

- 1.  $\lambda$  locus is not on  $F_8$  segment. P. 1.
- 2.  $P_1$  transduction of  $gal-Hfr_1$  P 2, p 3
- 3. Isolation of  $F_{13}$  sisters. P 4
- 4. Isolation of  $F_8$  sisters P. 5 P. 5' P. 6<sup>a</sup> P 7  
P 6<sup>b</sup>.
- Lac<sup>-</sup>  $F_{13}$ . 5. Isolation of  $F_{13} lac 52$  ( $m: onpG+$ ) : W 4112. P 8, P 9,
- w 9. 6. Isolation of  $F_{13} lac 87$  ( $m: onpG-$ ) : W 4147 P. 10 a P 10 b.
- 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~~  $4112 F_{13} \rightarrow 3086$ .  
Reversion test for homozygosity of  $Lac 52$ . : reversion test
- 10. Preparation of  $\lambda$  ref. B120. P. 11
- 11. Elimination of  $F_8$  from  $3350 F_8 1^- 2^- / ex++ F_8$  with  $\Delta O$  : p12a ~ p12c  
P13a.
- 12. Timing experiment of  $F_8$  P13b. :  $P_{int}^+ F^-$   
P14 P18 and  
d:  $F^-$
- 13. phosphatase<sup>-</sup> P15 and
- 14. Size of transducible marker of  $F_8$  P16  
Ind 4 & Anth.
- 15. Transduction of  $F_8, F_{11}$  by  $\lambda$  P 17
- 16. Infectivity of  $F$  from  $F_{13}$  : Separate transfer of  $F$  from  $F_{13}$

Test a possibility of location of  $\lambda_p^+$  on F<sub>8</sub> segment.

4/11 1960

REF:

1 2 3 4 5 6 7 8 9

Experimental design:

4520  $\rightarrow$  X 3110 ( $\lambda_p^+$ )  
( $\lambda_p^+$ )

1. pick F<sub>13</sub><sup>+</sup> by replica plating it on M6GalSm x 4573.
2. Test  $\lambda_p^+$  by cross-brushing with  $\lambda$  + 3110 on B-D.

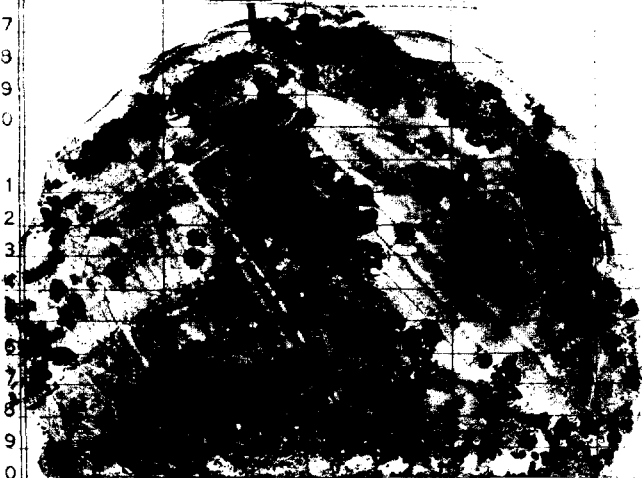
Result:

All the isolated F<sub>8</sub><sup>+</sup> show (20 isolates)  $\lambda_p^+$  trait.  
Sensitive to  $\lambda$  + does not produce  $\lambda$ .  
(see back page).

Conclusion:

F<sub>8</sub> segment does not carries  $\lambda_p$  locus on it.

Replicated plate.



on M6Gal Sm  
x 4573

Master plate.



4520 - x 3110 on D0

1  
2  
3  
4  
5  
6  
7  
8  
9  
0  
1  
2  
3  
4  
5  
6  
7  
8  
9  
0  
1  
2  
3  
4  
5  
6  
7  
8  
9  
0  
1  
2  
3  
4  
5  
6  
7

Unsuccessful.

Transduction of Hfr, from 2252 (Lp<sup>S</sup> Hfr, M<sup>-</sup>) to Gal<sup>-</sup>F<sup>+</sup>

21/11/1968

purpose: Does p<sub>1</sub> transduce Hfr? RE And Does it give F<sub>1</sub>?  
 If Hfr: Hfr or Lac  
 If F<sub>1</sub>: Hfr for Gal<sub>6</sub>  
 If F<sub>1</sub> can infect to F<sup>-</sup> by allel (see below)

1) Test Hfr, or F<sup>-</sup> need.

plate No.	Transductional event	# of transductants per plate on M Gal	Colonies which give Recombination reaction on M Lac Sm x 4573.	on M Gal Sm x 4573	
1					
2					
3					
4					
5					
6					
7	1	2252 x 3107	69	0	0
8	2	Gal <sub>7</sub>	72	0	0
9					
0					
1	3	2252 x 3102	33	0	0
2	4	Gal <sub>2</sub>	49	0	0
3					
4					
5	5	2252 x 3104	45	0	0
6	6	Gal <sub>4</sub>	40	0	0
7					
8	7	2252 <del>3103</del> x 3101	50	0	0
9	8	Gal <sub>1</sub>	52	0	0
0					

2) Test F<sup>+</sup> transfor.

Volume of lysate: 0.1 ml. ca. 10<sup>8</sup>/ml : final.  
 " " 4573: 0.5 ml. ca. 10<sup>7</sup>/ml : "

Mix them in 2 ml L-media + CaCl<sub>2</sub> (final, 0.005 M) to adsorb p<sub>1</sub> 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 for overnight.

After overnight shaking, test sex-compatibility of whole culture by cross-brushing, and purify it on B Lac & test sex-compatibility of 20 colonies of the isolates on M Lac.

cross-brushing method on M Lac. (x 4574)

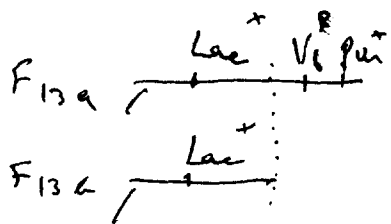
Cf.: # of transduction of S<sup>R</sup> marker to 3110 :  
 on M Gal Sm : 7, 19.

Procedure: Make transduction of Gal<sup>+</sup>: replicate it on M Gal Sm or M Lac Sm & one seeded with 4573, and see mating reaction on the plate





Expected trait of broth for F13.b

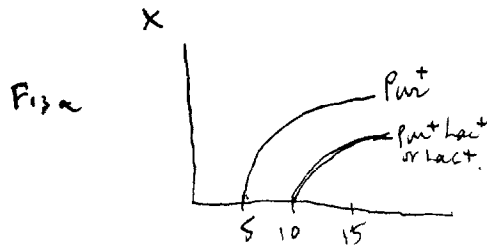
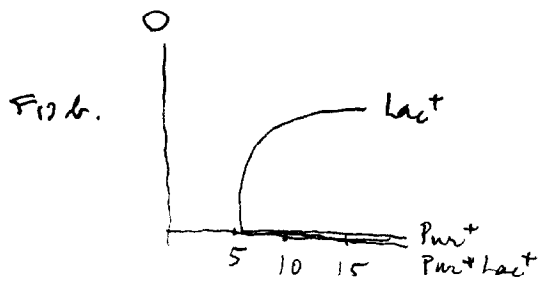


1.  $V_6$  Transfer.

$\rightarrow \times V_6^S : S$   
 $\rightarrow \times V_6^R : R.$

$\therefore$  hemige

2. Timing.



# Isolation of sisters $F_8$ from $F_8^+$

29/11/1968

REF:

- Principle:
- ① 4520  $\times$  W3350 : 3350  $F_8$   
 $\begin{array}{c} 1^- 2^- \\ \hline 1^+ 2^+ \end{array}$
  - ② UV 3350  $F_8$  — Look for  $gal^-$  ♂.  $\begin{array}{c} + + \\ \hline + + \end{array}$  Oxo.
  - ③ Same such strain and test transfer of other  $gal^-$  ma.  
 If it is  $F_8^+$  conjugate, it is  $1^-$  homo or  $1^-$  hemi.  
 " " "  $F_8^+$  sisters, it has deletion in it.  $1^-$  or  $2^-$ .

Method to differentiate between two. Cross it on Hgal

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

and look for Rec<sup>-</sup> reaction in cross.  $\times$  several  $gal^- F^-$ .

## 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.  $\sim 10^3 \sim 10^4$ .
3. Replicate on  $F^- 1^- S^R$  +  $F^- 2^- S^R$ . Look for Rec<sup>+</sup>  $F^-$ .

## Result:

a. 104  $gal^-$  colonies are isolated on Bgal, but 36 of out of 104 are mix<sup>+</sup>.  
 + 4 - (  $gal^-$  :  $\frac{36}{68}$  ), probably the isolates were  $gal^+$ .

$gal^-$	No. of $gal^-$ reaction in cross $\times$ $gal_2^-$ (4577) on Hgal Sm.	# of isolates
	$\times$ $gal_2^-$ (3991) " " "	2 ( $F_8^+$ )
	$\times$ $gal_4^-$ (3994) " " "	0
		0

# of  $gal^-$  colonies which does not gives any rec. reaction 66

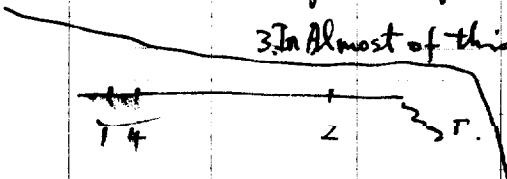
Further work : (Test transducibility of newly isolated  $gal^-$  to other  $gal^-$ .  
 Make 4354  $F_8^+$  a, 4354  $F_8^+$  b. adjacent (for example: 3, 5, 6, ... n. F  
 Tentative speculation from this result:  $\left. \begin{array}{l} \text{homologous} \\ \text{on Hgal Sm. + on M.} \end{array} \right\}$

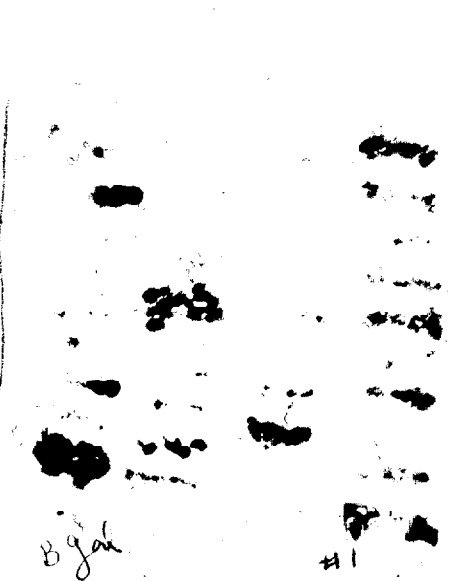
1.  $gal_2^-$  is linked with  $F_8^+$  and not closely linked with  $gal_1^-$  and

~~2.  $gal_1^-$  and~~

2.  $gal_1^-$  and  $gal_4^-$  is probably linked and is other end of  $gal_1^-$

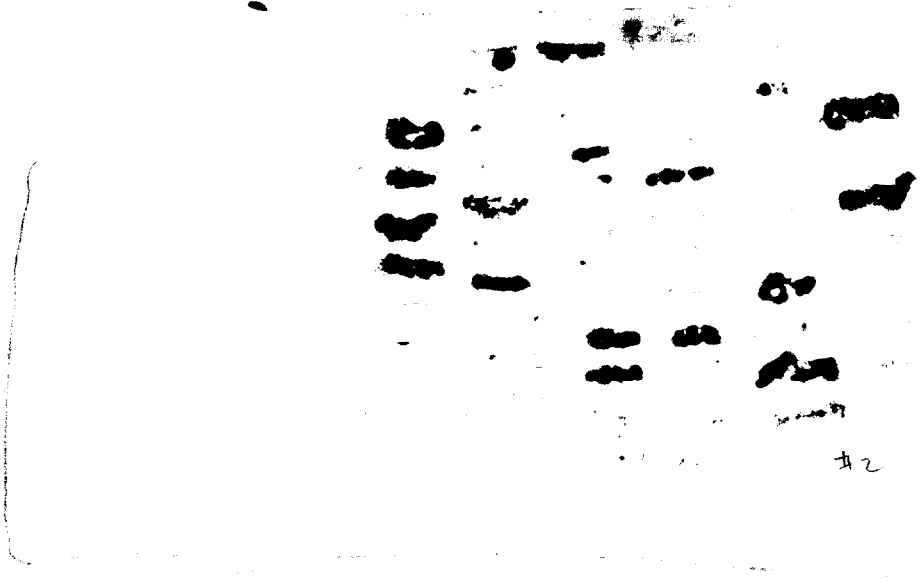
3. In almost of this case, whole  $gal^-$  segment will be eliminated, of U.V.-irradiation or by spontaneous loss of the segment from heterozygous aspicoid stage.



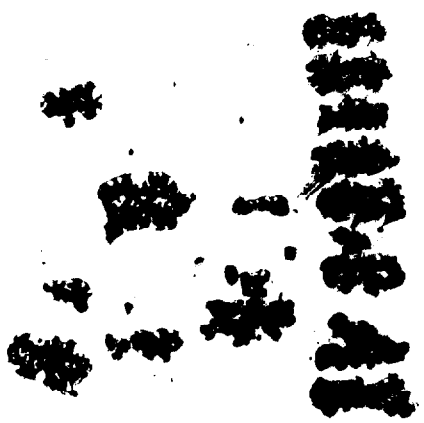


Bgal

#1



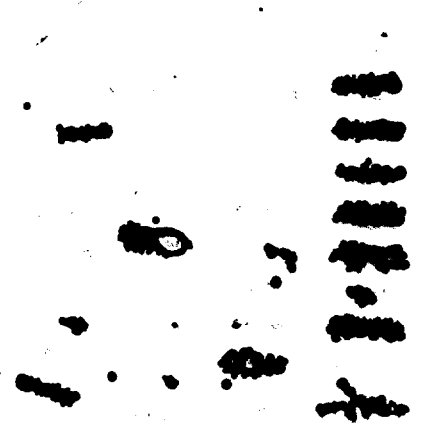
#2



MgalSm

x3994

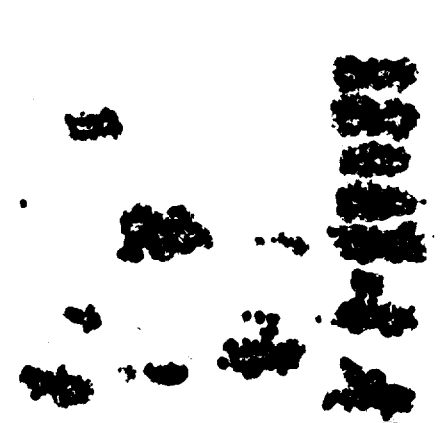
#1



MgalSm

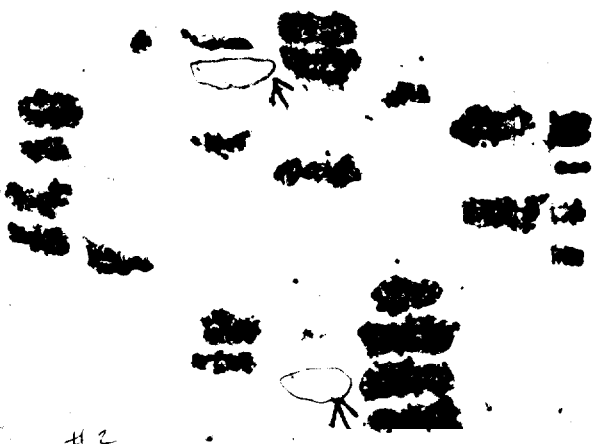
x4573

#1



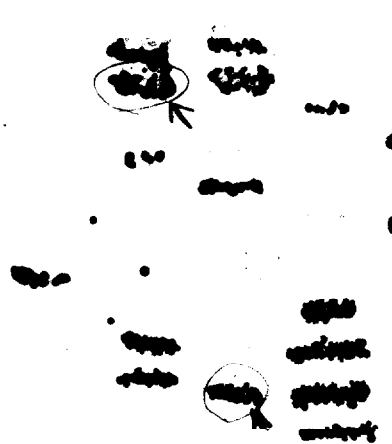
MgalSm x3991

#1



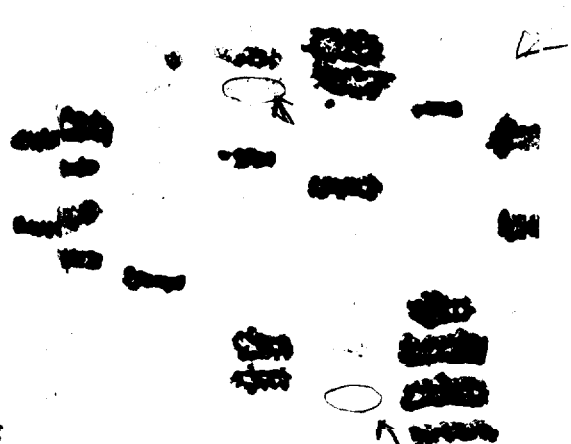
#2

x3994



#2

x4573



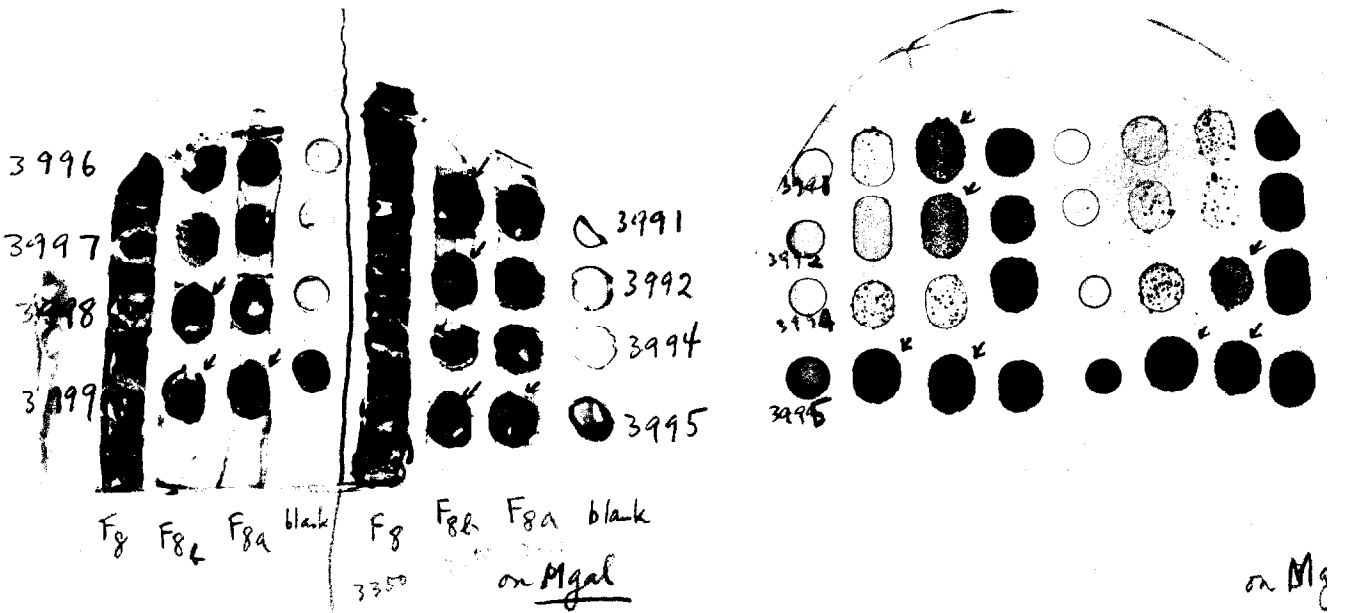
#2

x3991



29/10, '60

5'



Conclusion: parallelism can be seen in both plates, but less <sup>complementation</sup> (recombination) seen on Mgal than the M<sub>9</sub>; probably S<sub>m</sub> killed some F' male and make less F'.

∴ Use Mgal for this complementation experiment. or use excess F' S<sub>m</sub> for the purpose.

$$\begin{array}{r} 89001 \\ + \quad \quad \quad 467 \\ \hline \end{array}$$

9/19/60

Fga — X 4354

Fgb — X 4354

REF: Cf.

purpose: To get standard Fg sister strains.

Exptl. condition:

cultural age: overnight culture in 5ml peptone broth.

Infection:

0.1ml Fga or Fgb + 0.1ml 4354 + 5ml peptone &

Inoculate the mix at 37°C for overnight.

Streak on Bgal, pick gal<sup>+</sup> and ~~test for~~  
test sex-compatibility on Mgal x 3995.  
" x ~~3999~~ 3999.

Save a colony which shows male reaction.

Strains used:

3350 Fga and 3350 Fgb.

1-2<sup>-</sup>/ex Fga

1-2<sup>-</sup>/ex Fgb.

Result: (1) Infection

Fga — X 4354

Fgb — X 4354

# of colonies tested	F(Fga <sup>+</sup> ) obtained	# of colonies tested	F(Fgb <sup>+</sup> )
14	13 (same) relatively low. (probably this has shorter argmt)	15	15 (same)
See back page. #1			

(2) complementation.

(on Mgal. x F<sup>-</sup> S<sup>R</sup> gal<sup>-</sup> 9)

	1	2	4	5	6	7	8
W4354 Fga <sup>+</sup> (M <sup>-</sup> )	-	-	-	+	-	-	-
W4354 Fgb <sup>+</sup> (M <sup>-</sup> )	+	+	-	+	-	-	+
control W4520 (M <sup>-</sup> Fg <sup>+</sup> )	+	+	+	+	+	+	+
4354 Fg <sup>+</sup> (colony obtained from Fga infection) See back page. #1	-	-	-	+	-	-	-

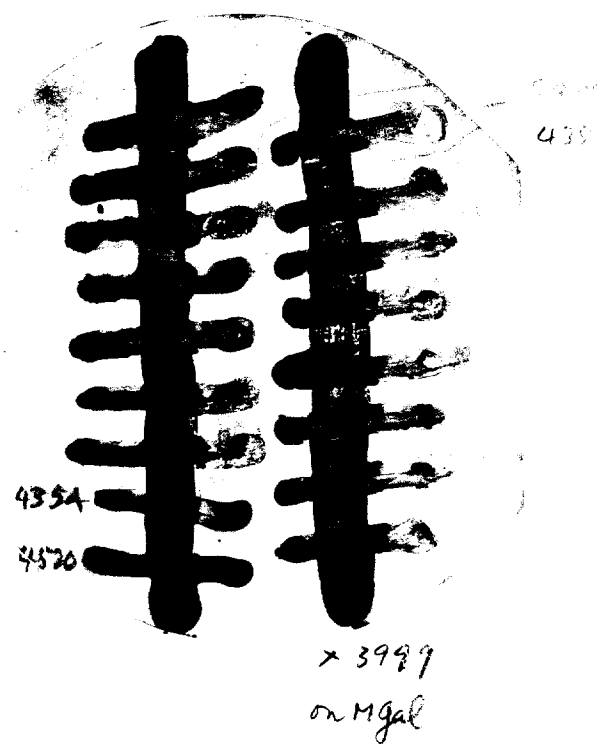
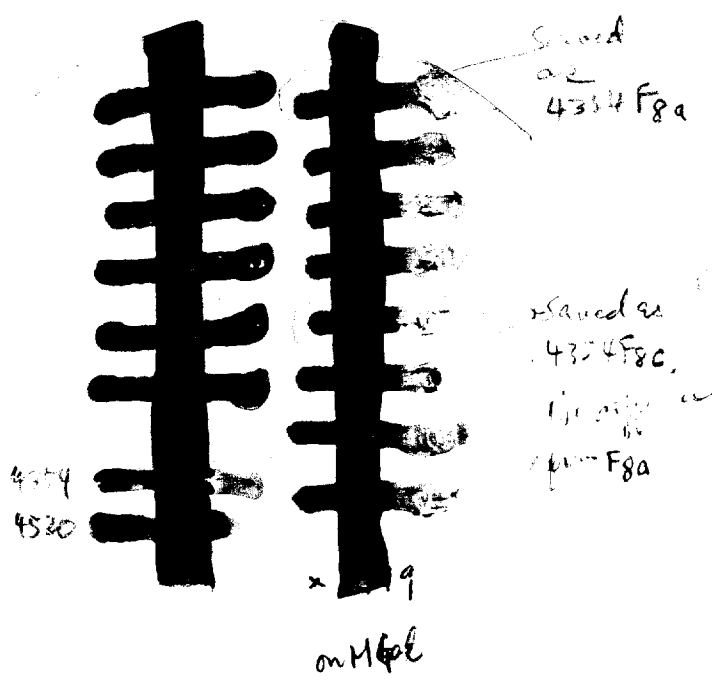
Conclusion: 3 groups can be classified.

— 9

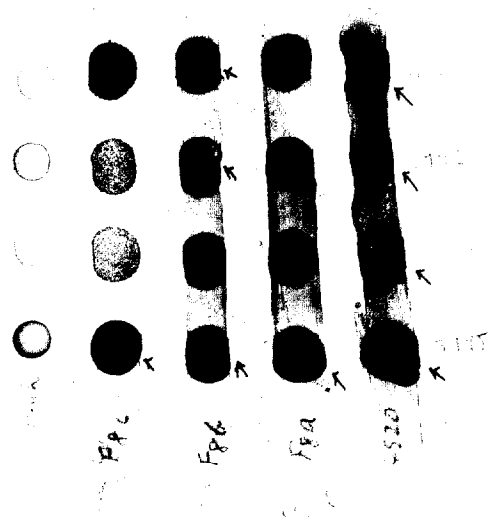
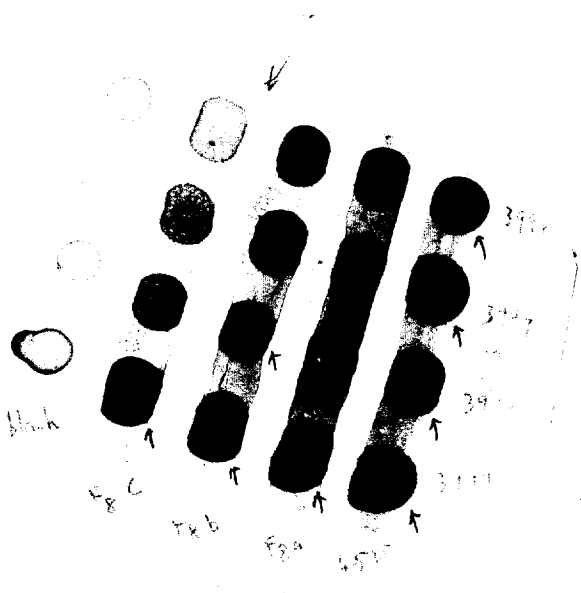
# 1

3350 F8a → X 4354

3350 F8b. → X 4354



# 2



Compare this with p.5

Retest on deletion of  $F_8^+$  :  $F_{9a}$  :  $F_{9b}$  &  $F_9$  (control).

62

Sex-direction of gal-segment by  $F_{9a}$ ,  $F_{9b}$ , and  $F_8$

1/11 ; 1960

REF:

purpose : To find transducible marker of  $F_{9a}$ ,  $F_{9b}$ ,  $F_8$ .

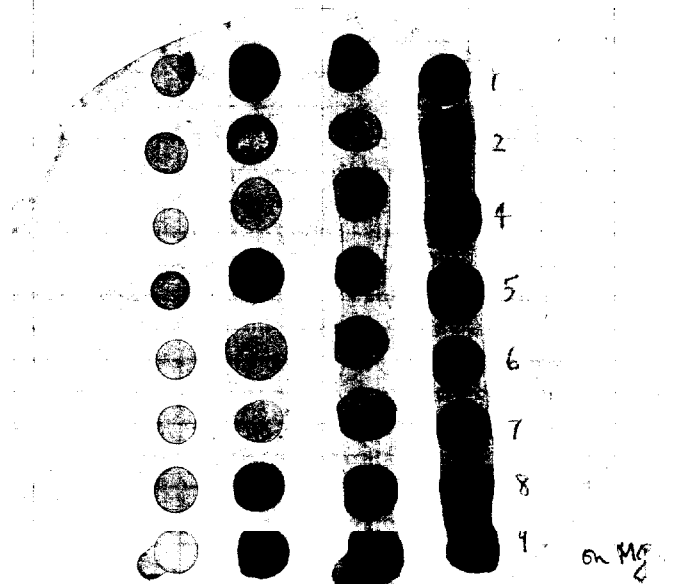
Exp. I : Mix  $F_8$  donor (2 drops) + gal<sup>-</sup>  $F_8^+$   $S^R$  (2 drops) + Salt  
Incubate the mix for overnight.  
Streak the mix on Bgal Sm.

	Result	cont	4354 $F_{9a}$	4354 $F_{9b}$
1	$F_8$ Donor			

1	3991	+		+		
2	3992	+		+		
3	3994	+		-		
4	3995	+	+	+		
5	3996	+	-	-		
6	3997	-	-	-		
7	3998	-	-	+		
8	3999	-	+	+		
			1, 2, 4, 6, 7, 8	2, 7, 9	4, 6, 7	1, 2, 22, 8, 9

See back page

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



Coat 4020-X



5 (6) +

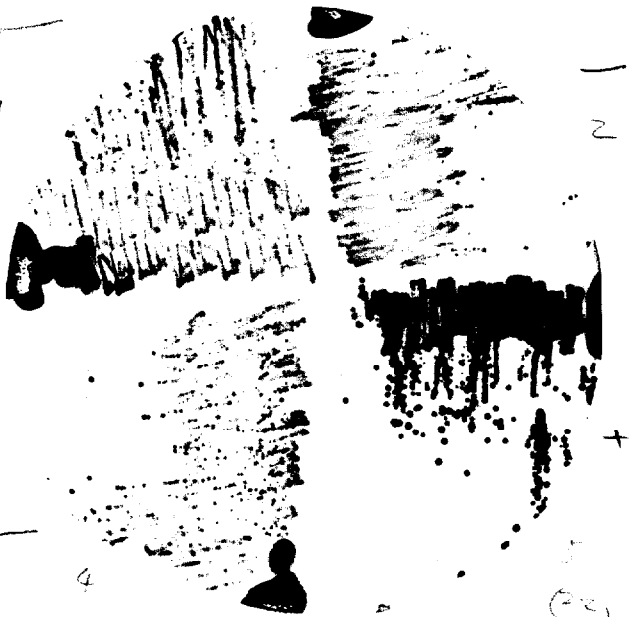
4520-X

Coat 20



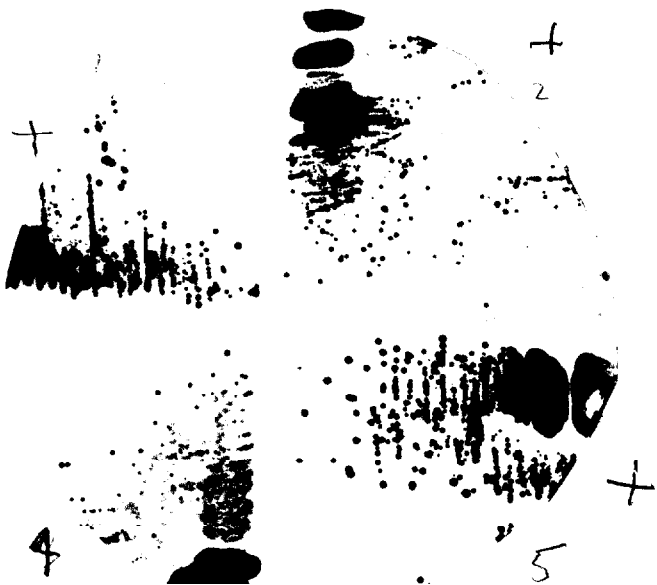
4354 F8a

4354 F8a (type)



4354 F8b

4354 F8b X

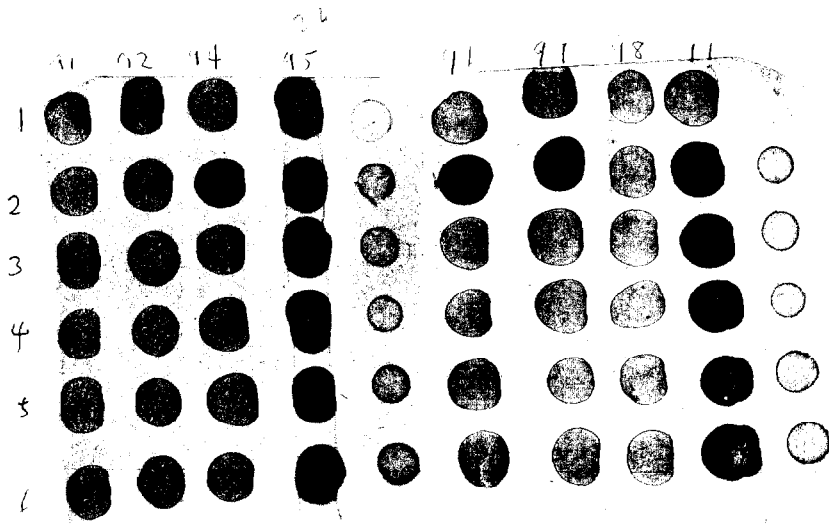


2nd isolation of gal-F<sub>8</sub> sisters.

1960

REF:

1	2	3	4	5	6	7	8	9
1	Strains used : 3350 F <sub>8</sub>							
2	Time of UV: 15 sec.							
3	on Bgal.							
4	① Survival :							
5								
6	Plate No.	Gal <sup>-</sup>	Gal <sup>v</sup>	Gal <sup>+</sup>				
7	<hr/>							
8	1	15	5	141				
9	2	19	7	114				
0	3	3	3	66				
1	4	4	2	87				
2	<hr/>							
3	② Inoculated on Bgal. a) Replicate it on Mgal Sm. x F <sup>-</sup> gal, gal s.							
4	b) Look for Rec <sup>+</sup> cells.							
5	55 gal <sup>-</sup> were isolated from the UV'd plates, and tested on their							
6	compatibility by cross x gal, ~ 9. on Mgal.							
7								
8								
9								
0								
1								
2								
3	Maleness	F <sub>8</sub> type	Number of F <sub>8</sub>	<del>Number</del>				
4	<hr/>							
5		Lowest gal						
6	♂	5, 9	44					
7	♂	5, 9, 4, 7, 6	2					
8	♀	No (F <sup>-</sup> )	89					
9		Σ	55					
0	<hr/>							
1								
2								
3								
4								
5								
6								
7								
8								



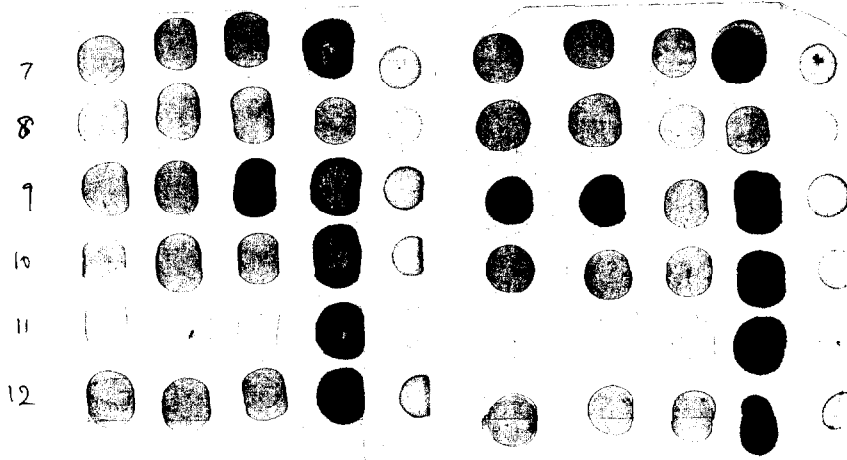
← 3350 F8c

a —  
distillation

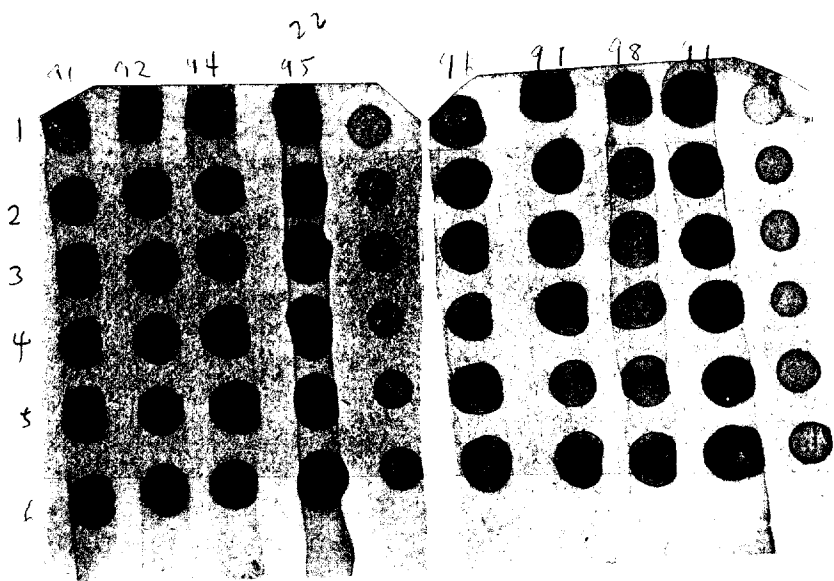
b : 1 2 8 4,

c : 1 2 4 6 7 8

d : 4 6 7 1

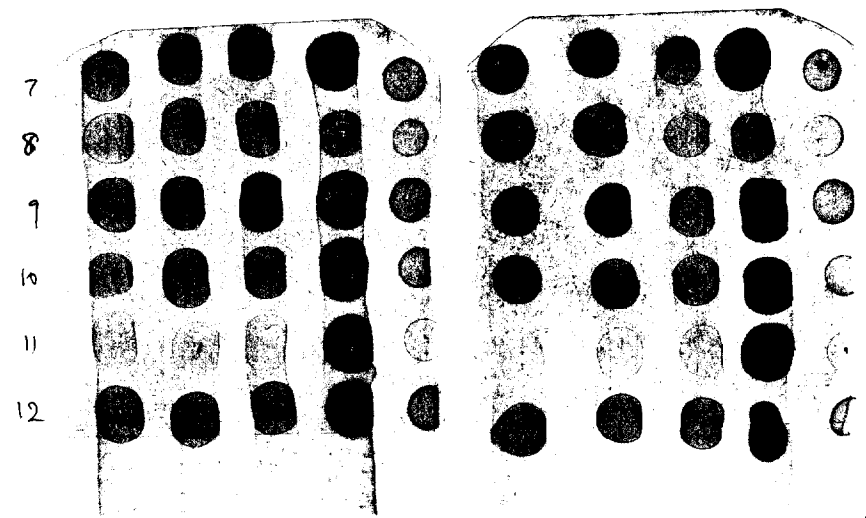


in MgCl



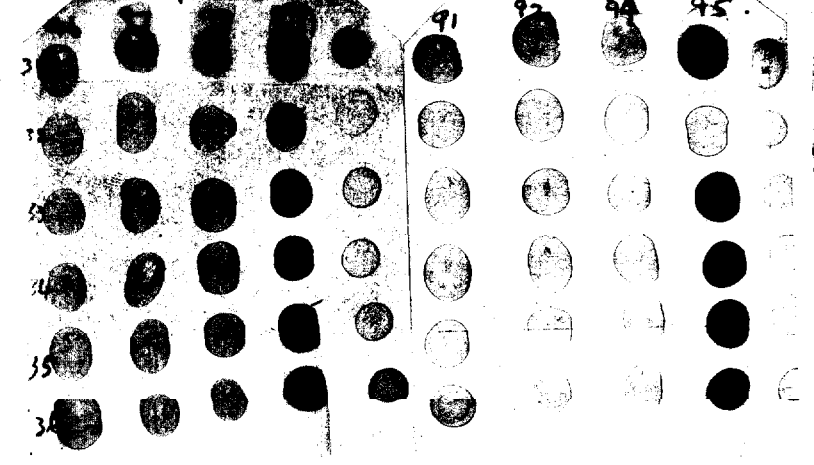
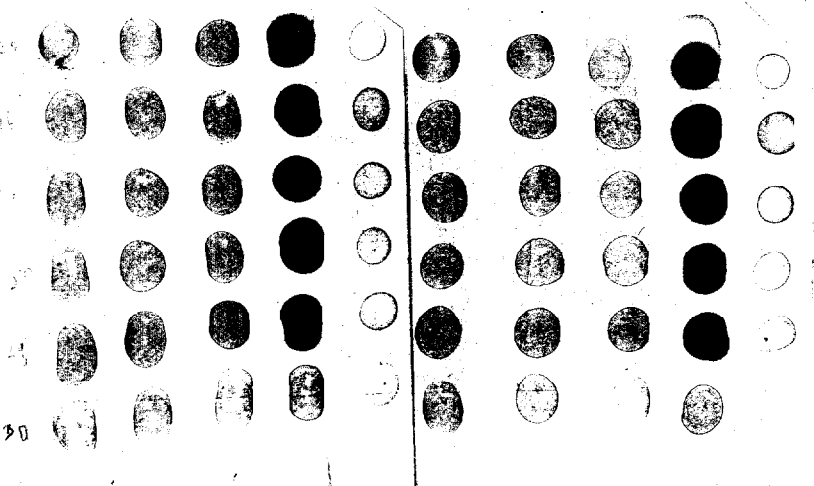
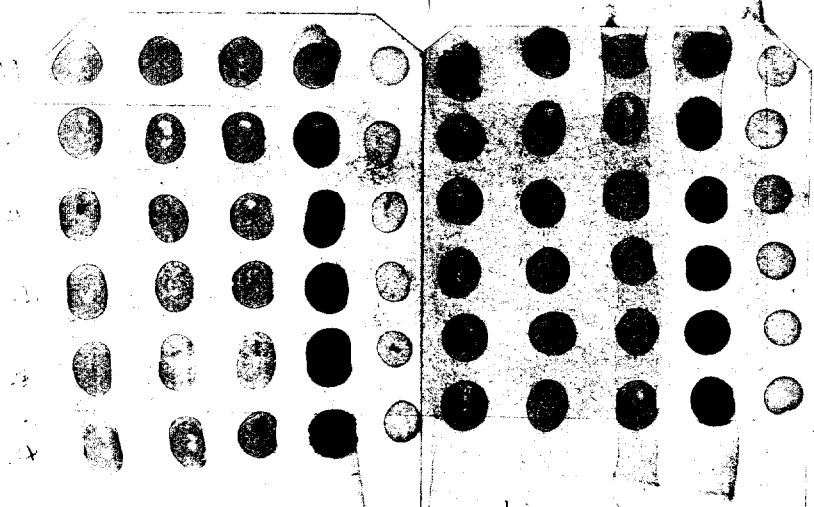
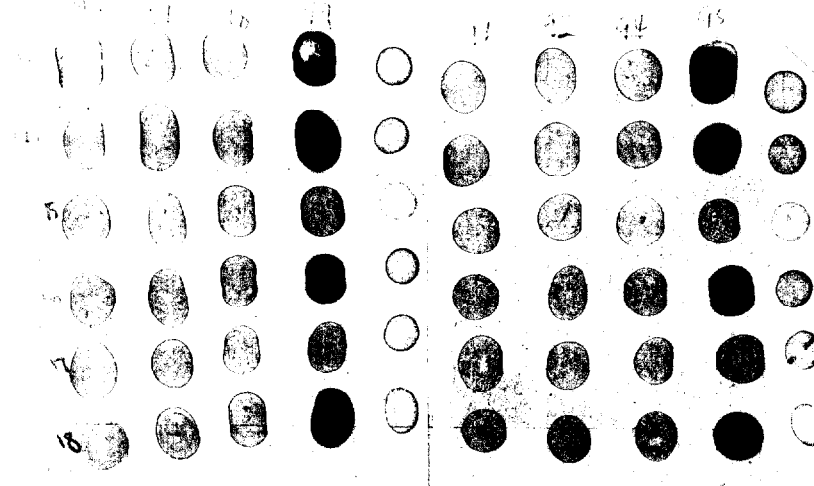
← 3350F8c

a — +  
 deletion  
 b : 1 2 8 4,2  
 c : 1 2 4 6 7 8 2  
 d : 4 6 7 1:



in Mgal





# Isolation of $F' lac^x$ from $F^- Lac^x$ .

5/8 : 1960

REF:

Strains used

1	2	3	4	5	6	7	8	9
Strain.	mutation	ONPG	$F_3$ donor					
W412	52 m	+	3747	HLac <sup>+</sup> F <sub>3</sub> V <sub>6</sub> <sup>R</sup> .				
W413	53 m	+						
W4147	87 m	-						
W4151	91 m	-						

Principle :

1. Isolate  $x/lac^+$  by 3747  $\times$ . Ratio 0.1:0.1:5ml; overnight 37°C
2. Pick out  $lac^x$  colony after streak from each ~~colony~~ <sup>purify it on B<sub>lac</sub></sup> ~~colony~~ <sup>streak on M<sub>lac</sub>. Select</sup> ~~colony~~ <sup>HLac<sup>+</sup> F<sub>3</sub> V<sub>6</sub><sup>R</sup>.</sup> ~~colony~~ <sup>streak it on B<sub>lac</sub></sup>
3. Test sex-compatibility by cross.

Experiment :

- A. Infection of F<sub>13</sub> } 0.1ml
1. Mix 0.1ml  $F^- Lac^-$ ,  $F_3^+ Lac^+$ , 5ml of peptone broth
  2. Incubate it for overnight at 37°C.
  3. Streak it on M<sub>lac</sub> to select ~~lac<sup>+</sup>~~ <sup>lac<sup>+</sup></sup>. save all 4 / lac<sup>+</sup>
  4. Streak it on complete, and make sure its purity.
- B. Isolation of  $Lac^x F_{13}$ .
- 1) Streak the transductant on B<sub>lac</sub>.
  - 2) Pick  $lac^x$  and restreak it on B<sub>lac</sub> again.
  - 3) Pick  $lac^-$  segregant and test male-ness.

Isolation of  $lac^- F_{13}$ .

12/11/1960

(4112  $F_{13}$ ) <sup>REF</sup>  $lac_{52}^- : m^+ : oMPG$   
W4151  $lac_{91}^- : m^+ : oMPG$

Continued from (p. 8. 5/11/60)

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 H<sub>2</sub>hac 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 H<sub>2</sub>hac).

$lac_{52}^-$ total	$lac^- F_{13}$	$lac^- F^-$	$lac^- F^+$
30	27	3	0

(2) 5  $lac^-$  colonies are streaked ~~and~~ on B<sub>2</sub>hac, and tested their sex. (replica plated on H<sub>2</sub>hac seeded with 4151).

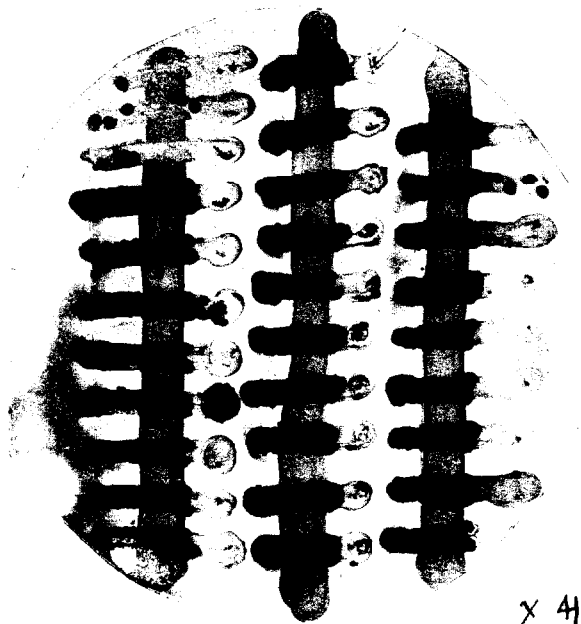
	$lac_{52}^-$ total (segregants)	$lac^- F_{13}$	$lac^- F^-$	$lac^- F^+$
Colony 1	56	56	0	0
Colony 2.	22	22	0	0
Colony 3	50	50	0	0
Colony 4	67	67	0	0
Colony 5.	93	93	0	0
Colony 6	3	1	0	2

(3) Test H<sub>1</sub> for Purine, T<sub>6</sub> r or s or T<sub>6</sub> v.

The newly isolated  $lac_{52}^- F_{13}$  is T<sub>6</sub> ~~var.~~. See back pg  
may not be V<sub>6</sub> var. maybe 1  
range mutant

(4) Test complementation +  $lac_{52}^-$ .  
x 4112, x 4113, x 4141, x 4151. T<sub>6</sub>.

Same thing as 412 Fis



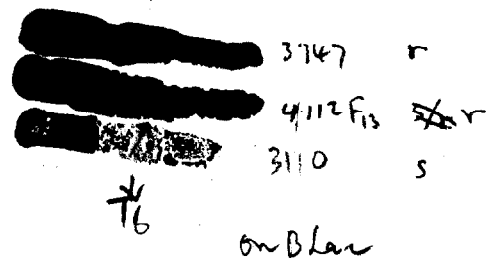
X 4151  
on M lae.



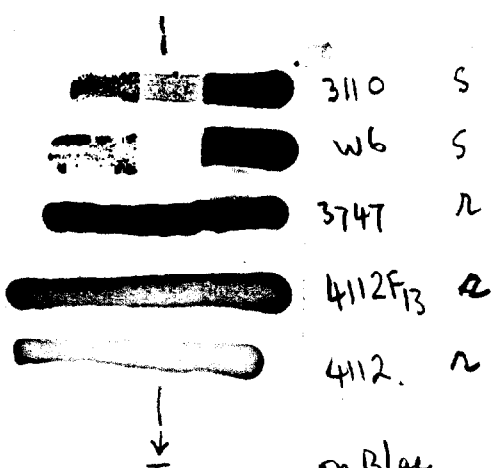
X 3747  
 X 412F13  
 X 412F33 blank  
 on M lae.

lae

- 4112 5
- 4113 5
- 4147 8
- 4151 9



\* T6  
on B lae



↓  
on B lae.

Complementation test using newly isolated  $Lac_{52F3}$ .

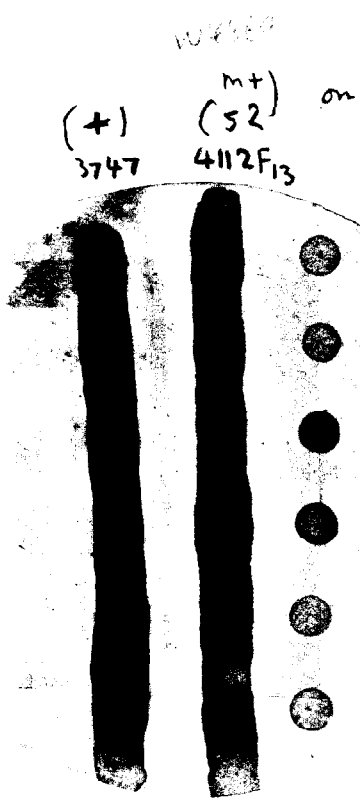
16/v : 1960

REF:

Type	1	2	3	4	5	6	7	8	9
	Strains tested.				purpose: Look for $Lac$ marker wh give complementation with $Lac$ Test on $M_{lac}$ .				
	lac mutation.				See back page.				
	Strain No.				complementation reaction. (on $M_{lac}$ ) after streak B $Lac$				
1	ONPG-	Δ 22	3147						
2									
3									
4									
5									
6									Streak $Lac$ spot on B and confirm it is rec reacting c.f. 9-b.
7		Δ 25	3151		##				
8		Δ 41	4101		##				
9		Δ 87	4147		##				
0		Δ 90	4150		##				
1		○ 93	4153		##				
2		○ 89	4149		##				
3		Δ 88	4148		##				
4		Δ 91	4151		##				
5		X 52	4112		-	Same mutation.	See p.9.		
6		○ 39	4287		##				
7		X 17	3152		+ Rec.				
8		X 18	3174		+ <del>low</del> ← 3174 does not give complementation reac with this!				
9		X 19	3156		##				
0		X 20	4286		+				
1		X 21	3153		+ Rec. ca. 100				
2		X 53	4113		+ Rec. ca. 100				
3		X 55	4115		+ Rec. ..				
4		X 56	4116		+ Rec. ..				
5		X 71	4131		##				
6		X 72	4132		- No lac. ← why? Ann's $Lac_{52F3}$ with 13				
7									

Δ contained in extreme f-type but not in  $Lac_{11D3}$  or 39

X Contained in  $Lac_{11D3}$

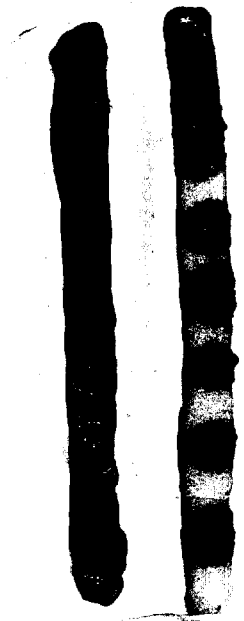


104389  
 (+) 3747  
 (52) 4112F13  
 on MLac

Strain No.	Loc	ONPG
4101	41	m -
4148	88	m -
3151	25	m -
4147	87	m -
4153	93	m -
4149	89	m -

contained in extreme f-type  
 but not in loc 11D<sub>3</sub> or 39.

contained in 39.



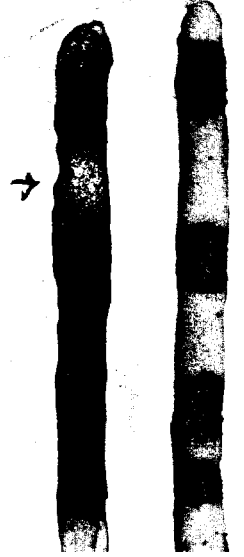
4287	39	m -
4286	20	m +
4131	71	m +
3156	19	m +
3152	17	m +
4150	90	m -
4113	53	m +

contains (23, 26, 60, 62, 95, 68, 81, 93, 97)

contained in 11D<sub>3</sub>.

contained in extreme f-type  
 but not in 11D<sub>3</sub> or 39.

contained in 11D<sub>3</sub>.



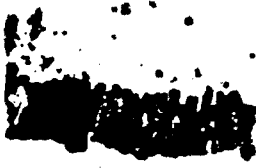
4113	53	m +
3174	18	m +
4115	55	m +
4132	72	f -
4116	56	m +
3153	21	m +

- ① Ayn's thesis says: 18 contains but there is some recombination.
- ② No recombination in x 3747. probably, there may be some site for mating reaction of this strain.

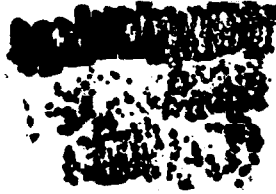
contained in 11D<sub>3</sub>.

o.d. steel

4101



4131



3153



4148



3156



3151



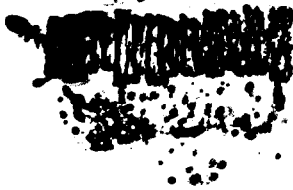
3150



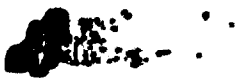
4147



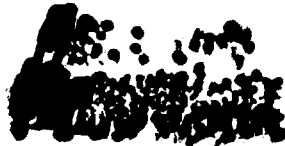
4150



4153



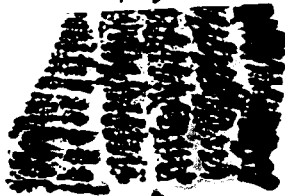
4113



4149



4115



4286



4116



streaked on B<sub>hac</sub>  
from <sup>Lac<sup>+</sup></sup> spot

Conclusion:

These Lac<sup>+</sup> spots on  
are real genetic exphen  
Lac locus, not spot

Test transfer of  $LacS_2$  to  $Lac^+ F^- S^R$   
3086

19/10/1960

REF: 9a, b, d.

- purpose of this experiment: To test whether  $LacS_2$  homozygous or hemizygous for  $LacS_2$  locus.
- Principle: If  $LacS_2$  is homozygous, there should be incorporation and segregation of  $LacS_2^-$  after infection of  $LacS_2$  to  $LacS_2^-$ . If this ratio of segregation is ca  $10^{-2}$ , it is possible to observe  $Lac^-$  segregant with this method.

$F_{13} LacS_2$  (0.2ml)  $\times$   $F^- Lac^+ S^R$  (0.1ml) (W3086)  $F^- M Mal, S^R$   
 for overnight culture in phage. purified on B  
 excess  $F_{13} F_{52}$ .  $Lac^+$  colony is picked.

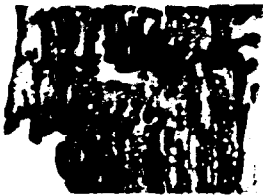
Seed the mix on B-lac Sm.

Cultural age: overnight grown culture of  $F_{13} LacS_2$  &  $F^- Lac^+$

Result. Seed  $10^8$ : 0.1ml / plate EMB Lac Sm. Further Experiment

plate No.	$Lac^+$ (lighter) (prob. $LacV$ )	$LacV$	$Lac^-$ (presumably it is segregant)	$F_{13}$	Notes
1	87 (9)	2	0	7	① streak each ho and confirm real $LacV$ .
2	97 (17)	3	1	8	② Test $Lac^-$ segre, a Test mal
3	75 (15)	3	0	10	b. Test mal ca
4	59 (7)	4	1	7	c. Test $LacV$
5	49 (2)	1	0	8	③ Test $F_{13}$ near
6	59 (7)	4	1	6	How much % of cells will become
7	75 (15)	3	0	5	(Rate of Inf. of $F_{13}$ on M1 x L (W)
8	97 (17)	3	1	3	
9	97 (17)	2	1	1	
10	0	-	1	1	





Continued from former page

3086

W4864 9d

Test  $Lac^-$  character transferred by 4112  $F_{13}(Lac_{52})$

26/4 1960

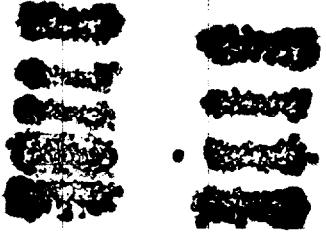
New  $F_{13}^+$   $Mal^-$ ,  $Sm^-$   $Lac_{52}$  (4866)

REF: T a. b. c.

1 2 3 4 5 6 7 8 9

1. Test  $Mal^-$  (3086 is  $Mal^-$ ) on B $Mal$  agar.

Result. all 5 are  $Mal^-$ .



2. Test  $Lac^-$  marker.

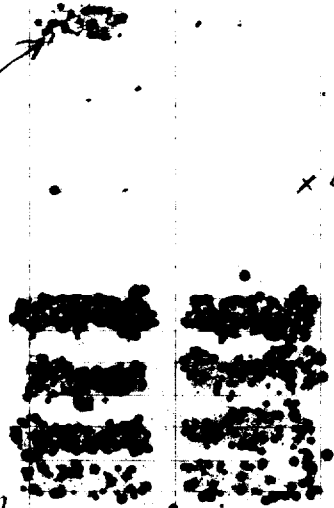
Show the  $lac^-$  is  $Lac_{52}$  and not others.

Replicate it on 4112 + 4151

This marker is  $Lac_{52}$  and all male.

- 1) Fertile in cross x  $F^- Lac_{91}$
- 2) Sterile in cross x  $F^- Lac_{52}$ .
- 3) Sterile in cross x  $F_{13}^+ Lac_{52}$

on B $Lac$  agar



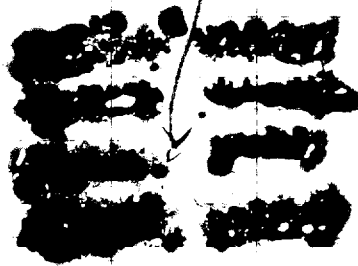
3. Test Hfr-ness of the transfer of the other markers.

$gal^+$   
H1: Gal  
H1: Mcl.  
Med: Ara<sub>2</sub>  
Lo: Lac.

reversion

Conclusion: fertility pattern is same as  $Hfr_{13}$ .

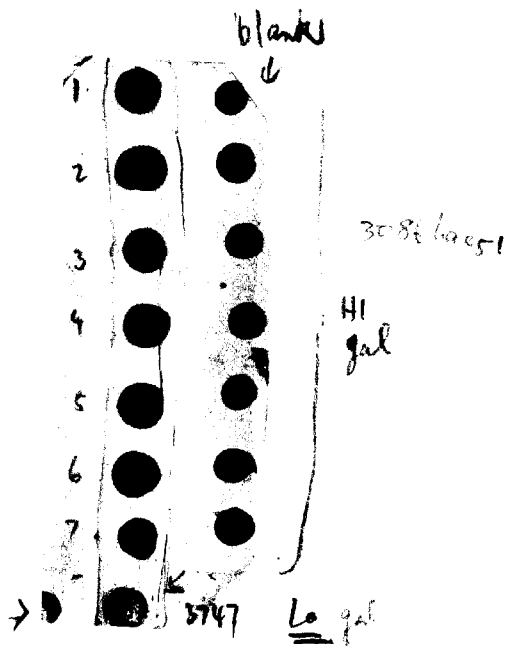
4.  $Gal^+$  recombinants does not segregate  $bet^-$  after streak the spots on B $Gal Sm$ .



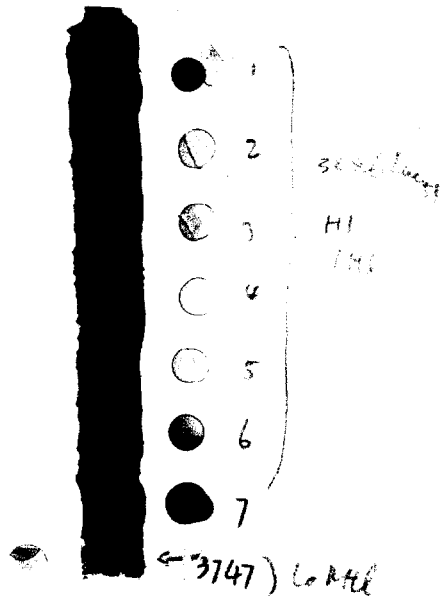
x 411

x 4151

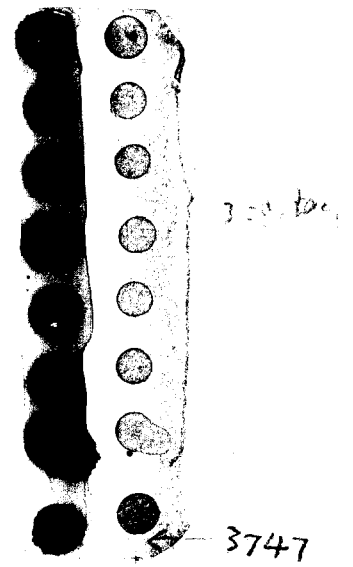
1  
2  
3  
4  
5  
6  
7  
8  
9  
0  
1  
2  
3  
4  
5  
6  
7  
8  
9  
0  
1  
2  
3  
4  
5  
6  
7  
8



x4573 (gal<sub>2</sub>)  
on Mgal



x4573 (Mtl)  
on M Mtl



x4573 (Ara<sub>2</sub>)  
on M Ara

Test homozygosity of  $Lac_{S2}$  of 4112  $F_{13}$  by reversion.

31/v 1960

REF: Cf. See transduction  
 $Lac_{S2}$  to  $Lac^+$   
308

1  
2  
3  
4  
5  
6  
7  
8

Principle: Test homo or hemi-zygosity of 4112  $F_{13}$  by reversion to  
If it segregate: homo:  
" " does not " : hemi.:

①

Method:

pick one reversion for separate colony.

3/v

1. Purify 4112  $F_{13}$  on B<sub>lac</sub>.
2. Pick 30 colonies into <sup>out</sup> EMB<sub>lac</sub> for ~~48 hrs~~ 48 hrs.
3. ~~Spot those colonies on B<sub>lac</sub> agar and incubate for 48 hrs.~~
4. (pick one  $Lac^+$  from each spot &) Purify  $Lac^+$  revertants on B<sub>lac</sub>. confirm purity of them.
5. <sup>Let each colony grow up in 5 ml penicillin</sup> Streak each revertants on B<sub>lac</sub>, and see segregate  $Lac_{S2}$ . Look for  $Lac^+$ , and restreak it on B<sub>lac</sub> again.
6. Test  $Lac^-$  type of segregants from the diploids.

Result:

Isolation No

	$Lac^-$	$Lac^+$	seg.		$Lac^-$	$Lac^+$	seg.		$Lac^-$	$Lac^+$	seg.
1		✓		9		✓		17		no	
2		no		10		no ?		18		✓	
3		✓		11		no		19		no	
4		no		12		✓		20		✓	
5		no		13		✓		21		no	
6		no		14		✓		22		✓	
7		✓		15		no		23		✓	
8		✓		16		✓		24		•	

②

Test  $Lac^+$  transfer. Test fertility for transfer of  $Lac_{S2}$  of  $Lac^+$  rev  
& cross x 4112  $S^R$  on M<sub>lac</sub> Sm. use spot test.

purpose: Is  $Lac^+$  reversion on exogenetic segment or endo;  
If exogenetic H<sub>1</sub>; If it is in endogenetic h<sub>o</sub>.

V: 13 (9)

h<sub>o</sub>: 10

21  
22  
23  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24

1  
2  
3  
4  
5  
6  
7  
8  
9

3747  
4112  $F_{13}$   
4112  $S^R$

Isolation of Lac F<sub>13</sub> (4147 F<sub>13</sub>)

12/6

12/6 1960

ONPG - 1

W4147 : Lac<sup>87</sup> F<sup>-</sup>  
W4112 : Lac<sup>52</sup> F<sup>-</sup> ONPG

1. One 4147 F<sub>13</sub> colony was streaked on Blac from Dgal.

~~2. One 4147 F<sub>13</sub> colony was streaked on Blac from Dgal.~~

Exp I. 4 Lac<sup>-</sup> segregants were tested on their sex.

x W4112.  
(Lac<sup>52</sup>:  
F<sup>-</sup>)

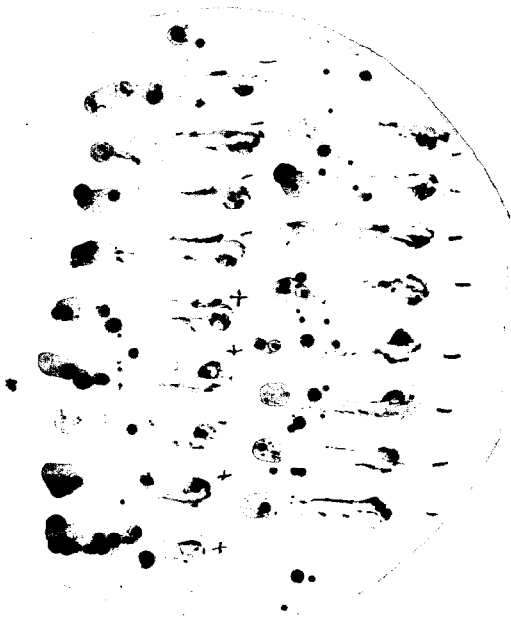
2. 5 Lac<sup>-</sup> colonies were streaked on Blac agar, and Lac<sup>-</sup> segregants were tested on their sex-compatibility.

Col. #	# of col tested	Lac <sup>87</sup> F <sub>13</sub>	Lac <sup>87</sup> F <sup>+</sup>	Lac <sup>87</sup> F <sup>-</sup>
1	5	1 (relatively low) Test : activity	4	0
2	12	0	4	8
3	10	0	10	0
4	3	0	1	2
5	17	0	4	13

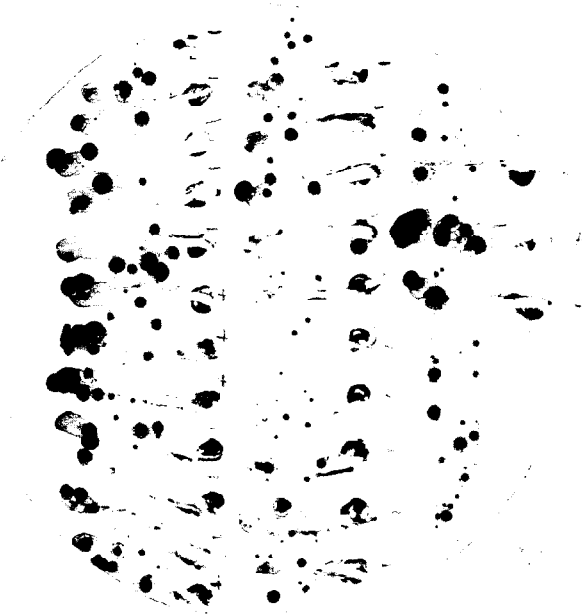
Conclusion:

The results of the  
sex-compatibility tests  
are not very clear.  
- Some Lac<sup>-</sup> segregants  
may be...

Fig X4147 (1ac87)



5  
+ 4 1-13



2 3 4  
+ 4, -8 + 0, -10 + 1, -2  
X4112 (1ac87)

Isolation of  $Lac^- F_{13}$

12/1

1960

REF: c.f. P. 8

1 2 3 4 5 6 7 8 9

continued from (p. 8. p. 105/169)

History: 1. 3747  $\rightarrow$  X 4147 ( $F^- X^+ Lac_{87}^-$ )

2. Pick  $Lac_{87}$  text

3. streak it + test fertility of  $lac^-$  segregants of it.  
X W4112 on Mlac.

6 colonies are tested on their compatibility.

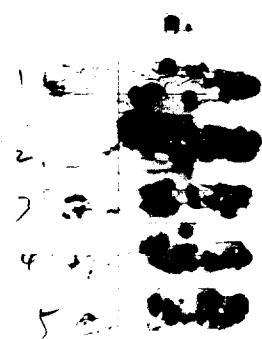
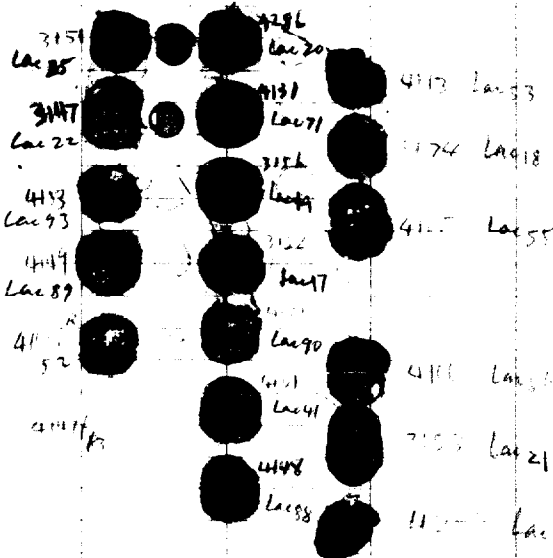
1 was  $F_{13}^+$   
F

4. Test complementarity by spot test on Mlac.

~~X 4151 (91) F~~

~~X 4152~~

4117  $F_{13}^- Lac_{87}^-$



x 4112 ( $Lac_{52}^-$ )  
on Mlac

Showing complementarity with  $Lac_{87}^- F_{13}$ .

Lac20  
Lac19  
Lac17  
Lac21

Lac53  
Lac54  
Lac56  
Lac57

Lac52  
Lac55  
Lac58

Preparation of  $\lambda$ -reference from B120.

30/12 1960

REF:

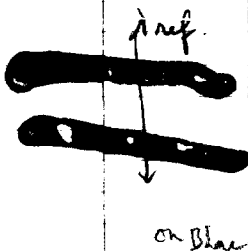
1 2 3 4 5 6 7 8 9 10

1. Make overnight grown culture in  $\lambda$  (8ml).
2. Add it to 10 ml  $\lambda$  ph. & incubate it at 37°C for 2hr on rotator.
3. C.f.g. resuspend bacteria in 4ml of  $\lambda$ O. Irradiate it with U.V. on Petri's dish for 10 seconds.
4. Add it into <sup>20 ml</sup> Phagey broth and incubate it for <sup>3</sup> hrs, at 37°C. (2:15 ~ 5:15 PM) on rotator.
5. ~~Keep~~ Keep it in refrigerator for overnight at 5°C. to complete lysis.
6. C.f.g. the lysate 3000 s.p.m. <sup>for 20 min;</sup>  $\wedge$  supernate saved.  
C.f.g. (8000 s.p.m. for 1.5 hrs) <sup>resuspend it in 1 ml of</sup>  $\lambda$ P-med. <sub>small. at 5°C. 1:40 ~ 3:10 pm.</sub>  
Use this as DNA source of  $\lambda$ . — assay titre of plaque forming ~~centre~~ centre.

	dilution vol.	# of Plaque
Titer	10 <sup>8</sup> : 0.1	

3110

4873





Effect of AO on F<sub>8</sub> gal<sup>+</sup> ~~str<sup>r</sup>~~-treated.  
(3350 F<sub>8</sub> 1<sup>-</sup> 2<sup>-</sup> / + +.)

2/8 / 1960

REF:

1	2	3	4	5	6	7	8	9	10
---	---	---	---	---	---	---	---	---	----

1  
2  
3  
4  
5  
6  
7  
8  
9  
0  
  
1  
2  
3  
4  
5  
6  
7  
8  
9  
0  
  
1  
2  
3  
4  
5  
6  
7  
8  
9  
0  
  
1  
2  
3  
4  
5  
6  
7  
8  
9  
0

1. 3 new isolates are grown in penicillin broth.
2. Inoculate ca. 10<sup>8</sup> cells/ml AO-N<sub>2</sub>B. : pH:7.6 : AO 30g/ml. 37°C overnight-treated.
3. Procedure:  
Seed 10<sup>-6</sup> ml of AO-treated and untreated culture onto B-Gal. and count the ratio of gal<sup>+</sup> and gal<sup>-</sup>.
4. Test sex-compatibility of gal<sup>+</sup> and gal<sup>-</sup> by replicating on ~~W4573~~ Hlae<sub>8</sub> seeded W4573. on it. Look for gal<sup>+</sup>F<sup>-</sup> and see segregation.

Treatment of 3350 F<sub>2</sub> with A0.  
1-2/++ F<sub>2</sub>

6/4 ; 1960

REF:

Purpose: 1. To show susceptibility of F<sub>2</sub> gal<sup>+</sup> to A0.  
2. Look for gal<sup>+</sup>F<sup>-</sup> segregant from it. Re-infect F to it and see.

Experimental condition:

Conc. of A0 : 207/ml ; N.B. 5ml pH. 7.6 3 tubes  
(0.2ml of 500x soln to 5ml of N.B.) as a control  
Inoculum size : 10<sup>8</sup> × 10<sup>2</sup> × 10<sup>2</sup> : 0.1/5ml. : ca 2 × 10<sup>3</sup> NO A0.  
Time of treatment : at 37°C. for overnight : 2:30 ~  
Score it on Bgal.

Result:

Test fertility by  
cross x<sup>+</sup>F<sup>-</sup> SR on MgalSm.  
W3995.

Tube	plate #	gal <sup>-</sup>		gal <sup>+</sup>		# of colonies on Bgal.			
		gal <sup>-</sup> F <sup>-</sup>	gal <sup>-</sup> F <sup>+</sup>	gal <sup>+</sup> F <sup>-</sup>	gal <sup>+</sup> F <sup>+</sup>	gal <sup>+</sup>	gal <sup>-</sup>		
A01	1	1	25	26	0	25	83	83	
	2	0	22	22	0	23	119	80	
	3						123	101	
	4						76	63	
	5						120	101	
						52 (54.9%)	428 (45.1%)	949	
A02	1	0	26	26	0	25	85	57	
	2						113	59	
	3						124	70	
	4						81	75	
	5						42	26	
						445 (60.8%)	287 (39.2%)	732	
A03	1	3	20	23	0	21	105	85	
	2	11	20	25	0	25	100	89	
	3						91	80	
	4						88	70	
	5						106	87	
$\Sigma$ A0		7	115	122	1	119	490 (54.4%)	411 (45.6%)	901
Untreated Control I	1	6	8	8	0	24	530	19	
	2	0	6	6	0	26	499	14	
	3	4	5	5	0	21	443	20	
	4	6	5	11			367	16	
	5						418	21	
		27	19	71	0	71			
II	1	36	1	37	0	35	2257 (96.1%)	90 (3.9%)	2347
	2	58	1	59	0	56			2998

Looks for F<sup>-</sup>gal<sup>+</sup> & Test segregation.

(from 3350 F<sub>2</sub>)

gal<sup>+</sup>F<sub>2</sub>

gal<sup>+</sup>F<sup>-</sup> or F<sup>+</sup>

A0 treated gal<sup>+</sup>F<sup>+</sup>

52

1 - F<sup>-</sup>

$\Sigma$  gal<sup>+</sup>F<sub>2</sub> : 355

105

1 - F<sup>-</sup>

gal<sup>+</sup>F<sup>-</sup> : 11

113

0 - F<sup>+</sup>

% : 0.84 %

85

0 - F<sup>+</sup>

Segregation of gal<sup>-</sup> from F<sup>-</sup> & F<sup>+</sup> strains derived from 3350 F<sub>8</sub> after treatment of AO.

20/4; 1960

REF:

	1	2	3	4	5	6	7	8	9	10
						on Blac.				
		Isolation No		# of colonies tested				# of colonies tested for		
			plate No	Lac <sup>+</sup>	Lac <sup>-</sup>	Lac <sup>+</sup>		sex-compatibility.		tester:
1		I	1	0	8	371	Segregate			3995
2			2	3	4	462				
3		Sex.	3	4	2	533				
4			4	3	1	19				
5		F <sup>-</sup>	5	4	2	631				
6			Σ	14	17	2016				
7										
8		II	1	0	0	629	No segregation			
9			2	0	0	397				
10		F <sup>+</sup>	3	0	0	691				
11			4	0	0	699				
12			5	0	0	627				
13			Σ	0	0	3033				
14										
15		III	1	4	3	777	Segregate			
16			2	3	2	421				
17		F <sup>-</sup>	3	1	1	514				
18			4	2	0	11				
19			5	5	1	30				
20			Σ	23	7	1753				
21										
22		IV	1	7	2	834	Segregate.			
23			2	5	2	635				
24		F <sup>+</sup>	3	0	3	821				
25			4	7	4	406				
26			5	6	3	613				
27			Σ	25	14	3309				
28										
29										
30										
31										
32										
33										
34										
35										
36										
37										
38										
39										
40										
41										
42										
43										
44										
45										
46										
47										
48										
49										
50										

Test sex-compatibility of gal<sup>+</sup> & gal<sup>-</sup> segregant from IV.

Are these all F<sup>+</sup> or segregate?

o This test give me answer about a question that: F<sup>+</sup> will be sick after treatment with AO. or F will be released into cytoplasm.

Tester: X 3995



segregants from  
gal<sup>+</sup>F<sup>+</sup> 3355 F<sub>12</sub> AD.  
↓  
Now it looks like F<sub>13</sub><sup>+</sup>

X3995  
on Mgal Sm.

Test behavior of  $gal^+ F^-$  obtained ~~from~~ with  
AO-treatment from 3350 F8.

; 1960

REF:

Purpose: <sup>2</sup> Are those <sup>3</sup>  $F^-$  strains <sup>4</sup> of or <sup>5</sup> simple  $F^-$ ? (Does it <sup>belong</sup> to  $F^-$  by  $F^-$ ?)  
(Does it segregate  $gal^-$ ?)

1. b/v's experiment gave me 4  $gal^+ F^-$  segregants. by AO-treatment of 3350 F8.

2. Infect F to them. Ratio of Mix.  
Pen 5ml +  $F^- gal^+$  0.1ml +  $F^+ W6$  0.2ml

(a) Make cross brush with  $5^R M^- F^-$  on Mgluc Sm. W3086. Incubate the mix for overnight.

(b) Streak <sup>the</sup> on DO. & Test compatibility on Mgal Sm X  $M^- F^- S^R$ .

3. Streak those  $F^-$  on Bgal. & see segregation of  $gal^-$ .  
Dil. volume:  $10^5:0.1$  / plate. on Bgal.  
5 plates for each. (original culture was grown in pen. for overnight at  $37^\circ C$ )

Result.

I. Segregation of  $gal^-$

	1	2	3	4
segregation of $gal^-$	seg.	No seg.	seg.	seg.
sex.	$F^-$	$F^+$	$F^-$	$F^+$ (transfer of $M^+$ ) to 3086 on Mgluc Sm
		backed to $F^-$ H1 gal <sub>2</sub>		backed to $F^-$ H1 gal <sub>2</sub>

II Infection of F. (Infectability)

(a) Cross brush the mixture of W6 and  $gal^+ F^- X^+$  on Mgluc Sm. (x 3086)

Isolation No.	+W6	-W6	+W6	-W6	+W6	-W6	+W6	-W6
Recombination								
Reaction with 3086 on Mgluc Sm	+	-	+	+	+	-	+	+
( $F^- M^- Hal^+ S^R$ ) by cross brushing method. transfer of $M^+$								

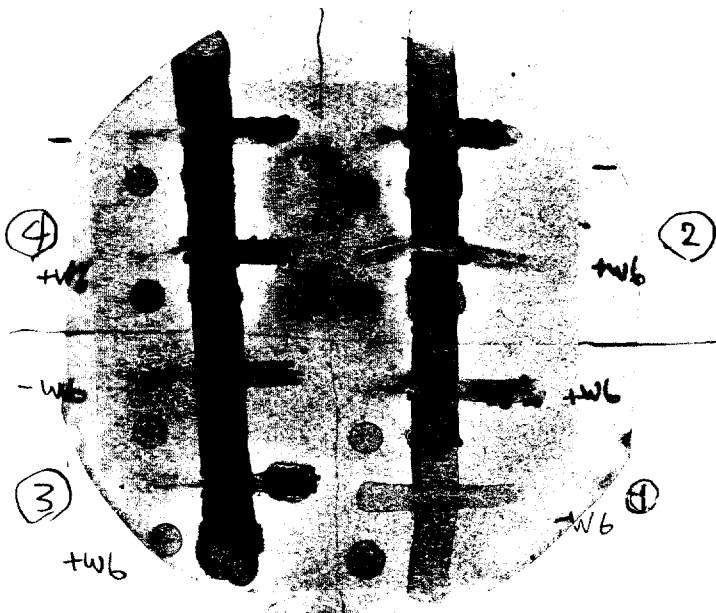
+W6: W6 was added as F-donor  
-W6: W6 was not added. = original  $F^- gal^+ 3350$

(b) Cross brush the isolates from mixture of W6 and  $gal^+ F^- X^+$  (from DO) (x 3086) on Mgluc Sm.

III Infectivity

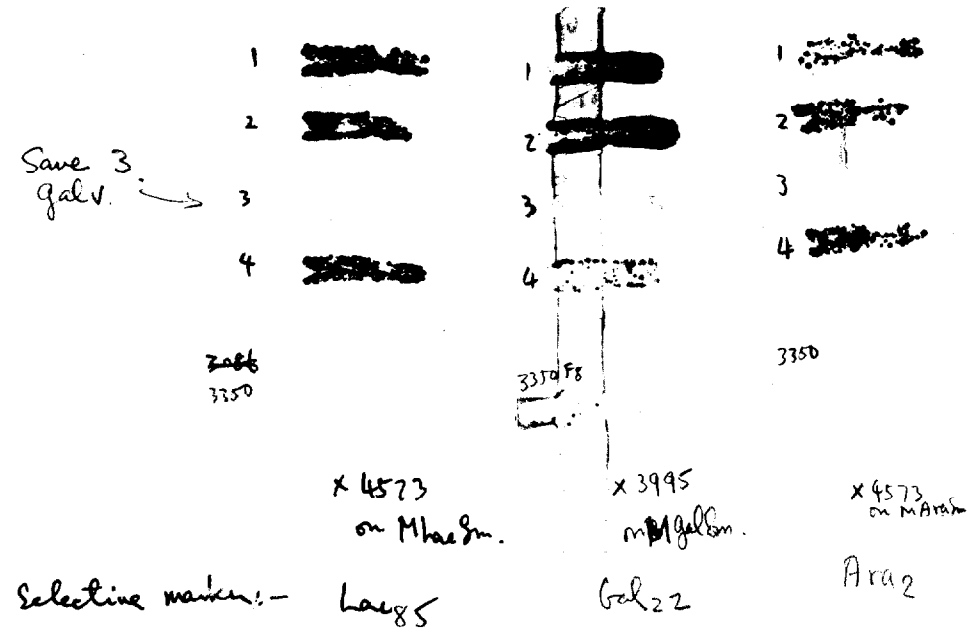
These strains are originally isolated as  $F^+$  or  $F^-$  from 3350/++ F8 with AD.  
 However, <sup>later on</sup> three of those strains back to F8. The phenomena to support the back mutation.

- a) 1, 2, 4 shows identical character of  $F_8$  after transfer to both. it recovers the fert:  $gal^+$  gradually.
- b). When these ~~columns~~ <sup>cells</sup> are ~~selected~~ plated and tested on mating types, it shows frustration of fertility. (transfer of  $gal^+$ )



on M glucose  
 X 3086  
 (M+ transfer)

Re - Test : Transfer of  $gal^+$  ( $gal_{22}$ ),  $Ara^+$ , and  $Lac^+$ .  
 3995

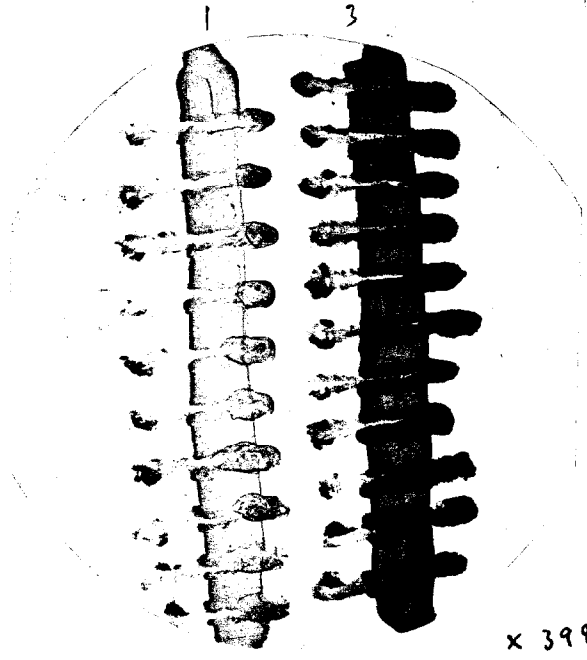


Test on Infectability. by F.

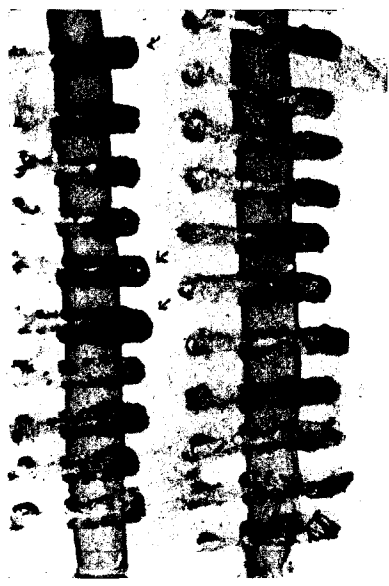
W6 - x(3350)A0  
F84?

12e

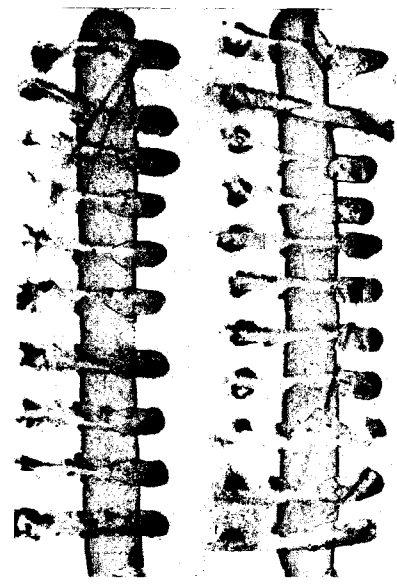
? remove!  
Rep. ...



x 3985  
on Hgal Sm



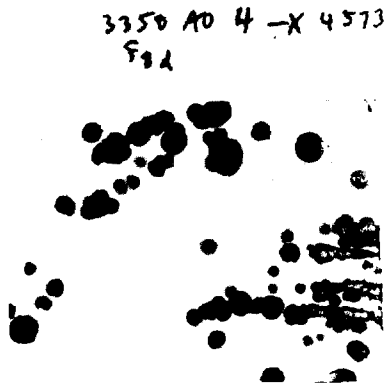
II  
I?



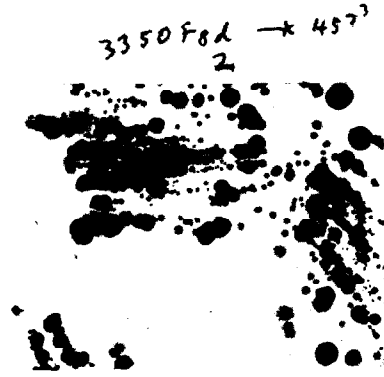
I  
IV?

on Hgal Sm.  
x 3975  
Zul22.

Transducibility of gal<sup>+</sup> (4573.)



on ~~gal~~ B gal. Sm.



on B gal Sm.

Transducible for gal<sub>2</sub>



Infectivity of  $F^+$  character of 3350FA.  
obtained by AO-method REF:

19/v; 1960.

	1	2	3	4	5	6	7	8	9	10
1		1. fertility of $F_x$ . (-x Lac; -x Hgal.;								
2										
3										
4		0.								
5										
6										
7										
8		2. Infectivity of F. to 4573.								
9										
0										
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										

All these  $F_x$  mutate back to  $F_8^+$  and infect  $F_8$  to  $F^-$

Timing experiment of F13

5/8 : 1960

REF:

1  
2 Strain : 3747  $M^{+} V_6^{R} Lac^{+}$  ; 4637  $V_6^{S} pur^{-} V_6^{S} Lac^{85} F^{-}$   
3 Cultural age : overnight grown cell (10ml per 100ml) : 11:00 AM — 3:00 PM at 37°C.  
4 0.2ml 4hr. on rotator.  
5 Media selected : DO, Mlac.  
6 (pur) (pur, Lac)  
7  
8 Ratio of mix. Ratio ♀ / ml : ♂ 9ml.  
9 Blending : gage 70 : 1 min. Top for mating :  
10 at 37°C.

Result	PH.	2:30	2:35	2:37.5	2:40	2:45	2:50	2:55
Time	Marker selected	Time after interruption (min.)						
Media	Marker selected	0	5	7.5	10	15	20	25
M Lac ( $10^{-3}$ ml)	Pur Lac	0, 0	0, 0	0, 0	0, 0	2, 1	0, 0	10, 18
DO ( $10^{-3}$ ml)	Pur.	3, 1	2, 0	0, 0	0, 1	9, 8	15, 9	101, 95
EMB Lac ( $10^{-6}$ ml) E		0, 0	0, 0	0, 0	0, 2	0, 0	0, 0	2, 0

Pick pur<sup>+</sup> colonies from DOs (10', 15', 20', 25') and inoculate it on DO.  
Test  $V_6^{R/S}$ ,  $F'$ , and  $Lac^{+}$  by replicating on Blec, Mlac Sm. ~~+~~  $pur^{-} S^{R} F^{-}$ ,  $V_6$  plate.

Media	Lac	# of Pur <sup>+</sup> Tested		F <sup>13</sup>	F <sup>13</sup>	F <sup>13</sup>	F <sup>13</sup>
		$V_6^{R/S}$	s				
DO	Lac <sup>+</sup>	0	9	9	19	170	170
DO	Lac <sup>-</sup>	1	3	3	2	22	22

- Further test:
- ① Test sex compatibility.  $V_6^{R/S}$   $pur^{-} S^{R} F^{-}$  → cross with  $V_6^{R/S}$   $pur^{-} S^{R} F^{-}$ . Replicate on  $pur^{-} S^{R} F^{-}$  or DO on gplate.
  - ② Is it  $V_6^{R/S}$  or R or s?
  - ③ If it is F<sup>-</sup>, infect F to it and see whether it becomes F<sup>+</sup> or F<sup>+</sup>.

1  
2  
3  
4  
5  
6  
7  
8  
9  
0

Infection of  $F^-$  to  $Pur^+ F^- Lac^-$  which obtained by

11/V. 1960 blending experiment.

REF:

1 2 3 4 5 6 7 8 9 10

• purpose of this experiment:

① Infect  $F^-$  to  $Pur^+ F^-$  obtained by interruption of  $F^-$  division and see what comes out.  $F^+$  or  $F'$ ?

② Does it segregate  $pur^-$  or  $V_6^R$  marker?

• Experiment:

1. pick 5  $Pur^+ F^-$  strains.

3747  
M  $V_6^R$

→ W4637  
 $F^- pur^- V_6^R Lac^+$

2. Test  $T_6$  resistance.

11/V

3. Infect  $F^-$  to

$Pur^+ F^-$   
2-5

$Pur^+ F^-$   
# 7

4. purify them by cross

x  $F^- pur^- S^R$   
W4628

on B<sub>12</sub> agar pick  $Lac^-$ . Test compatibility on ~~B<sub>12</sub>~~ Sm. by replica plating method. ( $F^-$  control W6)

• Result:

$F'$  or  $F^+$  obtained

No. of Isolates tested

Isolation No.	2	3	4	5	6	7
%	10	10	9	9	10	10

all of these isolates were  $F^-$ .

No recombination reaction on M<sub>1</sub> Gluc. Sm.

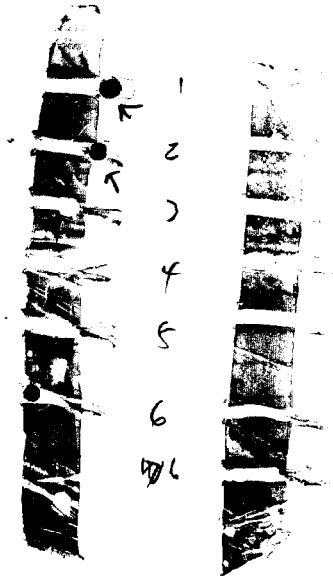
X 4628  $pur^- S^R F^-$

• Conclusion: Are these  $pur^+ Lac^- F^R$ ?

Take control  $F^- Lac^+ S^S$  and try this exp. again.

+wb

-wb



x3086

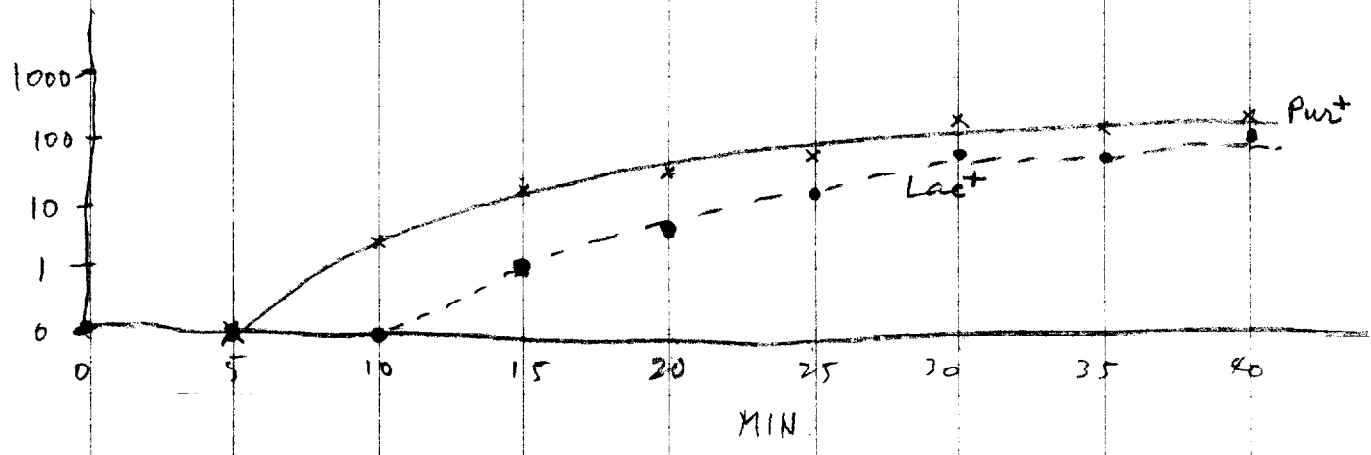
x3086

on 14lac8m

~~Make Strain W4354 F8<sup>+</sup>~~ REF: ~~W4354 F8<sup>+</sup>~~  
10/11/1960 Timing experiment.

	1	2	3	4	5	6	7	8	9	10
1	o Cultural age: 0.2 ml overnight, phasing grown culture / 10 ml phasing broth <span style="float: right;">on rotator at 37°C</span>									
2	o Strains used: <span style="float: right;">11:05 → 4:05</span>									
3						Ratio				
4						10 ml.				
5		3747	M U <sub>6</sub> <sup>R</sup> F <sub>13</sub>							
6		4637	Pur Lac <sub>85</sub> <sup>F-</sup>			1.1 ml.				
7	o Time of interruption: 0' 5' 10' 15' 20' 25' 30' 35' 40'									
8	o Experiment:									
9										
10										
11										
12										
13	Media selected	0'	5'	10'	15'	20'	25'	30'	35'	40'
14	Markers selected									
15										
16	DO Pur <sup>+</sup>	0, 0	0, 0	2	13	29	65	227	151	187
17	Dil. vol 10 <sup>3</sup> :0.1			1, 1	5, 8	18, 11	22, 43	111, 112	78, 73	97, 90
18			(Lac <sup>+</sup>	.0	4	6	23	60	48	63
19			Lac <sup>-</sup>	2	9	20	22	72	32	29
20	M Lac <sup>+</sup> Pur <sup>+</sup> Lac <sup>+</sup>	0, 0	0, 0	0, 0	0, 1	2, 4	5, 10	33, 61	37, 22	58, 75
21	Dil. vol 10 <sup>3</sup> :0.1				1	6	15	94	59	133

Interruption: gage 70 : 1 min. Temperature 37°C on rotator.  
4628 F<sup>-</sup> pur<sup>-</sup> SR<sup>0</sup>



Timing of F<sup>-</sup> presence.



Test on phosphatase activity, production of K-12.

26/V. 1962

Substrate: *o*-nitrophenyl phosphate

REF:

	1	2	3	4	5	6	7	8	9	10	
		Lo: phosphate medium.									
1		1.) Semiquantitative test:									
2							grow up <sup>W6</sup> cells in <i>l</i> <sub>p</sub> -medium.				
3							for overnight				
4							↓				
5							C.f.g				
6							↓				
7							resuspend it in 1ml H <sub>2</sub> O				
8							↓				
9							autolyse it with 2 drops of benzene.				
0							↓				
1							1ml of				
2							Add tris-buffer pH. 7.5 1M.				
3							+ 400 μ/ml <i>p</i> -nitrophenyl phosphate.				
4											
5											
6											
7											
8											
9											
0											
1											
2											
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9											
0											

Lo: phosphate medium.

1.) Semiquantitative test:

grow up <sup>W6</sup> cells in *l*<sub>p</sub>-medium.  
for overnight  
↓  
C.f.g  
↓  
resuspend it in 1ml H<sub>2</sub>O  
↓  
autolyse it with 2 drops of benzene.  
↓  
Add <sup>1ml of</sup> tris-buffer pH. 7.5 1M.  
+ 400 μ/ml *p*-nitrophenyl phosphate.

2.) qualitative test on agar plate:

Test on 1.5% agar + *l*<sub>p</sub>-agar medium.

Spot testing strain on the medium.

Incubate it for 3 hrs. 3:00 PM ~ 6:00 AM.  
↓  
or overnight 3:00 PM ~ 10:00 AM

Spot 400 μ/ml of *p*-NPP on that agar.

autolyzed with CHCl<sub>3</sub> → No autolysis.

Incubate it for 10' at 37°C

Conclusion:

- 1.) 4 mg/ml of *p*-NPP work well. or -*p*. med. (see back page)
- 2.) Selection <sup>by NPP.</sup> seems not work.

Low phosphate medium.

gr.	l.
✓ 3.0	Nacl
✓ 0.25	MgSO <sub>4</sub> · 7H <sub>2</sub> O
✓ 0.01	CaCl <sub>2</sub>
✓ 2.0	Na-Lactate
✓ 10.0	Difco-Bactopecton
✓ 12.0	Tris (hydroxymethyl) amino-methan

pH. 7.4

500 ml + 15.75 g/l

Ref. Nature 183, 1529, (1959)  
(No. 4674).

C.B.A. 3L, 570 (1959)



Test genetic marker of Garen's strains.

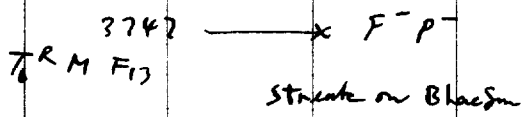
1960

REF:

1) Test markers.

Strain No	3 B <sub>lac</sub>	4 D <sub>gal</sub>	5 B <sub>gal</sub> B <sub>lac</sub> Sm	6 B <sub>gal</sub> B <sub>lac</sub>	7 H <sub>lac</sub>	8	9	10
	Lac	gal	Sm.	T <sub>G</sub>	Sax. (x4354)	Phosphatase		X
F <sub>7</sub>	-	-	R	S		-		+
F <sub>24</sub>	-	-	R	S		-		+
F <sub>13</sub>	-	-	R	S		-		+
F <sub>18</sub>	-	-	R	S		-		+
4573 P <sub>1</sub>	-	-	R	S		-		+
<del>4573</del> P <sub>2</sub>	+	+	R	S		+		+
3747	+	+	R	R	P-NPP; 4mg/ml.	+		+

2) Infect F<sub>13</sub> to P<sup>-</sup>



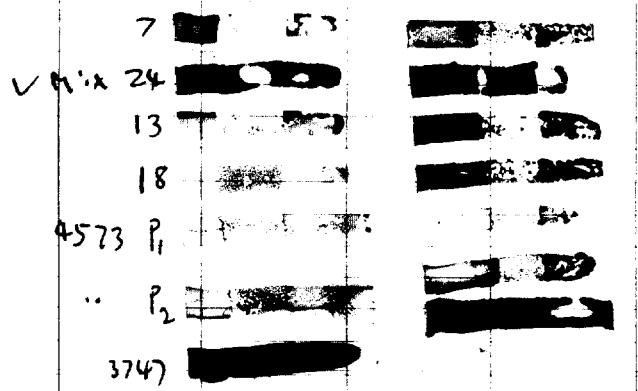
Result: Surprising enough, Lac<sup>-</sup> of those Garen's strain are not transmissible by F<sub>13</sub>.  
 all the colonies on B<sub>lac</sub>Sm shows Lac<sup>-</sup>!

Further test

1. Test maleness: transfer of lac x 4573 on H<sub>lac</sub>Sm. If it has F<sub>13</sub>, some Lac<sup>-</sup> of Garen's strain must transduce Plac<sup>+</sup> to 4573.
2. Test resistance to transduction of F<sub>13</sub> of Garen's strain x 3747 on H<sub>lac</sub>Sm.

Possibilities

- ① Garen's strain is resistance to F<sub>13</sub> infection
- ② Lac marker of Garen's are not transmissible by F<sub>13</sub>.



7  
24  
13  
18  
P<sub>1</sub>  
P<sub>2</sub>  
3747

B gal Sm.

on low P med.

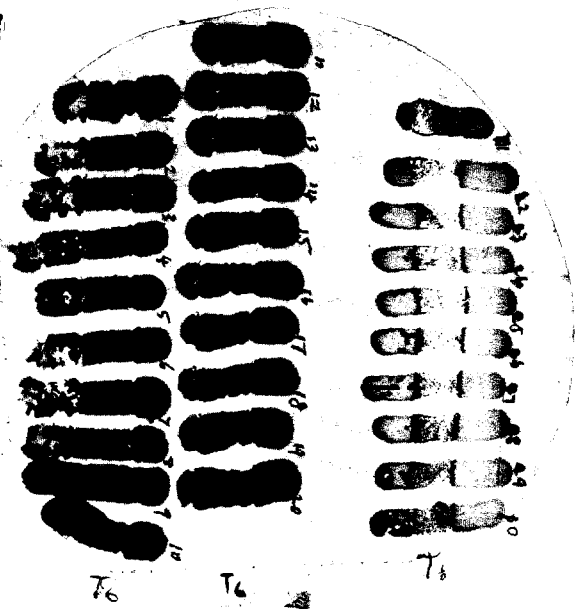
normal control  
 1cc | 1cc

normal control  
 1cc | 1cc

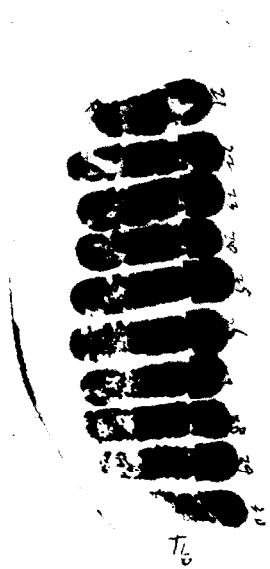
2247

F<sub>7</sub>  
 F<sub>29</sub>  
 F<sub>13</sub>  
 F<sub>18</sub>  
 P<sub>1</sub>

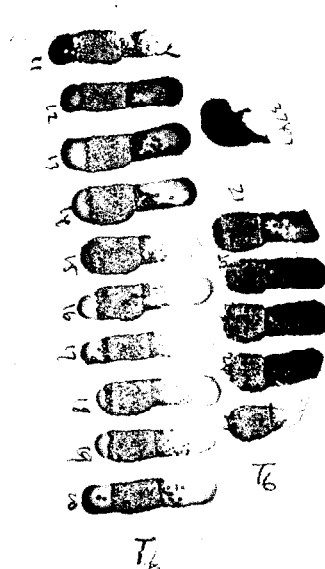
Co-phenyl med  
 p-phenyl-phosphate 4mg/kg



on base



T<sub>6</sub>



T<sub>6</sub>

on base

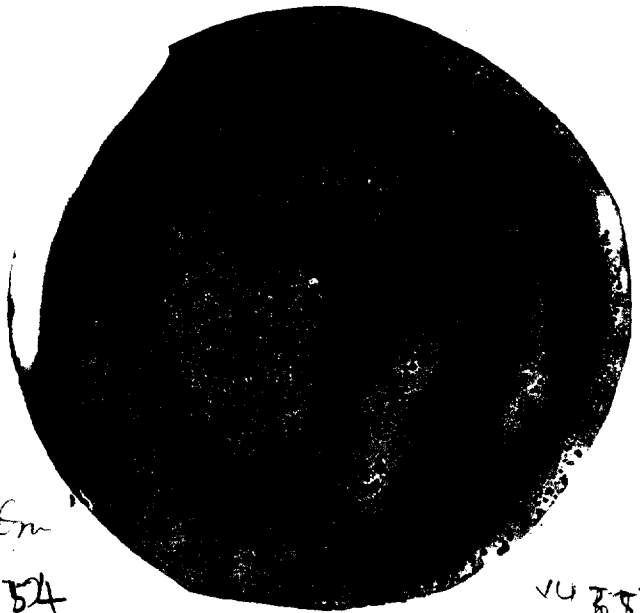
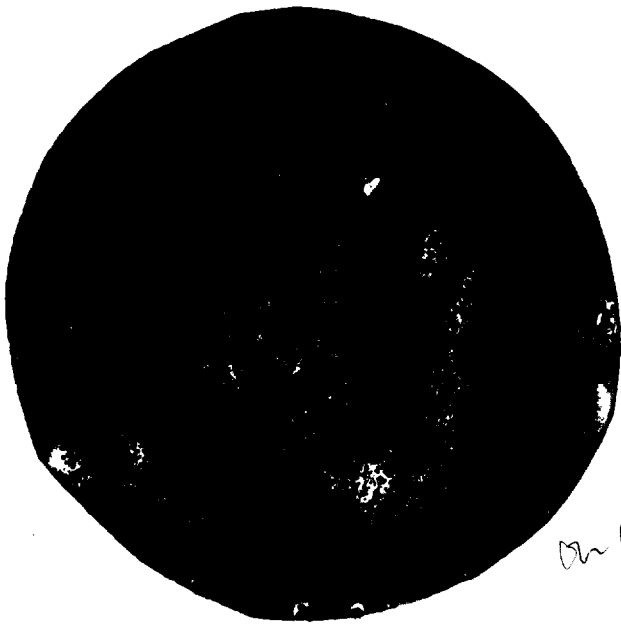
3747  
 on base

f73

3747 \*

f7

15c



on Mhaem  
X 4354

VU 354



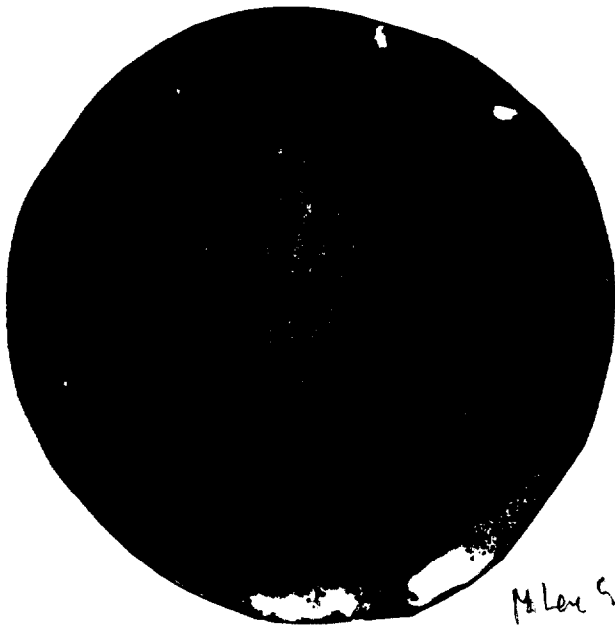
m BlacSm

BlacSm

F18

F24

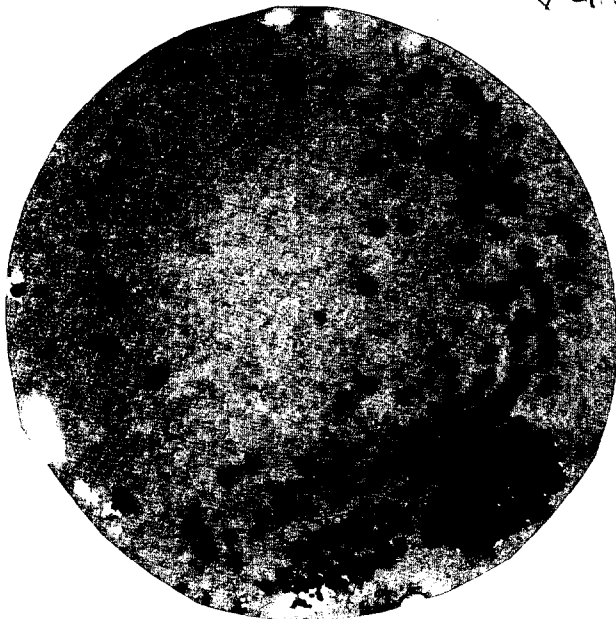
15 d



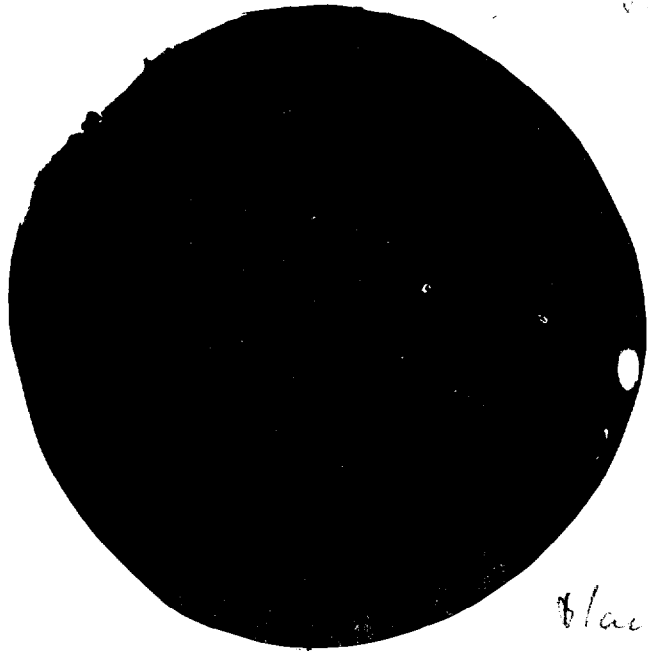
M. Loe S  
x 4750



M. Loe S  
x 4750



Blac S



Blac S

Test transducibility of F<sub>11</sub>, F<sub>15</sub>, F<sub>19</sub>  
on Try-transduction.

20/V 1960

REF:

1  
2  
3  
4  
5  
6  
7  
8  
9  
0

Tester : 4090 F<sup>-</sup> S<sup>r</sup> Try (Ind<sup>+</sup>) F<sup>-</sup> Strain : W4580 : F<sub>15</sub>  
4091 F<sup>-</sup> S<sup>r</sup> Anth (W4277 gal<sup>-</sup> W4278 gal<sup>-</sup>) W4582 : F<sub>19</sub>  
W4522 : F<sub>11</sub>

1.) Test Hfrness on Mlac Sm. by spot-test.  
of on Mlac. (See below)

1  
2  
3  
4  
5  
6  
7  
8  
9  
0

2) If H<sub>1</sub> : Test Co-transduction of Gal & Try  
Tester 4277 : gal<sup>-</sup> Anth<sup>-</sup> F<sup>-</sup>

Ratio of Mix. : 4277 0.1 ml : 4580 0.2 ml : 5 ml pen.  
" " : 4582 0.2 ml : 5 ml pen.  
" " : 4522 " : " "

Method :  
1. Streak the inoculated mix on B gal Sm.  
2. Replica-plate them on D.O. or Mglucose.

Transduction of	4520 x 4277		4522 x 4277		4580 x 4277		4582 x 4277	
	+	-	+	-	+	-	+	-
gal <sup>+</sup>	167	36	43	2	20	68	0	44
	169	25	24	1	1	54	0	51
Try (Anth <sup>-</sup> )	0	203	0	45	1	69	0	44
	0	174	0	25	1	54	0	51
gal <sup>+</sup> Try <sup>+</sup>	0	377	0	70	2	123	0	95
% of Try <sup>+</sup>	0		0		1.6		0	

4580 4354 4580 4354 4582 4580 4582 4582 4522 4522 4520 4520

4354 4090 4091

on Mlac on Mglucose

Conclusion : F<sub>15</sub> maybe transducible for Try. (Probably there is linkage in gal & Try)

# Transduction of $F_8$ or $F_{11}$ by $\lambda$ .

3/1/60

REF:

1	2	3	4	5	6	7	8	9	10
---	---	---	---	---	---	---	---	---	----

Purpose: 1) Is  $F_8$  or  $F_{11}$  transduced by  $\lambda$ . F may be on gal segment.  
2) Make  $F'$  which carries  $\lambda$  with it.

- Principle:
- 1) 4520 ( $F_8$ )  $\times$   $Lp^+ gal^- F^-$  W3097
  - 2) 4522 ( $F_{11}$ )  $\times$   $Lp^+ gal^- F^-$  : W3097.
  - 3) 4520 ( $F_8$ )  $\times$   $Lp^+ gal^- F^-$  + W6 (as F donor)
  - 4) 4522 ( $F_{11}$ )  $\times$   $Lp^+ gal^- F^-$  + W6 (as F donor)

Experiment:

(A) 1. UV-irradiate young culture of 4520 or 4522. 1ml of 4 hrs on rotator <sup>in 8-10% c.f.g</sup> & resuspend it in DO. Irradiate it for 10 sec. Add 8ml of pen. Incubate then for ca. 3 hrs. at 37°C on rotator, and keep it in refrigerator for overnight. at 5°C. to complete lysis. (4:00 ~ 6:45 PM)

Treat with 2. CHCl<sub>3</sub> and make cell free lysate of  $\lambda$ .  
3. Add those  $\lambda$ -lysates to W3097 with <sup>0.1ml</sup> & without <sup>1ml</sup> W6, and incubate it for overnight at 37°C. 1ml; pen 1ml. ~~Mix with the lysate.~~

4. Seed those transductants onto Mgal medium. Count gal<sup>+</sup> transductants and figure out <sup>rate of</sup> F-duction.  
5. Pick gal<sup>+</sup> and test maleness on Mgal Sm in cross  $\times$  3997 ( $F^+ gal^- S^A$ )

Stability of  $\lambda$ -lysates were tested at the same time 0.1ml / 5ul pen.

Experiment:

(B) Test rough estimate gal<sup>+</sup> F<sup>-</sup>  
1.) Mix 1ml pen.; 3097  $\times$   $\lambda$  4520 ;  $\lambda$  4522 ; 3997  
2.) Incubate the mix for 1 hr. (4:00 - 5:00 PM)  
3.) plate 0.2ml of the mix onto Mgal Sm.

Result:  $F_8 \lambda \times$  3997  $F_{11} \lambda \times$  3997

# of gal<sup>+</sup>/plate:

2	7
3	1
4	0
2	0
<hr/> Σ gal <sup>+</sup> 11	<hr/> Σ gal <sup>+</sup> 8

Mix : 3:00 PM.  
Add penicillin: 8:00 PM.  
10ml  
Incubate the for overnight.  
11:00 PM.  
 $\lambda$  lysate was sterile.







	10'	15'	20'	25'	30'	35'	40'	45'
n1	3	26	24	23	11	7	1	2
n1 V61	0	0	2	3	2	0	0	0
n1 V61 Lac1	0	1	2	0	0	0	0	0
n1 V61 Lac <sup>1</sup> Sex	0	<del>1</del>	<del>27</del>	<del>24</del>	<del>41</del>	57	57	59
Σ (tested)								
n1, V61 Lac <sup>1</sup> Sex		Pin	V61	Lac	Sex	<del>V61</del>	<del>Lac</del>	<del>Sex</del>
	0	0	0	0	0	0	0	1
Σ	3	28	26	26	50	54	58	59



Test of  $\phi$  segregation from  $\phi$  obtained by interrupted mating exp. REF:

10/11 1960

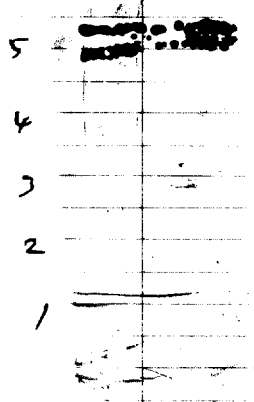
	1	2	3	4	5	6	7	8	9	10
1										
4										
15	20'	P <sub>int</sub> <sup>+</sup> V <sub>6</sub> M	Lac <sup>+</sup>	F <sup>-</sup>	Lac <sup>U</sup>					
27	25'	" V <sub>6</sub> M	Lac <sup>U</sup>	F <sup>-</sup>	Lac <sup>U</sup>					
39	25'	" V <sub>6</sub> M	Lac <sup>U</sup>	F <sup>-</sup>	Lac <sup>U</sup>					
4	25'	" V <sub>6</sub> M	Lac <sup>-</sup>	F <sup>-</sup>	Lac <sup>-</sup>					
52	45'	P <sub>int</sub> <sup>+</sup> Lac <sub>85</sub> <sup>-</sup> V <sub>6</sub> <sup>R</sup>	F <sub>13</sub> ?	Lac <sup>-</sup>						
8										
9										
10										
1										
2										
3										
4										
5										
6										
7										
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if all three colony are purified on B lac agar and retested

Isolated from (time of interruption)

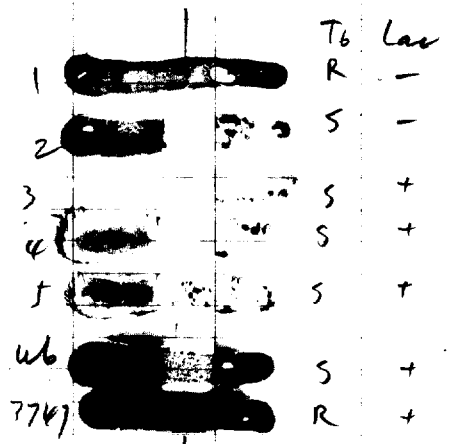
genetic marker

Retest.



\* 4147  
P<sub>int</sub><sup>-</sup>

miscellaneous



↓  
T<sub>6</sub>

B lac agar

Infectivity of F from 3747. to F<sub>3</sub>.

6/8 / 1960.





REF:

	1	2	3	4	5	6	7	8	9	10					
Principle.			3747	→	3876 F <sup>-</sup> G <sup>S</sup> Hfr <sub>3</sub> Mal <sup>S</sup> Lac <sup>+</sup> D <sub>3</sub> S <sup>R</sup>										
			F <sub>3</sub> Hfr <sub>6</sub>		x										
					W3637 F <sup>-</sup> G <sup>S</sup> M S <sup>R</sup>										
purpose:	Does F <sub>3</sub> contain F which is free from F <sub>3</sub> - Lac segment?									on <del>plate</del> M Lac Sm.					
			F <sub>3</sub> → 3876		→ Lac <sup>+</sup> F <sub>3</sub>										
			F → 3876		→ Lac <sup>-</sup> F <sup>+</sup>										
					→ Lac <sup>-</sup> Hfr <sub>3</sub> .										
Cultured age: PM 2:30 ~ pm 4:30	overnight culture														
28 hr.	Incubation size 1 ml / 10 ml penassay broth.														
	Ratio of mix 3747 10 ml : 3876 1.1 ml														
	at 37°C on rotator in penassay broth.														
Seed this mix on	B Lac Sm.														
										Dil. val: 10 <sup>-4</sup> :0.1					
Time	4:30		4:40		4:50		5:00		5:10						
Dil. vol	10 <sup>-4</sup> :0.1		10 <sup>-4</sup> :0.1		10 <sup>-4</sup> :0.1		10 <sup>-4</sup> :0.1		10 <sup>-4</sup> :0.1	10 <sup>-4</sup> :0.1					
Lac <sup>-</sup> /plate	308	325		359	283	296	296	260	274	233	191	155	182	180	134
Lac <sup>+</sup> /plate	0	0		0	0	5	2	11	16	28	20	36	36	69	23
% of Lac <sup>+</sup>															
F <sup>+</sup> /plate	0	0		0	0	5	2	11	16	28	20	36	36	69	23
% of F <sup>+</sup>															
Conclusion:	Separate infection of F from Lac <sup>+</sup> are not observed.														
	This data seems against <del>the</del> "defective-double F" hypothesis.														

Test on fertility of exogenetic and endogenetic segment.

26/4 ; 1960

REF:

	1	2	3	4	5	6	7	8	9	10
		Principle								
1			91			91	F <sup>-</sup>			
2										
3			52	F <sub>1</sub>		52	F <sup>-</sup>			
4										
5										
6										
7										
8										
9										
0						4112 F <sub>13</sub> (52/52)				Why did the low fertility F <sub>13</sub> ?
1										
2										
3										
4						4151/91 x 52				
5										
6										
7										
8										
9										
0						3747				
1										
2										
3										
4										
5										
6										
7										
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0										
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0										

on M<sub>1</sub>Lac<sup>+</sup>Sm