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W3703 → W6− (W6 4 Hfr.)

Ref: F of W64 does not REF. infect to K-12 F.

2/4/59 ; 1959

L Try Lac Mal Str.

1 2 3 4 5 6 7 8 9 10

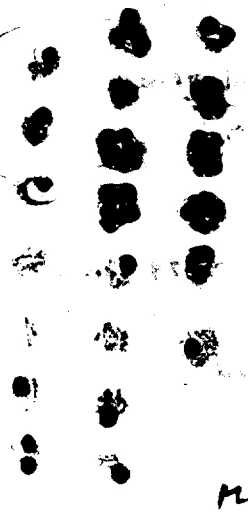
Method : W3703 1ml + W6− 0.1 ml + 5ml phage.
↓
Incubate 24hrs. at 37°C.
↓
Spread on M Lac + M
↓
Replica plate it on M Gal seeded 2979 on it.

Result : No F+, and no Hfr was observed.

M Lac + Meth.

M Gal
x 2979

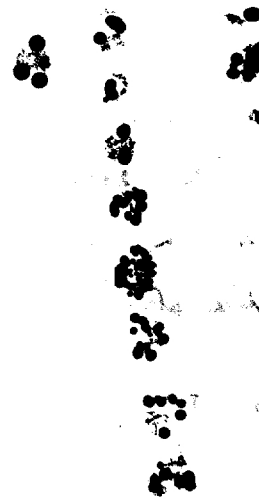
5 – x WB –



MLacβ,

x3828

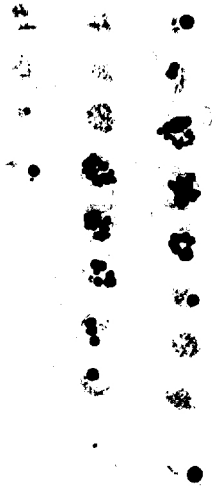
6 – x WB –



MLacβ,

x3828

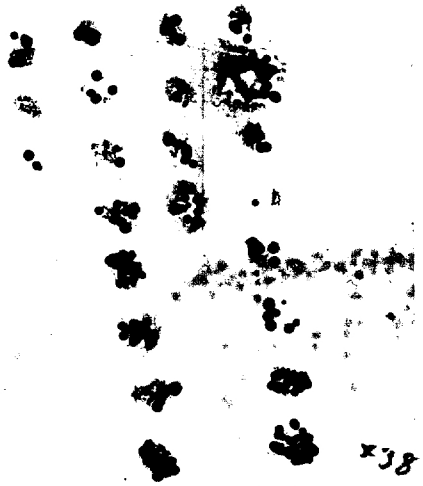
2 – x WB –



x3828

MLacβ,

4 – x WB –



x3828

MLacβ,

~~Infectivity~~ Infectability of F^R to F_8. purpose: Does F^R keep F_8 for a while? Temporally F_8^+

28/4 ; 1959

REF: P.44 P.45 P25.

Method : (cultural age) Overnight culture. (passage 5 ml.)

Ratio of mix : 1 : 1.

Experimental condition for F_8 infection: Shaked : 3 hrs. at 37°C on rotator.

(Spot them : Untreated.

Add Sm. 400γ/5m ml.

Keep them at 37°C for 1/2 hr. } : treated by Sm.

spot them.

Principle

$M\,Gal_4\,F_{8S} \times M\,F^R\,S^R$.
W4534 × $Gal_4^-\,S^R$.

	1	2	3	4	5	6	7	8	9	10
			1	2	3	~~8~~	4	5	6	7
								Shaked 3 hrs. 2 4r		
			3220 F^- MGal4	4534 F_8 MGal4	4526 $F^R M S^R$	~~3086 F− M+S^R~~	4534 + 4526 F^R	4534 + 3086 F^-	3220 + 4526	3220 + 3086
recombination	3994 (F^- Gal4 S^R)		−	−	−		++	++	−	−
	3086 (F^- M^- S^R)		−	−	−		−	−	−	−
Infectivity	3086 + 3994		−	++	−		++	++	−	−

Next step ① purify 4534 → 4526 & 4536 → 3086 on B Lac Sm. and test cross-compatibility by replica-method. (see next page. P76b)

on MGal and on MGal Sm.

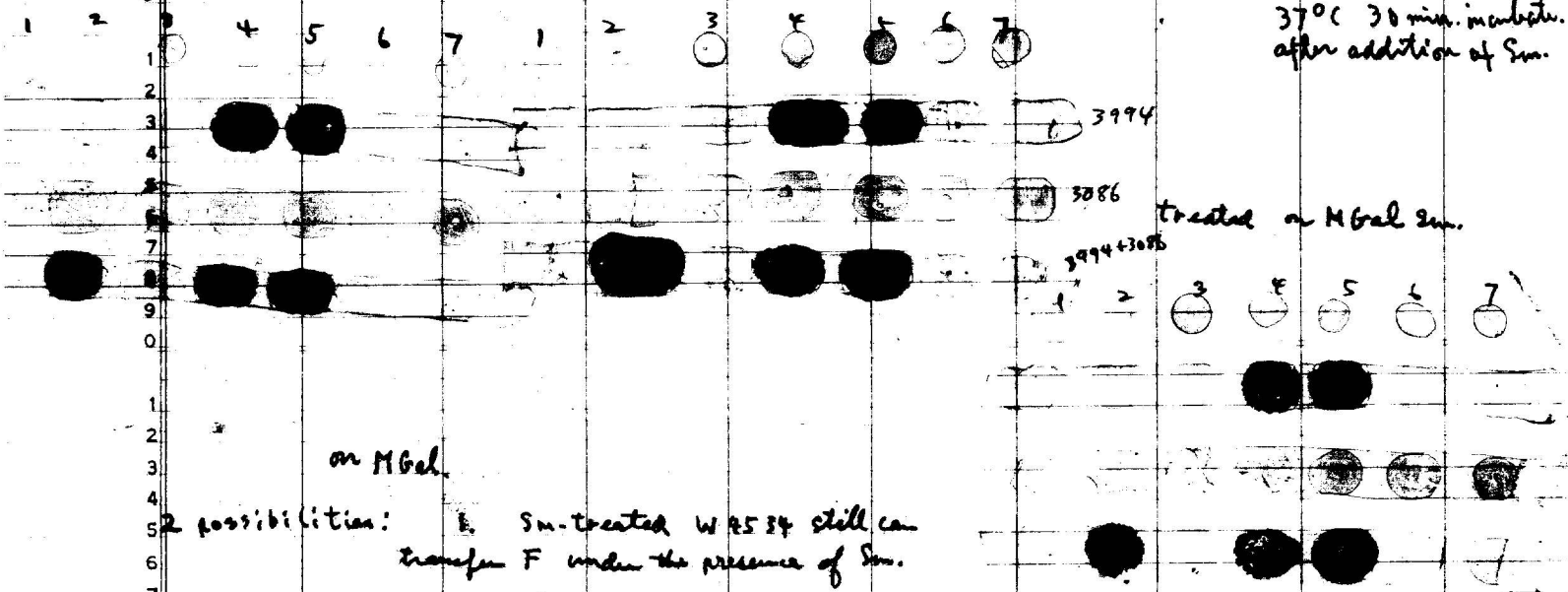
After treated by Sm.

on MGal on MGal Sm

Untreated.

untreated on MGal Sm

Sm. 37°C 30 min. incubation after addition of Sm.



on MGal.

2 possibilities: 1. Sm-treated W4534 still can transfer F under the presence of Sm.

2. F^R can carries F_8 for a while. and possesses F_8^+ character for a while. But it cannot continuous those traits.

Continued from former page

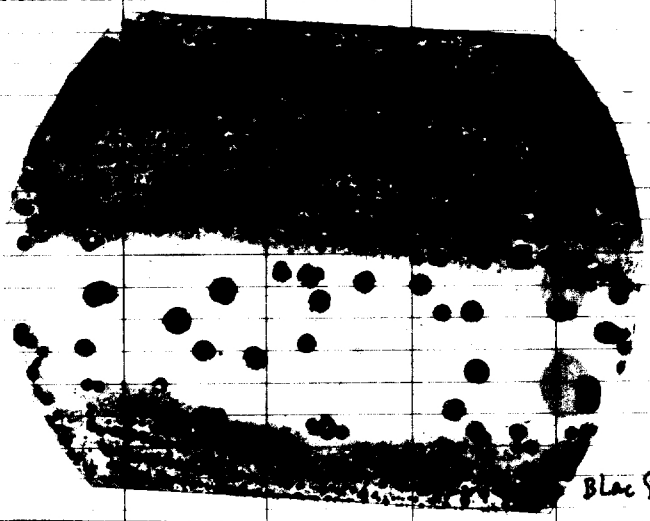
3D/4 ; 1959.

REF: cf. p76a : (Next step)

Method : ① # 4 and # 5 (4534 × 3086) were streaked on Blac Sm.
(4534 × 4526)
② Replica plate on Mlac B_1 seeded W3828, and see futile colony is there or not.

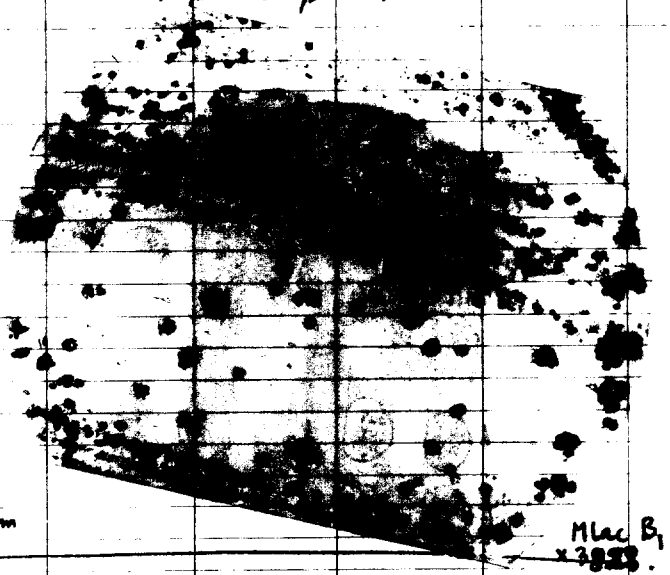
Control. 4534 × 3086

Master plate



Blac Sm

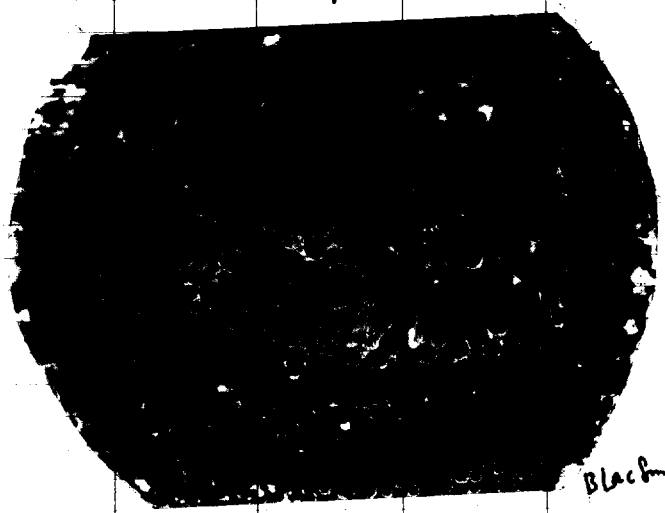
Replica plate.



Mlac B_1 × 3828.

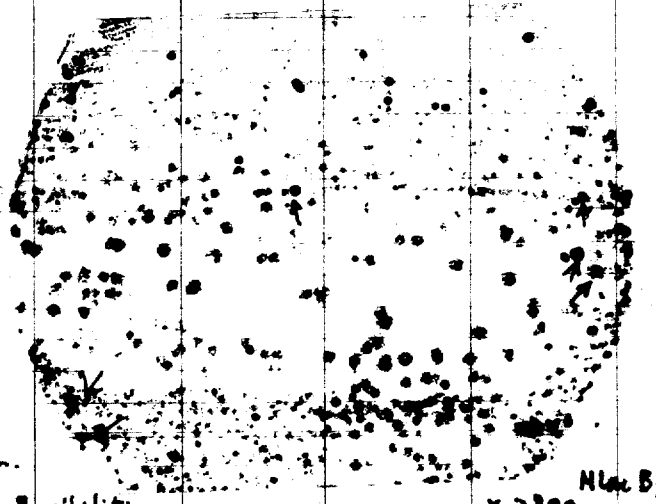
4534 × 4526

Master plate



Blac Sm.

Replica plate.

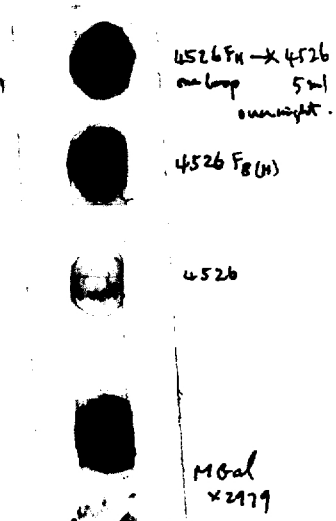


Mlac B_1 × 3828

Next step :
① Repeat this experiment with control.
② If it is host range mutant of F_8, it must be ~~[redacted]~~ infective to F^- and F^R. And also H1 for Gal ~~[redacted]~~ unlike Hfr_1. (See back page). This is H1 for Gal.

Possibilities :
① Is it mutant of F_8 (Host range mutant)
② Spontaneous (reversion) mutant of F^R to Hfr_1. (This seems unlike, because there is no recombination in 1, 2, 3 ; see p76a)
③ Recombination between F^R and F_8 and get Hfr_1 after recombination. F_8 + + + + + F^R

Cf. infect to F^-



Rough estimation of Host range hypothesis.

① Mix
W4526 F_{8H}. ——→ × W4526
one loopful — 5ml plenary.
plenary broth culture — overnight grown culture.

② Make spot test. on MGal. ×2979.

If black spot was obtained, it may be Host-range mutant of F_8.

Result and conclusion: 4526 F_H → ×4526 does not become black spot. Infectivity of $F_{(H)}$ is still very low to 4526 or not infective to F_8.

#2 —x W6–
#4 —x W6–
#5 —x W6–
C —x W6–

A0 #2
A0 #4
A0 #5
A0 C

27/4 ;1959 REF: cf. 74.

Experimental conditions: 0.1 : 0.1 : 5ml 0.1ml, A0 30γ/ml, 9 pm.
grown overnight at 37°C. Pen

Result: F+ of
1. A #2, #4, #5, C, are infective, and gives low fertile, probably F+ (See back page).
F+ of
2. (#2, #4, #5, C, are sensitive to acridine treatment.

#5

C A0

MlacB, X3806

F– ×3006

C A0

MlacB, X3828

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unsuccessful result.

Detection of recombinants from double F^+ strain.

$F_4 \times F_8$

1/VI ; 1959 #2 was used. Cf REF. p 42, p 50.

Principle.

F_8 F_4

\+ × + F_4

F_8 + +

↓

F_4 + +

F_8 + +

F_{48} F^+

↑ detcd F^+

point : Look for F^+ carefully. assumption : F_8 and F_4 is not allelic.

Method 1. Seed #2 on B Lac Sm. (1 ×10^{-2} × 10^{-2} · 0.1 ml) + (10^{-2} × 10^{-2} × 10^{-1} · 0.1 ml × 2.

2. Replica plate on M Gal Seeded 2979. on it.

3. Look for Lo colonies. 2985

	# of colonies tested	# of Y colonies steril (on M Gal + 2979)	Hfr colonies
	451	1	450
	437	0	437
	415	0	415
	395	0	395
	510	0	510
	372	0	372
	376	0	376
	333	0	333
	422	0	422
	533	(1) F^+ or F^-	532
Σ	4044	2	4042

detcd F^+ ← thick Gal marker

There two colonies are F^- (See bellow).

Possibility : 1. spontaneous reversion of F_8^+ or F_4^+ into F^+ (or F^-)

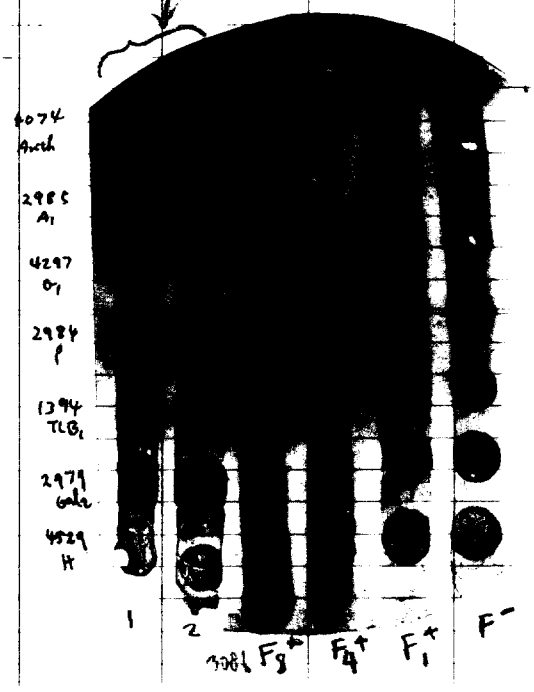
Result : F^+ was not observed (negative result was obtained)

Conclusion : F_4 and F_8 locus ① are allelic, and cannot recombine, or ② they are very close with each others or ③ does not make synapsis.

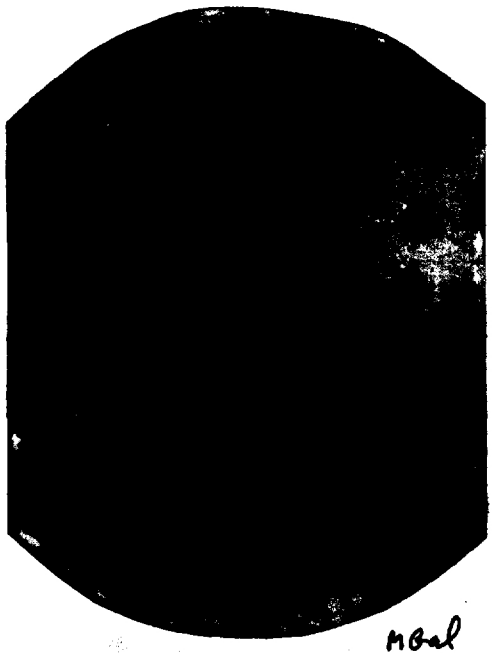
① F_8 F_4

② F_8 F_4

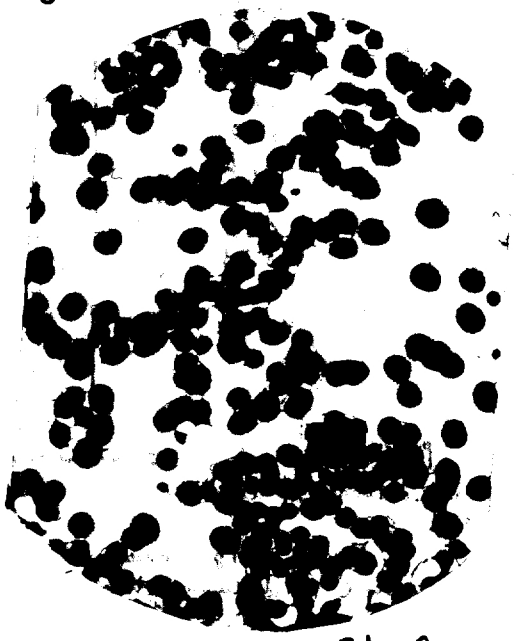
③ F_8 F_4



An example: Replica plating method gives clear spots in this case.



MGal
KL979



n Blac Sm

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Isolate Hfr_8 from W3208. unsuccessful result.

1/VI ~ ; 1959 REF:

Purpose : It is necessary to get Hfr_8.

Method : 1. Take W3208 from stab culture, and purify it on Blac.

2. Cross-brush the 10 colonies. (×2979 on MGal)

3. pick Hfr and | Treat by AO. and see it is resistant to AO or not.
infect F to 3086. and see is it infective or not.
Use F_8 control.

Method for infection:

① Wb F_8, or W3208 Hfr_8 1 ml : 3086 0.1 ml : Penney broth 1 ml.

② incubate it overnight at 37°C.

③ Purify on Blac Pm.

④ cross on MGal ×2979.

Result : This strain is infective to F^- ∴ F'_8 (not Hfr_8)

Ratio of infection : $Hfr_8 + F^-_{3086}$ $15/23 \times 100 = 65.2$ (%) ← (see back page)

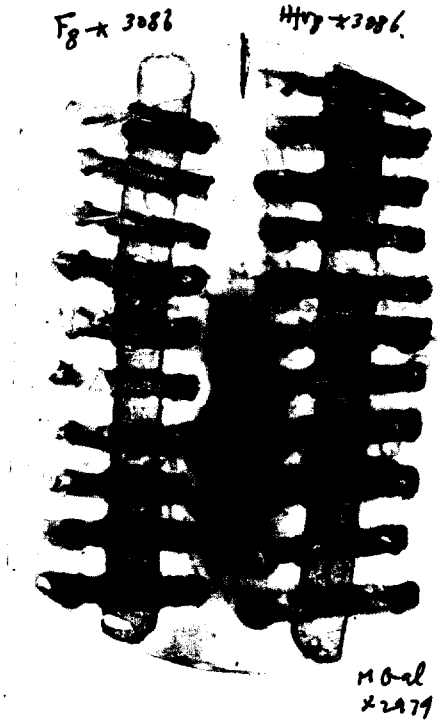
control. $F_8 + 3086$ $17/21 \times 100 = 81.0$ (%)

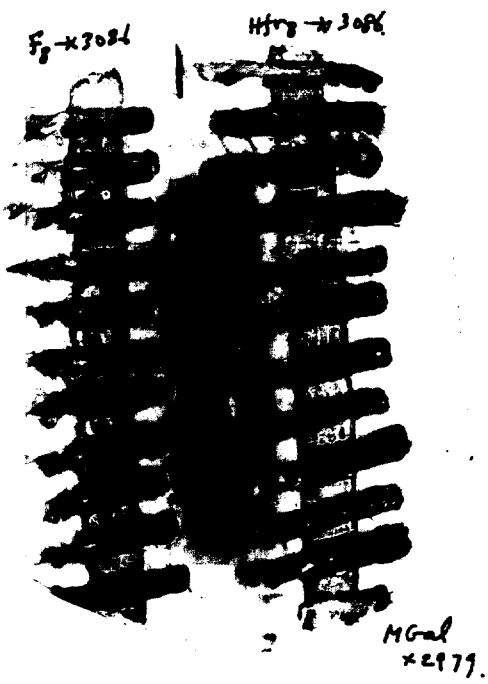
1/10 ×2979 on MGal.

Next step :

Treat W3208 with AO, and look for Hfr or use lyophilized culture for reisolation (no mutation) of Hfr_8.

Comparison of infectivity of F_8 to F-





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W4568

81

Characterization of Host range mutant of F_8.

4526 $F_{8.H.}$

4/21 ; 1959

REF: cf. P76a, b.

Purpose : Confirm this is "Host range mutant" of F_8, and this trait is heritable character determined by (itself)

1. Infect $F_{8H.}$ to W3735 ($S^s F^R$ H), and isolate W3735 $F_{8H.}$

· Mix 1 : 1 in passage (1ml : 1ml : 1ml)
4526 F_{8H} 3735 Bac.

· Purify it on BGal and replica plate it onto Blac Sm. and see which colony is W3735. or W4526.

· Pick W3735 (S^s) and cross-streak with W2979. on MGal

Purpose : ~~to~~ put more markers into F^R mutant to differentiate between F^R mutants.

2. Isolate Gal^-_6 from ~~W3735~~ W4526 ~~Sm~~.

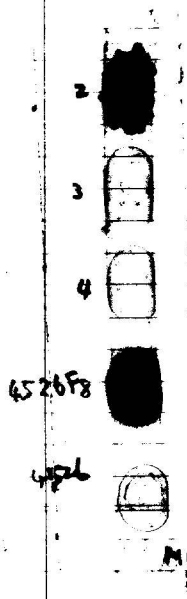
① HFT-6 ——× W4526
0.2ml 1ml overnight culture. Keep this one hour.

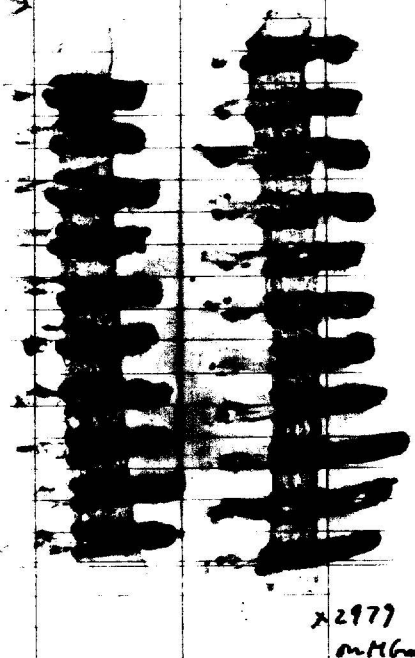
② Spread on B-O. and incubate it overnight.

③

3. Infectivity to F^-. 3735 $F^+_{8H.}$ —× 3086. ; Method : standard method was

Result: Infective (very efficient) to F^-. 100 %. (21/21)





3828 F_3 → Y10.

4/21 ,1959 REF:

1 2 3 4 5 6 7 8 9 10

1st trial.

Method : 1. Mix them 1 : 1.

2. Incubate it overnight.

3. ~~Streak~~ Streak it on Blac, and pick Lac+. Test str. transfer. ×2979. on Mtgl+B_1

Result : Strong competition was observed. 3828 has more selective advantage over Y10. Unsuccessful all F⁻. try again;

2nd trial:

Method : 1. Mix them 3828 (1ml) Y10 (0.5ml) 2 : 1, incubate it overnight. Ca. 24hrs.

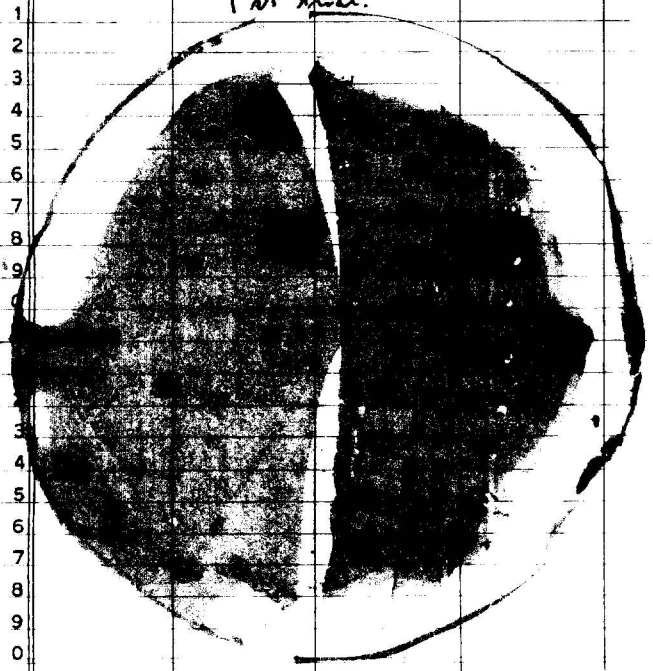
2. purify it on Blac.

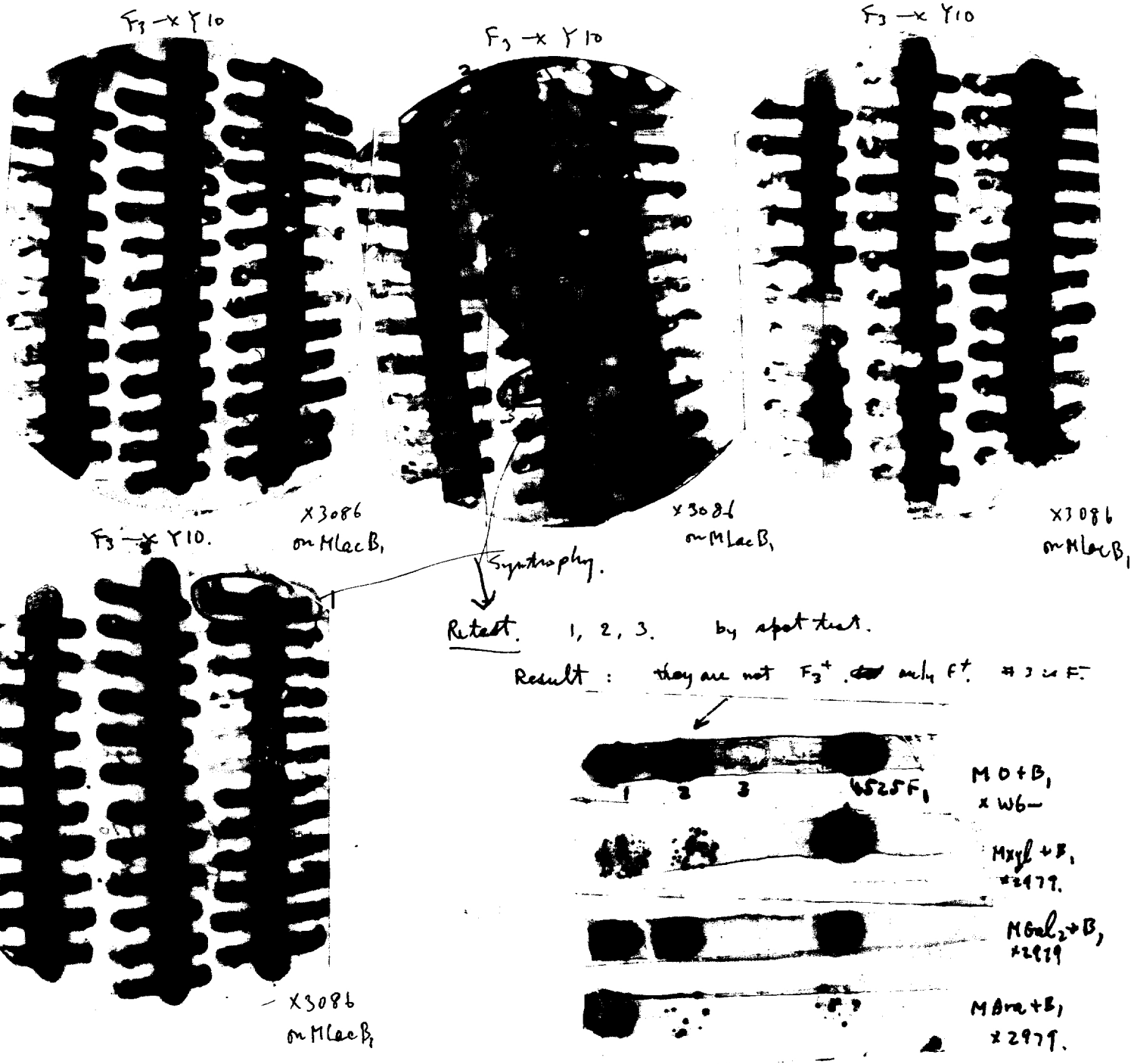
3. Cross brush Lac+ (Y10) against 3086 on MLac B_1. test at least 100 colonies!

Ratio : (See back page).
Hfr/♂. 0/139.

No F_3 were transfered to Y10.

3828 F_3 only gave F_1 not F_3.




F3 — x Y10
X3086
on MLacB1
F3 — x Y10
X3086
on MLacB1
F3 — x Y10
X3086
on MLacB1
F3 — x Y10.
X3086
on MLacB1
Synthophy.
Retest. 1, 2, 3. by spot test.
Result : they are not F3+ only F+ # 3 is F-
1
2
3
W525F1
MD+B1
x W6—
Mxyl+B1
x297?.
MGal2+B1
x297?
MBrn+B1
x297?.

3828 F_3 → × W6−

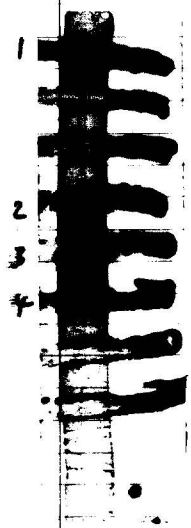
6/11 ; 1959.

REF:

Method 1. Mix them 1 : 1
2. Incubate + overnight at 37°C
3. purify it on Blac and test Lac+ on ala-compatibility, × 2979 on MXyl.

Result.
W6↗ was obtained after mixed culture with 3828 F_3 and W6−.
Is it O_3^+ or F^+?

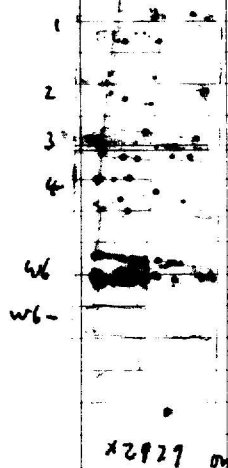
3828 F_3 → × W6−



× 2979 on MXyl.

4/8 = 50%

Retest



× 2979 on MXyl

W4576 (F_4 A_i^- Gal_6^-)

Infect F_4' and F_4'' to W4528.

(F_4'' (selected 10 times for infectivity)) F^- A_i^- Gal_6^-

8/VI ;1959 REF:

Purpose: Comparison of the infectivity of F_4 mutants.

1st comparison: Result.

	F_4^+/total (%)
W6 F_4' → W4528	0/34 (0)
W6 F_4'' → W4528	4/35 (11.4)

Select on BGal. Compare rate of infection. Test on #1 for ... (on ...)

(See back page)

2nd trials of infection of F_4 (non-selected F_4) to W4576 was not successful.
0/32 : 0% rate of infection: all F^-.

2nd Comparison

Spot W4528 F_4' and W4528 F_4'' on W4293 (F^- A_i^-) and W3996 (F^- Gal_6 S^R)

7/IV. 1st trials.

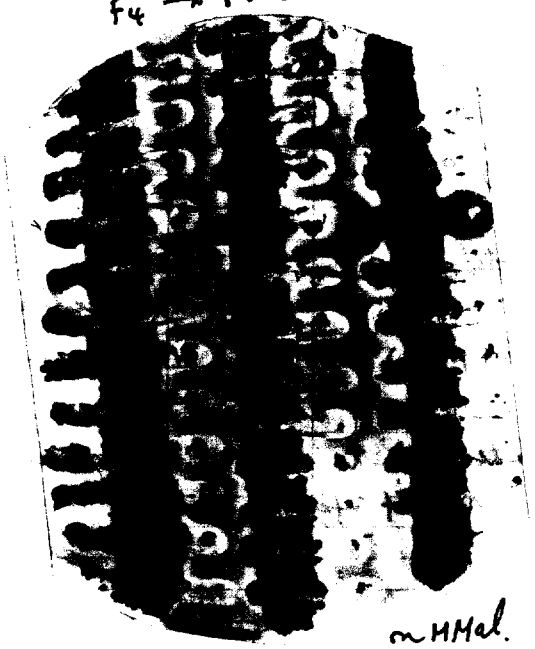
Method for selection of more infective F_4.

W6 F_4 one loopful (overnight culture) + W6 F^- 5 ml (ca. 10^8 cells/ml.)
↓ incubate overnight.
one loopful : + W6 F^- 5 ml (ca. 10^8 cells/ml.)
↓
Repeat this process.

This selection was done for 10 times: W6 F_4''.

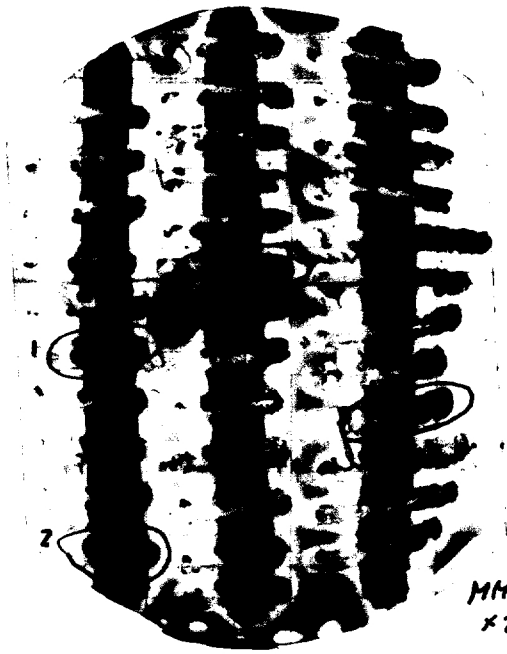
2nd trial: From 8/VI's experiment, the infectivity of F_4'' is still not enough. Try selection again using W4528.

F_4 — × 4528



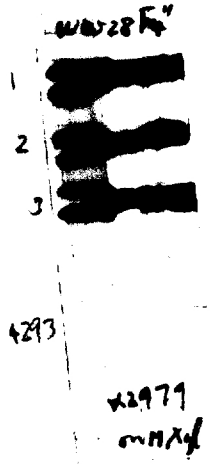
on MMal.
× 2979.

F_4" — × 4528



MMal
× 2979.

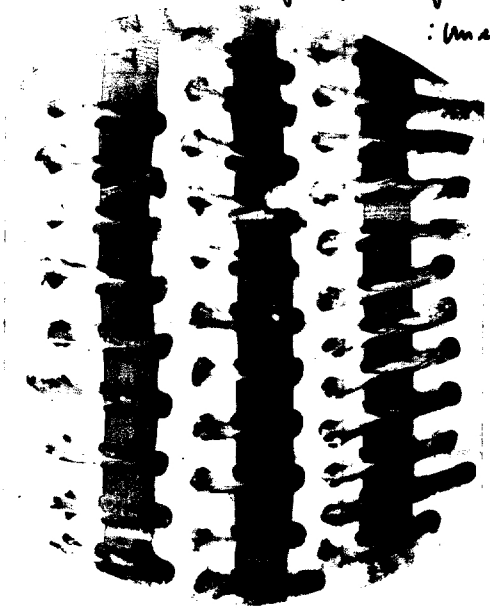
Retest of infected F_4"



×2979
on MMal

Mal, marker is reverse.
even though, F_4" can be tell
from highly fertility

2nd trials of infection of F_4 to ω4528
: unsuccessful :
May be some reason



Treat W4552 (3086 F_5^-)
with AO, and reinfect F_1 to it.

1959, Test the possibility of F_3 type F'. REF:

Purpose and Principle :

3086 F_5^- ~~and 3086 F_3^-~~ transfer their F to F^-. But the infected cell shows low fertility after infection. Then, what factor was left behind at the time of F transfer. If a factor which determines Hfr character cooperated with F, was left behind after treatment of these F'^+ strains, reinfection of F_1 to F^- obtained after treatment of the F'^+ appears either Hfr or F' mutant with high frequency.

Method of treatment : Usual method. conc of AO. 3 γ/ml. Pen. 5 u/ml. overnight at 37°C.

Result : Rate of F^-/11 = Ca. 1 % (See back page.)

Infect F_1 to 3086 F_5^- : W6 × 3086 F_5^- Use usual method for infection.

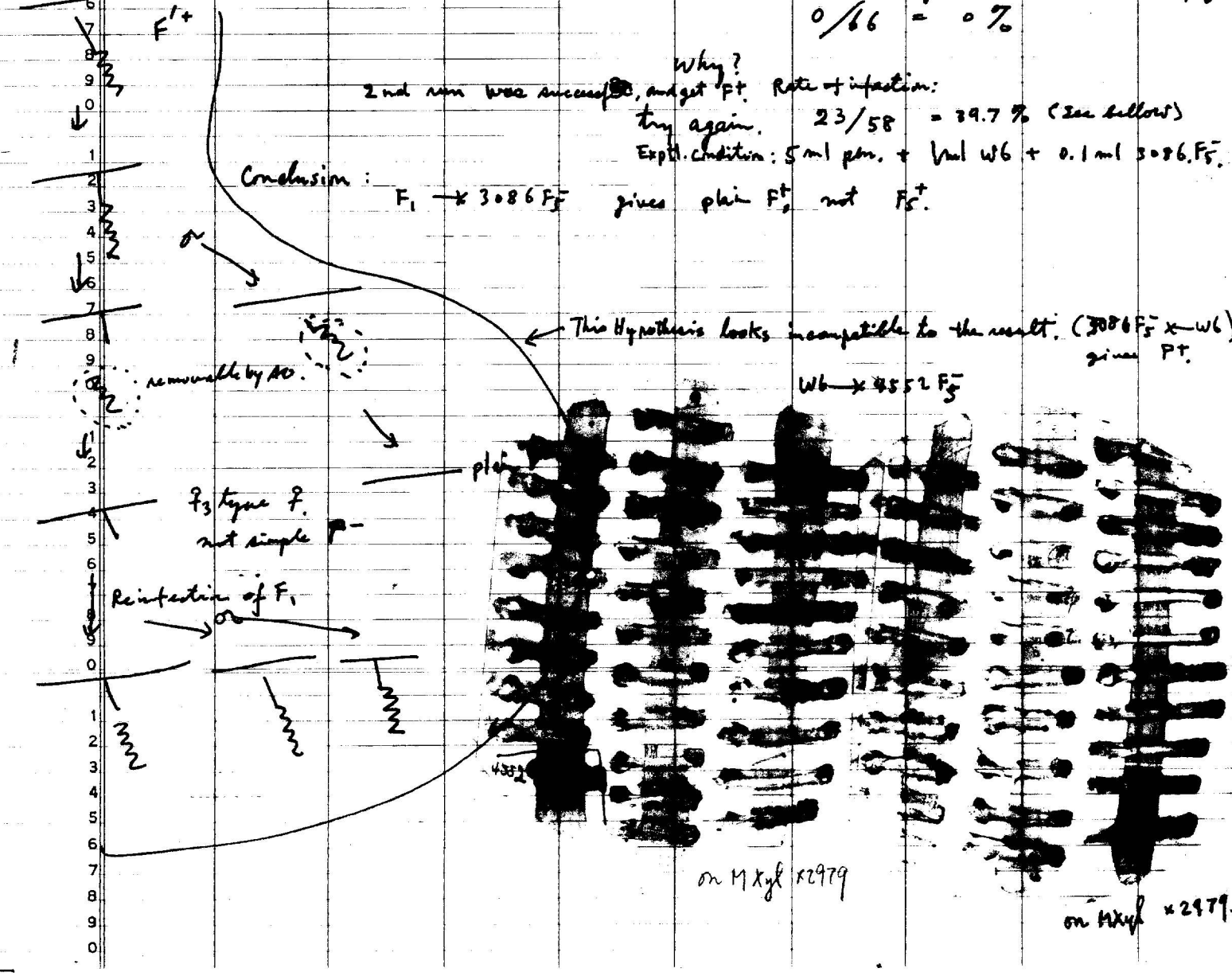
1st run was not successful. × 2979 on MXyl.

Result : Rate of infection : (see back page)
0/66 = 0 %

Why?

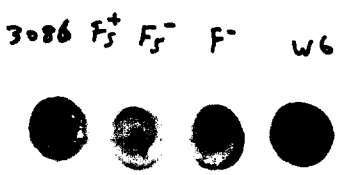
2nd run was successful, and get F^+. Rate of infection:
try again. 23/58 = 39.7 % (See bellow)
Expt. Condition : 5 ml pen. + 1 ml W6 + 0.1 ml 3086 F_5^-.

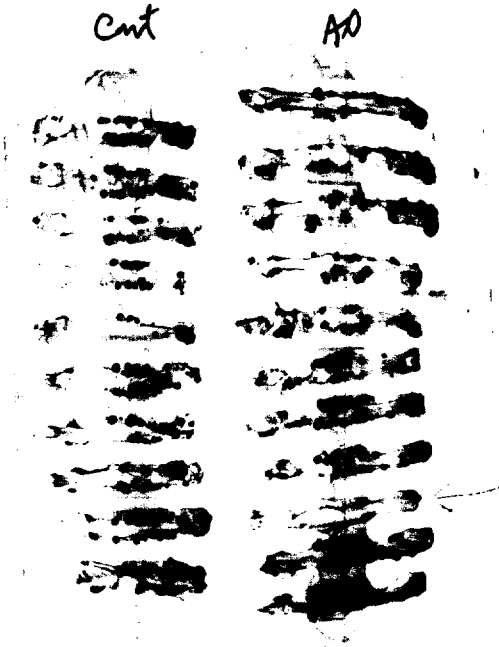
Conclusion :
F_1 × 3086 F_5^- gives plain F_5^+, not F_5^+.



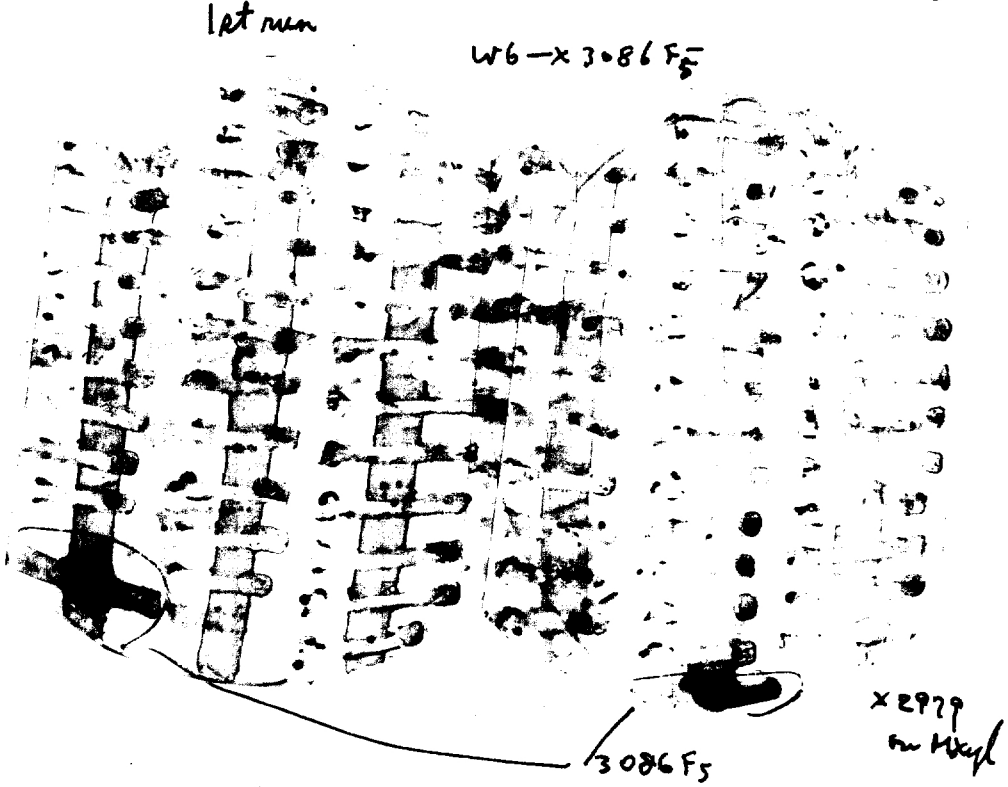
① cure from F_5.

3086 F_5





② Infect F_1 to 3086 F_5^-



Unsuccessful. compair infectivity of F_3' of W3642 F_1^+ to F^-

Take control (W6) F^+ M Mal$_5$ Gal$_2$ Lac$_1$

15/VI ; 1959. REF:

Exp. design. : W3642 F_1 → × 3086

W6 (control) → × 3086

Select on BGal Sm.

Replicaplate then on MXyl. × 2979.

Method:

Result:

	Exp. W3642 F_1 × 3086 # of colonies	# of Hfr_3 colonies (%) (select on MXyl.) × 2979	Control. W6 × 3086 # of colonies	# of Hfr_3 colonies (%) (select on MXyl. × 2979)
1	618	0 (0)	4 249	0 (0)
2	661	0 (0)	5 208	0 (0)
3	700	0 (0)	6 245	0 (0)
	Σ 3642 × 3086	0 (0)	Σ W6 × 3086	

Further experiment:

Use 3133 for F receptor.

Agglutination of F' strains.

18/4 ; 1959

REF: Peter ^ says He also observed this phenomena.

1	2	3	4	5	6	7	8	9	10

o Starting point : W4293 F_4 showed agglutination. but F^- was not
(A; Gal$^-$) (λ^+ Sugar$^-$ S^R)

young culture : 2hrs. shows no distinct difference between them.

Experiment :

1. Inoculate

Hfr_2 F_2 , Hfr_4 , F_4 , Hfr_5 , F_6 ; F^- ; F^+

4321 , ~~W3384~~ W6 F_2 , W4321 , W6 F_4 , 4536 , 3086 F_6 , W6 ; 3086 ; W6, W3086 F^+

and incubate them for overnight. All culture was obtained from stock collection in cold room.

In overnight culture, agglutination was not observed.

2. Inoculate 0.2 ml of the overnight culture into 5 ml. phrassay broth. and shake it on rotator at 37°c for 2hrs. 10:00 AM — 12:AM.

Result : Agglutination was not observed in Hfr, F', F^+ and F^-. after 2 hrs, 4hrs. incubation.

Conclusion : Agglutination of F'^+ cells are sometimes observed, but not always does it. Presumably, it is influenced by unknown experimental conditions sensitively.

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W4526 F_8^- (Infectability to F_1)

19 REF: cf. P 89

	1	2	3	4	5	6	7	8	9	10

Principle :

Control { W6 → × W4526 (F^R) 0/26
W6 → × W3086 22/28

W6 → × W4526 F_8^- (1) 10/31
W6 → × W4526 F_8^- (2) 10/29
W6 → × W4526 F_8^- (3) 11/29

Ratio.

Mixed culture.
Overnight:
W6 1 ml. & 0.1 ml.
Plate at 37°C.
Purify on BLac Sm.

W6 → × F^R control.

W6 → × 3086

W6 MLac ×3828

MLac ×3828 W6

W6 MLac ×3828

MLac ×3828 W6

W6 MLac ×3828

MLac ×3828 W6

2nd trial on : Take all F_3 apart. from (W4583 3828 F_3^+)

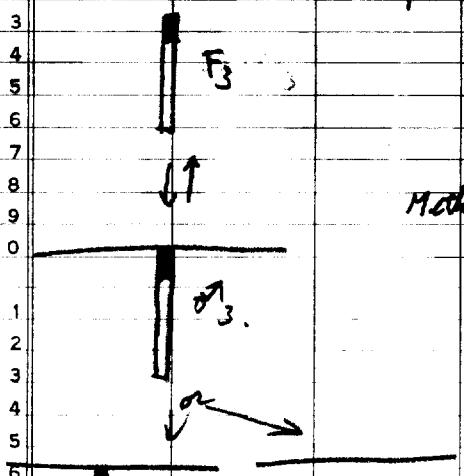
" Isolation of $\mathcal{F}_3$ from F_3^+.

5/4 ; 1959

REF:

If it does work, this may be apply to other F^+.

A possibility.



Most of them may become plain F^- but some of them may become $\mathcal{F}_3$ type

Method for detection of $\mathcal{F}_3$ among many F^- colonies.

Principle : F^+ M^- Lac$^-$: W1816.

$F_1 \times \mathcal{F}_3$ M^+ Lac$^-$: W3828 F_3^- (arised from W3828F_3 by AO treatment)

$M^+ \times$ F^- M^- : W3086

Method :

① treat W3828 F_3 with AO (30γ/ml gonococcy broth; 5ml, 48 hrs) treated for

② Seed it on BLac.

③ ~~Replica plate it on Mlac Seeded W3086~~

Cross-brush the treated W3828F_3 against ~~and Mlac seeded W3086~~ W3086 on Mlac.

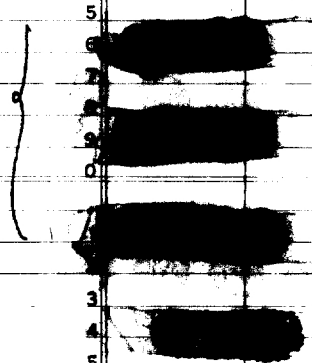
Results: $\mathcal{F}$: 51 / tested 92 : 55.4% See : back page.

④ Inoculate those ~~male~~ female clones into plasmy.

⑤ Make spot test as P.82. using W3086, W1816. on Mlac. Use control of $\mathcal{F}_3$: W3876 ; F^- W3828.

Result: All of the females are all $\mathcal{F}_3$, not F^-! See back page.

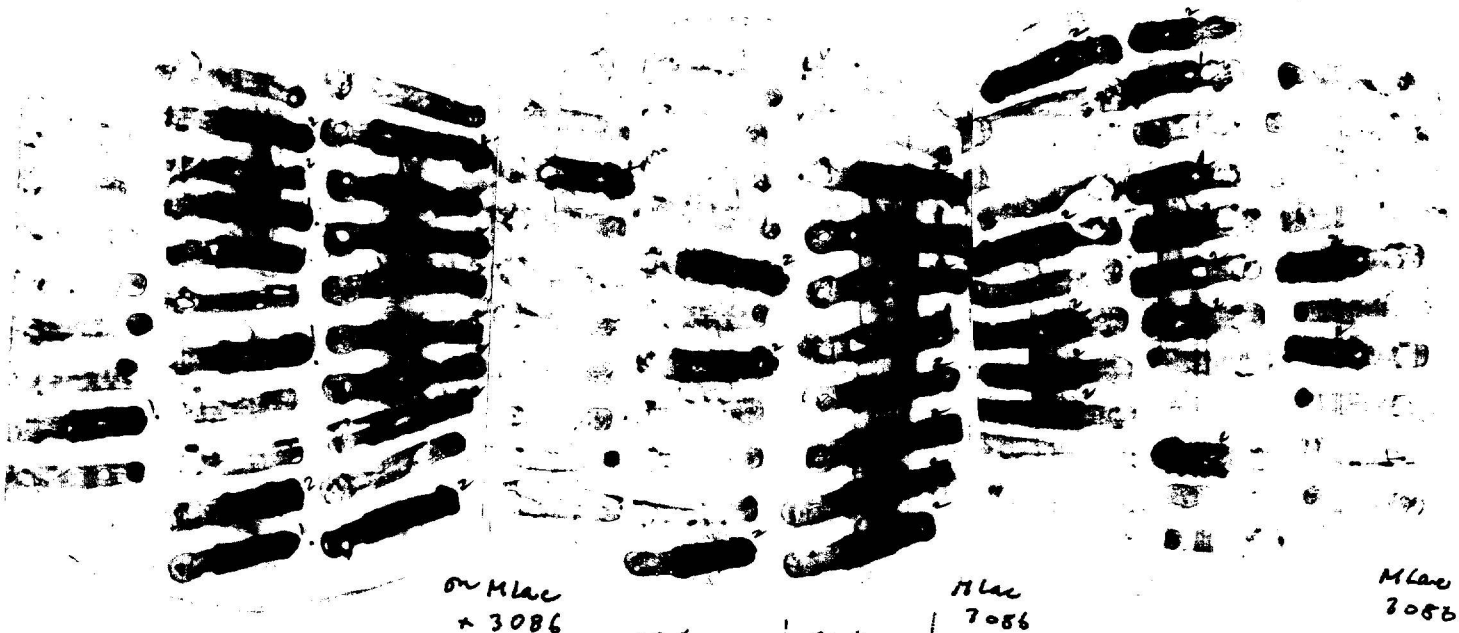
not very clear cut, but still higher than F- (control)



W3828 F_3^- #1			+ 3086
W3828 F_3^- #10		H1	+ 3086
W3876 : $\mathcal{F}_3$ control			+ 3086
W3833 : $\mathcal{F}^-$ control.			+ 3086

on M Lac

AO-treated ($W3828\ F_3$) W4583



on Mlac × 3086

Mlac 3086

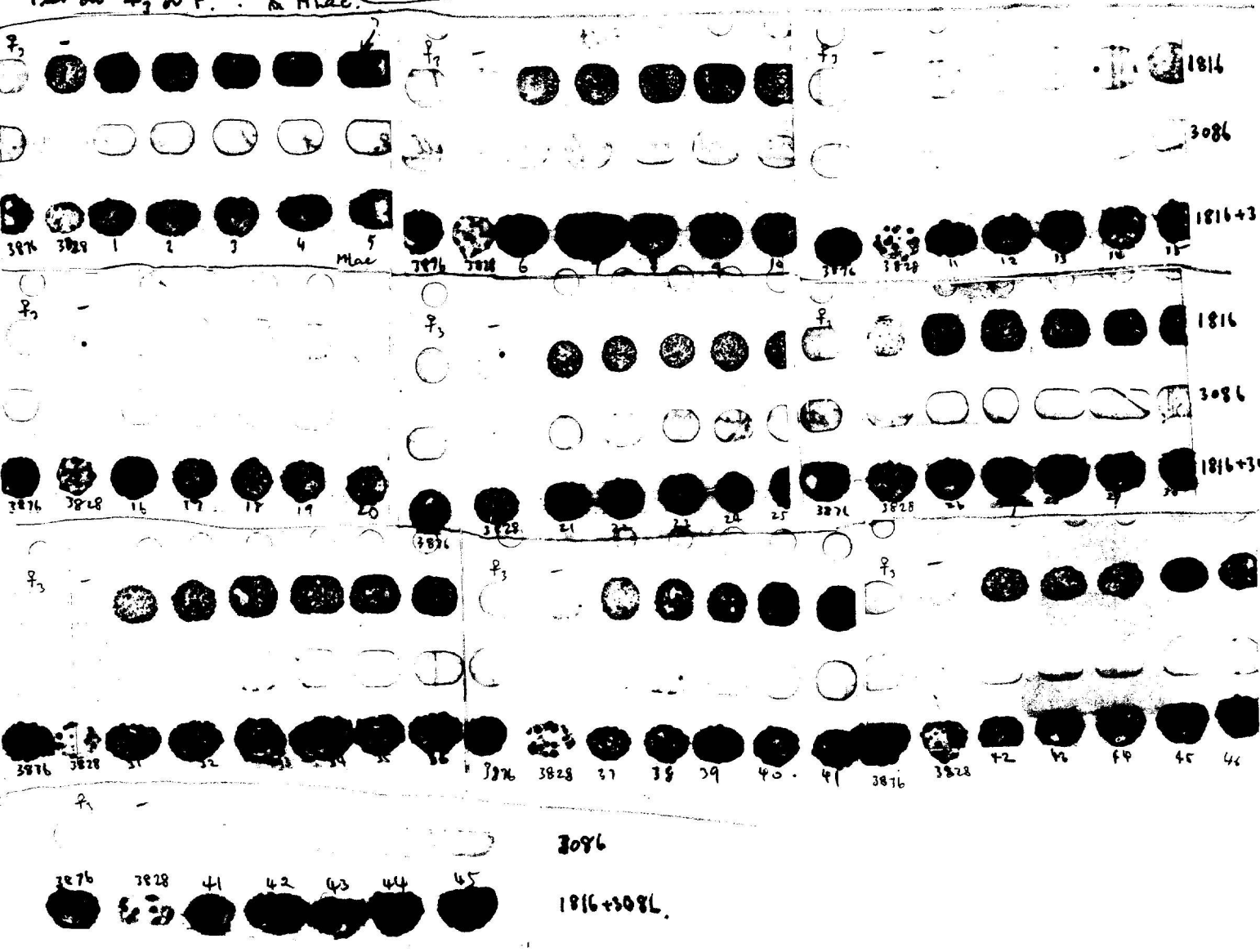
State I (Hi for Lac.) : 10

State II (Lo for Lac) : 32

♀ : 51

Test on $♀_3$ on F⁻ : on Mlac.

State I: 9	State II: 32	Mlac 3086 F⁻: 50



Cross $F_2 \times F_4$

with F_4 Hi for F_2

select Ara_1 and Ara_2 on M Ara.

20/4 – 23/4 1959.

REF:

1. Purify W6 F_2, W6 F_4, W3086. on B Lac.
2. Make overnight culture of them.
3. Add 0.2 ml of the culture into pen (5 ml) and incubate 4 hrs.
4. Mix W6 F_2, W6 F_4, W3086. and shake it for 2 hrs. at 37°C.

	1 ml	1 ml	0.1 ml.

4:30 ~ 6:30 PM.

① W6 F_2 × W6 F_4 → × 3086

② W6 F_2 → × 3086

③ W6 F_4 → × 3086

5. Seed it on B Lac Sm. and incubate them.
   dilution: $10^{-2} \times 10^{-2}$ 0.1 ml/plate.
6. Replica on the M Ara seeded W2979 and WK5-50 ($Ara_2 F^-$) [10^8 cells 0.3 ml/plate] ($Ara_1 F^-$)

Result: Fertility of W6 F_4 in transfer of Ara_1 is very low as Peter said; therefore it is very hard to tell is it F^+ or not, however, rate of infection under this condition was confirmed.

Control F_2 × W3086

total no. of colonies.	F_2^+ (%)
341	230 (67.5)
325	198 (61.0)
394	258 (65.5)
346	208 (60.2)
322	194 (60.3)
Σ	

Control F_4 × W3086

269	152.	(56.5)
155	89	(57.5)
260	132	(50.4)
323	182	(56.4)
304	189	(62.2)
Σ		

F_4 . F_2 → W3086

		41		L_0
444	77 (F_2)	(17.3)	54	(12.2)
304	72 (F_2)	(23.7)	47 F_4	(12.3)
341	66	(19.4)	37	(11.1)
239	45	(18.8)	36	(15.0)
305	48	(15.7)	37	(12.1)
Σ				

Effect of U.V. to infectivity of F'

23/11 — 25/11 ; 1959 REF:

Experimental conditions: 1. Purify all mutants on B Gal. before use.
2. Use young culture: 4hr at 37°C in water bath. (overnight culture 0.2ml / 5ml per. → incubate for 4hrs)

Principle:

W4534 (M⁻ Gal⁻ Sᵗ Fg)
W3637 (M⁻ Sᴿ Sᵗ F⁻)
X
W3104 (Gal Sᵗ F⁻)

Exp 1.

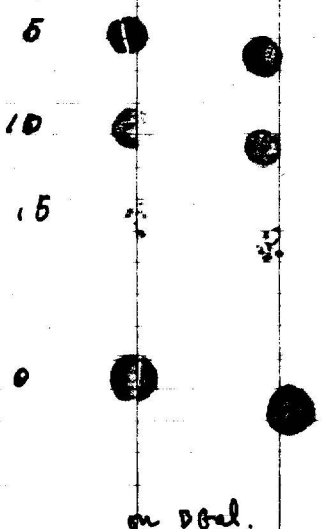
Time of irradiation:

	F_g infection	0.1 ml. Colony count. /plate
0	+++	—
5		—
10	+++	too much
15	+++	ca. 10^4 /plate

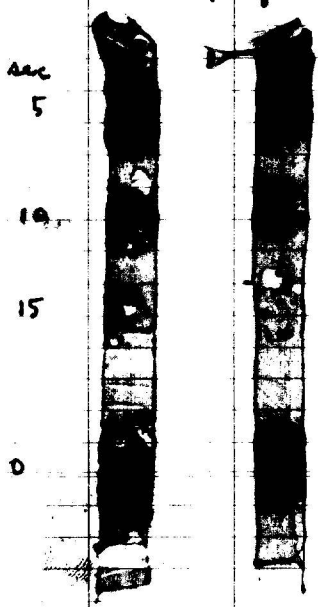
Method:
Survival of Fg. : 0.1ml W4534 UV + (1ml : W3637 / 1ml : W3104) → incubate it overnight. → spot on MGal.
survival of all: 0.1ml /plate.

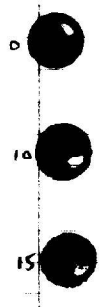
Exp 2. Qualitative test

Survival test.



Test for Infection.





Method: ① 1ml : W4534. : broth culture : ca. 5m diameter :
② U.V.-irradiated additionally.
③ Spotted on BGal., MGal.

4534 → (UV.) × W3104

Infection of F' by Killed cell.

Sm. EtoH, $CHCl_3$, CCl_4.

28/11 : 1959

REF:

Principle :

F_8 :
- 4534 — F_8 — M Gal_4 — $4p^s$
- 3637 — F^- — M — $4p^s$
- 3104 — F^- — Gal_4 — $4p^s$

F_4'' :
- ~~4528~~ F_4'' — F_4'' — A_1 Gal_6 — $4p^s$
- 3106 — F^- — Gal_6 — $4p^s$
- 2985 — F^- — A_1^- — 4^+

Method : ① Treat w4534, and w4528 F_4'' with various agents.

1 ml of F'-donor possess overnight grown culture

Agent used:
- one drop $CHCl_3$
- CCl_4
- 0.1 ml Sm (x100) (1 mg/ml : Sm) (usually use 0.1 ml (100 ml))
- 1 ml of Et OH (95%)

} keep it for 15 min. at room temp. 1 hr.

② Expel the treated agent by centrifugation or bubbling air.

$CHCl_3$ & CCl_4 is adsorbed into plastic Petri's dish, therefore it is easily removable by this procedure.

Et. OH is removed by centrifugation (once).

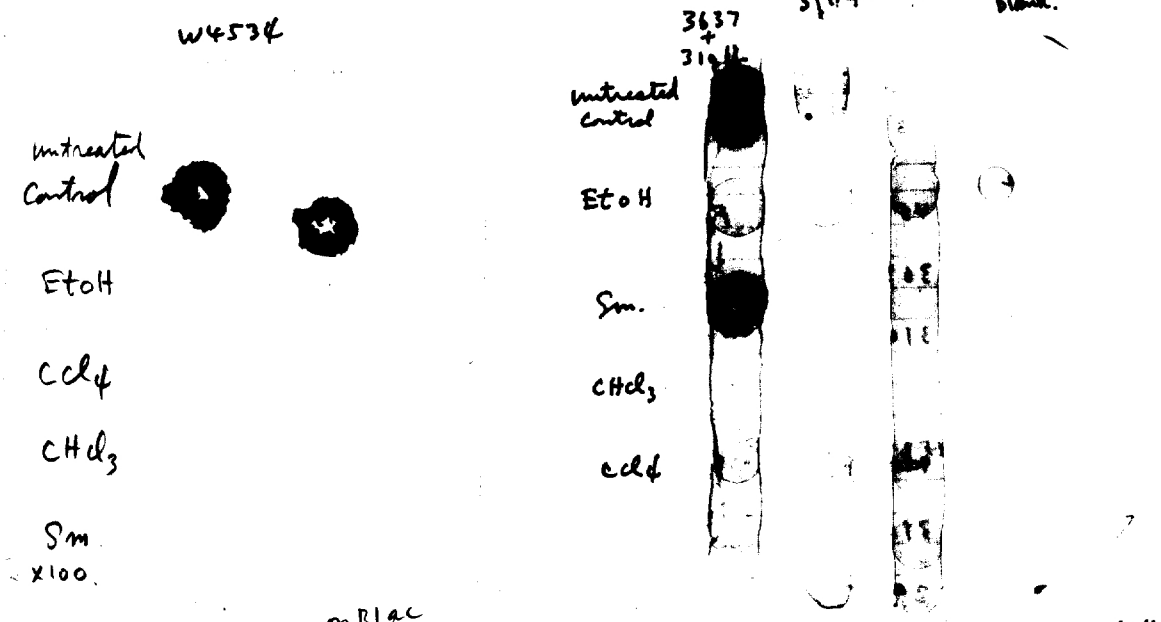
Sm is removed by centrifugation (twice).

Centrifuge → discard the supernate.
Add 5 ml pen.
↓
Centrifuge → discard the supernate
Add 1 ml pen. and suspend it.

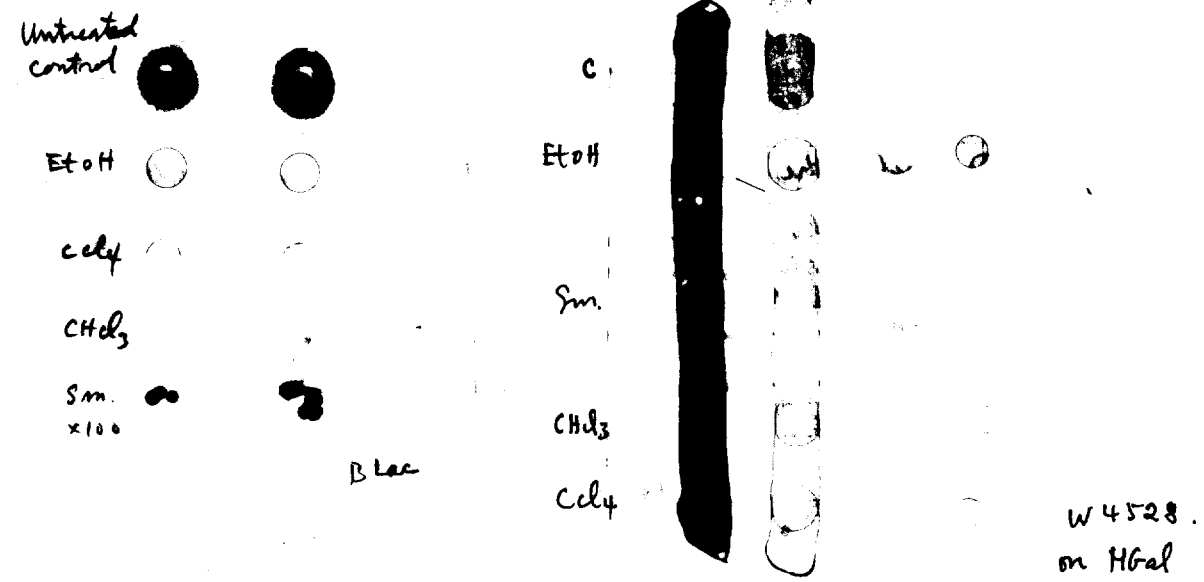
Result : Streptomycin looks good. Almost no decrease.

Next step: Check various concentration of Sm, and various time of treatment.

W4534 ($MGal_4Gb^5 F_8$)



W4528 ($A_1 Gal_6 F_4''$)



Confirmation : Infection of F_8 to F^- by Streptomycin-killed cell.
(24 hrs treatment) at 37°C.

28/11 ;1959 REF: 1mg/ml Sm.

Principle :

W4534 M Gal+ F_8 → F^+ * 5R Mal, 5R
W3086 M F^+ *
W3994 Gal+ F^-

Method : ① Add 0.2ml of Sm to 5ml overnight culture of W4534 (ca 10^8 cells/ml), 2ml. Plenary. 10 times conc.
Control : non-addition of Sm. 2ml.

② Incubate it overnight at 37°C.

③ Wash twice with broth. (2ml) 5min : centrifugation.

④ Spot it on MGal streaked W3086 + W3994 on it.

5ml overnight culture → 2ml | 2ml
Sm 0.2ml
Incubate them for overnight at 37°C.
Test their infectivity by spot-test.

Survival test by spotting on Blac.

Infectivity of F_8

W4534

treated by Sm (overnight) ×10 : 24hr

untreated

W6- {

on Blac.

Control. 3220 →
4534 untreated control →
4534 Treated with Sm. ×10 for usual use. 24hrs. →

3086 M | 3994 Gal+ | 3086 ≠ 3994 (on MGal)

Conclusion : Sm-killed cells still have some F^+ infecting ability to F^-, but it decreases into about < 1 ~ 0.1%.

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Transferring ability of F^{+} to infect F' to F^{-} after killing by Sm.

(Short time treatment:)

28/VI ~30/VI ; 1959

REF:

Method[1] 2 3 4 5 6 7 8 9 10

Conc. of Sm:

200U ×200 : 0.2 ml Sm sol'n. : ~~2 mg~~ / 1 ml

100U. ×100 : 0.1 ml Sm. soln. : 2 mg / 1 ml.

Time : ½ hr. & 2 hr.

½ ; 3:15 ~ 3:30

2 : 3:15 ~ 5:15

System :

W3994 F^{-} Gal_6 S^R) × $\frac{F_8}{}$ W4534 F

$W3^{+}_{086}$ F^{-} M S^R

W 2985 F^{-} A_1^{-}) × $\frac{F}{}$ W4576

$W3^{+}_{106}$ F^{-} Gal_6

Method :

1. Add Sm 200U. & 100U. /ml. separately.
2. Incubate them for ½ hr. & 2 hr.

Survival test : 0.1 ml / plate : after washing : Sm = sample as used as F donor.

W4728(F_6^{+})

	100U-30 min,	200U—30 min.	100U ···· 2hr.	200U ··· 2hr.
1	3	0	0	0
2	0	0	0	0
W4534				
4	0	0	1	0
5	0	0	0	0

Infectivity test.

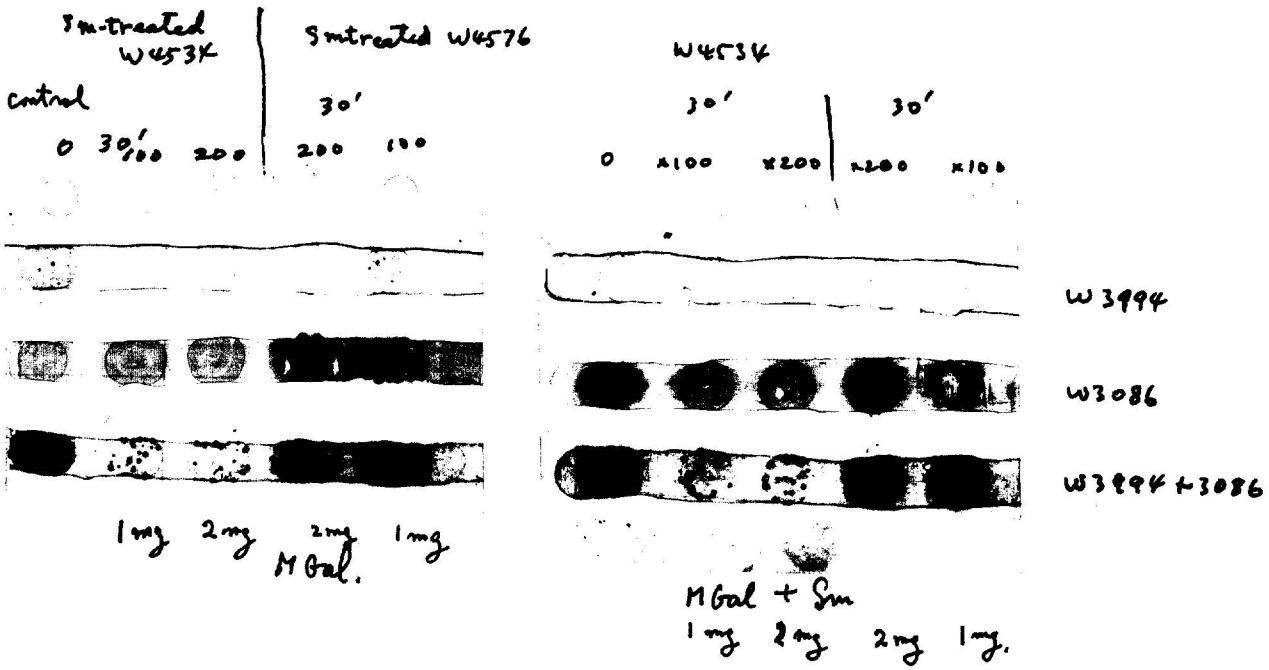
W4728 F_6^{+}

	2hr		30'		Untreated control	W4534 2hr					
	×200	×100	200	100	0	Untreated control	100	200	Untreated Control	100	200
Blank	1										
2985	3										
	4					3994					
3106	6										
	7					3086					
2985 + 3106	8										
						3994 + 3086					

on MGal.

on MGal

on MGal Sm


Sm-treated W4573K
Sm treated W4576
control
30'
0 30' 200 200 100
W4573K
30'
30'
0 x100 x200 x200 x100
W3994
W3086
W3994+3086
1mg 2mg 2mg 1mg
MGal.
MGal + Sm
1mg 2mg 2mg 1mg.