Segregation of F'^{+} from double F'^{+}.

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

Isolating of various infected strains.

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.

WLF4 → × W3086 F8	# of colonies counted	F_4 (+++)	F_8 (+)
①	46	33	13
②	89	66	23
③1	67	17	50
③4	48	47	① stail. ← spontaneous loss? or after [redacted] between two different F4
②8	36	36	0

② By spot test

Pick typical segregants for expression of F_4 type and F_8 type and test comprementality 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 F4 type.

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

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

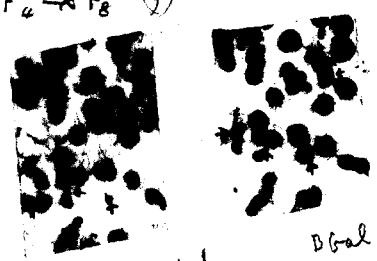
Further experiment : Does these segregant segregate the other F'^{+} after multiplications? How about F_4 & F_8.

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

$F_4 \rightarrow F_8$ (1)

master



m Mxyl

B Gal

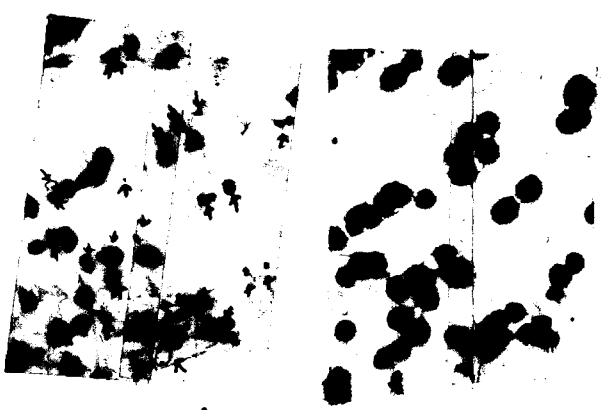
$F_4 \rightarrow F_8$

(31)

Replica master

↑ No cross

F_8 F_4



Mxyl

B Gal

Further test for

(34)

F_8 type

see front page; compare with the fertility.

on H Gal

sel: Gal_2

on H Gal

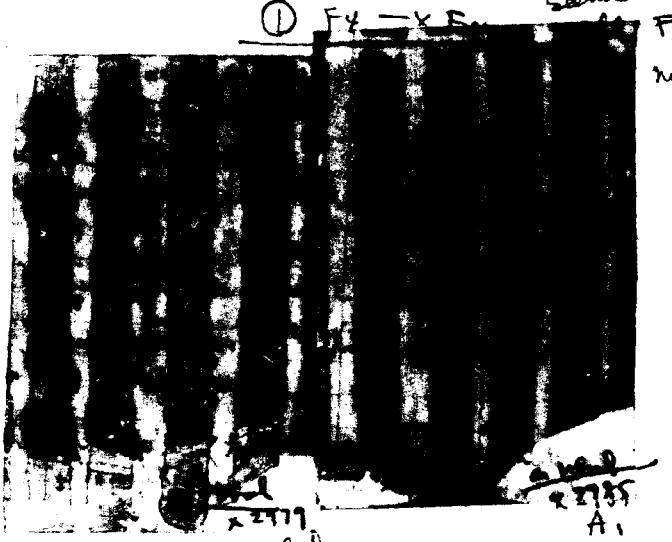
sel: A_1

(1) $F_4 \rightarrow F_8$

Same

F_8 type.

not segregate



x 2979

Gal_2

x 2985

A_1

Isolation of (F_4^R) from 3086 F_4

F_4^-

5/14 ; 1959 REF:

Method :

1). Inoculate 3086 F_4 to penassy 5 ml.

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

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

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

5). Incubate it overnight

Ca. 200 colonies per plates → 6). Replica plate it on MGal seeded W2985 (A_1^-) on it.

of ♀ col: Ca: 2~3 colonies/plate 7). Pick ♀ colony and inoculate it into broth.

8) Retest the fertility in cross times W2985 on MGal.

9). Test infectability to F_1^0 or F_4 or F_8.

Result :

1. # of colonies tested : Ca. 4000
2. # of ♀ colonies obtained : >2 (~~all of them~~ only 3 was F^- the other are F^+)
   recombination
3. # of ♀ colonis obtaind : see p. 52. and the back page.
   after testing by cross brushing method
4. Rate of F^- obtained

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Isolation of more-infective F' from F4.

Mutant "Rapid infection"

7/10 1959 REF:

• Experimental schedule.

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

↓ incubate overnight

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

↓

Repeat 10 times.

• Method : ① st 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 penassy.

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

1st : 5:30 p.m.

↓

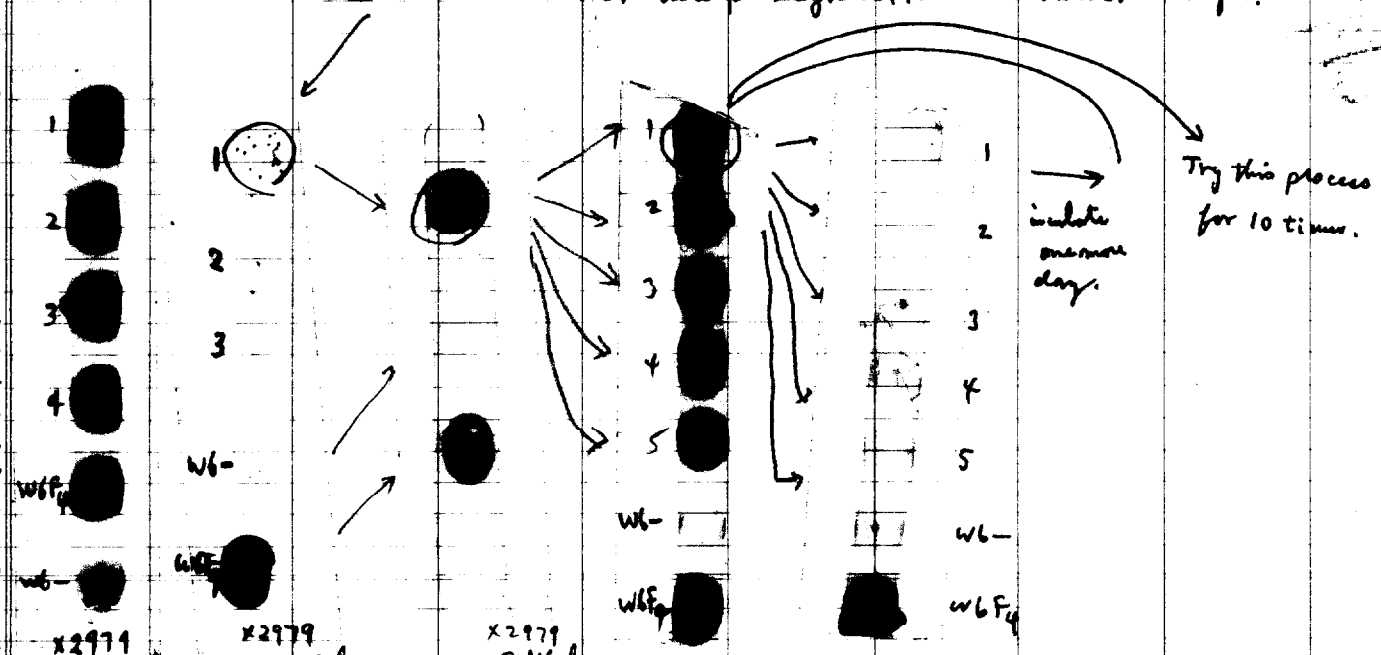
Incubate overnight. — Test fertility by spot test. Save it in cold room.

↓

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⁻ ①.

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



x2979 on MGal

x2979 on MGal

x2979 on MGal

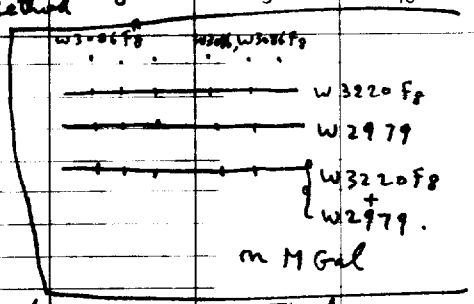
1st infection. 2nd infection. → overnight culture

x2979 on MGal

Test for immunity of F8 ♀ strains to the infection of F8

9/4 ; 1959 REF: See p 49.

Method



Principle:

$M\,Gal_4 \xrightarrow{F_8} \times$ $M\,Gal_4^+$ F_8^R?
(W3220 F8)
×
$M^+\,Gal_2^-$ F^-.
(W2979)

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

Unsuccessful result

might be 3220F8 → × 2979 → M Gal

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

1). ~~W3220~~ W6 F8 + 3086 F_8^R → Replica plate on M Gal Seeded → [W2979. on it.
on Sm B Gal

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

2	5	42	control. W3086
↑	↑	↑	↑
W6 F8	W6F8	W6F8	W6F8.

Results : All the clones, #2, #5, #42, are infected by F8, in otherword, these ♀ strains are plain F^- in the sense of immunity. But looks more resistant to the infection of F8. See back page.
(Compair with control)
#5 is most promissing.

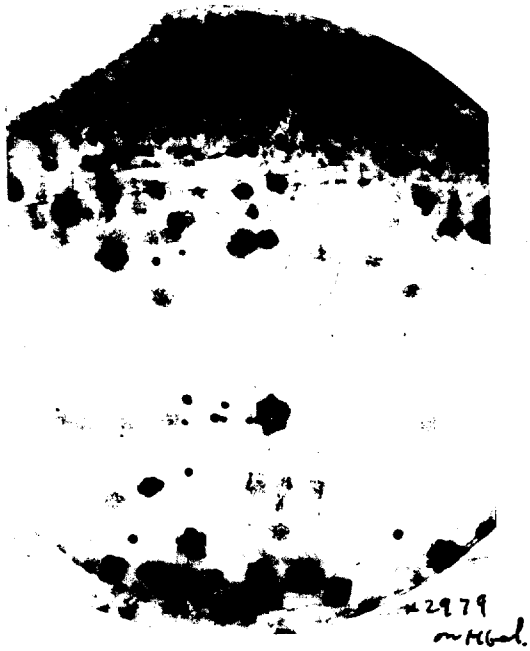
control

$F_8 \rightarrow$ 3086
×2979
on M Gal.

$F_8 \rightarrow$ #5

×2979
on M Gal.

$F_8 \rightarrow$ #2



×2979
on M Gal.

$F_8 \rightarrow$ #42

×2979
on M Gal.

next step : Infect F_1 to these 2 strains and see F_8 coming up or not.
(See p. 61)

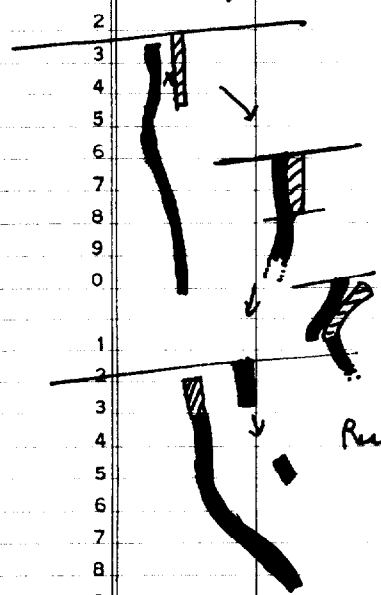
Test for transfer of Hfr_3 to F^-.

W3234 (Lp^+, ~~Hfr_3~~ Mal_5 - $MGal_2$ Lac_1)

W1394 (TLB, F^- S^R) × W6 F^- on MLac

Test this possibility

Procedures.



① Purify W3234 on BLac check S^s before use.

② old culture of W3234 1ml + W1394 ~~[illegible]~~ 0.1ml + 5ml phasing. and incubate it overnight.

③ Streat it on BLac Sm.

④ Replica plate it on MLac seeded W6 - on it.

Result: negative result See bellow.

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

13 NOV. 1959

REF: Cf. p 62.

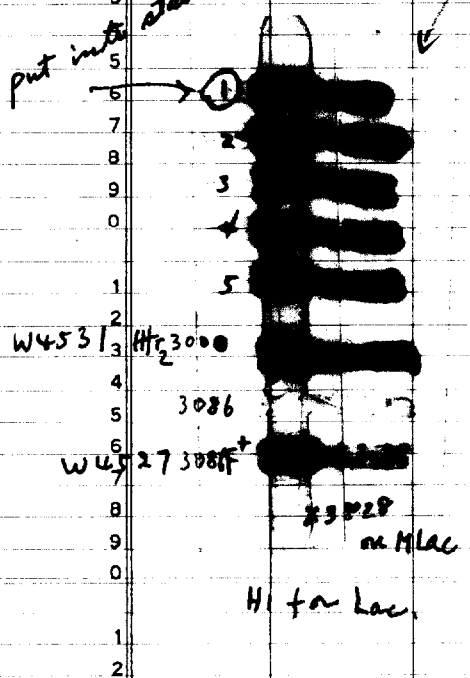
Method:

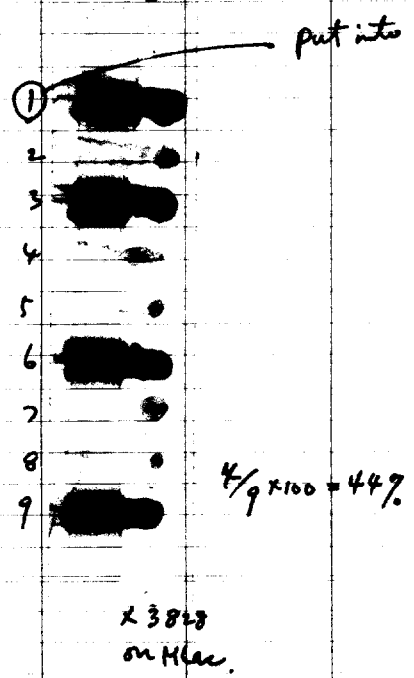
1. 3000 5 ml overnight culture + 0.1 ml phage W3086 ●
   ↓
   Incubate it overnight at 37°C.
   ↓
   Seed on B Lac Sm.
   ↓
   Replica plate on M Lac seeded W3828 mit.
   ↓
   Look for Recombination + colonies from it.

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 M Lac.

   See ~~back page~~.

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





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

12/4 ; 1959 REF:

1. Wash W6 F4 once by H_2O. and suspend it 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 penassy broth.
: 0.1 ml. 1 ml

- Time of irradiation :

  0, 10 sec, 15, 20, 25,

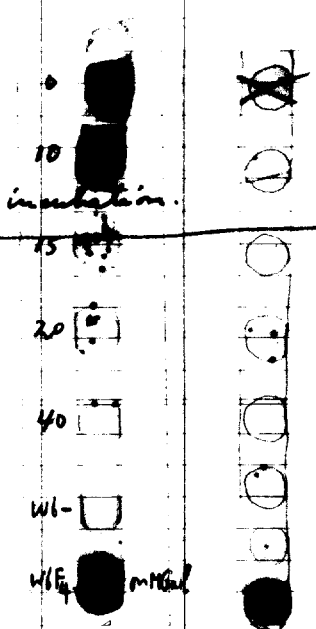
- Spread 0.1 ml of $\times 10^{-2}$-diluted suspension over EMB. : survival test.

Result :

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

thickness of the layer irradiated are Ca. 2 mm.

Same number ~~20 times~~ as much as 0.1 ml. ~~than~~ used in infection.



0 ← ? Try again. Something was wo

before incubation

after incubation.

10
15
20
25
60
W6−
W6 F4 on MGal

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 ♀ strains obtained from F4 by U.V. irradiation

11/V ; 1959. REF: cf. 952

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

W6F4 1ml + ♀ strain 0.2ml + 1ml penassy

↓

Incubate it overnight at 37°C.

↓

Purify it on Blac Im.

↓

Replica plate them on MGal seaded W2988 on them
F⁻A₁⁻

Results:

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

Not immune to the infection of F4.

These ♀ strains are same as F⁻ in the sense of ~~[illegible]~~ not immune to the infection of F4.

78 may be more resistant than plain F⁻.
(3086)

control.

W6F4 → x 3086

x2979
on MGal.

W6F4 → x #39.

x2979
on MGal.

W6F4 → x #69.

x2977
on MGal.

W6F4 → x #78

x2979
on MGal.

↑
#78 maybe promising.

Mix #78 and #5, or #2, or #42 comes from F8, and test the fertility.
(F4)

see p.54

Try all the combinations.

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

14/V ; 1959 REF: Cf. P.48, P53.

Test of 1 2nd segregants. 3 4 5 6 7 8 9 10

1. These strain used are obtained from P. 53 - experiment (from master plate) (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 $F_{1,8}$.

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 arise ♀ 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 fertility than F^+. They may still have F_4 in it.

[See back page.]

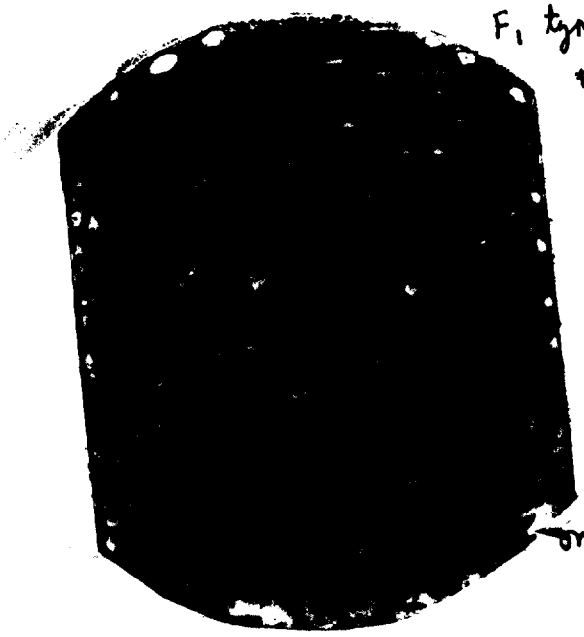
Segregant from $F_{8,1}$. (Example.)

F_8 type #1 (F_1 type) F^- type #1

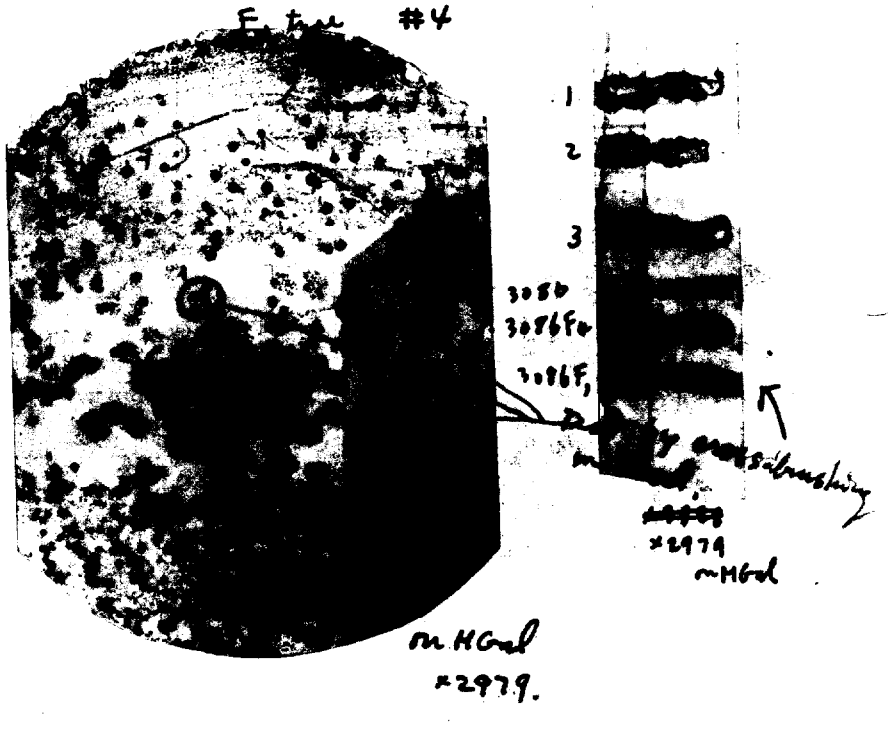
on MGal ×2979 on MGal ×2979.

○ Check ♀ (F^-) arised after infection of F_8 to F^+. It might be the result obtained 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.

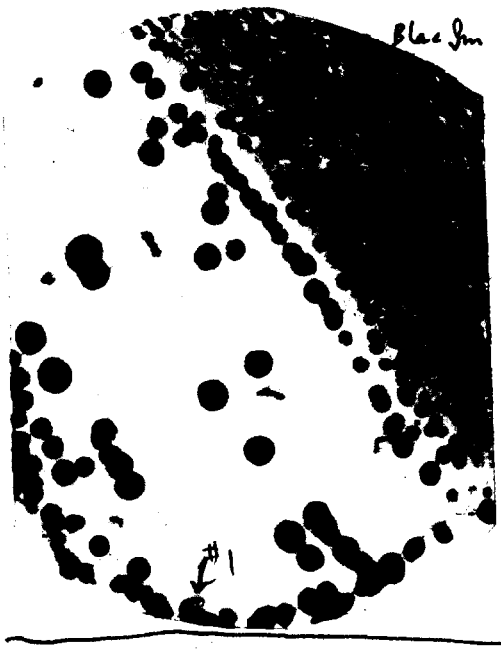
F_1 and F_8 are not miscible, but F_1 and F_4 are missible.

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 to F_1.)

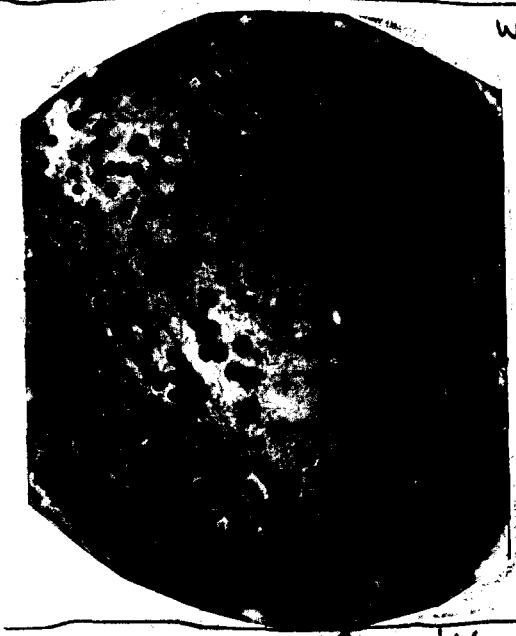
Interpretation: Both kinds of Fs are spread into the bacterial populations, and they are distributed with different proportion of particles.

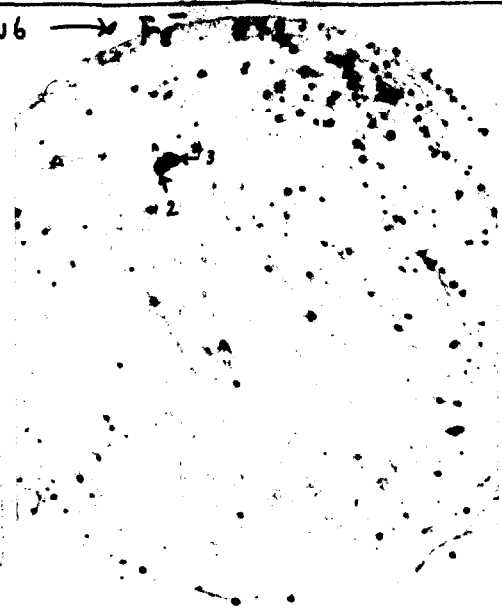
Master plate W6 → F_8^- # 2. continued.



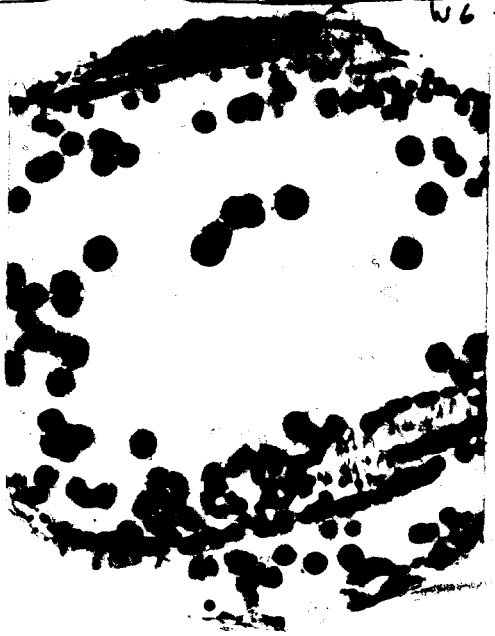
×2979 m Kbal

W6 → F_8^-





W6 → F_8^- #2.

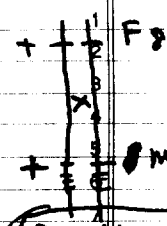


Infection of F_1 to F_8^- W6 $\xrightarrow{F_1}$ x F_8^- 3086.

14/V ; 1959. REF: Cf. P58. see P.68. 2, 5, 42.

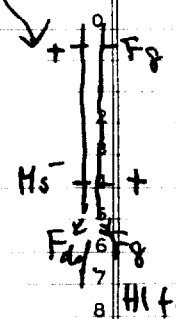
#2, #5, #42.

Principle



1. Mix W6 1ml + F_8^- (M 5 Mal.) 0.1ml + 5ml pheasy.
2. Incubate it overnight.
3. Purify it on Blac Sm.
4. Replica plate it on M Gal seeded 2979 on it.
5. Pick Hfr colonies and retest by cross brushing, or spotting method.

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



1. 3086F- is more infectable by F_1 than 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 brushing method) (see below) spot test
2. Recombine these defective F'.
3. Retest using more larger scale.
4. check is it F_3 type male or Standard F^+ type or F' type

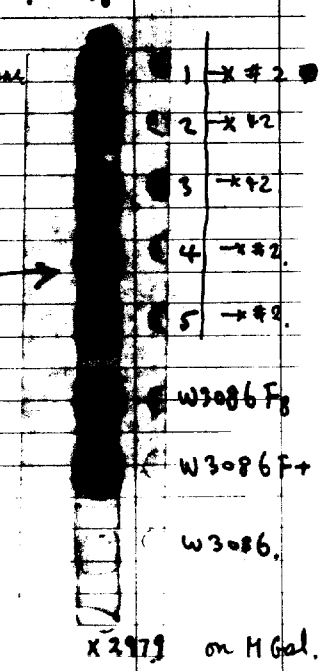
3849 Tryp.
2985 A_1
4297 G_1
2984 P
1394 TLB_1
2979 Gal
4539 H·Gal

3086F? Hfr? 3086F_8 3086+ 3086
#4 W4544

on M Gal

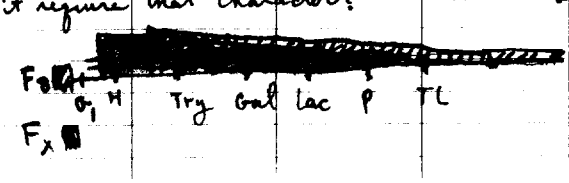
put into stab. Test infectivity of this F'? and test pattern of fertility on various markers!

F_1 → F_8^-



H1 for Histidine : This Hfr type mutant seems differ from F_8, however, it is quite useful because H1 transfers character for histidine, it is linked to antigenic characters.

How does it require that character? H1-marker seems shift a little to Histidine side.



W6 → × 3086
control.

Master plate

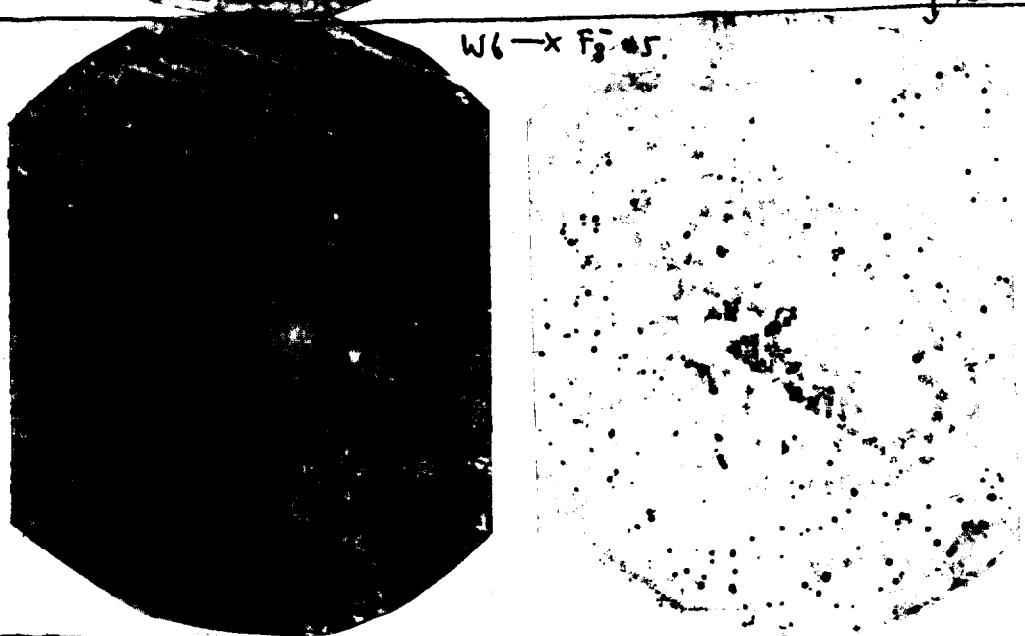
Replica plate.

× 2979
on MGal

on Blac Sm.

compare
ratio of infection of F_1

W6 → × F_8^- #5.



× 2979
MGal

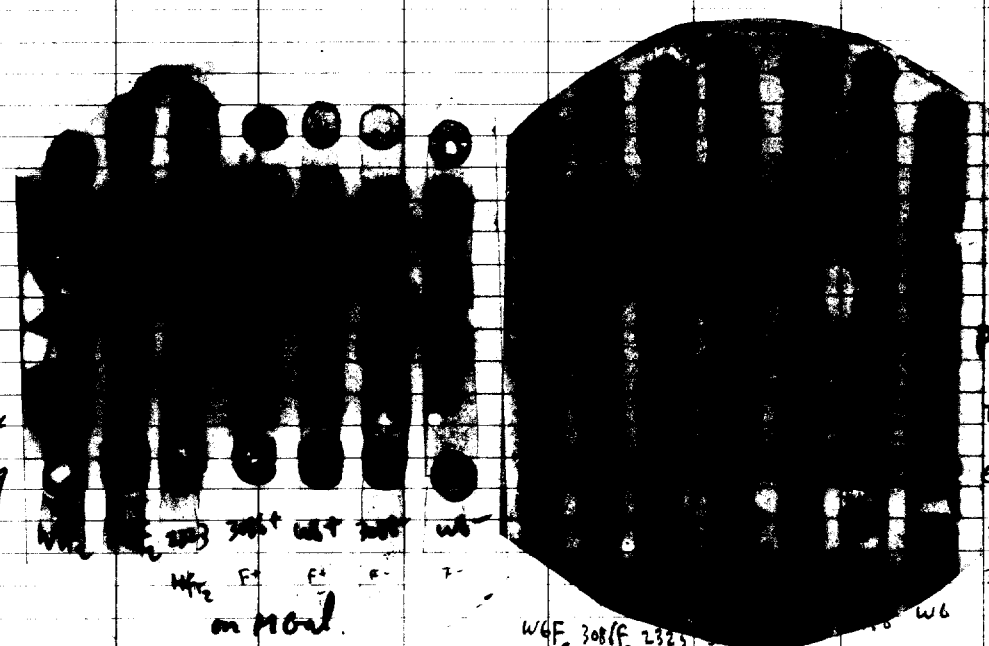
Comparison of Hfr_2 and F_2 strains.

17/V, 1959 REF: 4.957

conclusion

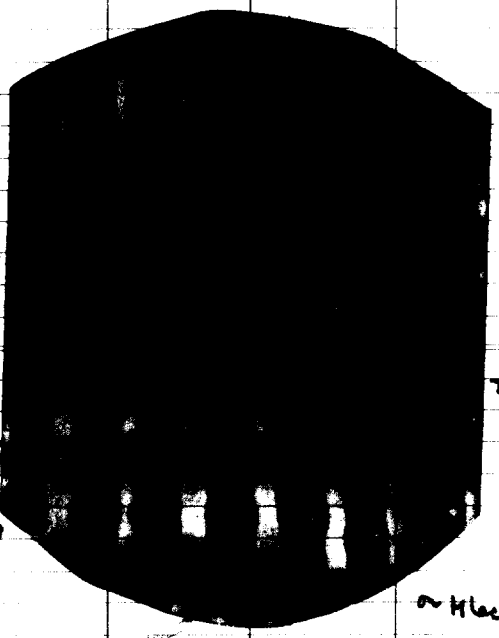
F_2 shows quite same quality as Hfr_2 in transfer of chromosome.

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



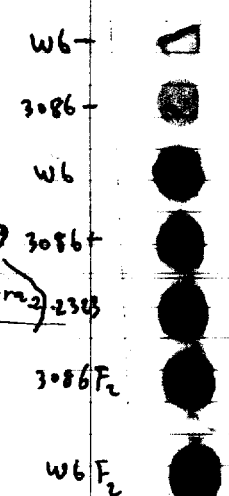
Syntrophy.

This is mistake: 2979 was spotted on MLac B_1



← Syntrophy

← mistake
W2979 was spotted by mistake.



Selected $H^+ Ara_2^+$

on $MAra_2$
x 2979

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P.63

(W4537: Hfr_5 [redacted])

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

16/V; 1959

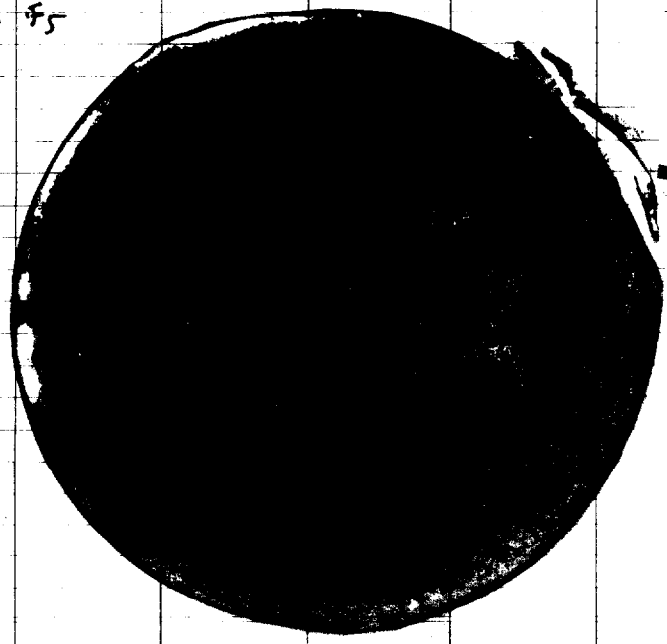
REF:

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

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

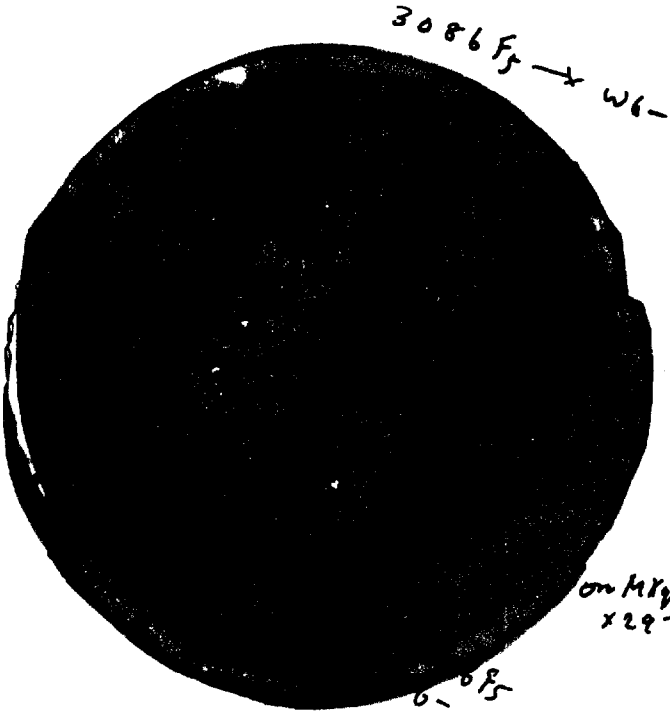
W4537
$Hfr_5 \rightarrow F_5$

on B Lac Sm



on MXyl.
× 2979.

3086 F_5
1
2
3086+
3086-
× 2979
on MXyl.



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

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

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

↓

? If a factor which controls high fertility was left ~~out~~ back, infection of F_1 to 3086 F_5^- may ~~give~~ results Htr_5 type cell.

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

17/V ; 1959 REF:

Principle : M^- Gal_4^- F_8 (W3220 F_8) W4534
M^- + F^- (W6 F^-) W4354
+ Gal_4^- F^- W3994.

Select on MGal.

Purpose : Does this system work well ?

Result : ok.

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

F^- M^- Gal^+ M^- Gal^+ F_8
W3220 W4534 F_8 donor

W3994 (Gal_4 F^-)

W6− (M F^-)

W3994 + W6−

Use young culture.

MGal

Developed method to look for colony of F.

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

Unsuccessful result.

Recombination between F_8 defective mutants (polysacharide defect.)

19/V ; 1959.

REF:

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

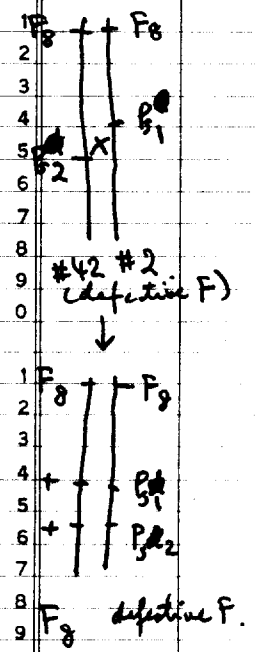
~~Isolation~~ strain used ; #2, 5, 42, (W3086 ⊕) -.

Combination.

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

5 6 7
2, ; 5 ; 42 ;

9 10 11 8
2, - ; 5, - ; 42, - ; - only



• Experimental conditions.

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

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

↓

Incubated overnight at 37°C

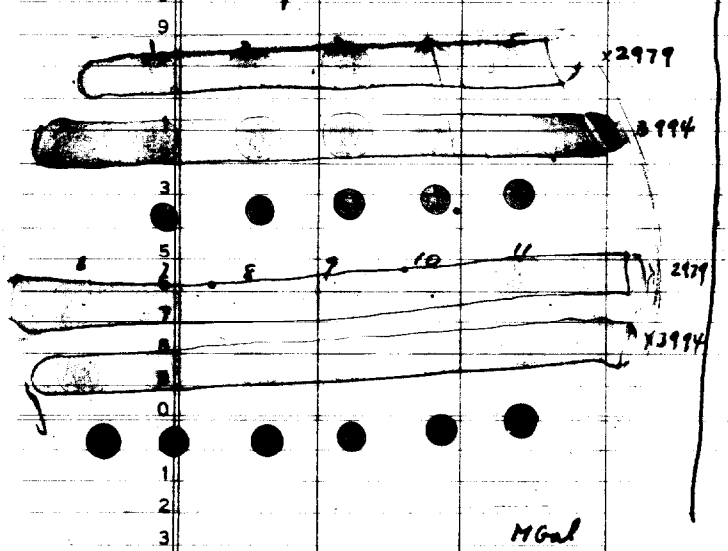
↓

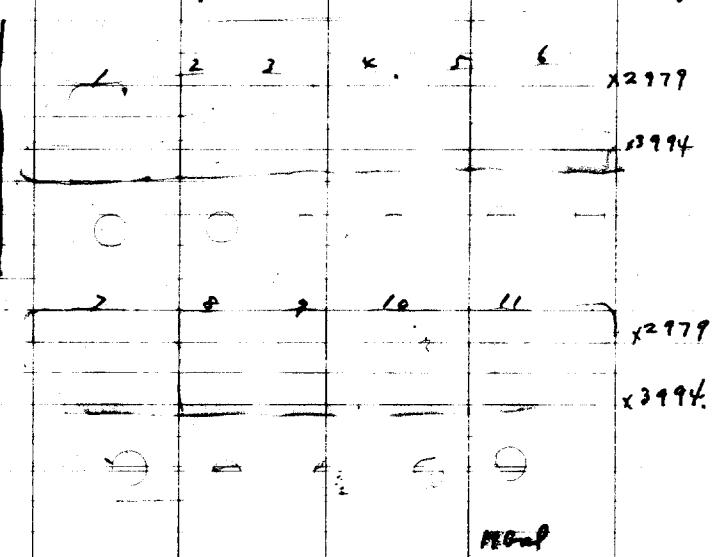
Spotted on 2979 & 3994 on MGal. (Control : W3086 F, W3086 F

Sel: M^+ Gal_4^+ or Gal_2^+

• Interpretation for this negative result.
Cell contacts or Conjugations does not occur between those defective F_8^{1+} just like F^-.
After incubation.

before incubation





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

21/V., 1959 REF:

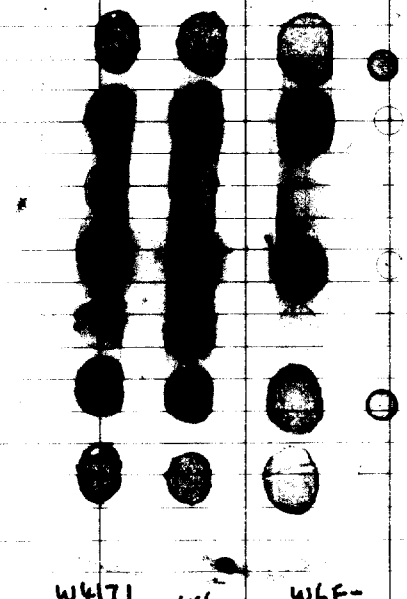
purpose: Is it F+ or F'+? Test H1 marker.

method: 1. purify W4171 on 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 ~~this recombinant F'~~ F of W4544 infective? Is it Removable by AO treatment?

Principle: 3086 $F'_{Rec.}$? ⟶ x W6−

(I) Infectivity method: 1 ml 3086 $F'_{Rec.}$ + 0.1 ml W6− + 5 ml phassy.

↓ Incubate overnight

↓ purify on BMal.

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

This strain carries F_1 or F', and is not standard Hfr. Is $O_3^{\rightarrow}$ type Hfr? What was left behind F_1, then?

(II) Treat W4544. by AO.

F in W4544 is sensitive to acridine treatment. (see back page)

Result: AO 13/13 F^-/F^+ ; control 2/11 F^-/F^+

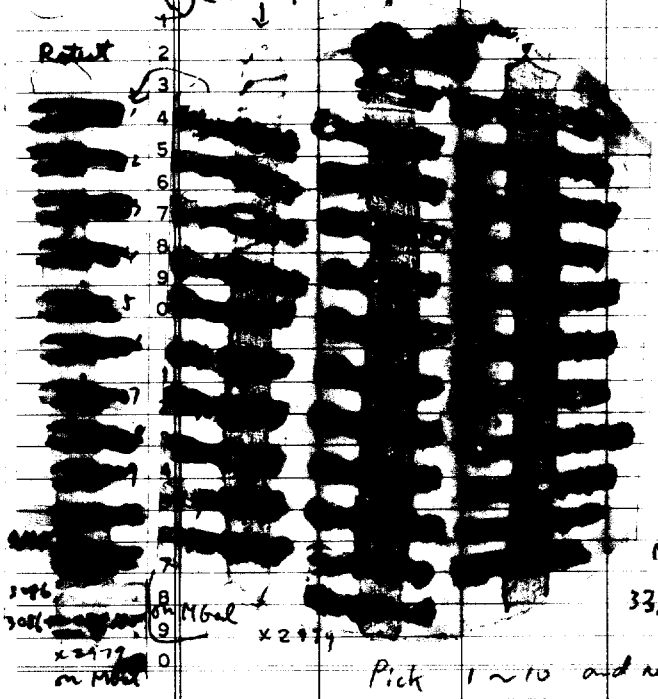
Result.

(I) Infectivity W4544 $\xrightarrow{F_1?}$ x W6−

Retest

[redacted]

3086 on MGal x 2979



x 2979 on MGal

Rate of infection

33/33 x 100 = 100 %.

Pick 1 ~ 10 and retest

Result

1. W4544 infect F to F^-.

Effect of UV to infectivity of F'.

22/V.; ; 1959 REF:

Experimental conditions.

overnight culture. W4534 (M⁻ Gal₄⁻ Fg Lp⁵)
W6− (F⁻ M⁻)
W3994 (F⁻ Gal₄⁻)

U.V. 6.5 cm.

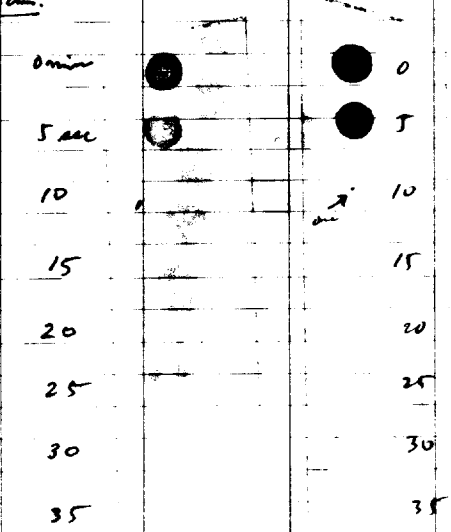
1 ml of W4534 → diameter 6 cm. (Penassay culture)

Spread it onto petri-dish.

Take one drop of sample after each UV. irradiations.

Spot it on BGal and on MGal streaked a mixture of W3994 + W6− (1:1).

Survival test.



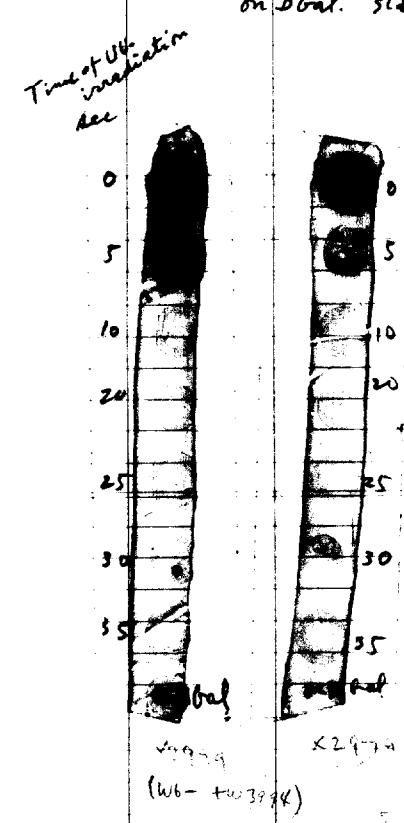
Conclusion:

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

↑ on BGal. ↗ stained.

Fast green 5% Ac. Ac. Et-OH.

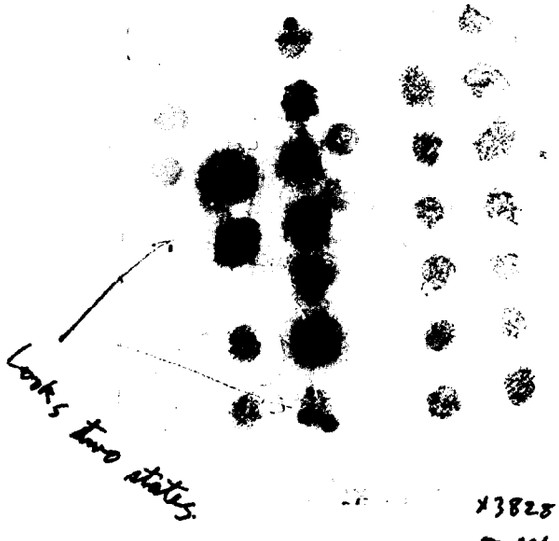
Petri method



Replica plated on M Lac B, from Blac Sm

Untreated Control.

AO treated



Looks two states.

X3825 on M Lac B,

F8 × F4. Ref. P42, P50

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

1. Take doubly infected compound male. (# 1, 2, 31) See p. 42
2. Make colony on B Gal
3. Replica plate it on M Gal. seeded ~~[illegible]~~ ~~[illegible]~~ ~~W4529.~~ ~~F⁻ H Gal~~ W3861 F⁻ A2⁻
4. Look for H1 colonies to ~~Histidine~~ A2.
5. Pick the colonies H1 for ~~histidine~~ A2. Infect the F' to the other F⁻.
6. Confirm stability and uniformity of the progeny. (non-segregational.) (Heritable.

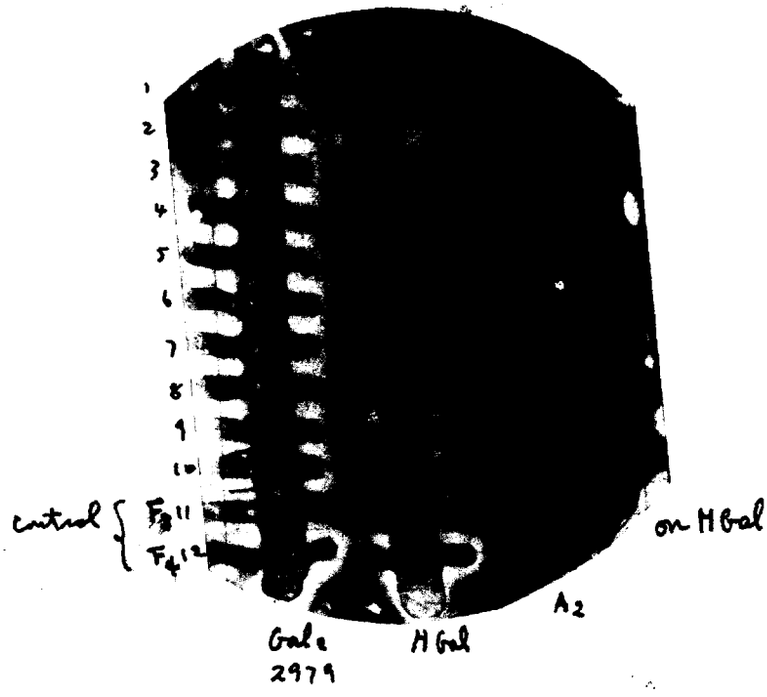
Replica | Cross brushing.

Ca 500 colonies per plate.

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

A2 : F4

Xyl : F8.



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

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

F_4 —x F_1^+

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

W4518 W4527

1. purify both strains before using.
   Purified on Blac. and inoculated into 5 ml penassy.

2. Ratio of mix: 1 : 1 (1 ml + 1 ml) overnight culture.
   1 ml 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_{41}. and suspend it into penassy. Streak it on Blac Sm. F^- A_1^-

7. Replica plate it on M Gal seeded W2985 (A_1^- F^-).

Results and conclusions.

1. F_1^+ is infectable by F_4. and gives $F_{4.1}$ and F_4 probably F_1.

2. The percentage of infectivity of F_4 to F_1^+ is ca. 1 %.

on M Gal.
x2979

6/669 x 100 = 0.90 %

Ca. 1 %

Scheme. $F_4 \times F_1^+$

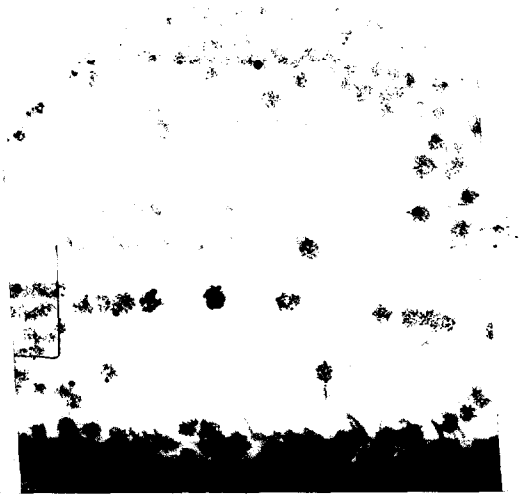
$\downarrow$

F_{41}^+

$\swarrow \quad \searrow$

$F_4^+ \quad F_1^+$

Segregant F_1 type.



Double F type F_{41}. # 1

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

W6 $\xrightarrow{F_1}$ × 3086 F_8^d

20/V ; 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 donor cultural age : overnight culture.

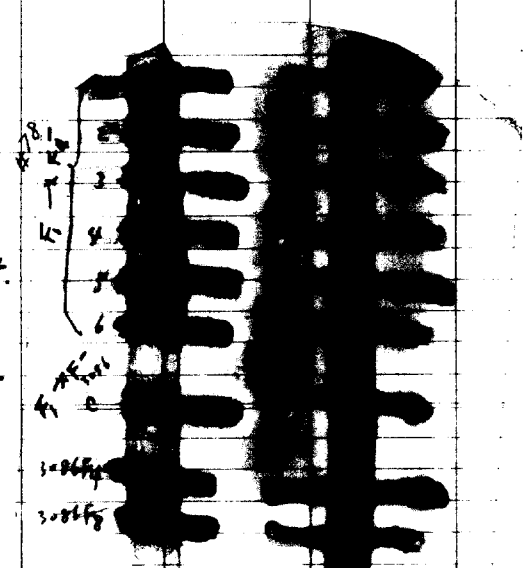
Control W6 ⟶ 3086.

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

Replica plate. Retest the Hfr colonies by Crosslinking method Σ

	No of colonies tested	Hfr colonies (H1 for Gal.)	Hfr colonies	
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 2 ×	1	3
	158	0		
	168	2 3 4 ×	1	
	162	1 5	1	
	Σ 1119	5		

~~Retest by crosslinking 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 ♀3 type female by itself, or F_1 can mutate into F' by spontaneous mutation, and becomes 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 M Gal 2, 4, 5, c. H1 marker by spot test (see back page)

U.W.
MICROBIAL
GENETICS

~~W3898~~ → W03086
W3924

23/4 ; 1959.

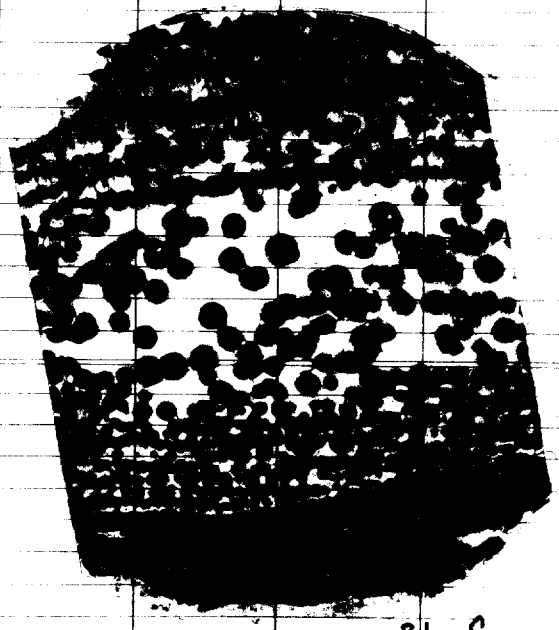
Hfr x Lp⁵ Th Mal Lac V₁ P100.

REF:

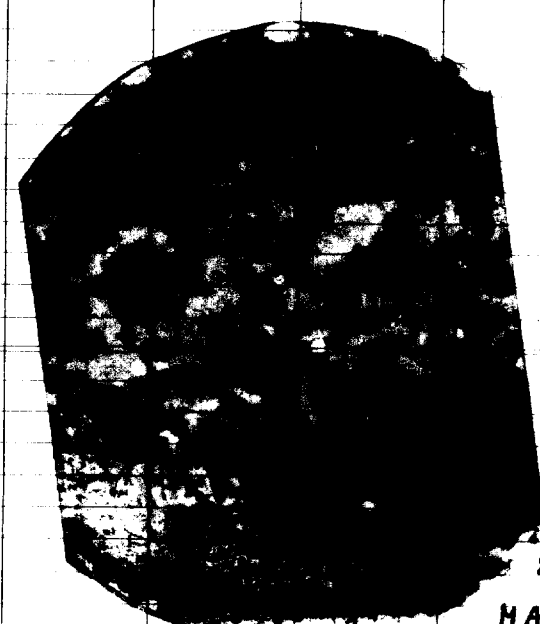
Jacob's Hfr.

Method : Just same as standard method.

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



Blac Sm.



x 2979.
MAra

MAra
x 2979