Preparation of λ reference from B120.

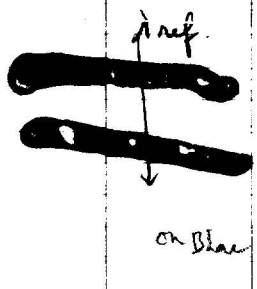
30/IV 1960 REF:

1. Make overnight grown culture in Pen. (8 ml).
2. Add it to 10 ml pen. & incubate it at 32°C for 2 hr on rotator.
3. C.f.g. resuspend bacteria in 4 ml of PO. 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. resuspend it in 1 ml of P. med. Small. 1:40 ~ 3:10 pm.

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

Titre	dilution vol.	# of Plaque
	10⁸ : 0.1	



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12a

Effect of AO on F8 gal+ ~~str~~ treated.

(3350 F8 1⁻ 2⁻ / + +.)

2/17 / 1960 REF:

1. 3 new isolates are grown in penassay broth.
2. Inoculate ca. 10^8 cells/ml AO- N~~C~~B. : 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 ~~C~~Hfr seeded W4573. on it. Look for gal+ F- and see segregation.

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Treatment of 3350 F_8 with AO.

1-2⁻/++ F_8

6/V ; 1960 REF:

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

Experimental condition:

Conc. of AO : 200γ/ml ; N.B. 5 ml pH. 7.6 3 tubes.
(0.2 ml of 5000γ 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^- SR on MGalSm.

Result:

Tube		plate #	gal⁻ F⁺	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	79
		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	5	0	6	26	0	26	499	14
		3	4	1	5	21	0	21	443	28
		4	6	5	11				367	16
		5							418	21
	II	1	22	1	19	71	0	71	2257 (96.1%)	90 (3.9%) 2347
		2	36	1	37	35	0	35	data 3999.	
			58	1	100	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 & Test
113	1
85	0 — F^+.

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

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12c

Segregation of gal⁻ from F⁻ & F⁺ strains derived from 3350 F_8 after treatment of AO.

20/4 ; 1960 REF:

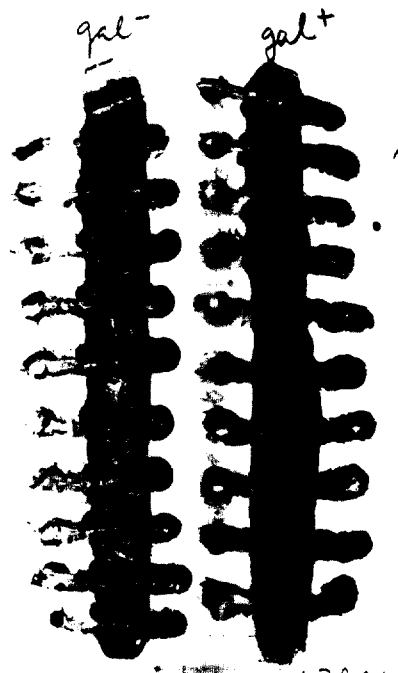
Isolation No	plate No	# of colonies tested on Blac. Lac⁻	Lac⁻	Lac⁺	# of colonies tested for sex-compatibility.	tester:
I	1	0	8	371	Segregate	3995
Sex.	2	3	4	462		
	3	4	2	533		
F⁻	4	3	1	19		
	5	4	2	631		
	Σ	14	17	2016		
II	1	0	0	629	No segregation	
	2	0	0	397		
F⁺	3	0	0	691		
	4	0	0	699		
	5	0	0	627	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

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

Are those all F+ or segregate?

o 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
↓
Nearly all [illegible] F_{13}^{+}

X 3995
on Mgal Sm.

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12d

Test behavior of gal⁺ F⁻ (d gal⁺F⁺) obtained with AO-treatment from 3350 F8.

; 1960 REF:

Purpose : [2]Are [3]those F⁻ [4]strains [5]df or [6]simple F⁻? (Does it become to F'9 by F×10 / Does it segregate gal⁻?)

1. b/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 SᴿM⁻F⁻ on Mglu Sm. W3086. } Incubate the mix for overnight.
   (b) Streak the on DO. & Test compatibility on MGal Sm × M⁻F⁻Sᴿ.
3. Streak (d) those F⁻ on BGal. d, 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⁻

Segregation of gal⁻	1	2	3	4
Sex.	seg. F⁻	No seg. F⁺ backed to F' H1 gal₂	seg. F⁻	seg. F⁺ (transfer of M⁺) to 3086 on Mglu Sm backed to F' H1 gal₂

II Infection of F. (Infectability)

(a) Cross brush the mixture of W6 and gal⁺F⁻X⁺ on Mgluc 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⁻Sᴿ) by cross brushing method. transfer of M⁺	+	−	+	+	+	−	+	+

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

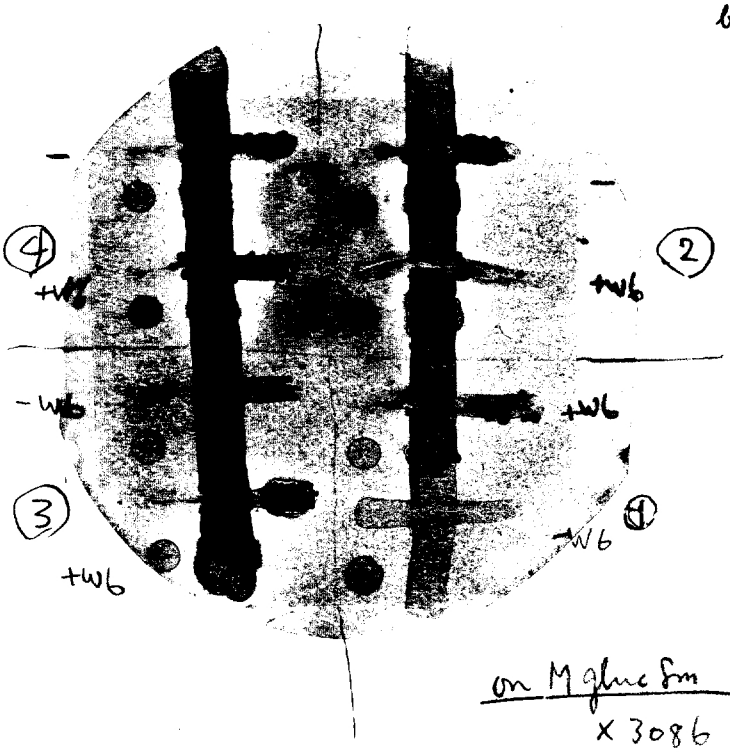
(b) Cross brush the isolates from mixture of W6 and gal⁺F⁻X⁺ (from DO) (x 3086) on Mgluc. Sm.

III Infectivity

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

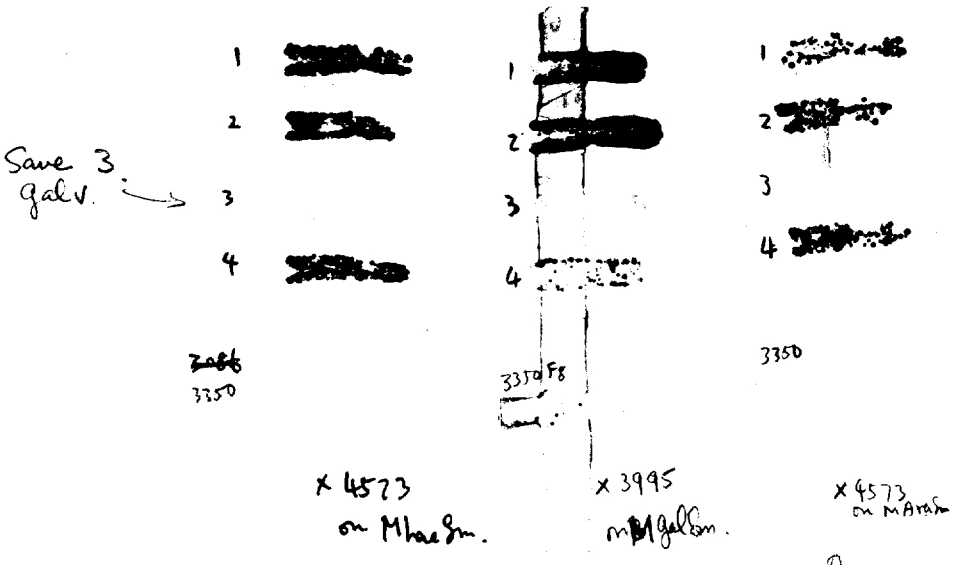
a) 1, 2, 4 shows identical character of Fg after transfer to broth. it recovers the fertility gradually.

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



on M gluc Sm
× 3086
(M+ transfer)

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



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

Test on Infectability by F.

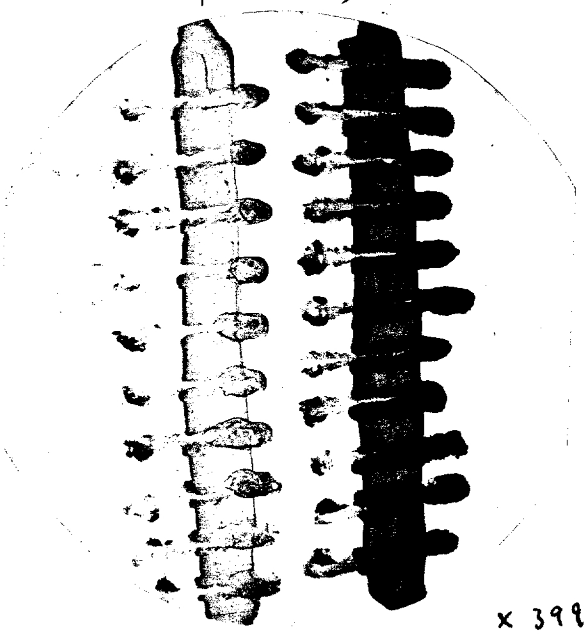
Wb → X (3350) AO
F8A ?

? reverse ?

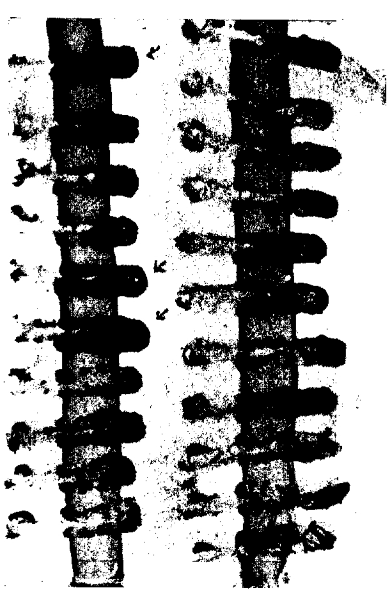
Rep...

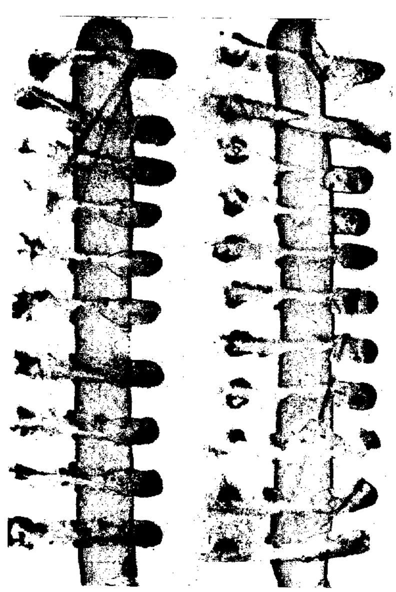
Test ...

1 3



x 3995
on H gal Sm



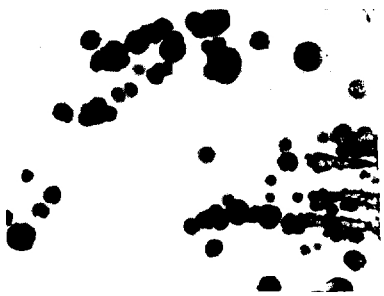


III	IV	I	II
II ?	I ?	IV ?	III ?

on H gal Sm.
x 3995
gal22.

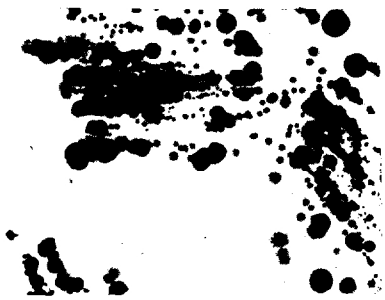
Transducibility of gal_2^+ (4573)

3350 AD 4 — X 4573
Fgal



on Dgal Su.

3350 Fgal 2 — X 4573



on Bgal Su.

Transducible for gal_2

Infectivity of F+ character of 3350 Fx.

19/v/1960. obtained by AO-method REF:

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

2. Infectivity of F. to 4573.

All those Fx mutate back to F8+ and infect F8 to F−

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Timing experiment of F13

5/8 : 1960 REF:

Strain : 3747 : M Pur⁺ V_6^R Lac⁺ ; 4637 ♀ S^S pur⁻ V_6^S Lac_{85} F⁻

Cultural age : overnight grown cell 0.2 ml / 10 ml penassay : 11:00 AM —4hr.— 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 :

Media \ Time	Marker selected	PM. 2:30	2:35	2:37.5	2:40	2:45	2:50	2:55
		Time after interruption (min.)						
		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⁺ 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. ~~~~ pur⁻ S^R F⁻, V_6 plate.

# of Pur⁺ Tested		10	15	20	25
V_6 r/s	r				
	s				
Lac	+	0	9 (F⁺ 9, F⁻ 0)	19 (F⁺ 19, F⁻ 0)	170 (F⁺ [illegible], F⁻ 0)
	−	1 (F⁻)	3 (F⁻ 3, F' 0)	2 (F⁻ 2, F' 0)	22 (F⁻ 20, F' [illegible], F⁺ [illegible])

Further test : ① Test sex compatibility. → Replicate on pur⁻ S^R F⁻ on DO Sm. V_6 ver Pur ver ∔ V_6^R → cross brush with V_6 W6628
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 conjugation and see what comes out. F$^+$ or F'?

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

• Experiment:

1. pick 5 pur$^+$ F$^-$ strains. 3747 MV_6^R ——× W4637 F$^-$ pur$^-$ V_6 Lac$^-$ S$^-$

2. Test T_6 resistance.

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

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

• Result:

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

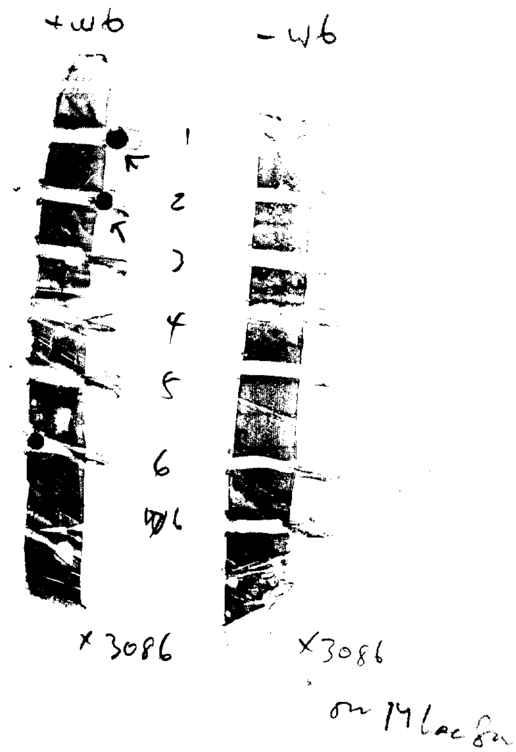
Isolation No.	2	3	4	5	6	7
	0/10	0/10	0/9	0/9	0/10	0/10

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$^-$ S^S and try this exp. again.


+wb
-wb
1
2
3
4
5
6
x3086
x3086

~~Make Strain W4354 F8a + a W4354 F8a+~~

10/V; 1960 Timing experiment. REF:

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

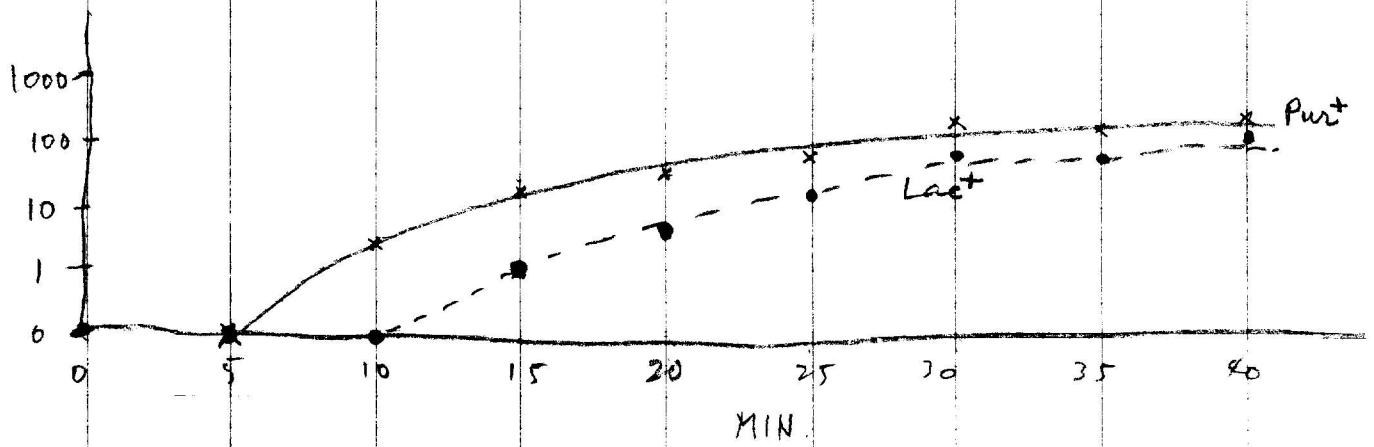
		Ratio
3747	M U_6^R F_{13}	10 ml.
4637	Pur Lac_{85} $\frac{S}{6}$ F^-	1.1 ml.

- 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 / 5, 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 / 1	2, 4 / 6	5, 10 / 15	33, 61 / 94	37, 22 / 59	58, 75 / 133
Dil. vol	10^3 : 0.1									

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

4628 $F^- pur_1^-$ SR^S



Timing of F recs.

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19 REF:

1	2	3	4	5	6	7	8	9	10
		b :	4671	≠8	20	39	16	27	
		c							
		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

↓

autolyse 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.

↓ d. overnight 3:00 PM ~ 10:00 AM

Spot 400γ/ml of p-NPP solution on that agar.

autolyzed with $CHCl_3$ → No autolysis.

↓ ↓

Incubate it for 10' at 37°C "

Conclusion :

1). 4 mg/ml of p-Npp works 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. 3L, 570 (1959)

Test genetic marker of Gara's strains.

; 1960 REF:

1) Test markers.

Strain No	3 Blac Lac	4 Dgal gal	5 Bgal Blac Sm.	6 Dgal Blac T6.	7 Mlac. Sex. (x4354)	8	9 Phosphatase	10 X
F7	−	−	R	S			−	+
F24	−	−	R	S			−	+
F13	−	−	R	S			−	+
F18	−	−	R	S			−	+
4573 P1	−	−	R	S			−	+
P2	+?	+?	Conc. Bf	P-NPP, 4mg/ml.			+	+
~~4573~~ 3747	+	+	R	R			+	+

2) Infect F13 to P⁻

3747 ——× F⁻P⁻

T6R M F13

Streak on Blac Sm

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

Further test

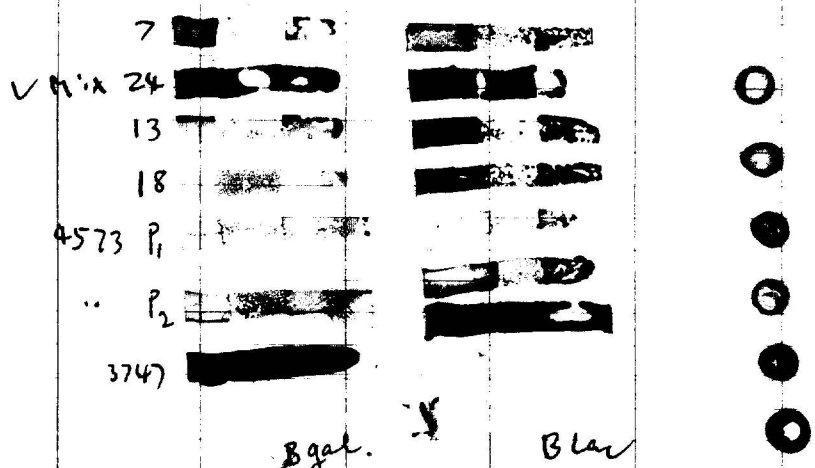
1. Test maleness: transfer of lac × 4573 Lac 85. on Mlac Sm

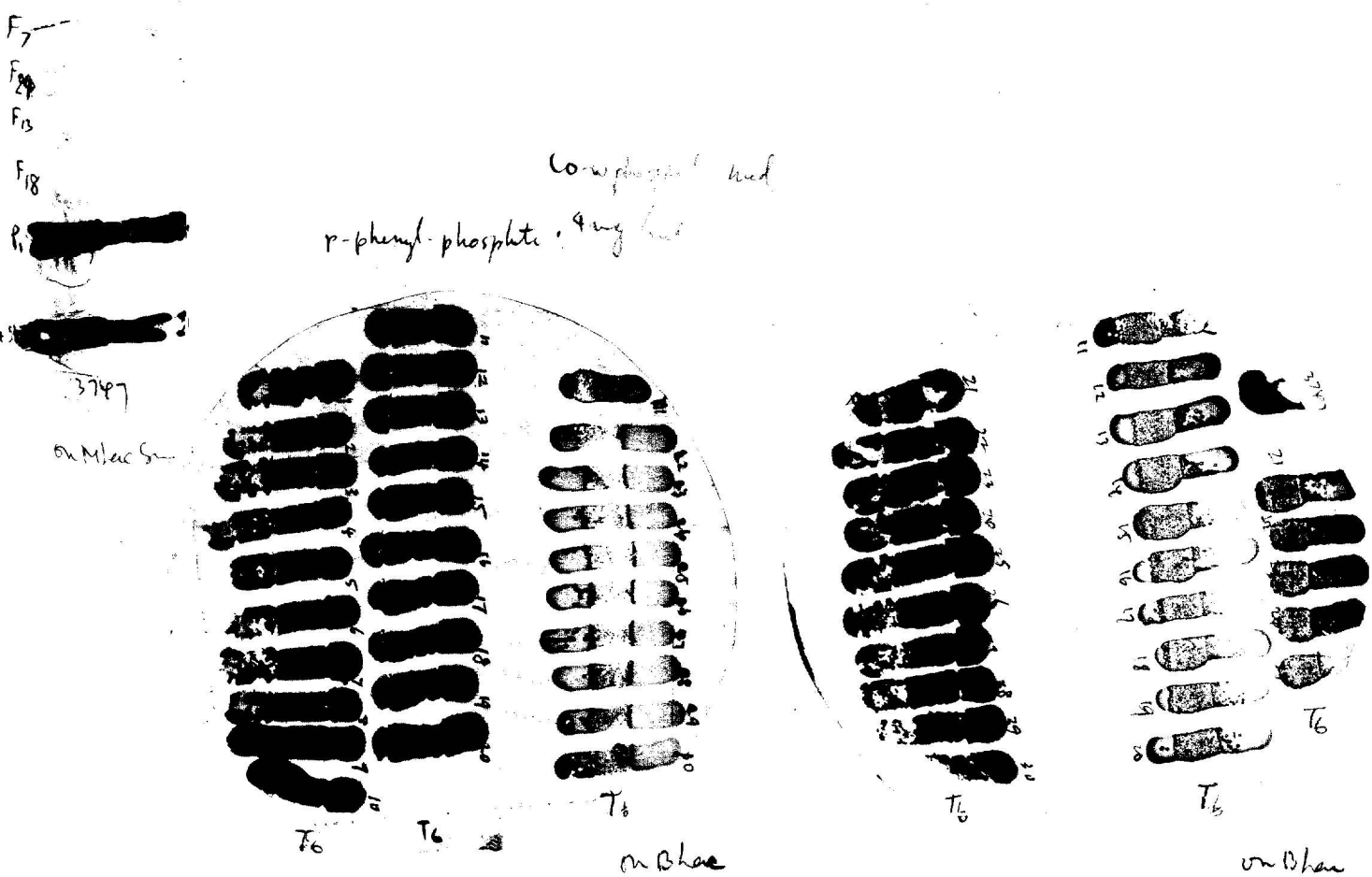
If it has F13, Some Lac⁻ of gara's strain must transduce Blac⁺ to 4573.

2. Test resistance to transduction of F13. of gara's strain × 3747 on Mlac Sm

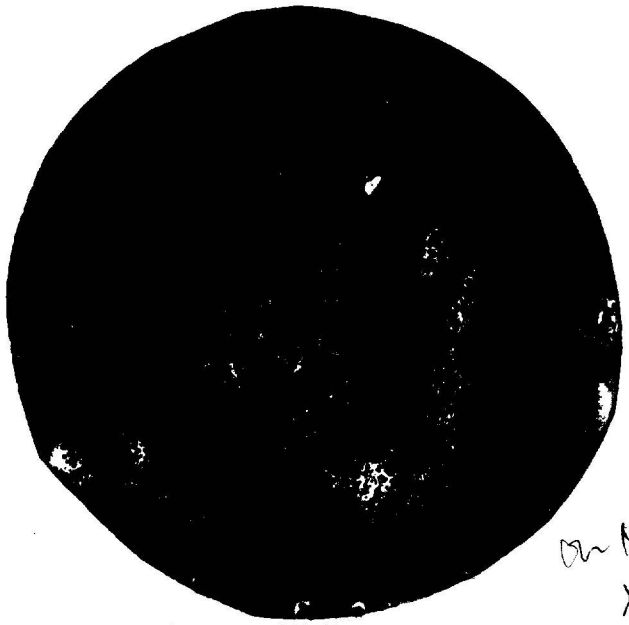
Possibilities ① Gara's strain is resistance to F13 infection

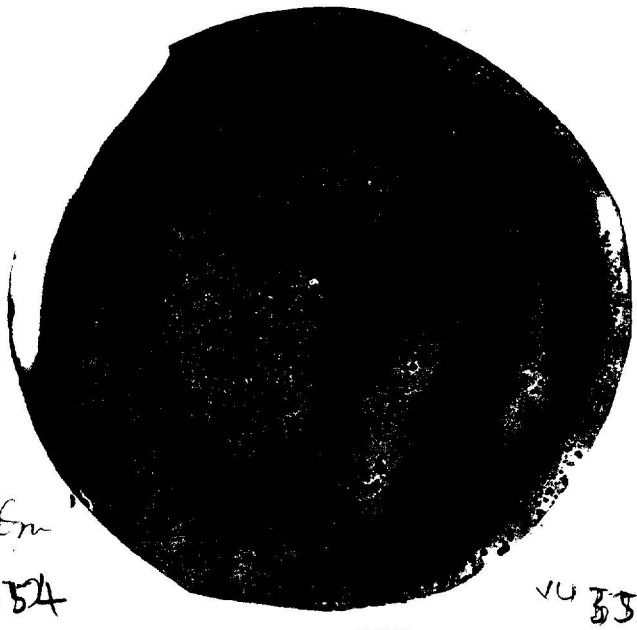
② Lac marker of gara's are not transducible by F13.

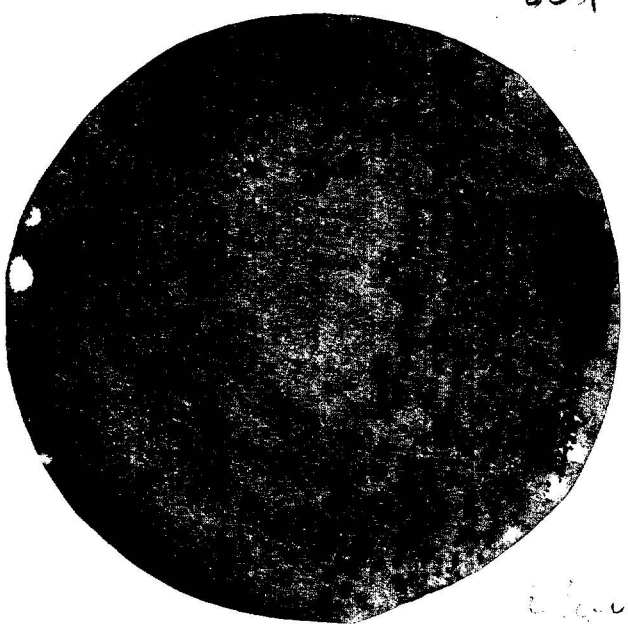



F7
F13
F18
3747
p-phenyl-phosphate
T6
T6
T6
T6
T6

F13 3247 ✱ F7 15c



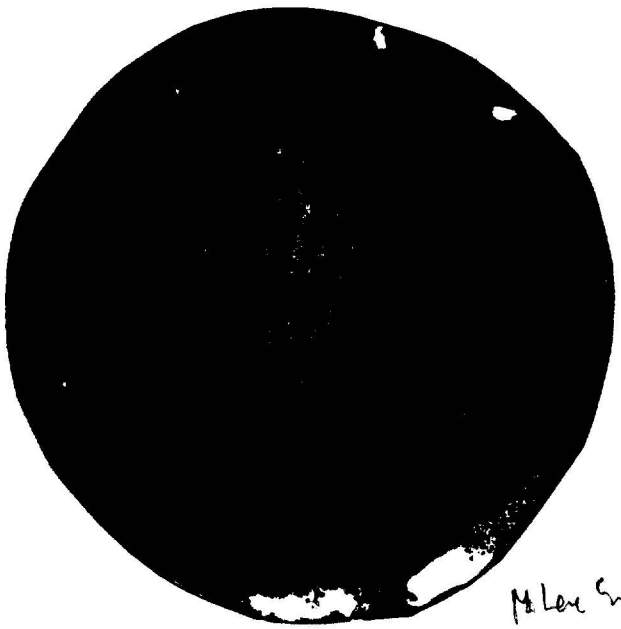


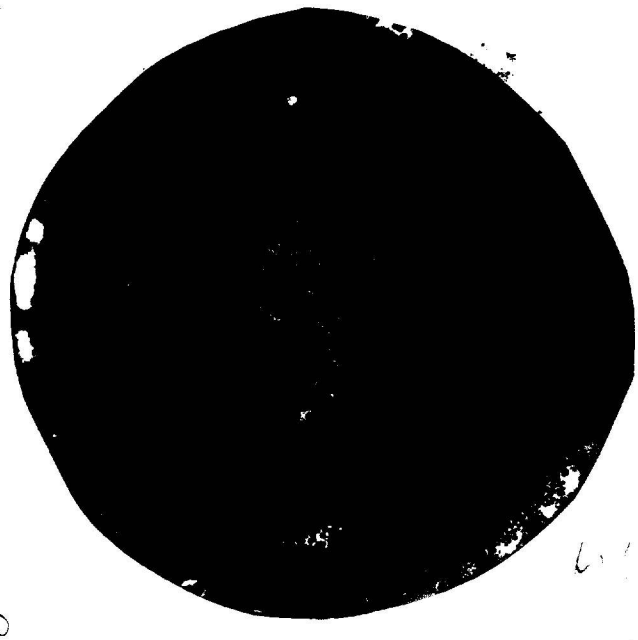


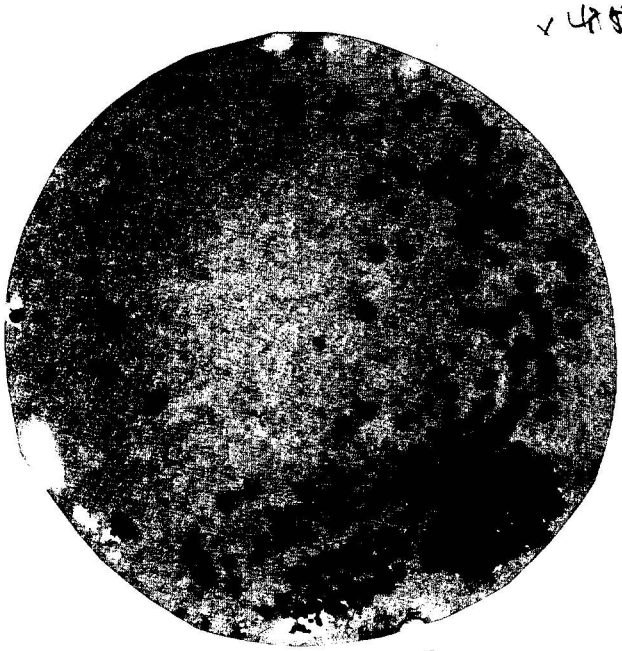
F18

F24

15 d







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 $F^- S^R$ Arth { W4277 gal$_7^-$ W4582 : F_{19}
W4278 gal$_6^-$ W4522 : F_{11}

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

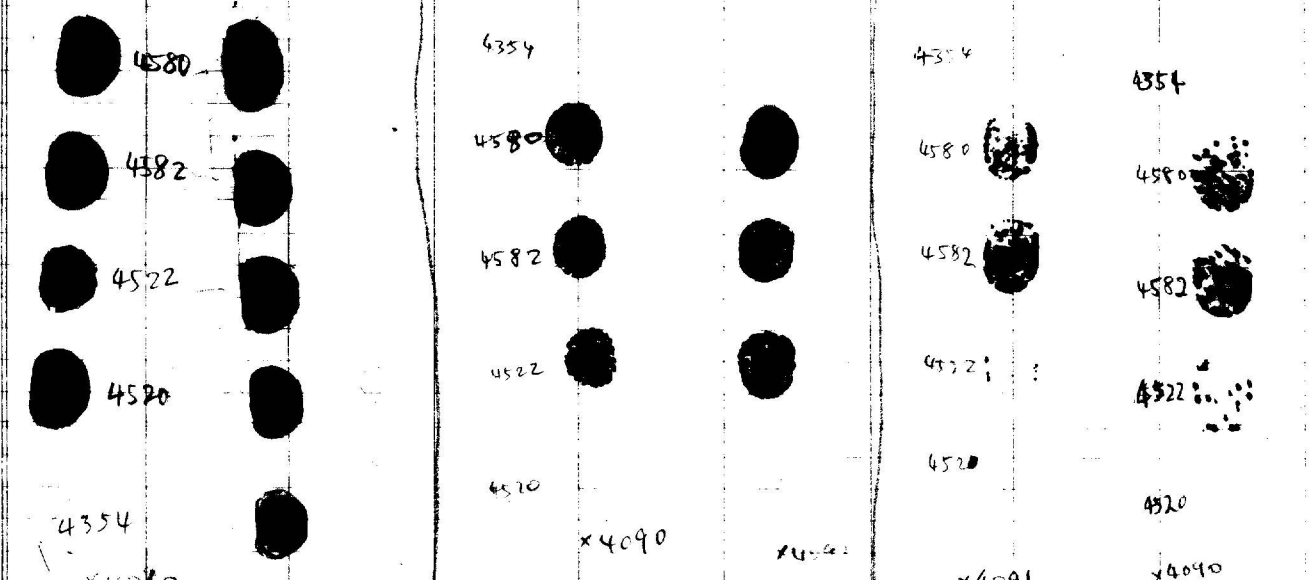
2) If HI. : Test Co-transduction of Gal & Try
Tester 4277. : gal$_7^-$ Arth. F^-

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 Bgal Sm.
2. Replica-plate them on DO. ~~or Mglucose~~.

Transduction of	4520 × 4277			4522 × 4277			4580 × 4277			4582 × 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(Arth$^+$)	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.

3/4 ; 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. 5ml of 4 hrs on rotator; c.f.g of resuspend it in DO. Irradiate it for 10 sec. Add 8 ml of phen. Incubate them 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.)

2. Treat with CHCl$_3$ and make cell free lysate of λ.

Mix : 3:00 PM. 3. Add those λ-lysates to W3097 with or without W6, and incubate it for overnight at 37°C. (0.1 ml : 1×1 ; phen 1 ml.)

Add penassay: 8:00 PM. 10 ml

Incubate them for overnight. 11:00 PM.

λ lysate was sterile.

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

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 phen.

Experiment :

(B) Test rough estimates

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

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
Σ gal$^+$	11	8

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18a

Time analysis of F_{13}-transfer.

21/6 ; 1960 REF:

Strains used:
3747 : M F_{13} $T6^R$
93:11 D_3 Pur⁻ Lac_{11D_3} F^-

↓ T_6 on BLac

Inoculate 0.2 ml / 10 ml pen. → Incubate it on rotator for 3 hrs. 1:00 : 3:00 p.m. → Mix ♂ & ♀.

Media selected	Markers selected	# of Recombinations / plate. 0'	5'	10'	15'	20'	25'	30'	35'	40'
M glucose 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 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 1182
M Lac + Pur + BA (Ad) 10^3 : 0.2	Lac.	0	0	0	1	9	18	216	557	306
	T_6									

45'

Pur⁺ colonies are isolated and tested on their $T6^R$ as by replicating them on EMBlac - which are attacked with T6 lysate.

Look for appearance of $T6^R$s.

	45'
Pur	293
Pur Lac	295
Lac	0.1

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

	0	5'	10'	15'	20'	25'	30'	35'	40'	45'
Lac⁻ V_6^{Var}	—	—		16	9	17	13	7	2	2
Lac⁺ V_6^{Var}	—	—		1	28	23	42	57	58	57
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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Test on Mating type of Recombined cells from interrupted mating experiment.

27/4 ; 1960

REF:

Method

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

Time of interrupting mating.

	10' & 15'			15'			20'			25'			30'			35'			40'			45'		
	Di	Lac	Sex	Vi	Lac	Sex	Vi	Lac	Sex	Vi	Lac	Sex	Vi	Lac	Sex	Vi	Lac	Sex	Vi	Lac	Sex	Vi	Lac	Sex
1	s	−	♀	m	−		m	+	♂	s	−	♀	m	+	♂	m	+	♂	m	+	♂	m	+	♂
2	s	−	♀	m	−		ms	+	♀	s	−	♀	m	+	♂	r	+	♂	m	+	♂	m	+	♂
3	s	−	♀	m	−		m	+	♂	m	+	♂	m	+	♂	m	+	♂	m	+	♂	r	+	♂
4	s	−	♀	m	−		ms	+	♀	m	+	♂	s	−	♀	m	+	♂	m	+	♂	m	+	♂
5	s	−	♀	r	−		ms	−	♀	m	−	♀	m	+	♂	m	+	♂	m	+	♂	m	+	♂
6	s	−	♀	s	−		ms	−	♀	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	m	−	♀	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	−	♀	m	+		s	−	♀	ms	−	♀	m	+	♂	m	+	♂	m	+	♂	m	+	♂
23	r	−	♀	m	+		s	−	♀	m	+	♂	m	+	♂	m	+	♂	m	+	♂	m	+	♂
24	s	−	♀	m	+		s	−	♀	r	−	♀	r	+	♂	m	+	♂	m	+	♂	s	−	♀
25	s	−	♀	m	+		m	+	♂	s	−	♀	m	+	♂	m	+	♂	m	+	♂	r	+	♂
26	s	−	♀	m	+		m	+	♂	s	−	♀	s	−	♀	r	+	♂	m	+	♂	m	+	♂
27	s	−	♀	m	+		r	+	♂	s	−	♀	m	+	♂	s	−	♀	m	+	♂	m	+	♂
28	s	−	♀	m	+		m	+	♂	s	−	♀	m	+	♂	s	−	♀	m	+	♂	m	+	♂
29	s	−	♀	m	+		m	+	♂	s	−	♀	r	−	♀	r	+	♂	m	+	♂	m	+	♂
30	m	+	♂	m	+		m	+	♂	s	−	♀	r	+	♂	s	−	♀	s	+	♂	m	+	♂
				m	+		m	+	♂	r	+	♂	s	−	♀	r	+	♂	m	+	♂	s	−	♀
31				m	+		m	+	♂	m	+	♂	m	+	♂	m	+	♂	m	+	♂	m	+	♂
32				r	+		m	+	♂	s	−	♀	s	−	♀	m	+	♂	m	+	♂	m	+	♂
33				s	−		m	+	♂	m	+	♂	m	+	♂	m	+	♂	r	+	♂	m	+	♂
34				m	+		m	+	♂	m	+	♂	m	+	♂	m	+	♂	s	−	♀	m	+	♂
35				m	+		m	+	♂	m	+	♂	r	+	♂	m	+	♂	m	+	♂	m	+	♂
36				s	−		m	+	♂	m	+	♂	m	+	♂	s	−	♀	r	+	♂	m	+	♂
37				s	−		s	−	♀	s	−	♀	s	−	♀	m	+	♂	m	+	♂	m	+	♂
38				s	−		s	−	♀	s	−	♀	m	+	♂	m	+	♂	m	+	♂	m	+	♂
39				m	+		s	−	♀	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 | V_6 |	0	0	2	3	2	0	0	0
n | V_6 | Lac |	0	1	2	0	0	0	0	0
n | V_6 | Lac | Sex |	0	~~2~~ 1	~~2~~ 27	~~4~~ 24	~~5~~ 41	57	57	59
Σ (tested)								
Pun, V_6 | Lac | Sex |		Pun	V_6 | o Sex		~~V_6 Lac Sex~~			
	0	0	0	0	0	0	0	1
Σ.	3	2[illegible]	[illegible]9	50	54	44	58	[illegible]

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continued from former page.

infect F.

10/VI 1960 REF:

	20'	25'	30'	35'	40'	45'
	V6 Lac Sex	V6 Lac Sex	V6 Lac Sex	V6 Lac Sex	V6 Lac Sex	V6 Lac Sex
41	m + ♂					
42	s [illegible] ♀	s − ♀	m + ♂	m + ♂	m + ♂	m + ♂
43	mr − ♀	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 V6 maleness~~

Lac	V6	maleness
~~−~~	~~s~~	~~♀~~
~~+~~	~~s~~	~~♀~~
~~−~~	~~m~~	~~♀~~
~~+~~	~~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.

Σ Lac V6 maleness

	Lac	V6	maleness	10'	15'	20	25'	30'	35'	40'	45'
only. F1 is not transfe[rred]	+	m	♀	0	0	(1)	(2)	0	0	0	0
whole eg	+	m	♂	0	1	25	20	34	50	54	54
	−	m	♀	0	0	0	1[illegible]	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

check (20: 1; 25': 2)

Save (45': 1)

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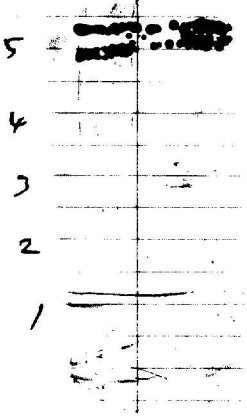
18d

Test of ♥ segregation from Z. obtained by interrupted mating exp.

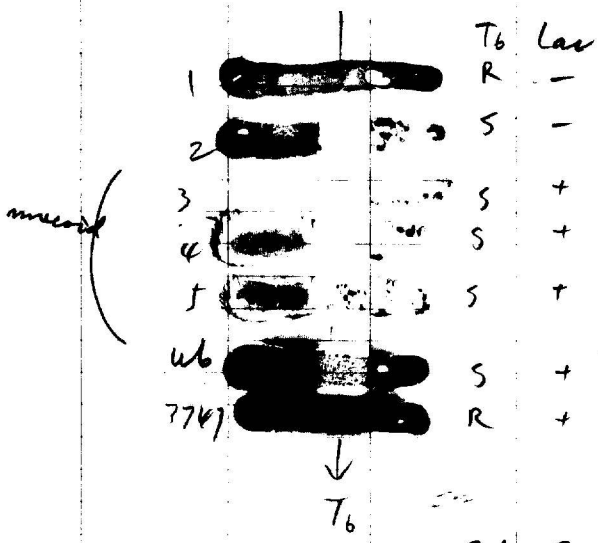
10/VI 1960 REF:

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^-	Lac_V
2	25'	" V_6m	Lac_V^+ F^-	Lac_V
3	25'	" V_6m	Lac_V^+ F^-	Lac_V
4	25'	" V_6m	Lac^- F^-	Lac^-
5	45'	Pur^+	Lac^-_{85} V_6^R F_{13}?	Lac^-



× 447
Pur^-



B Lac Sm.

Infectivity of F from 3747 to F_3.

W
6/8/ ; 1960.

REF:

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

purpose: Does F_{13} contain F which is free from F'~~ma~~-Lac segment? or ~~M~~ 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
28 hr. Inoculum size 1 ml / 10 ml penassay broth.
Ratio of mix 3747 10 ml : 3876 0.1 ml
at 37°C on rotator in penassay broth.

Seed this mix on BLac Sm. Dil. vol: 10^{-4} : 0.1 ~~ml~~

Time	4:30 0'			4:40 10'		4:55 15'		5:10 20'		5:25 25'		5:40 30'		5:55 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	0	0		0	0	5	2	11	16	28	20	36	36	69	23
% of Lac^+															
F^+/plate	0	0		0	0	5	2	11	16	28	20	36	36	69.	23
% of F^+															

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

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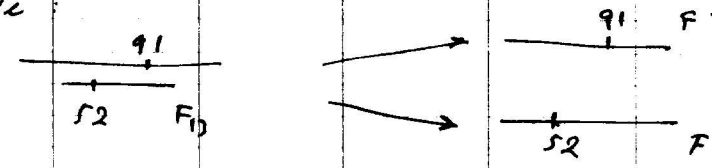
Test on fertility of exogenetic and endogenetic segment.

26/x ; 1960

REF:

Principle :



4112 F_{13} (52/52)

4151/91 x 52

3747

4112 S^R • 4151 S^R

on Mlac Sm