Segregation of F'lt from double F'+

30/10 : 1959 REF: cf. P. 42

Isolating of Varicose infected strains.

1 2 3 4 5 6 7 8 9 10

① Purify double F clone on Blac. (See: P 42. 23/10 ; 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.

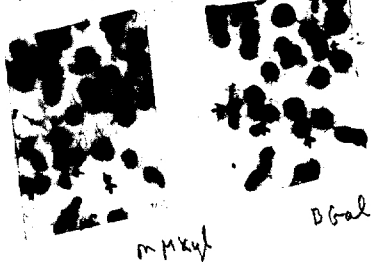
Conclusion : These results seems parallele to the spot test done on 23/10 See P.

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

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

Replica master

$F_a \rightarrow F_8$ (2)



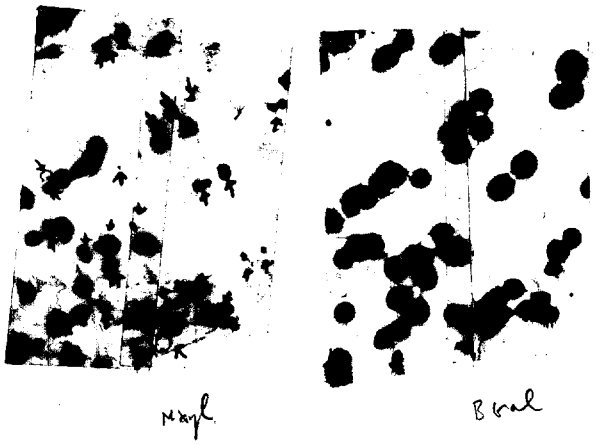
mMXyl BGal

$F_4 \rightarrow F_8$

(3)

Replica master

F_8 F_4 No sound



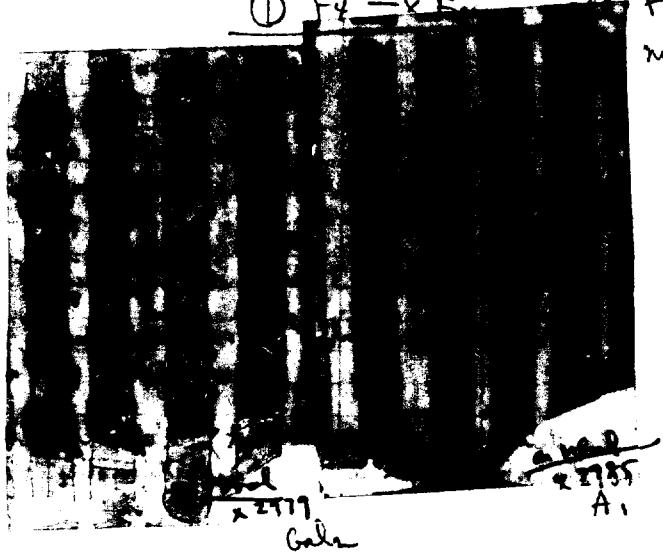
MXyl BGal

Further test for Fetype see front page; compare with the fertility.

(1) $F_4 \rightarrow F_{\text{same}}$ F_8 type. not segregate.

mMGal mMGal

sel: Gal_2 sel: A_1



x2779 x2735

Gal_2 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_4^R)

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 M Gal seeded w 2985 (A_1^-) on it.

of F_4^R gal: Ca. 2 ~ 3 colonies/plate 7). Pick F_4^R colony and inoculate it into broth.

8) Retest the fertility ~ cross times w 2985 on M Gal.

9). Test infectability to F_1^s or F_4 or F_8.

Result :

1. # of colonies tested : Ca. 4000
2. # of F_4^R colonies obtained : 72 (~~all of them~~ only 3 were F^- the other are F^+) recombination -
3. # of F_4^R colonies obtained : after testing by cross brushing method

See p. 52. and the back page.

4. Rate of F^- obtained

U.W.
MICROBIAL
GENETICS

Isolation of more-infective F' from F4.

Mutant "Rapid infection"

7/10 1959 REF:

1 2 3 4 5 6 7 8 9 10

• **Experimental schedule:**

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

↓ incubate overnight

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

↓

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.

} 4 tubes for each.
1 tube for W6 F4
1 tube for W6 F-

1st : 5:30 p.m.

↓

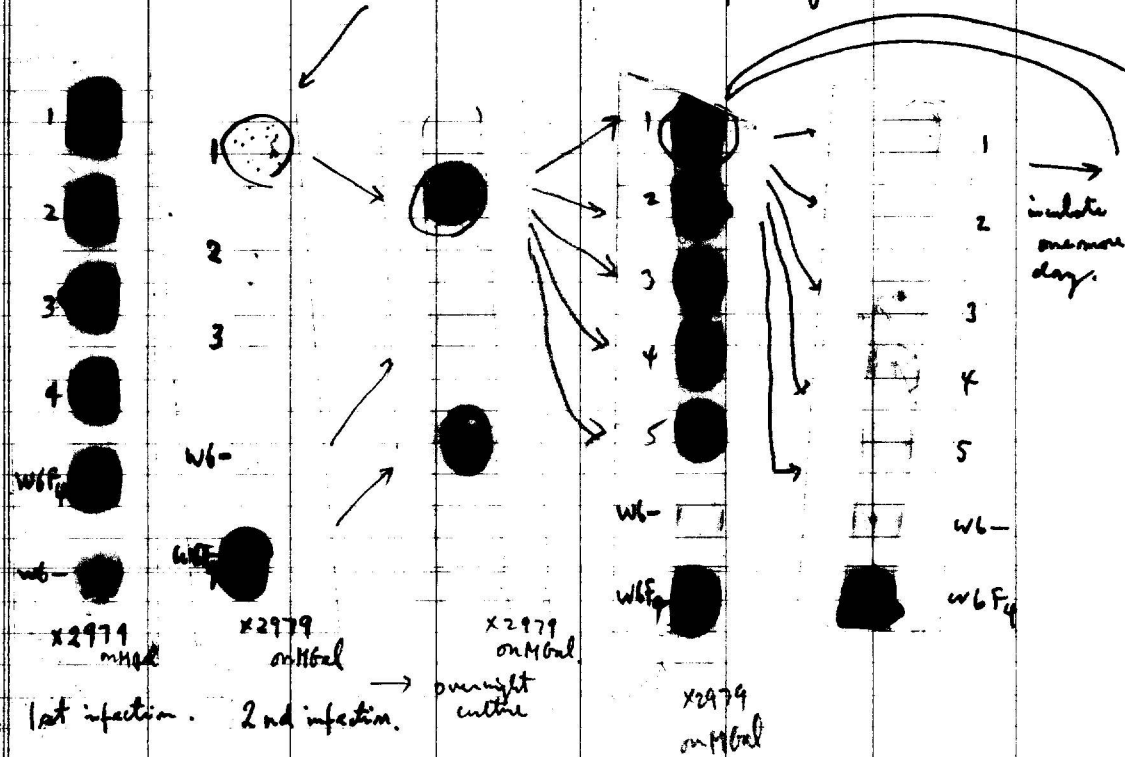
Incubate overnight. — Test fertility by spot test. Save it in cold room.

↓

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 $^+$ strains)

9/4 ; 1954

REF: See p 49.

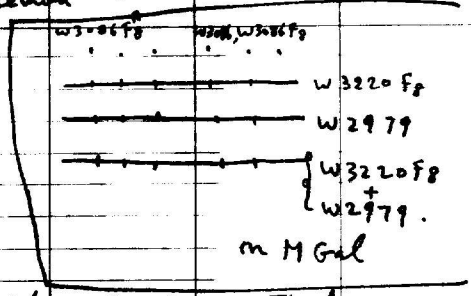
Principle: F_8

$M\ Gal_4^+ \longrightarrow \times$ ⑤ $M\ Gal_4^+\ F_8^{R?}$
(W3220 F_8)

$\times$

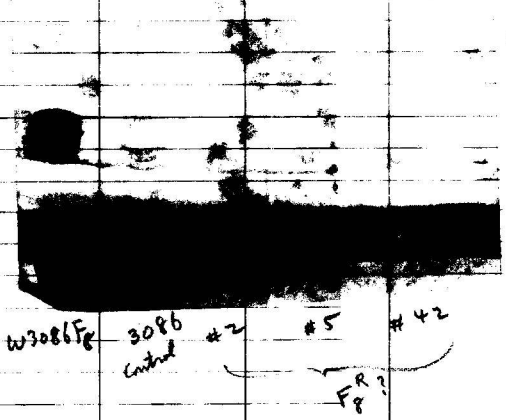
$M^+\ Gal_2^-\ F^-$
(W2979)

^{7}Method



o Difficulty is Gal_4 and Gal_2 are not allelic with each others. Therefor, background gives many a black spot. But it may be possible to differentiate between infectable spot and noninfectable 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 ⟶ Replica plate on M Gal Streaked → [W2979. on it.
on sm B Gal

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

2	5	#2	control.
↑	↑	↑	↑
W6 F8	W6 F8	W6 F8	W3086 W6 F8.

Results : All the clones, # 2, # 5, #42, are infected by F_8, in otherward, these F_8 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

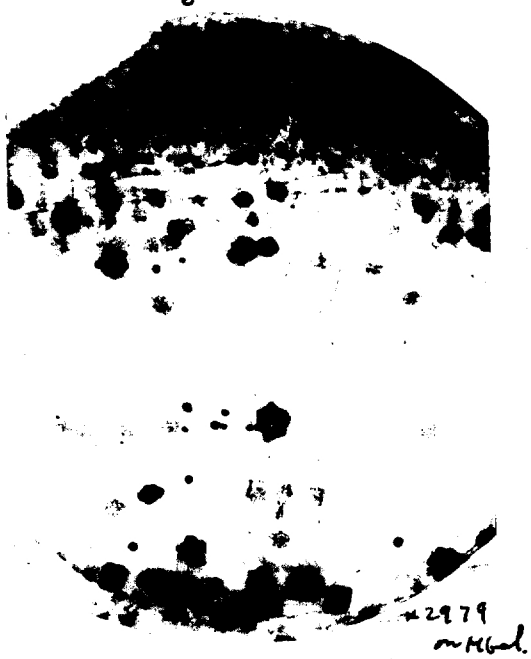
$F_8 \rightarrow$ 3086

×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)

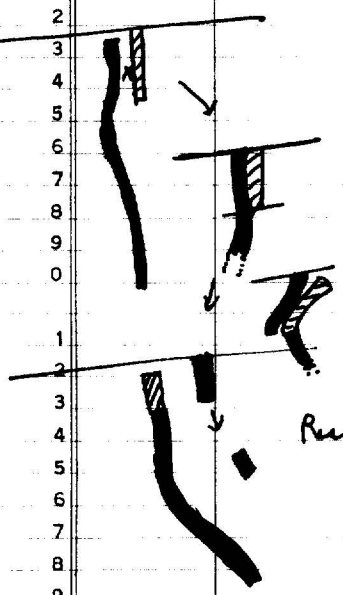
Test for transfer of Hfr$_3$ to F$^-$.

W3234 (G$^+$ ~~Hfr$_3$~~ Hals- MGal$_2$ Lac$_1$)

19

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

Test this possibility by Procedures.



① Purify W3234 on Blac Check 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) from Adelberg) W4531

13 NOV. 1959

REF: Cf. p 62.

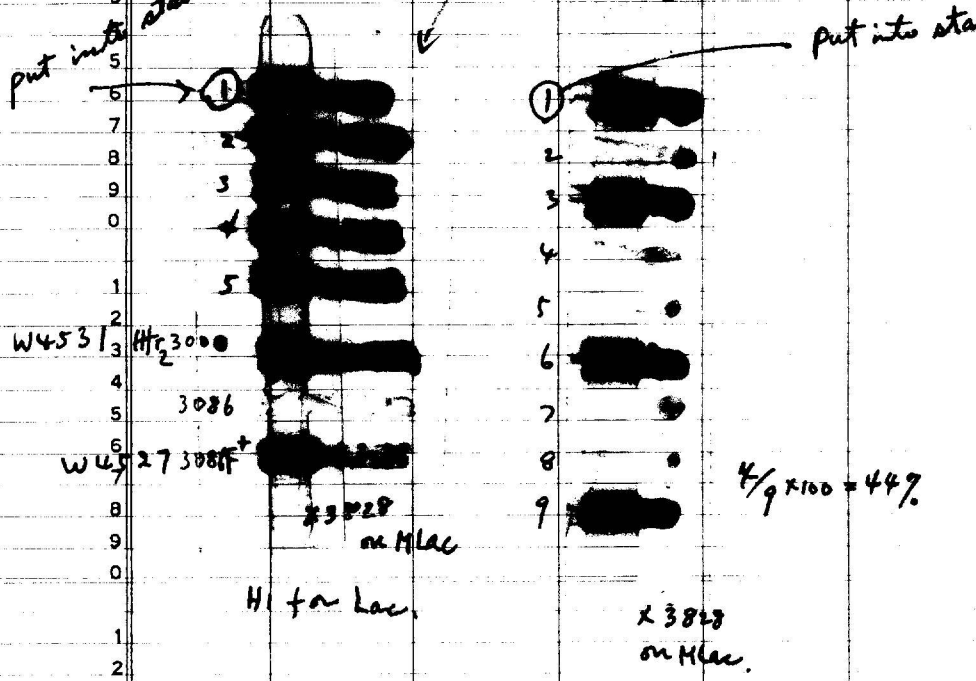
Method:

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 Mlac seeded W3828 mit.
   ↓
   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 Mlac.

   See ~~back page~~.

3. Infect F_2 to W6−, ~~and~~ 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/x. ; 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.
[redacted] :	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 ... the thickness of the layer irradiated are ca. 2 mm.

0 ← ? try again. Something was wr

10

15

20

40

W1−

W6F4 mMal

W6−

W6 F4 on MCal

before incubation — after incubation.

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

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

11/4 ; 1949. REF: cf. P52

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

W6F4 1ml + F strain 0.2ml + 1ml phosphate

↓ Incubate it overnight at 37°c.

↓ Purify it on Blac Sm.

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

Results:
all of these F strains are infectable by F4. (See back page.)

Not immune to the infection of F4.

These F 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⁻.
(3086)

control.

W6 Fu → × 3086

×2979
on MGal.

W6Fu → #39.

#2979
on MGal.

W6 Fu → #69.

×2977
on MGal.

W6 Fu → # 78

×2979
on MGal.

↑
78 maybe promising.

Mix #78 and #5, or #2, or #42 comes from Fo, and test the fertility.
(Fu)

see p.54

Try all the combinations

Segregation of F_1 and F_4 from double F. ($F_{1,8}$ / $F_{4,8}$)
(2nd segregation.)

14/V ; 1959

REF: cf. P.48, 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 sector F_8 type.

From $F_{1,4}$.

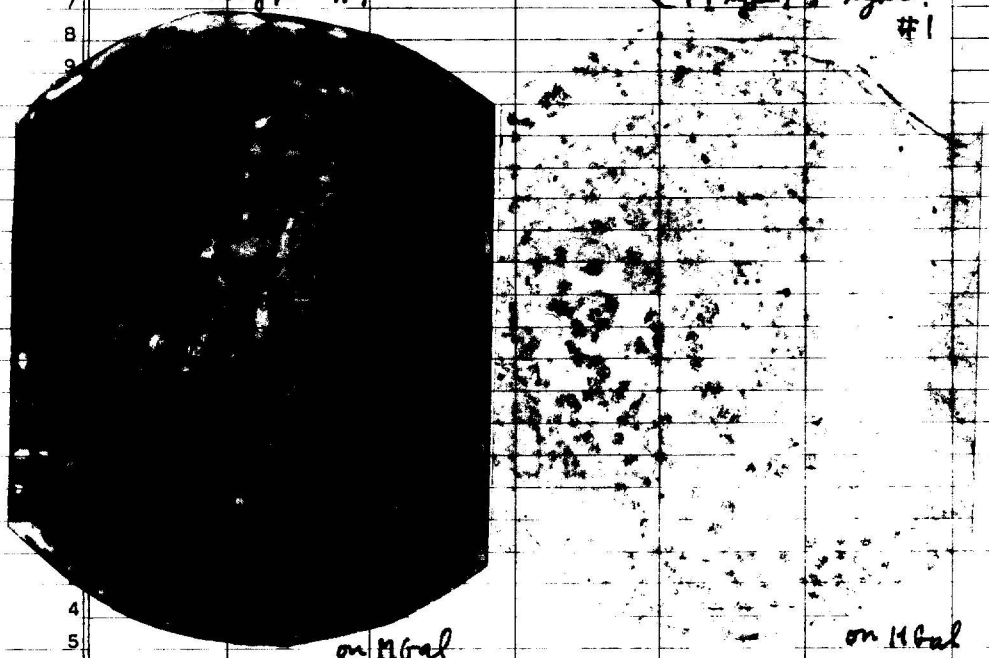
3. F_4 type segregant gives F_4 type with very few exceptions.
4. F_1 type segregate few F_4 and many F_1. But some colonies show higher futility (than F^+). They may still have F_4 in it.

[See back page.]

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

F_8 type #1

(F_1 type) F^- type. #1

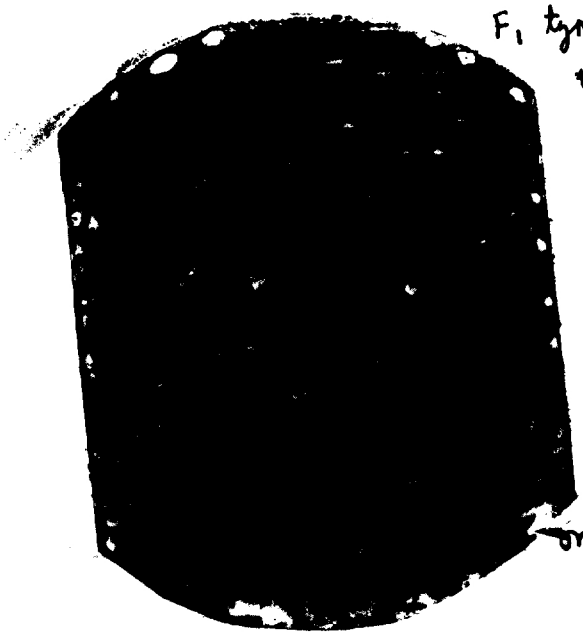


on MGal ×2979

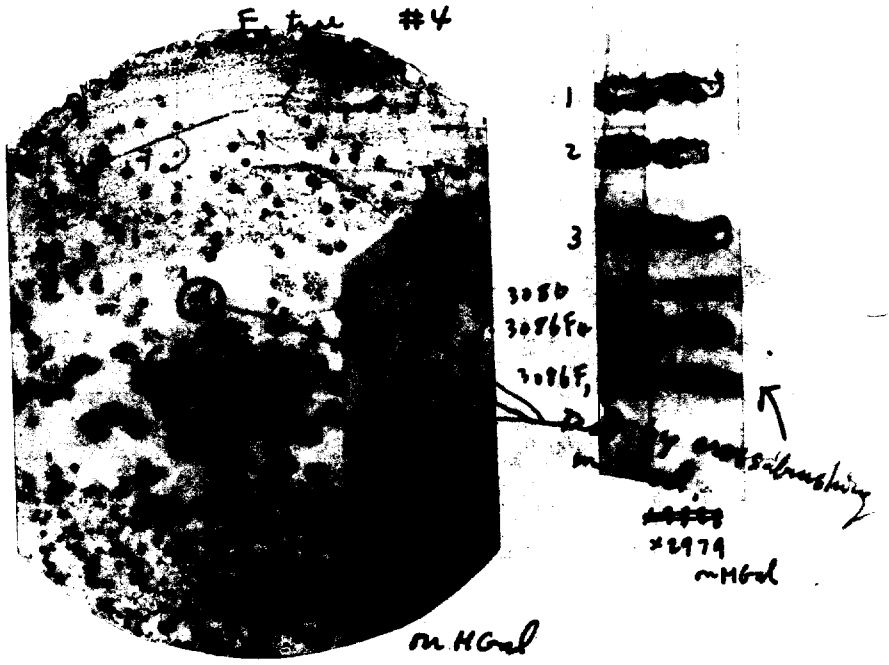
on MGal X 2979.

○ Check if (F^-) arised after infection of F_8 to F^+. It might be the result from exclusion between F^+ and F' 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.

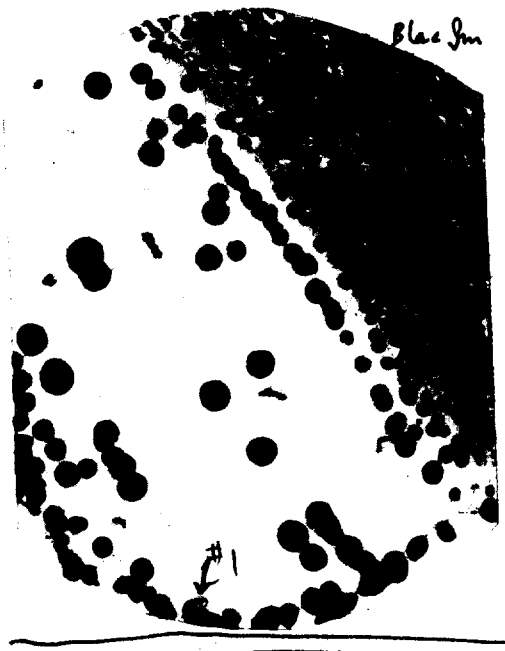
F_1 and F_8 are not miscible, but F_1 and F_4 are missible.

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~~ assemble to F_1)

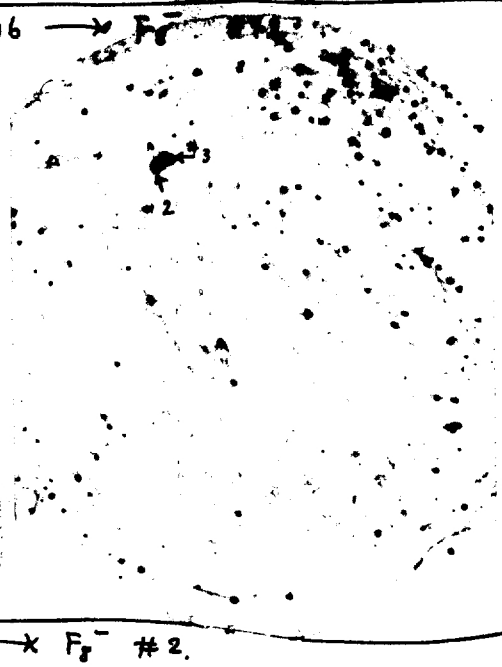
Interpretation: Both kinds of Fs are spread into the bacterial populations, and they are distributed with different proportion of particles.

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

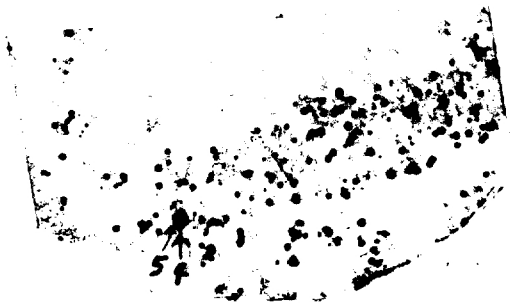


×2979 on Kodak

W6 → F_8^- #2.



W6 → F_8^- #2.

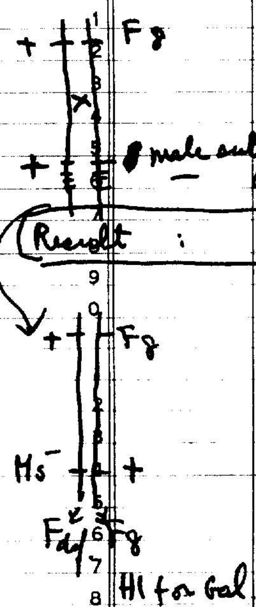


Infection of F_1 to F_8^- W6 $\xrightarrow{F_1}$ × F_8^- 3086. 2, 5, 42.

14/V ; 1959.   REF: cf. p.58. See P.68

#2,⁶ #5, #⁷42.

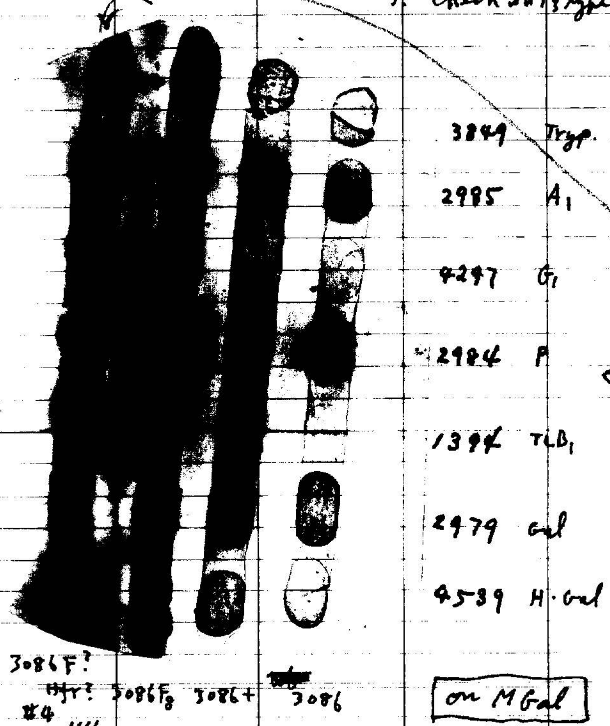
Principle
1. Mix W6 1ml + F_8^- (M 5% Mal.) 9/ml + 5 ml penassay.
2. Incubate it overnight.
3. Purify it on BLac Sm.
4. Replica plate it on M Gal seeded 2979 on it.
5. Pick Hfr colonies and retest by cross breeding, or spitting method.



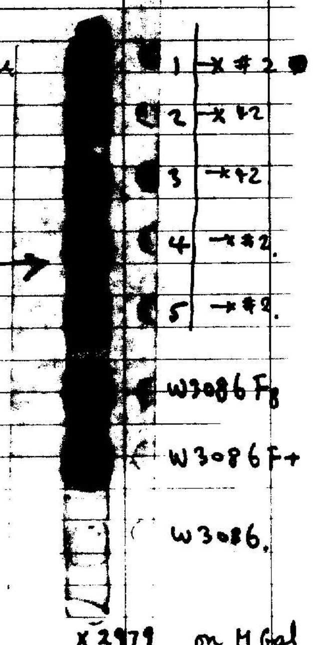
Result : Seems promissible!   Ratio is quite low, but gives Hfr colonies by infection of F_1 to F_8^-.

1. 3086F⁻ is more infectable by F_1 than 3086F_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.~~

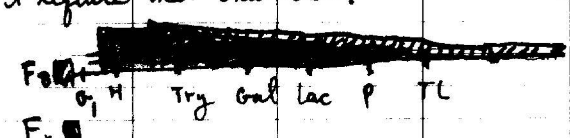
Further experiment:
1. Pick Hfr colonies and test by (cross breeding method) spot test (see bellow)
2. Recombine ~~these~~ these defective F'.
3. Retest using more larger scale.
4. check it F_8 type male or Standard F^+ type or F' type



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

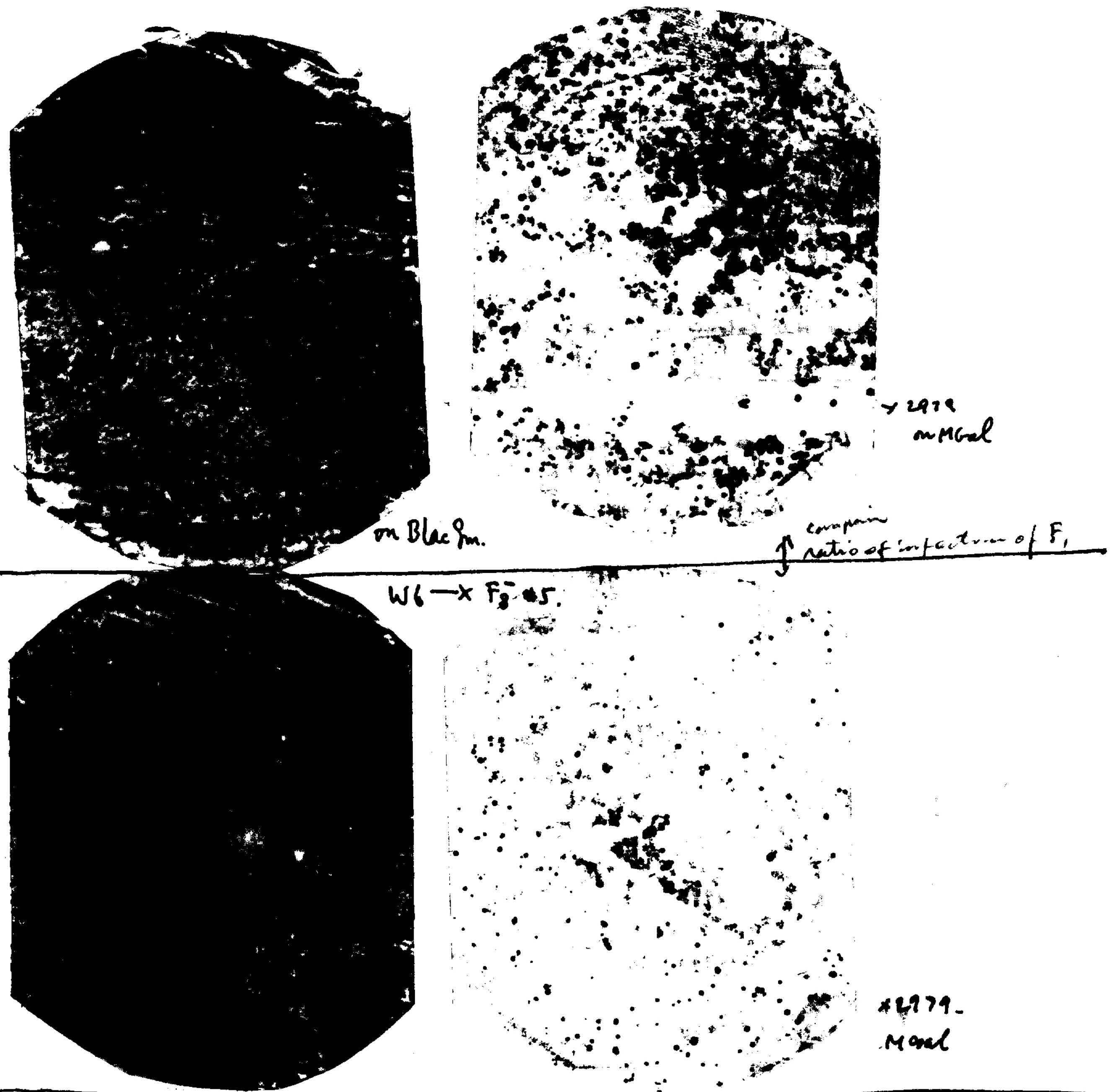


Hl for Histidine : This Hfr type mutant seems differ from F8, however, it is quite useful because Hl transfer ~~g~~ character for histidine, it is linked to antigenic characters.
How does it require that character?   Hl-marker seems shift a little to Histidine side.



W6 → x 3086
control.

Master plate — Replica plate.



Comparison of Hfr$_2$ and F$_2$ strains.

17/V, 1959 — REF: 4. 857

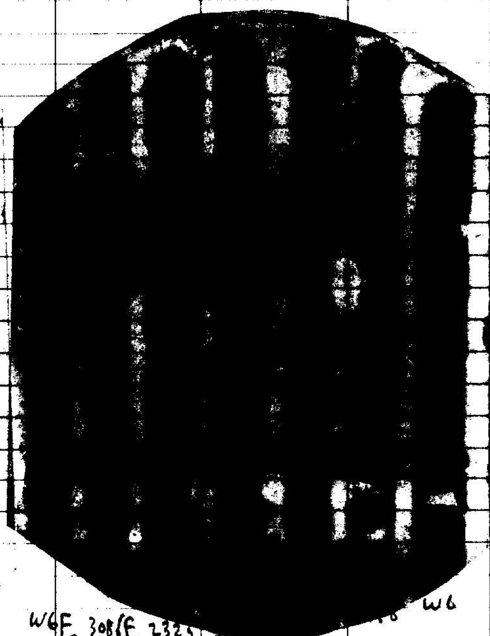
conclusion

B$_1$ | TL ? Ara$_2$ P Lac Gal Try
H1 H1 H1 H1 H1.

F$_2$ showes quite same quality as Hfr$_2$ in transfer of chromosome.

3849
2985
9297
2984
1384
2979
Gal

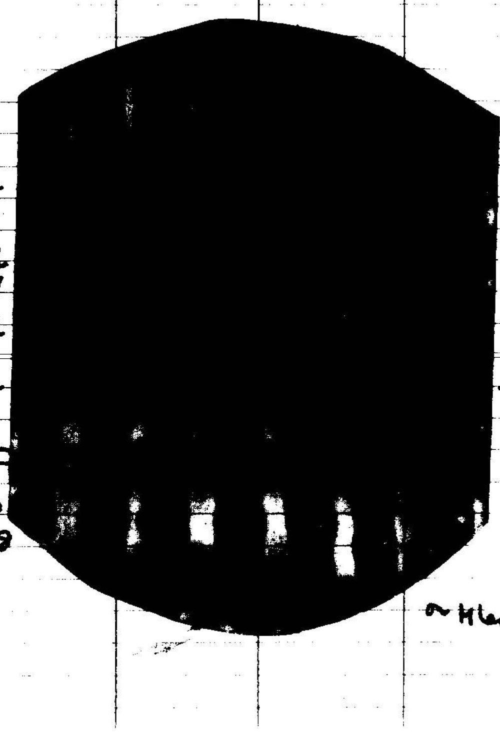
on MGal.



Try Syntrophy.
A$_1$
G$_1$
P
TL
This is mistake: 2979 was spotted on MLac B$_1$
3828 Lac.
3950 Ar

on MLac B$_1$

3849
2985
2984
9297
2984
1384
3828
2950
2829



← Syntrophy
TLB$_1$
← mistake W2979 was spotted by mistake.

on MLac

W6−
3086−
W6
3086+
Test Ara$_2$ 2323
3086F$_2$
W6F$_2$

Selected H$^+$ Ara$_2^+$

on MAra$_2$
× 2979

Isolation of F_5 from Hfr_5 [redacted] (W4537: Hfr_5 [redacted])

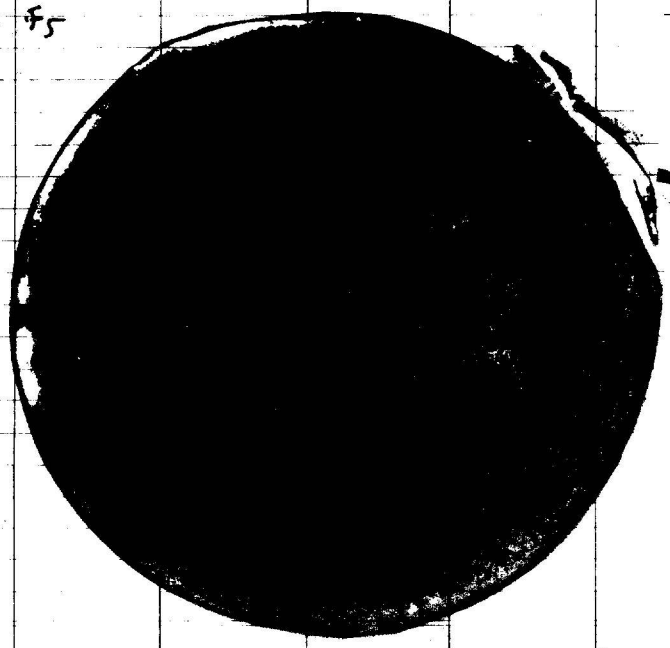
16/V ; 1959 REF: (W6-, 3086)

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

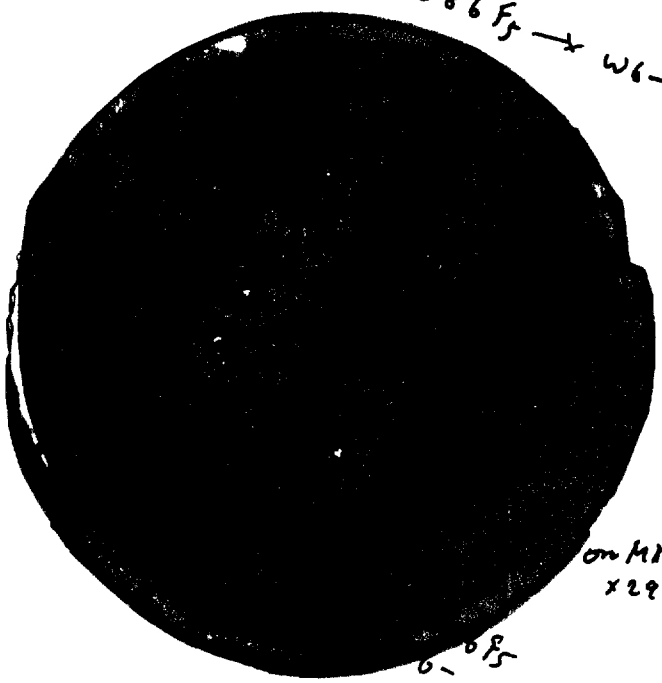
1. Replica plated on MXyl seeded 2979 on it, and looked for Hfr colony formit.
2. Test infected F'_5 by cross streaking method. (check H1 for Xyl)
3. Infect F_5 to W6-. see back page :
4. Compair fertility-pattern to original Hfr_5 (Hfr_5 H1 for Th, Xyl, A,)

W4537
Hfr_5 → F_5

on B Lac Sm



on MXyl.
×2979.



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

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

3086 F5 —cure by Ac.→ 3086 F5⁻ ← F1

↓

? If a factor which controls high fertility was left back, infection of F1 to 3086 F5⁻ may ~~give~~ results Hfr5 type cell.

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

17/V ; 1959 REF:

Principle : M⁻ Gal₄⁻ F_8 (W3220 F_8) W4534
M⁻ + ↓ F⁻ (W6 F⁻) W4354
+ Gal₄⁻ F⁻ W3994.

Select on MGal.

Purpose : Does this system work well ?

Result : Ok.

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

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

W3994 (Gal₄ 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 one colony from the spot, and inoculate it into plenary. (5 ml)
4. Infect the F to 3994 F⁻ (mix culture of it with 3994 and purify it)
5. Test the infectivity by spot test.

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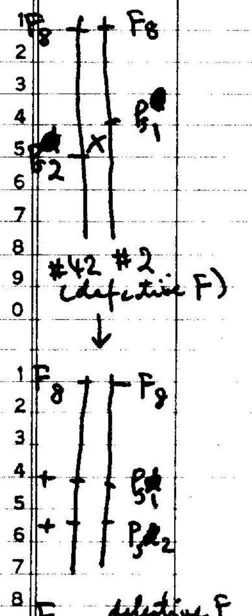
Unsuccessful result.

Recombination between F_8 defective mutants (polysaccharide defect.)

18/V ; 1959. REF:

1	2	3	4	5	6	7	8	9	10



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

~~Isolate~~ strain used ; #2, 5, #42, (W3086 F) -.

Combination.

1: 2, 5, 42. ; 2: 2, 5 ; 3: 2, 42 ; 4: 5, 42 ;

5: 2, ; 6: 5 ; 7: 42 ;

9: 2, - ; 10: 5, - ; 11: 42, - ; 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.

↓

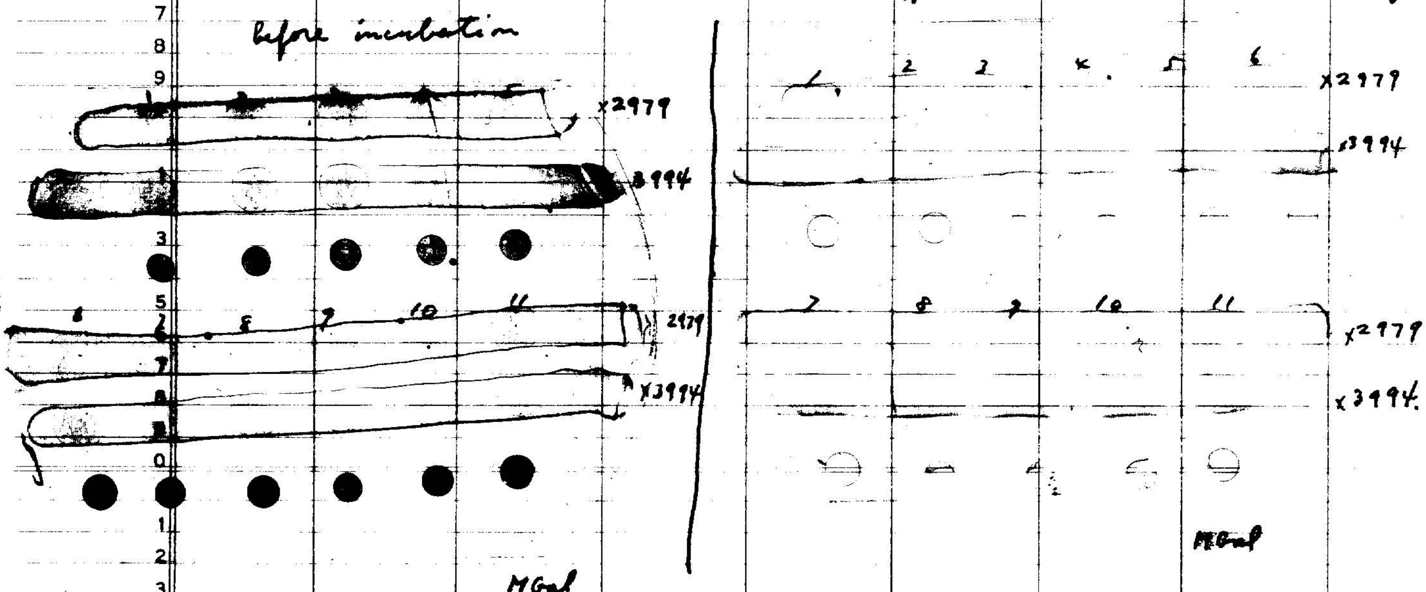
Incubated overnight at 37°C.

↓

Spotted on 2979 + 3994 on MGal. (control : W3086 F / W3086 F)

Sel: M^+ Gal_1^+ or Gal_2^+

- Interpretation for this negative result.
Cell contacts or Conjugations does not occur between those defective F_8^{+} (just like F^-) After incubation.



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

21/V., 1959 REF:

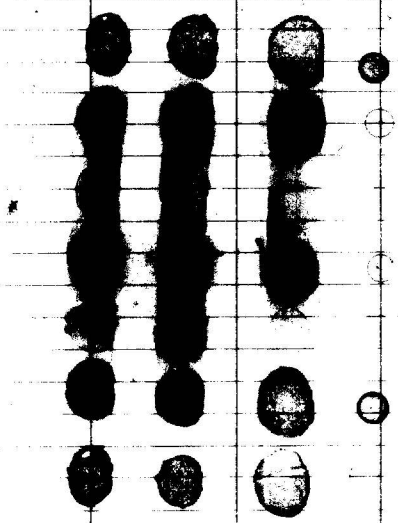
purpose: Is it F+ or F'+? Test H1 marker.

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 :

1 ml 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)

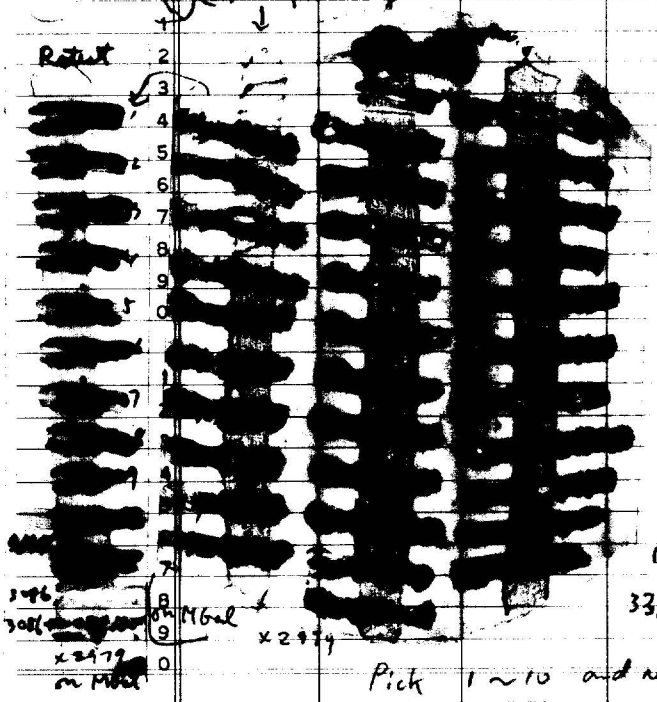
Result: AO F^-/F' 13/13 ; Actd F^-/F' 2/11

Result.

(I) Infectivity W4544 $\xrightarrow{F'_1}$ × W6−

↓

Result



Rate of infection

33/33 × 100 = 100 %.

MGal

× 2979

Pick 1 ~ 10 and retest

Result

1. W4544 infect F to F⁻.

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Effect of UV to infectivity of F'.

22/V. ; ; 1959 REF:

Experimental conditions.

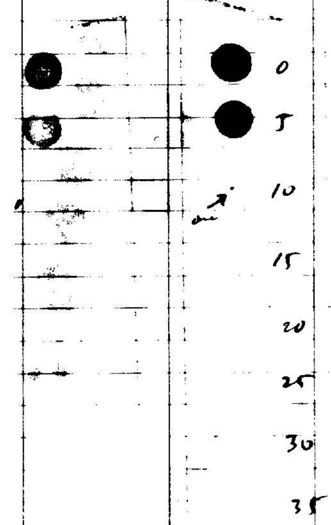
overnight culture. { W4534 (M⁻ Gal4⁻ Fg ?p³)
W6− (F⁻ M⁻)
W3994 (F⁻ Gal4⁻)

U.V. 6.5 cm.

1ml* of W4534 → Spread it onto petri-dish. diameter 6cm.
(* Penassay culture)

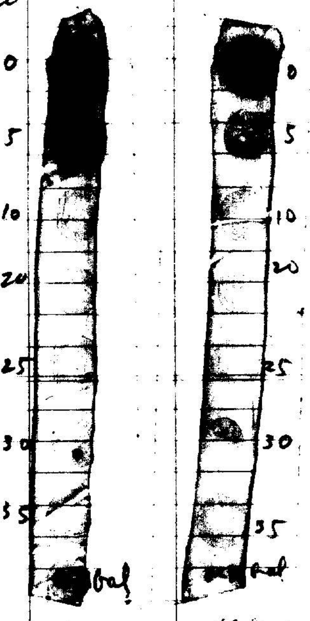
Take one drop of sample after each UV irradiations.
Spot it on BGal and on MGal streaked a mixture of W3994 + W6− (1:1).

Survival test.



Conclusion:

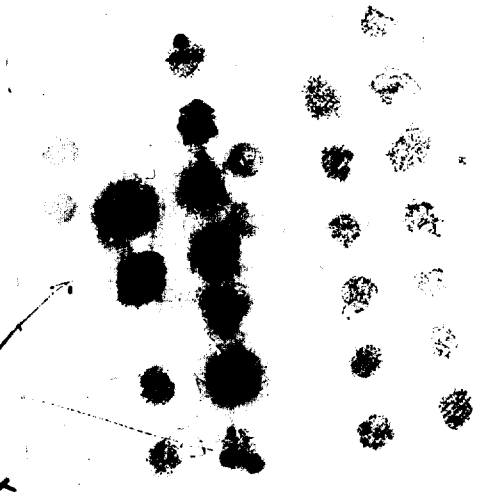
F' itself or mechanism for infection of F' are sensitive to UV as well as host cell.



Replica plated on MLac B, from Blac Sm

Untreated control.

AO treated



looks two states.

×3828 on MLac B,

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⁻ A, Xyl, Mal, Gal, Try, Pur, Lac ... P.)

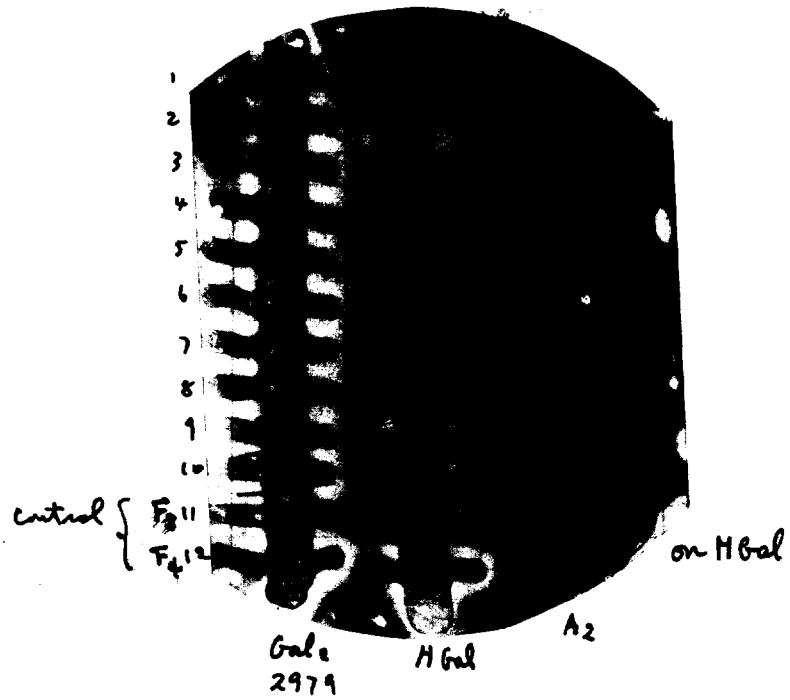
1. Take [redacted] doubly infected compound male. (# 1, 2, 31) see p. 42
2. Make colony on B Gal
3. Replica plate it on ~~H...~~ M Gal. seeded [redacted] → W3861 F⁻ A2⁻ (~~W4529.~~ ~~F⁻...~~)
4. ~~Look~~ Look for HI colonies to ~~Histidine~~ A2.
5. Pick the colonies of HI for ~~histidine~~ A2. Infect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

Replica — Cross brushing.

Ca 500 colonies per plate.

		plate No.	# of Hfr reacting
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] 3.8 Fg.)

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 B Lac. and inoculated into 5ml phenex.
2. Ratio of mix: 1:1 (1ml + 1ml) overnight culture.
   1ml 4527 (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 phenex. Streak it on B Lac 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 $F_4 \rightleftarrows F_{4.1}$ probably
2. The percentage of infectivity of F4 to F_1^+ is ca. 1 %.

on Mgal.
W2979

6/669 x 100 = 0.90 %

Ca. 1 %

Scheme. $F_4 \times F_1^+$

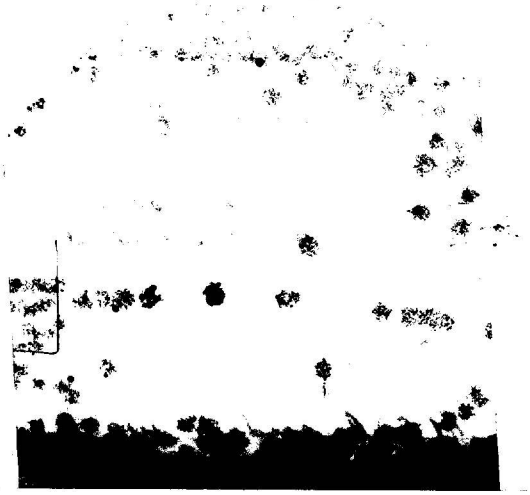
$\downarrow$

F_{41}^+

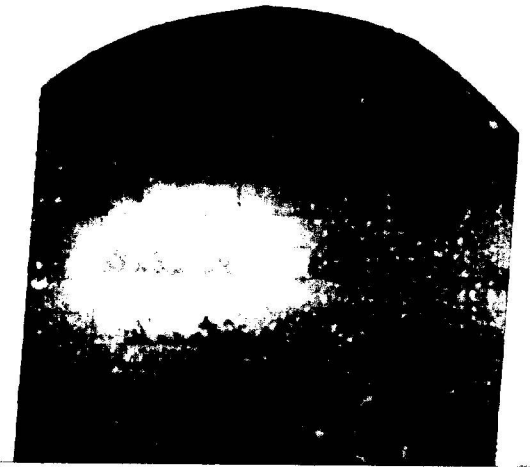
$\swarrow \quad \searrow$

$F_4^+ \qquad F_1^+$

Segregant F_1 type.



Double F type F_{41}. # 1



W6 —F1→ × 3086 F_4^d Control W6 —F1→ × 3086.

W6 —F1→ × 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 (Gal⁻) (Select Gal⁻)

Replica plate.

Retest the Hfr colonies by Cross-streaking method

	No of colonies tested	Hfr colonies (Hi for Gal.)	Hfr clones	
F_1 W6 × F^- 3086	286	1 ? 5	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 3 4 ×	1	
	162	1 5	1	
	Σ 1119	5		

Retest by cross-streaking method

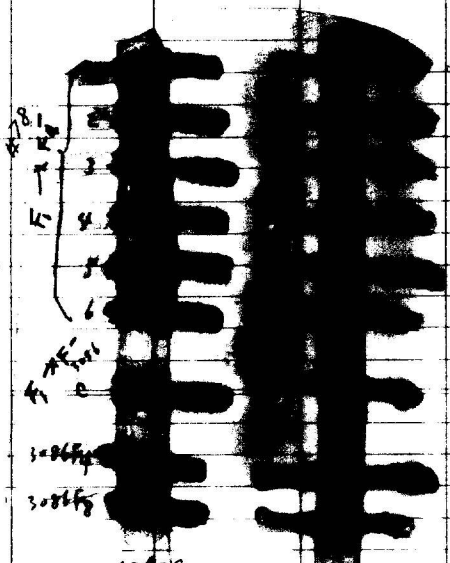
1. Test infectivity and accessibility to AO-treatment.
2. Remove F and reinfect F_1 to it. See what happens.

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.



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

~~W3898~~ → W3086
W3924

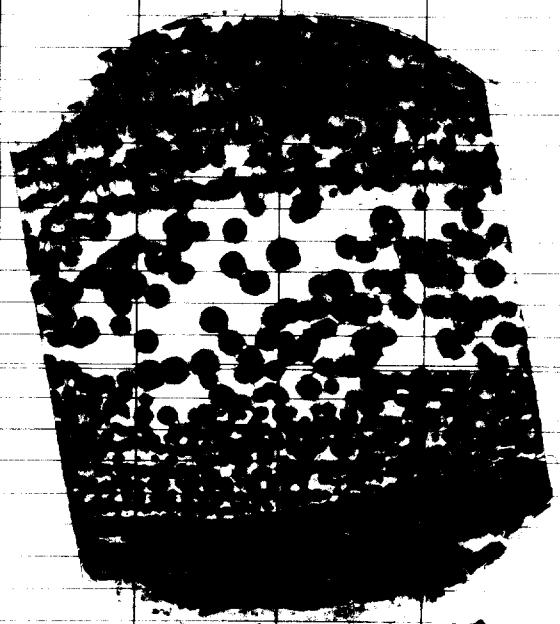
23/4 : 1959.

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

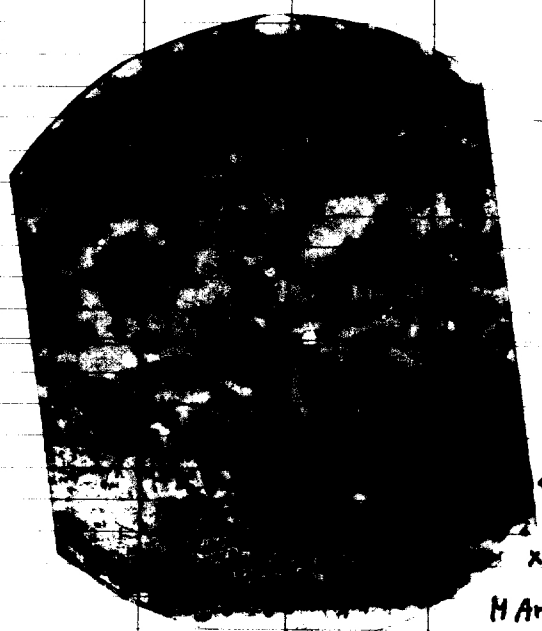
REF:

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