Hfr
3200 F13 × W3086 W

12/IV : 1959

REF:

Method

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

↓

Incubate overnight (20 hrs)

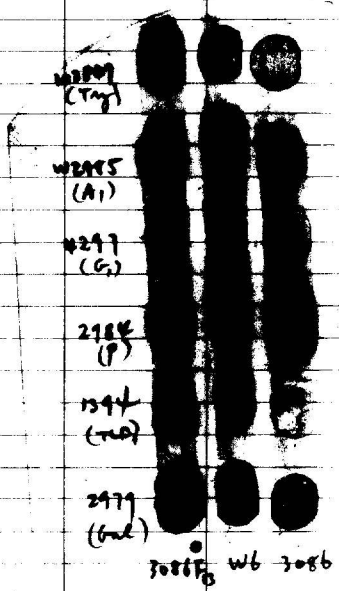
↓

purify it on Blacsm-agar.

↓

Replica plate it on MGal seeded W2979 on it.

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



3086 F'_{13} W6 3086

on MGal

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Comparisons on infectivity of F'+ and corresponding Hfr.

27

16/IV : 1959.

REF: cf. p.33. infectivity.

· cultural age : Overnight culture (Penassay)

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

· Experimental method:

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

↓ Incubate it overnight.

↓ Purify it on MMal Sm agar.

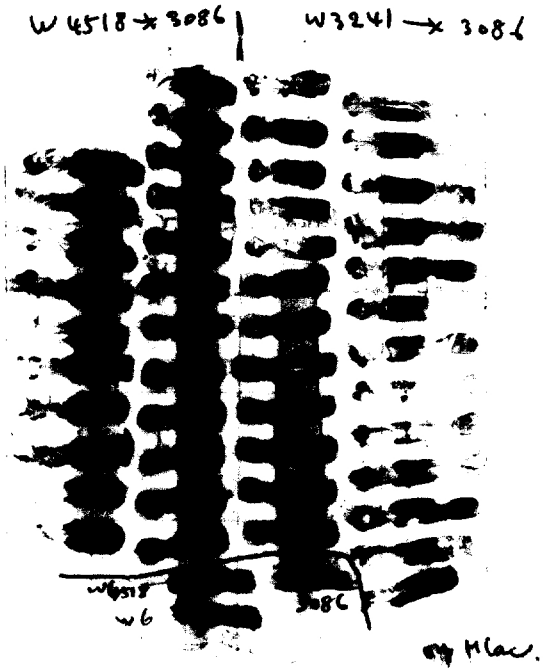
↓ Test sex-compatibility

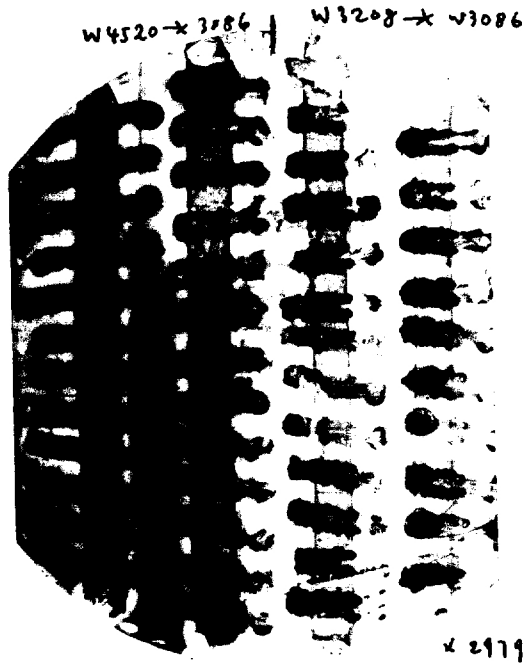
Result:

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

This was not Hfr of W4321

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





m Hlac.
K2985
A1

K 2979
m Hgal.

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

3086 F_8 —x W6 •

F_8 —x F_1

Is it co-exist or exclud) with each others? P.29

REF: P.48

16/IV ; 1959.

1	2	3	4	5	6	7	8	${}_9F_4$ —x F_{10}

3086 F_8 : 1ml : W6 F_1 0.1ml : 5ml penassy.

↓

Incubate it for 24 hrs at 37°C.

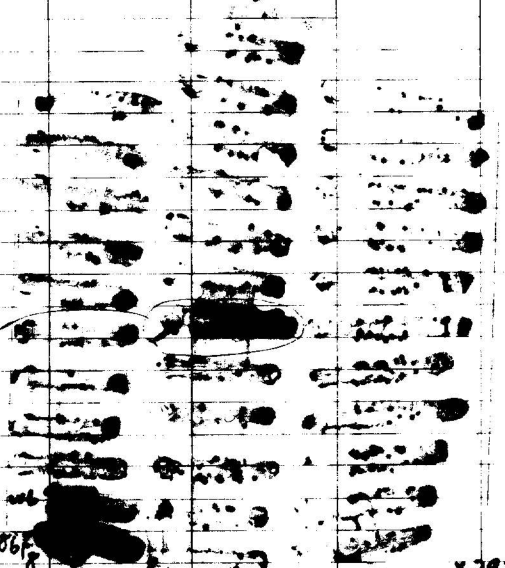
↓

Purify it on B Mal

↓

Test Mal^+ colonies on sex. in cross times 2979. on MGal.

3086 F_8 —x W6



x 2979 on MGal

F^+/total tested : $1/32 \times 100 = 3.12$

test infectivity of 3086 F_8 F_1 and F_8 to F^- strain (P 29?)

Does this clone still contain F_1?

Conclusion : F_8 is infective to W6 F^+.

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

3086 F_8

W6(F1 F8) ⟶× 3086

28/VI, 1959. REF:

Purpose of this experiment: Does W6F1F8 still retains F1 or not?
Cf. P.28.

W6F1F8
Control 1. or W6F1 ——× W3086
control 2. or W6F8

1 ml + 0.1 ml + 5 ml penassay

↓

incubate overnight.

↓

Purify it on Blac Sm.

↓

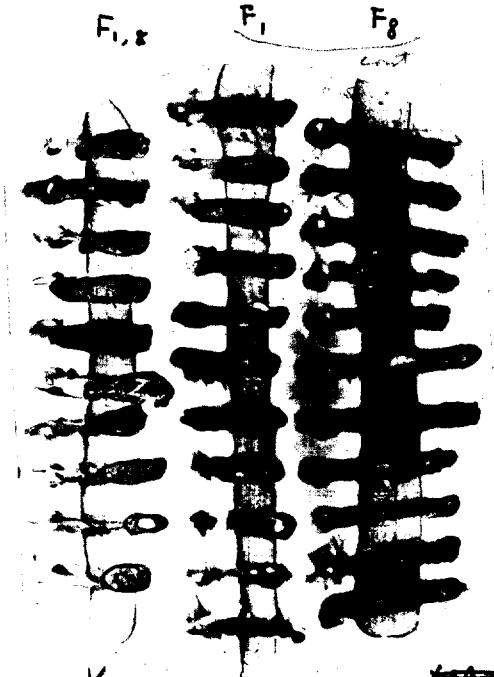
Cross-brush it in rows × 2979 on M Gal.

		F8/total (%)	F1/total (%)
Experiment:	W6F1 F8 ⟶× 3086	6/30 (20)	0/30 (0)
Control:	W6F1 (W6) ⟶× 3086	0/34 (0)	17/34 (50)
	W6F8 ⟶× 3086	30/34 (88)	0/34 (0)

Conclusion: W6F1,8 (obtained by infection of F8 to F1+ ; W3086F8 ⟶× W6) does not contain F1, or not segregate in the time of infection.

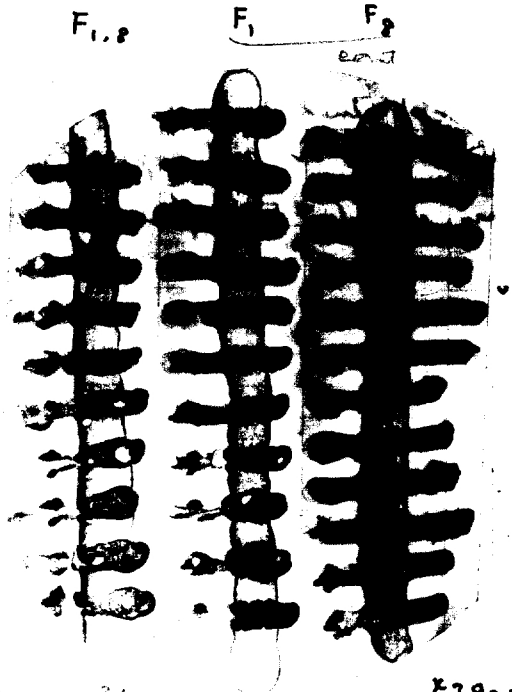
~~F1 and~~ F8 may exclude F1 after infection. F8 > F1 stronger than.

F donor : W6 F_1 , W6 F_8 , W6 $F_{8,i}^{2}$

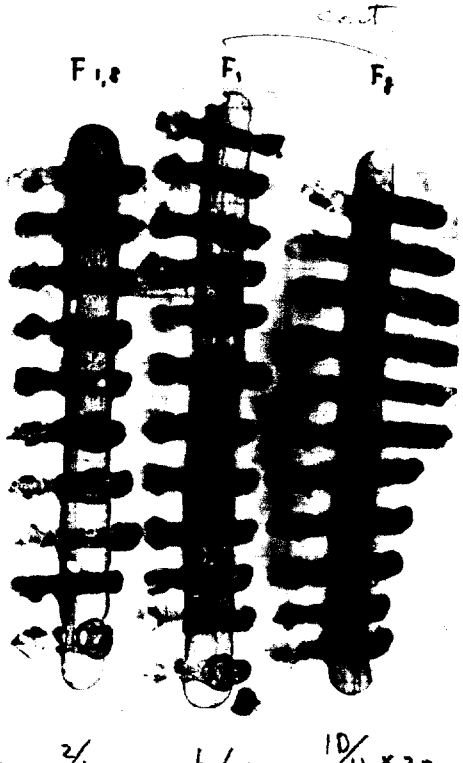


Y/10 5/11 10/11 ~~X 3828~~ on Mlec

F recipient : 3086.



3/10 6/11 10/12 X 3828 on Mlec



2/10 6/12 10/11 X 3828 on Mlec.

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

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Direct demonstration of true recombination, Not syntrophy!

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

20/IV ; 1959. REF:

1	2	3	4	5	6	7	8	9	10

1. Mix W6F8 1ml + W2979 1ml + 5ml pen. Ca. 10^8 cells/ml.
↓
incubate 1 hours at 37°C.
↓
dilute it into 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}.
and spread it on MGal and BGal.
↓
Incubate 40 hrs and see what happens.

See : back page.

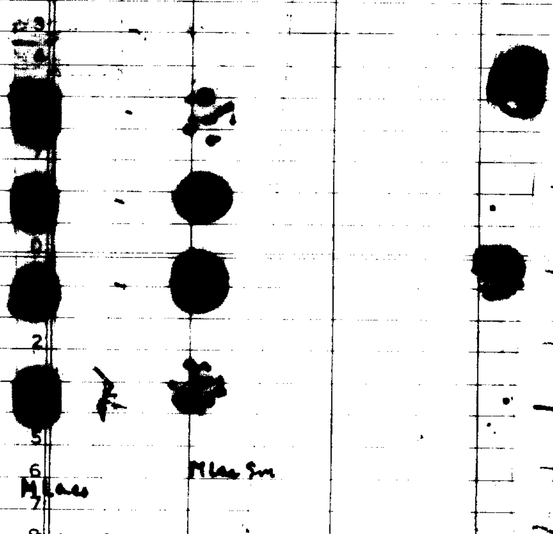
	Degree of dilution						surviving cont.
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-6}
# of Colonies	too many	too many	too many	150	32	6	17

2. pick cells from black spot and ~~see if it Gal⁺ or not~~ streak on BGal, and test nutritional requirement on ~~M~~Gal (~~Replica plate from BGal to Gal~~)

		isolation	on BGal	~~on MGal~~ Replica plate	Aux/Prot. Test nutritional requirement.	% of X
W6F8 × 2979	on MGal	BGal	almost Gal⁺	tested Gal⁺	0/22	0.0
W6F8 × 2985 A⁻	on MLac	Bgal.	All gal⁺		4/21	16.0
W1394F8 × 2979	on MGal	BGal.	2/3 gal⁺.	tested Gal⁺	2/23	8.0

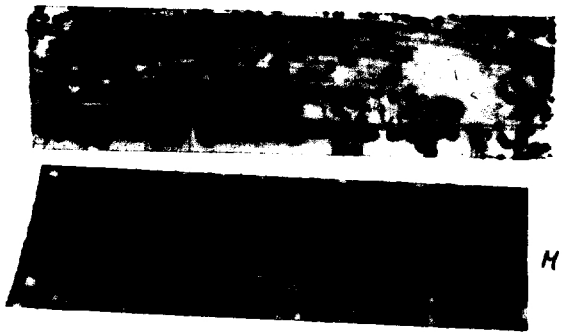
young culture. 4 Spot it on MLac SMB1 seeded 3828
old culture

SM destruct male capacity of S^S F' male but not S^r F' male.

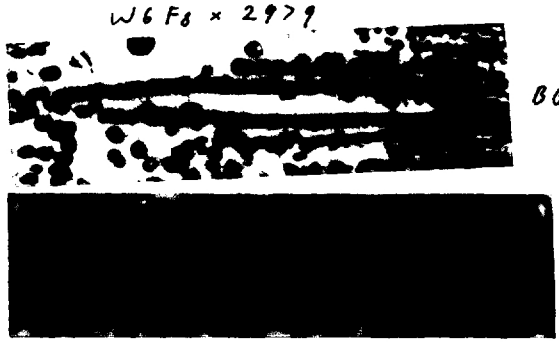


Conclusion : ① This phenomenon is not syntrophy but true recombination.
② ~~This~~ recombination process (F8 × F⁻S^R) is inhibitted by SM-treatment. (It may be interpreted by incorporation of S^S loci to F-S^R)
③ F4 × F⁻ S^R is not inhibitted by Sm.
This phenomenon may be interpreted by incorporation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

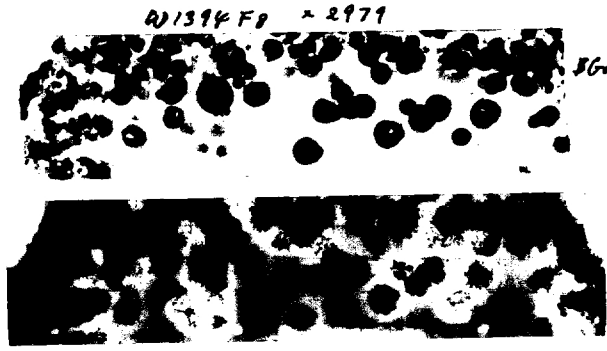
W6 F_4 x W2985



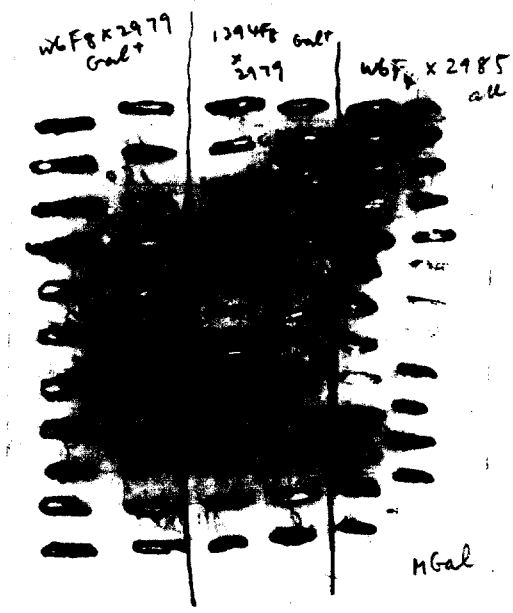
W6 F_8 x 2979



W1394 F_8 x 2979



Test of the syntrophy.



Conclusion:
almost of three Gal+ colonies are X^+.

Therefore, this phenomena are not syntrophy but recombination.
Granted.

Preliminary experiment for exclusion of F particles.

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31

W6 F_4 $\xrightarrow{F_4}$ × 3086 F_8

W6 F_8 $\xrightarrow{F_8}$ × 3086 F_4.

16/IV. 1959

REF: cf. p 42 ~ p 50.

Ratio of mix.

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

↓

Incubate overnight at 37°C

↓

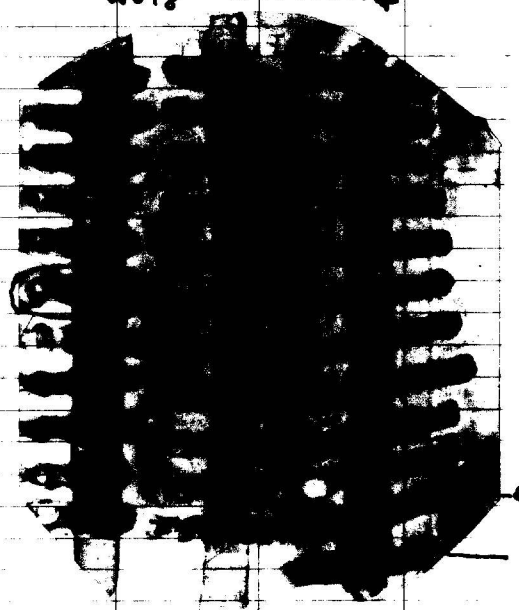
Purify it on B Hard SM.

↓

Test Mal⁻ colonies for alk-compatibility.

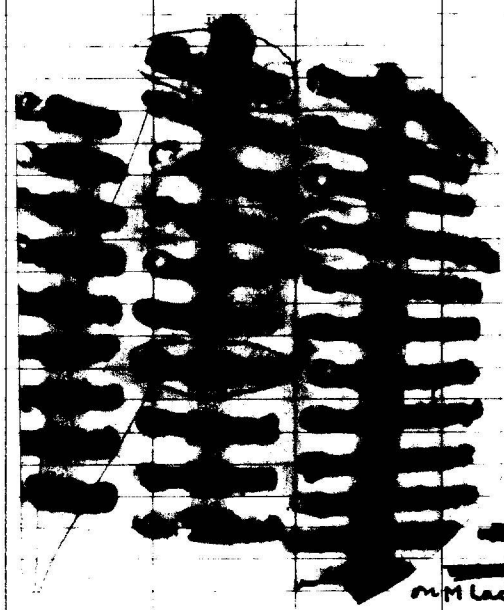
The method used in this experiment is not suitable to know the difference between two different F'+ Use spot method for this purpose. (See back page)

W6 F_8 → × W3086 F_4



on H Gal
× 2979
Gal⁻

W6 F_4 → × W3086 F_8



on M Lac
× 2985
A_1^-

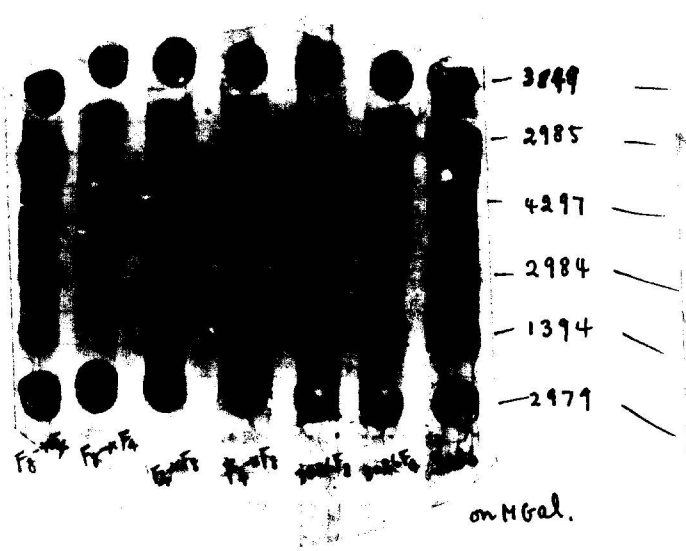
test each H1 markers. Retest H1 markers: See back page

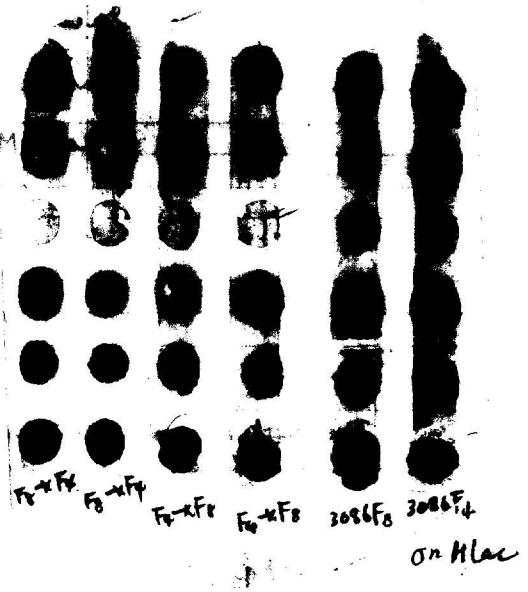
conclusion : F_4 is stronger than F_8. F_4 seems expel F_8 after infection

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

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

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





F_4 F_4 F_4 F_4 F_8 F_4 F^-

$F_4 \times F_8$

$F_8 \times F_4$

control.

Phenotype F_4 F_4 F_4 F_4 F_8 F_4 phenotype

$F_4 \rightarrow \times F_8$

$F_8 \rightarrow \times F_4$.

control

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Isolation of F_2 from Hfr$_2$. 32

4068 Hfr$_2$ ⟶ × 3086. F⁻ M Mal 5^R

16/IV. 1959. REF:

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

3086 : old broth

Ratio of mix.

4068 /ml : 3086 0.1 ml : Penassay 5 ml.

↓

Incubate the at 37°C. for overnight.

↓

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

↓

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

Hfr$_2$ 4068 — × 3086

Retest.

Master plate.

Replica plated on MLac seeded ×3828

on MLac Sm.

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Treatment of W1922 F_8 by AO.

22/IV. ; 1959 REF:

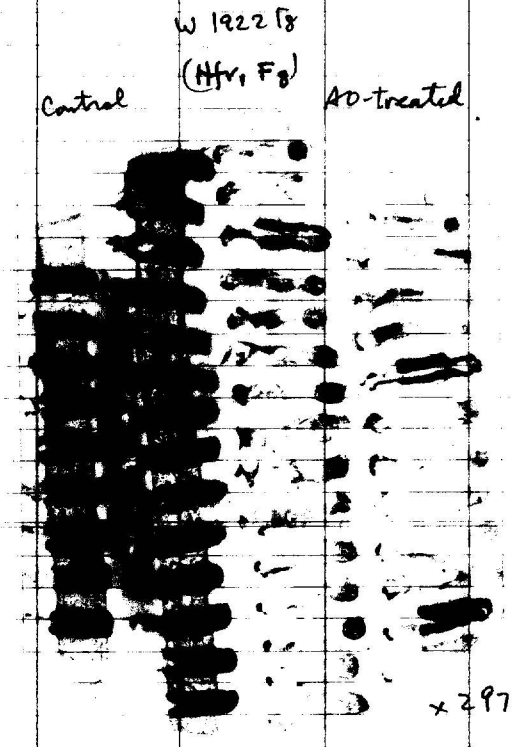
Time of treatment : Ca. 40 hrs. at 37°C. Conc. of AO 30γ/ml
5 ml pancreas.

Purpose : W1922 is Hfr. Has W1922 F_8 Hfr, or not. If Hfr, is in W1922 F_8
it will gives Hfr, after treatment. tester : W2979 on MGal.

Result:

	AO treated W1922 F_8	Untreated W1922 F_8
γ/total	23/27	0/23
% of γ	90	0

Conclusion :
W1922 F_8 does not give aroused Hfr, after AO-treatment. Only gives F^-.



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36

FR. from F8 3086.

28/N — 1957

REF: cf. p35
p37 51 49

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

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Interrupted mating experiment of W6F8.

18/VI i 1959. REF: H is ~~low marker~~ ...

~~F⁻ : W4352 F⁻ Lp R Try Pro~~

F⁻ : W4352 F⁻ Try P M Gal₆ Lac₅₉ V_6^R Lp^+ — Make S^R mutant.

Hfr : W4520 F8 H.
(W6F8)

~~Method~~.

Selective marker:	Medium	Purpose
Try	M Glucose + Meth + Prol	Try incorporation.
Try & Prol	M Gal + Meth.	II Which is earlier Try or Prol.
Try ~~Meth~~ Lac.	M Lac + Meth + Prol	Which is earlier Try or Lac.
	M " + Meth + Prol + Trp	I Lac incorporation
Try & Gal	M Gal + Meth + Prol	Which is top. Try or Gal.
Gal	" " " + Tryp	Gal.

Method:
1. Purity W4352 on Blac agar. (It was very small colonies).
2. Inoculate purified colony into penassay and incubate it at 37°C for overnight.
3. Transfer it into penassay broth (10ml) and inoculate ~~it~~ W4520 into 10ml penassay and shake it for ~~4~~ hrs. (11:00 — 2:45)
4. Mix them (1:1) ~~and~~ take a sample, and dilute it into chilled H2O. ($\times 10^{-3}$, & 10^{-5} dil: ca 10^6 & 10^4 0 time, 0 time ...)
5. Spread over each selective medium ~~at~~ each time.

Selective Medium	Time / Selective marker	0 (#of cells ×100.) after mix.	5	10	15	20	25	min.
	actual time	2:45	2:50	2:55	3:00	3:05	3:10	
Gal-M-P	Try	0	0	0	0	0	0	
Gal-M-P	Try-~~Gal~~	0	0	0	0	0	1	
Lac-M-P-Try	~~Try~~ Lac	0	0	0	0	0	0	
~~Gal~~-M-P	Try - Lac	0	0	0	0	0	0	
Gal-M-Po T.	Gal.	3 ($\times 10^{-2}$)	1	?	5	9	0	
Blac ~~survival count~~	+ survival count F8 # of 4520	0	0	0	0	0	0	# of cells used in Reg one ($\times 10^2$).
	#	0	0	2	0	1	0	
	F⁻ # of 4352	7	12	33	15	12	7	
	#	12	24	38	18	27	11	

Method of dilution.

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

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

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

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40

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

24/10 ; 1954.

REF:

Cultural age : ca. 5 hr. on rotator.

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

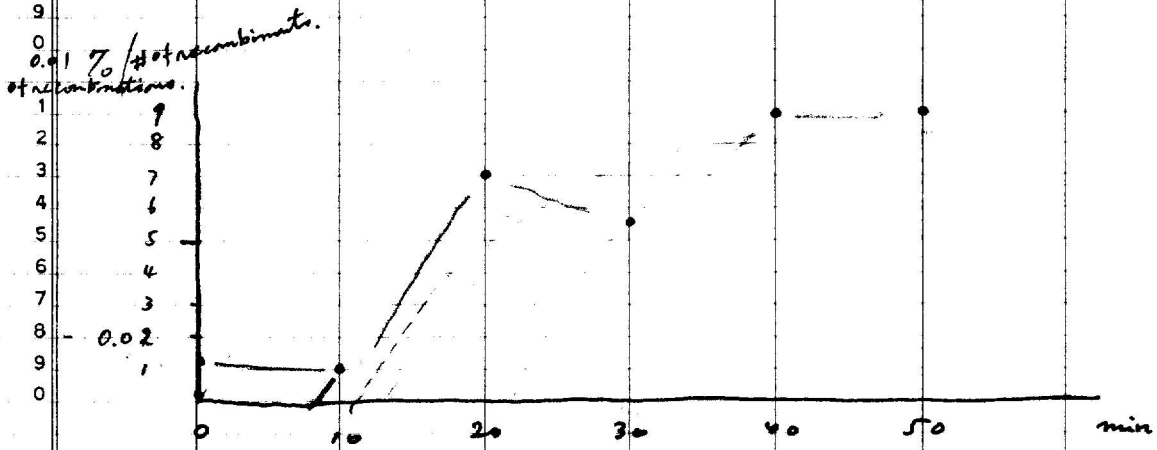
Plateau.

selective media : M Lac Sm.

Strain : W6 F_8 ♀. W3828.

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

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



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41

W6 F8 × W3828.

Is there 2 states in W6 F8 ?

23/10. 1959 REF:

W6 F8 × W3828. on MLac Sm, & MLac.

M⁻ F8 Lac⁻ S^R.

Hypothesis: Interpretation for low fertility of W6 F8 on Sm-media.

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

: If it is true, all of W6 F8 colonies show Hi on MLac, and only (colonies) few W6 F8 shows Hi fertility in the cross on MLac Sm. (W6 F8 only shows the state II phenotype on (MLac) Sm-media) and almost of the W6 F8 colonies are low fertile.

Method:

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

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

3) Incubate it for 30 hrs.

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

Result.

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

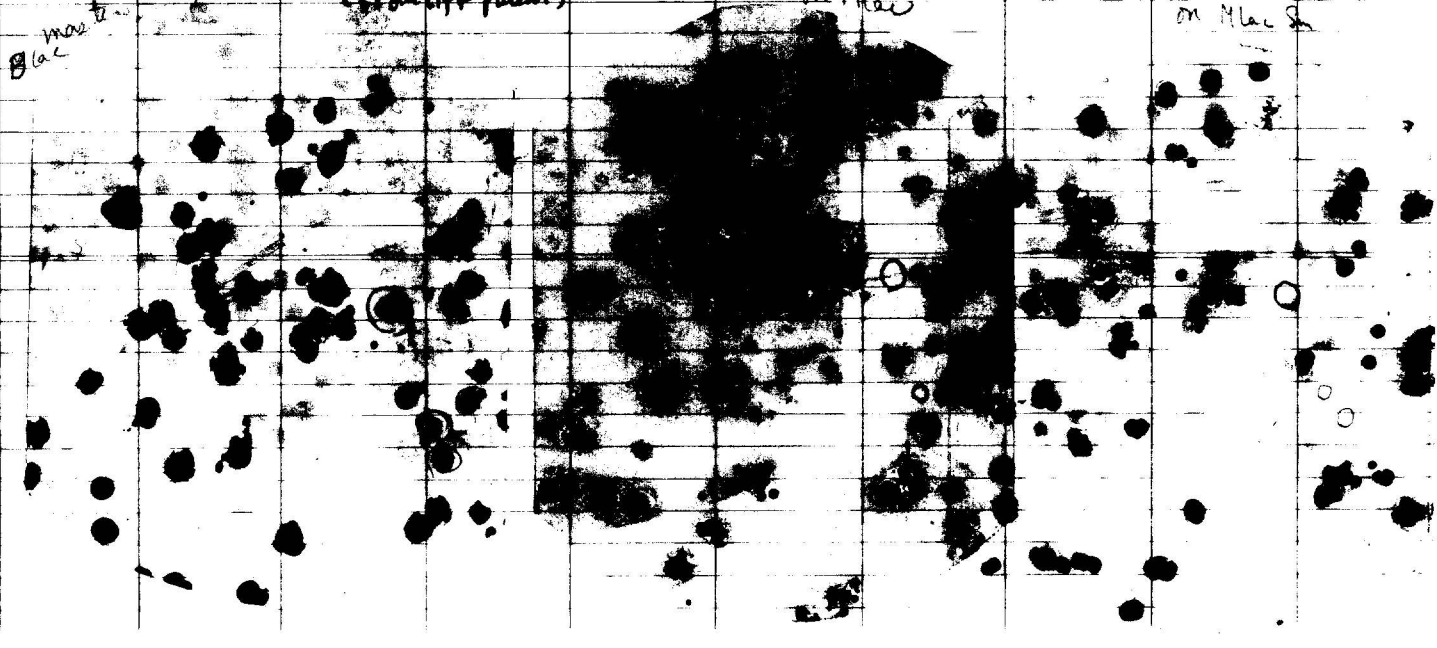
all state I ?

F⁻ or F^R =

Conclusion:

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

(from Hfr parent)



me example.

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42

2nd trial of infection of F_4 —× F_8.

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

- Method : (W6) (W3086)
  Mix F_4 1ml : F_8 1ml : 5ml phmassy. Control F_8 only.
  ↓
  Incubate it overnight.
  ↓
  Purify it on B Mal Sm.
  ↓
  Inoculate Mal⁻ Sm^R colonies into 1ml phmassy. tubes. and let it grow for 5 hrs at 37°C
  ↓
  Spot them on (W3086, W2985, W2979) m. Gal.
  (A_1) (Gal)
  and compair Hi recombination pattern.

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

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

- Result :

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

Double F strains. (F_4 + F_8 in W3486)

- Conclusion :
  1. F_4 is infectable to F_8^+ cell, and sometimes expel F_8 from the host cell and sometimes make double F strain.
  2. These fertility ▶ may depends on the segregation of two types F^+. (∵ If A_1 becomes higher than parent Gal will become lower or vice versa, not both.) See segregation of F characters by spot test and replica plating method.

[redacted]

efficient method : application of Replica plating method.

W6 F_4 —× 3086 F_8. Use Xyl to detect F_4
Seed on B Mal Sm.
Replica plate on M Xyl seeded W2979.

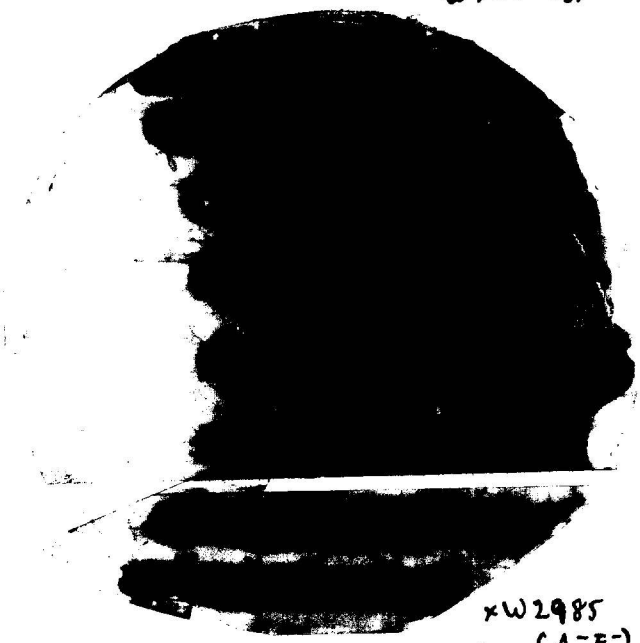
(F_4 is Hi for Xyl but F_8 is very low for Xyl, therefore it is easily differentiate between F_4 and F_8.)

on M Xyl W2979

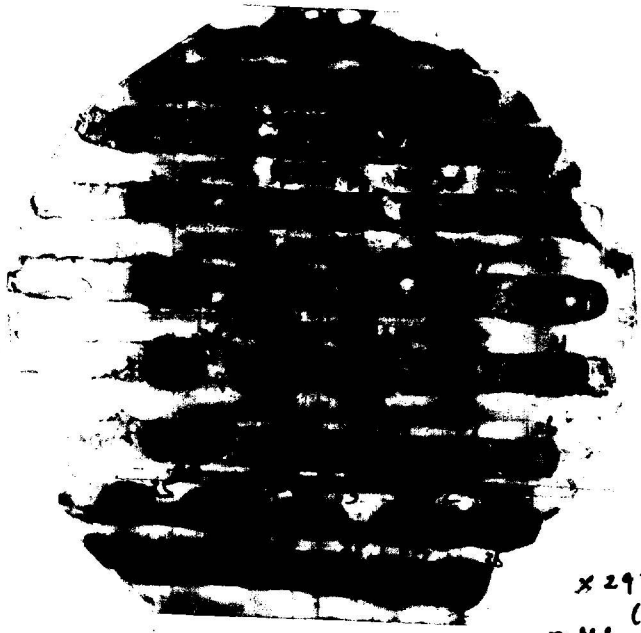
Take HI colony, on Xyl-transfer and see segregation of the markers by replica plating in same way.

X2979 (Gal⁻) 2985 (A_1) on M Gal

Control W3086 F_8.



× W2985 ($A_1^- F^-$) on MGal

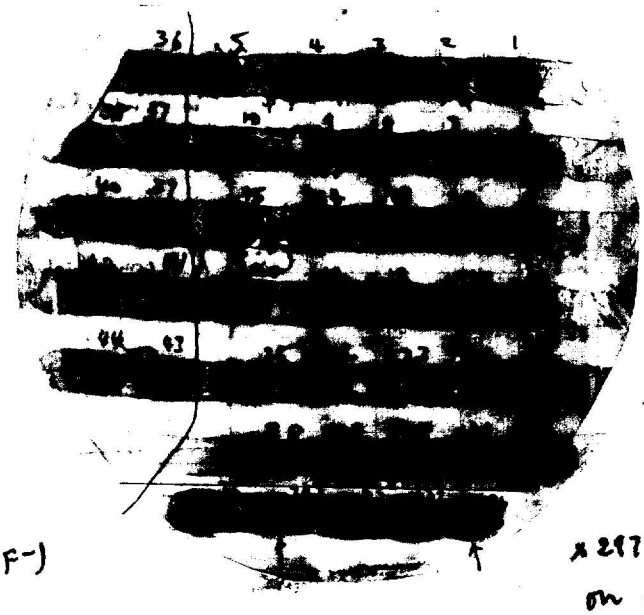


× 2979 (Gal$^-$ X$^+$) on MGal.

infection. Look for double F.

W6 F_4 × W3086 F_8

× W2985 ($A_1^- F^-$) on MGal



× 2979 (Gal$_2^-$ X$^+$) on MGal

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

2nd trial of infection of F_8 to F_4^+

W6 F_8 —× W3086 F_4^+.

23/IV 1954

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

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

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

Result:

			Hi for A_1 only (F4 type)	Hi for Bal_2 only (F8 type)	Hi for both (double F)
	W6 F_8 —× 3086 F_4		35/35 (100)	0/35	0/35
Control	3086 F_4 only.		35/35 (100)	0/35	0/35

All the colonies of 5 tested

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

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

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

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

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

W4525 (W3644 F_8^+)

1/N : 1959.

REF ~~TLB,Mal, SR f_3.~~ Cf p55, p70, p23

W4525.

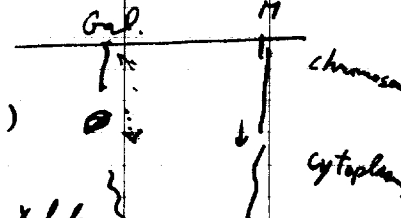
① purify F_8^+ f_3 : (W3644 F_8). on B lac.

② Inoculate it into penassay and let it grow for overnight. (Single colony)

③ Make colonies on B lac. and replica plate it on M Gal plates seeded ~~W3220 F M Gal~~ W2979 ~~and~~ or W6 F-.

Purpose :

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



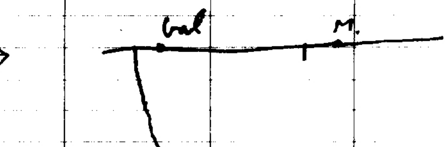
Result :

Only one state of fertility was observed. (see below) in both cross. (x M^-, x Gal_2^-)

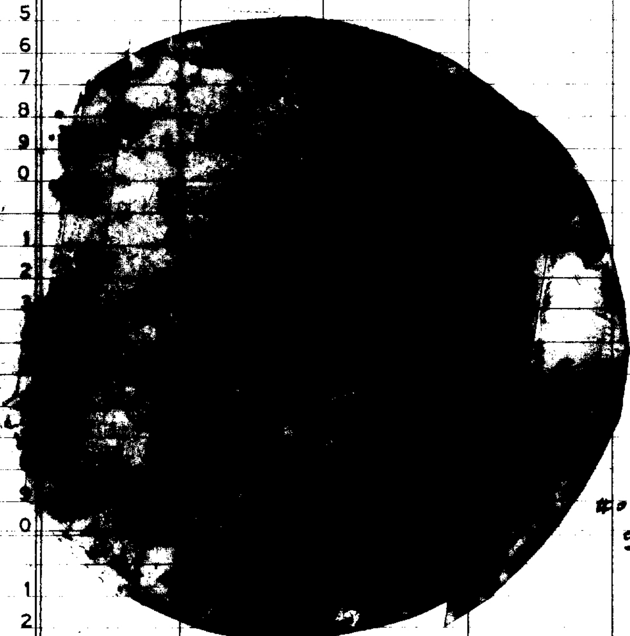
Conclusion : F8 does not fixed on Rfd3 locus. Only attach at the Xyl locus

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

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

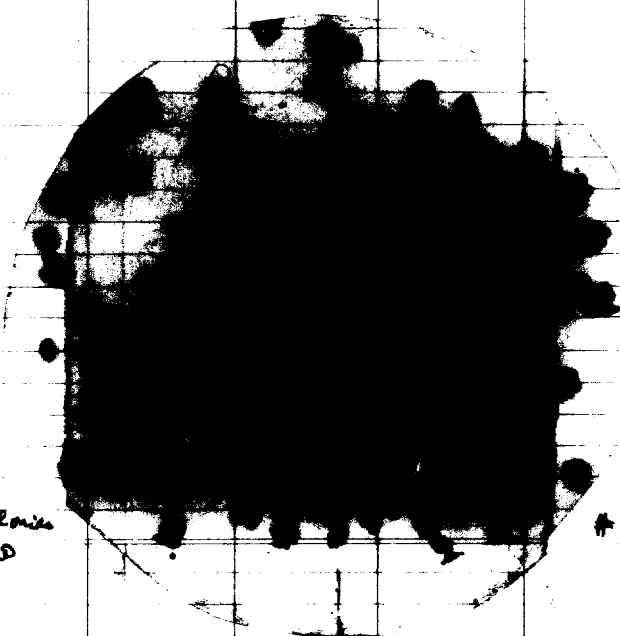


W4525



# of colonies 220

x 2979 on M Gal



# of colonies 220

x W6F- on M lac

Minimal cell number for infection of F' to F⁻

5/11 ; 1959 REF:

1. Overnight culture (penassay culture) of W6 F8 and W3086 are inoculated into penassay medium. (/ml culture/5ml penassay).
2. Incubate it for 2 hrs. at 37°C. (To make new culture.)
4. Mix them in the ratio 1 : 10^6 cells/ml

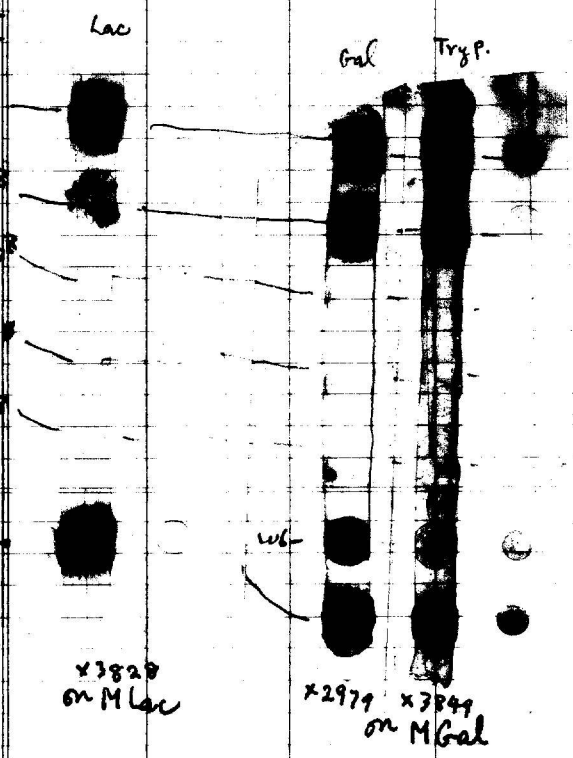
No of tube	1	2	3	4	control
W6 F8 :	$\times 10^{-2}$ 2×10^6 ↓ 0.1ml 2×10^5 +	10^{-2} 2×10^4 ↓ 0.1ml 2×10^3 +	$\times 10^{-2}$ 2×10^2 ↓ 0.1ml	$\times 10^{-1}$ 2×10^1 ↓ 0.1ml 2×10^0 +	0 0
W3086 Ca. 10^8	1 ml	1 ml	1 ml	1 ml	1 ml.

5. Add 5m into the mixture. at the final concentration of 1000 unit /ml. to kill F8 donor. 2 hrs after mixing, and incubate it overnight. 4:00 pm. 5/11/59.
   2g/5ml H_2O $\times 10^{-1}$: 0.2ml/1ml pen. final : 0.8 mg/ml.
6. Survival count of W6 F8. & W3086.

	W6 F8	W3086
	$\times10^{-2}\times10^{-2}\times10^{-1}\times10^{-1}$ 0.1ml/plate	$\times10^{-2}\times10^{-2}\times10^{-1}\times10^{-1}$ 0.1ml.
	230, 189	206, 185
Cell Numbers used : Original cell #.	2 x 10^8 cells/ml	: 2 x 10^8 cells/ml.

number of F8 donor

W6 F8	3086
# of F'	# of F'
2×10^5	10^8
2×10^3	10^8
2×10^1	10^8
2×10^0	10^8
~~0~~	10^8
W6 F8	—



Summary of experimental conditions.

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

Inoculum size : (within 2 hrs.

Minimal cell number for infection of F'

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

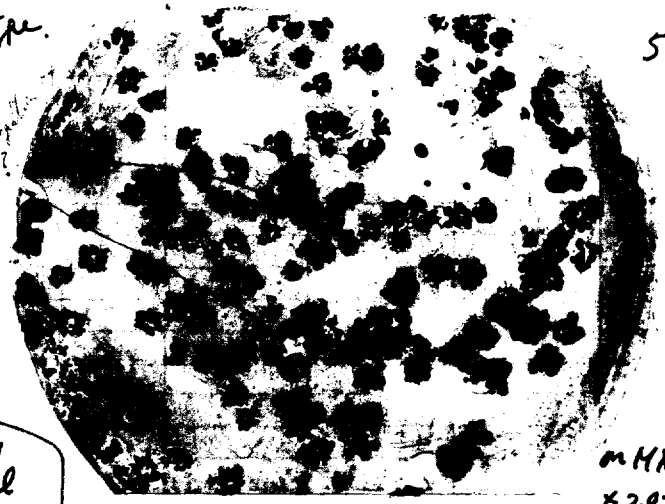
2000 ~ 20

Ca. 100.

F4 → F8.

④ type.

×2979 on MGal



on MXyl. ×2979.

F4 → F8.

⑧ type

×2979 on MGal

on MXyl ×2979.

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

Cf. ♯42

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

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