Quantitative measurements of the transfer of F' to F- by Sm-killed F^+ cells.

30/11 : 1959 REF:

Principle: W4534 (F_8) → 3086 × W4573.

Method: 1. Treat W4534 with Sm. ×10 conc. than usual Sm. (1 mg/ml)
×10 diluted Sm. 0.2 ml / 2 ml culture
· cultural age : 24 hrs.; aerassy grown; Wash twice with broth:
Time of treatment 9:00 pM → 1: pM. (next day)
at 37°C

2. Mix → Incubate it overnight.

Experiment: Sm treated W4534 + 3086 (ca. 10^6 cells/ml) — 2 hr. culture & overnight grown culture 0.5 ml/5 ml per.
control: Untreated W4534 + 3086 (ca. 10^6 cells/ml.)
and incubate them for overnight.

Check: survival: 0.1 ml/plate Sm-killed cell; 10^{-6} ml/plate untreated cell. Incubate it for 2 hrs more

2' Test survivor.

3. Seed it on Blac Sm, and select W3086. ×10^{-5} ml & ×10^{-6} ml.

4. Replica plate on MGal needed W4573. on it.

Result :

1. # of survivors: (for overnight) : colony forming activity.

0.1 ml/plate Sm-treated	Sm-Untreated (×10⁻⁶) 10^{-6} ml/plate.
0	460
0	516
0	540 ?
0	520
0	575
Σ 0	

2. # of (F_8) infections.

Sm treated: F_8^+ / # of colonies tested (%) 10^{-6} ml/plate.	Control Sm-Untreated: F_8^+ / # of colonies tested. (%) 10^{-5} ml/plate
1 709 : / 2104 (33.8)	1 59 / 68 (87)
2 584 : / 2390 (24.4)	2 65 / 51 (88)
3	3 60 / 69 (87)
4	4
5	5
Σ (%)	Σ (%)

Sm untreated control, F_8^+ is survivor. Therefore, it shows strong competition with 3086. But Sm-treated group is not. It is clear to see their difference.

% of infections per survivors:

W4534 : overnight culture in Penassay broth. + Sm 10 μg/ml
5 ml. < 2 ml : Sm-treated
2 ml : untreated control. (X10 much than usual use.)

↓

Incubate it overnight at 37°C.

↓

Wash twice by Centrifugation. (with penassay). (10 min. for each centrifugation)

↓

Suspend it into penassay. 1.5 ml.

↓

Test survivors of W4534? No
0.1 ml/plate Sm treated
0.1 ml. × 10^{-6}/plate untreated.

Add. young culture. (2 hr : ① overnight culture 0.5 ml + 5 ml/plate
of W3086. incubate it 2 hrs more
(F' recipient). ② dilute × 10^{1}; add 0.1 ml to
Add 10^{6} cells into the 1 ml of W534

↓

Incubate it for overnight at 37°C.

↓

Seed W3086 on Blac Sm. and incubate them.

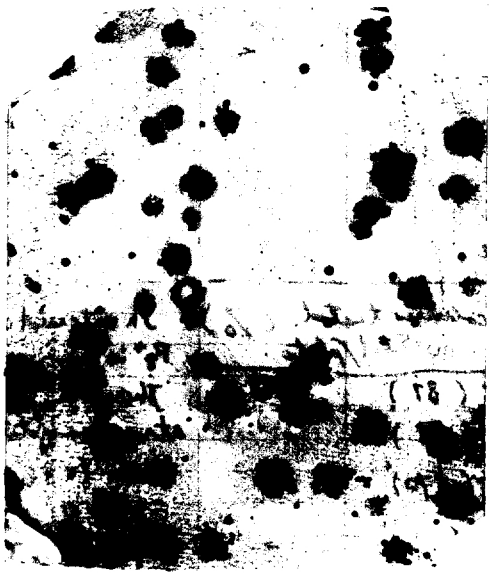
↓

Replica plate them on MGal seeded W4573 on it.

↓

count the percentage of $F8^{+}$ infected.

untreated control.
W4534 × W3086



untreated control.
× W4573
on MGal

Sm killed W4534 × 3086
Treated control.

on MGal
seeded W4573

2nd trial for isolation of F_3^+.

(W3642F_1): F^+ H Mal_S Gal_2 Lac_1.

2/24 ~ ;1959 REF: See p122

W4554.

Principle.

① W3642 F_1 → × 3133.

W6 → × 3133

② select on DO.

③ Replica plate on MLac seeded W3086. and look for H1 for M.

Results.

W3642 F_1 → × 3133			W6 → × 3133		
# of Colonies	Hfr	%		Hfr	%
627	1		586	0	
613	2		673	0	
676	3		517	1 ?	
693	20		684	13	
1742.	2		509	0	

④ Test the fertility again. (× 3086, on MLac) by cross-streaking method

⑤ purify #2 and #6 on BLac and reisolate 3133 F_3.

#2 #6. 55 colonies of 3133 F_3 are reisolated and tested on the fertility in transfer of Mt. Best non of them are Hfr. (see back page)

∴ It means the 3133 F_3^+ is very unstable after infection of the F' to F⁻. It segregate many F^+

⑥ Try Replica plating method to for reisolation of 3133 F_3.

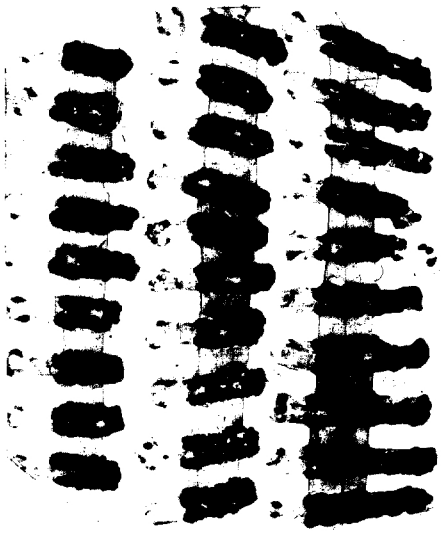
3642 F^+ → × 3133

3133

× 3086

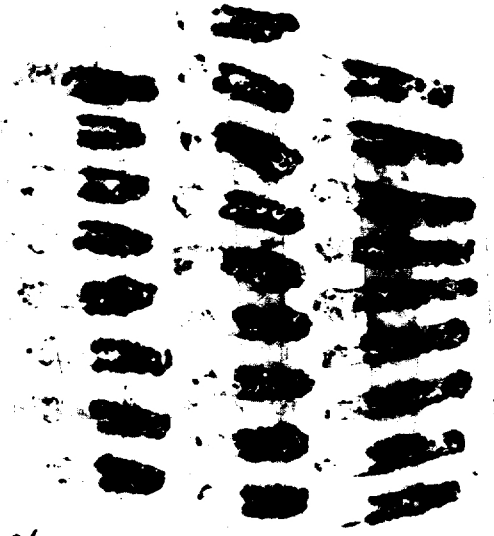
Purification of 3133 F_3.

#6



×3086
on Mlac.

#2

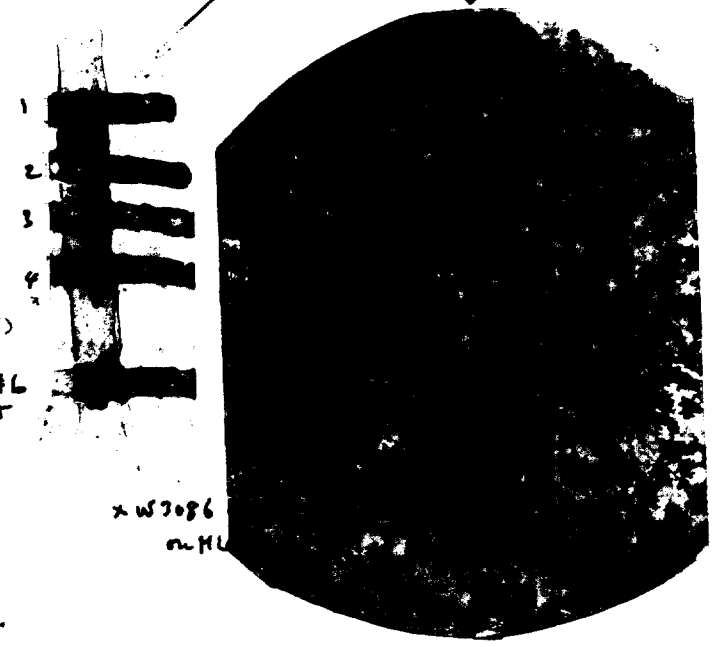


×3086
on Mlac

Reisolation of 3133 F_3^+ by cross-streaking method was not successful. Use replica plating method for 3133 F_3^+ isolation.

Seeded W3086
on Mlac.

these reisolated F_3 looks reasonably stable.



#L
ent

×W3086
on ML

Test W3747. (derived strain from W3213)

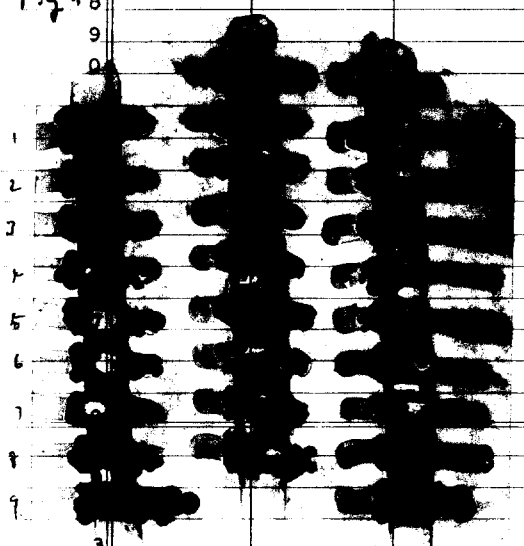
17/11 ;1959 $Lp^R M^- V_6^R Hfr_{13}$. REF: Hfr_{13}.

J.L says: Alan found that it is HI for Lac (× 3133 on MLac) but the many of these recombinants show Lac^v. This seems quite peculiar phenomenon, it may (linked transfer of Lac and F' dependent on Hfr marker. (Hfr may linked with Lac.)

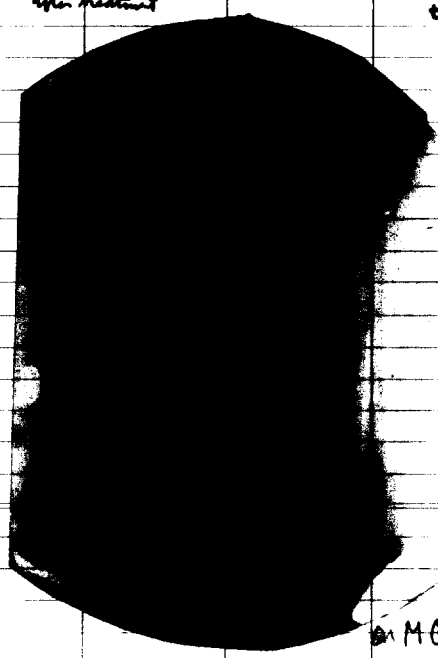
1. Purify W3747 on Blac and test the fertility of each picked colonies from the Blac (× on MLac)
2. Look for Hfr_{13} and pick recombinant from cross streaked location. Purify it on Blac.
   Result: All the colonies are Hfr. (Fig 1)
3. See variegated colonies on the Blac plate.
   Result: It segregate Lac$^+$, Lac$^-$, and Lac^v. (See bellow Fig. 2) several colonies/plate are Variegated.
4. Pick 12 Lac^v, suspend it into water, and streak it on Blac, Gal, Mal, Mt/Xyl, to know the size of incorporated chromosomal segment.
5. Pick Lac$^+$ and Lac$^-$ and cross × F$^-$, and see the relation between F and Lac marker.
6. Treat #17 with AO. and conform the infectivity of Hfr-character.
   AO 20′, and AO 30′.
   W3747 → × W3086.
   Result: This strain W3747, transfers the Hfr-character into F$^-$ (3086). Therefore, it is not Hfr but F$^+$ (Hfr for Lac.) (see back page).
   Hfr 16/28 total ×100 = 57 (%)
2. AO does not work in this experiment. (25/25 were Hfr. after treatment. untreated control: 27/27 Hfr. tester 3828

Fig 1

W3747



Anth
A_1
G_1
P
TLB_1
H



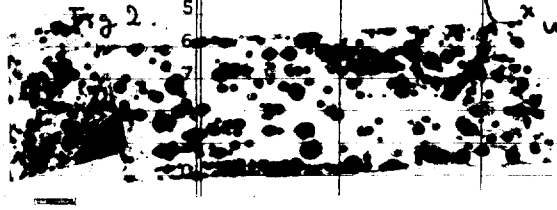
on M Glucose

Lac v.

Blac

Fig 2.

on MLac
× W4573

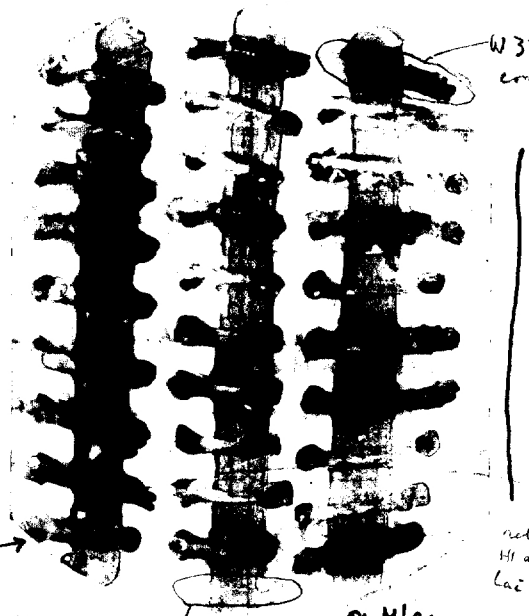


on Blac cont.

Tests on infectivity of F_{13} to 3086

$Hfr_{13}\ M^-V_6^R\ Lp_4^R$ $\quad F^-M^-Mal_1\ S^R$

W3747 → × W3086



This might be related with infectivity of Hi and Lo colony in the Lac property.

This colony is hi for Gal, instead of Lac! This means there are two states. Hi for Lac or Hi for Gal. See below.

Data from cross-bunching method — Rate of conversion

Hi : Lac	16/28	(57 %)
Lo : Lac	1/28	(3.7 %)
total	17/28	(60.8 %)

Data from Replicaplating

Hi : Lac	101/576	(17.6%)
Lo : Lac	34/576	(5.9%)

F_{13}

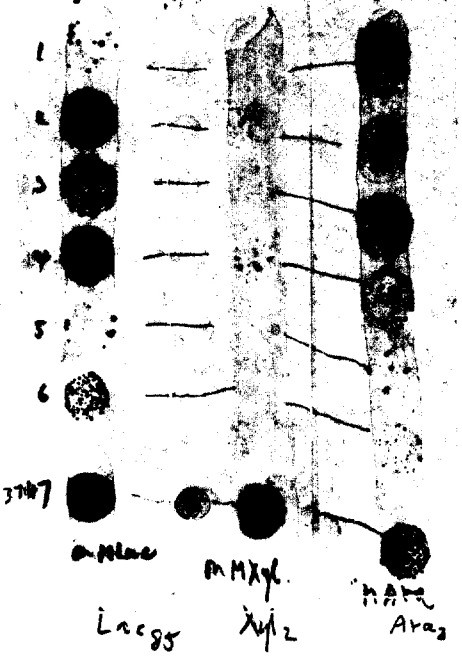
W3747 → × W3086 :

on Blac Sm → replicated on Mlac seeded W3828.

Test of Hi and Lo colonies of W3086 F_{13} on the transfer of various markers.

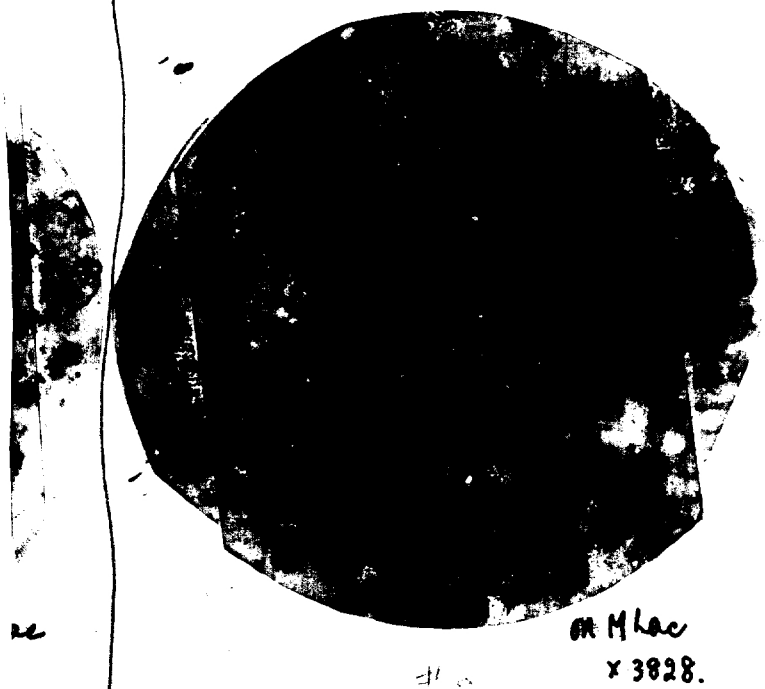
3086 F_{13} → × W4573.

2, 4 : Hi Lac + 1, 3, 5, 6 Lo : Lac.



Hi : Lac is low for all markers, but Lo : Lac is Hi Gal, relatively Hi for Ara_2.

It is not so strict. Hi for both



Save one as W3086 F_{13}.

ity on the futility of Lac and Gal transfer, However, It may be interpreted by the mixture of both types of clones.

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104

Fertility of the segregants from a sectored-colony arised from the cross W3747 × 3828.

3°/9 1959 REF:

Method: 1. ~~Spot W3743 × 3828~~. Cross-streak W3743 × 3828 on Mlac.
2. Purify the black ~~spot~~ area on Blac.
3. Pick sectored colony and resuspend it into water (1 ml) and streak it on Blac again.
4. Pick (Lac⁺ and) Lac⁻ colonies, respectively, and test their fertility by cross × 3086. (H⁻ F⁻) on Mlac.
5. Result.

HI for H: Hfr 1/33

Lo for H: 32/33

Control. W4 —

3743×

×3133 ×4573 on Mlac

Conclusion: Lac⁻ Segregants from diploid colony are all of not ♀ unlike as usual Hfr.

all the sugar markers are checked by replica plating method
All, Lac⁻, Mal⁺, Mtl⁺ Gal⁺ (See back page)

#1 #2

Control 3744 3133

on Mlac ×3086

3744 3133

on Mlac ×3086

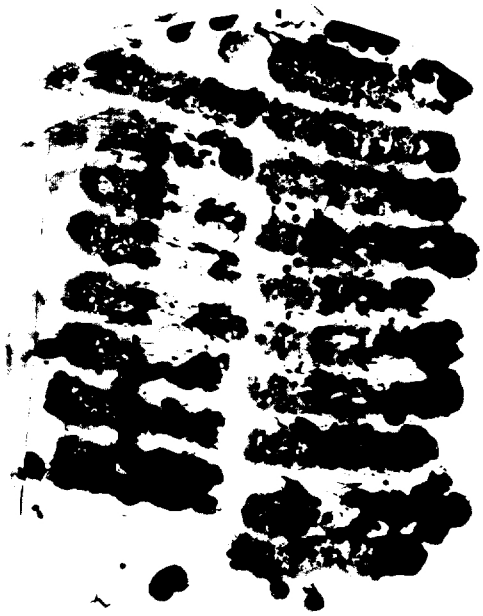
Hfr? Save this strain F⁺ 19 Lac⁻ Sm^R

Three 2 kinds of colonies, HI for H Lo for H may be interpreted by two states of colonies.

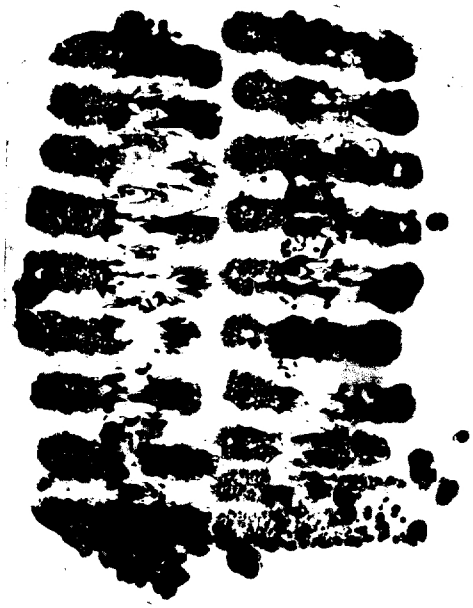
HI Lac is Lo the others, but Lo Lac is HI the others. (esp. Gal)

Hypothesis; ① Transduction of Lac result HI Lac but it will mutate to Lo Lac.

#2.



#1



Sex-compatibility of Lac⁻ segregants from Lac+ colonies obtained from the cross W3747 x W3133.

30/VI ; 1959 REF:

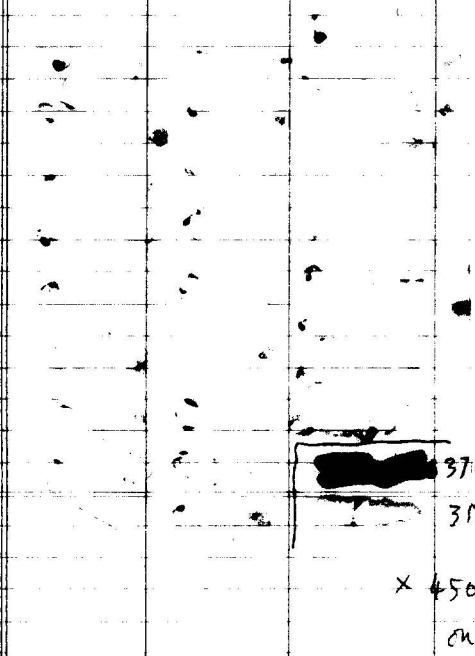
Method : 1. Cross W3747 x W3133 on MLac agar. by spot test.
2. Purify it on Blac agar.
3. Pick Lac+ and repurify on Blac.
4. Pick Lac⁻ segregants and cross-brush it with W4503 (Pur⁻F⁺) on MLac agar.

Result.
all of the Lac⁻ segregants were F⁻. (see bellow.)

Lac+ : #1. 0/26 all 26 were F⁻ (0 %)
Lac+ : #2. 0/27 " 27 " " (0 %)

Conclusion: This result seems contradict from the former result.
Try again.

#1



× 4506 (Pur⁻F⁻) on MLac

#2

× 4506 (Pur⁻F⁻) on MLac.

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L06

Transduction of Lac-loci by F_{13} (W3747)

$S^S F_{13} M 4^S V_6^R$

29/VI ; 1959 REF:

Method : 1. Mix W3747, W4573. 1 ml + 1 ml + 1 ml fresh broth.

2. Incubate it overnight.

3. Dilute and seed onto EMBlac Sm.

4. Count the ratio of numbers of Lac^+ and Lac^- colonies on it.

5. Test compatibility of those colonies

Marker selected: Sm^R

Results:

1. Rate of infection of Lac^+ loades into Lac^-_{85} strains.

plate No.	Lac^-	Lac^+	% of Lac^+
1	20	9	31.2
2	28	2	6.67
3	13	2	13.3
4	14	5	26.3
Σ	75	18	19.4 %

on Blac Sm.

(: rate of infection of Lac^+ to Lac^-)

Conclusion : This rate (19.4 % : totals colonies tested) is very high.
Other kind of transfer of Lac^+ marker occured. (mechanism of not recombination) to F^-

2. Test the Compatibility of the Lac^- and Lac^+ colonies isolated from above experiment.
Look for Lac^- F_{13} comes from splitting of F_{13} from Lac by

Method : Replica plate on MXyl seeded W4506 on it. (save it)

Result: All Lac^+ is compatible with F^- (x F^-Pur^- : W 4506), but fertility is lower than control : Parent. (Test fertility of Lac^+ transfer).
All Lac^- are F^- (Steril in cross x W4506) on MXyl.
41 (Lac^+) all σ^+ : 88 (Lac^-) all σ^-. (see back page)

3. Length of the transduced-segment by F_{13}.

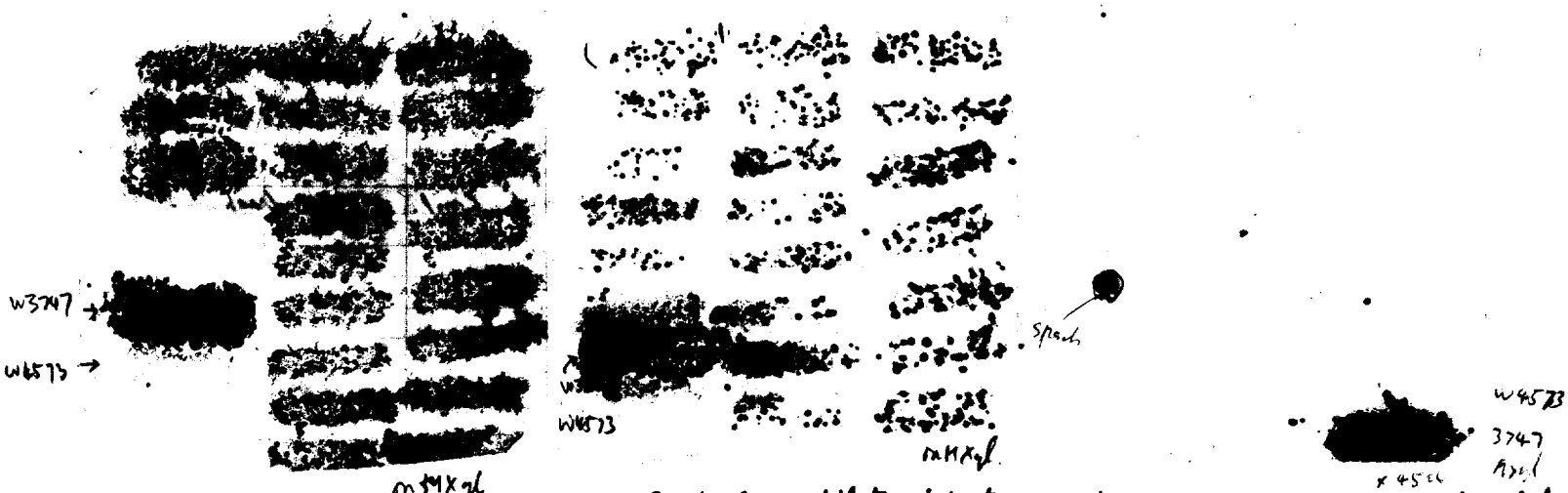
V_6	Lac$_{85}$	Gal_2	Ara_2	Xyl$_2$	Mtl.	Mal$_1$	M.	# of colonies tested.	
S	+	-	-	-	-	-	+	41	Lac. transduced.
S	-	-	-	-	-	-	+	88	F^- Parental type.

No other combinations of these markers were found.

Conclusion : Size of genetic materials transferred is very small.
It looks only Lac is transferred into F^-, even no selective marker was used for near Lac-region.

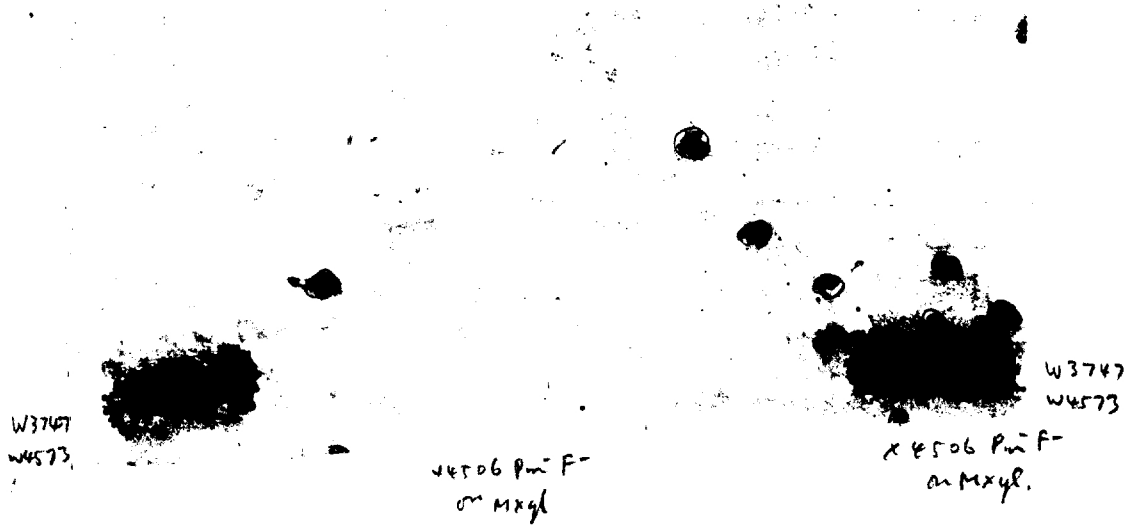
Next step : Use reverse transduction of Lac^+_{85} to Lac^- other origin M^- F^-
Use W6 F_{13} and W4541 ($F^- S^R$ Lac Xyl$_2$ Mtl Mal$_1$ Gal_2 V_6 P)

1 (Lac$^+$) 2 (Lac$^+$) 3 (Lac$^-$)



○ The low fertility of Lac$^+$ clones from donor (W3747) may be interpreted by two states. (Lo for Lac is Hi for the other markers, but Hi for Lac is low for other markers.) Test ● Lac-transfer. of Lac$^+$.

4 (Lac$^-$) 5 (Lac$^-$)



Transfer of F' of $^{V}F_8^+$ (W4534 Sm-killed) to F⁻; 1 mg/ml Sm.

1/VII ; 1959

REF:

1	2	3	4	5	6	7	8	9	10

• Principle:

W4534 (Sm-killed cells.) $\xrightarrow{F_8}$ ~~W4534~~ W4573 + W3994

W4573: F^- Ara_2 X_{y1_2} MtI Gal_2 Lac_{85} Mal_1 (φ^+)

W3994: F^- Gal_4 (φ^+) S^R

Gal_4^+ and Gal_2^+

Gal^s M Gal_4

on BGal + BGal Sm

• Further experimental design.

① (Use S^R φ^s for all strain)
W3104 (S^R) φ^s Gal_4 F^-
W3102 (S^R) φ^s Gal_2 F^-
No S^R.

② If Gal_2^- Gal_4^- S^s F_8 was used for F_8-donor, it is better.

• Possibility:

These infective F' of Sm-killed W4534 may be streptomycin-resistant F_8'.

• Experiment:

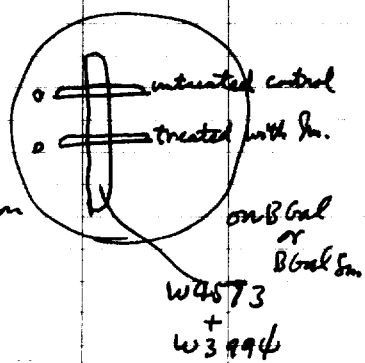
Method: ① add 0.1 ml (there is a sign of Sm (use: 0.1 ml to 100 ml)) to 1 ml of overnight culture of W4534.

② Incubate it at 37°C for 2 hrs.

③ Wash it with 5 ml of Pensasy once.

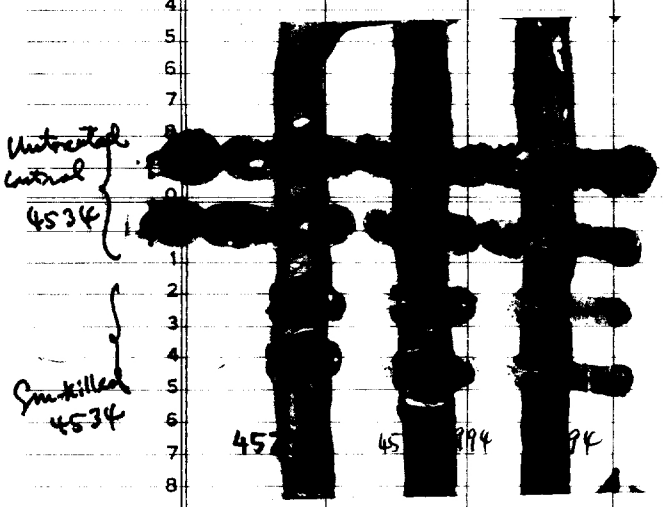
④ Cross-brush it with the mixture of W4573 and W3994 on BGal and BGal Sm.

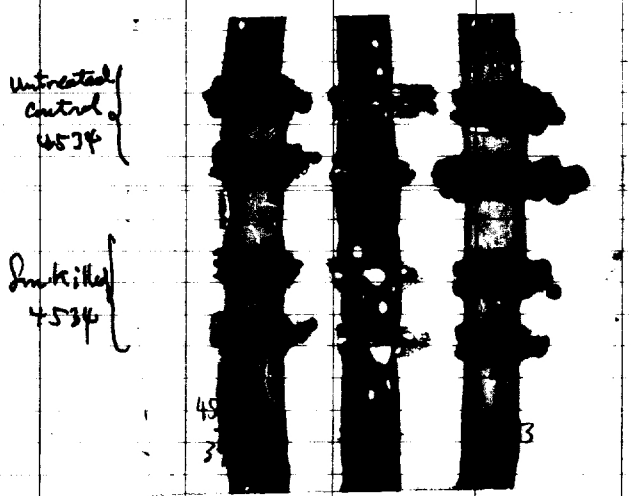
⑤ Incubate it overnight.



• Expectation:

If Sm-treated W4534 cells are completely killed, and still have some infectivity of F_8, it may gives gal^+ recombinant at the contact area by infection and chromosome transfer from each other.





(Treat W3747 with Sm.)
Transduction of Lac-marker to F⁻ by Sm-killed F13.
(and isolation of Sm-resistant F13 from it.)

2/4 :1959

Method (Add)

1. (Treat) W3747 with Sm. (×10 conc. ; overnight-treated in phrasey-Sm) overnight grown culture in penassay broth.
2. Incubate it for overnight at 37°C. (Sm-treated cell ● shows no growth but untreated control is ca. 10 times turbid)
3. Wash it once with 2 ml of Penassay broth and resuspend it into 1 ml of Penassay broth, and test the fertility ~~infectivity~~, by cross-streaking method. and by standard recombination method.

Result : See back page

● Cross-streaking method : Cross-brush it with [redacted] 3828, and W4573. on MLac, Blac, and BLac Sm. use untreated W3747 as a control.

● Semi-plating method :

1. Spread W4573 on MLac, and add 0.1 ml W3747-Sm-killed cell to ● half of the plate, other half is control. Also try to make control.

Result :

+ Sm killed W3747	control
377	0

see back page

2. Incubate it 40 hrs.

Result :

● Standard method for testing fertility of Sm-killed cells.

	# of Recombinants / 10^{-6} ml
Untreated control :	542
	: 579
	: 609
Sm treated	: 63
	109
	62

1. Add 0.2 ml of Sm-killed cells to W4573 : 1 ml. Use untreated W 3747 as a control. Add 1 ml of fresh broth to it.
2. Incubate it for 2 hrs. at 37°C. (11:45 AM ~ 1:45 PM.)
3. Dilute them in adequate concentration, and seed it on MLac.

Untreated control : ~~Use~~ Use 10^{-6} ml. / plate.

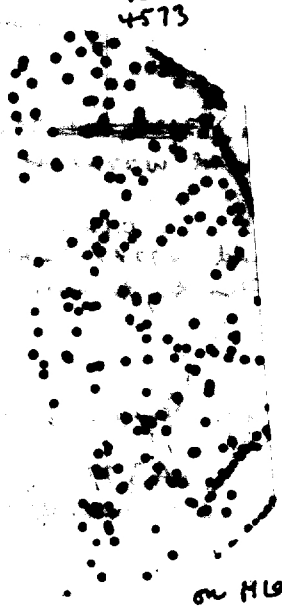
Sm-killed cell : " 10^{-1} ml / plate.

● ~~Sm~~ Survival test

		colonies / plate
Untreated control :	Use 10^{-7} ml / plate.	248 ; 303 (3×10^9)
Sm-killed cell :	" 10^{-1} ml / plate.	0 ; 1 (0 or 10)

W 4573 only

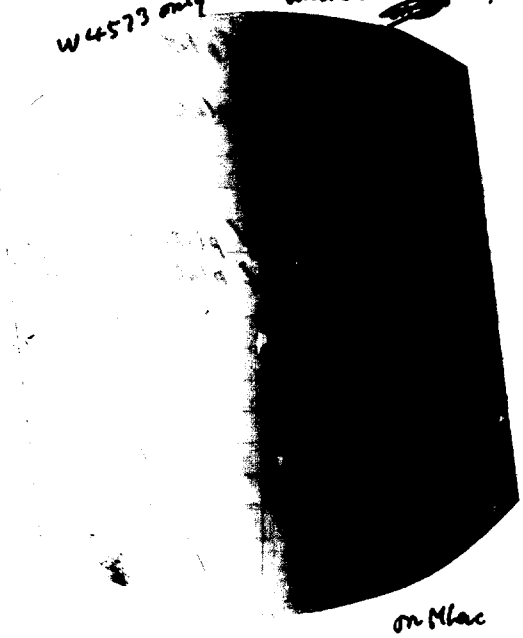
Sm-killed W3747 (0.1ml) x 4573



on Mlac

W4573 only

Untreated W3747



on Mlac

x 4573.

on Blac Sm.

x W 4573.

on Blac

Untreated W3747

Sm-killed W3747

x4573

on Mlac Sm

on Mlac.

Untreated W3747

Sm killed W3747.

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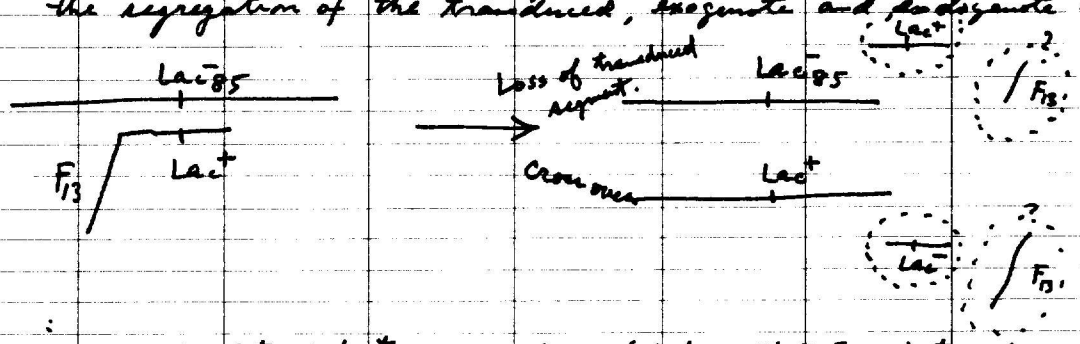
109

Test stability of transduced marker "Lac+"

7/VII ; 1959 REF:

Principle : Lac+ is dominant marker, therefore, diploid strain exhibits Lac+ phenotype as well as haploid Lac+.

If it is ~~[illegible]~~ hemizygous diploid at Lac loci, it must show the segregation of the transduced, exogenote and zygogenote marker.



Method :

Test unstability of Lac+ of newly isolated W4411 F_{13}. Lac+. (see 15_3 6/VII)
Use 5 strains

1. Dilute it in adequate ~~[illegible]~~ cell number. and plate it on Dlac, incubate it overnight.
   use 10^{-7} ml / plate.

2. Count total number of colonies and Lac⁻ colonies

Ref : These culture is A independently isolated ~~[illegible]~~ by transduction of Lac+ to 4411 from W3747. Lac+ colonies are inoculated into pimasay broth and incubated ca. 7 hrs ~~[illegible]~~ at 37°C. Those cultures were used for this experiment.

Result :

Esolation number :		# of total colonies obtained	# of Lac⁻ colonies	% of Lac⁻
2	2	0	0	
		0	0	
3	3	186	1	
		134	0	0
		173	0	0
4	4	377	3	0.7.9.6
		384	2	0.5.2.1
		394	2	0.5.0.8
5	5	668	2	0.3.0.0
		714	1	0.1.4.0
		683	2	0.2.9.3
1	1	10	0	
		4	0	
		0	0	

1 , 2 was too few colonies

Further work : Test sex-compatibility of Lac⁻ colonies which are segregated from the transduced Lac+ probably Diploid for Lac.

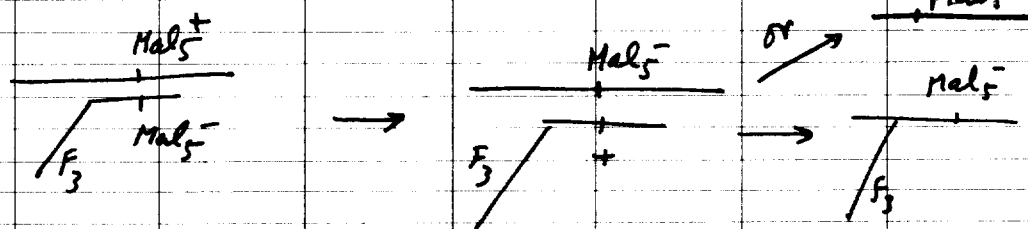
Result : #3 of 1/7 F⁻ #5 of 1/5 F⁻

Recombination between Mal_5^- and F_3.

7/6/11 ; 1959 REF:

Principle: F_3 may transfer Mal_5^- in the transduction, but Mal_5^- is recessive. Look for segregation of Mal_5^- from 3133 F_3 (Mal_5^-/Mal_5^+)

Scheme:



Method: ① Dilute 3133 F_3^+ : Use 10^{-6} ml/plate ; and seed it on B Mal.

② Incubate it overnight at 37°C.

③ Look for Mal^-.

Control 3133		Experiment. 3133 F_3	
Mal+	Mal−	Mal+	Mal−
244	0	841	0
224	0	920	0
239	0	856	0
222	0		
287	0		

allelism test using 3828 F_{13}. (Lac_{1a})→(Lac^{3229})
Called $11D_3$.

6/11; 1959 REF:

	1	2	3	4	F⁻ x⁺ 5	6	F⁻ x⁺ 7	8	9 History of 10 $11D_3$.
very unstable	Lac_1	: Permease			W 3238	Lac_{61}	: W4121		Lac_1 T87
	Lac_3	: Lac^- gal⁻ mal⁻ Gal⁻			W 2243				Lac_1 W518
									" W1578
	Lac_5	: Lac^- Mal⁻			W2245 (S^R)				" W1321
	Lac_2	: β-galactosidase			W3112 (S^R)				Lac_1 W1687
	4	: β-galactosidase			W4287	W3127			Lac_{1a} W3120
	Lac_7	: Permease and β-galactosidase			W 2247				Lac^{3229} W3229
	Lac_{11}	(11)			W 3230				Lac^{3229} W3133
	Lac_{12}	(12)			W 3089 (Mal S)				Lac_{11D_3} W3828

Principle : W3828 F_{13} × F⁻ Lac⁻ on MLac.

Method :

1.) Purify all Lac⁻ mutants on Blac agar.

2.) Inoculate purified colony into 5 ml/ passage broth.

3.) ~~Water~~ Spot test on each strains. for transfer of Lac from W3828 F_{13}.

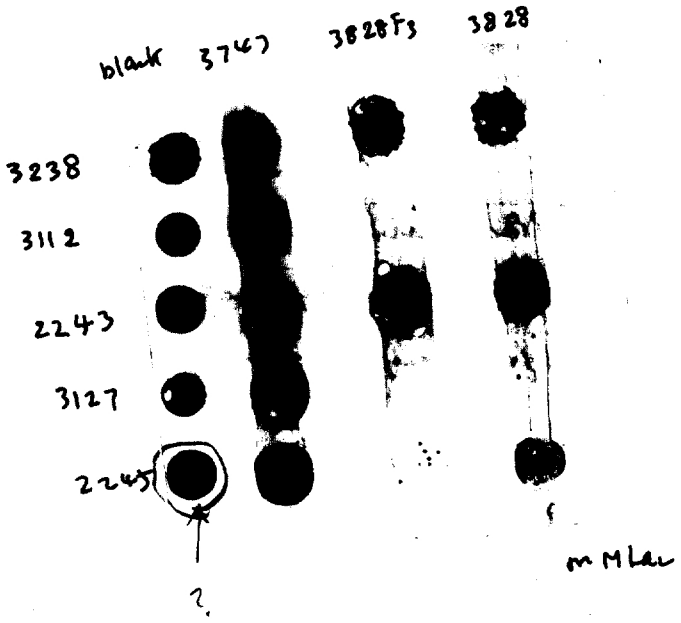
Result :

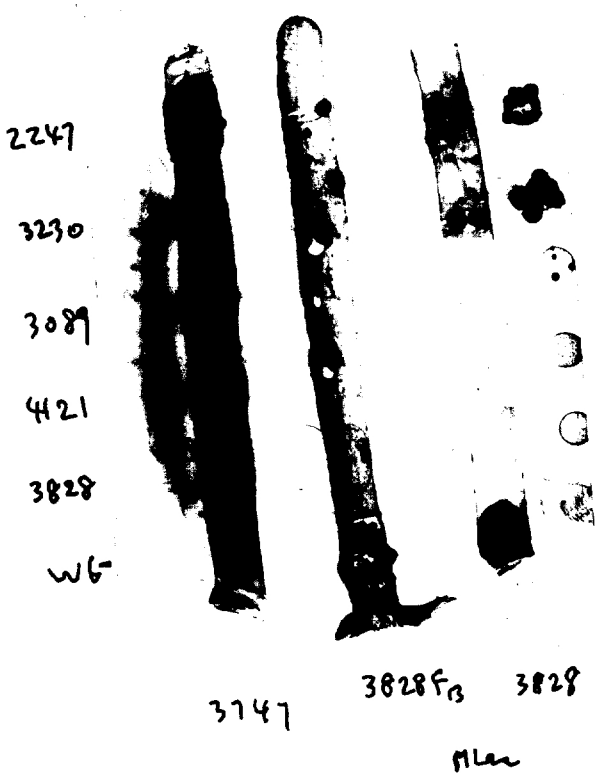
Lac	W-	3828	3828 F_{13}	3747	blank
1	3238	(+) –	(+) –	+++	(+)
2	3112	–	–	+++	–
3	2243	? +++	? +++	! +++	+++? remain
4	3127	–	–	+++	–
4	2245	+	+	+++	+++ ?
7	2247	–	–	+++	–
11 (1a)	3230	–	–	+++	–
12 (1b)	3089	–	–	+++	–
61	4121	–	–	+++	–

on MLac 4 on MLa Sm.

see back page :

Conclusion : W3828 F_{13} may be wrong, not F_{13}^- but F⁻


blank
3747
3828F3
3828
3238
3112
2243
3127
2245
?
mMLau


2247
3230
3089
4121
3828
WG-
3747
3828F13
3828
MLau

Transduction of Lac-loci by F_{13}.

[W6 F_{13} ⟶× W4541
W4511 F_{13} —× W2594]

3863 $Pa\bar{c}$ F^-

7/VII 1959 REF:

1	2	3	4	5	6	7	8	9	10

Principle :

One way : W6 F_{13} (transducible) ⟶× W 45[illegible]. ($F^- S^R Lac_{85} Xyl_2 Mtl Mal_1 Gal_2 V_6 P$)
M F_{13}.

back : Lac^+ [illegible] W4411 F_{13} ⟶× W 2594 ($F^+ M Lac\ S^R\ U_1^R$)
P [illegible] Lac^+ P Xyl_2 Mtl Mal, Gal_2 V_6

Method :

1. Mix 1 : 1 : 1 (fresh broth) and incubate it overnight at 37°C.
2. Purify them on Blee 9m. count the ratio of Lac^+ and Lac^-. Use 10^{-3} ml/plate
3. Test on all markers of Lac-transduced W4541. and W2594.

Ratio of Lac^+ and Lac^- (+/−)

W6 F_{13} × W4541

; 239/249 (51.1) ; 224/267 (45.6) ; 255/266 (49.0)
; 285/213 (52.4) ; 209/242 (46.4)

F S Lac Xyl Mtl Mal_1 Gal_2 V_6 P M

Plate	1.	2	3	4	5	6	7	8	Σ	%
r +	40	40	35	38	37	38	38	40	306	97.2
.. + ..	1	0	2	1	3	0	0	2	9	2.86

W4411 F_{13} ⟶× W2594 ① Ratio of Lac^+ / Lac^- Partial^R ; 178/260 (40.6) ; 152/237 (38.1) ; 125/229 (35.3)
; 135/206 (39.6) ; 201/258 (43.8) :

② Pick Lac^+ and test other markers.

Plate No.	F	S	Lac	Xyl_2	Mtl	Mal_1	Gal_2	V_6	P	M	T_1	No of colonies	%
2	r	+	+	+	[illegible]±	+	[illegible] S^s (r:1) (s:1)	+	−	r		53	
4	r	+	+	+	+	+	S^s (r:4)	+	−	r		48	
8	r	+	+	+	+	+	S^s (r:2)	+	−	r		39	
2	r	+	+	+	+	+	(−:1) S^s (r:2)	+	−	r		39	
	r	+	+	+	+	+	S^s {r:3 s:2}	+	−	r		53	
1	r	+	+	+	+	+	S^s {r: s:}	+	−	r		40	
6	r	+	+	+	+	+	(−:1) S^s {r:3 s:}	+	−	r		39	
7	r	+	+	+	+	+	S^s {r:1 s:}	+	−	r		38	
5	r	+	+	+	+	+	S^s (r:2)	+	−	r		44	

Total Tested 393 — 93.4

	S^r	Lac^+	Xyl_2^+	Mtl^+	Mal_1^+	Gal_2^+	$V_6^{s^s}$	P	M	T_1^r	: 367	93.4	
	r	+	+	+	+	Gal_2^-	$V_6 S^s$	+	M^-	T_1^r	: 2.	0.508	Lac^- 0.508
	r	+	+	+	+	+	V_6^r	+	M^-	T_1^r	: 19	4.83	Lac^+ 99.492 %
	r	+	+	+	+	+	V_6^s	+	M^-	r	: 5	1.27	

V_6^s + 24
Gal_2^- 2

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H2a

19

REF:

	plate 7	1	2	3	plate 8	5	6	plate 9	8	9	10
		V6	Lac	Gal	V6	Lac	Gal	V6	Lac	Gal	
1		I	H1	H1	I	H1				H1	
2		I	I					I			
3		r	H1								
4		I									
5								r	H1	acap. tr.	
6											
7											
8											
9											
0											
11											
2						Endomix?					
3					r	H1 Excpl					
4					I						
5											
6											
7											
8											
9											
20											
21											
2											
3											
4											
5											
6											
7											
8											
9											
30											
31					r	Lo					
2					I	H					
3	Gal-										
4											
5											
6											
7											
8											
9											
40											
41											
2											
3											
4											
5											
6											
7											
8											
9											
50											

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REF:

	plate 4	2	3	plate 5	5	6	plate 6	8	9	10
	V6	Lac	Gal	V6	Lac	Gal	V6	Lac	Gal	
1	I	HI	HI	I	HI	HI				
2										
3										
4										
5										
6										
7										
8										Gal –
9										
0										
11										
2	I									
3	r	Lo								
4	I	HI								
5										
6				R	Lo					
7										
8										
9										
20							R	Lo		
21										
2							R	(HI)	except	
3							R	Lo		
4										
5										
6										
7										
8										
9										
30										
	I									
31	r	Lo								
2	I	HI								
3										
4	I								except	
5	r	Lo								
6	I	HI					(S)	Lo		
7										
8										
9										
X0										
X1										
2										
3										
4										
5										
6										
7	I									
8	r									
9										
0										

Crossover?

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19

REF:

Plate 3	1	2	3	4	5	6	7	8	9	10
	V6	Lac	Gal		V6	Lac	Gal			
1	I	H	H	51						
2				52						
3				53						
4										
5										
6										
7										
8										
9										
10										
11										
12										
13	r	Lo								
14										
15										
16										
17										
18										
19										
20										
21	S	Lo								
22										
23										
24										
25										
26										
27										
28										
29										
30										
31										
32	r	H1								
33										
34	r	Lo								
35										
36										
37										
38										
39										
40										
41										
42										
43										
44										
45										
46										
47										
48										
49										
50										

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112 d

resistant: sensitive;
V_6 : r. S. I. Intermediate
Lac : H (HI) ; L (Lo)
REF: Gal:

19

Plate #①	1	Fertility	3	Plate-2.	5	MLac ×45 73	MLac. ×V573	8	9	10
Clone #	V_6	Lac	Gal		V_6	Lac	Gal		V_6 Lac	Gal
1	I	H	H		I	H	H	51		
2		"	"			"	"	52		
3		"	"			"	"	53		
4		"	"			"	"			
5	r	Lo	"			"	"			
6		"	"			"				
7		"	"		S	Lo	"			
8		"	"				"			
9		"	"				"			
0			"				"			
11			"							
12			"							
13	r	Lo								
14										
15										
16										
17										
18										
19										
20										
21										
22										
23										
24										
25										
26										
27										
28										
29										
30										
31										
32										
33										
34										
35										
36										
37					r	Lo				
38										
39										
40										
41										
42										
43										
44										
45										
46										
47										
48										
49										
50										

Transduction of Lac-segment to F⁻ pur⁻ by F_{13}.

4411 F_{13} → 93:85. (pur $V_6^R S^R$ F⁻ Lac85 [illegible])p⁺

REF: W4637 4697 ?(8 101 85)9

7/VII ;1959

Purpose: Is pur⁺ transduced into F⁻ by F_{13}.

Procedure: 1. Mix 4hrs culture of Parents and incubate it overnight at 37°C.

2. Plate out it on BLac Sm. after optimal dilutions.

3. Count ratio of Lac⁺ and Lac⁻. and pick Lac⁺ (1400) and streak it onto BLac Sm.

4. Replica plate it on DO, DO + pur. + Prol. Bgal

Results

1. Rate of infection.

Lac⁺	Lac⁻	% of Lac⁺
63	720	
45	662	
44	685	
60	684	
50	703	
57	503	
319	3857	Σ4176

7.42%

2. Transfer of marker by replica. Selected

All colonies showed X⁺.

(Hi Gal)	(Hi Lac)	F_{13}	V_6	Gal	Prol⁰	Pur	Lac⁺ S^R	Number of colonies tested.
Hi	Hi	F_{13}	r	+	+	+	+ r	149

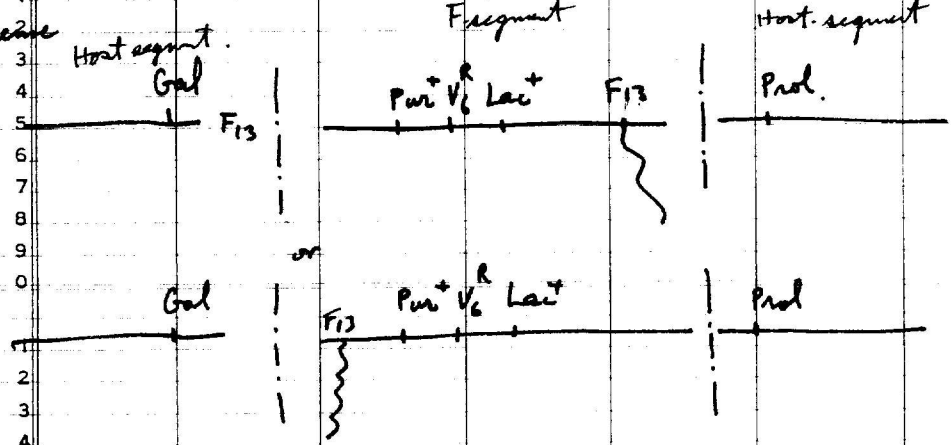
all colonies can grow on DO. no the other kind of colonie were found.

Conclusion:

Pur. is also transduced with F_{13}.

Rate of multiplication of the frequent recom relatively low in pur⁻ stock.

Scheme



Tests of Sex-compatibility and Lac-marker of segregants from "Diploid colonies" which obtained by transduction of Lac$^+$.

8/VII. ; 1959 REF:

Ref.
1. Lac loci of W4411 is Lac 85.
2. W4411 was originally F$^-$.

Method
1. Streak all Lac$^-$ colonies on BLac.
from # 4 ; 7 : # 5 , 5.

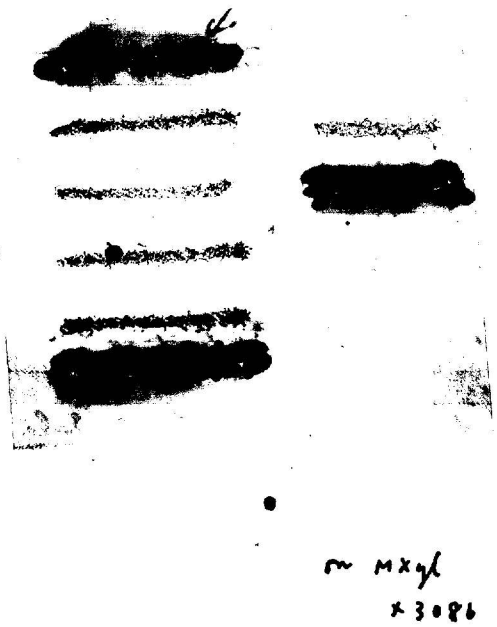
2. Replica plate it on Mlac seeded W3086 on it. ● Sel: Lac M.
Replica plate it on Mlac seeded W4573 on it.

Result:

Segregants from # 4		Segregants from # 5	
♂ / ♀(F$^-$)	(%)	♂ / ♀(F$^-$)	(%)
1 / 7	(14.3%)	1 / 5	(20.0%)

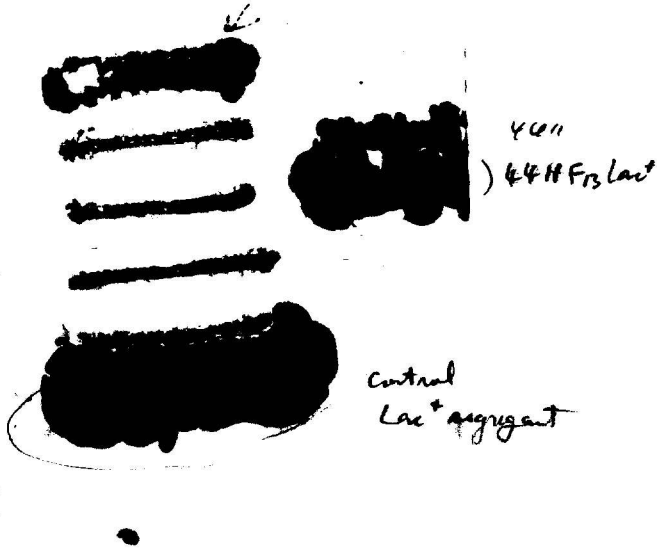
Ref: fertile Lac$^-$ grows better than infertile Lac$^-$ on BLac

From 4411 F13
#5



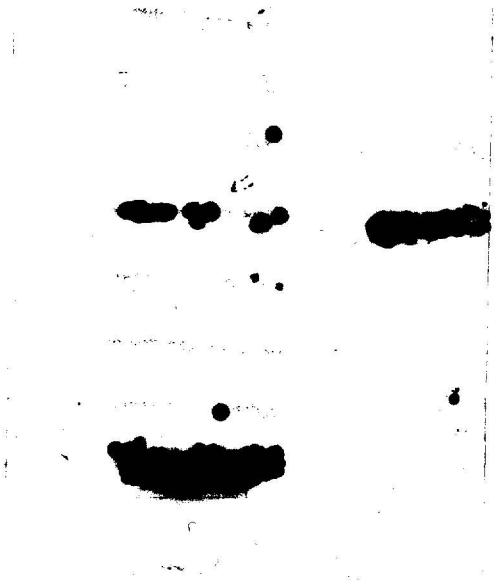
on MXyl
×3086

#5



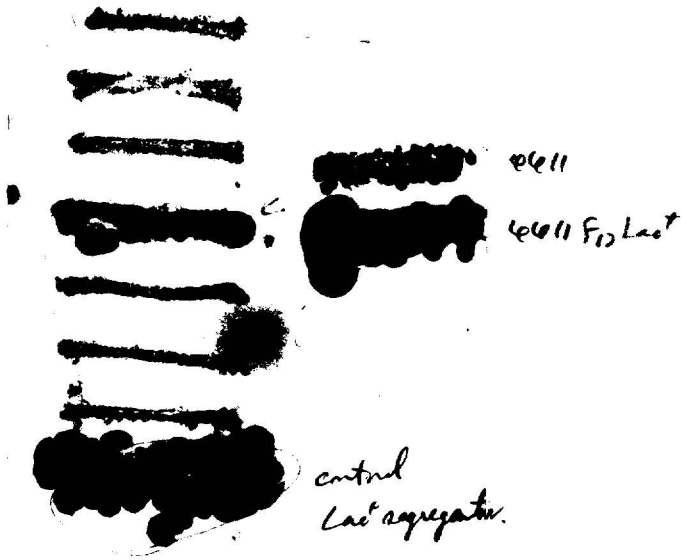
on Blac

#4



on MXyl
×3086

#4.



on Blac

Treatment of Lac$^+$ transduced F_{13} with AO.

8/VII ,1959 REF: Cf. P117, 127, 118

Purpose : Is F_{13} sensitive to AO-treatment?

Strain : W4411 F_{13} Lac$^+$. (This is diploid in Lac loci.) Cf. P114

W6 F_{13}

AO : 50γ/ml Med : Nutrient broth pH. 7.6. : 5 ml.
overnight treated at 37°C. — 0.1 ml ca. 10^6 cells/ml.

Result :

1. 4411 Lac$^+$ F_{13}

a. Lac marker :

		AO-treated	Control
Lac$^+$/	1	13/29	6/27
[redacted]	2	9/25	6/24
total	3	15/27	8/28
Σ		37/81	20/79
		% (35.7)	(25.3)

b. F_{13}. Tested by Lac$^+$ transfer.

		AO-treated	Control
F^-/total	1	0/16 (×4573 (Mlac))	0/21 (×4573 (Mlac))
	2	0/19	0/12
	3	0/16	0/18
Σ			

2. W6 F_{13}

F_{13}

		AO treated		Control	
		[redacted] ×4573	[redacted] ×4573	Mlac ×4573	Mlac ×4573
F^-/total	1	2/25	2/25	0/26	0/26
	2	1/27	1/27	0/28	0/28
	3	1/23	1/23	0/23	0/23
Σ. F^-/total		4/75		0/77	
%		5.34		0.00	

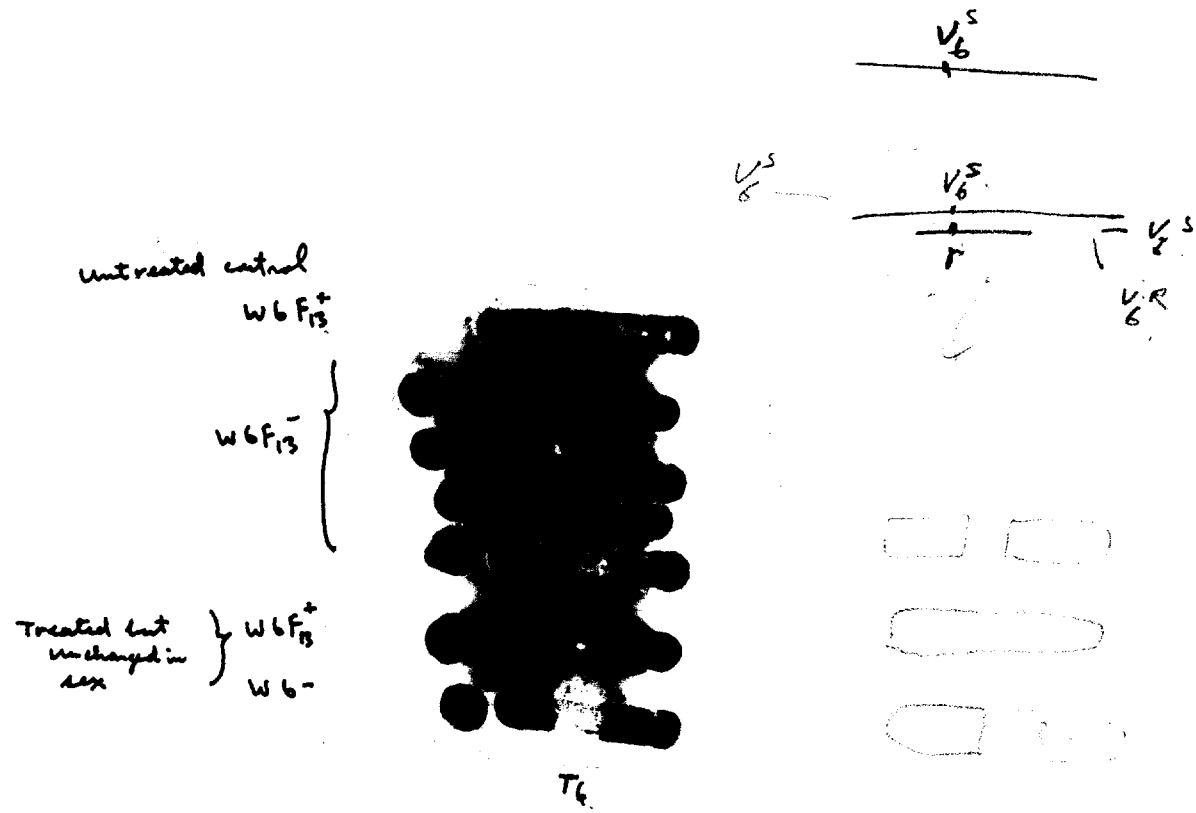
Test T_6-resistance.

3/4 : T_6^S

1/4 : Intermediate.

Conclusion :

1. Acridine does work to Lac-V_6 segment at the same time to attack F_{13}, and result loss of V_6^R segment.
2. But the rate is relatively lower than F^+.


untreated control
W6 F13+
W6F13−
Treated but unchanged in sex
W6F13+
W6−
T4
V6S
V6S
V6S
V6S
V6R

Treatment of Lac⁺-transduced W4611 with AO.

19 REF:

Purpose: I_3 V_6 Lac⁺ F_{13} loci sensitive to AO-Treatment.

strain W4611 F_{13}: F_{13} Lac⁺ / Lac_{85} V_6^S / V_6^R Hal, λ_{vl_2} [redacted] Htr1 S^R

Experimental method:

1. Inoculate 0.1 ml of overnight culture of W4611 F_{13} into AO-NSB, pH. 7.6 : AO 50 γ/ml : 6×10^6 cells/ml.
2. Incubate it overnight.
3. Seed it on BLac₃ and incubate them overnight at 37°C.
4. Count Lac⁺ and Lac⁻

	AO-Treated W4611 Lac⁻ F_{13}	Untreated control.
Lac⁺ / Lac⁻ (% of Lac⁻)	97/242 (27.8%) 349	267/623 (30.0%) 890
	105/273 (~~30.9~~ 27.8) 378	249/702 (26.2%) 951
	91/283 (24.3) 374	237/585 (28.9%) 822

Conclusion:

AO-treated group gives slightly lower ratio of Lac⁺, but almost same as untreated control.

This result seems not conclusive.

Further Test: Use Lac⁺ heterogenote which contains high rate of Hat and not segregate progeny.

Elimination of Lac-F_{13} segment with AO-treatment. (I)

16/VII ; 1959 REF: 2594F_{13} Lac⁺ (M S^R V_1^R Lac V_6 ...
c.f 118, 127 & 115

1 2 3 4 5 6 7 8 9 10

Purpose: Is Lac-F_{13} segment sensitive to Acridine treatment.

Strain: W2594(F_{13} Lac⁺) cf. P112.
Purify a Lac⁺ colony on BLac, ~~and~~ pick a Lac⁺, and suspend it into 1ml distilled water. (ca. $10^7 \sim 10^8$ cells/ml)
Use the 10^{-3} ml for AO treatment.

AO treatment: inoculum size: Ca. $10^5 \sim 10^4$ cells/5ml. ; Medium: NSB-conc. pH7.6
AO conc.; 30γ/ml : 37°C, overnight treatment.

Procedure:
Seed 10^{-2}ml of AO-treated and untreated culture onto BLac agar, and count the ratio of Lac⁺ and Lac⁻ colonies.

Result:

Tube A

	AO-treated (30γ AO)					Untreated control				
	Lac⁺	Lac⁻	Lac V.	Σ	% of Lac⁻	Lac⁺	Lac⁻	Lac V.	Σ	% of Lac⁻
Plate # 1	52	110	3	165	66.7	250	66	1	317	20.8
2	65	150	1	216	69.5	289	54	1	344	15.7
3	65	171	1	237	72.2	246	82	1	329	24.9
4	71	117	2	190	61.7	243	73	3	319	22.9
5	38	102	4	144	71.0	220	58	2	280	20.7
Σ - %	291	650	11	952	68.2 %	1248	333	8	1589	21.0 %

~~% of Lac⁻~~

Tube B. (30γ AO)

	AO-treated					Untreated control				
	Lac⁺	Lac⁻	Lac V.	Σ	% of Lac⁻	Lac⁺	Lac⁻	Lac V.	Σ	% of Lac⁻
1	12	59	0	71		382	46	3	431	
2	31	107	1	139		374	42	3	419	
Σ - %	43	166	1	210	79.0 %	756	88	6	850	10.3 %

Further work

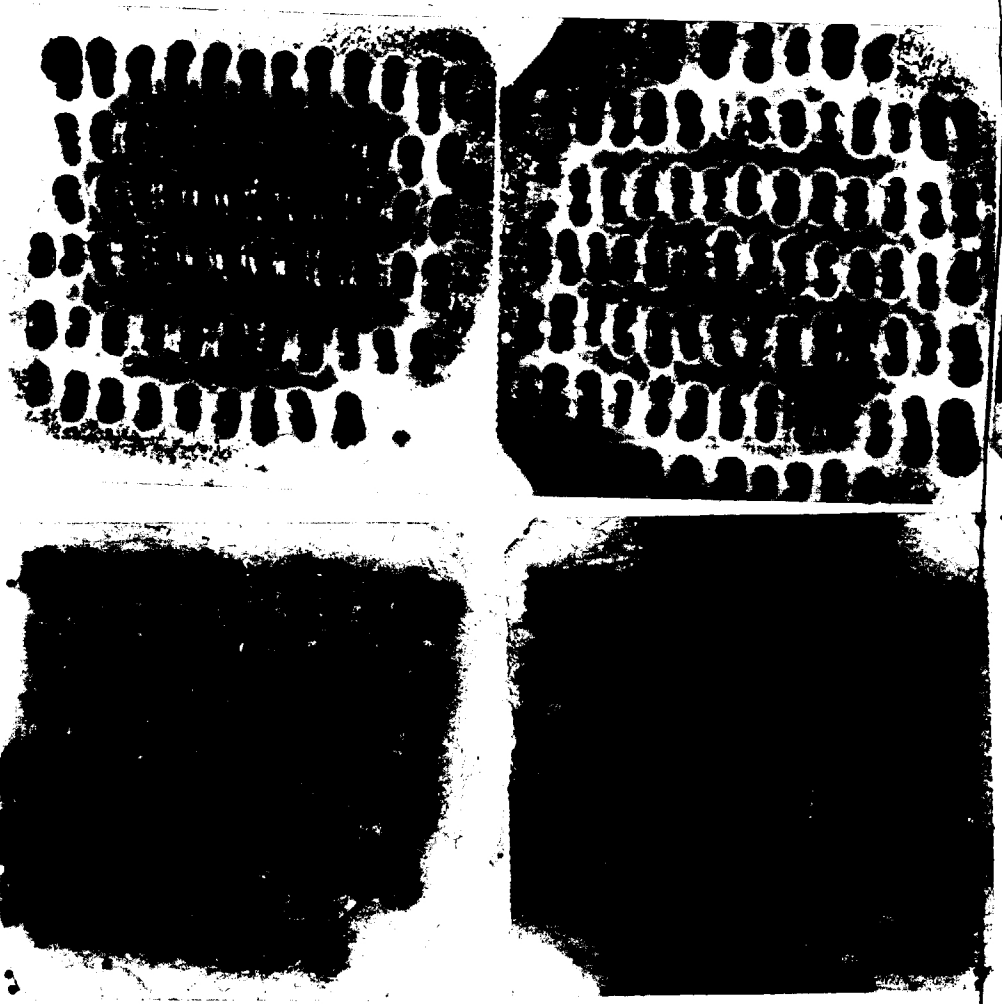
1. Test [illegible] V_6 r or s. or intermediate.
2. Test F_{13} by Gal-transfer.
pick Lac⁺ Lac⁻ of Untreated and treated
↑ These all Lac⁻ gives Lac⁺ papillae on BLac.

Conclusion:
AO-can eliminate exogenote with very high frequency.

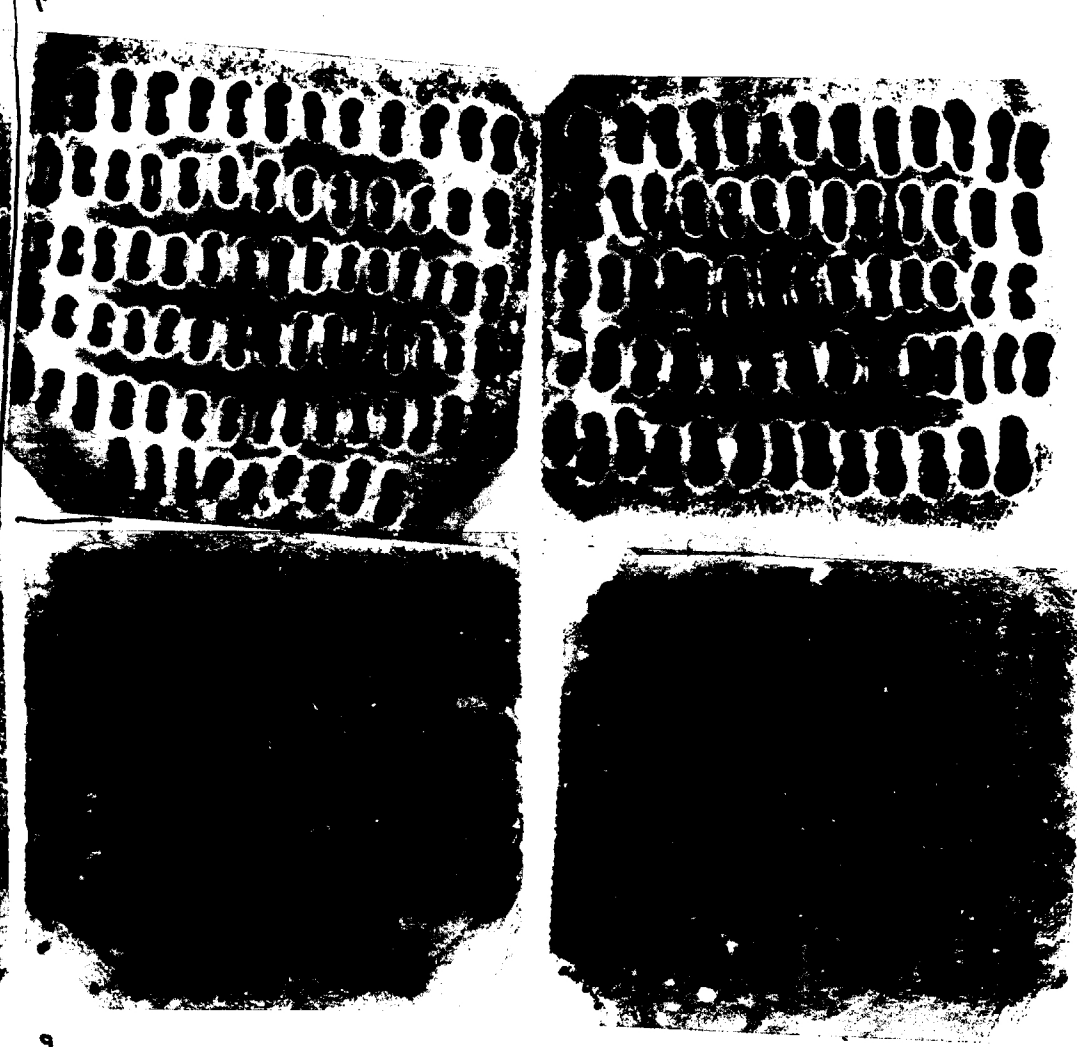
History of 2594 (Lac$_{II}$)

X87	Lp^S $Gal4^-$
W'518	M Lac$_I$ Gal_4 Lp^S V_1^R
W'1578	Lp^S F^- M Lac$_I$ Gal_4 V_1^R
W'1321	Lp^+ F^- M Lac$_I$ Gal_4 S^R V_1^R
W'1607	F^- Lp^+ M Lac$_I$ Gal_4 S^R V_1^R
W'2594	F^- Lp^S M Lac$_I$ S V_1

AO-Treated Lac+ colonies



Untreated control
Lac+ colonies



Elimination of Lac-F_{13} segment with AO-treatment. (Continued.)

22/VII ; 1959. REF: Cf 1274. 115. 117

Continued.

6 streined with AO. 7 treated : 8 W 2598 9 F_{13} (His^R Ura^R lac^+-Vir^S_{13}) 10 Lac^-_{11}

Purpose : Test sex-compatibility : Does AO remove F_{13} with Lac ?

Principle : Pick Lac^+ and Lac^- colonies from the plates obtained after seeding treated and untreated cultures with AO.

Method : Replicate the streaked clones on Mod seeded with 0.2 ml broth culture of 4503 (Gal^0_2 Lac_{85} Ara_2 $Mal_{1,2}$ Tyl_2 Mtl S^R F^-)

Ref. This Lac^- is unstable. It gives several papillae per one streak. on O Lac agar.

Result :

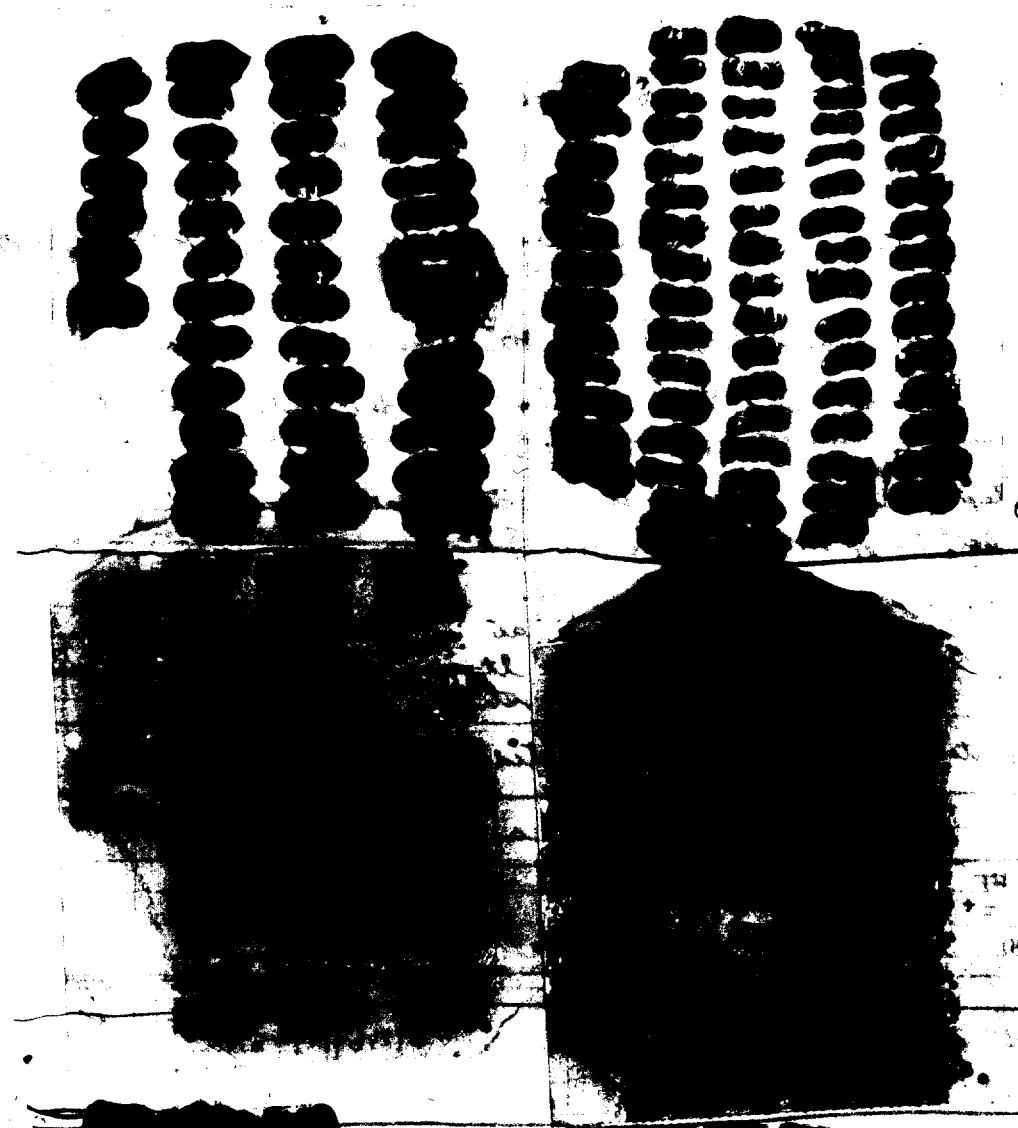
	Isolated from AO treated culture								Isolated from untreated control.							
	Lac^+				Lac^-				Lac^+				Lac^-			
	F^+		F^-		F^+		F^-		F^+		F^-		F^+		F^-	
Gal	HI	Lo		Σ	HI	Lo		Σ	HI	Lo		Σ	HI	Lo		Σ
	148	2	0	149	13	9	116	138	146	0	0	146	104	12	0	116
%	98.6	1.3	0		9.42	6.52	84.1		100	0	0		89.8	10.3	0	

Conclusion : ① Acridine acts on F_{13} as well as Lac. (84.1 % of Lac^- is F^-)

② No F^- is observed in AO treated Lac^+. (This may contain $Lac^+/-$ and Lac^+)

③ In control experiment, no F^- was found. (0% in both Lac^+ and Lac^- colonies.

a untreated control Lac⁻ each streaks has papillae. ∴ lac, is unstable.

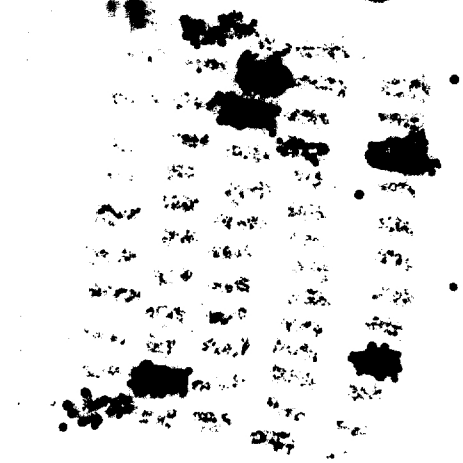


on Blac.

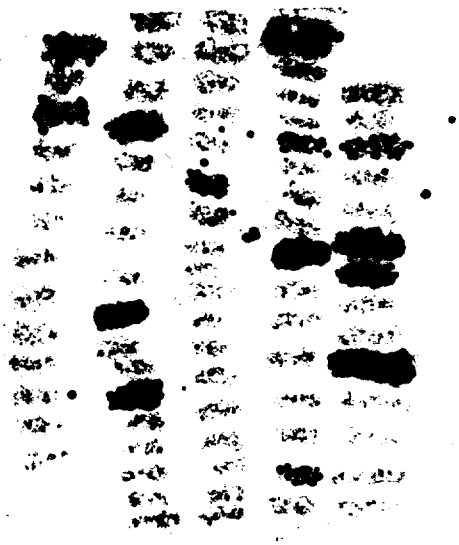
on MGal x 4573

AO-treated, lac⁻
each streaks has papillae.

on Blac



on MGal x 4573



on MGal x 4173

Rate of multiplication of F_{13}-Lac segment.

(Infection of Lac-F_{13} segment to F^-)

W4573 F^- Gal$_c$ Lac$_{85}$ Mal$_c$ X$_{yl_c}$ MTL Ara$_c$ S^R.
W3747 M[illegible] F_{13}

18/11 ; 1959

Purpose ; Is Lac-F_{13} segment multiplies more than cell? ~~directional~~

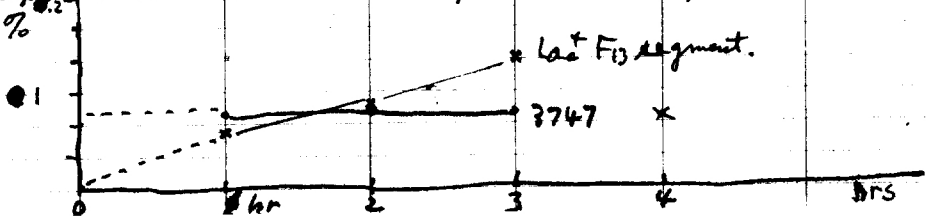
1. Make overnight culture of W4573 and W3747.

2. Make fresh culture of these strain by inoculating the 0.2 ml of the culture into 10 ml penassay broth and shaking it on rotator at 37°C.
   P.M. 9:10 ~ 1:00. P.M.

Time	1:00 0 hr	2:00 1 hr	3:00 2 hr	4:00 3 hr	4 hr
	10^{-5} ml	10^{-7} ml	10^{-7} ml	10^{-7} ml.	
				+ / −	
BLac		16/1011	0/125	2/164	~~3/164~~
		8/890	2/151	5/151	~~5/151~~
		15/964	1/123	2/129	~~3/129~~
multiplication of Lac marker : Σ		39/2865	3/399	9/444	
original Lac$^+$	Lac$^+$+Lac$^-$	2909	402	453	
	%	1.34	0.746	1.99	%
		Ara$^+$/Ara$^-$			
BAra		8/1011	0/125	0/164	
		6/890	0/151	3/151	
		8/964	0/123	0/129	
Σ		22/2865	0/399	3/444	
multiplication of Ara. cell itself.	Ara$^+$+Ara$^-$	2887	399	447	
	%	0.762	0.	0.671	%
		X$_{yl}^+$/X$_{yl}^-$			
BXyl		8/1011(8)	0/125(0)	0/164 (2)	
		6/890(2)	0/151(2)	3/151 (2)	
		8/964(7)	0/123(1)	0/129 (2)	
			17/2865	3/399	6/444
Difference multiplication of Lac$^+$-segment.	Multiplication of Lac$^+$ (Lac$^+$−Ara$^+$)		2882	402	450
	%		0.592	0.746	1.33. %

Result : After 3 hr incubation, F_{13} becomes approximately double.

Further experiment : Use 1/10 of mixture of F_{13} and F^-.



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120

Transfer of F_{13} to W3127, W3112,

5/VA ; 1959 REF:

Purpose : For cistron-analysis.

Method :

1. Spot W3747 on W3127, W3112, and W3230. on MLac.
2. Purify them on BLac and incubate it overnight at 37°C.
3. Look for Lac_v. and purify Lac_v on BLac.
4. Pick Lac^- derived from Lac_v, and test the compatibility. Streak them on BLac. Replicate it on BLac. and make copy.
5. Replica plate on MLac seeded various Lac indicator strains on it.

Indicator strain colonies

Indicator strain. Lac_{1a} (11) W3230 ⟷ W3112 : Lac_2

" ⟷ W3127 : Lac_4.

Isolation of F_{13}^+ W3127: Result :

1. All Lac^- colonies which isolated from sectored colonies, and purified on BLac are very unstable and gives many papillae on the streaked colonies. After replicating these streaks, they gives various kinds of fertility according to Lac^- mutations. This unstability is still not understood that is Lac^+ appeared by segregation or reversion of Lac^-.

Then, the streaked are purified on BLac again, and picked Lac^- from the and tested the fertility by cross breeding (it gives almost Lac^+!) on MLac with W3230. However, they show very unstable Lac^- character on MLac again. (see back page).

But, 2 of them showed fertile cross times W3230! (Lac_{1a}) on MLac.

② Test V_6-resistance. (See back page).

Keep it.

W3112: Result.

① Test 174 of Lac^- segregated from Lac^+ diploids. (picked 2 Lac^- from each Lac_v colonies) by replicating on MLac seeded W3230 (Lac_{1a})

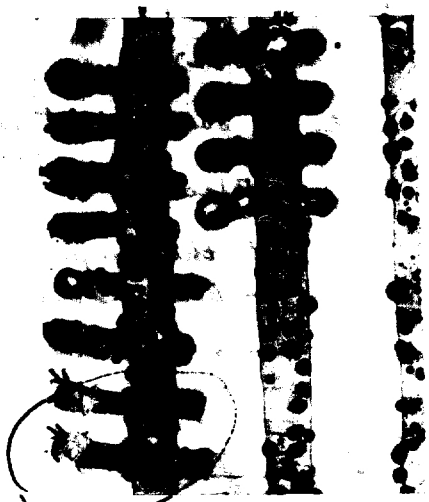
Σ	Hi Lac	to Lac	F^- (probably, because Lac^-_2 is unstable) (test with than 3127)
174	88	80	6.

② one of the isolated clone shows Hi fertility, and shows mixed clone in resistance to T_6. (see p.126)

Result: ③ Test fertility, all Lac^- are tested in their compatibility

marker transferred	Gal	Lac_{85}	Lac_{88}
Hi	72	0	58 (80.6 %)
Lo	0	0	14 (19.4 %)
on	MGal (Gal-transfer)	MLac ↑	MLac Lac transfer

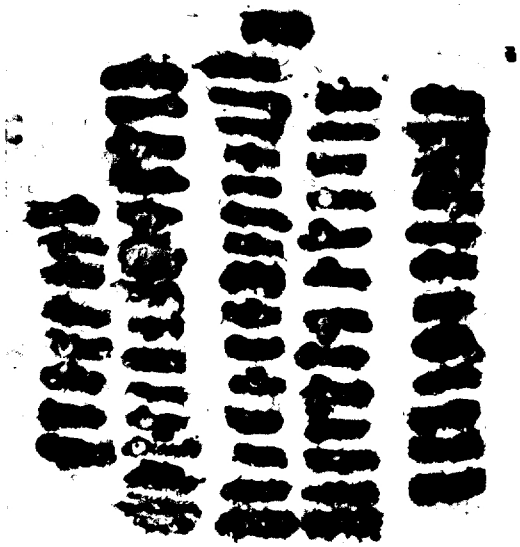
Lac_{85} include or overlapped with Lac_2 (W3112)



× 3230 on Hlac

Segregants from Hac z F_{13}/+ F−

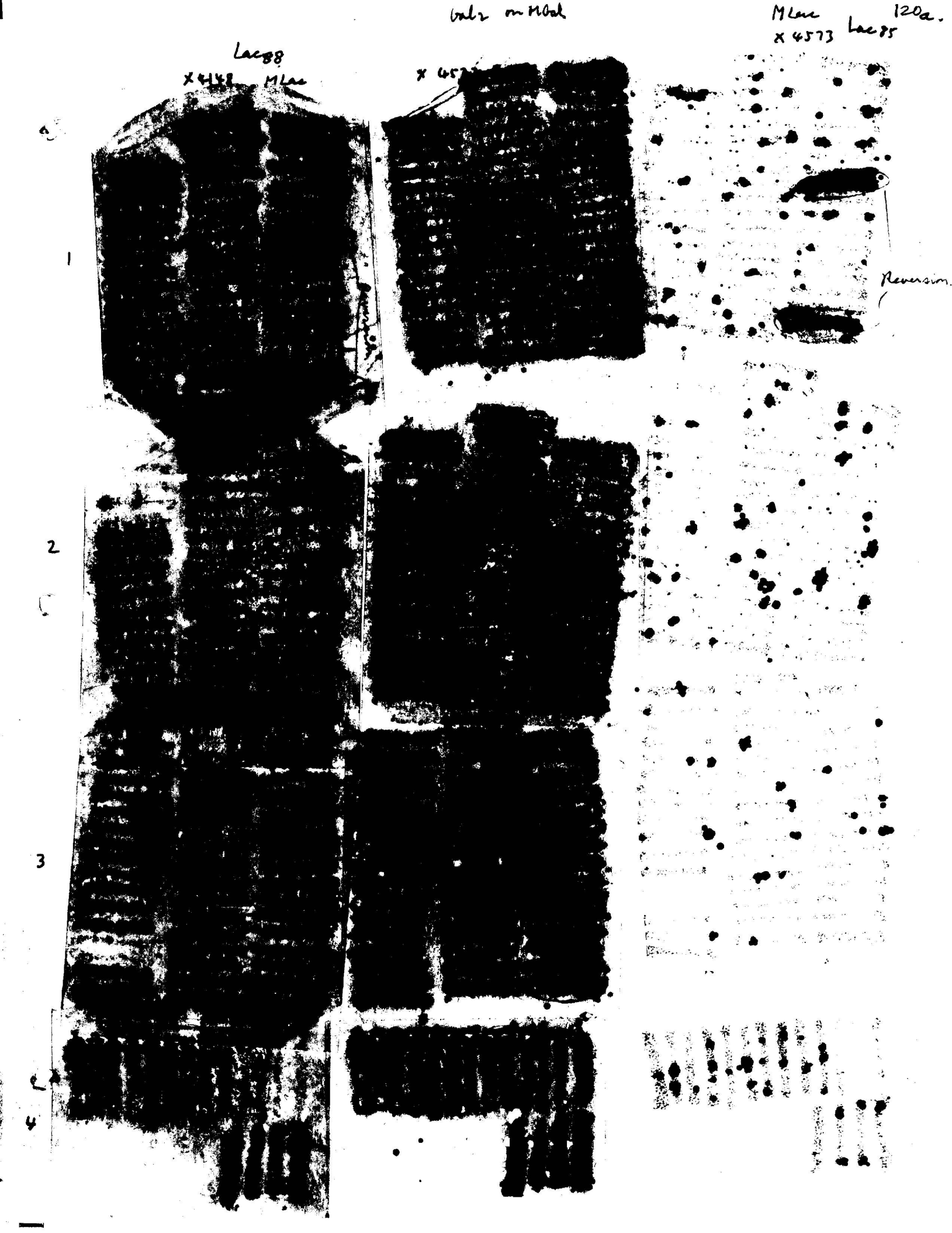
W3112 F_{13}



on Hlac

× seeded W 3230

on Blac


Lac88
MLac
Lac88
MLac
Lac85
Reversion
1
2
3
4

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121

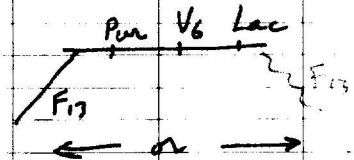
Timing experiment of transduction of Lac F_{13} segment. I

20/VII ; 1959 REF:

Strain : ♀ : AC: 93:85 : Pur⁻ V_6^R S^R F^- Lac 85

♂ : W3747 H- V_6^R F_{13}.

Purpose : Test the direction of Lac-F_{13} transfer from F_{13}^+ to F^-



Experimental design:

- Use 2 hrs old culture of both strains (ca 10^8 cells/ml) AM 9:00 ~ AM 11:00; overnight culture 0.5 ml / 50 ml pep. incubate it (2 hrs on rotator.
- Mix 10 ♂ : 1 ♀.

Result :

- Inoculum size : Recombination plate : 10^{-5} ml /plate.
  Survival counting plate : 10^{-7} ml./plate

Media / Time	Marker selected	0	2.5 min	5 min	7.5 min	10 min	15 min	20 min
MLac + Sm	Pur, Lac	0	0	0	0	0	0	0
		0	0	0	0	0	0	0
MLac + Pur + Sm (Adenin)	Lac	0	0	0	0	0	0	Lac⁺ → 2
		0	0	0	0	0	0	0
MO + Sm.	Pur.	0	0	0	0	0	0	0
		0	0	0	0	0	0	0

# of Recombinants.

EMB-Lac (10^{-7} ml)	− / +	− / +	− / +	− / +	− / +	− / +	− / +
	0/13	0/11	1/15	1/19	2/11	2/32	1/23
	0/12	4/12	3/13	1/18	0/16	5/26	1/20

blank (10^{-6} ml /plate) 1̄7 / 1̄10 ; 2̄6 / 1̄37 ; 1̄7 / 1̄41 :

① # of cells used { ♂ Initial : 1×10^8 cells; final : 2×10^8 cells
♀ : Ca 1×10^7 cells

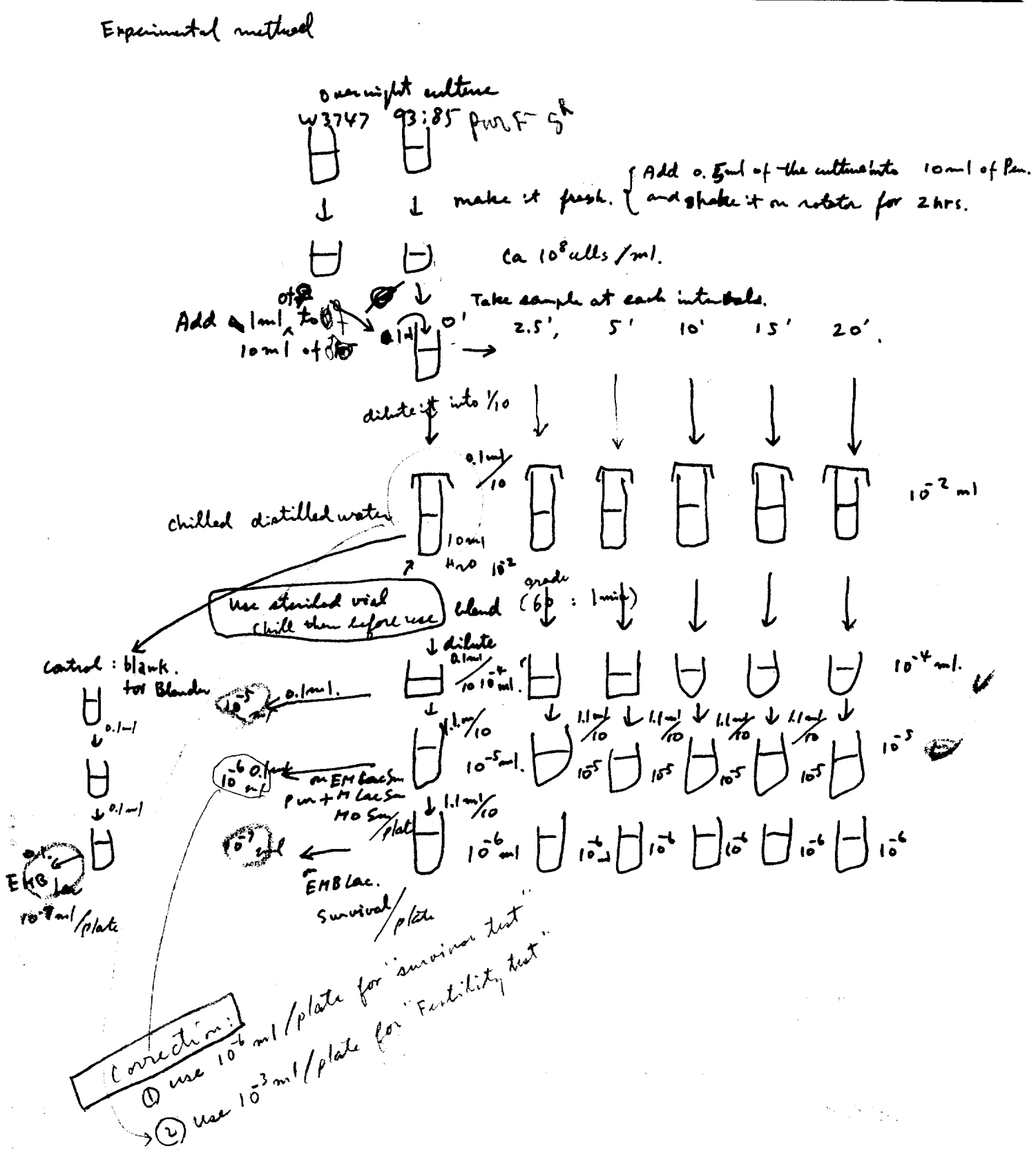
② Rate of Recombination :

Rate of Recombination in this experiment :

Rects / ♀ ca. $\frac{1 \times 10^2}{\text{after 20 mins}}$ ♀ cell (Lac⁺)

1. Test F_{13} by Gal-transfer
2. Test Pur⁻ → both strain is purine⁻.

Experimental method



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Isolation of 3133 F_3^- with AO method.

122

20/VII ; 1959

REF:

AO : 30γ/ml : NSO conc. standard for AO method pH 7.6.

Inoculum size ca 10^5 cells/ml. overnight treatment.

3133 F_3^+ [redacted]

3133 F_3^-

3133 F_{10}^+ [redacted]

3133 F_3^- [redacted]

×40- on plate

Result.

AO-treated		Untreated control.	
−	+	−	+
1/210		0/359.	

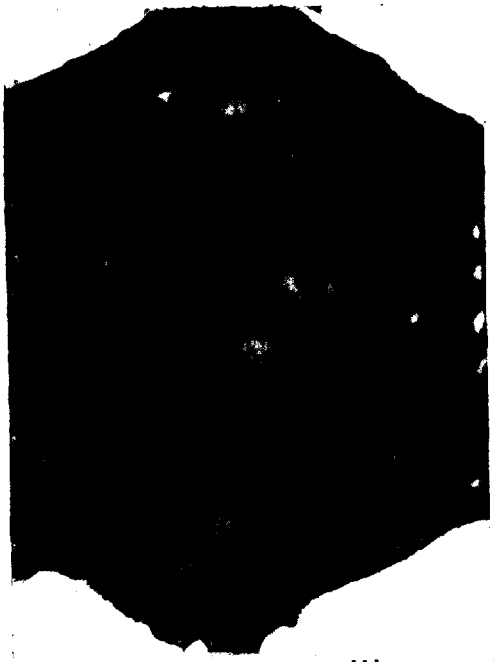
See back page

Further work

① Save this.

② Test F_3 type F^- or plain F^-.

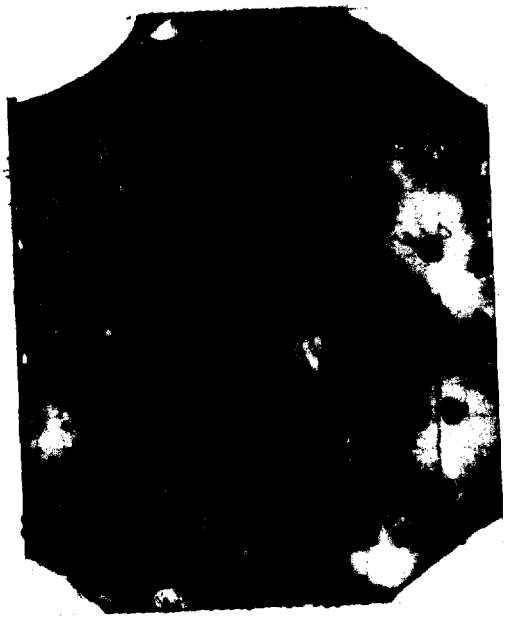
untreated control

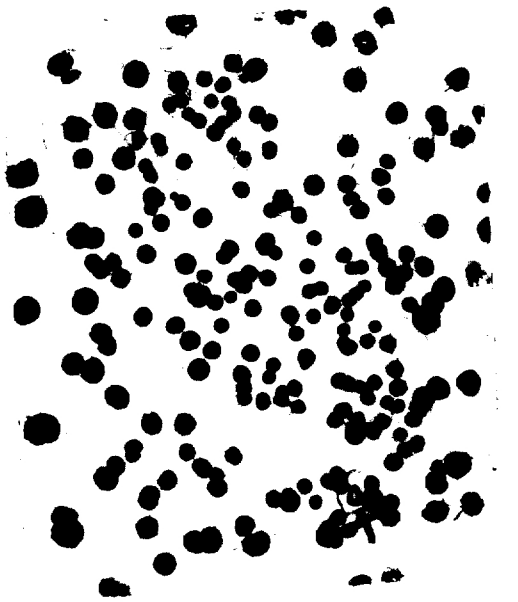


Blac

Mlac x W6—

Treated with AO

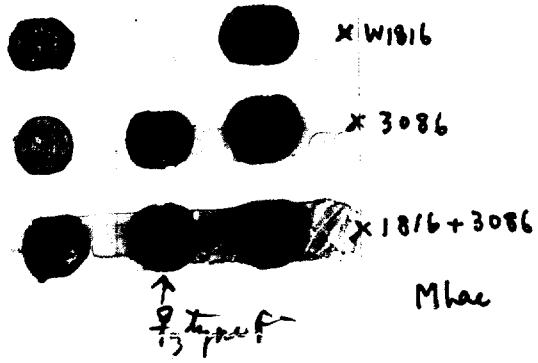




Mlac x W6—

Blac

3828 3133F_3^- 3133F_3



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123

Infection of Lac- F_{13} segment. (II).

21/VII 1959 REF:

Principle[2]: W3747[3] —F_{13} Lac+[4]→ W4573[5]

Strain: Log-phase 3747 + 4573 { 1:00 PM. → 3:00 PM. 0.2 ml / 10 ml pen. Made fresh on rotator

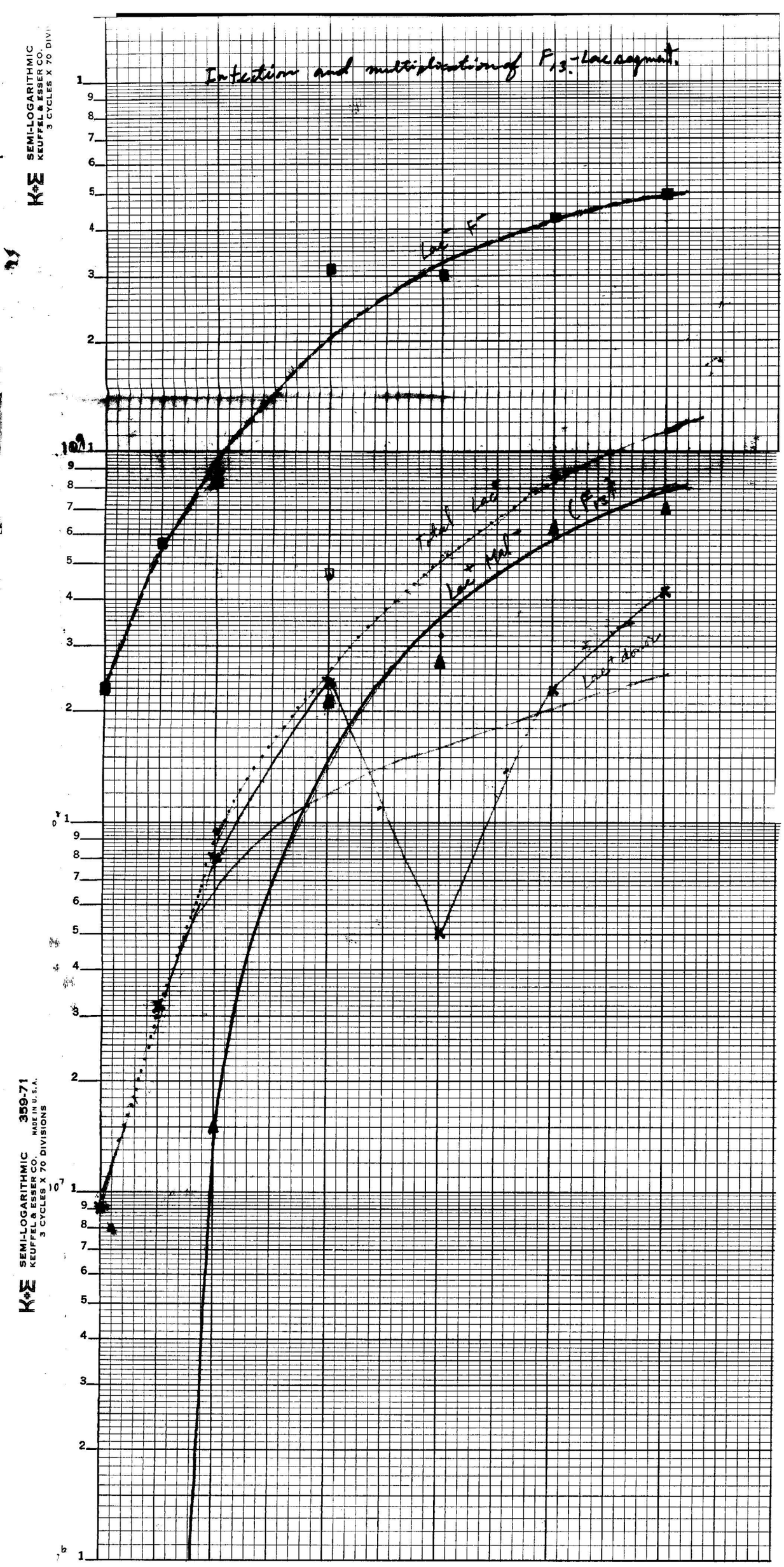
① Mix: Ratio 1.1 ml + 10 ml Ca 10^8 cells/ml.

② Shake on rotator. Take samples at each time from the mixed culture. Dilute it with H_2O into 10^{-6} to ~10^{-7}. Seed on B Lac. Incubate them for overnight at 37°C

③ Replica plate them on BXyl, BMtl, BAra, and ~~Other~~ MGal Sm seeded 93:85 on it.

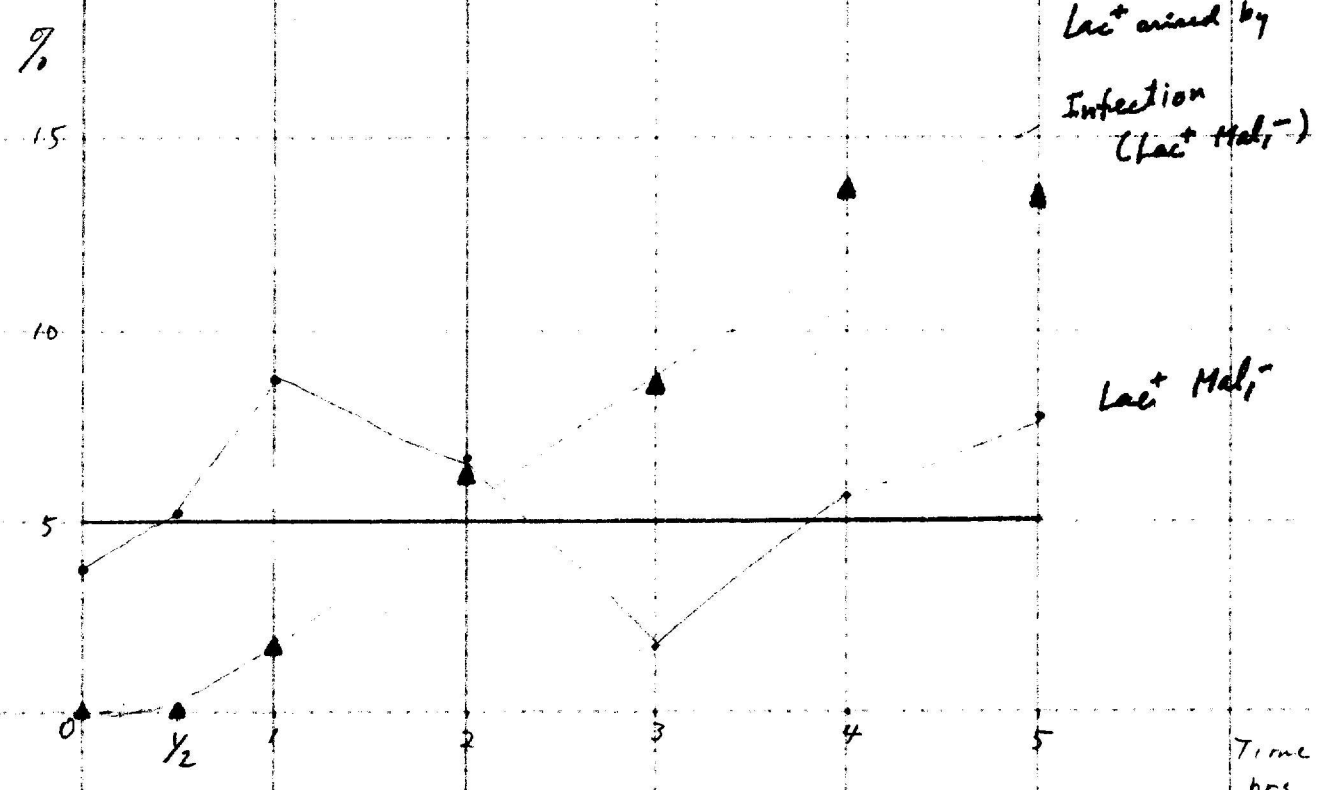
		1	2	3	4	5	6	7	8	9	10
Time		3:05	3:35	4:05	5:05	6:05	7:05	8:05			
Inoculum size		10^{-6} ml/plate	10^{-6} ml	2×10^{-7}	10^{-7}	10^{-7}	10^{-7}	10^{-7}			
Time		0 hr	1/2 hr	1 hr	2 hr	3 hr	4 hr	5 hr			(hrs.)
on B Lac		+ / −	+ / −	+ / −	+ / −	+ / −	+ / −	+ / −			
	1	1/39	5/111	0 3/38	3 13/68	1 3/68	14 20/61	12 24/95			
	2	2/38	6/92	~~1~~ 4/48	7 9/68	6 7/58	11 17/100	15 21/93			
	3	2/45	3/117	1 7/38	5 8/71	8 9/77	8 14/113	12 19/101			
	4	1/49	10/119	1 5/42	4 8/66	3 5/58	14 17/75	23 30/96			
	5	3/59	8/126	[illegible]	4 8/63	7 8/40	15 18/83	11 17/102			
	Σ	9/230	32/565	19/166 (+5 95 [illegible])	46/336 (+10)	32/271	86/432	111/487			
		(239)	(597)	(185)	(382)	(333)	(518)	(598)			
	%	3.77	5.36	10.3	12.0	9.65	16.6	18.5			
on B Mal		△	△	△	△	△	△	△			
	1	0 1/39	0 5/111	0 3/38	3 10/68	3 0/68	15 5/61	12 12/95			
	2	0 2/38	0 6/92	1 3/47	7 2/68	6 1/58	11 6/100	13 8/93			
	3	0 3/45	0 3/117	1, 6/38	5 3/71	8 1/77	8 6/117	11 8/101			
	4	0 1/49	0 10/119	1, 4/42	4 4/66	3 2/58	14 3/75	23 7/96			
	5	0 3/59	0 8/126		3 5/63	7 1/40	15 3/83	11 6/102			
Δ Infection of Fi Lac+		△	△	△ (15) (+5 80)	△	△	△	△			
		0, 9/230	0, 32/565	3, 16/166	22, 24/336	27, 5/308	63, 23/432	70, 41/482			
		(239)	(597)	(182)	(360)	(308)	(455)	(523)			
	%	0, 3.77	0, 5.37	1.65, 8.80	6.12, 6.67	8.82 1.66 ?	13.8 5.06	13.4 7.83			

Conclusion: Lac-segment multiply about 2 times ~~make~~ more than host cell after 5 hrs. at 37°c on rotator.


Infection and multiplication of P13-Lac segment.
SEMI-LOGARITHMIC
KEUFFEL & ESSER CO.
3 CYCLES X 70 DIVISIONS
359-71
MADE IN U.S.A.

Rate of Multiplication of Lac- F_{13} segment.

3747 ⟶ 4573
M V_6 F_{13} Gal$_2$ Lac$_{g5}$ Ara$_2$ X gl$_2$ Mtl Mal$_1$ Su.



Multiplication of Lac-F_{13} segment. (continued)

23/VII 1959 REF:

Make cultures fresh : 1. Inoculate 0.2 ml of the overnight broth culture of W3747 and W4573 into 10 ml of Penassay broth. and incubate it for 2hr on rotator at 37°C.

Dilute 2. Dilute W3747 into 10^{-6} : Use 10^{-7} ml of W3747 for F_{13}-Lac donor. count number of cells. used.

Mix 3. Mix 1 ml of W4573 and 10^{-6} ml of W3747, and 10 ml penassay broth. (ca 10^3 cells + ca 10^2 cells + 10 ml per)

4. ~~Shake on rotator~~ Incubate it at 37°C for overnight.

Result :

I] Initial ratio

Survival count :

	# of colonies in 10^{-6} ml / plate	Inoculum size :	Ratio (Initial ratio) of Lac^+ and Lac^-
W3747	73	8 cells /ml	ca 10^{-7}
	85		
	84		
W4573	115	1×10^7 /ml.	
	88		
	107		

5. Inoculate 0.1 ml of the mixed culture into 10 ml of Penassay broth. count the Lac^+ and Lac^-.

6. Incubate it overnight at 37°C.

II] After 24hrs incubation : Seed 10^{-7} ml / plate. (EMB Lac agar).

plate #	Lac^+	/ Lac^-
1	0	485
2	0	504
3	0	496
4	0	485
5	0	471

III] After 48 hrs incubation.

Inoculate 0.1 ml of the mixed culture into 10 ml Penassay-broth, and incubate it overnight at 37°C.

Plate #	Lac^+	/ Lac^-
1	1	95
2	2	106
3	0	101
4	1	137
5	0	106

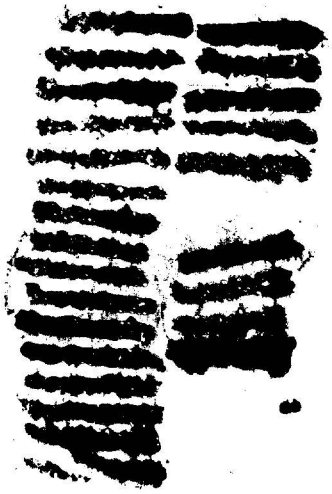
The segregants are tested for these 4 colonies.

2 Mal_3^+ ? 2 Xyl^+

2 Mal_1^- 2 Xyl^-

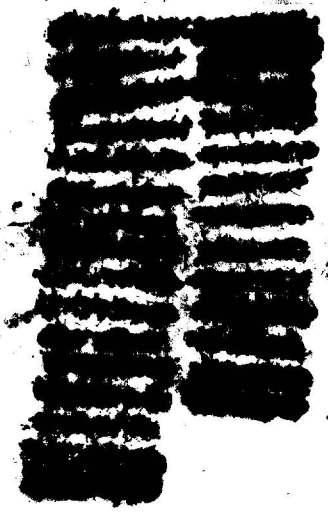
Selective marker : Lac - Meth.

10'

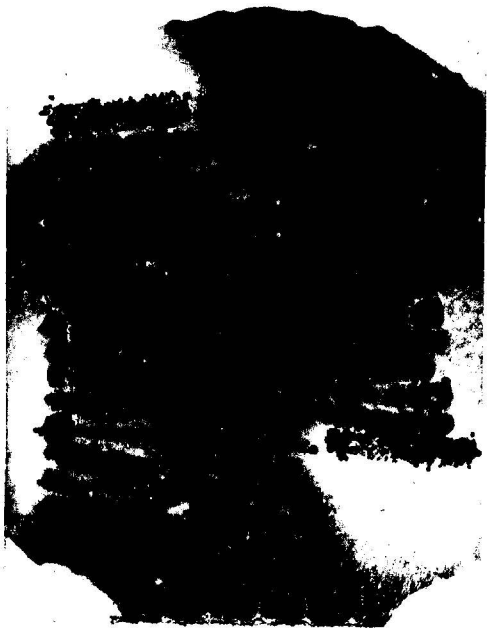


15'

15'



20'



20'

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1205 Q

Time experiment of Lac - F_{13} transfer.

25/VII ; 1959 REF: 125 b.

1 2 3 4 5 6 7 8 9 10

○ F_{13} ♂ : 3747 ; ♀ 93:85 ; Cultural age : 2 hrs aerotator at 37°C

0.5 ml overnight culture Pasteurly grown / 10 ml Penassay.

: Ratio ♀ 1 ml : ♂ 9 ml.

○ Method : exactly same as former experiment except inoculum size of the mixed culture onto selective medium. (See P. 121.)

○ blending ; Gage : 7 ; 1 min. Temp. for mating : 35°C.

Result :

Media \ Time	Marker selected	Time after interruption (min.): 0	2.5	5	7.5	10	15	20
M Lac Sm (10^{-3} ml)	Pur Lac	0	0	0	0	0	8	67
		0	0	0	0	0	7	75
M Lac -Pur- Sm Adenine B_1 (10^{-3} ml)	Lac	0	0	0	0	0	8	93
		0	0	0	0	1	5	41
M Gal Sm. (10^{-3} ml)	Pur.	0	0	0	1	5	43	154
		0	0	0	1	9	40	182
EMB - Lac. (10^{-6} ml)		149	163	124	152	125	177	196
		141	149	130	113	121	187	180
Blank (before blending) (10^{-6} ml)		143						
		127						
		121						

# of Recombinants per plate.

① # of cells used ♂ : Ca. 1.5×10^8
♀ : Ca. 1×10^7

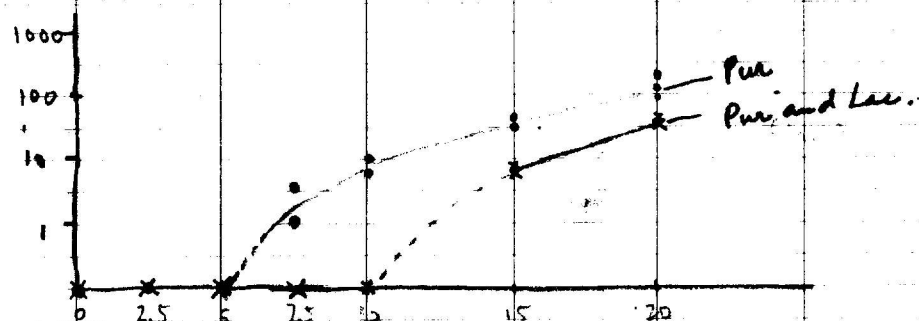
② Frequency of Pur^+ transfer within 20 min. ca. 10^{-2} ♂ and ♀

1 %.

Conclusion : ① Purine looks first, and Lac is next.

② Test Lac, Pur^-, and Ade. of these recombinants.

There is no marker to test F_{13} cells.



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markers

~~125~~ 126

Test ~~Pur~~, Lac, ~~Pur~~, F_{13} of the recombinants obtained from timing experiment in cross W3747 x 93:85.

30/VII ,1959 REF:

Purpose: Reconfirm which end is earlier (from the markers of these) recombinants (cf. p.125).

Method: (1.) Streak them on M-0 + Sm. and incubate it overnight at 37°C.

(2.) Replica plate it on BLac V_6, ~~Pur~~, DO, MGal seeded with W4573.

Result

1. All colonies grown on M Lac Sm are Lac^+

Time (min)	15'	60'
# of colonies Tested	14	125

2. Recombinants grown on M gal Sm are Lac^+ or Lac^-.

Time (min)	7.5	10	15	20'
Lac^+	0	0	16, 6) 22	49, 34) 83
Lac^-	2	21	29, 23) 52	26, 11) 37

Conclusion from 1. 2. Pur is first, Lac is second.

Pur^- Lac^-

Pur^+ Lac^+

Which side of the fragment is it attaching

Conclusion:

Pur^+ Lac^+ F_{13}

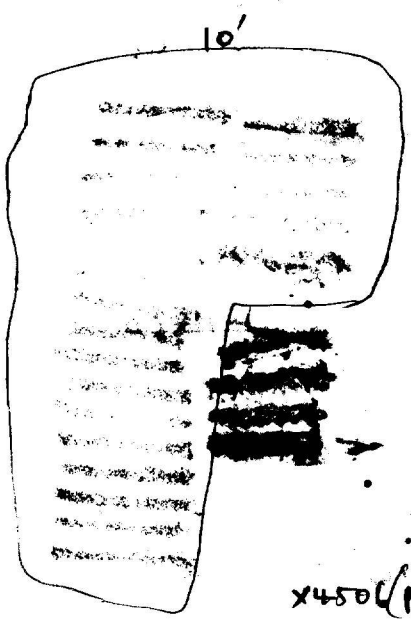
Method: (3) Replica plate on M Lac seeded Pur^- F^- on it. (Test purine transfer) W4506.

Principle: If it is F_{13}^+ the Lac^- strains can transfers pur^+ to F^-, but if it is F^-, they can't.

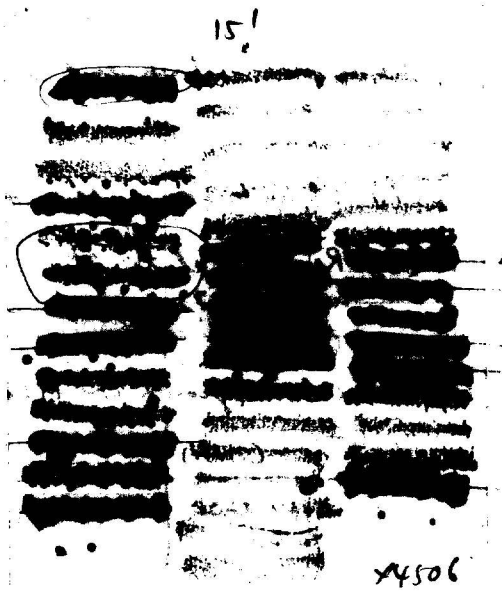
Result:

1. purine is first : Pur – Lac – F_{13}
2. F_{13} is latter than Lac.

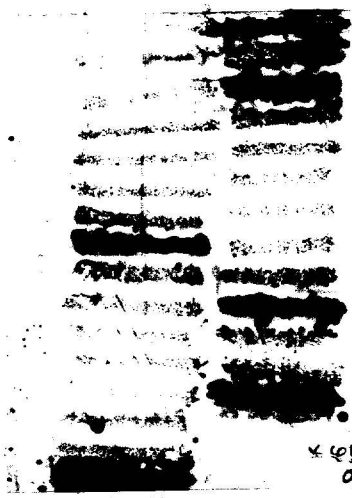
Selected on Lac - Pur transfer



X4506 (pur F⁻)
on M Lac

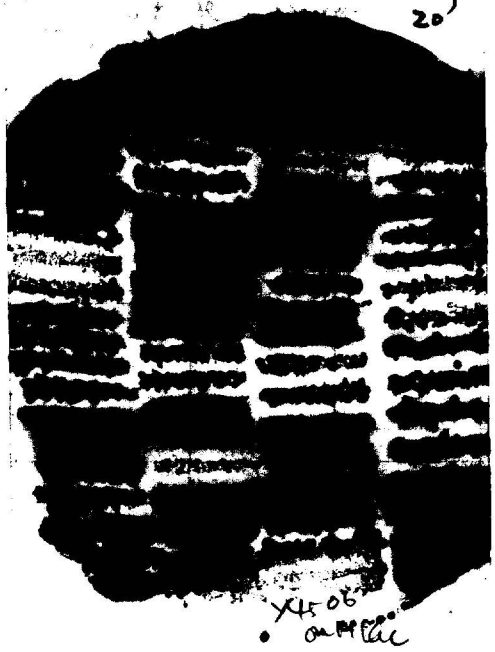


X4506
on M Lac



X4506
on M Lac

M Lac



X4506
on M Lac