1. Lp locus is not on F_8 segment. P.1.

2. P_1 transduction of gal-Hfr_1 P2, P3

3. Isolation of F_{13} sisters. P4

4. Isolation of F_8 sisters P.5 P.5' P.6a P7 P6b.

Lac_X^- F_{13}. 5. Isolation of F_{13} Lac_{52} (m: ONPG+) : W4112. P8, P9,

W 4847 6. Isolation of F_{13} Lac_{87} (m: ONPG-) : W 4147 P.10a P10b.

7. Iso

8. complementation reaction of various Lac^- F^- strains with Lac_{52}^- F_{13}. P.9-a

9. Transduction of Lac_{52} to Lac^+ strain (3086) 4112 F_{13} → 3086.

Reversion test for homozygosity of Lac_{52}. : reversion test

10. Preparation of λ ref. B120. P.11

11. Elimination of F_8 from 3350 F_8 1^-2^-/ex++ F_8 with AO : p12a ~ p12c

12 Timing experiment of F_{13} P13a.

P13b. : Pm^+ F^-

P14 P18a~d d: F^-

13. phosphatase$^-$ P15a~d

14. Size of transducible marker of F_8 P16

Ind 4 & Auth.

15. Transduction of F_8, F_{11} by λ P17

16 Infectivity of F from F_{13} : Separate transfer of F from F_{13}

Test a possibility of location of $4p^+$ on F_8 segment.

4/14 1960

REF:

Experimental design:

4520 $(4p^+)$ ⟶ × 3110 $(4p^s)$

1. pick F_{13}^+ by replica plating it on MGalSm × 4573.
2. Test $4p^+$ by cross-linking with λ & 3110. on B-O.

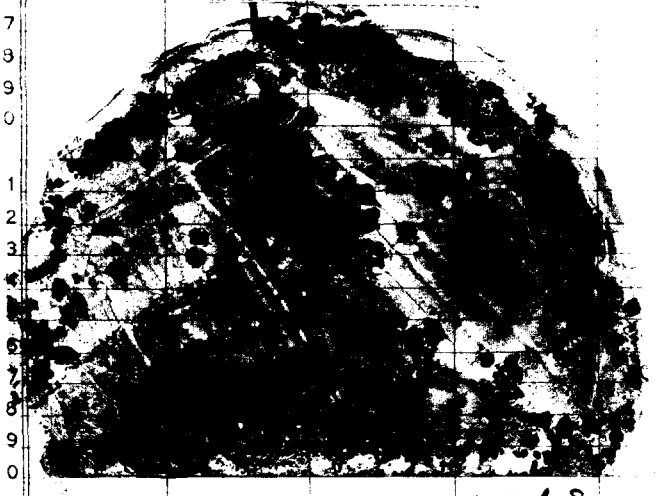
Result:

All the isolated F_8^+ show (20 isolates) $4p^s$ trait.
Sensitive to λ & does not produce R.
(see back page).

Conclusion:

F_8 segment <u>does not carries 4p locus</u> on it.

Replicated plate.



on MGal Sm
× 4573

Master plate.

4520 — × 3110 on DO

Unsuccessful.

U.W.
MICROBIAL
GENETICS

2

Transduction of Hfr, from 2252 (Lp^s Hfr, M$^-$) to Gal$_x^-$ F

21/VII 1960 purpose: Does p_1 transduce Hfr, ? And Does it give F'

If F': H1 for Gal
If Hfr: H1 for Lac

If F$^+$, it can infect F$^-$ to F$^-$ by cell c
(see below)

1) Test Hfr, & F'ness.

plate No.	Transductional event	# of transductants per plate on M Gal	Colonies which give Recombination reaction. on M Lac Sm × 4573.	on M Gal Sm × 4573
1	2252 → × 3107 Gal$_7$	69	0	0
2		72	0	0
3	2252 → × 3102 Gal$_2$	33	0	0
4		49	0	0
5	2252 → × 3104 Gal$_4$	45	0	0
6		40	0	0
7	2252 → × 3101 Gal$_1$	50	0	0
8		52	0	0

2) Test F$^+$ transfer.

Volume of lysate : 0.1 ml Ca. 10^7 p_1/ml : final.
" " 4573 : 0.5 ml Ca. 10^7/ml : "

Mix them in 2 ml L-media + $CaCl_2$ (final, 0.005 M) to adsorb p_I to 4573. and keep it at 37°C for 3 hrs. And then add 10 ml of L-broth to
3:35 ~ 6:30 at 37°C
Shake it on rotator f overnight.

° After overnight shaking, test sex-compatibility of whole culture by cross-brushing, and purify it on BLac & test sex-compatibility of 20 colonies of the isolates
× 4574
on M Lac.
. cross-brushing method on M Lac. (× 4574)

Cf. : # of transduction of S^R marker to 3110 :
on M Gal Sm : 7, 19.

Procedure : Make transduction of Gal$^+$: replicate it on M Gal Sm or M Lac Sm
are seeded with 4573, and see mating reaction on the plate

Make P_1 lysate on 2252 M Hfr, Lp^s.

16/III 1960 REF:

order of addition: original 10^8 → diluted into NB 1/10

1. 3 P_1 (1×10^8 /ml.) : Use 0.05 ml, (5×10^6) : grown on
& 0.01 ml (1×10^6) : "
1 2252 : Use 1 ml. : Ca. 10^8 suspended into NB.
2 $CaCl_2$: 0.5M : 0.01 ml / 1 ml of broth culture.

↓ Keep the mix at 37°C for 20' 1:35 ~ 1:55.
(Let P_1 adsorb. to W2252)

2. Add 0.5 ml of the mix to 2 ml of soft L-agar + $CaCl_2$ 0.5M : 0.01 m.
↓ Make double layer on L-agar;

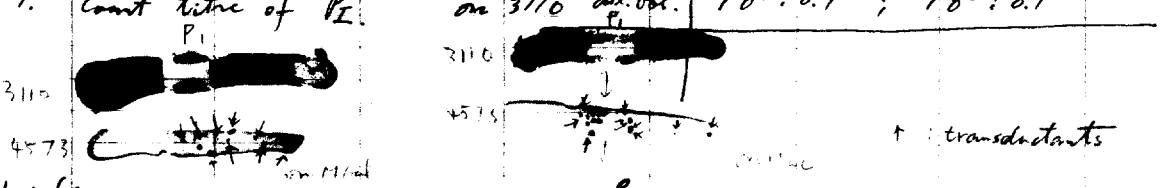
3. Incubate it for 8 hrs. : at 37°C. 2:00 ~ 10:00. PM.
plates made: 1 : 0.05 ml; 2 : 0.01 ml.

4. Add 3 ml of NB to each plate and extract P_1 with soft agar. add to 10 ml Blank & Homogenize it with pipette. Keep it ~~at [illegible]~~ (in refrigerator to extract P_1.

5. Take supernate, ~~and~~ centrifuge, and chloroform.

6. Save it in vials.

7. Count titre of P_I. on 3110 dil. vol. | 10^4 : 0.1 ; 10^6 : 0.1



P_1
3110 [redacted]
4573 [redacted]

P_1
3110 [redacted]
4573 [redacted]

↑ : transductants

2nd trial: Pensacy grown,
0.1 ml of P_1 lysate (from 2252) + 0.5 ml of 2252 + 2 ml of soft-L agar
Conc. of $CaCl_2$: 0.005M : final. conc.
↓ pore on L-agar and incubate it at 37°C for 8 hrs. ; 1:30 ~ 9:30.

C.f. L-mix shows pH. 6.4. :- Add 1 M NaOH : 0.4 ml / 10 L-
to make pH 7.0.

Isolation of brother F'_{13}s from F'_{13}.+ with UV.

29/4 ; 1960

Lac — Pur V_6 — Lac REF

Principle:

① Irradiate W4637 F_{13}^+ : F^- Pur^- V_6^S Lac^{85-} / Pur^+ V_6^R F_{13}

② Look for Pur^- ♂. Blac.
by replicating on / Expectation: Elimination of Pur^+ segm
Exogenote F_{13}.
DO, and MLacSm seeded 4573.

③ Test size of new F_{13} brother: V_6 resistance. | If it is V_6^S Homogenote: R/S
V_6^R " : R/R
Heterogenote: R
Progeny × V_6^R

Design:

Ratio of mix: 1:1
optimal age; overnight

1. Make W4637 F_{13}.
   a) by infection. 3747 → 4637.
   Streak on ~~Blac~~ Mac, pick 5.
   b) purify it on ~~Blac DO~~ B Lac
   Test X^+ on 9:3. Save ~~Lac~~ pur^+.
   c) Test malenes by cross × F^- ~~using plication~~. 4573 on M

Survival: Too much.
irradiation: 10 sec
Inoculum size: ca. 10^8~10^7.
Repeat 1 ~~exponential~~ mult: 0.2 ml / plate.
Increase 3 U.V.-Dose
Irradiation: 15 sec, 20 sec
survival:

Let it form colony on Blac,
2. Irradiate it with U.V. and replicate it :-
   a) onto DO ~~+ Blac~~ + BLac.
   b) onto MLac Sm seeded 4573.
   save X^- Lac^- or X^- Lac^+ or F^- Lac^+
3. Infect it the next F_{13} brother to 4574, and test mar
transduced to F^- (esp. V_6) Test malenes too, by replicating onto ML
seeded F^- M^- (W4354)

Experiment:

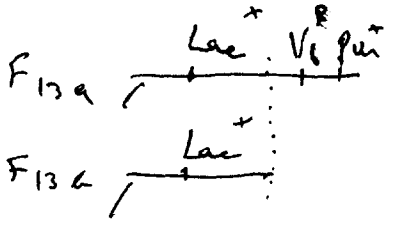
~~Alt~~ Almost of X^- Lac^+ a
Lac v.

	Lac^+ X^-	# of Lac^- X^-	Lac^+ / plate EMB.	♂ Lac^+	F^-	Pur^-	Pur^+	V_6^R	V_6^S
	1	0	75						
	0	2	64						
	0	0	52						
	0	1	106						
Σ	1	3	297						

29 X^- Lac^+ colonies are picked from UV'ed plate (Blac), and tested their L
and their malenes replicating on T_6 plate, DO, DO + Pur B, and MLac × 457.
Save V_6^S Lac v F_{13}^+

Further experiment: ① Make timing experiment, and check time for
transfer of Lac^+

Expected trait of brother from $F_{13,b}$

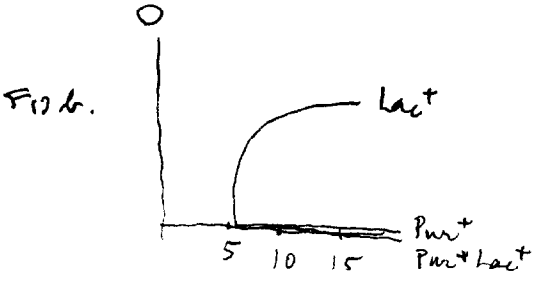


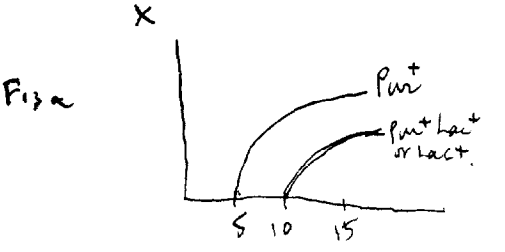
1. V_6 Transfer.

$\longrightarrow\!\!\times$ V_6^S : S ∵ hemiz

$-\!\!\times$ V_6^R : R.

2. Timing.





Isolation of sisters F_8 from F_8^+.

29/11 ; 1960 REF:

Principle : ① 4520 → W3350 : 3350 F_8 $1^-2^-/++$

② UV 3350 F_8 — Look for gal^- of. + + ⊖ x0.

③ Save such strain and test transfer of other gal^- ma

If it is $F_8'^+$ exconjugants, it is 1^- homo or 1^- hemi. $1^-/2^-$ $1^-/2^-$

" " " F_8^+ sisters, it has deletion in it. 1 or 2

Method to differentiate between two. Cross it on Mgal and look for Rec^- reaction in cross. x several gal^- F^-.

F^-	1^-	S^R
F^-	2^-	S^R
F^-	3^-	S^R
.	4^-	.
.	5^-	.
.	6^-	.
.	7^-	.
.	8^-	.

Experiment :

1. Use overnight culture of 3350 F_8.
2. Irradiate with UV. for 10 sec. /plate : survival : too much. gal^- colonies are picked and inoculated on Bgal. ~10^3~10^4.
3. Replicate on $F^-1^-S^R$ + $F^-2^-S^R$. Look for Rec^+ F'.

Result :

a. 104 gal^- colonies are isolated on Bgal, but 36 out of 104 are mix+ + + - . (gal^- : 68), probably the isolates were gal v.

b.

			# of isolates
gal^-	No. of gal^- which gives recombination reaction in cross x	gal_2 (4579) on MgalSm.	2 ($F_{8a}^{\bullet}$
	" " " "	x gal_1 (3991) " " "	0
	" " " "	x gal_4 (3994) " " "	0
	# of gal^- colonies which does not gives any rec. reaction		66

Further work : (Test transducibility of newly isolated gal to other gal^-_x. Make 4354 F_8a, 4354 F_8b. and repeat (for example : 3, 5, 6, ... F_{8a} + same cross on MgalSm. + on M

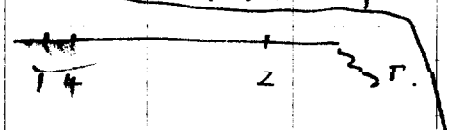
Tentative speculation from this result :

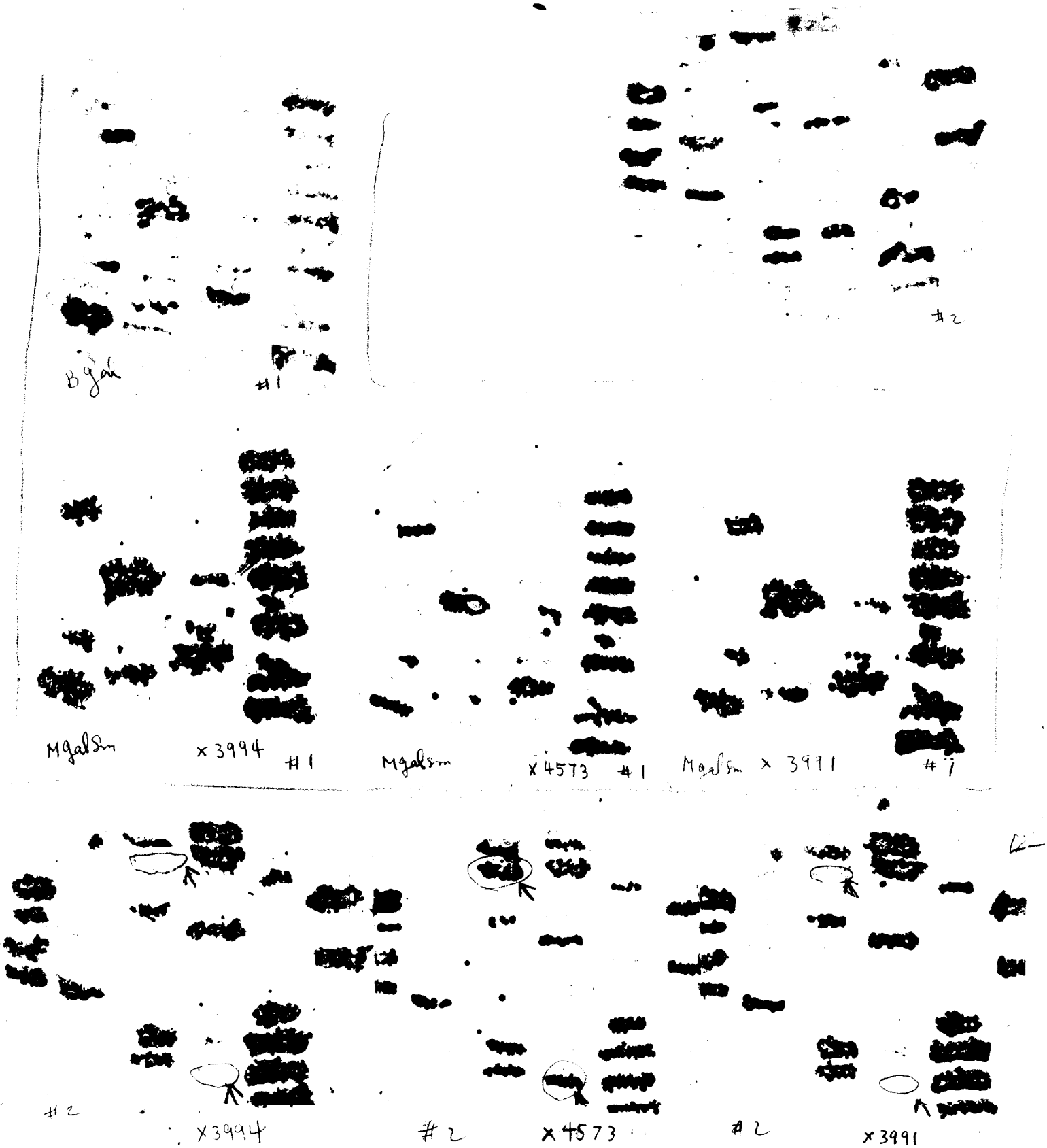
1. gal_2 is linked with $F_a^{\bullet}$ and not closely linked with gal_1 and

~~2. gal_1 and~~

2. gal_1 and gal_4 is probably linked and is other end of gal_2

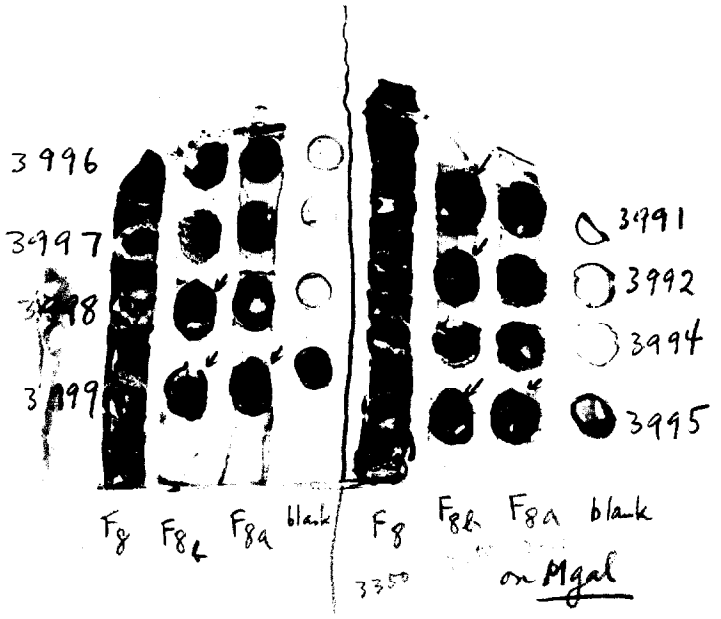
3. In Almost of this case, whole gal-segment will be eliminated from F_8^+ after U.V.-irradiation or by spontaneous loss of the segment from heterozygous diploid stage.

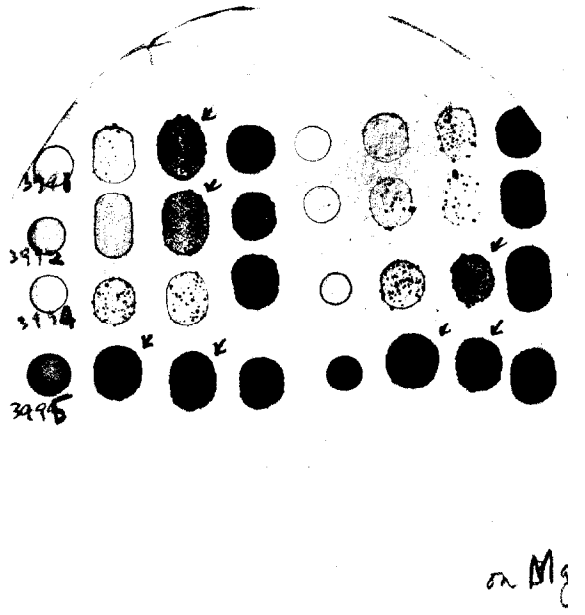



B gal
#1
#2
Mgalsm
x3994
#1
Mgalsm
x4573
#1
Mgalsm
x3991
#1
#2
x3994
#2
x4573
#2
x3991

29/IV; '60

5'





Conclusion: parallelism can be seen in both plates, but less (complementation recombination) rea on Mgal Sm than Mgal; probably Sm killed some F' male and make less F'.

∴ Use Mgal for this complementation experiment. or use excess F' S^s for th purpose.

8 9 5 2 1 , 4 6 7

+ −

F_{8a} —— x 4354

9/1960 F_{8b} —— x 4354 REF: c.f.

purpose : To get standard F_8 sister+ strains.

Exptl. condition :
cultural age : overnight culture in 5 ml penassy broth.
Infection :
0.1 ml F_{8a} or F_{8b} + 0.1 ml 4354 + 5 ml penassy b
↓
Incubate the mix at 37°c for overnight.
↓
Streakiton B gal, pick gal+ and ~~[illegible]~~
Test sex-compatibility on M gal x 3995.
or x ~~[illegible]~~ 3999.
↓
Save a colony which shows male reaction.

Strains used :

3350 F_{8a} and 3350 F_{8b}.

$1^- 2^-$/ex F_{8a} $1^- 2^-$/ex F_{8b}.

Result : (1) Infection

F_{8a} —— x 4354		F_{8b} —— x 4354	
# of colonies tested	♂ (F_{8a}^+) obtained	# of colonies tested	♂ (F_{8b}^+)
14	13 (save one)	15	15 (sa

relatively low : 1 (save one)
(probably this has shorter segment)

See back page. #1

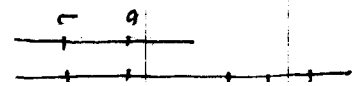
(2). complementation. (on M gal. x $F^- S^R$ gal$^-$ ~ 9)

		1	2	4	5	6	7	8	
	W4354 (M^-) F_{8a}^+	−	−	−	+	−	−	−	+
	W4354 (M^-) F_{8b}^+	+	+	−	+	−	−	+	+
control	W 4520 ($M^- F_8^+$)	+	+	+	+	+	+	+	+
	4354 F_8 c+	−	−	−	+	−	−	−	+

(colony obtained from F_{8a} infection
See back page. #1)

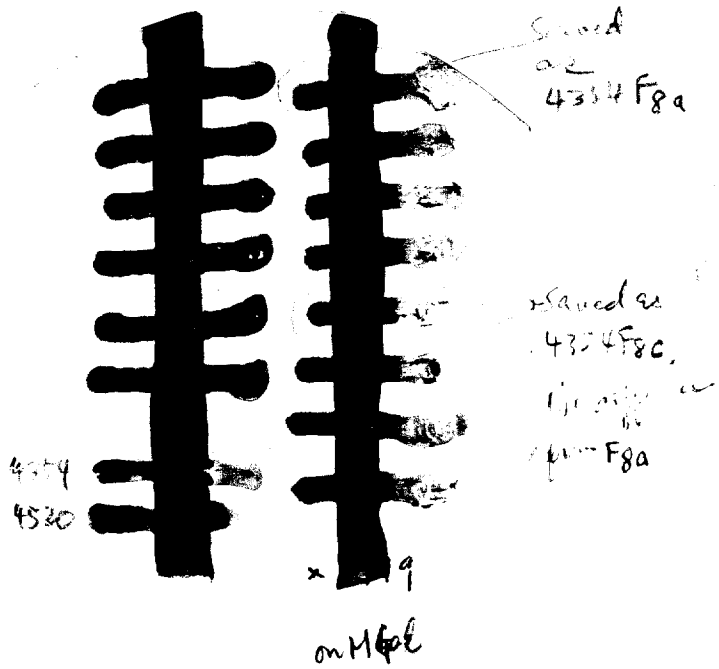
Conclusion : 3 groups can be classified.

5 9

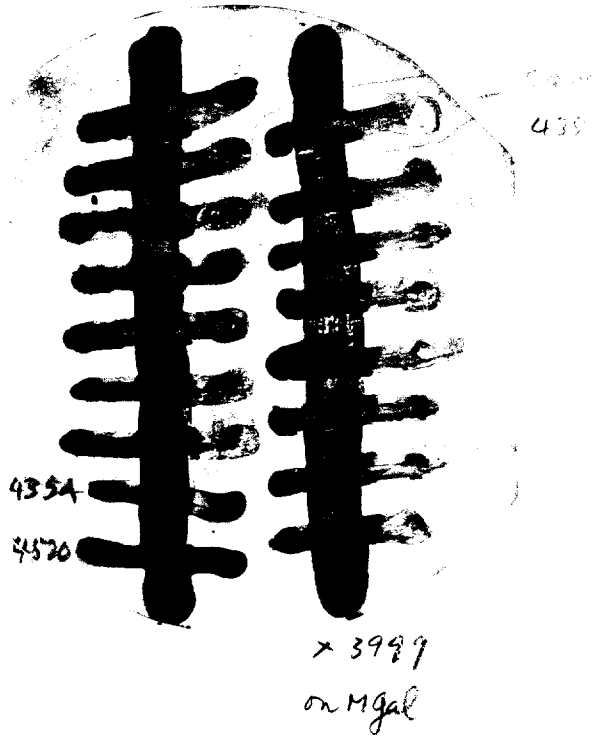


1

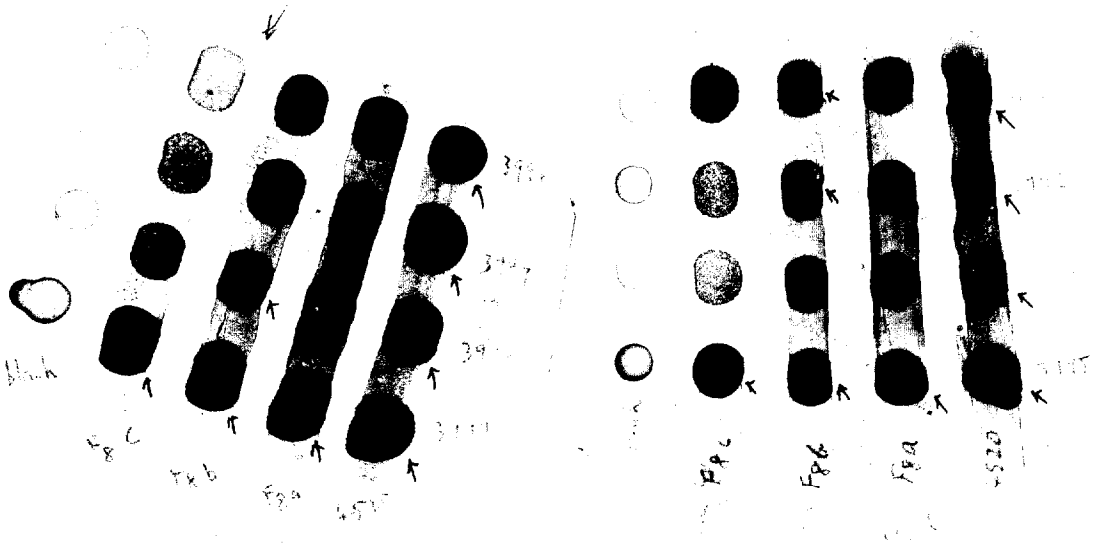
3350 F8a → x 4354



3350 F8b. → x 4354



2



Compare this with p.5

Retest on deletion of F_8^* ; F_{8a} ; F_{8b} & F_8 (control). 6b

Sex-duction of gal-segment by F_{8a}, F_{8b}, and F_8

1/VI ; 1960 REF:

purpose : To find transducible marker of gal* by F_{8a}, F_{8b}, F_8.

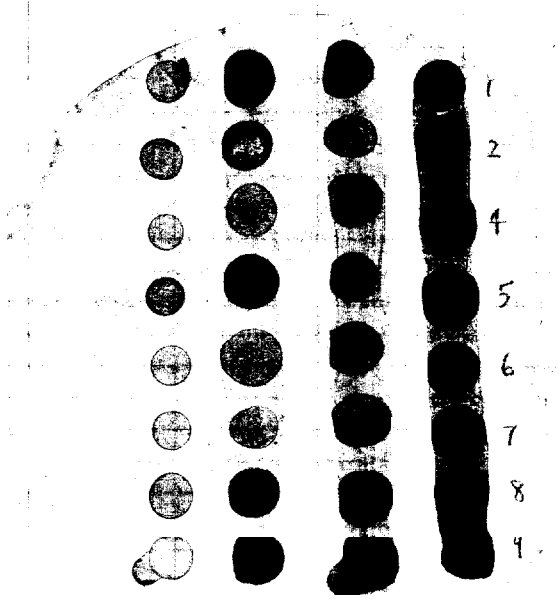
Exp I : Mix F_8 donor (2 drops) + gal$^-$ λ^- S^R (2 drops) + Sal

Incubate the mix for overnight.

Streak the mix on Bgal Sm.

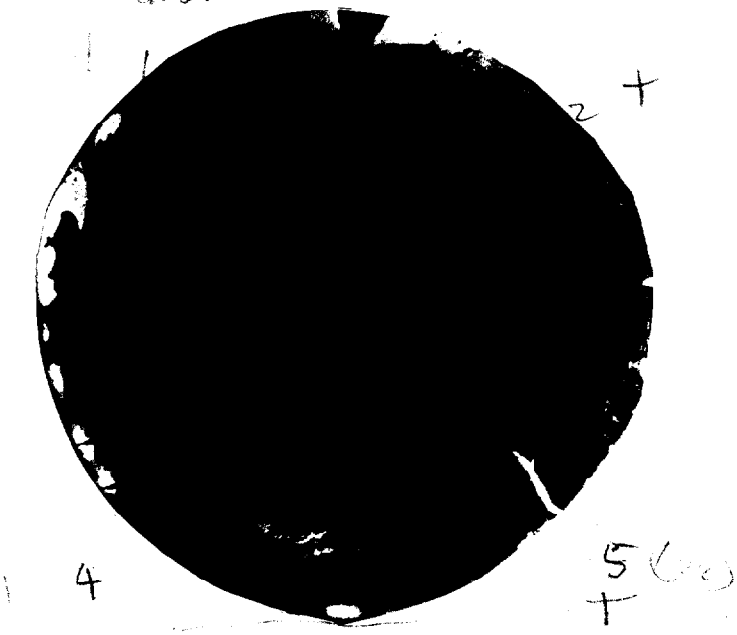
Result				
F_8 Donor	cont 4520	4354 F_{8a}	4354 F_{8b}	
3991	+		+	
3992	+		+	See back page
3994	+		−	
3995	+	+	+	
3996	+		−	
3997	+	−	−	
3998	+	−	+	
3999	+	+	+	
		− +	− +	
		1,2,4,6,7,8 2,2,9	4,6,7 1,2,22,8,9	

Exp II : Make spot test on Mgal & Mgal Sm.



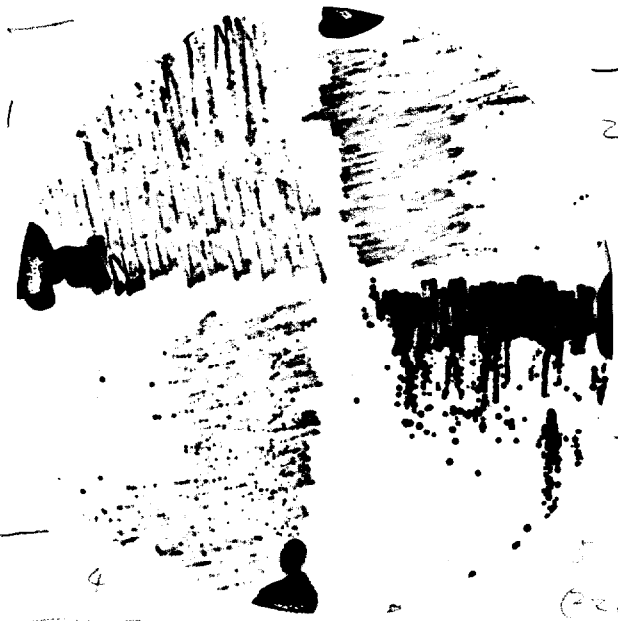
on Mg

Cont 4520-X



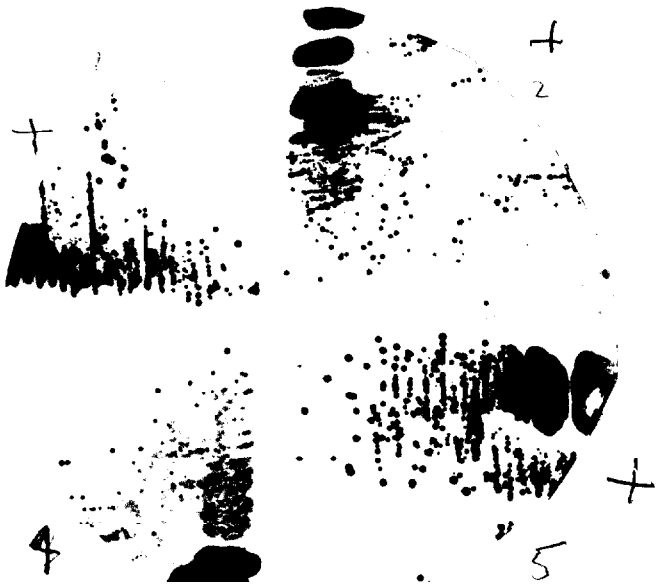
4520-X

4354 F8a



4354 F8a (type)

4354 F8b



4354 F8b X

2nd isolation of gal-F_8 sisters.

; 1960 REF:

Strains used : 3350 F_8

Time of UV.: 15 sec.
on B gal.

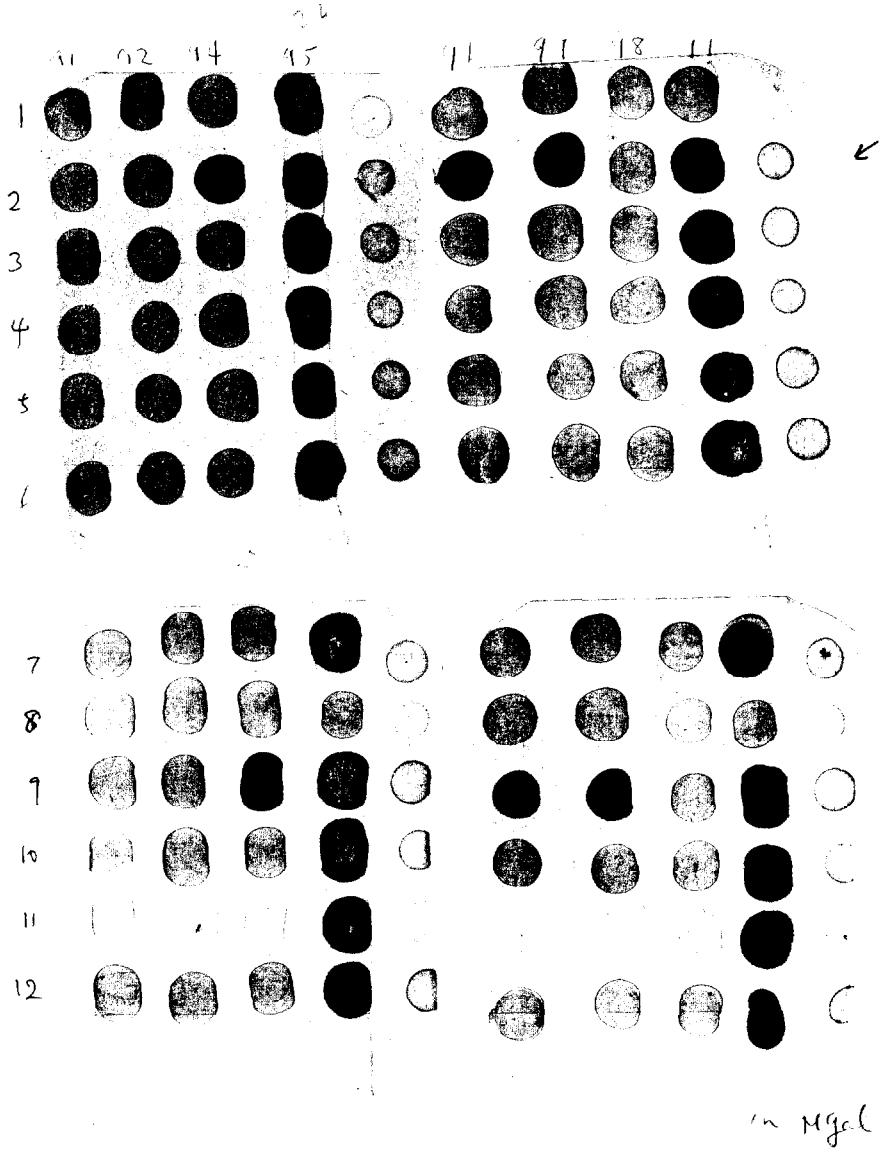
① Survival :

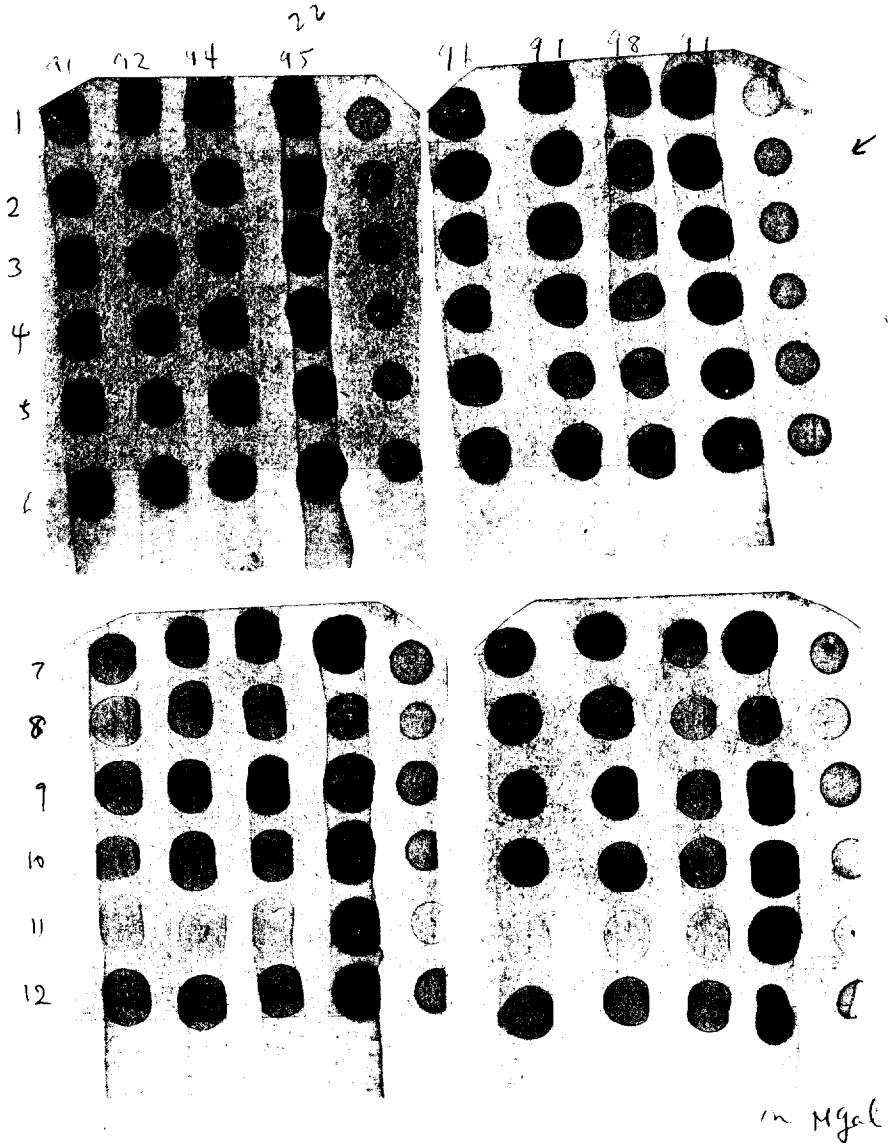
Plate No.	Gal⁻	Gal v	Gal⁺
1	15	5	141
2	19	7	164
3	3	3	66
4	4	2	87

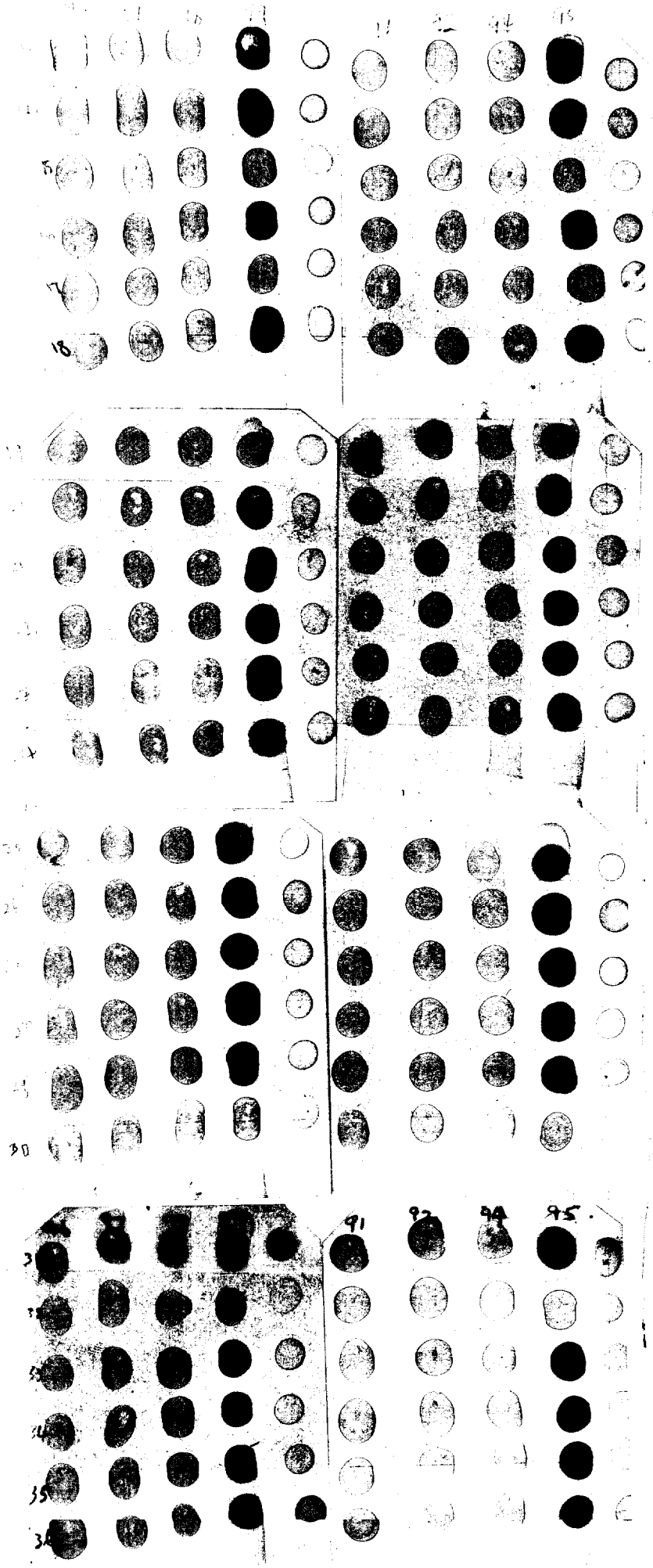
② Inoculated on B Gal. a) Replicate it on M Gal Sm. × F^- gal, gal gals.
b) Look for Rec.⁺ cells.

55 ~~43~~ gal⁻ were isolated from the UV'ed plates, and tested on their compatibility by cross × gal, ~ 9. on M gal.

Maleness	F_8 type	Number of F_8
	Covers gal	
♂	5, 9	44.
♂	5, 9, 4, 7, 6	2
♀.	No (F^-)	9
	Σ	55


3350 F8C
a —
deviation
b 1 2 8
c 1 2 4 6 7 8
d 4 6 7
in Mgal


22
91 92 94 95
91 91 98 94
1
2
3
4
5
6
7
8
9
10
11
12
3350 F8C
a —
deletion
b 1 2 8
c 1 2 4 6 7 8
d 4 6 7
+
4,2
in Mgal



Isolation of F' Lac^-_x from F^- Lac_x.

5/V : 1960 REF:

Strains used

Strain.	mutation	ONPG	F_{13} donor 3747 $MLac^+ F_{13}$. V_6^R.
○ W4112	52 m	+	
W4113	53 m	+	
○ W4147	87 m	−	
W4151	91 m	−	

Principle :

1. Isolate $^-_x/_{ex}$ Lac^+. by 3747 → X. {Ratio 0.1 ml : 0.1 : 5 ml ; overnight 37°c streak on MLac. Select purify it on BLac : ~~[illegible]~~.
2. pick one Lac^-_x along after streak from each ~~of~~ Lac_V colonies. Streak it on BLac
3. Test sex-compatibility by cross.

Experiment :

A. Infection of F_{13} {
1. Mix 0.1 ml F^- Lac^-, 0.1 ml F_{13}^+ Lac^+, 5 ml of penassy broth
2. Incubate it for overnight at 37°c.
3. Streak it on MLac to select ~~[illegible]~~ Lac^+. (save all 4 $/_{ex}$ Lac^+
4. Streak it on complete, and make sure its purity.

B. Isolation of Lac^-_x F_{13}. {
1) Streak the transductant on BLac.
2) pick Lac_V and restreak it on BLac again.
3) pick Lac^- segregant and test male-ness.

W4864

Isolation of Lac⁻ F_{13}.

12/V ; 1960 (4112 F_{13}). REF: W4112 Lac_{52} : m ; ONPG
W4151 Lac_{91} : m : ONPG

Continued from. (p.8 5/V '60)

History : 3747 → 4112 : 4112 F_{13} (Lac_{52}/+ F_{13})
Streaked on BGal : picked one colony and restreaked on B
Lac⁻ segregants were tested on their sex-compatibility by c
x 4151 on MBlac plate (I) The other 5 colonies are streaked
again.

Experiment:

(1). 30 Lac⁻ colonies from 4112 Lac_{52}/+ F_{13} were picked and tested the sex. (x 4151 on MLac).

Lac^-_{52} tested	Lac^- F_{13}	Lac^- F^-	Lac^- F^+
30	27	3	0

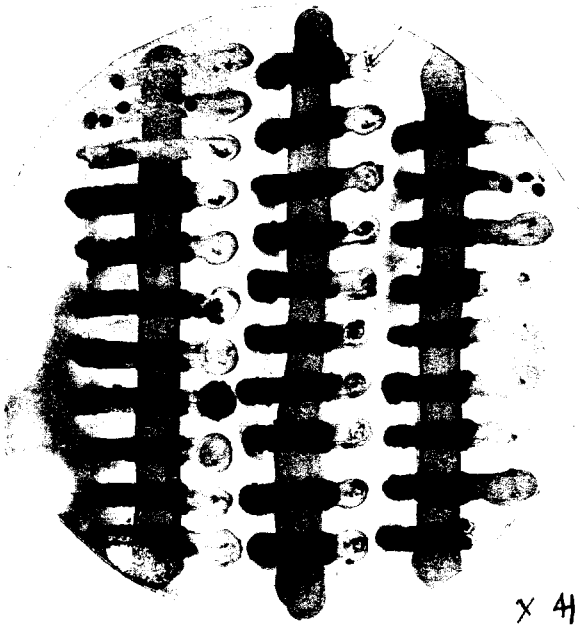
(2) 5 Lac v colonies are streaked ~~and~~ on BLac, and tested their sex. (replica plated on MLac seeded with 4151).

	Lac^-_{52} tested (Segregants)	Lac^- F_{13}	Lac^- F^-	Lac^- F^+
Colony 1	56	56	0	0
Colony 2.	22	22	0	0
Colony 3	50	50	0	0
Colony 4	67	67	0	0
Colony 5.	93	93	0	0
Colony 6	3	1	0	2

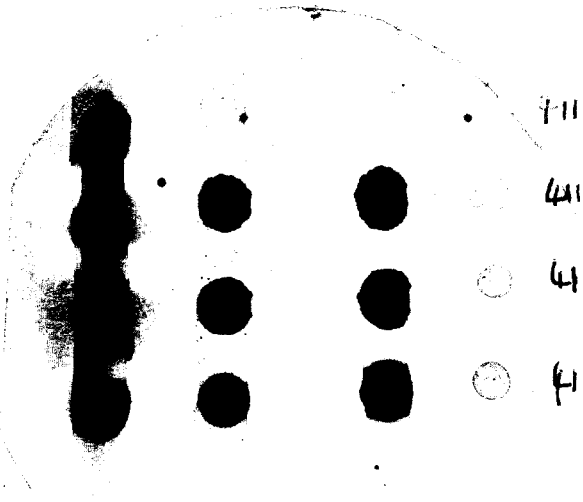
(3) Test H_1 for Purine, T_6 r or s or T_6 v.

The newly isolated Lac_{52} F_{13} is T_6 [redacted]. See back pa
may not be V_6 var. maybe
range mutant
(4) Test complementation + Lac_{52}.
x 4112, x 4113, x 4141, x 4151. T_6.

Save this as 4112 F_{13}



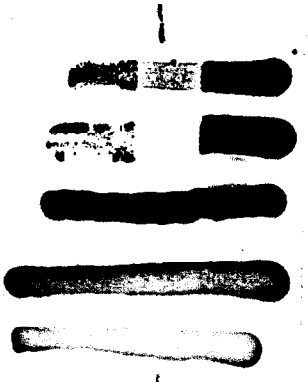
x 4151
on M Lac.



x 3747 x 4112F_{13} x 4112F_{13} blank
on M Lac.

Lac
4112 5
4113 5
4147 8
4151 9

3747 r
4112F_{13} ~~s~~ r
3110 s
T_6
on B Lac



3110 s
W6 s
3747 r
4112F_{13} r
4112. r
on B Lac

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MICROBIAL
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W4864 9-a

Complementation test using newly isolated $Lac_{52}F_{13}$.

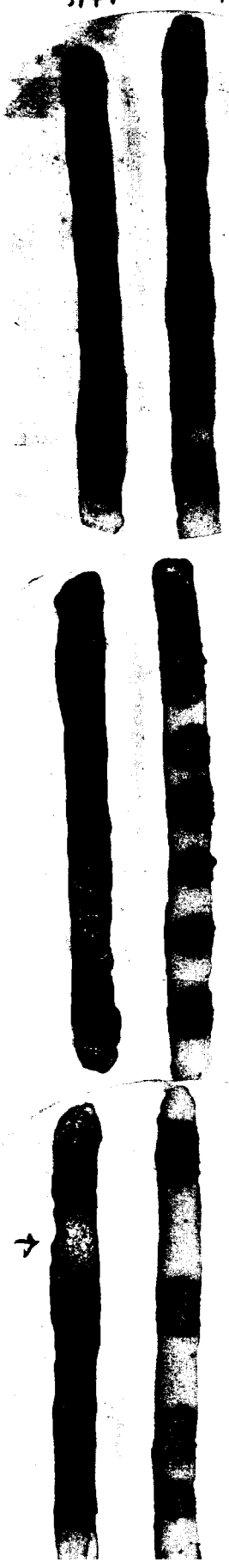
16/V :1960 REF:

• purpose: Look for Lac⁻ marker wh give complementation with Lac
• See back page. Test on MLac.

Type	ONPG	Strains tested. Lac⁻ mutation.		Strain No. F⁻	complementation reaction. (on MLac)		after streak BLac
m	−	△	22	3147			
m	−	△	25	3151	+++		Streak Lac⁺ spot on B and confirm it is reco reactions c.f. 9-b.
m	−	△	41	4101	+++		
m	−	△	87	4147	+++		
m	−	△	90	4150	+++		
m	−	○	93	4153	+++		
m	−	○	89	4149	+++		
m	−	△	88	4148	+++		
m	−	△	91	4151	+++		
m	+	×	52	4112.	−	Same mutation.	See p.9.
~~f:m~~							
f:m	−	○	39	4287	+++		
m	+	×	17	3152	++ Rec.		
m	+	×	18	3174	+ ~~[illegible]~~ few ← 3747 does not give complementation reac with this!		
m	+	×	19	3156	++		
m	+	×	20	4286	+		
m	+	×	21	3153	++ Rec. Ca.100		
m	+	×	53	4113	++ Rec. Ca.100		
m	+	×	55	4115	++ Rec. ..		
m	+	×	56	4116	++ Rec. ..		
m	+	×	71	4131	++		
~~m~~ f	−	×	72	4132	− No Lac. ← ~~Why? Ann's Lac 52 [illegible] with 73.~~		

△ contained in extream f-type but not in Lac_{11D3} or 39

× Contained in Lac_{11D3}.

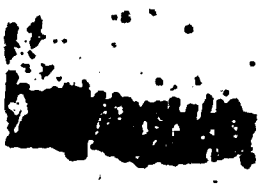


(+) 3747 | (m+) (52) 4112F13 on MLac

Strain No.	Lac	ONPG	
4101	41	m −	Contained in extreme f-type but not in Lac11D3 or 39.
4148	88	m −	
3151	25	m −	
4147	87	m −	
4153	93	m −	Contained in 39.
4149	89	m −	
4287	39	m −	Contains (23, 26, 60, 62, 95, 68, 89, 93, 97)
4286	20	m +	Contained in 11D3.
4131	71	m +	
3156	19	m +	
3152	17	m +	
4150	90	m −	Contained in extreme f-type but not in 11D3 or 39.
4113	53	m +	Contained in 11D3.
4113	53	m +	
3174	18	m +	① Ann's thesis says: 18 contai[illegible] but there is some recombination. ② No recombination in x 3747. probably, there may be some [illegible] for mating reaction of this strain
4115	55	m +	
4132	72	f −	
4116	56	m +	Contained in 11D3.
3153	21	m +	

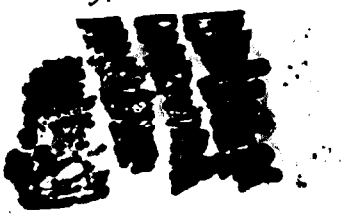
[illegible]

4101



4131

3153

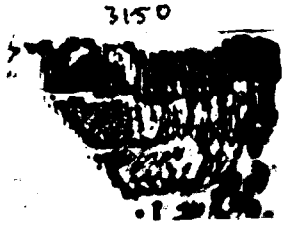


4148

3156

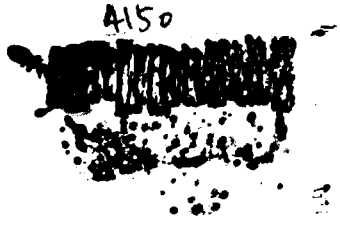
3151

3150

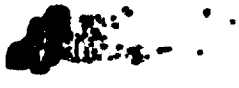


4147

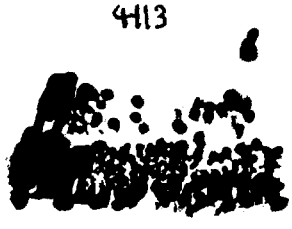
4150



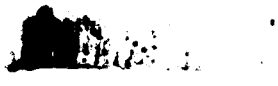
4153



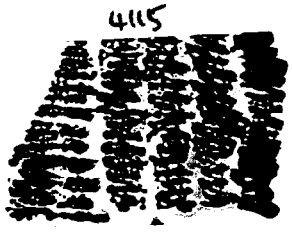
4113



4149

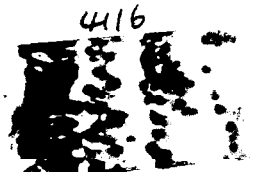


4115



4286

4116



streaked on Blac
from Lac+ spot

: conclusion:

These Lac+ spots on
are real genetic exchan
Lac locus, not synt

U.W. MICROBIAL GENETICS

Test on homozygosity of Lac_{52} W4864

9c

Test transfer of Lac_{52} to $Lac^+ F^- S^R$.
3086

19/V. ; 1960 REF: 9a, b, d.

- purpose of this experiment: To test whether Lac_{52} homozygous or homizygous for Lac_{52} locus.

- Principle: If Lac_{52} is homozygous, there should be incorpo and segregation of Lac_{52}^-, after infection of Lac_{52}^- to
If this ratio of segregation is ca 10^{-2}, it is possi to observe Lac^- segregant with this method

F_{13} Lac_{52} 0.2 ml $\longrightarrow$ F^- Lac^+ S^R 0.1 ml. +5 ml per. (W3086) F^- M Mal, S^R purified on B Lac^+ colony w picked.

for overnight culture in penassy. excess F_{13} F_{52}.

↓

Seed the mix on B Lac Sm.

cultural age: overnight grown culture of F_{13} Lac_{52} & F^- Lac^+
Seed 10^3: 0.1 ml / plate EMB Lac Sm.

- Result.

plate No.	Lac^+ (lighter) + (prob. Lac V)		Lac V	Lac^- (presumably it is segregant)	F_{13}
1	87	(9)	2	0	7
2	97	(17)	3	1	8
3	75	(15)	3	0	10
4	59	(7)	4	1	7
5	49	(2)	1	0	8
6	59	(7)	4	1	6
7	75	(15)	3	0	5
8	97	(17)	3	1	3
9	97	(7)	2	0	4
10	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Further Experiment

① streak each La and confirm real Lac V.

② Test Lac^- segre
a Test mal-
b. Test mele ca
c. Test Lac,

③ Test F_{13} ness
How much % of cells will become
(Rate of Inf. of F_{13}.
on Ml
x L
(W

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continued from former page

3086 ×——— W4864 9d

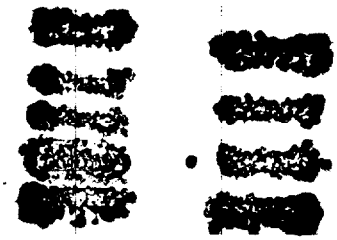
Test Lac⁻ character transduced by 4112 F_{13}.(Lac$_{52}$;

26/V ; 1960 New F_{13} M Mal$_1$ Sm Lac$_{52}$ (4866) REF: 9a, b, c.

1. Test Mal$_1^-$ (∵ 3086 is Mal$_1^-$) on BMal agar.

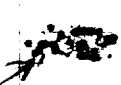
Result. all 5 are Mal$_1^-$.

[redacted]



2. Test Lac⁻ marker.
Show the Lac⁻ is Lac$_{52}$ ~~but~~ and not others.
Replicate it on 4112 + 4151

This marker is Lac$_{52}$ and all male.
∵ 1) fertile in cross × F⁻Lac$_{91}$
2) Sterile in cross × F⁻Lac$_{52}$.
3) Sterile in cross × F_{13}^+ Lac$_{52}$
on BLac agar

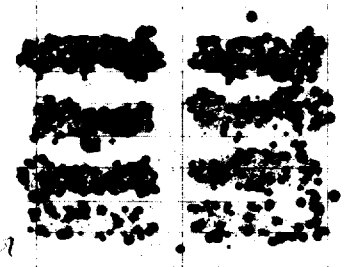


3 Test Hfr-ness of the transfer of the other markers.

gal⁺ H1 : Gal
H1 : Mtl.
Med : Ara$_2$
Lo : Lac.

reversion

Conclusion : fertility pattern is ~~as~~ same as Hfr$_{13}$.

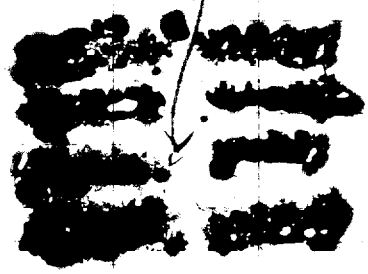


4. Gal⁺ recombinants does not segregate Gal⁻ after streak the epets on BGal Sm.

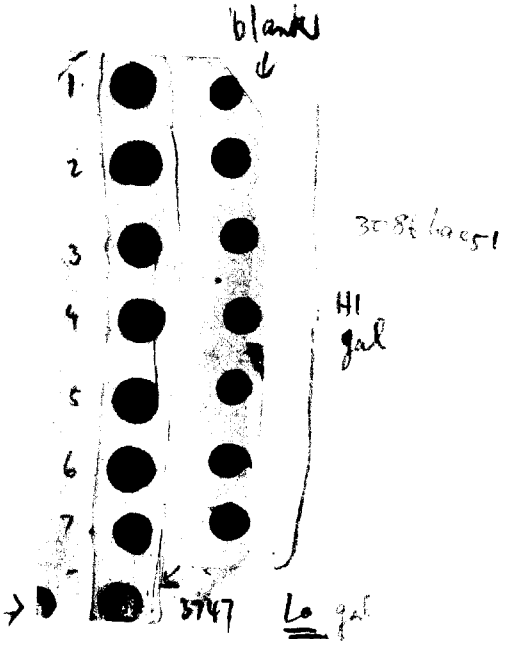
[redacted]

× 411

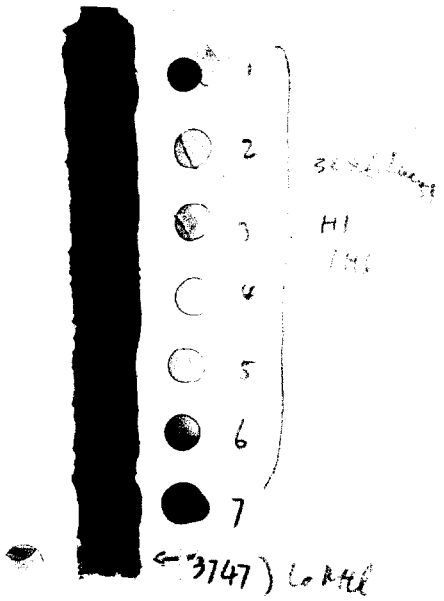
[redacted]



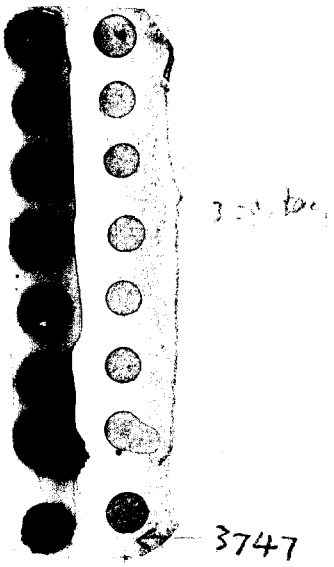
× 4151



×4573 (gal2⁻)
on M gal



×4573 (Mtl)
on M Mtl



×4573 (Ara2⁻)
on M Ara

Test homozygosity of Lac_{52} of $4112F_{13}$ by reversion

31/v 1960

REF: Cf. See transduction Lac_{52} to Lac^+ 308

Principle : Test homo or hemi-zygosity of $4112F_{13}$ by reversion to

If it segregate : homo :

" " does not " : hemi. :

① Method:

pick one reversion from separate colony.

31/v

1. Purify $4112F_{13}$ on BLac.
2. Pick 30 colonies into ~~penassy broth (1 ml)~~ onto EMB Lac. and let it g for ~~overnight~~ 48 hrs
3. ~~Spot those colonies on BLac again and incubate it for 48 hrs.~~
4. pick one Lac^+ from each spots & Purify Lac^+ revertants on BLac. confirm purity. the L
5. Let each colony grow up in 5 ml penassy and Streak each revertants on BLac, and see segregat Lac_{52}. Look for LacV., and restreak it on BLac again.
6. Test Lac^- type of segregants from the diploids.

Result:

Isolation No	Lac^-	LacV	seg		Lac^-	LacV	seg		Lac^-	LacV	seg
1		V		9		V		17		no	
2		no		10		no ? V		18		V	
3		V		11		no		19		no	
4		no		12		V		20		V	
5		no		13		V		21		no	
6		no		14		V		22		V	
7		V		15		no		23		V	
8		V		16		V		24		[illegible]	

② Test Lac^+ transfer. Test fertility for transfer of Lac_{52} of Lac^+ reve

cross x $4112S^R$ on MLac Sm. Use spot test.

perpose : Is Lac^+ reversion on exogenotic segment or endog

If it is in Exogenote H1 ; If it is in endogenote ho :

V : 13 (9)

[illegible] 10

21 [illegible] 12 [illegible] 2

22 [illegible] 13 [illegible] 3

23 [illegible] 14 [illegible] 4

15 5

3747 [illegible] 16 [illegible] 6

$4112F_{13}$ 17 7

$4112S^R$ 8 [illegible]

17 9 [illegible]

Isolation of Lac F_{13} (4147 F_{13})

12/6 ; 1960 ONPG - M

W4147 : Lac_{87} F^-
W4112 : Lac_{52} F^- ONPG

1. One 4147 F_{13} colony was streaked on BLac from BGal.

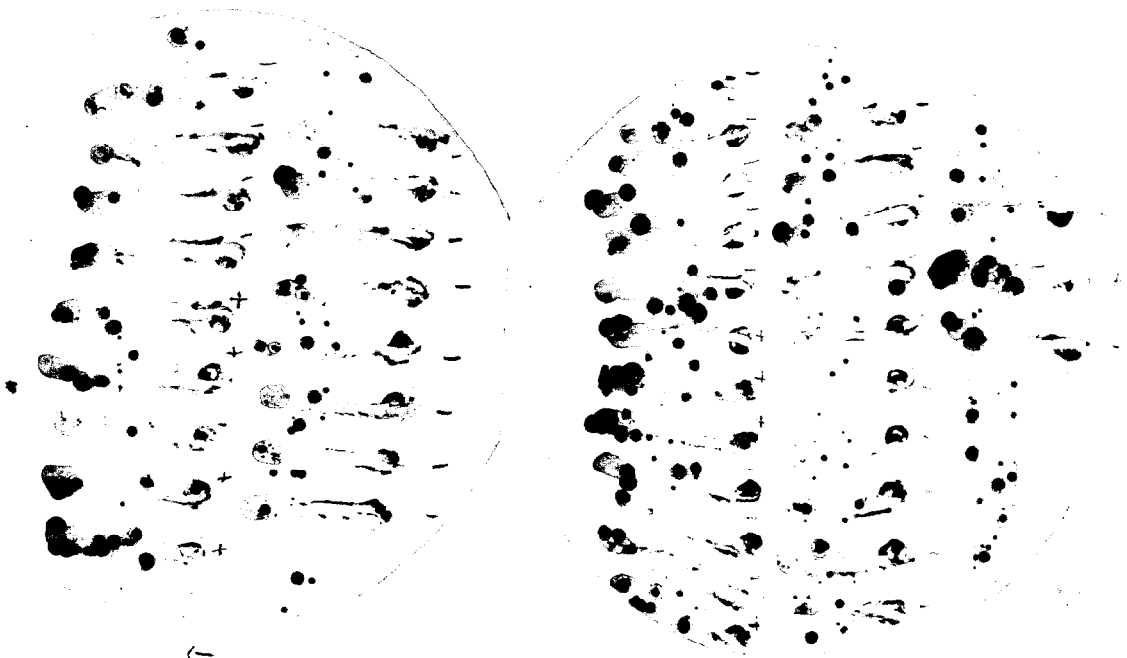
Exp I. 4 Lac^- segregants were tested on their sex.
× W4112.
(Lac52 F-

2. 5 Lac_V colonies were streaked on BLac agar, and Lac^- segregants were tested on their sex-compatibility.

Col. #	# of col. tested	Lac^-_{87} F_{13}	Lac^-_{87} F^+	Lac^-_{87} F^-
1	5	1 (relatively low) Test infectivity.	4	0
2	12	0	4	8
3	10	0	10	0
4	3	0	1	2
5	17	0	4	13

Conclusion:

F13 X4147 (lac87)



5 1

+ 4 ; − 13

2 3 4

+4, −8 + 0, −10 +1 ; −2

X4112 (lac[illegible])

Isolation of Lac^-_{87} F_{13}

12/v 1960 REF: c.f. P. 8

continued from (p. 8, p10 5/v/60)

History: 1. 3747 → x 4147 (F^- X^+ Lac^-_{87})

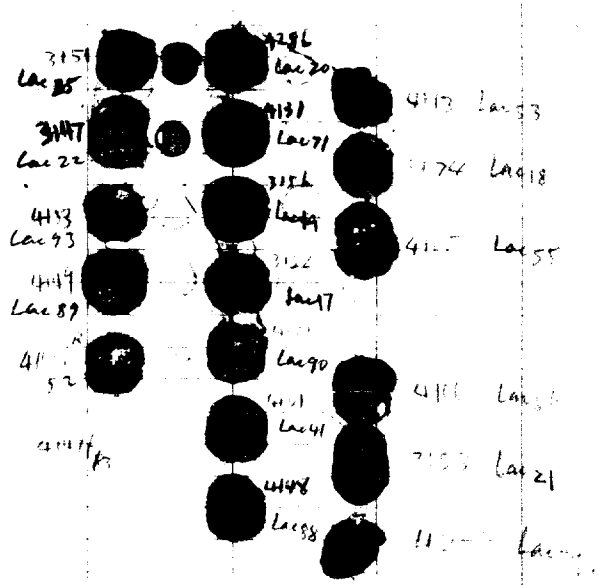
2. Pick Lac_{87}/ex+

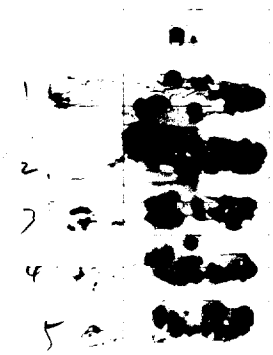
3. Streak it + test fertility of Lac^- segregants f it.
x W4112 on MLac.

6 colonies are tested on their compatibility.
1 was F_{13}^+.
F

4. Test complementality by spot test on MLac. ~~x 4151 (41) F~~

4147 F_{13} Lac 87 F_{13}





x 4112 (lac 52) F^- on MLac

Strong complementation with Lac_{87}^- F_{13}.

Lac 20	#	#	+
Lac 19		Lac 53	Lac 18
Lac 17		Lac 55	
Lac 21		Lac 56	