1. 4 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 P 7, 7' P 6a.
5. Isolation of F_{13} Lac 52 (m: ONPG+) : W4112. P 8, P 9,
6. Isolation of F_{13} Lac 87 (m: ONPG-) : W 4147 P. 10 a P10 b.
7. Iso

Lac^-_X F_{13}.

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. P. 9c
   Reversion test for homogenicity of Lac 52. : reversion test P. 9d P 9e
10. Preparation of λ ref. B120. P. 11
11. Elimination of F8 from 3350 F8 1-2-/ex++ F8 with AO : p12a ~ p12c, f
12. Timing experiment of F_{13} P 13a.
    P 13b. : Pur^+ F^-
    P 14 P 18 and Lac d: F^- rev.
13. phosphatase − P 15a~d
14. Size of transducible marker of F8 P 16
    Ind 4 + Auth.
15. Transduction of F_8, F_{11} by λ P 17
16. Infectivity of F from F_{13} : Separate transfer of F from F_{13} P. 19

Test a possibility of location of 4p+ on F8 segment.

4/14 1960

REF:

Experimental design:

4520 (4p+) ⟶ × 3110 (4p s)

1. pick F13+ by replica plating it on MGal Sm × 4573.
2. Test 4p+ by cross-streaking with λ of 3110. on B-O.

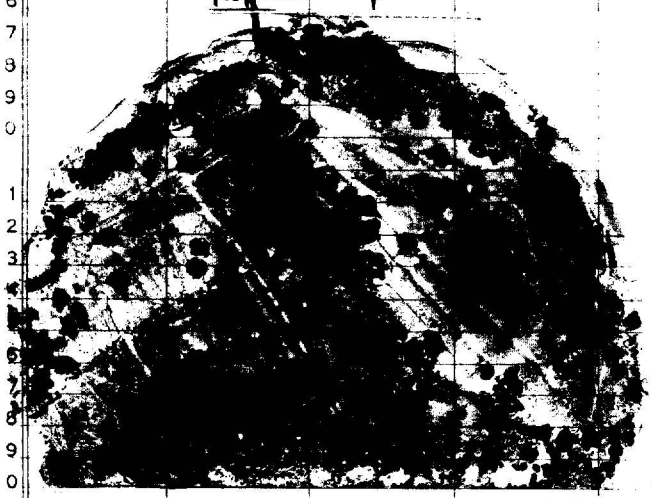
Result:

All the isolated F8+ show (20 isolates) 4p s trait.
Sensitive to λ & does not produce λ. (see back page).

Conclusion:

F8 segment <u>does not carries 4p locus</u> on it.

Replicated plate.



on MGal Sm
× 4573

Master plate.

4520 — × 3110 on DO

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Unsuccessful.

2

Transduction of Hfr, from 2252 ($4p^s$ Hfr, M^-) to Gal_x^- F$^-$.

21/II, 1960 purpose: Does P_1 ~~—~~ transduce Hfr_1? RE And Does it give F', F^+ or Hfr_{Tr}?

If$_4$ F': $_5$Hi for Gal$_6$
If Hfr: Hi for Lac

$_7$If F$^+$ can infect F$^-$ to F$^-$ by cell contact?
(see below)

1) Test Hfr, ~~&~~ F' recs.

~~#~~ 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. 10^9 P.I./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_1 to 4573. and keep it at 37°C for ~~2~~ 3 hrs. And then add 10 ml of L-broth to it. Shake it on rotator for overnight.

3:35 ~ ~~6:35~~ 6:30 at 37°C

○ After overnight shaking, test sex-compatibility of whole culture by cross-streaking, and purify it on BLac & test sex-compatibility by of 20 colonies of the isolates
× 4574
on M Lac.
. cross-streaking method on MLac. (× 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 which are seeded with 4573, and see mating reaction on the plate

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Make P_1 lysate on 2252 M Hfr, λ^s.

16/III 1960 REF:

order of addition:

1.
- 3 P_1 (original 10^9 → diluted (in NB 1/10) 1×10^8 /ml.) : Use 0.05 ml , (5×10^6) : grown on 3110
  & 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 ml
↓ Make double layer on L-agar;

3. Incubate it for 8 hrs. : at 37°C.
2:00 ~ 10:00. p.m.

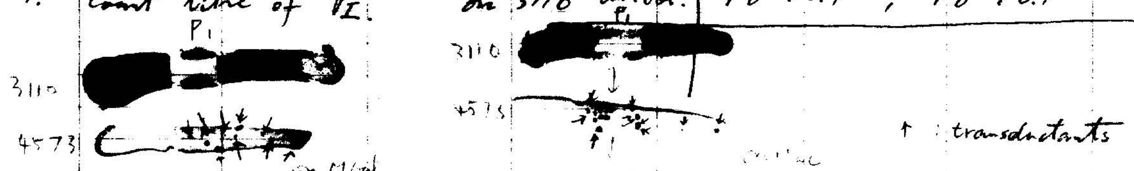
plates made:
1 : 0.05 ml
2 : 0.01 ml.

4. Add 3 ml of NB to each plate and extract P_1 with soft agar. add it to 10 ml blank & Homogenize it with pipette. Keep it ~~at room temp. for ... to extract it.~~ (in refrigerator to extract P_1.

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

6. Save it in vials.

7. Count titre of P_1 on 3110 dil. vol. | 10^4 : 0.1 ; 10^6 : 0.1



2nd trial:
0.1 ml of P_1 lysate (from 2252) + 0.5 ml of 2252 + 2 ml of soft-L agar
Penassay grown,
Conc. of $CaCl_2$: 0.005M : final. conc.
↓ pour 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 1M NaOH : 0.4 ml / 100 ml L-broth
to make pH 7.0.

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

29/4 ; 1960

Lac
Pur V6 REF

Principle :

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

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

③ Test size of new F_{13} brother: / If it is V_6^S Homogenote : R/3 S/S
V_6 resistance. V_6^R : R/R S/R
Heterogenote : R S.
$\times V_6^R \times V_6^S$ Progeny become.

Design :

1. Make W4637 F_{13}. a.) by infection. 3747 → × 4637.
Streak on ~~Mac~~ Mlac pick 5.
b.) purify it on ~~Blac Do~~ B lac
Test X^+ on 9:2. Save ~~Lac~~ pur^+.
c) Test maleness by cross × F^- ~~Lac~~ 4573 on MLacSm.

Ratio of mix : 1 : 1
optimal age ; Overnight

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.

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

save X^- Lac^+ or X^- Lac^+ or F^- Lac^+
3. Infect it the next F_{13} brother to 4574, and test markers transduced to F^- (esp. V_6) Test maleness too, by replicating onto MLac seeded F^- M^- (W4354)

Experiment :

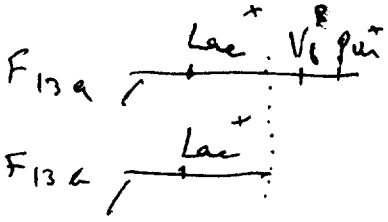
~~All~~ Almost of X^- Lac^+ are Lac v.

Lac^+ X^-	# of Lac^-	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 V_6, Pur and their maleness replicating on T_6 plate, DO, DO + Pur B, and MLac × 4573.
Save V_6^S Lac v F_{13}^+

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

Expected trait of broth from F_{13}, b

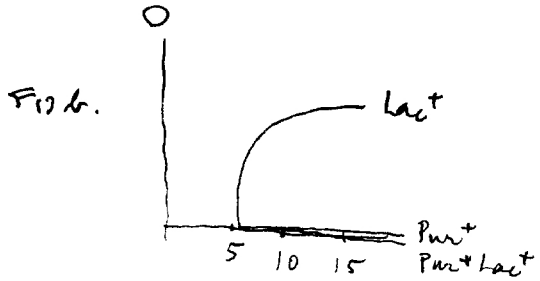


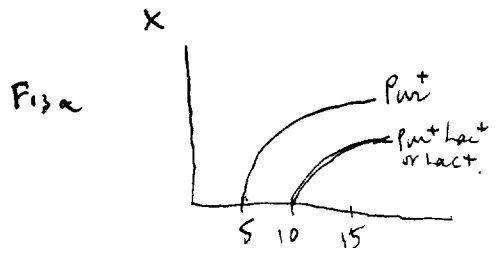
1. V_6 Transfer.

→ × V_6^S : S ∴ hemizygous.

→ × V_6^R : R.

2. Timing.





Isolation of sisters F8 from F8⁺.

29/iii ; 1961 REF:

Principle : (1). 4520 ⟶ W3350 : 3350 F8 $1^-2^-/++$ 1^- 2^- / + + exo.

(2). UV 3350 F8 —— Look for gal⁻ ♂.

(3) Save such strain and test transfer of other gal⁻ markers.

If it is F8⁺ exconjugants, it is 1^- homo or 1^- hemi. $^{+/2^-}$ $^{+/2^-}$

" " " F8⁺ sisters, it has deletion in it. 1 or 2 and etc.

Method to differentiate between two. Cross it on Mgal

F⁻ 1⁻ S^R — and look for Rec.⁻ reaction in
F⁻ 2⁻ S^R — cross. x several gal⁻ F⁻.
F⁻ 3⁻ S^R
. 4⁻ .
. 5⁻ .
. 6⁻ .
. 7⁻ .
. 8⁻ .

Experiment :

1. Use overnight culture of 3350 F8.
2. Irradiate with UV. for 10 sec. / plate : survival : too much. ~10³~10⁴.
Gal⁻ colonies are picked and inoculated on Bgal.
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 mixture of + + −. (gal⁻ : ~~72~~ 68), probably the isolates were galv.

b. ~~which gives recombination~~

	# of isolates /68.
gal⁻ No. of gal⁻ reaction in cross x gal₂ (4579) on MgalSm.	2 (F°8a, F°8b)
" " " " x gal₁ (3991) " " "	0
" " " " x gal₄ (3994) " " "	0
# of gal⁻ colonies which does not gives any rec. reaction	66

Further work : (Test transducibility of newly isolated gal to other galₓ. Make 4354 F8a, 4354 F8b. and repeat same cross (for example : 3, 5, 6, ……n. F⁻S^R) on MgalSm. + on Mgal.)

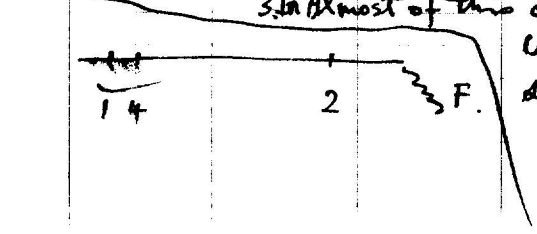
Tentative speculation from this result :

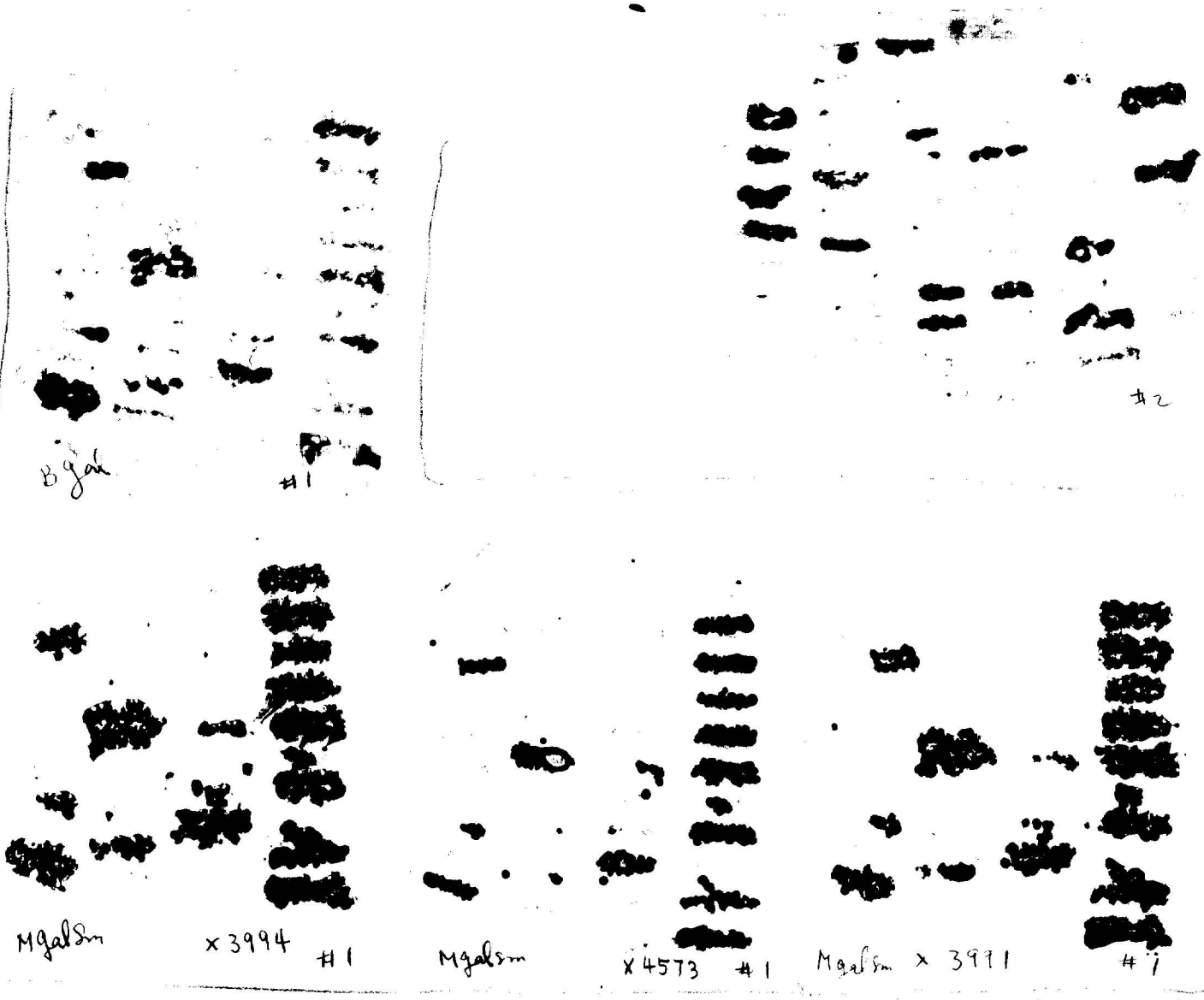
1. gal₂ is linked with F°, and not closely linked with gal₁ and gal₄.

~~2. gal₁ and~~

2. gal₁ and gal₄ is probably linked and is other end of gal-segment.

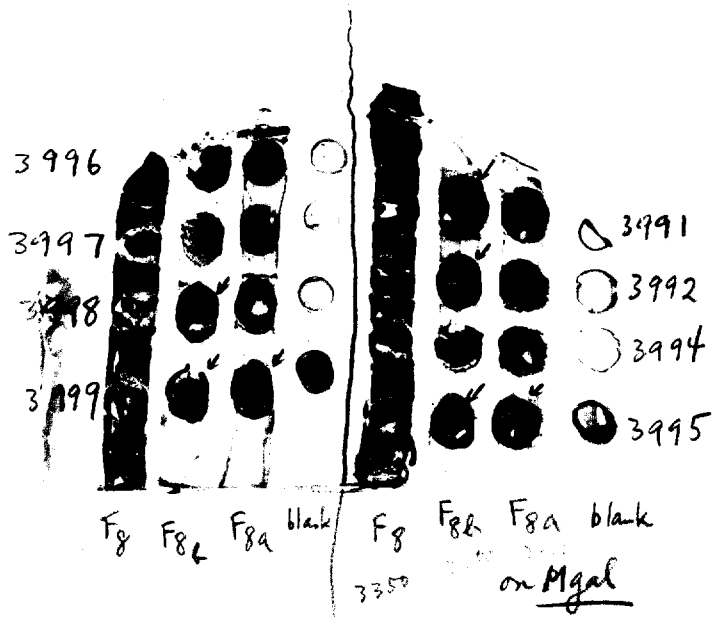
3. In Almost of this case, whole gal-segment will be eliminated further after U.V.-irradiation or by spontaneous loss of the segment from heterozygous diploid stage.

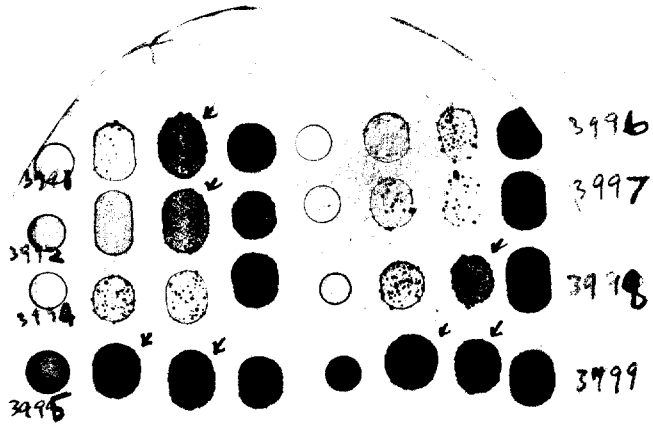



Bgal
#1
#2
MgalSm
x3994
#1
MgalSm
x4573
#1
MgalSm
x3991
#1
#2
x3994
MgalSm
#2
x4573
MgalSm.
#2
x3991
MgalSm

29/IV; '60

5'





Conclusion: parallelism can be seen in both plates, but less complementation (recombination) reaction 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'ss for the purpose.

8 9 2 1 , 4 6 7

+ −

F_8a —— × 4354
F_8b —— × 4354

9/10/1960 REF: cf.

purpose : To get standard F_8 existent strains.

Expt'l. condition
cultural age : overnight culture in 5 ml penassay broth.
Infection :
0.1 ml F_8a or F_8b + 0.1 ml 4354 + 5 ml penassay broth.
↓
Incubate the mix at 37°c for overnight.
↓
Streaketon Bgal, pick Gal+ and ~~[illegible]~~
Test sex-compatibility on Mgal × 3995.
" × ~~4023~~ 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 × 4354		F_8b × 4354	
# of colonies tested	♂(F_8a^+) obtained	# of colonies tested	♂(F_8b^+) obtained
14	13 (save one)	15	15 (save 1).

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

See back page. #1

(2). complementation. (on Mgal. × $F^- 5^R$ gal$^-_{1-9}$)

		1	2	4	5	6	7	8	9
W4354 (M^- 1)	F_8a^+	−	−	−	+	−	−	−	+
W4354 (M^-)	F_8b^+	+	+	−	+	−	−	+	+
control W 4520 ($M^- F_8^+$)		+	+	+	+	+	+	+	+
4354	F_8 c$^+$	−	−	−	+	−	−	−	+

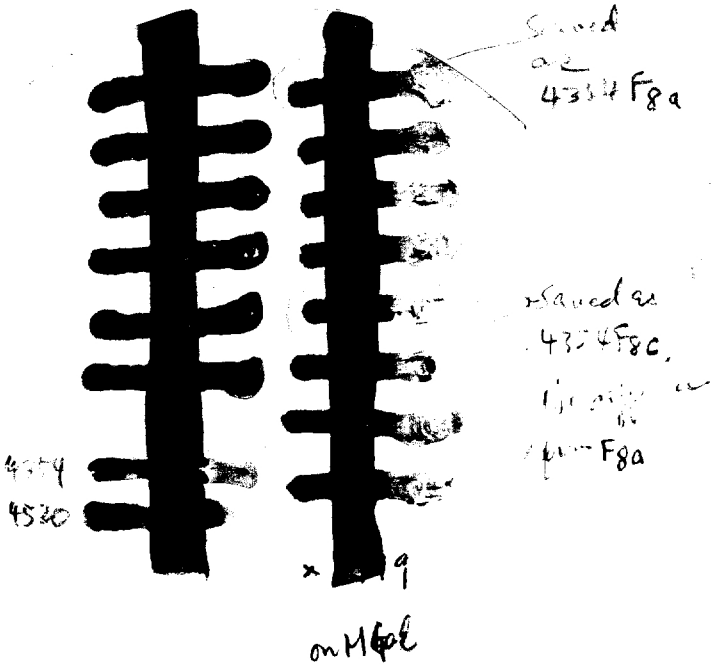
(colony obtained from F_8 a infection
See back page. #1)

Conclusion : 3 groups can be classified.

5 9
5 9 1 2 8
4 6 7

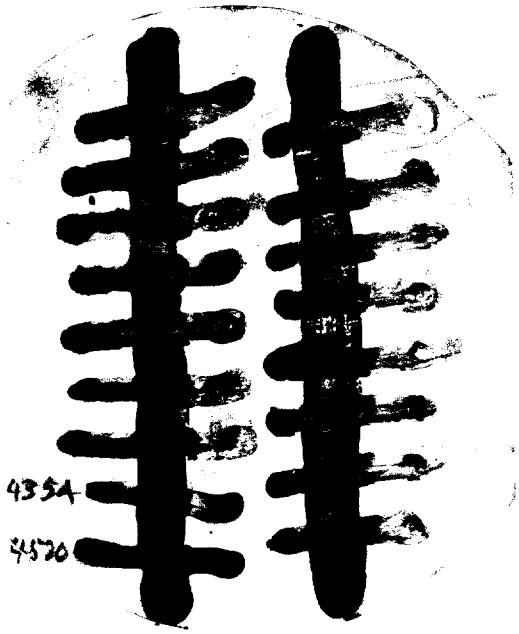
1

3350 F8a → × 4354



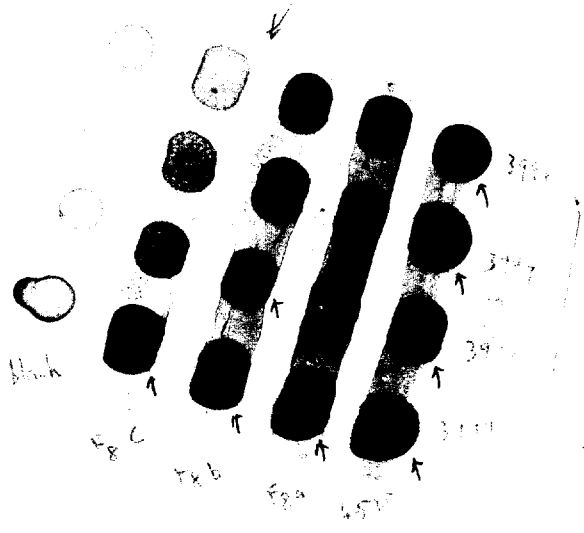
on MGal

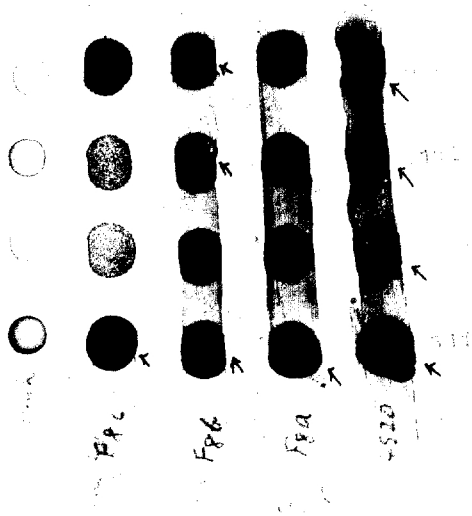
3350 F8b. → × 4354



× 3999
on MGal

2





Compare this with p.5'

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

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

1/VI ; 1960 REF:

1	2	3	4	5	6	7	8	9	10

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

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

↓

Incubate the mix for overnight.

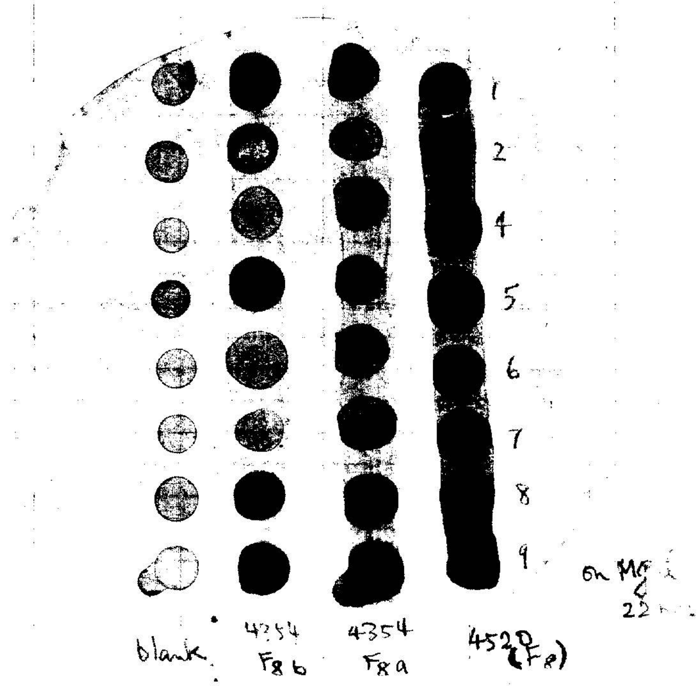
↓

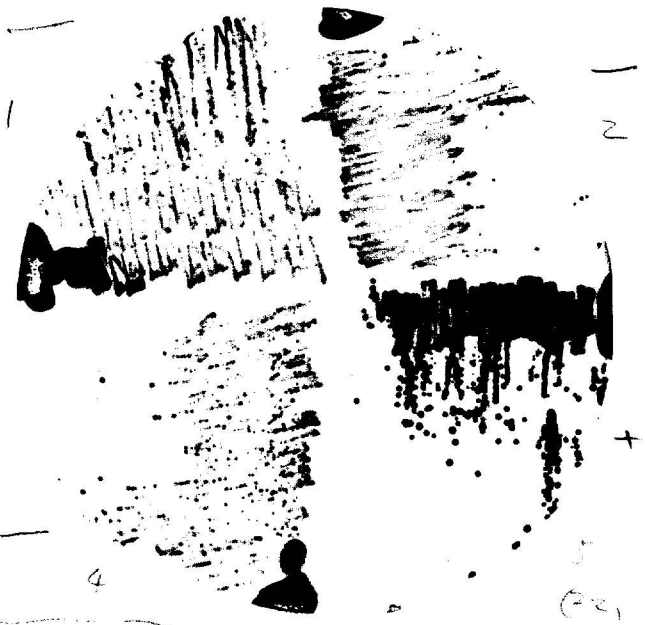
Streak the mix on B gal Sm.

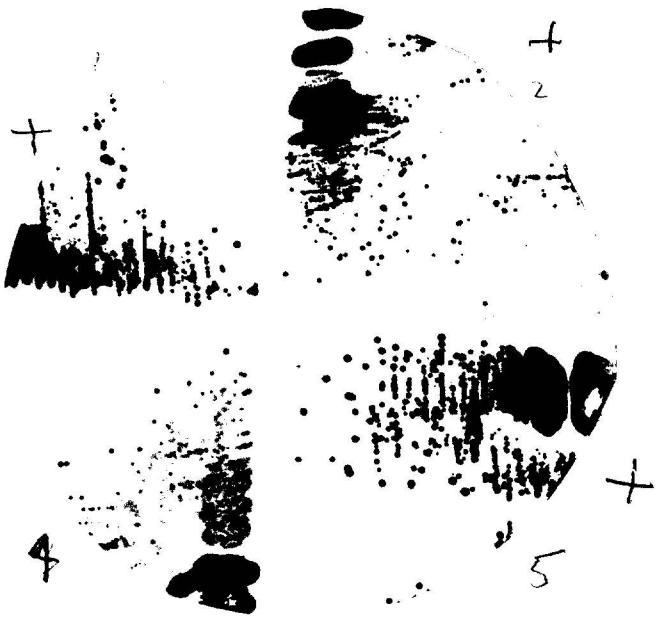
Result: F_8 Donor	cont 4520	4354 F_{8a}	4354 F_{8b}
3991	+		+
3992	+		+
3994	+		−
3995	+	+	+
3996	+	-	−
3997	+	-	-
3998	+	-	+
3999	+	+	+
		− 1,2,4,6,7,8 / + 22,9	− 4,6,7 / + 1,2,22,8,9

See back page.

Exp II : Make spot test on M gal & M gal Sm.







2nd isolation of gal- F8 sisters.

; 1960 REF:

Strains used : 3350 F8
Time of UV.: 15 sec.
on B gal.

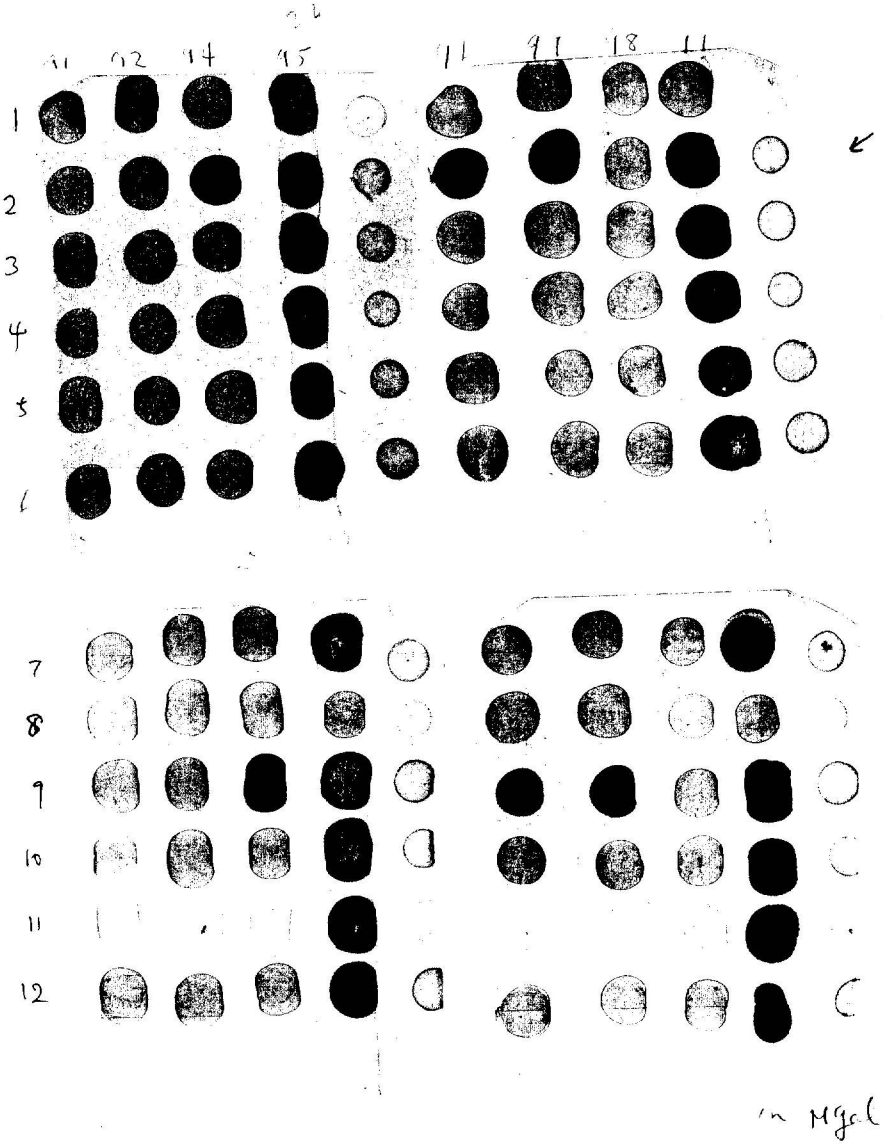
① Survival :

Plate No.	gal⁻	gal u	gal⁺
1	15	5	141
2	19	7	114
3	3	3	66
4	4	2	87

② Inoculated on B Gal. a) Replicate it on M Gal Sm. × F⁻ gal₁, gal₂, gal₅.
b) Look for Rec.⁺ cells.

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

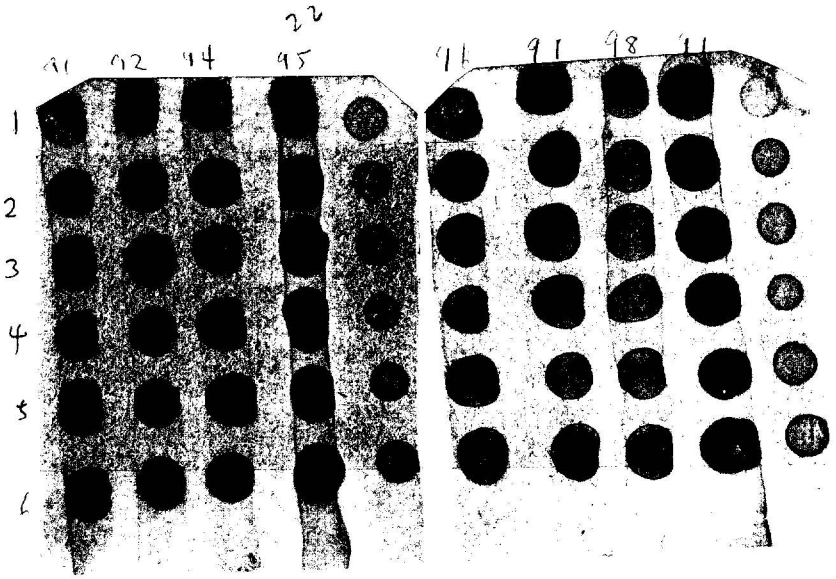
Maleness	F8 type	Number of F8
	Covers ▲ gal	
♂	● 5, 9	44
♂	5, 9, 4, 7, 6	2
♀.	No (F⁻)	● 9
	Σ	55

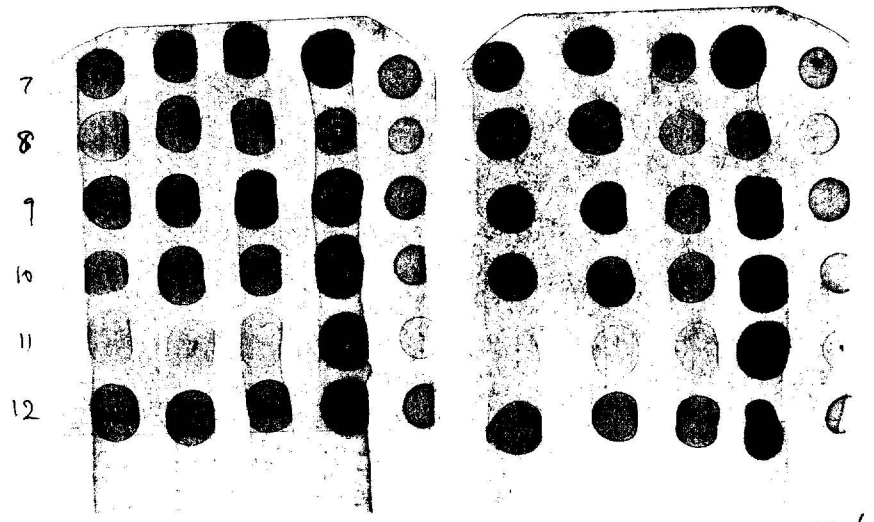


↙ 3350 F8C

	—	+
a	deletion	
b	1 2 8	4, 22, 6, 7, 9
c	1 2 4 6 7 8	22, 9
d	4 6 7	1, 3, 22, 8, 1

in Mgal

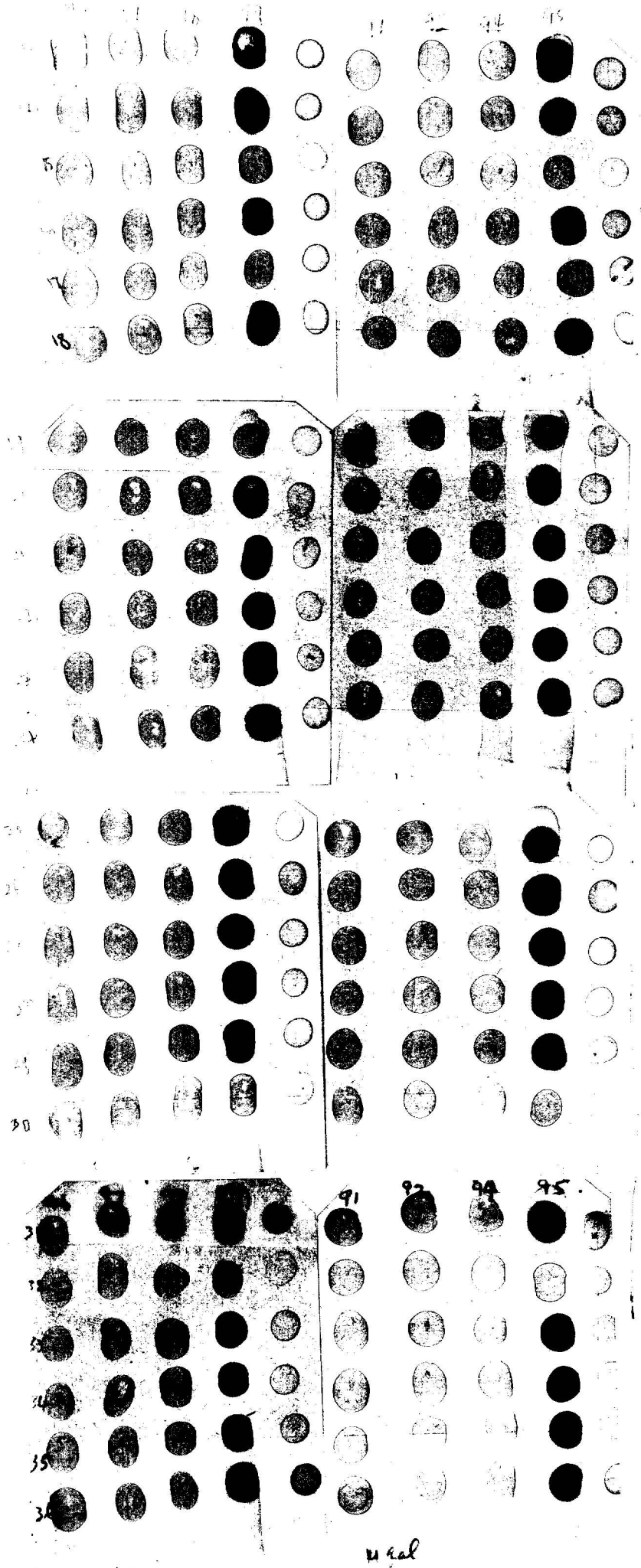




in Mgal

↙ 3350 F 8C

	− deletion	+
a		
b	1 2 8	4,22,6,7,9
c	1 2 4 6 7 8	22,9
d	4 6 7	1,2,22,8,9



11 gal

Isolation of F′ Lac_X^- from $F^- Lac_X$.

5/V : 1960 REF:

Strains used

Strain.	mutation	ONPG
○ W4412	52 m	+
W4413	53 m	+
○ W4147	87 m	—
W4151	91 m	—

F_{13} donor 3747
M Lac^+ F_{13} . V_6^R.

Principle :
1. Isolate $_X^-/_{ex}Lac^+$. by 3747 → X. {Ratio 0.1 ml : 0.1 Fe : 5 ml ; overnight 37°C streak on MLac. Select −/+ purify it on BLac : [illegible].
2. pick out Lac_X^- clony after streak from each Lac V colonies. Streak it on BLac.
3. Test sex-compatibility by cross.

Experiment :

A. Infection of F_{13}
1. Mix 0.1ml F^-Lac^-, 0.1ml $F_{13}^+ Lac^+$, 5 ml of penassy broth.
2. Incubate it for overnight at 37°C.
3. Streak it on MLac to select 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 Blac.

Lac⁻ segregants were tested on their sex-compatibility by cross × 4151 on MBlac plate (I). The other 5 colonies are streaked on Blac again.

Experiment:

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

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

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

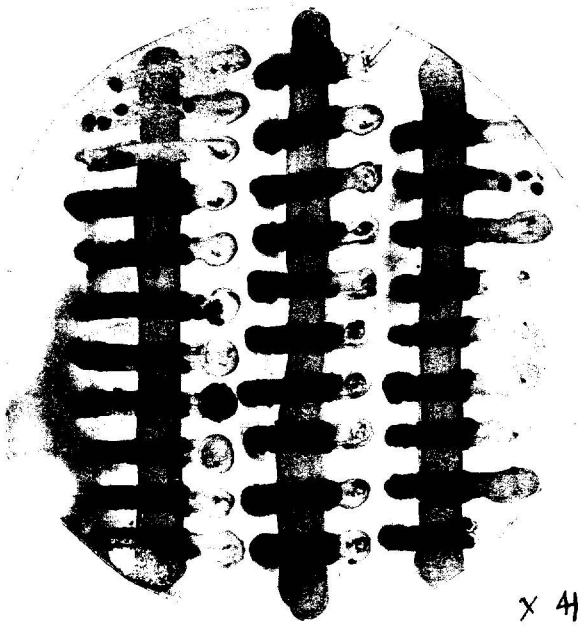
	Lac^-_{52} tested (Segregants)	Lac⁻ F_{13}	Lac⁻ F⁻	Lac⁻ F⁺
Colony 1	56	56	0	0
Colony 2.	22	22	0	0
Colony 3	50	50	0	0
Colony 4	67	67	0	0
Colony 5.	93	93	0	0
colony 6	3	1	0	2

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

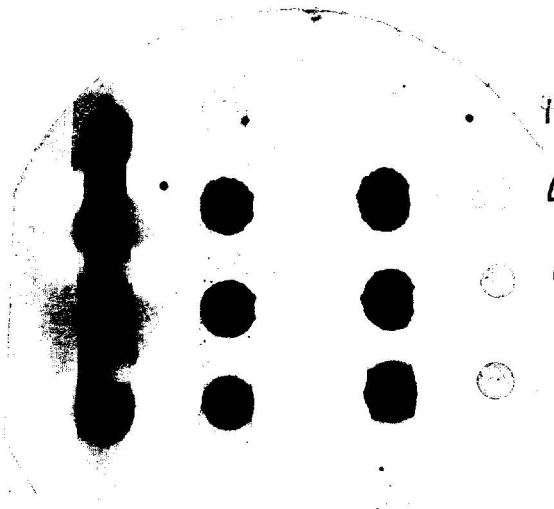
The newly isolated Lac_{52} F_{13} is T_6 ___. See back page
may not be V_6 star. maybe host range mutant to T_6

(4) Test complementation + Lac_{52}.
× 4112, × 4113, × 4147, × 4151. T_6.

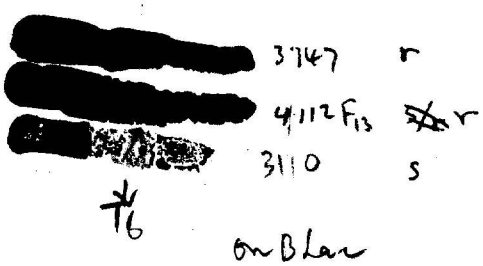
Save the ... F_{13}



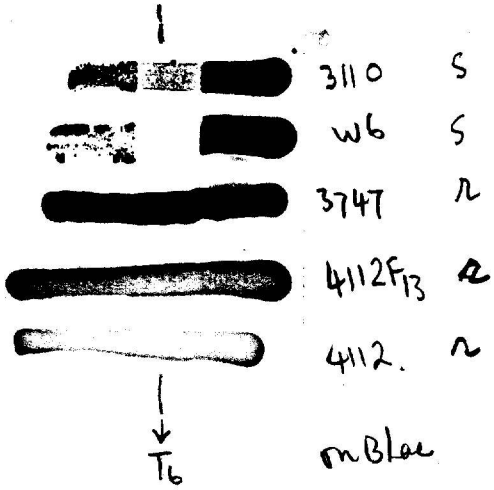
× 4151
on M lac.



× 3747 × $4112F_{13}$ × $4112F_{33}$ blank
on M lac.



on B lac



on B lac

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MICROBIAL
GENETICS

W4866 9°-a

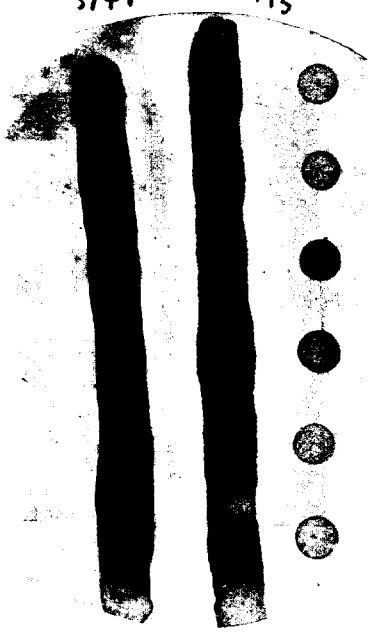
Complementation test using newly isolated $Lac_{52}F_{13}$.

16/V. :1960 REF:

• purpose: Look for Lac⁻ marker which give complementation with $Lac_{52}F_{13}$
• See back page. Test on MLac.

Type	ONPG		Strain No.	F⁻ Lac mutation.	complementation reaction. (on MLac)	after streak on BLac
m	−	Δ	22	3147		
m	−	Δ	25	3151	++	Streak Lac spot on BLac. and confirm it is recombination reaction c.f. 9-b.
m	−	Δ	41	4101	++	
m	−	Δ	87	4147	++	
m	−	Δ	90	4150	++	
m	−	O	93	4153	++	
m	−	O	89	4149	++	
m	−	Δ	88	4148	++	
m	−	Δ	91	4151	++	
m	+	X	52	4112.	− Same mutation.	See p.9.
~~f-m~~						
f-m	−	O	39	4287	++	
m	+	X	17	3152	++ Rec.	
m	+	X	18	3174	+ ~~weak~~ few ← 3747 does not give complementation reaction with this!	
m	+	X	19	3156	++	
m	+	X	20	4286	+	
m	+	X	21	3153	++ Rec. Ca 100	
m	+	X	53	4113	++ Rec. Ca. 100	
m	+	X	55	4115	++ Rec. ..	
m	+	X	56	4116	++ Rec. ..	
m	+	X	71	4131	++	
~~m~~	+ f −	X	72	4132	− No Lac. ← ~~Why? Many with F13. Lac 52 H+ Lac~~	

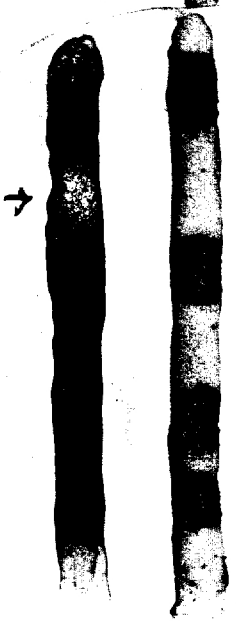
Δ contained in extream f-type but not in Lac_{11D3} or 39
X Contained in Lac_{11D3}
O Contained in Lac 39.



on MLac

Strain No.	Loc	ONPG	
4101	41	m −	Contained in extreme f-type but not in Loc II D_3 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 II D_3.
4131	71	m +	
3156	19	m +	
3152	17	m +	
4150	90	m −	Contained in extreme f-type but not in II D_3 or 39.
4113	53	m +	Contained in II D_3.

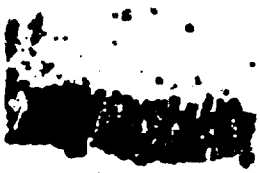


4113	53	m +	Contained in II D_3.
3174	18	m +	① Ann's thesis says : 18 contains 52 but there is some recombination. ② No recombination in x 3747. probably, there maybe some selection for mating reaction of the strain
4115	55	m +	
4132	72	f −	
4116	56	m +	Contained in II D_3.
3153	21	m +	

Selected on MLac.

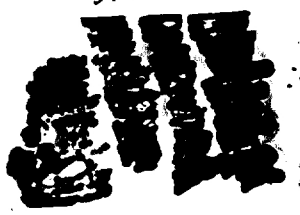
→ 3174

4101

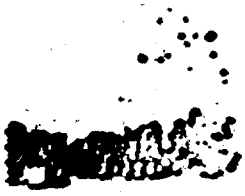


4131

3153



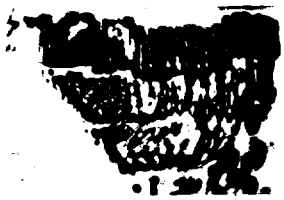
4148



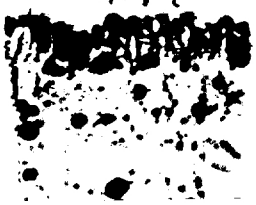
3156

3151

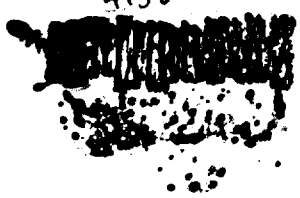
3150



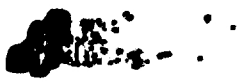
4147



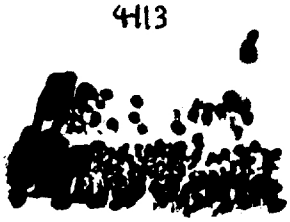
4150



4153

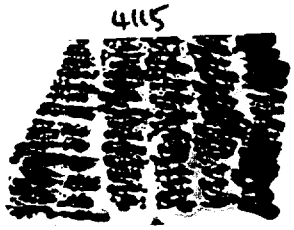


4113



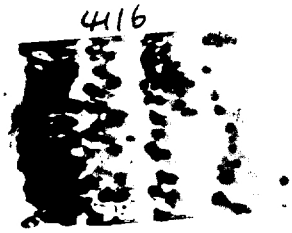
4149

4115



4286

4116



streaked on Blac
from Lac+ spot

: conclusion :

There Lac+ spots on Mlac are real genetic exchange in Lac locus, not segregation

U.W. MICROBIAL GENETICS

Test on homozygosity of Lac_{52} W4864

9c

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

11/V. ; 1960

REF: 9a, b, d.

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

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

F_{13} Lac_{52} 0.2ml ⟶ × $F^- Lac^+ S^R$ 0.1ml (W3086) + 5ml gen. F⁻ M Mal, S^R purified on B Lac. Lac^+ colony was picked.

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

↓

Seed the mix on B lac Sm.

cultural age: overnight grown culture of F_{13} Lac_{52} & $F^+ Lac^+ S^R$.

Seed 10^{-3}: 0.1ml / plate EMB Lac Sm.

• Result.

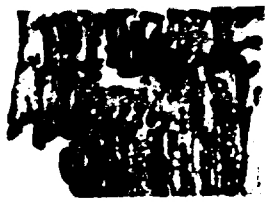
plate No.	Lac^+ (lighter) + (prob. Lac^V)		Lac^V	Lac^- (presumably it is segregant)	F_{13}
1	87	(9)	2	0	7
2	97	(17)	3	1	8
3	75	(15)	3	0	10
4	59	(7)	4	1	7
5	49	(2)	1	0	8
6	59	(7)	4	1	6
7	75	(15)	3	0	5
8	97	(17)	3	1	3
9	87	(9)	2	0	4
10	91	(10)	0.3.1%	1 0.6%	12
Σ	776	(108)	25	5	

Further Experiment

① Streak each Lac^V and confirm real Lac^V.

② Test Lac^- segregants
a Test mal^-
b. Test melt capacity
c. Test Lac_1

③ Test F_{13} nos.
How much % of the cells will become F_{13}.
(Rate of Infection of F_{13}.
on M lac × Lac_{91} (W4151)



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MICROBIAL
GENETICS

continued from former page

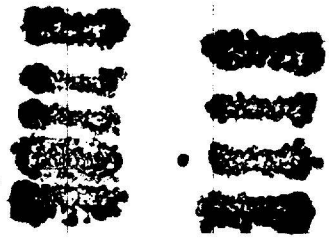
3086 × W4864 9d

Test Lac⁻ character transduced by 4112 F_{13} (Lac_{52}).

26/V ; 1960 New F_{13} M Mal, Sm Lac_{52} (4866) REF: 1 a. b. c.

1. Test Mal_1^- (: 3086 = Mal_1^-) on Bmal agar.

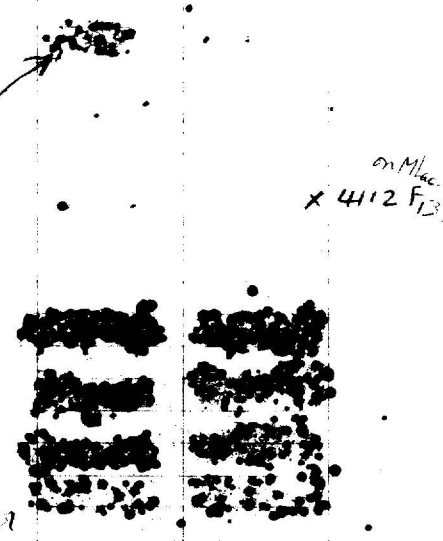
Result. all 5 are Mal_1^-.



2. Test Lac⁻ marker.
Show the Lac⁻ is Lac_{52} and not other.
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 Mlac agar



on Mlac
× 4112 F_{13}

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

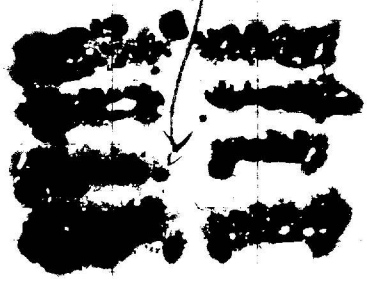
gal⁺ H1 : Gal
H1 : Mtl.
Med : Ara_2
Lo : Lac.

reversion

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

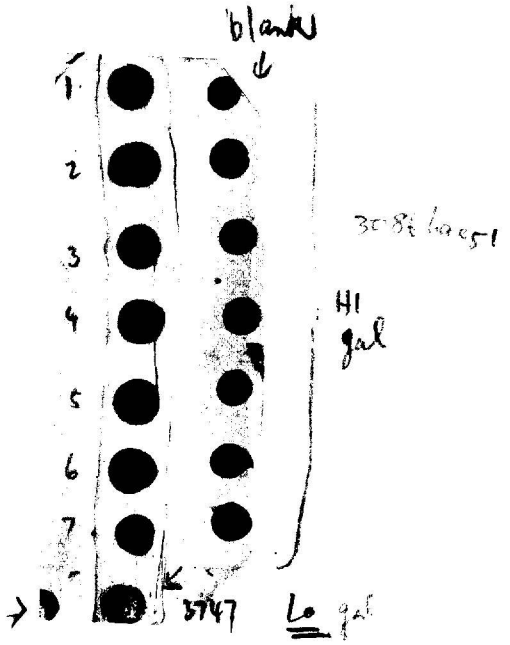
4. Gal⁺ recombinants does not segregate Lac⁻ after streak the cpate on B/Gal Sm.

× 4151 (91)

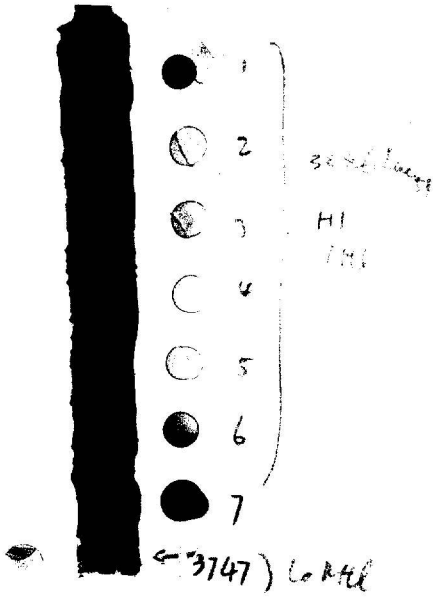


on Blac

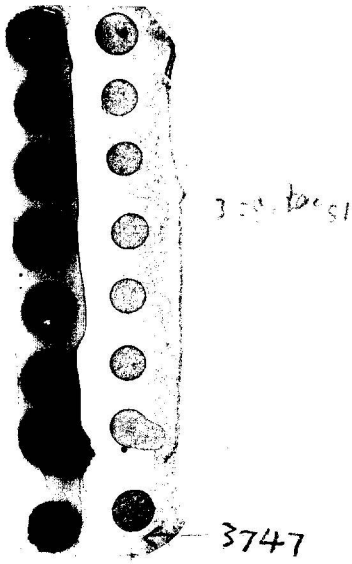
× 4112 (52)



×4573 (gal$_2^-$)
on M gal



×4573 (Mtl)
on M Mtl



×4573 (Ara$_2^-$)
On M Ara

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

31/V 1960

REF: Cf. See translocation of Lac_{52} to Lac^+ 10 3086.

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

If it segregate: homo:

" " does not " : hemi.:

(1) Method: pick one reversion from separate colony.

31/V 1. Purify 4112 F_{13} on BLac.

2. Pick 30 colonies into ~~nutrient broth (Lauth)~~ onto EMB Lac and let it grow for ~~overnight~~ 48 hrs

~~3. Spot those colonies on Blac again and incubate for 48 hrs.~~

pick one Lac^+ from each spot a

4. Purify Lac^+ revertants on BLac. confirm purity of the Lac^+ colony.

Let each colony grow up in 5 ml nutrient broth and

5. Streak each revertants on BLac, and see segregation of Lac_{52}. Look for Lac V., and restreak it on BLac again.

6. Test Lac^- type of segregants from the diploids.

Result:

Isolation No

	Lac⁻	Lac v	seg		Lac⁻	Lac v	seg		Lac⁻	Lac v	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		•	

(2) Test Lac^+ transfer. Test fertility for transfer of Lac_{52} of Lac^+ revertants, & cross x 4112 S^R on MLac Sm. use spot test.

purpose: Is Lac^+ reversion on exogenote segment or endogenote

If exogenote H1 ; If it is in endogenote no:.

V: 13 (9)

no: 10

3747

4112 F_{13}

4112 S^R

on MLac

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GENETICS

10a

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

12/6 ; 1960

W4147 : Lac_{87} F^- ONPG − M.
W4112 : Lac_{52} F^- ONPG + M.

1. One 4147 F_{13} colony was streaked on Blac from Δgal.

[redacted]

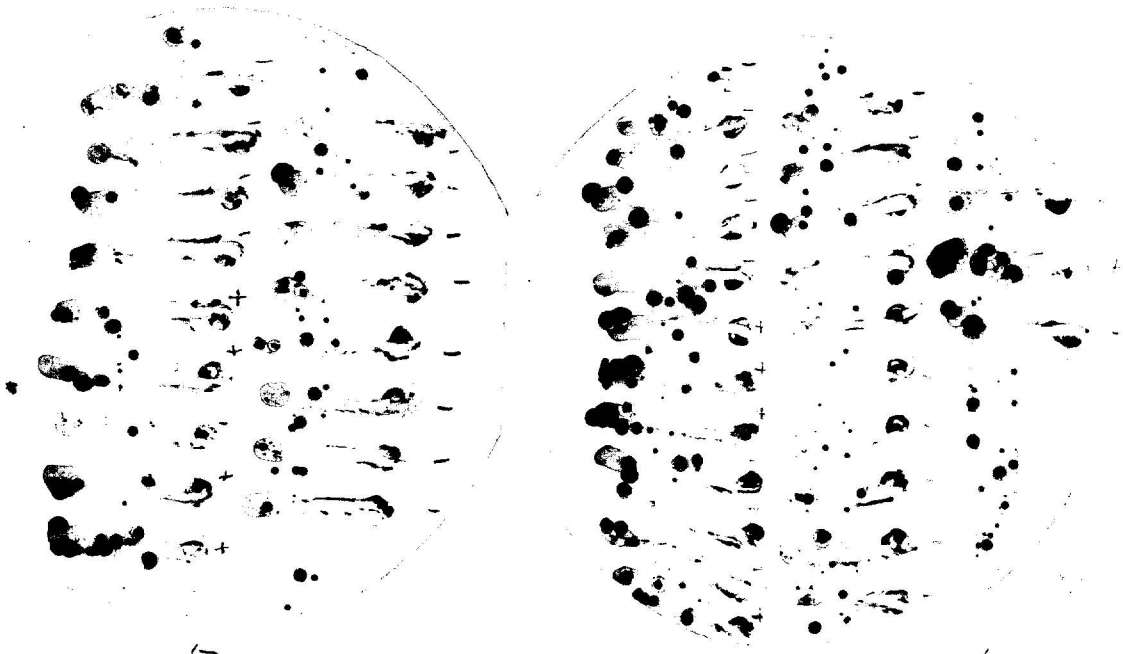
Exp I. 4 Lac^- segregants were tested on their sex.
× W4112.
(Lac_{52})
F^-

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

Cl. #	# 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 x 4147 (lac87)



5 1

+4 ; −13

2 3 4

+4, −8 +0, −10 +1 ; −2

x4112 (lac⁻)

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

12/v 1960

REF: c.f. P. 8

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

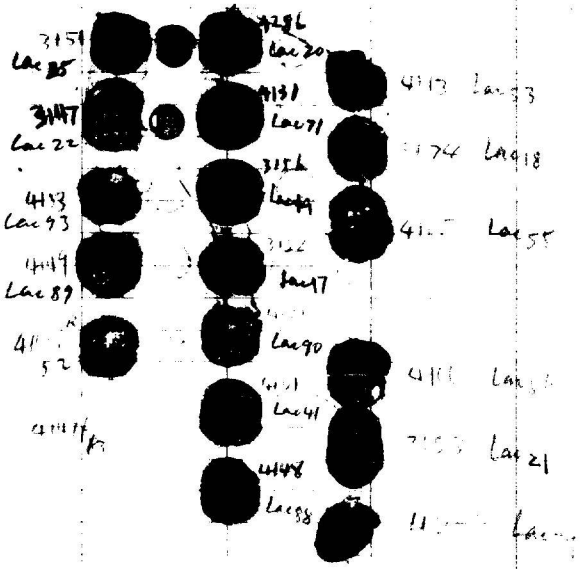
History: 1. 3747 → × 4147 (F^- λ^+ Lac^-_{87})

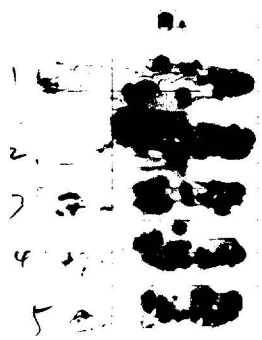
2. Pick Lac_{87} /ex+

3. Streak it + test fertility of Lac^- segregate from it.
× W4112 on MLac.

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

4. Test complementality.
by spot test.
on MLac.





× 4112 (F^- lac 52)
on MLac

Showing complementation with Lac^-_{87} F_{13}.

+	+	+
Lac 20	Lac 53	Lac 18
Lac 19	Lac 55	Lac 39
Lac 17	Lac 56	Lac 40
Lac 21	Lac 25	41
	Lac 22	