Preparation of λ-reference from B120.

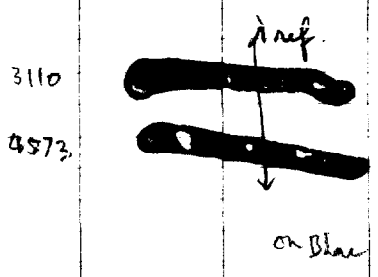
30/IV 1960 REF:

1. Make overnight grown culture in Pen. (8 ml).
2. Add it to 10 ml pen. & Incubate it at 37°C for 2 hr on rotator.
3. C.f.g. resuspend bacteria in 4 ml of DO. Irradiate it with U.V. in Petri's dish for 10 seconds.
4. Add it into 20 ml Penassay broth. and incubate it for 3 hrs. at 37°C. on rotator. (2:15 ~ 5:15 PM)
5. ~~C.f.g.~~ Keep it in refrigerator for overnight at 5°C. to complete lysis.
6. C.f.g. the lysate 3000 r.p.m. for 20 min; supernate saved.

   C.f.g. 16000 rpm for 1.5 hrs. at 5°C. Servall. resuspend it in 1 ml of P-med. 1:40 ~ 3:10 pm.

   Use this as DNA source of λ. — assay titre of plaque forming centre.

	dilution vol.	# of Plaque
Titer	10^8 : 0.1	



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Effect of AO on F8 gal+ strain treated.
(3350 F8 1- 2-/+ +.)

2/V / 1960 REF:

1. 3 new isolates are grown in penassy broth.

2. Inoculate ca. 10^4 cells/ml AO-NB. : pH: 7.6. : AO 30γ/ml. 37°C overnight-treated.

3. Procedure:
Seed 10^{-6} ml of AO-treated and untreated culture onto B-Gal. and count the ratio of gal+ and gal-.

4. Test sex-compatibility of gal+ and gal- by replicating on MLac Sm. seeded W4573. on it. Look for gal+ F- and see segregation.

Treatment of 3350 F_8 with AO.

$1^-2^-/++$ F_8

6/4 ; 1960 REF:

Purpose: 1. To show susceptibility of F_8 gal^+ to AO.
2. Look for gal^+F^- segregant from it. Reinfect F to it and see.

Experimental condition:

Conc. of AO : 200 γ/ml ; N.B. 5 ml pH. 7.6 3 tubes.
(0.2 ml of 5000 γ soln to 5 ml of N.B.) as a control NO AO.
Inoculum size : ~$10^8 \times 10^{-2} \times 10^{-2}$: 0.1/5 ml. : Ca 2 × 10^3
Time of treatment : at 37°C. for overnight : 2:30 ~
Score it on BGal. Test fertility by cross X^+ W3995 F^- 5^R on MGalSm.

Result:

Tube	plate #	gal⁻ gal⁻F⁺	gal⁻ gal⁻F⁻	gal⁻ F⁻ gal⁺	gal⁺ gal⁺F⁺	gal⁺ gal⁺F⁻	gal⁺ F⁻ gal⁺	# of colonies on BGal. gal⁺	gal⁻	
AO 1	1	1	25	26	25	0	25	83	83	
	2	0	22	22	22	1	23	119	80	
	3							123	101	
	4							76	63	
	5							120	101	
								521 (54.9%)	428 (45.1%)	949
AO 2	1	0	26	26	25	0	25	85	57	
	2							113	57	
	3							124	70	
	4							81	75	
	5							42	26	
								445 (60.8%)	287 (39.2%)	732
AO 3	1	3	20	23	21	0	21	105	85	
	2	1	20	21	25	0	25	100	89	
	3							91	80	
	4							88	70	
	5							106	87	
Σ AO		7	115	122	118	1	119	490 (54.4%)	411 (45.6%)	901
Untreated Control I	1	6	2	8	24	0	24	530	19	
	2	6	0	6	26	0	26	499	14	
	3	4	1	5	21	0	21	443	20	
	4	6	5	11				367	16	
	5							418	21	
		22	8	19	71	0	71			2347
II	1	36	1	37	35	0	35	2257 (96.1%)	90 (3.9%)	2347
	2	58	1	60	106	0	106			

Look for F^- gal^+ (from 3350 F_{12}) & Test segregation.

gal^+F_8	gal^+F^- or F^+
52	1 — F^-
105	1
113	1
85	0 — F^+.

AO treated gal^+F^+ : 2
Σ gal^+F_8 : 355
gal^+F^- : 1
% : 0.84 %

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Segregation of gal⁻ from F⁻ & F⁺ strains derived from 3350 F8 after treatment of AO.

20/4 ; 1960

REF:

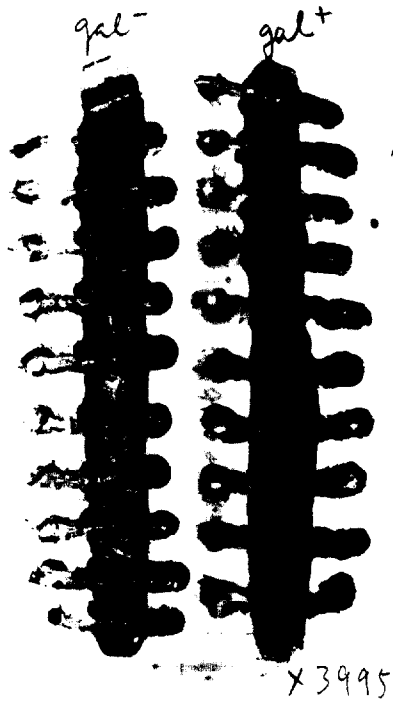
Isolation No		plate No	# of colonies tested on Blac. Lac⁺	Lac⁻	Lac⁺	# of colonies tested for ex-compatibility.	tester:
Sex. F⁻	I	1	0	8	371	segregate	3995
		2	3	4	462		
		3	4	2	533		
		4	3	1	19		
		5	4	2	631		
		Σ	14	17	2016		
F⁺	II	1	0	0	629	No segregation	
		2	0	0	397		
		3	0	0	691		
		4	0	0	699		
		5	0	0	627		
		Σ	0	0	3033		
F⁻	III	1	4	3	777	segregate	
		2	3	2	421		
		3	9	1	514		
		4	2	0	11		
		5	5	1	30		
		Σ	23	7	1753		
F⁺.	IV	1	7	2	834	segregate.	
		2	5	2	635		
		3	0	3	821		
		4	7	4	406		
		5	6	3	613		
		Σ	25	14	3309		

Test ex-compatibility of gal⁺ & gal⁻ segregant from IV.

Are those all F⁺ or segregate?

∘ This test give me answer about a question that: F' will be sick after treatment with AO. or F will be released into cytoplasm.

Tester: X 3995



segregants from
gal⁺F⁺ 3350 F_{13} AD.
↓
Now it becomes F_{13}^{+}

X 3995
on MGal Sm.

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

; 1960 REF:

Purpose: Are those F$^-$ strains df or simple F$^-$? (Does it become to F$'$ by F$^+$ x) (Does it segregate gal$^-$?)

1. 6/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 S^{R}M$^-$F$^-$ on Mglu Sm. W3086.

Incubate the mix for overnight.

(b) Streak the on DO. & Test compatibility on Mgal Sm x M$^-$F$^-$S^R.

3. Streak (4) 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°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 Mglu Sm
		backed to F$'$ H1 gal$_2$		backed to F$'$ H1 gal$_2$

II Infection of F. (Infectability)

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

Isolation No.	1 +W6	1 −W6	2 +W6	2 −W6	3 +W6	3 −W6	4 +W6	4 −W6
Recombination Reaction with 3086 on Mglu Sm (F$^-$M$^-$Mal$^-$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

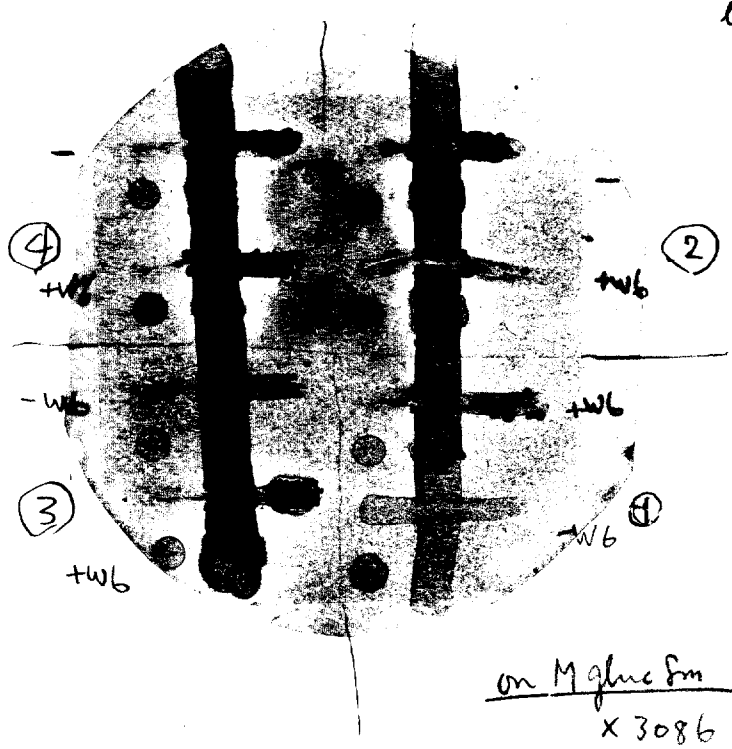
(b) Cross brush the isolates from mixture of W6 and gal$^+$F$^-$x$^+$ (from DO) (x 3086) on Mglu Sm.

III Infectivity

These strains are originally isolated as F^+ or F^- from 3350/++ F_8 with AO.
However, later on three of those strains back to F_8. The phenomena to support the back mutation.

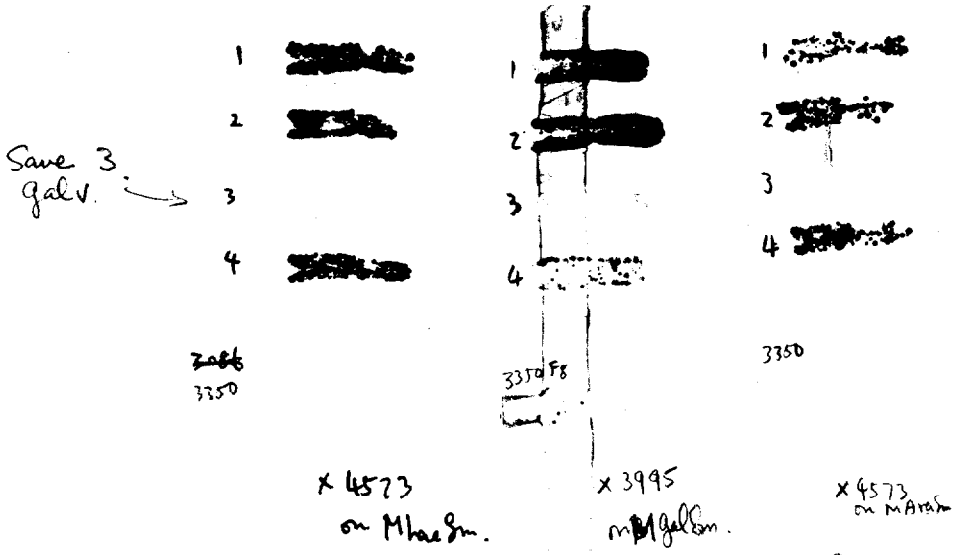
a) 1, 2, 4 shows identical character of F_8 after transfer to broth. it recovers the fertility gradually.

b). When these cells are plated and tested on mating type, it shows fractuation of fertility. (transfer of gal_{22})



on M glucSm
x 3086
(M+ transfer)

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



x 4573 on MlacSm.
x 3995 on MGalSm.
x 4573 on MAraSm

Selective marker:- Lac_{85} Gal_{22} Ara_2

Test on Infectability. by F.

Wb → x (3350) AO
F8d ?

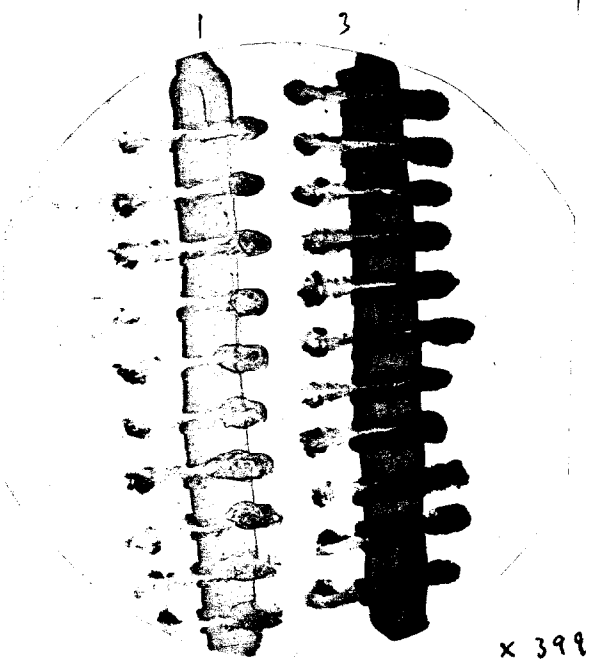
12e

? reverse ?

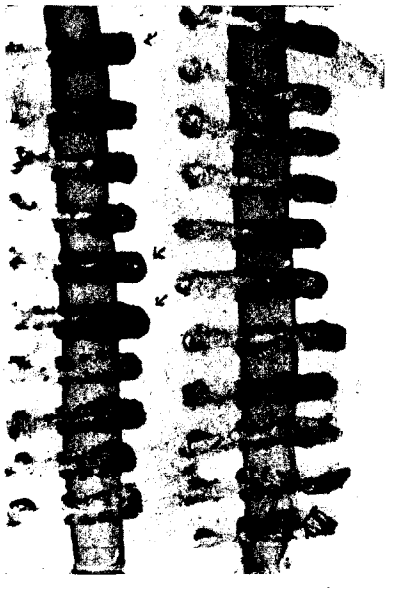
Repeat

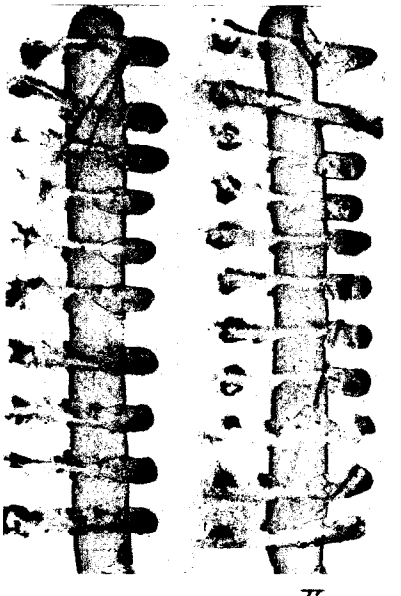
Test #

1 3



x 3995
on Mgal Sm





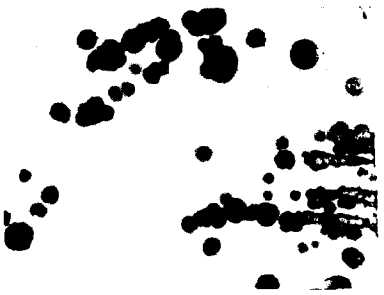
III IV I II

II ? I ? IV ? III ?

on Mgal Sm.
x 3995
gal22.

Transducibility of gal_2^+ (#573.)

3350 A0 4 → X 4573
Fgd



on Bgal Sm.

3350 Fgd 2 → 4573

on Bgal Sm.

Transducible for gal 2

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12 f

Infectivity of F^+ character of 3350 FX.
obtained by AO-method

19/v; 1960.

REF:

1. fertility of Fx. (→ Lac ; → Mgal. ;

2. Infectivity of F. to 4573.

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

Timing experiment of F13

5/v : 1960 REF:

Strain : 3747 M Pur⁺ V_6^R Lac⁺ ; 4637 L_p^S pur⁻ V_6^S Lac$_{85}$⁻ F⁻

Cultural age : overnight grown cell 0.2 ml / 10 ml penassy : 11:00 AM —— 4 hr. 3:00 PM. at 37°C. on rotator.

Media selected : DO, MLac. (pur) (pur, Lac)

Ratio of mix. Ratio ♀ 1 ml ; ♂ 9 ml.

Blending : gage 70 : 1 min. Temp for mating : at 37°C.

Result :

Time / Media	PM.	2:30	2:35	2:37.5	2:40	2:45	2:50	2:55
		Time after interruption (min.)						
	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) ε		0, 0	0, 0	0, 0	0 2	0, 0	0, 0	2 0

Pick Pur⁺ colonies from DOs (10', 15', 20', 25') and inoculate it on DO. Test V_6 R/s, F', and Lac⁺/⁻ by replicating on BLac, MLac Sm. ~~[illegible]~~ pur⁻ S^R F⁻, V_6 plate.

# of Pur⁺ Tested

V_6 r/s	r					
	s					
Lac	+	0	9 (F+ 9, F− 0)	19 (F+ 19, F− 0)	170 (F+ 170, F− 0)	
	−	1 (F− 1)	3 (F− 3, F' 0)	2 (F− 2, F' 0)	22 (F− 20, F' 1, F+ 1)	

V_6 var Pur var → cross brush with V_6 W4628

Further test : ① Test sex compatibility. + V_6^R → Replicate on Pur⁻ S^R F⁻ on DO on ~~[illegible]~~ Sm.

② Is it V_6 r/s or R or s ?

③ If it is F⁻, infect F to it and see whether it becomes F' or F⁺.

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

11/V. ;1960 REF:

• purpose of this experiment:

① Infect F to $pur^+ F^-$ obtained by interruption of F_{13} duction 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 $F^- pur^- V_6 Lac_{85}$

2. Test T_6 resistance.

11/V 3 Infect F to $Pur^+ F^-$ 2 ~ 6 + Pur^+ Fx^+ #. 7

4. purify them on Bhac agar. pick Lac^-. Test compatibility by cross × $F^- pur^- S^R$ W4628 on BM-Sm. by replica plating method. (F^+ control W6)

• Result:

F' or F^+ obtained / No of Isolates tested $Pur^+ Lac^-$

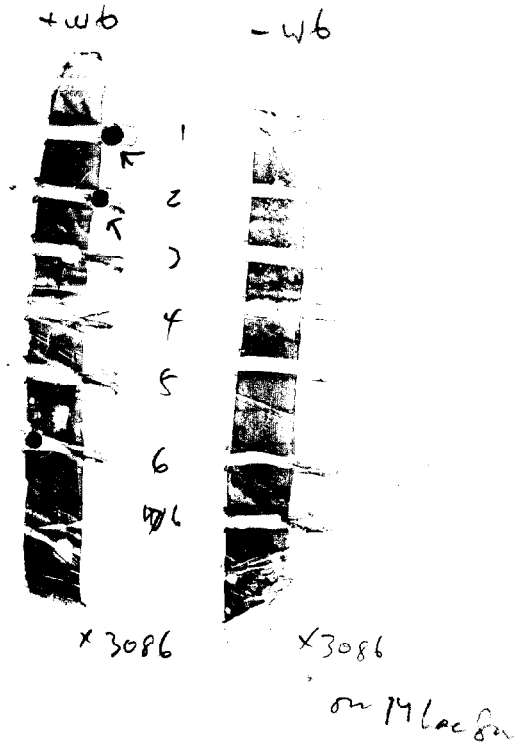
Isolation No.	2	3	4	5	6	7
	0/10	0/10	0/9	0/9	0/10	0/10

all of these isolates were F^-.

No recombination reaction on M Gluc. Sm. × 4628 $pur^- S^R F^-$

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

Take control $F^- Lac_{85}^- S^S$ and try this exp. again.


+wb
-wb
1
2
3
4
5
6
wb
x3086
x3086
on 14 lac 8n

~~Make Strain W4354 F8 a+ 4 W4354 F8 b+~~

10/V; 1960 Timing experiment. REF:

- cultural age:
  0.2 ml overnight, pneumo grown culture / 10 ml pneumo broth → on rotator at 37°C
  11:05 → 4:05
- Strains used:

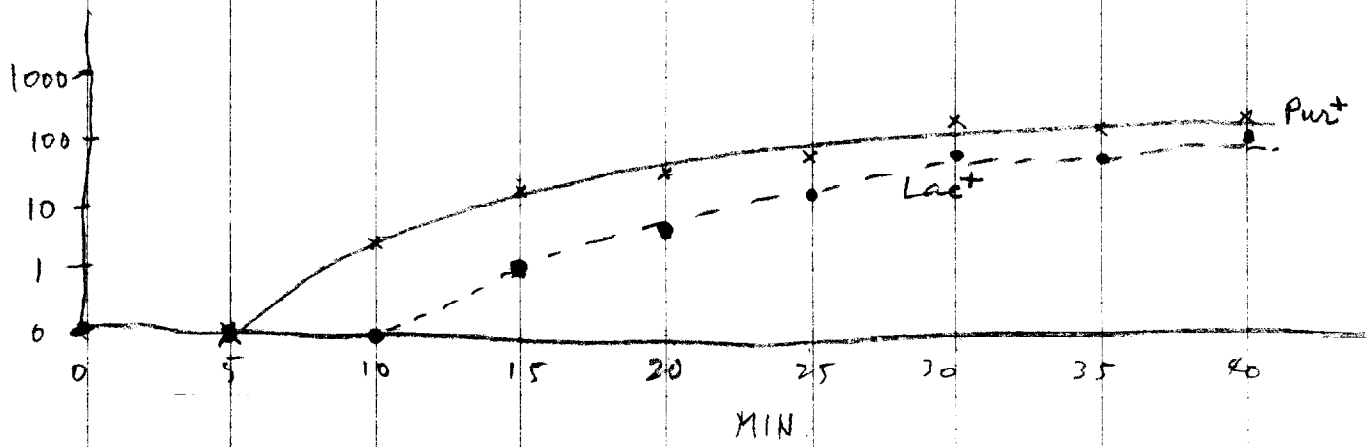
		Ratio
3747	M V_6^R F_{13}	10 ml.
4637	Pur Lac_{85} V_6^S F^-	1.1 ml.

- Time of interruption: 0' 5' 10' 15' 20' 25' 30' 35' 40'.
- Experiment:

Media	Markers selected	Time of interruption. 0'	5'	10'	15'	20'	25	30'	35'	40'
DO	Pur^+	0, 0	0, 0	2 / 1, 1	13 / 6, 8	29 / 18, 11	65 / 22, 43	227 / 111, 112	151 / 78, 73	187 / 97, 90
Dil. vol	10^3 : 0.1		(Lac^+	.0	4	6	23	60	48	63
			Lac^-	2	9	20	22	72	32	29
M Lac	Pur^+ Lac^+	0, 0	0, 0	0, 0	0, 1	2, 4	5, 10	33, 61	37, 22	58, 75
Dil. vol	10^3 : 0.1				1	6	15	94	59	133

Interruption: gage 70 : 1 min. : Temperature 37°C on rotator.

4628 F^- pur_1^- S^R ⊕



Timing of F ness.

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19 REF:

	1	2	3	4	5	6	7	8	9	10
			b :	4671	38	20	39			
1										
2			c							
3										
4			c							
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Unsuccessful result

Test on phosphatase ~~activity~~ production of K-12.

26/V. 1960 Substrate : o-nitrophenyl phosphate

REF:

Lo : phosphate medium.

1.) Semiquantitative test : grow W6 cells in Lp-medium. for overnight

↓

C.f.g

↓

resuspend it in 1ml H_2O

↓

autolyze it with 2 drops of toluene.

↓

Add 1ml of tris-buffer pH. 7.5 1M. + 400γ/ml p-nitrophenyl phosphate.

2.) qualitative test on agar plate :

Test on 1.5 % agar + Lp- ~~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 400γ/ml of p-NPP soln on that agar.

autolyzed with $CHCl_3$ → 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 by pNPP. seems not work.

Low phosphate medium.

gr.	/l.
3.0	NaCl
0.25	$MgSO_4 \cdot 7H_2O$
0.01	$CaCl_2$
2.0	Na-Lactate
10.0	Difco-Bactopepton
12.0	Tris (hydroxymethyl) amino-methan

pH. 7.4

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

B.B.A. 31, 570 (1959)

Test genetic marker of Garan's strains.

; 1960 REF:

1) Test markers.

Media used	3	4	5	6	7	8	9	10
	B Lac	B gal	B gal Sm / B Lac Sm	B gal / B Lac	M Lac.			
Strain No	Lac	gal	Sm.	T6.	Sex. (x 4354)		Phosphatase	X
F7	−	−	R	S			−	+
F24	−	−	R	S			−	+
F13	−	−	R	S			−	+
F18	−	−	R	S			−	+
4573 P1	−	−	R	S			−	+
P2	+ ?	+ ?	Conc. of	P-NPP's 4 mg/ml.			+	+
~~4573~~ 3747	+	+	R	R			+	+

2) Infect F13 to P⁻

3747 ⟶ x F⁻ P⁻

T6R M F13

Streak on B Lac Sm

Result: Surprising enough, Lac⁻ of those Garan's strain are not transducible by F13. all the colonies on B Lac Sm shows Lac⁻!

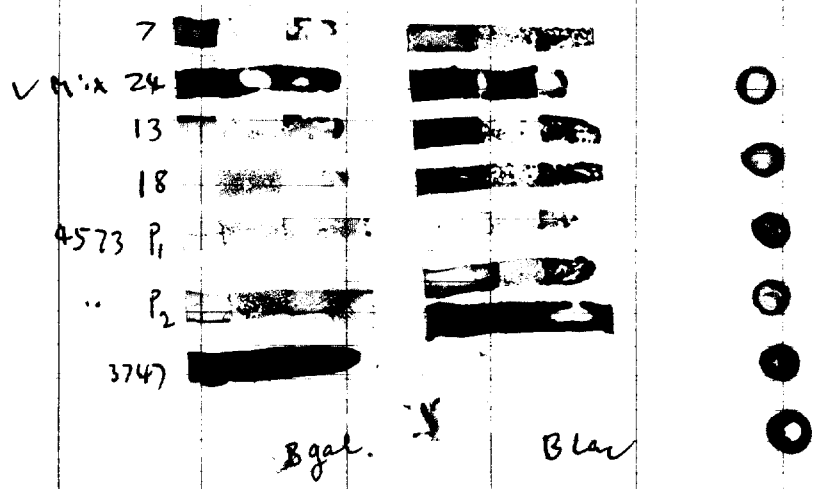
Further test

1. Test maleness: transfer of Lac x 4573 Lac 85. on M Lac Sm

If it has F13, Some Lac⁻ of garan's strain must transduce P Lac⁺ to 4573.

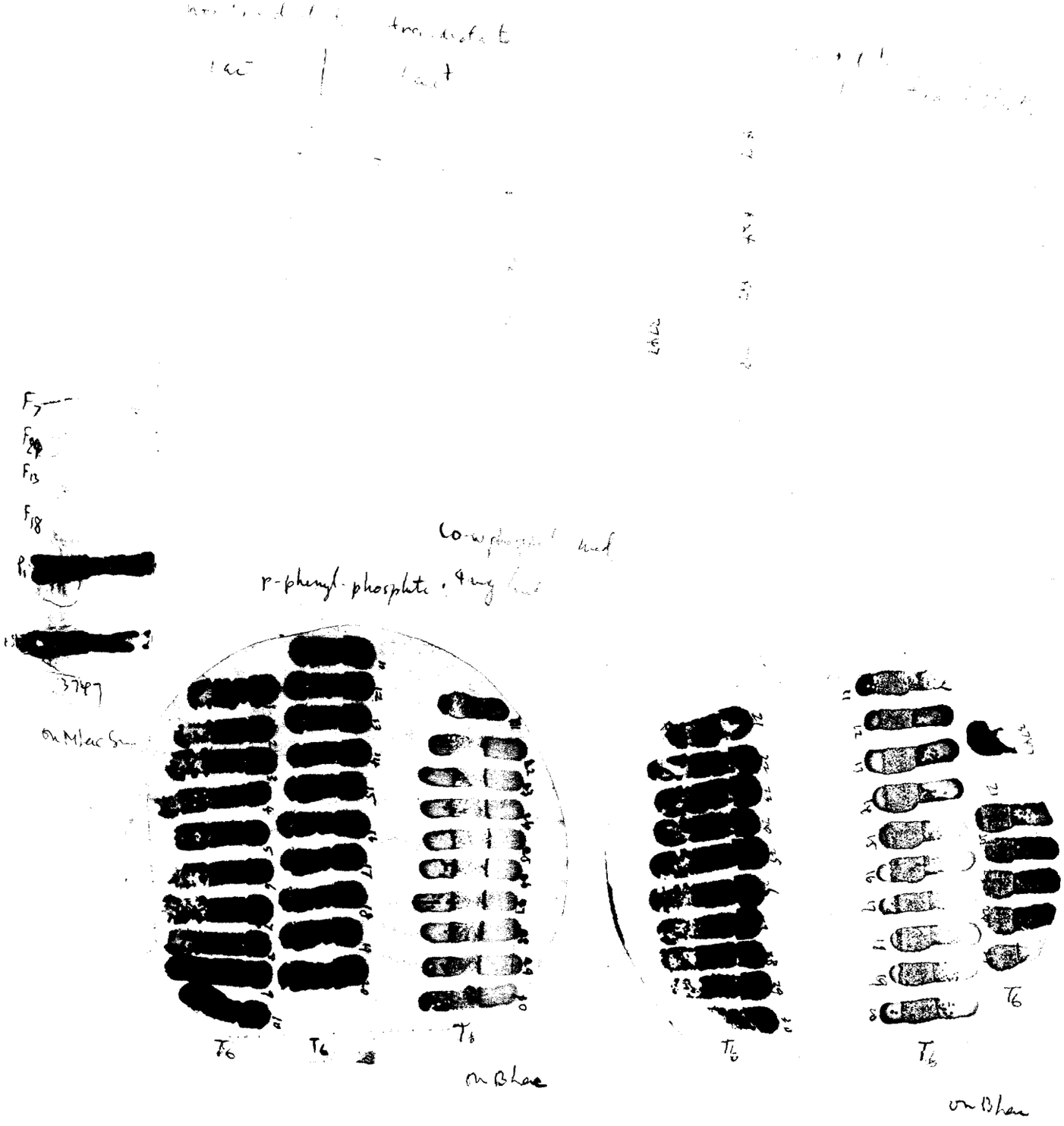
2. Test resistance of garan's strain to transduction of F13. x 3747 on M Lac Sm

Possibility
① Garan's strain is resistance to F13 infection
② Lac marker of garan's are not transducible by F13.



7
24
13
18
P1
P2
3747

on Low P med.



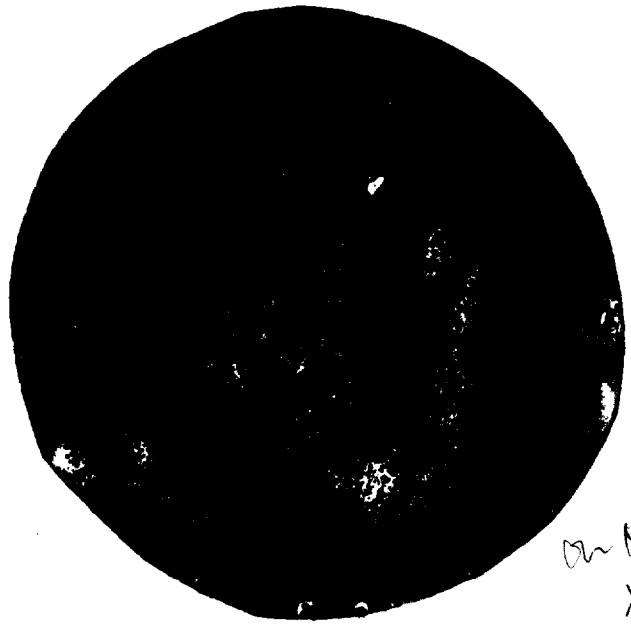
F7
F13
F18
3747
on Mlac Sm
p-phenyl-phosphate
T6
T6
T6
on Blac
T6
T6
T6
on Blac

3747 ✗

F13

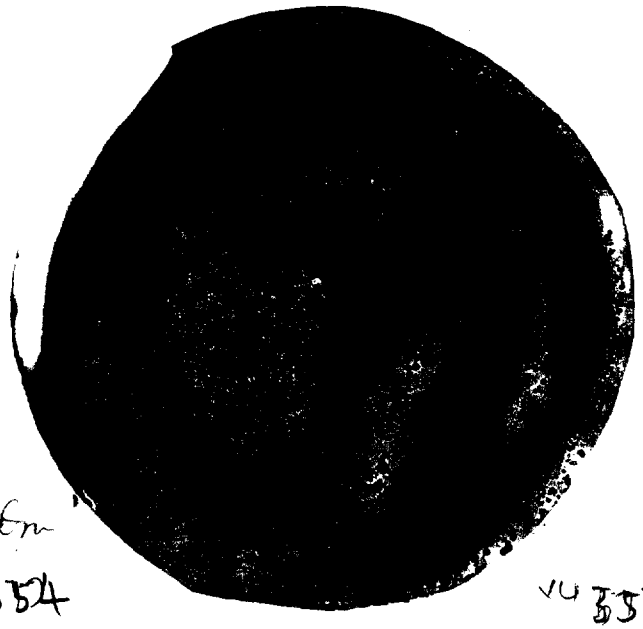
F7

15c



Ow M Lac Sm

X 4354

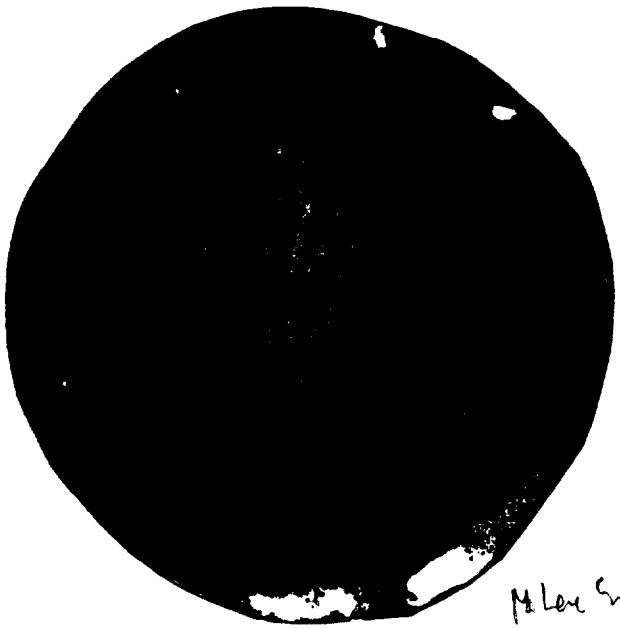


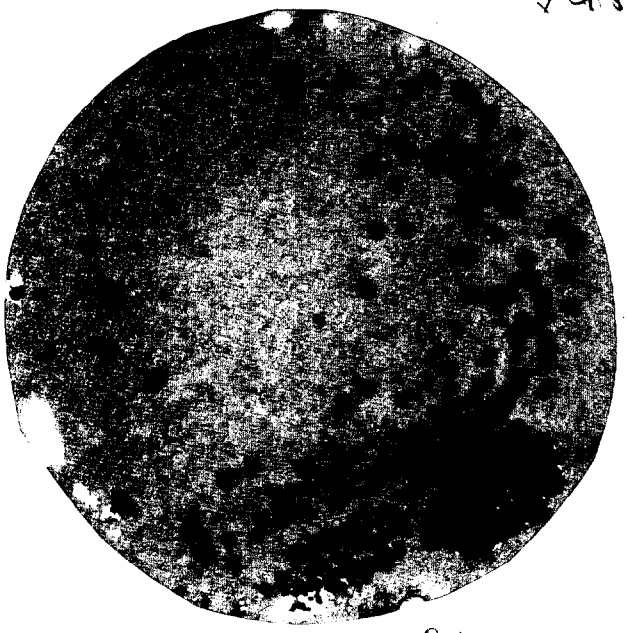
VU 354

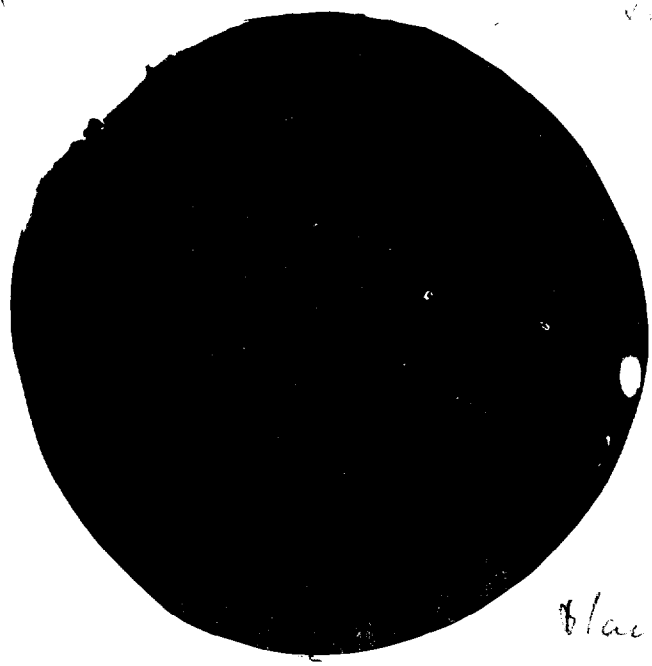
M Blac Sm

[illegible]

F18 F24 15d







Test transducibility of F_{11}, F_{15}, F_{19}.
on Try-transduction.

20/V : 1960 REF:

Tester : 4090 $F^- S^R$ Try (Ind_4) F'Strain : W4580 : F_{15}
4091 $F^- S^R$ Anth {W4277 gal_7^- W4582 : F_{19}
der W4278 gal_6^- W4522 : F_{11}

1.) Test Hfonness on MLac Sm. by spot-test.
& on MLac. (See below)

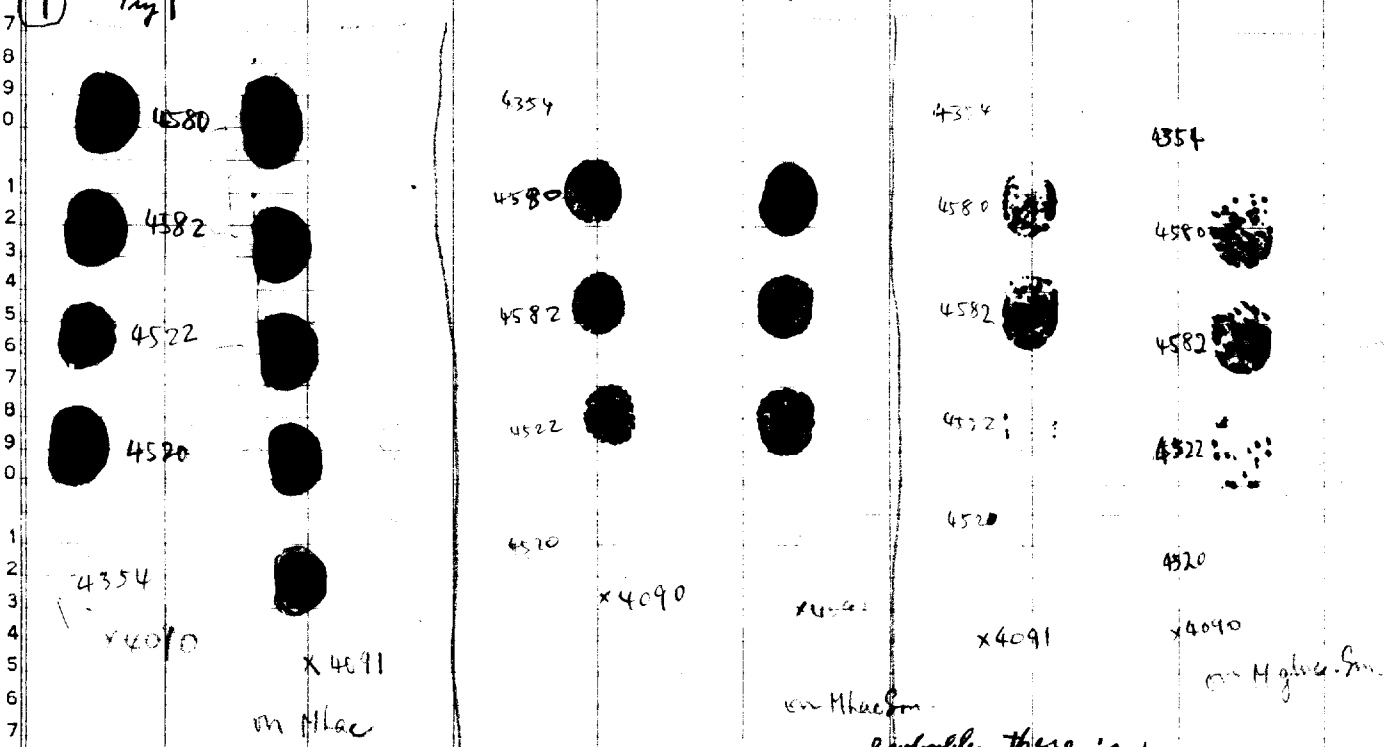
2) If H1. : Test Co-transduction of Gal & Try
Tester 4277. : gal_7^- Anth. F^-

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 incubated mix on B gal Sm.
2. Replica-plate them on DO. ~~or Mglucose~~.

Transduction of	4520 → x 4277			4522 → x 4277			4580 → x 4277			4582 → x 4277		
	+	−		+	−		+	−		+	−	
gal_7	167	36	89.2%	43	2	95.7%	2	68	2.4%	0	44	0%
	169	25		24	1		1	54		0	51	
Try (Anth)	0	203	0%	0	45	0%	1	69	1.6%	0	44	0%
	0	174		0	25		1	54		0	51	
gal^+ Try^+	0	377		0	70		2	123		0	95	
% of Try	0			0			1.6			0		

(1)



Conclusion : F_{15} may be transducible for Try. probably there is linkage in gal & Try.

Transduction of F_8 or F_{11} by λ.

31/1 ; 1960 REF:

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

Principle : 1) 4520 (F_8) → $Lp^+ gal_7^- F^-$: W3097

2) 4522 (F_{11}) → $Lp^+ gal_7^- F^-$: W3097.

3) 4520 (F_8) → $Lp^+ gal_7 F^-$ + W6 (as F donor)

4) 4522 (F_{11}) → $Lp^+ gal_7 F^-$ + W6 (as F donor)

Experiment :
(A)

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

2. Treat with $CHCl_3$ and make cell free lysate of λ.

3. Add those λ-lysates (0.1 ml) to W3097 (1 ml) with or without W6 (1 ml ; pen 1 ml.), and incubate it for overnight at 37°C. ~~Mix W6 therefore λ.~~

Mix : 3:00 PM.
Add penassy 10 ml : 8:00 PM.
Incubate them for overnight. 11:00 PM.

4. Seed those transductants onto M gal medium.
Count gal^+ transductants and figure out rate of F-duction.

λ lysate was steril.

5. Pick gal^+ and test male-ness on M gal Sm in cross × 3997 ($F^- gal_7 S^R$)

Sterility of λ-lysates were tested at the same time 0.1 ml / 5 ml pen.

Experiment :
(B) Test rough estimation

1.) Mix 1 ml pen. ; 3097 ($gal_7 F^-$) × — λ 4520 / λ 4522 ; 3997 ($gal_7 F^- S^R$)

2.) Incubate the mix for 1 hr. (4:00 — 5:00 PM)

3) plate 0.2 ml of the mix onto M gal Sm.

Result :

	F_8 λ → × 3997	F_{11} λ → × 3997
# of gal^+/plate :	2	7
	3	1
	4	0
	2	0
Σgal^+	11	8

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18a

Time analysis of F_{13}-transfer.

21/v ; 1960 REF:

Strains used:

3747 : M F_{13} V_6^R — [redacted]

93:11D_3 Pur⁻ Lac_{11D_3} F⁻ —

↓ T_6 on BLac

Inoculate 0.2 ml / 10 ml pen. → Mix ♂ & ♀.

Incubate it on rotator for 3 hrs.
1:00 : 3:00 p.m.

Media selected	Markers selected	# of Recombinations / plate. 0'	5'	10'	15'	20'	25'	30'	35'	40'
M glue, 10^3:0.2	Pur.	0 0	0 0	1 2	15 15	24 117	294 305	499 546	564 662	332 439
	Σ			3	30	141	599	1039	1226	771×2 1542
M Lac 10^3:0.2	Pur Lac	0 0	0 0	0 0	3 4	54 75	124 70	551 504	478 439	279, 312
	Σ				7	129	194	1055	917	591×2 1182
M Lac + Pur + Bl (Ad) 10^3:0.2	Lac.	0 [illegible]	0 [illegible]	0 [illegible]	1	7	18	216	557	306
	T_6									

	45'
Pur	293
Pur Lac	295
Lac	0.1

Pur⁺ colonies are isolated and tested on their V_6^R or S by replicating them on EMBLac - which are streaked with T6 lysate.

Look for apperance of V_6 R/s.

Pur⁺ colonies are picked & Tested on T_6 and Lac.

	0	5'	10'	15'	20'	25'	30'	35'	40'	45'
Lac⁻ V_6^{Var}	—	—	[illegible]	16	9	17	13	7	2	2
Lac⁺ V_6^{Var}	—	—		1	28	23	42	57	58	58
Lac⁻ V_6^S	—	—	1	4	16	10	0	0	0	0
~~Lac⁻ V_6^{Var}~~										
Lac⁺ V_6^S	—	—	0	0	0	0	1	0	0	1
Lac⁺ V_6^R										3

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18b

27/4. ; 1960

Test on Mating type of Recombined cells from interrupted mating experiment.

REF.

Method

1. Recombinants are picked on DO from recombination plates
2. Replica plate it on ~~[illegible]~~ ; M gal Sm , M Lac Sm. on which 4573 is needed. 4. ~~[illegible]~~. × 4647 (on DO Sm) (0.5 ml/plate) Make heavy inoculum,
3. Keep it at 37°C for 2 days. and make reading.

Time of interrupting mating.

	10' & 15'			15'			20'			25'			30'			35'			40'			45'		
	U_6	Lac	Sex	U_6	Lac	Sex	U_6	Lac	Sex	U_6	Lac	Sex	U_6	Lac	Sex	U_6	Lac	Sex	U_6	Lac	Sex	U_6	Lac	Sex
1	s	−	♀	m	−		m	+	♂	s	−	♀	m	+	♂	m	+	♂	m	+	♂	m	+	♂
2	s	−	♀	m	−		s	−	♀	s	−	♀	m	+	♂	r	+	♂	m	+	♂	m	+	♂
3	s	−	♀	m	−		m	+	♂	m	+	♂	m	+	♂	m	+	♂	m	+	♂	r	+	♂
4	s	−	♀	m	−		s	−	♀	m	+	♂	s	−	♀	m	+	♂	m	+	♂	m	+	♂
5	s	−	♀	r	−		s	−	♀	m	−	♀	m	+	♂	m	+	♂	m	+	♂	m	+	♂
6	s	−	♀	s	−		s	−	♀	r	+	♂	m	+	♂	m	+	♂	m	+	♂	m	+	♂
7	s	−	♀	s	−		m	+	♂	r	+	♂	m	+	♂	r	+	♂	m	+	♂	m	+	♂
8	s	−	♀	s	−		s	−	♀	m	+	♂	m	+	♂	m	+	♂	m	+	♂	m	+	♂
9	s	−	♀	s	−		m	+	♂	m	+	♂	m	+	♀	m	+	♂	m	+	♂	m	+	♂
10	s	−	♀	m	−		s	−	♀	s	−	♀	m	+	♂	m	+	♂	m	+	♂	m	+	♂
							s	−	♀	s	−	♀	m	+	♂	m	+	♂	m	+	♂	r	+	♂
11	r	−	♀	m	−		m	+	♂	r	+	♂	r	−	♀	m	+	♂	m	+	♂	m	+	♀
12	s	−	♀	r	−		m	+	♂	m	+	♂	s	−	♀	m	+	♂	m	+	♂	m	+	♂
13	s	−	♀	m	−		s	−	♀	m	+	♂	m	+	♂	m	+	♂	m	+	♂	m	+	♂
14	s	−	♀	m	−		m	+	♂	m	+	♂	m	+	♂	m	+	♂	m	+	♂	m	+	♂
15	s	−	♀	r	−		m	+	♂	m	+	♀	m	+	♂	m	+	♂	m	+	♂	m	+	♂
16	s	−	♀	m	−		m	+	♂	m	+	♂	r	+	♂	m	+	♂	m	+	♂	m	+	♂
17	s	−	♀	m	−		m	+	♂	m	+	♂	m	+	♂	m	+	♂	m	+	♂	m	+	♂
18	s	−	♀	m	−		s	−	♀	r	−	♀	m	+	♂	s	−	♀	r	+	♂	m	+	♂
19	s	−	♀	m	−		m	+	♀	m	+	♂	m	+	♂	m	+	♂	m	+	♂	m	+	♂
20	s	−	♀	m	−		r	−	♀	m	+	♂	s	−	♀	s	−	♀	m	+	♂	m	+	♂
21	s	−	♀	m	+		s	−	♀	m	+	♂	r	+	♂	m	+	♂	m	+	♂	m	+	♂
22	s	−	♀				s	−	♀	s	−	♀	m	+	♂	m	+	♂	m	+	♂	m	+	♂
23	r	−	♀				s	−	♀	m	+	♂	m	+	♂	m	+	♂	m	+	♂	m	+	♂
24	s	−	♀				s	−	♀	r	−	♀	r	+	♂	m	+	♂	m	+	♂	s	−	♀
25	s	−	♀				m	+	♂	s	−	♀	m	+	♂	m	+	♂	m	+	♂	r	+	♂
26	s	−	♀				m	+	♂	s	−	♀	s	−	♀	r	+	♂	m	+	♂	m	+	♂
27	s	−	♀				r	+	♂	s	−	♀	m	+	♂	s	−	♀	m	+	♂	m	+	♂
28	s	−	♀				m	+	♂	s	−	♀	m	+	♂	s	−	♀	m	+	♂	m	+	♂
29	s	−	♀				m	+	♂	s	−	♀	r	−	♀	r	+	♂	m	+	♂	m	+	♂
30	m	+	♂				m	+	♂	s	−	♀	r	+	♂	s	−	♀	s	+	♂	m	+	♂
							m	+	♂	r	+	♂	s	−	♀	r	+	♂	m	+	♂	s	−	♀
31							m	+	♂	m	+	♂	m	+	♂	m	+	♂	m	+	♂	m	+	♂
32							r	+	♂	s	−	♀	s	−	♀	m	+	♂	m	+	♂	m	+	♂
33							s	−	♀	m	+	♂	m	+	♂	m	+	♂	r	+	♂	m	+	♂
34							m	+	♂	m	+	♂	m	+	♂	m	+	♂	s	−	♀	m	+	♂
35							m	+	♂	m	+	♂	r	+	♂	m	+	♂	m	+	♂	m	+	♂
36							s	−	♀	m	+	♂	m	+	♂	s	−	♀	r	+	♂	m	+	♂
37							s	−	♀	s	−	♀	s	−	♀	m	+	♂	m	+	♂	m	+	♂
38							s	−	♀	s	−	♀	m	+	♂	m	+	♂	m	+	♂	m	+	♂
39							m	+	♂	s	−	♀	m	+	♂	m	+	♂	m	+	♂	m	+	♂
40							s	−	♀	s	−	♀	r	+	♂	m	+	♂	m	+	♂	s	+	♂

	10'	15'	20'	25'	30'	35'	40'	45'
n|	3	26	24	23	11	7	1	2
n| V6|	0	0	2	3	2	0	0	0
n| V6| Lac|	0	1	2	0	0	0	0	0
n| V6| Lac' Sex|	0	~~27~~ 1	~~24~~ 27	~~44~~ 24	~~57~~ 41	57	57	59
Σ (tested)								
Pm, V6' Lac' Sex,		Pm	V6 Lac Sex		~~V6 Lac Sex~~			
	0	0	0	0	0	0	0	1
Σ	3	[illegible]	[illegible]	50	54	64	58	[illegible]

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18c

continued from former page.

10/VI 1960 REF:

infect F. and see F...

	1	2	3	4	5	6	7	8	9	10
				20'	25	30'	35'	40'	45'	
				V_6 Lac Sex	V_6 Lac Sex	V_6 Lac Sex	V_6 Lac Sex	V_6 Lac Sex	V_6 Lac Sex	
41				m + ♂						
42				s − ♀	s − ♀	m + ♂	m + ♂	m + ♂	m + ♂	
43				r − ♀	s − ♀	r + ♂	m + ♂	m + ♂	r + ♂	
44				s − ♀	m + ♂	m + ♂	m + ♂	m + ♂	m + ♂	
45				m + ♂	s − ♀	m + ♂	m + ♂	m + ♂	m + ♀	
46				s − ♀	s − ♀	m + ♂	m + ♂	m + ♂	m + ♂	
47				s − ♀	m + ♀	m + ♂	m + ♂	m + ♂	r − ♂	save.
48				s − ♀	s − ♀	s + ♂ F+? lo	m + ♂	m + ♂	m + ♂	
49				m + ♂	s − ♀	m + ♂	m + ♂	m + ♂	m + ♂	
50				m + ♂	m + ♂	s − ♀	m + ♂	m + ♂	m + ♂	
51				s − ♀	s − ♀	m + ♂	m + ♂	m + ♂	m + ♂	
52				m + ♂		s − ♀	m + ♂	r − ♀	m + ♂	
53						s − ♀	m + ♂	m + ♂	m + ♂	
54						s − ♀	m + ♂	m + ♂	m + ♂	
55						m + ♂	m + ♂	m + ♂	m + ♂	
56							s − ♀	m + ♂	m + ♂	
57							r + ♂	m + ♂	r + ♂	
58							m + ♂	m + ♂	m + ♂	
59							m + ♂	m + ♂	m + ♂	
60							m + ♂		m + ♂	
							r + ♂		m + ♂	
							m + ♂		m + ♂	
							m + ♂		m + ♂	

Σ	Lac	V_6	Maleness
2	−	s	♀
3	+	s	♀
4	−	m	♀
5	+	m	♀

Conclusion :

1. Lac⁺ is linked with maleness. all the Lac⁻ is F⁻ in early stage of mating. At the latter stage (45') Lac⁻ F_{13}, Lac⁺ F⁻ is also segregate out.

2.

check

Save

Σ	Lac	V_6	Maleness	10'	15'	20	25'	30'	35'	40'	45'
only. Fis not transferred	+	m	♀	0	0	1	2	0	0	0	0
whole seg	+	m	♂	0	1	25	20	34	50	54	54
	−	m	♀	0	0	0	1	0	0	0	0
	−	m	♂	0	0	0	0	0	0	0	0
	+	r	♀	0	0	0	0	0	0	0	0
can be mixed up	+	r	♂	0	0	2	4	7	7	3	5
Rec.	−	r	♀	0	0	2	2	2	0	1	0
Rec.	−	r	♂	0	0	0	0	0	0	0	1
Pur-only	−	s	♀	3	26	24	23	11	7	1	2
	−	s	♂	0	0	0	0	0	0	0	0

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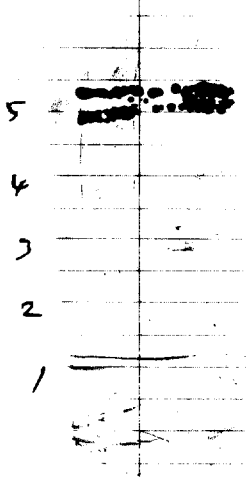
18d

Test of segregation from ♀ obtained by interrupted mating exp.

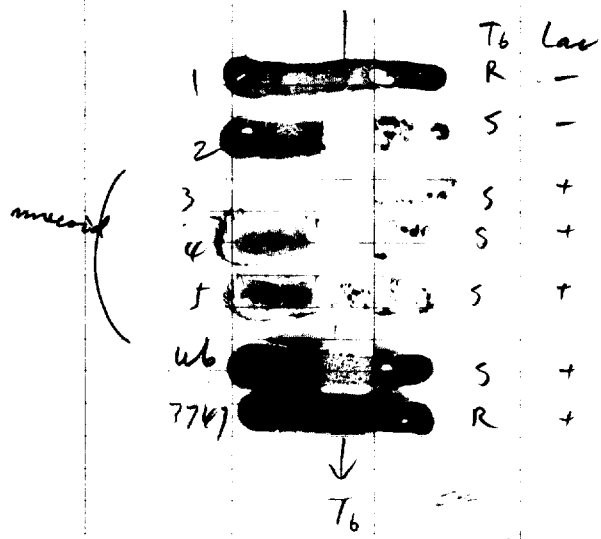
10/VI 1960 REF:

cf. all these colony are purified on B Lac agar. and retested

Isolated from (time of interruption)	genetic marker				Retest.
1	20'	Pur^+	V_6m	Lac_v^+ F^-	Lacv
2	25'	"	V_6m	$Lacv^+$ F^-	Lacv
3	25'	"	V_6m	$Lacv^+$ F^-	Lacv
4	25'	"	V_6m	Lac^- F^-	Lac^-
5	45'	Pur^+	Lac_{85}^-	V_6^R F_{13}?	Lac^-



+ 4447
Pur^-



B Lac Sm.

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Infectivity of F from 3747. to $\mathcal{F}_3$.

6/8 / ; 1960.

REF:

Principle. 3747 → 3876 F^- Cp^S Hfr_3 Mal_5 Lac_{11D_3} S^R.
F_{13} M V_6^R ×
W3637 F^- Cp^S M S^R.

purpose: Does F_{13} contain F which is free from F'~~[illegible]~~-Lac segment? on ~~[illegible]~~ MLac Sm.
F_{13} → ×3876 → Lac^+ F_{13}
F → ×3876 → Lac^- F^+ → Lac^- Hfr_3.

cultural age: PM 2:30 ~ pm 4:30 overnight culture
28hr.
Inoculum size 1 ml / 10 ml penassy broth.
Ratio of mix 3747 10 ml : 3876 1.1 ml.
at 37°C on rotator in penassy broth.

Seed this mix on BLac Sm.

Dil. vol: 10^{-4} : 0.1 ~~[illegible]~~

	4:30			4:40		[illegible]		[illegible]		[illegible]		[illegible]		[illegible]	
Time	0'		~~[illegible]~~	10'		15'		20'		25'		30'		35'	
Dil. vol	10^4:0.1			10^4:0.1		10^4:0.1		10^4:0.1		10^4:0.1		10^4:0.1		10^4:0.1	
Lac^-/plate	308	325		359	283	296	296	260	274	233	191	155	182	180	134
Lac^+/plate % of Lac^+	0	0		0	0	5	2	11	16	28	20	36	36	69	23
F^+/plate % of F^+	0	0		0	0	5	2	11	16	28	20	36	36	69.	23

Conclusion: Separate infection of F from Lac^+ are not observed.
This data seems against ~~[illegible]~~ "defective-double F" hypothesis.

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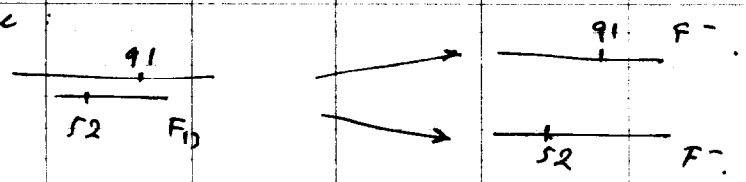
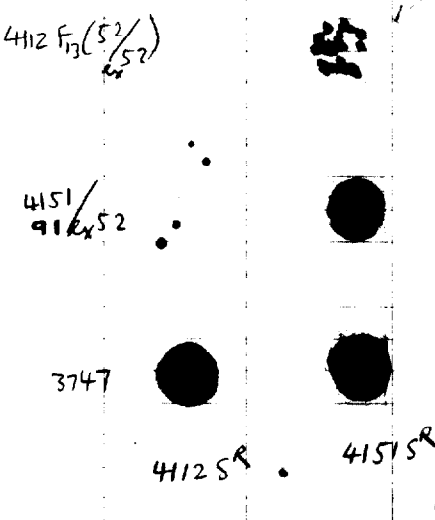
Test on fertility of exogenotic and endogenotic segment.

26/x ; 1960

REF:

Principle :