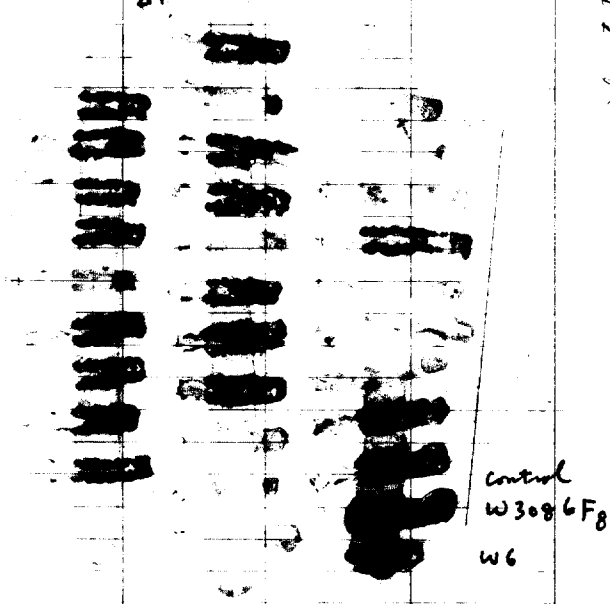
Experimental conditions.

Ratio: W6 1 ml + 0.1 ml $3086\,F_8^-$ + 5 ml pln.

" + $3086\,F_{15}^-$ + 5 ml pln.

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

W6 → × W3086 F_8^- #1

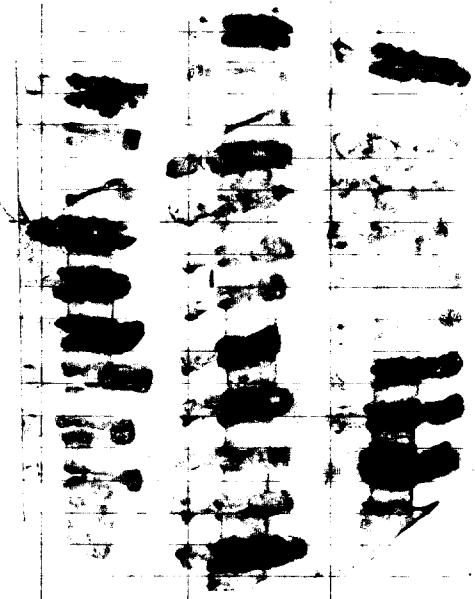


control W3086 F_8 W6

× 2979 on M Gal

this bo[illegible] due to the [illegible] impression technique not spontaneous mutation

W6 → × W3086 F_{15}^- #17



control W3086 F_8 W3086 F_{15}^- #17

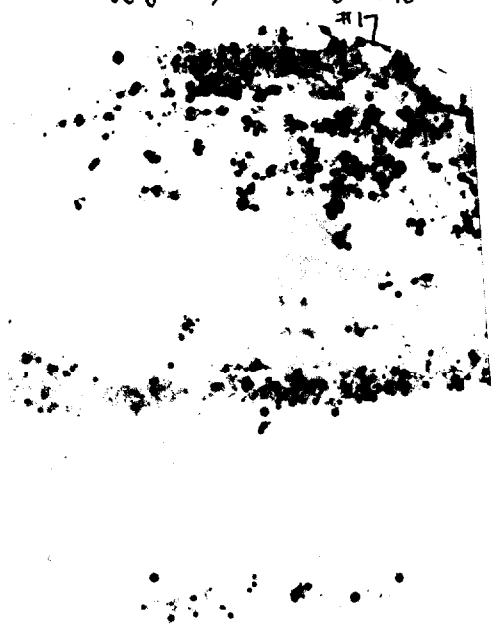
× 2979 on M Gal.

Conclusion:

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

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

W6 → W3086 F_{15}^{-}
#17



ordinary FP

X2979
or Moal

W6 → W3086 F_{8}^{-}
#1

3086 F_{11} ——x W6 F−

3086 F_6 ——x W6 F−

12/11 ; 1959 REF: Cf P15

Method

1.0 ml of 3086 F_{11} or 3086 F_6 (24hr old) + 0.1 ml of W6F− (20 hr.) + 5 ml pen.

↓

Incubate overnight

↓

Purify it on BMal, and pick Mal+ and test Hi for Gal (on MGal x 2979)

↓

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

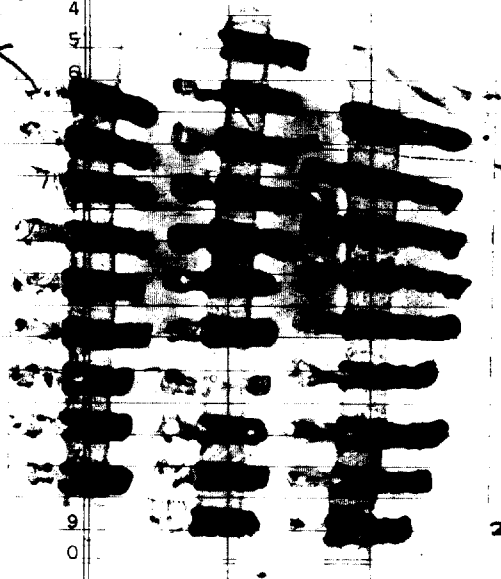
Result:

Rate of transfer. Hfr_{11}/total

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

Put into stab. W6 F_{11}.

3086 F_{11} —x W6F−

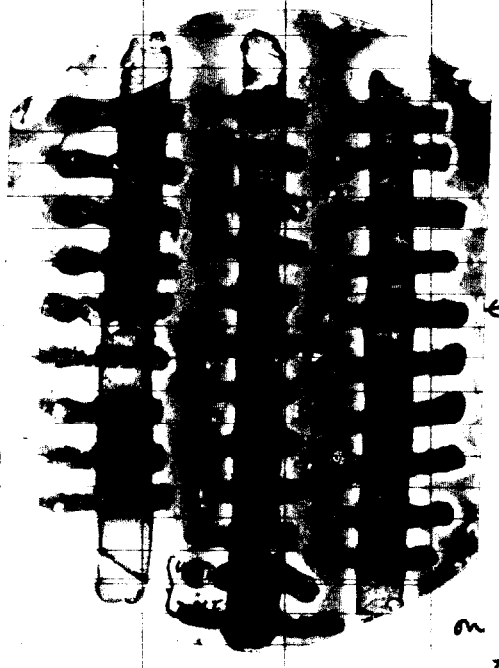


W6F− } Control
3086 F_{11}

27/28

on MGal x 2979.

3086 F_6 —x W6F−



Put into stab W6 F_6

on MGal x 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 :

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, G, 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

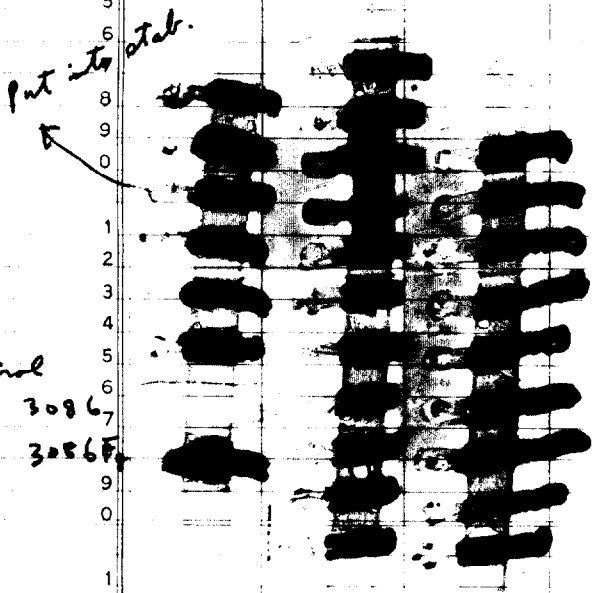
~~W1922 F_8~~

(~~Hfr, F_8~~) × 3086.

Conclusion :

F_8 in W1922 F_8 has infectivity to F^-.

It is in cytoplasm of W1922.



× 2979 on M Gal

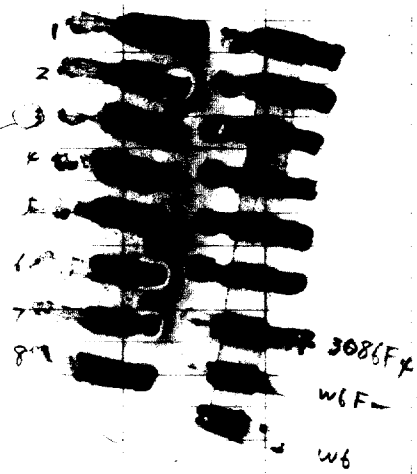
3086 F_4 ⟶ × W6 F−
M S^R Mal F_4

13/IV ; 1959.

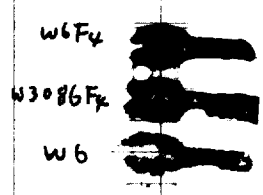
REF:

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 for A_1

Put into stab.
W6 F_4.



× 2985 (A_1^- F^-)
on Mlac



× 2979
on MGal.

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

W1394 F8
F8 TLB1 SR

12/IV 1959

REF: does F8 be removed by AO treatment?
How about Hfr1 makes 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.

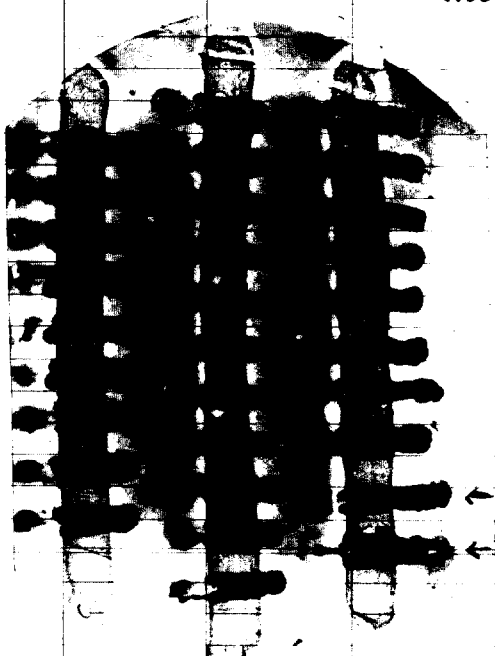
3. test fertility of Gal and TL.
(x2979) on MGal ; (x1922) on MLac SMB1. It does not grow in AO. (It may be too conc.) Try again.

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.

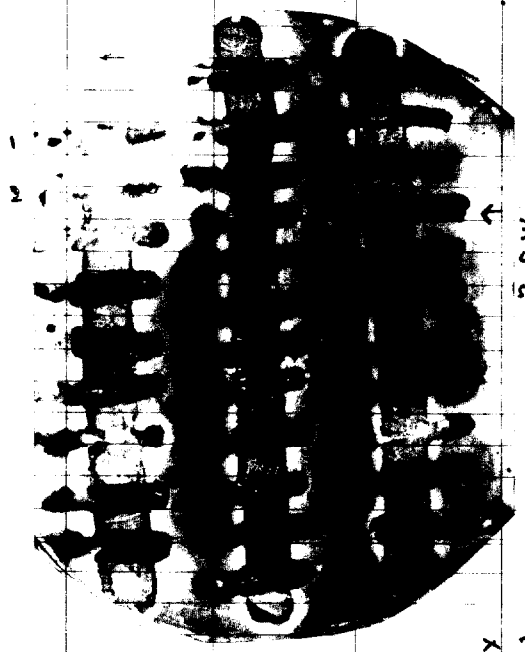
2) test their fertility on MGal and TL
(x 2979) on MGal (x 1922) on MLac SMB1

untreated control W1394 F8 ~~W1922 F8~~



x 2979 on MGal

AO treated W1394 F8 ~~W1922 F8~~



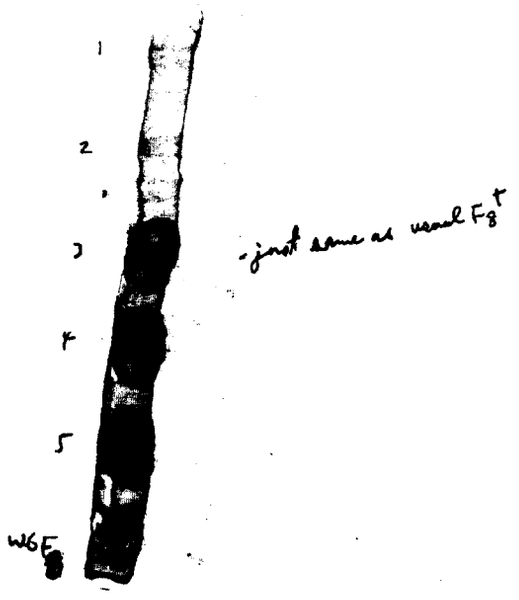
x 2979 on MGal

% 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 bit more unstable.
How about W6 F_8^+. Is it stable or unstable?

Method :

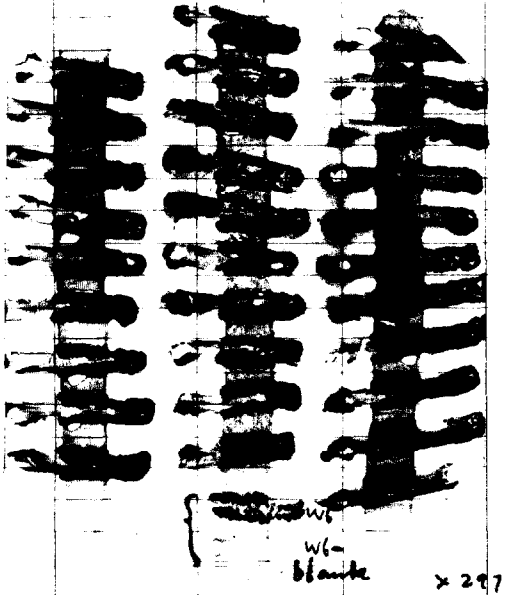
1. Purify W6 F_8^+ on Blac. and suspend single colonies into water.
2. Purify it on Blac and test compatibility of each colonies. (on MGal x 2979.)

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).

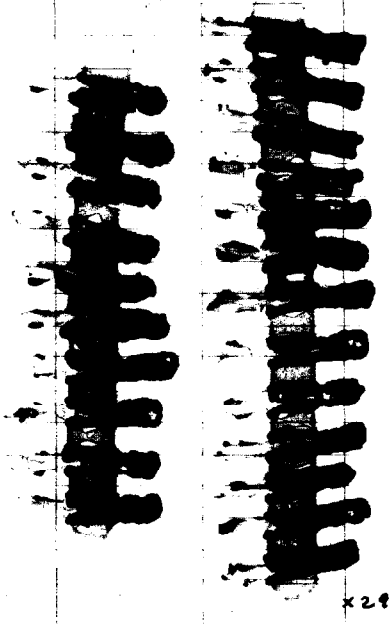
Untreated W6 F_8^+



W6-blanks

x 2979 on MGal

Untreated W6 F_8^+



x 2979 on MGal.

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

Treatment of W6F8 by AO.

15/IV. 1959. REF:

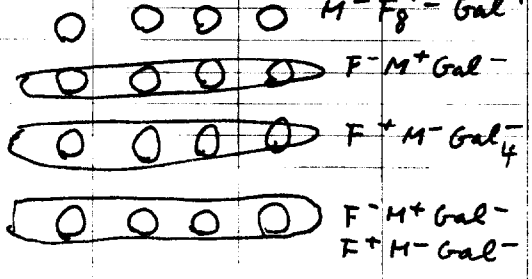
Purpose : Does W6 F8 produce $♀_3$ type F⁻ after treatment of AO?

Principle :

M⁻ F_8'⁻ Gal⁺
F⁻ M⁺ Gal⁻
F⁺ M⁻ Gal⁻

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

Method



(W6 F_8⁻)
(2979)
(3046.6)

on MGal

Principle :

M⁻ ♀ × — Gal⁻ M⁻ F⁺
×
Gal⁻ on MGal.

- Use single colony as a starting W6F8.

1. Purify W6 F8.
2. Treat W6 F8 by AO. Inoculum size : Ca 10^8 cells/ml. AO : 30γ/ml per.
3. Test their sex-compatibility by crossstreaking 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

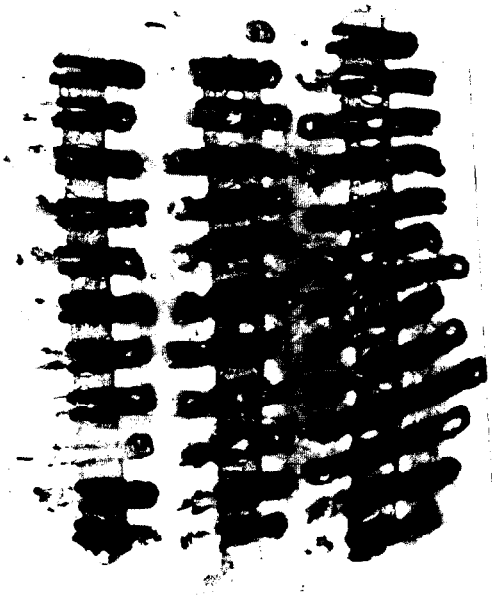
% of ♀ obtained : 8.35

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

see back page

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

W6 F8 AO treated



W6 F8 AO treated.

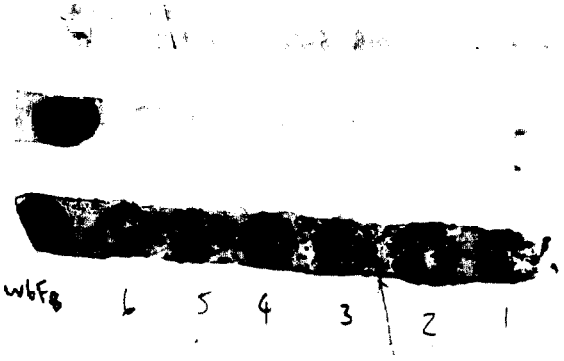
♀ : 6.
♂ : 66.

$^{6}/_{72} \times 100 = \underline{8.35}$ % ♀.

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

x2979
on MGal.

blank.



3046 F+ M- Gal-1

2979 F- M+ Gal-2

3046+2979

on MGal.

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W 4526
F^8 TLB, Mal_5 S^R f_3.

23

W6 F_8 —— x W3644
F^- TLB, Mal_5 S^R f_3.

12/10. 1959 REF: cf. 55, 70, 46.

← W6 F_8

← W3644

on BMal on BlacSM.

W6 F_8 1ml + W3644 0.2ml + Penassy 5ml.
↓
Incubate it for overnight at 37°C.
↓
Purify on Blac SM.
↓
Test their sex-compatibility (x 2979) on M Gal.

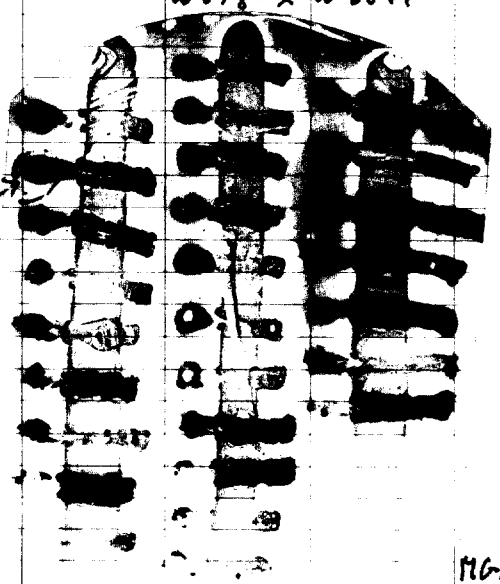
Result.

F_8 is transfered to f_3 (W3644). W3644 F_8 shows Hi-trait after infection.

Further experiment:
Compair Hi marker.

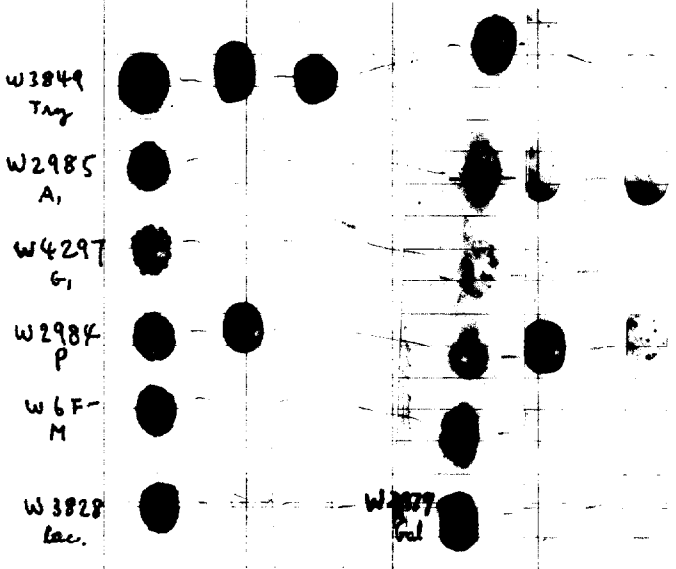
Conclusion: F_8 is transmissible to f_3 and gives high frequency recombinations. The rate is about 50% after growth for overnight culture in penassy.

W6 F_8 → x W3644



Put into stab. 3644 F_8.

MGal
x 2979



$13/27 \times 100 = 48.2\ \%$

Conclusion:
W3644 F_8 is not so hi in Meth (but higher than TLTh).
Hi: Tryptophane, Hi for Gal, Moderate: Prol, M.

W3644 F_8 1394 W3644 on MLac. W3644 F_8 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

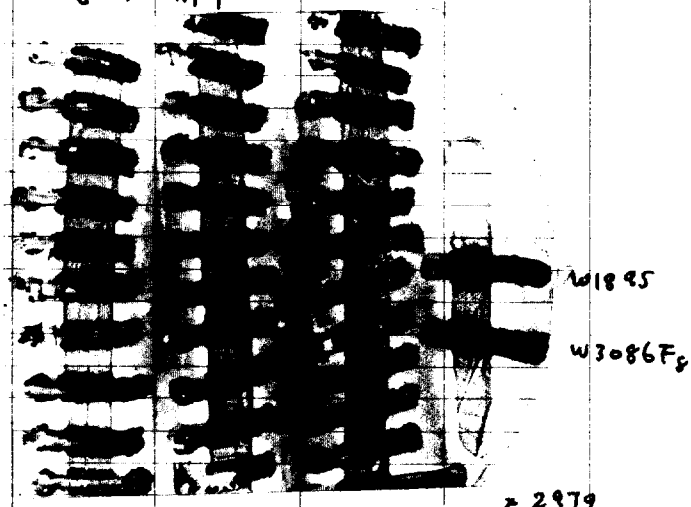
↓

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 on B Gal Sm. → Replica plate it on M Gal added F^- Gal⁻ F^- M⁻.