# Quantitative measurements of the transfer of F′ to F− by Sm-killed $F_8^+$ cells.

30/11 : 1959 REF:

**Principle**: $Lp^s$ MGal$_{4}$ $F_8$ W4534 $\xrightarrow{F_8}$ × 3086 × W4573.

Method: 1. Treat W4534 with Sm. ×10 conc. than usual Sm. (~~[illegible]~~ 1mg/ml)

×10 diluted Sm. 0.2 ml / 2 ml culture

- cultural age : 24 hrs.; penassy grown; Wash twice with broth:
- Time of treatment 9:00 pm → 1: pM. (next day)

at 37°C

2. Mix → Incubate it overnight.

2' Test survivor.

- 0.1 ml/plate Sm-killed cell
- $10^{-6}$ ml/plate untreated cell

Experiment: Sm treated W4534 + 3086 (ca. $10^6$ cells/ml) 2 hr. culture ← overnight grown culture → 0.5 ml/5 ml pb. ↓ Incubate it ~~overnight~~ for 2 hrs more

Check : survival:

control : Untreated W4534 + 3086 (ca. $10^6$ cells/ml.)

and incubate them for overnight.

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

4. Replica plate on MGal seeded W4573 on it.

Result :

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

| 0.1 ml/plate Sm-treated | control Sm-Untreated (×10) $10^{-6}$ 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 45 / 51 (88) |
| 3 | 3 60 / 69 (87) |
| 4 | 4 |
| 5 | 5 |
| Σ (%) | Σ (%) |

In untreated control, $F_8^+$ is survive. 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 Penassy broth. + Sm 10 γ/ml
(×10 much than usual use)

5 ml. < 2 ml : Sm-treated
2 ml : untreated control.

↓

Incubate it overnight at 37°C.

↓

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

↓

Suspend it into penassy. 1.5 ml.

↓

Test No of survivors of W4534
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/pen) incubate it 2 hrs more
② dilute × $10^{-1}$ ; add 0.1 ml to 1 ml of W4534
Add $10^6$ cells into the of W3086. (F' recipient).

↓

Incubate it for overnight at 37°C.

↓

Seed W3086 on BLac Sm. and incubate them.

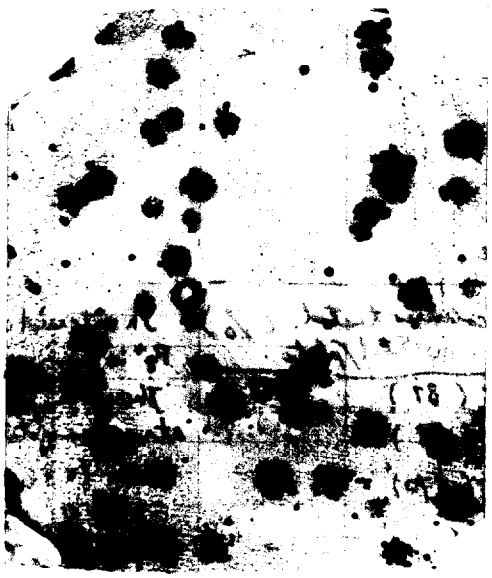
↓

Replica plate them on MGal seeded W4573 on it.

↓

count the percentage of $F_8^+$ infected.

untreated control.
W4534 → W3086
untreated control.



X W4573
on MGal

Sm killed W4534 → X 3086
Treated control.

on MGal
seeded W4573

2nd trial for isolation of $F_3^+$.

(W3642F₁): $F^+$ H $Mal_5$ $Gal_2$ $Lac_1$.
||
W4554.

2/vi ~ ; 1959 REF: see p122

Principle.

① W3642 $F_1$ → x 3133.

W6 → x 3133

② select on DO.

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

Results.

W3642 $F_1$ → x 3133 W6 → x 3133

| # of colonies | Hfr | % | | Hfr | % |
|---|---|---|---|---|---|
| 627 | 1 | | 586 | 0 | |
| 613 | 2 | | 673 | 0 | |
| 676 | 3 | | 517 | 1 ? | |
| 693 | 10 | | 684 | 1 ? | |
| 1742. | 2 | | 509 | 0 | |

④ Test the fertility again. (x 3086, on MLac) by cross-brushing 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 $M^+$. But non of them are Hfr. (see backpage)

∴ 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 for reisolation of 3133 $F_3$.

3642 $F^+$ → x 3133

1
→ 2
3
→ 4
5
→ 6
7

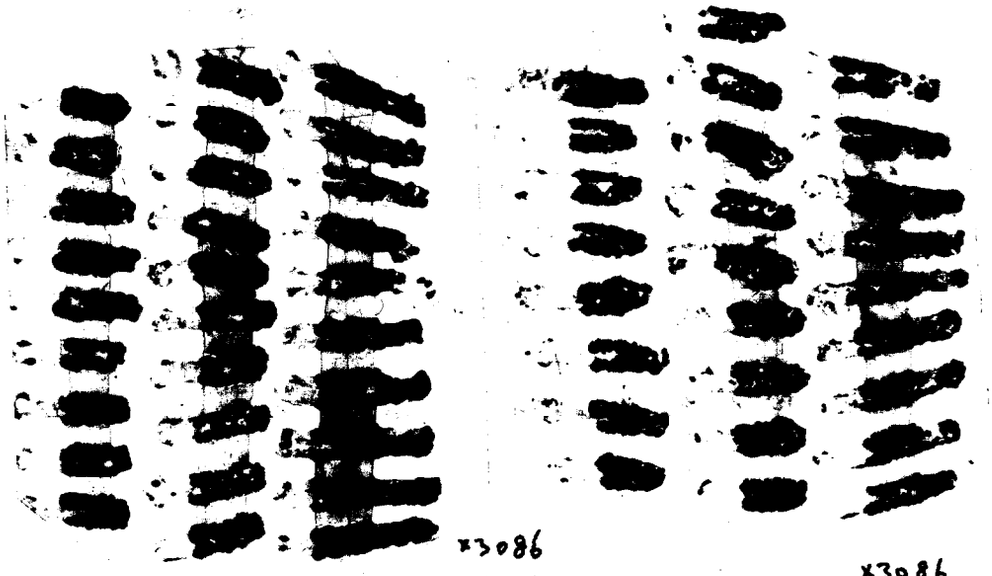
3133

x 3086

Purification of 3133 $F_3$.

#2

#6



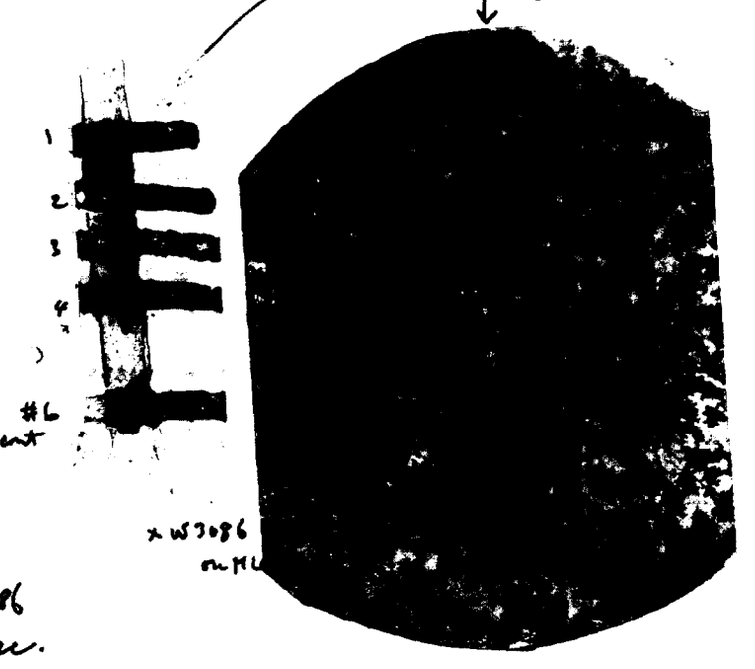
×3086 on Mlac.

×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.




#6 cut

×W3086 on Ml

# Test W3747: (derived strain from W3213)

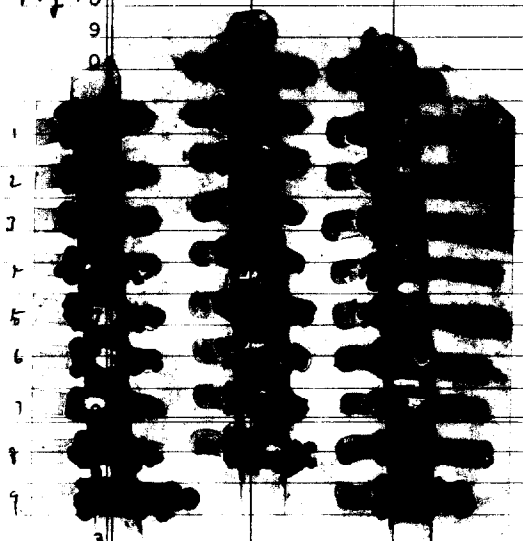
17/VI ;1959 $Lp_4^R M^- V_6^R$ $Hfr_{13}$. REF: $Hfr_{13}$.

J.L says: Alan found a strange Hfr that it is HI for Lac (x 3133 on MLac) but the many of these recombinants show Lacv. This seems quite peculiare phenomenon, it may depended to linked transfer of Lac and F'. or depended on Het marker. (Het may linked with Lac.)

1. Purify W3747 on BLac and test the fertility on each picked colonies from the BLac (x 4578 on MLac)
2. Look for $Hfr_{13}$ and pick recombinant from cross-fenched lesion. 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 Lacv. (See bellow Fig. 2) several colonies/plate are Var-Lac.
4. Pick 12 Lacv, suspend it into water, and streak it on BLac, Gal, Mal, Mt/Xyl, to know on the size of incorporated chromosomal segment.
5. Pick Lac+ and Lac− and cross x F−, and see the relation between F and Lac marker
6. Treat #19 with AO. and conform the infectivity of Hfr-character.
   AO 20γ, and AO 30γ. W3747 → x W3086.
   Result: This strain W3747, transfers the Hfr-character into F− (3086). Therefore, it is not Hfr but (HI for Lac.) (see back page).
   Hfr 16/28 total x100 = 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

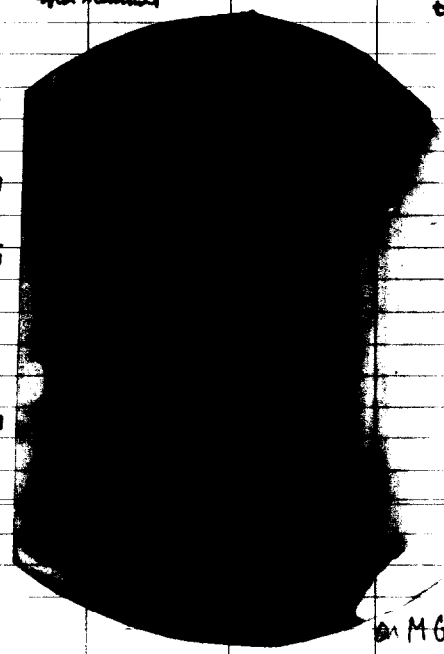
$A_1$

$G_1$

P

$TLB_1$

H



on M Gulcose

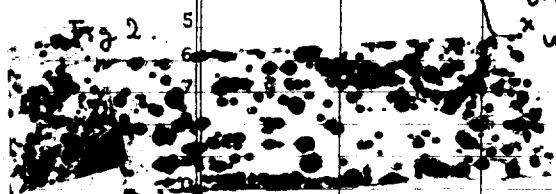
Lac v.

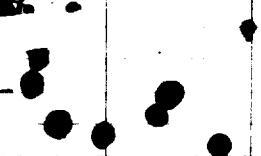
BLac

on MLac

x W4573

Fig 2.





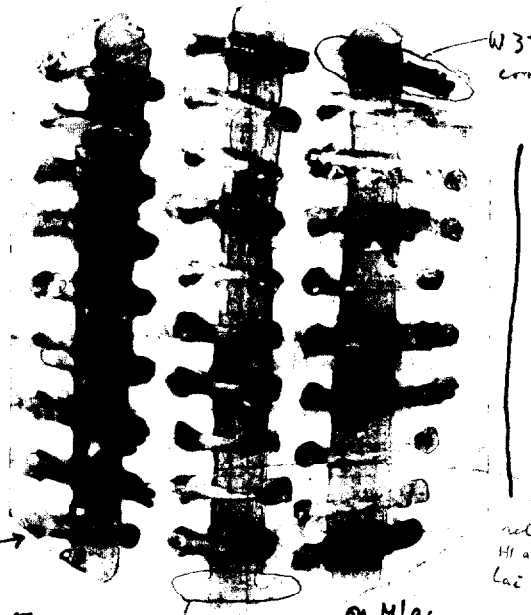
on BLac

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 segregation of Hi and Lo colony in the Lac progeny.

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

| | control | W3086 | Rate of conversion |
|---|---|---|---|
| Hi : Lac | — | 16/28 | (57 %) |
| Lo : Lac | — | 1/28 | (3.7 %) |
| total | | 17/28 | (60.8 %) |

on MLac
× 3828

Data from Replicaplating

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

$F_{13}$

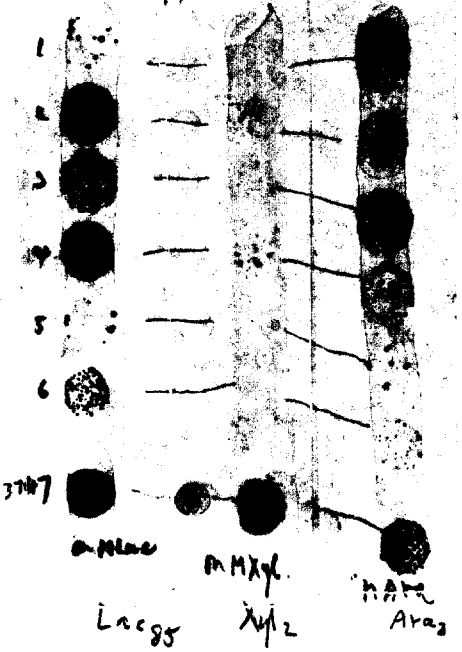
W3747 → × W3086 :

on BLac Sm → replicated on MLac seeded W3828.
futility of infested colonies by $F_{13}$.

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

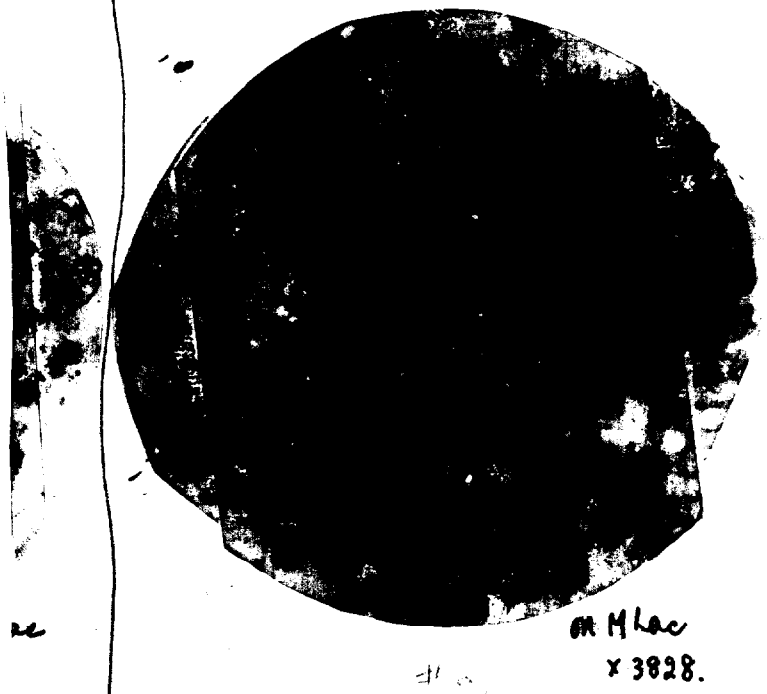
3086 $F_{13}$ → × W4573.

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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fertility of the segregants ~~from~~ of a sectased-colony arised from the cross W3747 x 3828.

30/4 1959 REF:

3743x

Method: 1. ~~Spot W3743 x 3828~~ Cross-brush W3743 x 3828. on Hlac.
2. Purify the black area on Blac.
3. Pick sectased colony and suspend it into water (1ml) and streak it on Blac again.
4. Pick (Lac+ and) Lac- colonies, resuspectibly, and test their fertility by cross x 3086. (M-F-) on Hlac.
5. Result.

Hl for M: Hfr 1/33

Lo for M: 32/33

Conclusion: Lac- Segregants from diploid colony ~~are~~ are all ♂ not ♀ unlike as usual Hfr.

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

#19

control. W6-

x3133 x4573

on Hlac

#1 #2

control

3734
3133

on Mlac
x3086

3774
3133

on Mlac
x3086

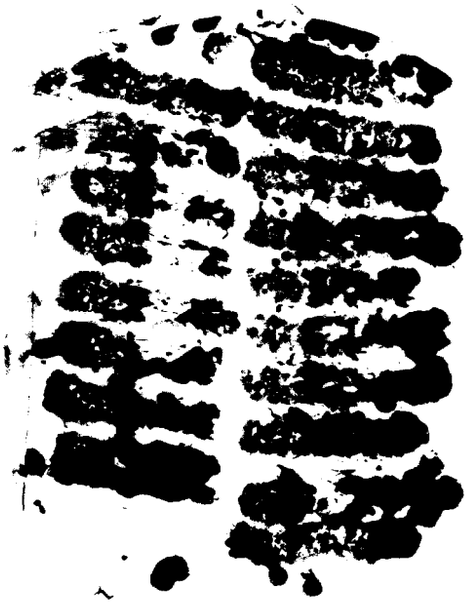
Hfr? Save this it may be $F^+_{13}$ Lac- $S^R$

These 2 kinds of colonies, Hl for M Lo for M may be interpreted by two state of colonies.

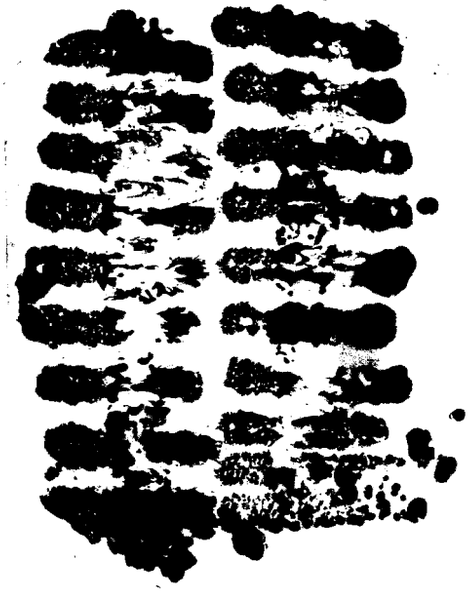
Hl Lac is Lo the others, but Lo Lac is Hl the others. (exp. Gal)

Hypothesis:
Transduction of Lac result Hl Lac but it will mutate to Lo Lac.

#2.



#1



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

30/VI ; 1959

REF:

Method : 1. Cross W3747 × 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

3747

3133

× 4506 (Pur⁻ F⁻) on MLac

#2

3747

3133

× 4506 (Pur⁻ F⁻) on MLac.

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# 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 $Lac^+$ loci 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 % : total colonies tested) is very high.
Other kind of transfer of $Lac^+$ marker occurred. (mechanism of not recombination) to $F^-$

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

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 ♂ : 88 ($Lac^-$) all ♀. (see back page)

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

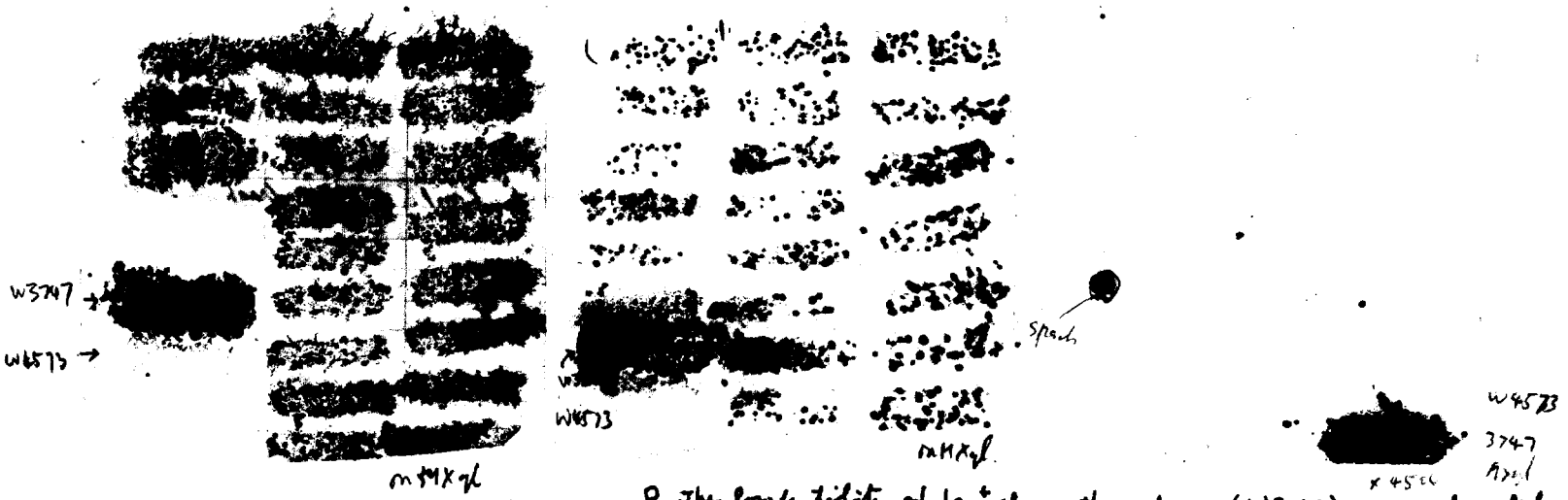
| $V_6$ | $Lac_{85}$ | $Gal_2$ | $Ara_2$ | Xyl$_c$ | Mtl. | ● | $Mal_1$ | M. | # of colonies tested. | |
|---|---|---|---|---|---|---|---|---|---|---|
| S | + | - | - | - | - | | - | + | 41 | Lac. transformed. |
| S | - | - | - | - | - | | - | + | 88 | $F^-$ Parental type. |

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)

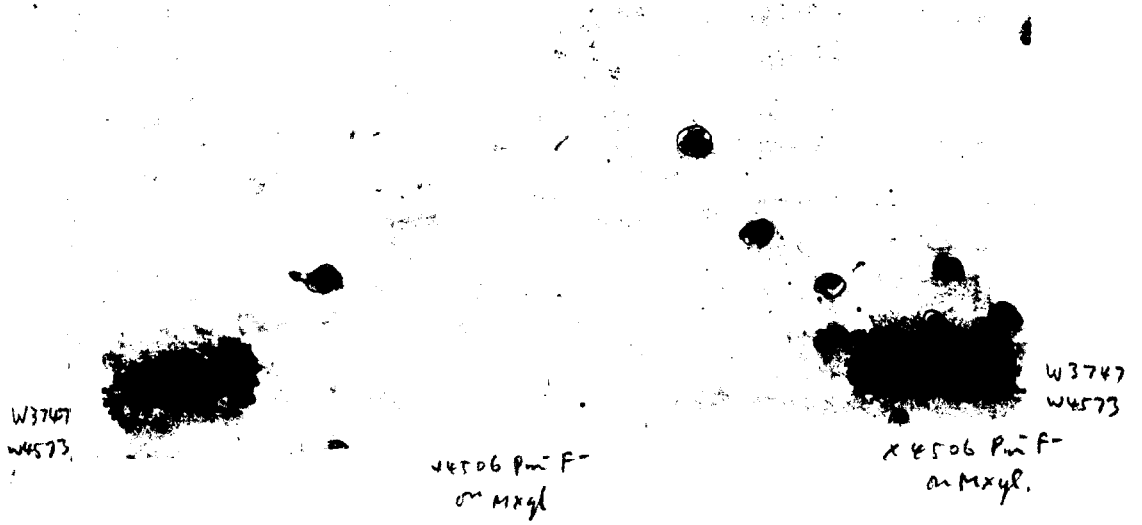
1 (Lac$^+$) 2 (Lac$^+$) 3 (Lac$^-$)




○ The low fertility of Lac$^+$ clones than donor (W3747) may be interpreted by two states. (Lo for Lac is Hi for the other marker, but Hi for Lac is Low for other marker.)

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 (↑↓ $Gal_4^+$ and $Gal_2^+$)

W4573: $F^-$ $Ara_2$ $X_{1/2}$ MtI $Gal_2$ $Lac_{85}$ $Mal_1$ $(\varphi^+)$

W3994: $F^-$ $Gal_4$ $(\varphi^+)$ $S^R$

$\varphi^s$ M $Gal_4$

on BGal + BGal Sm

• Further experimental design.

① (Use $S^R\varphi^s$ for all strain)
W3104 ($S^R$) $\varphi^s$ $Gal_4$ $F^-$
W3102 ($S^R$) $\varphi^s$ $Gal_2$ $F^-$
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 (use: 0.1 ml to 100 ml) of Sm to 1 ml of overnight culture of W4534.

② Incubate it at 37°C for 2 hrs.

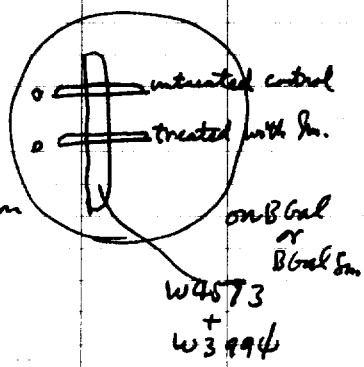
③ Wash it with 5 ml of Pensasy once.

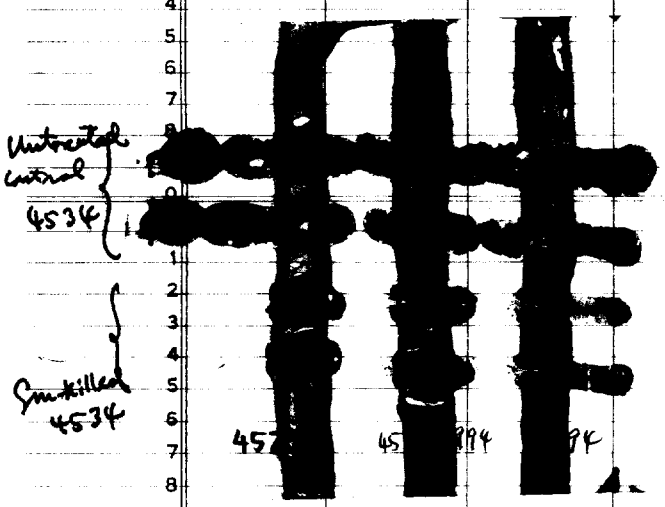
④ Cross-brush it with the mixture of W4573 and W3994 on BGal and BGal Sm.

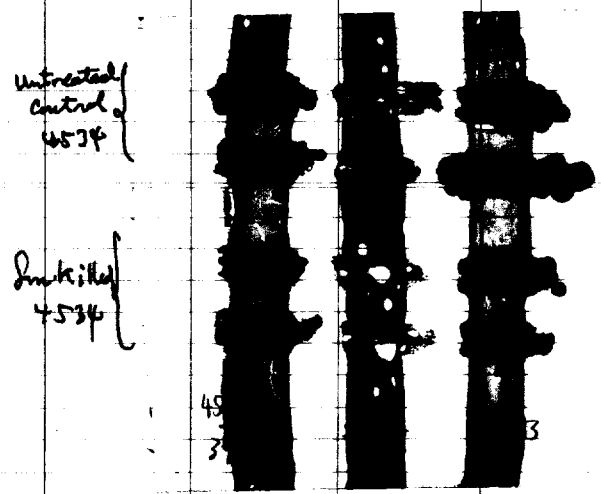
⑤ Incubate it overnight.

• 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 $F_{13}$.

2/VII :1959 (and isolation of Sm-resistant $F_{13}$ from it.)

Method

(Add)

1. (Treat) W3747 with Sm. (x10 conc. ; overnight-treated in penassy-Sm) overnight grown culture in penassy broth.

2. Incubate it for overnight at 37°C. (Sm-treated cell shows no growth but untreated control is ca. 10 times higher turbidity)

3. Wash it once with 2 ml of Penassy broth and resuspend it into 1 ml of Penassy broth, and test the fertility ~~and infectivity~~, by cross-brushing method. and by standard recombination method.

Result : See back page

- Cross-brushing method : Cross-brush it with ~~[illegible]~~ 3828, and W4573. on MLac, BLac, and BLac Sm. use untreated W3747 as a control.

Result :

| + Sm killed W3747 | control |
|---|---|
| 377 | 0 |

see back page

- 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.
  2. Incubate it 40 hrs.

Result :

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

| | # of Recombinants $10^{-4}$ ml |
|---|---|
| Untreated control : | 542 |
| | 579 |
| | 609 |
| Sm treated : | 63 ($10^{-1}$ ml) |
| | 109 |
| | 62 |

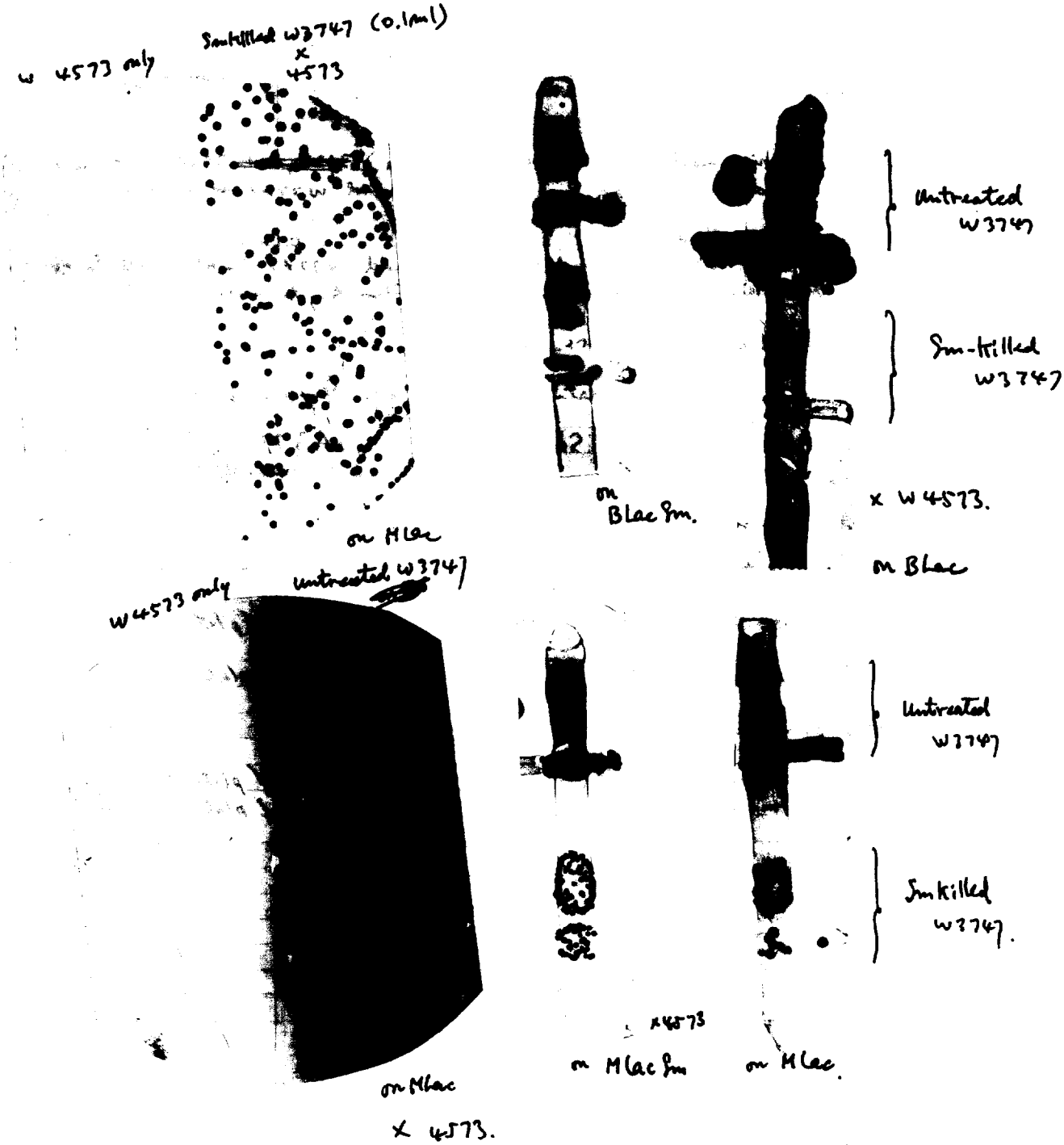
1. Add 0.2 ml of Sm-killed cells to W4573 : 1 ml. Use untreated W3747 as a control. Add 1 ml of fresh broth to it.
2. Incubate it for 2 hrs. at 37°C. (11:45 AM ~ 1:45 PM.)
3. Dilute them in adequate concentration, and seed it on MLac.

   Untreated control : ~~[illegible]~~ use $10^{-4}$ ml. / plate.

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

- ~~[illegible]~~ Survival test

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

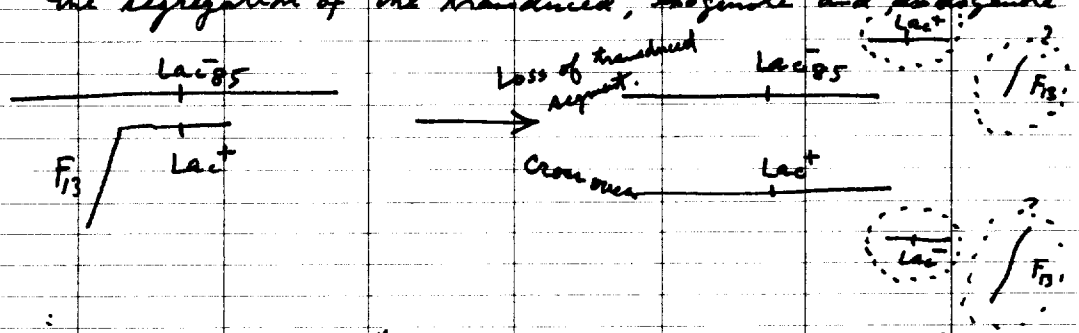

W 4573 only
Sm-killed W3747 (0.1ml)
x
4573
on MLac
W4573 only
Untreated W3747
on Mlac
x 4573.
on Blac Sm.
Untreated
W3747
Sm-killed
W3747
x W 4573.
on Blac
Untreated
W3747
Sm killed
W3747.
x4573
on MLac Sm
on MLac.

# Test stability of transduced marker "Lac+".

7/VIII ; 1959 REF:

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

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




Method :

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

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

2. Count total number of colonies and Lac⁻ colonies

Ref : These culture is ^ independently isolated ~~[redacted]~~ transduction of Lac+ to 4411 from W3747. Lac+ colonies are inoculated ca. 7 hrs into peptone broth and incubated ~~[redacted]~~ at 37°C. These 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.

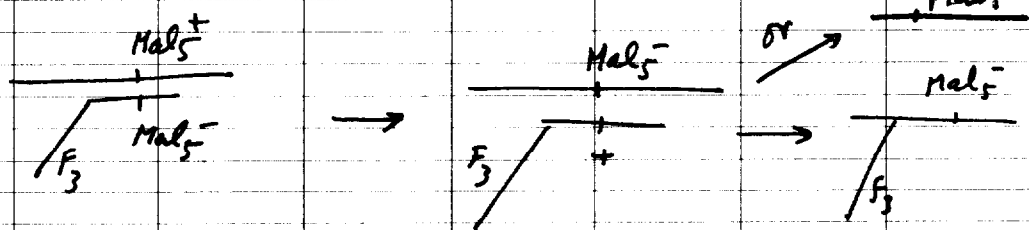
Result : #3 of 1/7 F⁻ #5 of 1/5 F⁻

# Recombination between $Mal_5^-$ and $F_3$.

7/4/4 ; 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 $F_{13}$. $(Lac_{1a}) \rightarrow (Lac^{3229})$

Called $11D_3$.

6/VII; 1959 REF:

| | 1 | 2 | 3 | 4 | 5 $F^- x^+$ | 6 | 7 $F^- x^+$ | 8 | 9 History of $11D_3$. |
|---|---|---|---|---|---|---|---|---|---|
| very unstable: | $Lac_1$ | : Permease | | | W 3238 | | $Lac_{61}$ : W4121 | | $Lac_1$ Y87 |
| through | $Lac_3$ | : $Lac^-$, $gal^-$, $mal^-$, $Gal^-$ | | | W 2243 | | | | $Lac_1$ W518 |
| | $Lac_5$ | : $Lac^-$, $Mal^-$ | | | W2245 ($S^R$) | | | | " W1578 |
| | Lac 2 | : β-galactosidase | | | W 3112 ($S^R$) | | | | " W1321 |
| | 4 | : β-galactosidase | | | W4287, W 3127, | | | | $Lac_1$ W1607 |
| | $Lac_7$ | : Permease and β-galactosidase | | | W 2247 | | | | $Lac_{1a}$ W3120 |
| | $Lac_{1a}$ | (11) | | | W 3230 | | | | $Lac^{3229}$ W3229 |
| | 1b | (12) | | | W 3089 (Mal S) | | | | $Lac^{3229}$ W3133 |
| | | | | | | | | | $Lac_{11D_3}$ 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 pancreas broth.

3.) ~~Make~~ Spot test on each strains. for transfer of lac from W3828 $F_{13}$.

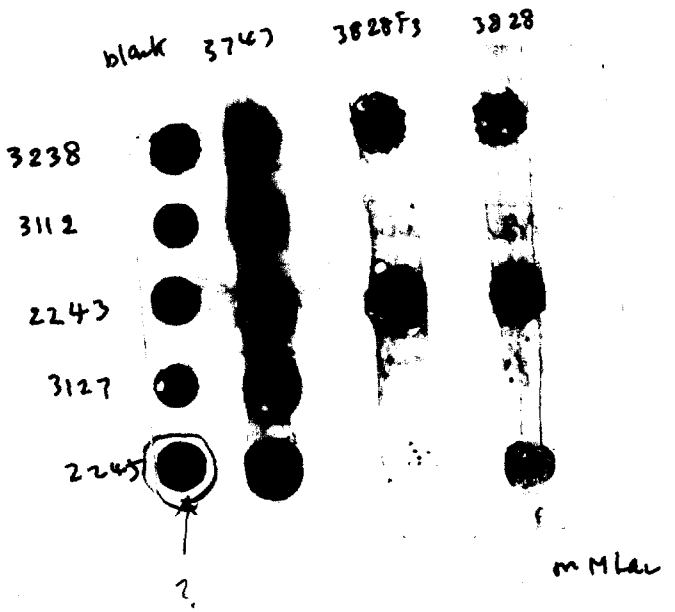
Result :

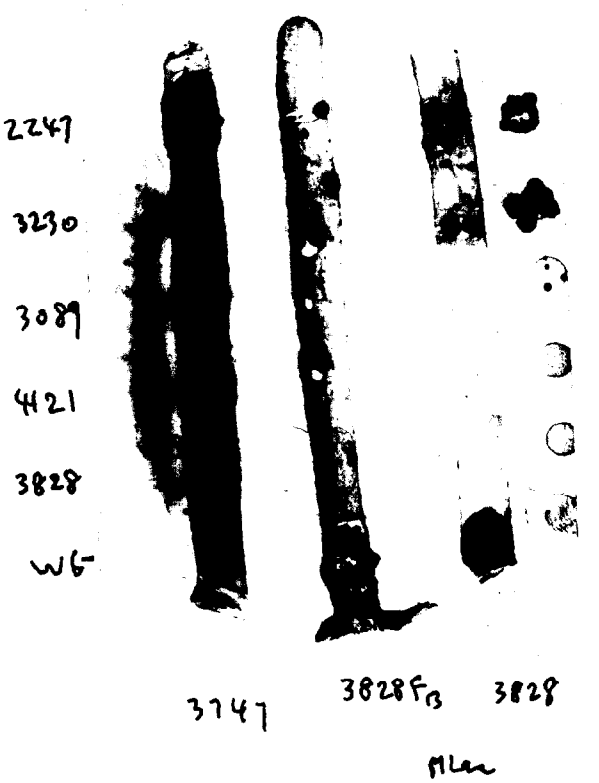
| Lac | W- | 3828 | 3828 $F_{13}$ | 3747 | blank. |
|---|---|---|---|---|---|
| 1 | 3238 | (+) − | (+) − | +++ | (+) |
| 2 | 3112 | − | − | +++ | − |
| 3 | 2243 | ? +++ | ? +++ | ? +++ | +++? reversion |
| 4 | 3127 | − | − | +++ | − |
| 4 | 2245 | + | + | +++ | +++ ? |
| 7 | 2247 | − | − | +++ | − |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

on MLac & 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
?
mMLac


2247
3230
3089
4121
3828
WG-
3747
3828F3
3828
MLac

# Transduction of Lac-loci by $F_{13}$.

{ W6 $F_{13}$ → × W4541
W4511 $F_{13}$ → × W2594 }
$Lac^+$

3863 $Pa^-$ $F^-$

7/VII 1959 REF:

Principle :

One way : W6 $F_{13}$ (transducible) → × W4541 ($F^-S^R Lac_{85} Xyl_2 Mtl Mal_1 Gal_2 V_6 P$
M $F_{13}$.

back : $Lac^+$ W4411 $F_{13}$ → × W2594 ($F^-M Lac S^R V_1^R$)
$Lac^+$ P $Xyl_2$ Mtl $Mal_1$ $Gal_2$ $V_6$

Method :

1. Mix 1 : 1 : 1 (fresh broth) and incubate it overnight at 37°C.
2. Purify them on Blue Gm. count the ratio of $Lac^+$ and $Lac^-$. Use $10^{-6}$ ml/plate
3. Test on all markers of Lac-transduced W4541. and W2594.

W6 $F_{13}$ → × W4541

1. Ratio of $Lac^+$ and $Lac^-$ (+/−) (49.0)
; 239/249 (51.1) ; 224/267 (45.6) ; 255/266
; 235/213 (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 | + | − | − | − | − | 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 $Lac^+$ / $Lac^-$ ; $\overset{+}{178}/\overset{-}{260}$ (40.6) % ; $\overset{+}{152}/\overset{-}{237}$ (39.1) % ; $\overset{+}{125}/\overset{-}{229}$ (35.3) %
Partial$^R$ ; $\overset{+}{135}/\overset{-}{206}$ (39.6) ; $\overset{+}{201}/\overset{-}{258}$ (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 1 | | r | + | + | + | + | + | $S^r$ (r:1) (s:1) | + | − | r | 53 | |
| 4 2 | | r | + | + | + | + | + | $S^r$ (r:4) | + | − | r | 48 | |
| 8 3 | | r | + | + | + | + | + | $S^r$ (r:2) | + | − | r | 39 | |
| 2 6 | | r | + | + | + | + | + (−:1) | $S^r$ (r:2) | + | − | r | 39 | |
| 8 | | r | + | + | + | + | + | $S^r$ {r:3, s:2} | + | − | r | 53 | |
| 1 0 | | r | + | + | + | + | + | $S^r$ {r:1, s:1} | + | − | r | 40 | Total Tested |
| 6 1 | | r | + | + | + | + | + (−:1) | $S^r$ {r:3, s:1} | + | − | r | 39 | 39 3 |
| 7 2 | | r | + | + | + | + | + | $S^r$ {r:1} | + | − | r | 38 | |
| 5 4 | | r | + | + | + | + | + | $S^r$ (r:2) | + | − | r | 44 | |

$V_1^s + V_1^r$ 24
$Gal_2^-$ 2

| Σ | $S^r$ | $Lac^+$ | $Xyl_2^+$ | $Mtl^+$ | $Mal_1^+$ | $Gal_2^+$ | $V_6^{s'}$ | P | $M^-$ | $T_1^r$ | : 367 | 93.4 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 7 | r | + | + | + | + | $Gal_2^-$ | $V_6^{s'}$ | + | $M^-$ | $T_1^r$ | : 2. | 0.508 | $Lac^-$ 0.508 |
| 8 | r | + | + | + | + | + | $V_6^r$ | + | $M^-$ | $T_1^r$ | : 19 | 4.83 | $Lac^+$ 99.402 % |
| 9 | r | + | + | + | + | + | $V_6^s$ | + | $M^-$ | r | : 5 | 1.27 | |

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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 | | | | | | | | | | |
| 3 | r | H1 | | | | | | | | |
| 4 | I | | | | | | | | | |
| 5 | | | | | | | r | H1 | exception | |
| 6 | | | | | | | | | | |
| 7 | | | | | | | | | | |
| 8 | | | | | | | | | | |
| 9 | | | | | | | | | | |
| 0 | | | | | | | | | | |
| 11 | | | | | | | | | | |
| 2 | | | | | | Endomirist | | | | |
| 3 | | | | r | H1 | except | | | | |
| 4 | | | | I | | | | | | |
| 5 | | | | | | | | | | |
| 6 | | | | | | | | | | |
| 7 | | | | | | | | | | |
| 8 | | | | | | | | | | |
| 9 | | | | | | | | | | |
| 20 | | | | | | | | | | |
| 21 | | | | | | | | | | |
| 2 | | | | | | | | | | |
| 3 | | | | | | | | | | |
| 4 | | | | | | | | | | |
| 5 | | | | | | | | | | |
| 6 | | | | | | | | | | |
| 7 | | | | | | | | | | |
| 8 | | | | | | | | | | |
| 9 | | | | | | | | | | |
| 30 | | | | | | | | | | |
| 31 | | | | r | Lo | | | | | |
| 2 | | | | I | H | | | | | |
| Gal- 3 | | | | | | | | | | |
| 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 | | | | | | | R | (HI) | | exceptional |
| 2 | | | | | | | R | Lo | | |
| 3 | | | | | | | | | | |
| 4 | | | | | | | | | | |
| 5 | | | | | | | | | | |
| 6 | | | | | | | | | | |
| 7 | | | | | | | | | | |
| 8 | | | | | | | | | | |
| 9 | | | | | | | | | | |
| 30 | | | | | | | | | | |
| | I | | | | | | | | | |
| 31 | r | Lo | | | | | | | | |
| 2 | I | HI | | | | | | | | |
| 3 | | | | | | | | | | |
| 4 | I | | | | | | | | | exceptional |
| 5 | r | Lo | | | | | | | | |
| 6 | I | HI | | | | | (S) | Lo | | |
| 7 | | | | | | | | | | |
| 8 | | | | | | | | | | |
| 9 | | | | | | | | | | |
| X0 | | | | | | | | | | |
| X1 | | | | | | | | | | |
| 2 | | | | | | | | | | |
| 3 | | | | | | | | | | |
| 4 | | | | | | | | | | |
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| 9 | | | | | | | | | | |
| 0 | | | | | | | | | | |

Crossover?

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19 REF:

| Plate 3 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 9 | $V_6$ | Lac | Gal | | $V_6$ | 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 | | | | | | | | | | |
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| 27 | | | | | | | | | | |
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| 29 | | | | | | | | | | |
| 30 | | | | | | | | | | |
| 31 | | | | | | | | | | |
| 32 | r | Hi | | | | | | | | |
| 33 | r | Lo | | | | | | | | |
| 34 | | | | | | | | | | |
| 35 | | | | | | | | | | |
| 36 | | | | | | | | | | |
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| 50 | | | | | | | | | | |

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resistant; sensitive; Intermediate

$V_6$ : r. S. I.

Lac : H (HI) ; L (Lo)

Gal :

REF:

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| Plate #① | 1 | 2 | 3 | Plate-2. | 5 | MLac ×4573 | MLac ×7573 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Clone # | $V_6$ | fertility 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 | | | | | | | | | | |
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| 34 | | | | | | | | | | |
| 35 | | | | | | | | | | |
| 36 | | | | | | | | | | |
| 37 | | | | | r | Lo | | | | |
| 38 | | | | | | | | | | |
| 39 | | | | | | | | | | |
| 40 | | | | | | | | | | |
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| 47 | | | | | | | | | | |
| 48 | | | | | | | | | | |
| 49 | | | | | | | | | | |
| 40 | | | | | | | | | | |

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

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

7/VII ; 1959 REF: W4637 4647 ?(101:85)

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$^+$ (149) and streak it onto BLac Sm.

4. Replica plate it on DO, DO + pur. + Prol. B Gal

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. All colonies show X$^+$.

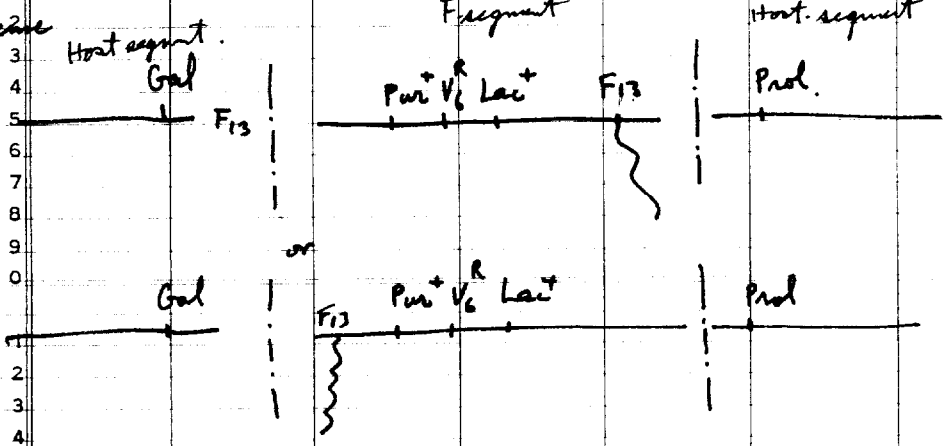
| (H1 Gal) | (H1 Lac) | $F_{13}$ | $V_6$ | Gal | Prol | Pur | Selected Lac$^+$ $S^R$ | Number of colonies tested. |
|---|---|---|---|---|---|---|---|---|
| H1 | H1 | $F_{13}$ | r | + | + | + | + r | 149 |

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

Conclusion:

Pur. is also transferred with $F_{13}$. Rate of multiplication of the fragment seems 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.

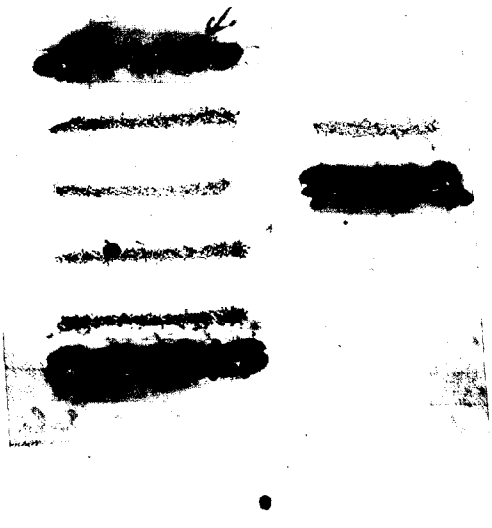
2. Replica plate it on MLac seeded W3086 on it. • Sel: Lac M.
Replica plate it on MLac seeded W4573 on it.

Result:

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

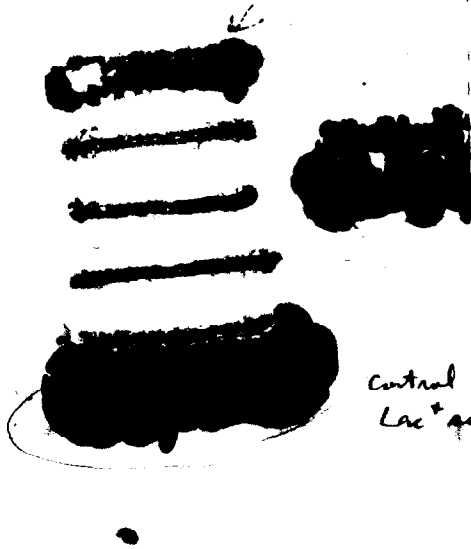
Ref: fertile Lac− grows better than infertile Lac−, on BLac

From 4411 $F_{13}$
#5



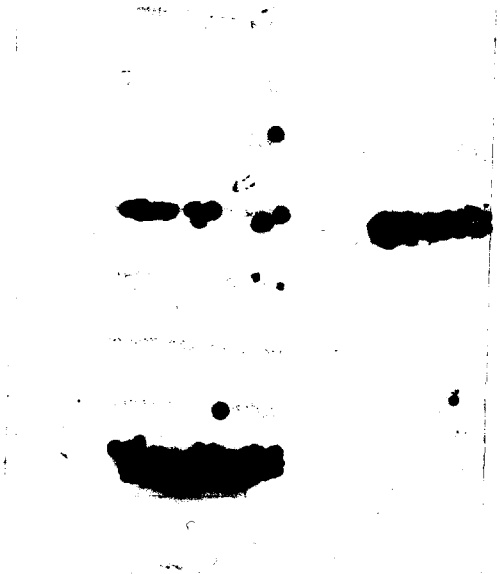
on MXyl
×3086

#5



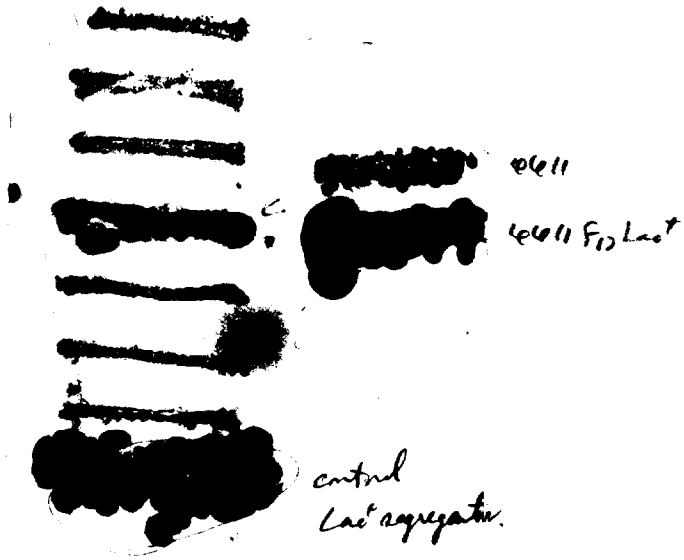

on Blac

#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$^+$/total | 1 | 13/29 | 6/27 |
| | 2 | 9/25 | 6/24 |
| | 3 | 15/27 | 8/28 |
| Σ | | 37/81 | 20/79 |
| | | % (35.7) | (25.3) |

Treated by Lac$^+$ transfer.

b. $F_{13}$.

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

## 2. W6 $F_{13}$

$F_{13}$

| | | AO treated | | Control | |
|---|---|---|---|---|---|
| | | on Gal ×4573 | on Mlac ×4573 | Mgal ×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 | |

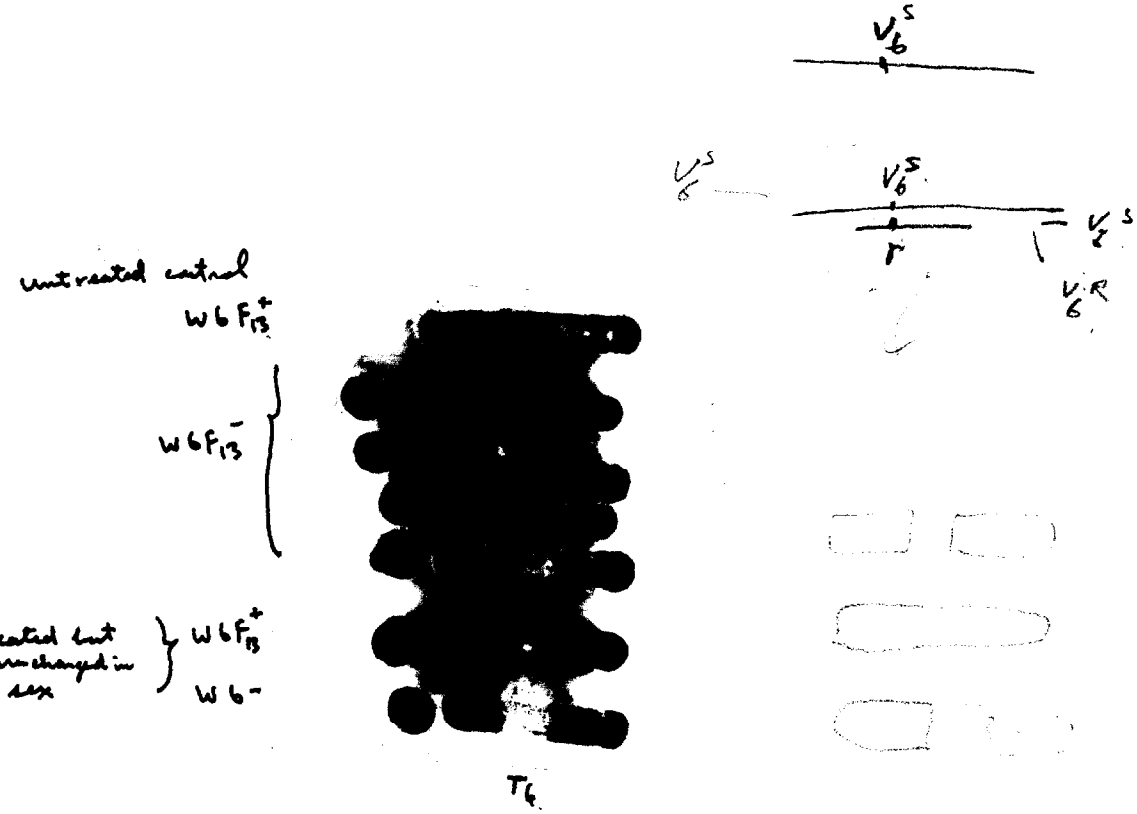
Test $T_6$-resistance.

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+
W6 F13−
Treated but unchanged in sex
W6 F13+
W6−
T6
V6S
V6S
V6S
V6R

# Treatment of $Lac^+$-transduced W4411 with AO.

19 REF:

Purpose: Is $V_6$ $Lac^+$ $F_{13}$ loci sensitive to AO-treatment.

strain W4411 $F_{13}$: $F_{13}$ $Lac^+/Lac_{85}$ $V_6^S/V_6^R$ $Mal_1$ $Xyl_2$ ~~[illegible]~~ $Mtl$ $S^R$

Experimental method:

1. Inoculate 0.1 ml of overnight culture of W4411 $F_{13}$ into AO-NSB.
   pH. 7.6 : AO 50 γ/ml: $6 \cdot 10^6$ cells/ml:
2. Incubate it overnight.
3. Seed it on $BLac_3$ and incubate them overnight at 37°C.
4. Count $Lac^+$ and $Lac^-$

| | AO-treated W4411 $Lac^+$-$F_{13}$ | Untreated control. |
|---|---|---|
| $Lac^+/Lac^-$ (% of $Lac^-$) | 97/242 (27.8%) 349 | 267/623 (30.0%) 890 |
| | 105/273 (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 Het and not segregat[illegible] progeny.

# Elimination of Lac-$F_{13}$ segment with AO-treatment. (1)

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

**Purpose:** Is Lac-$F_{13}$ segment sensitive to Aoridine treatment.

**Strain:** W2594($F_{13}$ Lac+) cf. P112.
Purify a Lac+ colony on BLac, ~~and~~ pick a Lac+, and suspend it into 1 ml 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/5 ml. ; Medium: NSB-conc. pH7.0
AO conc.; 30γ/ml : 37°C, overnight treatment.

**Procedure:**
Seed $10^{-6}$ ml of AO-treated and untreated culture onto BLac agar. and count the ratio of Lac+ and Lac- colonies.

Result:

| | | AO-treated (30γ AO) | | | | | Untreated control | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Tube A | | Lac+ | Lac- | Lac V. | Σ | % of Lac- | Lac+ | Lac- | Lac V. | % 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 ō % | 291 | 650 | 11 | 952 | 68.2 % | 1248 | 333 | 8 | 1589 | 21.0 % |
| Tube B (30γ AO) | 1 | 12 | 59 | 0 | 71 | | 382 | 46 | 3 | 431 | |
| | 2 | 31 | 107 | 1 | 139 | | 374 | 42 | 3 | 419 | |
| | Σ ō % | 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 exogenate, with very high frequency.

History of 2594 ( $Lac_{11}$ )

Y87 $Lp^S$ $Gal_4^-$

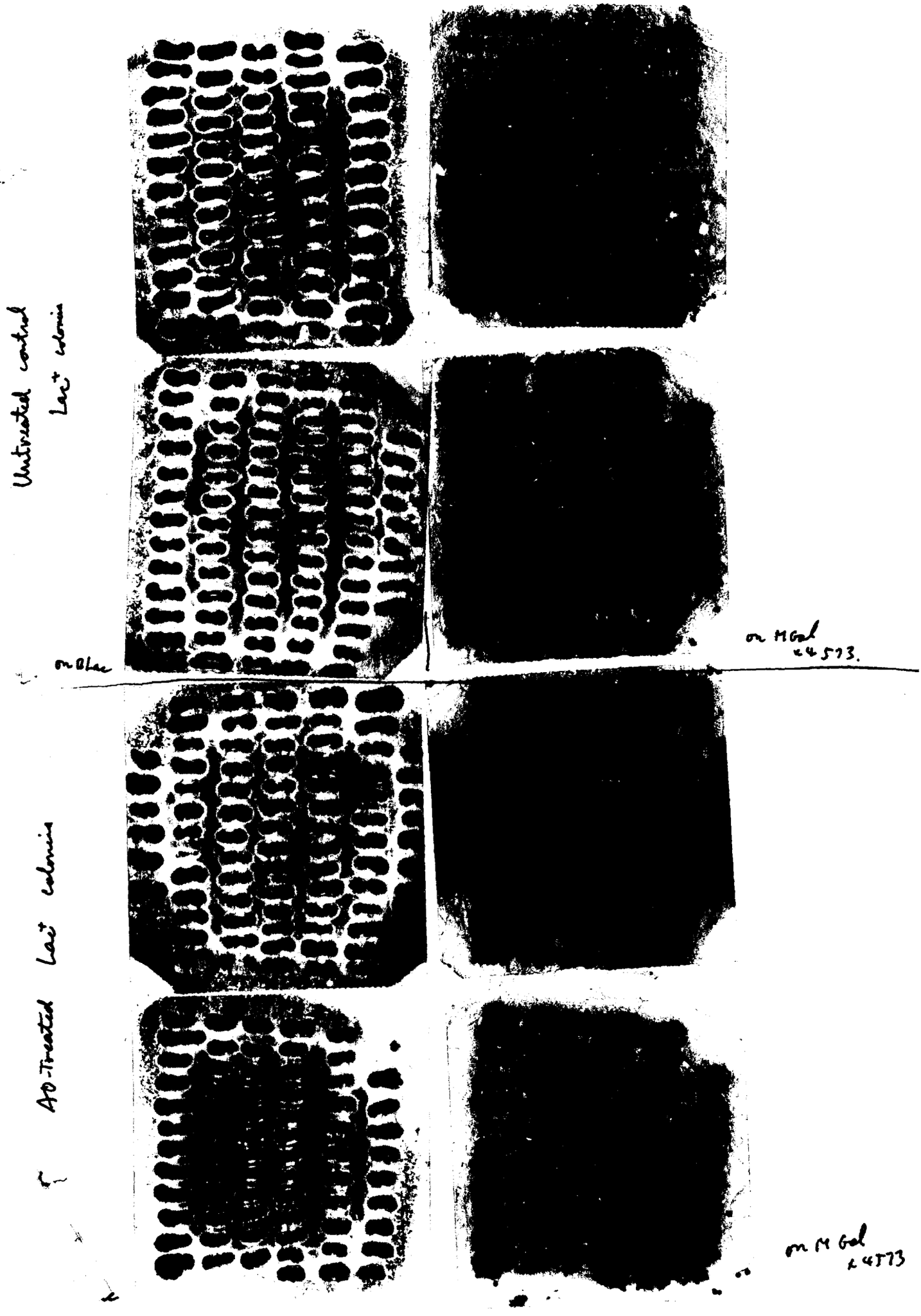
W518 M $Lac_1$ $Gal_4$ $Lp^S$ $V_1^R$

W1578 $Lp^S$ $F^-$ M $Lac_1$ $Gal_4$ $V_1^R$

W1321 $Lp^+$ $F^-$ M $Lac_1$ $Gal_4$ $S^R$ $V_1^R$

W1607 $F^-$ $Lp^+$ M $Lac_1$ $Gal_4$ $S^R$ $V_1^R$

W2594 $F^-$ $Lp^S$ M $Lac_1$ S $V_1$


Untreated control
Lac+ colonies
on B Lac
on M Gal
x4573.
AO-Treated Lac+ colonies
on M Gal
x4573

# Elimination of Lac-$F_{13}$ segment with AO-treatment. (continued.)

22/VII ; 1959. REF: Cf 1274.115.117

Strained treated with AO: W2594 $F_{13}$ ($MS^R V_1^R Lac^{+/-} V_6^{r/s}$) Lac$_{11}$

Continued.

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 M Gal seeded 0.2 ml broth culture of W 4573 ($Gal_2^0$ $Lac_{85}$ $Ara_2$ $Mal_1$ $Xyl_2$ MtI $S^R$ $F^-$)

Ref. This Lac$^-$ is ~~[illegible]~~ unstable. It gives several papillae per one streak. on D 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 | Gal HI | Lo | | Σ | Gal HI | Lo | | Σ | Gal HI | Lo | | Σ |
| | 147 | 2 | 0 | 149 | 13 | 9 | 116 | 138 | 146 | 0 | 0 | 146 | 104 | 12 | 0 | 116 |
| % | 98.6 | 1.3 | 0 | | 9.43 | 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 contains Lac$^{+/-}$ and Lac$^+$)

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

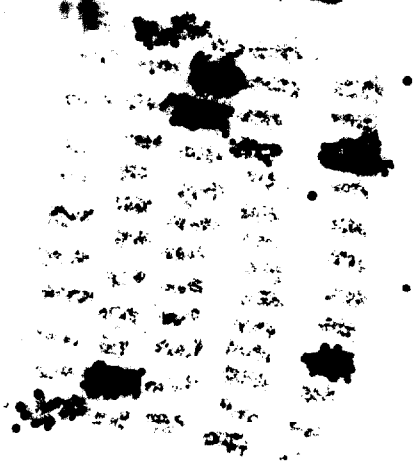
a untreated control Lac⁻ each streaks has papillae. ∴ lac, is unstable.

on Blac.

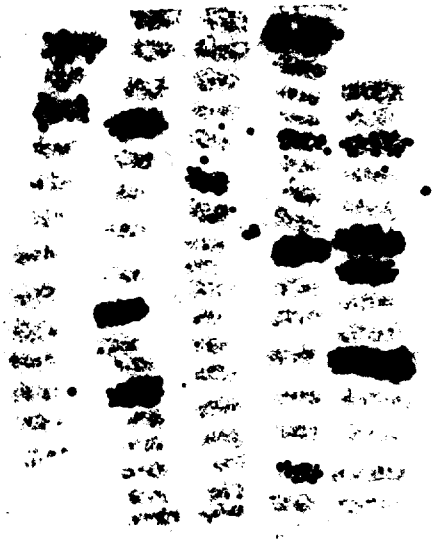
on MGal x4573

AO-treated, lac⁻
each streaks has papillae.

on Blac



on MGal x 4573



on MGal x4173

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# Rate of multiplication of $F_{13}$-Lac-segment.
## (Infection of Lac-$F_{13}$ segment to $F^-$)

W4573 $F^-$ $Gal_2$ $Lac_{85}$ $Mal_1$ $Xyl_2$ $Mtl$ $Ara_2$ $S^R$.
W3747 $M$ $O_6^R$ $F_{13}$

18/III ; 1959

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

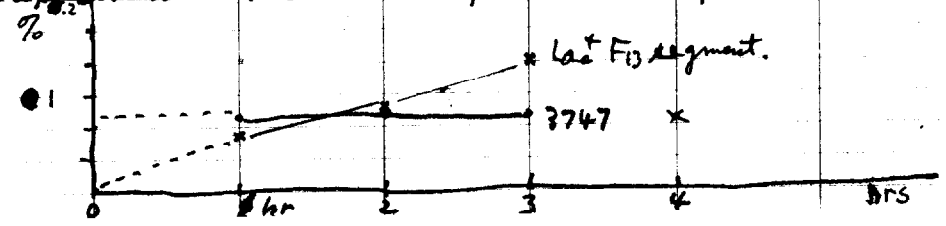
1. Make overnight culture of W4573 and W3747.

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

| | Time | 1:00 0 hr $10^{-5}$ ml | 2:00 1 hr $10^{-6}$ ml | 3:00 2 hr $10^{-7}$ ml | 4:00 3 hr $10^{-7}$ ml + − | 4 hr |
|---|---|---|---|---|---|---|
| BLac | 1 | | 16/1011 | 0/125 | 2/164 | ~~2/164~~ |
| | 2 | | 8/890 | 2/151 | 5/151 | ~~5/151~~ |
| | 3 | | 15/964 | 1/123 | 2/129 | ~~2/129~~ |
| multiplication of Lac marker : | Σ 5 | | 39/2865 | 3/399 | 9/444 | |
| | 6 | $Lac^+ + Lac^-$ | 2904 | 402 | 453 | |
| original $Lac^+$ | 7 | % | 1.34 | 0.746 | 1.99 | % |
| | 8 | | | | | |
| | 9 | | | | | |
| | σ | | $Ara^+/Ara^-$ | | | |
| B Ara | 1 | | 8/1011 | 0/125 | 0/164 | |
| | 2 | | 6/890 | 0/151 | 3/151 | |
| | 3 | | 8/964 | 0/123 | 0/129 | |
| | 4 | | | | | |
| | Σ 5 | | 22/2865 | 0/399 | 3/444 | |
| multiplication of Ara. | 6 | $Ara^+ + Ara^-$ | 2887 | 399 | 447 | |
| cell itself. | 7 | | | | | |
| | 8 | % | 0.762 | 0. | 0.671 | % |
| | 9 | | | | | |
| | σ | | $Xyl^+/Xyl^-$ | | | |
| BXyl | 1 | | 8/1011(8) | 0/125(0) | 0/164 (2) | |
| | 2 | | 6/890(2) | 0/151(2) | 3/151 (2) | |
| | 3 | | 8/964(7) | 0/123(1) | 0/129 (2) | |
| | 4 | | | | | |
| | 5 | | 17/2865 | 3/399 | 6/444 | |
| | 6 | | | | | |
| Difference | 7 | Multiplication of $Lac^+$ | 2882 | 402 | 450 | |
| multiplication of $Lac^+$-segment. | 8 | | | | | |
| | 9 | ($Lac^+ - Ara^+$) % | 0.592 | 0.746 | 1.33. | % |
| | σ | | | | | |

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

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



# 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. $Lac_{1a}$ (11) W3230 ⟷ W3112 : $Lac_2$

" ⟷ W3127 : $Lac_4$.

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

1. All $Lac^-$ colonies which isolated from sectored colonies, 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
Under that $Lac^+$ 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 ($Lac_{1a}$)

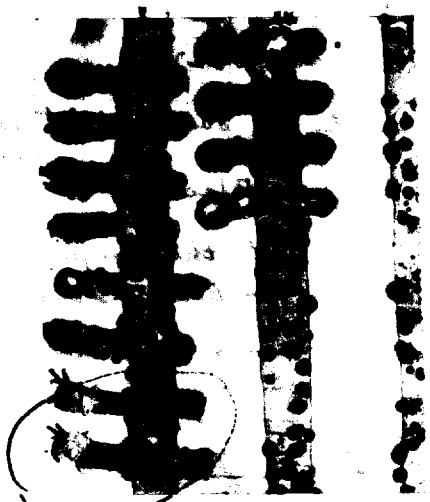
| Σ | HI Lac | Lo Lac | F⁻ (probably, because $Lac_2$ is unstable) |
|---|---|---|---|
| 174 | 88 | 80 | 6. (but better than 3127) |

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 |

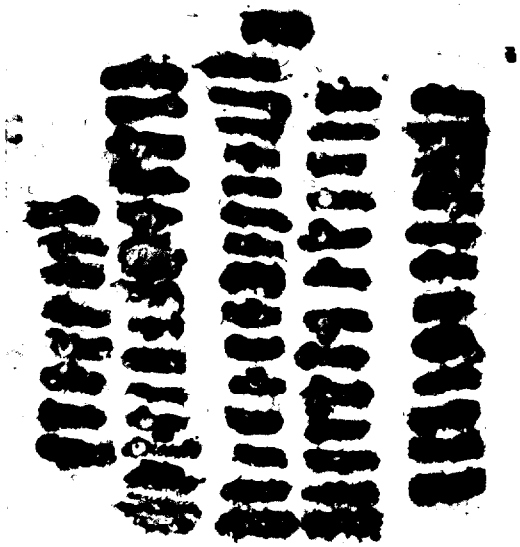
$Lac_{85}$ include or accompanied with $Lac_2$ (W3112)



X 3230 on MLac

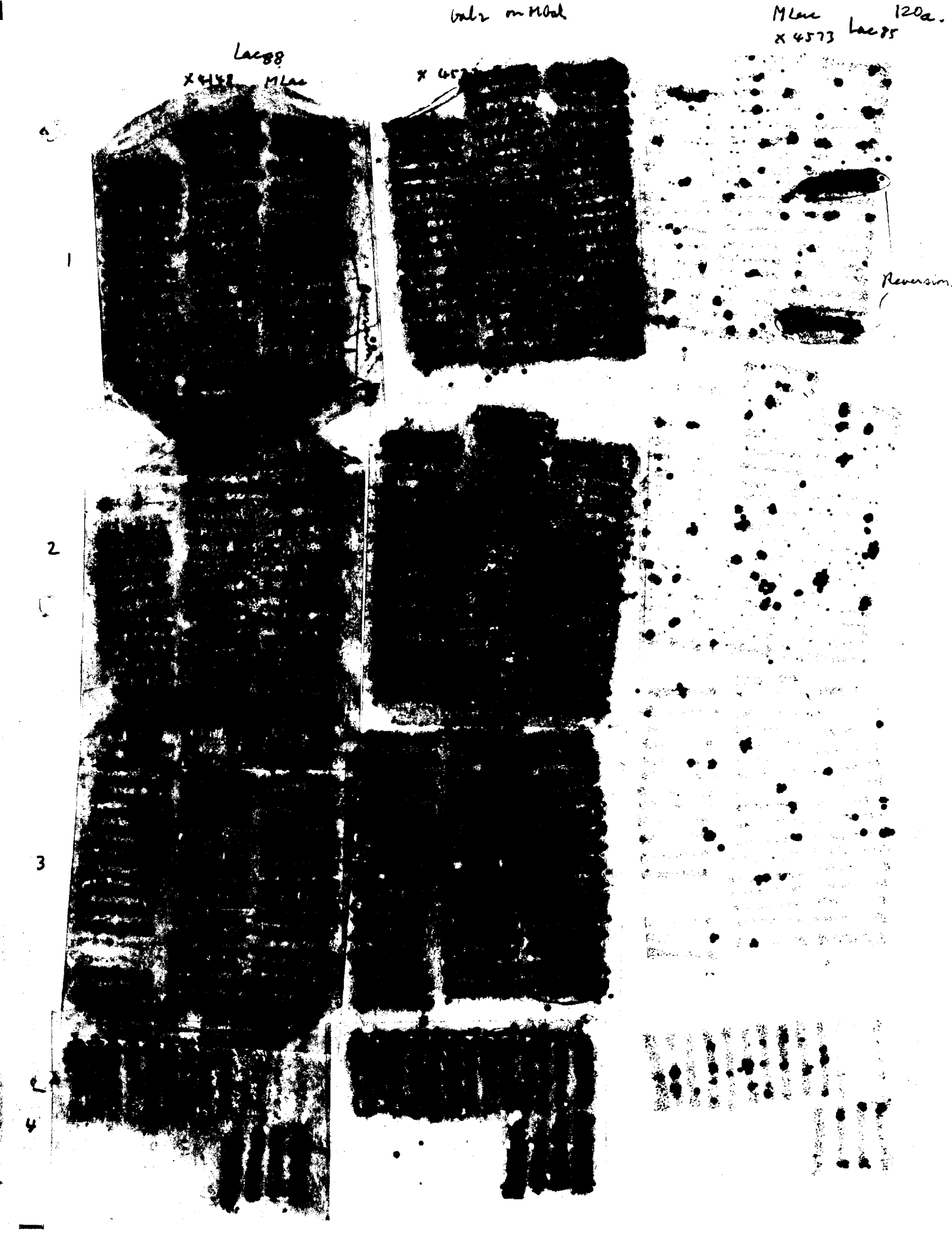
Segregants from Lac 2 F13/+ F-

W3112 F13



on MLac
x seeded W 3230

on BLac


Lac88
Lac85
Reversion

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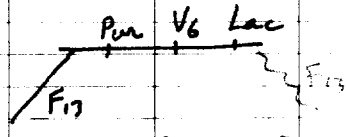
# Timing experiment of transduction of Lac $F_{13}$ segment. I

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⁻.




← or →

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 / 10 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 |
|---|---|---|---|---|---|---|---|---|---|
| # of Recombinants. 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 | 0 / 0 | 0 / 0 | 0 / 0 | Lac⁺ → 2 / 0 |
| MO + Sm. | | Pur. | 0 / 0 | 0 / 0 | 0 / 0 | 0 / 0 | 0 / 0 | 0 / 0 | 0 / 0 |
| EMB-Lac ($10^{-7}$ ml) | | | − + 0/13 / 0/12 | − + 0/11 / 4/12 | − + 1/15 / 3/13 | − + 1/19 / 4/18 | − + 2/11 / 0/16 | − + 2/32 / 5/26 | − + 1/23 / 1/20 |

blank ($10^{-6}$ ml /plate) $\frac{-}{17} / \frac{+}{110}$ ; $\frac{-}{26} / \frac{+}{137}$ ; $\frac{-}{17} / \frac{+}{141}$ :

① # of cells used { ♂ initial : $1 \times 10^8$ cells; final : $2 \times 10^8$ cells; ♀ : ca $1 \times 10^7$ cells

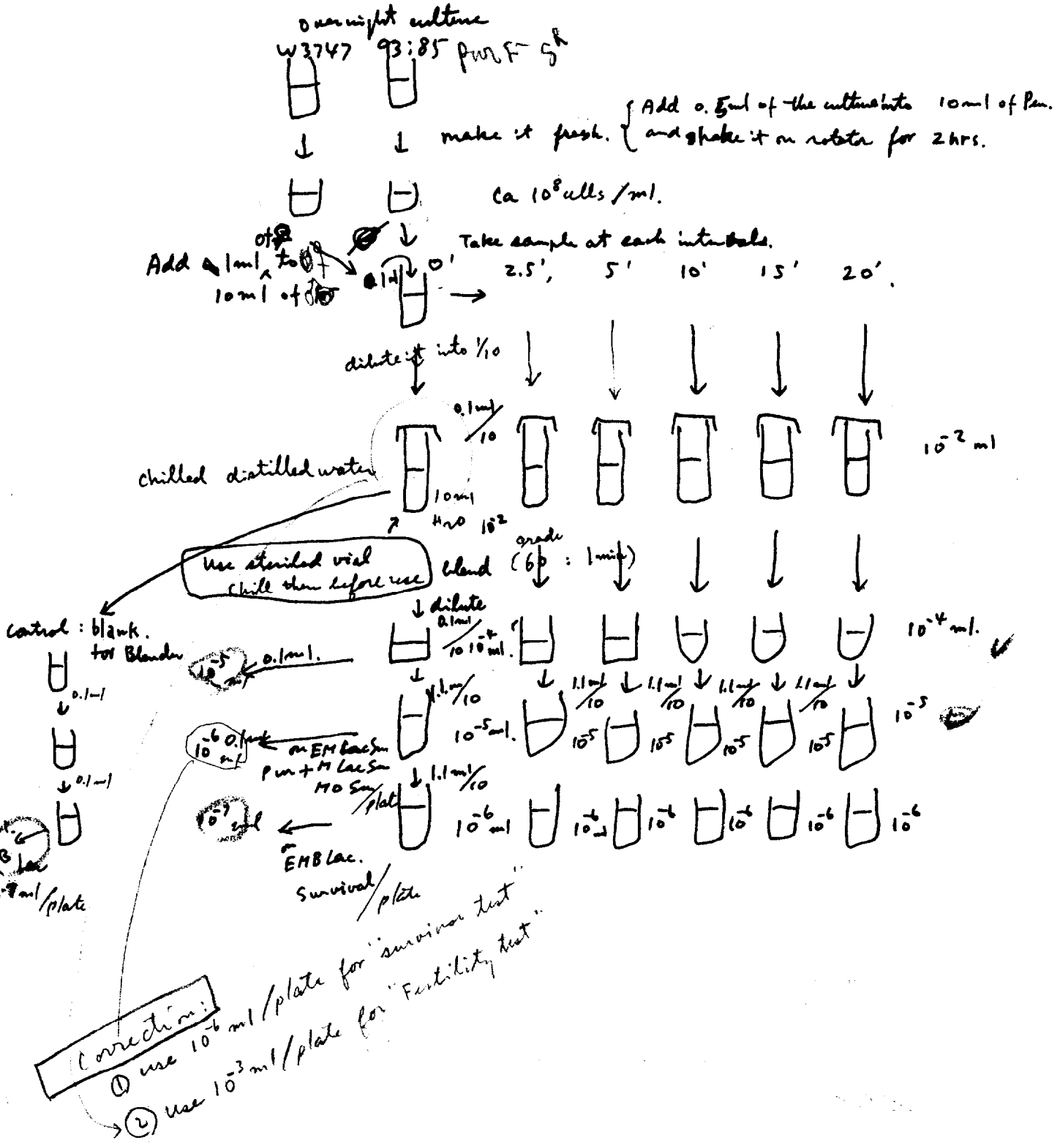
② Rate of Recombination :

Rate of Recombination in this experiment :

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

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

Experimental method



# Isolation of 3133 $F_3^-$ with AO method.



20/VII ; 1959 REF:

AO : 30γ/ml : NSB conc. standard for AO method PH 7.6.

3133 $F_3^+$ [redacted] Inoculum size ca $10^5$ cells/ml. Overnight treatment.

3133 $F_3^-$

3133 $F_3^+$ [redacted]

3133 $F_3^-$ [redacted]

×W6- on Mac

**Result.**

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

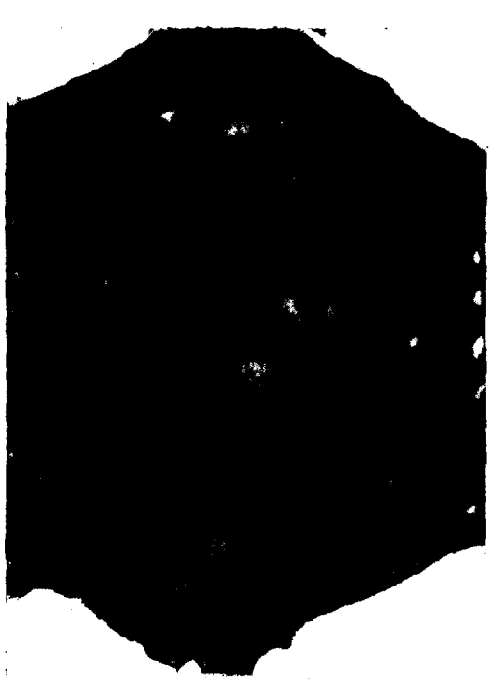
See back page

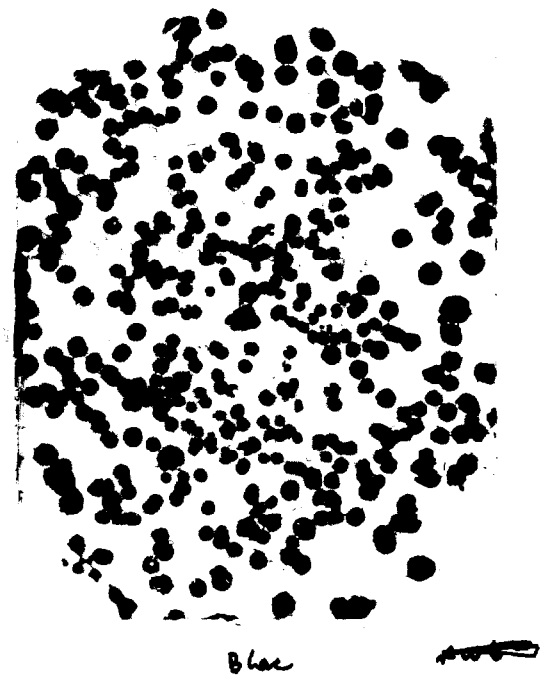
Further work

① Save this.

② Test $F_3$ type $F^-$ or plain $F^-$.

untreated control

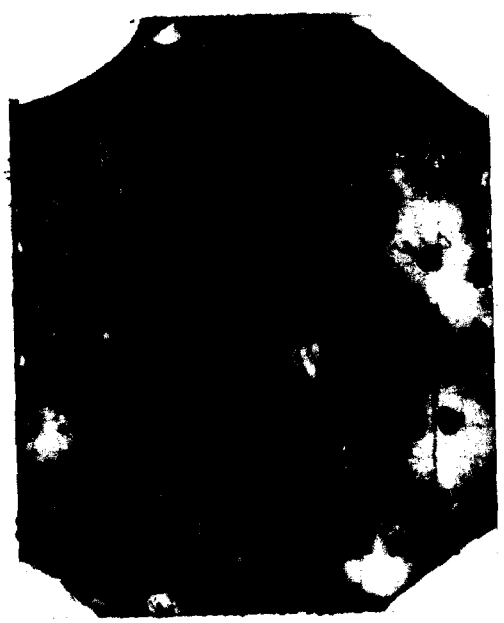


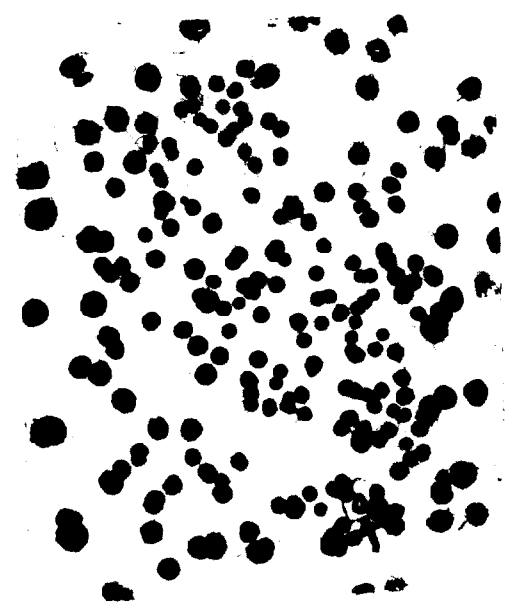


Mlac x W6—

Blac

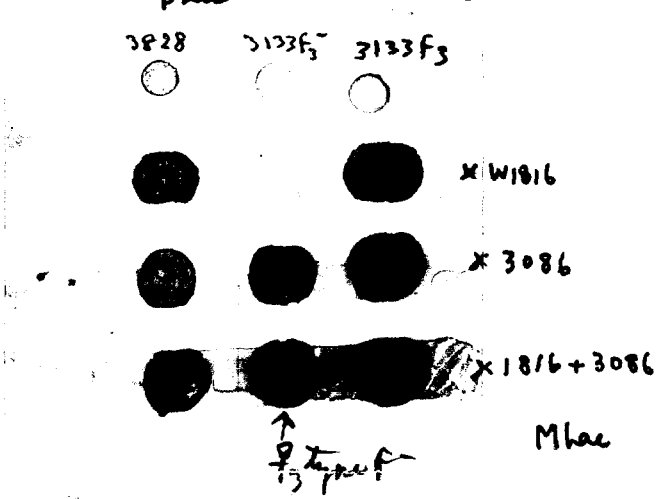
Treated with AO





Mlac x W6—

Blac



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

21/VII 1959 REF:

Priciple: W3747 $\xrightarrow{F_{13}Lac^+}$ W4573

Strain:

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

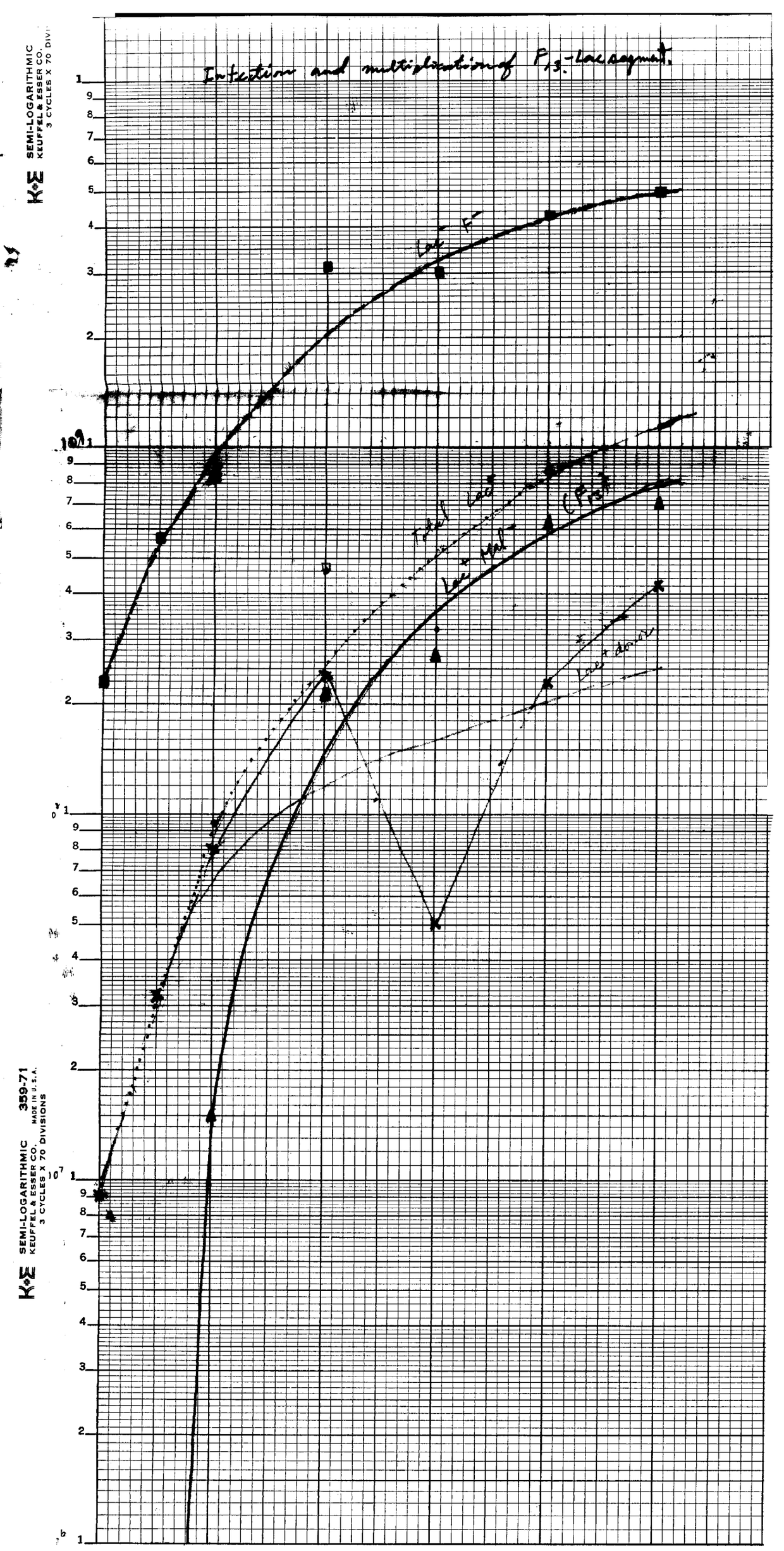
① Mix: Ratio 1.1 ml + 10 ml  Ca $10^8$ cells/ml.

② Shake it 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 then on BXyl, BMtl, BAra, and MGal Em seeded 93:85 on it.

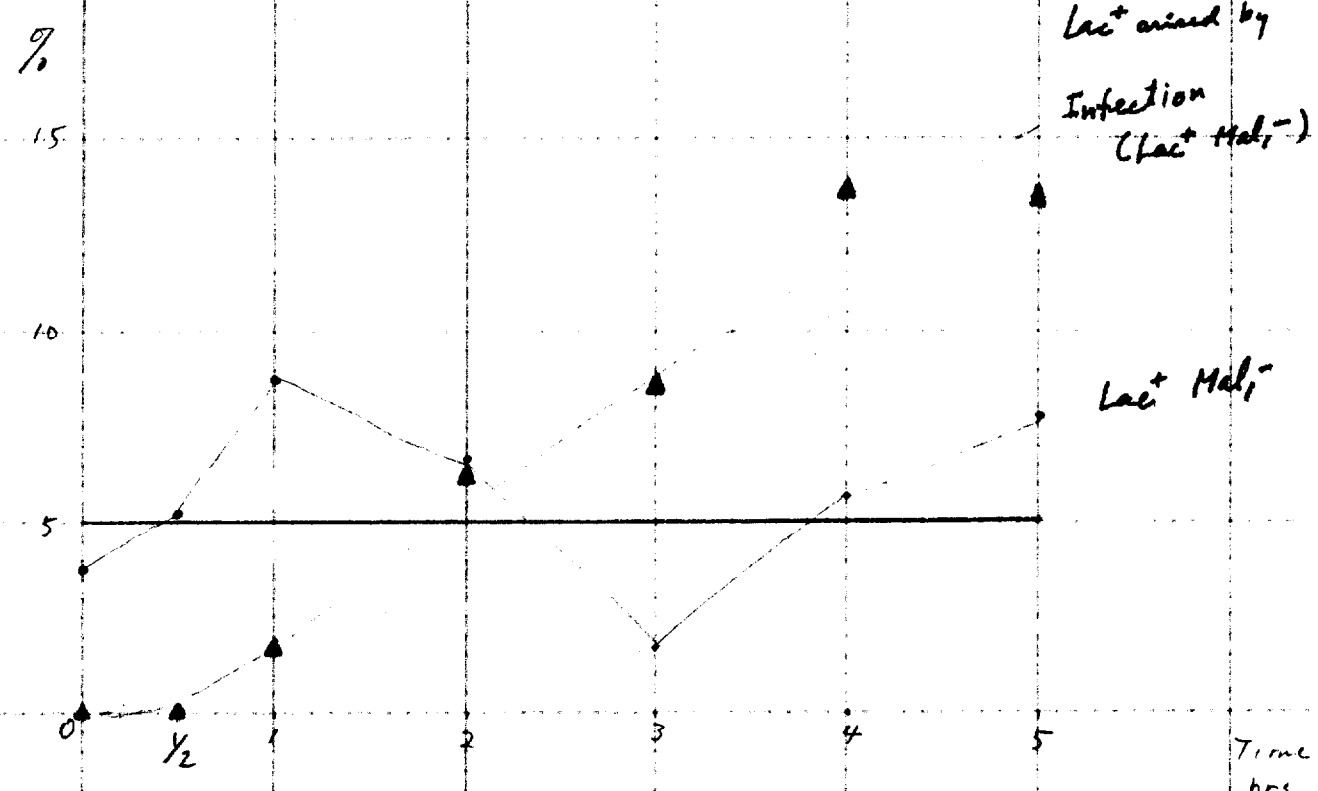
| Time | | 3:05 | 3:35 | 4:05 | 5:05 | 6:05 | 7:05 | 8:05 |
|---|---|---|---|---|---|---|---|---|
| Inoculum size | | $10^{-6}$ ml/plate | $10^{-6}$ ml | $2\times10^{-7}$ | $10^{-7}$ | $10^{-7}$ | $10^{-7}$ | $10^{-7}$ |
| Time | | 0 hr | 1/2 hr | 1 | 2 | 3 | 4 | 5 (hrs.) |
| | | + − | + − | + − | + − | + − | + − | + − |
| 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 | 13 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 | ×5 95 19/166 | ×10 46/336 | 32/301 | 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 | | Δ | Δ | Δ | Δ | Δ | Δ | Δ |
| | 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 2/45 | 0 3/117 | 1, 6/38 | 5 3/71 | 8 1/77 | 8 6/113 | 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 $F_{13}$ lact | | Δ | Δ | Δ 15 ×5 80 | Δ | Δ | Δ | Δ |
| | | 0, 9/230 | 0, 32/565 | 3, 16/166 | 22, 24/336 | 27, 5/301 | 63, 23/432 | 70, 41/482 |
| | | (239) | (597) | (182) | (360) | (301) | (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 more than host cell after 5 hrs. at 37°C on rotator.


Infection and multiplication of $F_{13}$-Lac segment.
Lac⁻ F⁻
Total Lac⁺
Lac⁺ Mal⁻ ($F_{13}$)
Lac⁺ donor
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 $V_6$ $F_{13}$ $Gal_2$ $Lac_{g5}$ $Ara_2$ $Xyl_2$ Mtl $Mal_1$ Sm.



# 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 → 10-1 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 | $1 \times 10^7$ /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 characters.

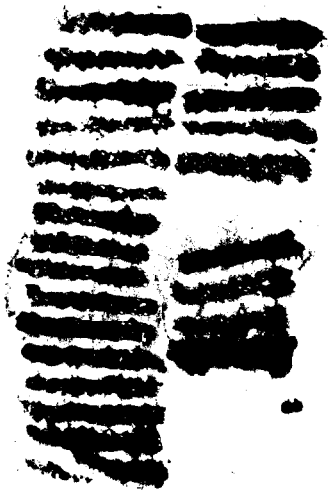
2 $Mal_3^+$ ? 2 $Xyl^+$

2 $Mal_1^-$ 2 $Xyl^-$

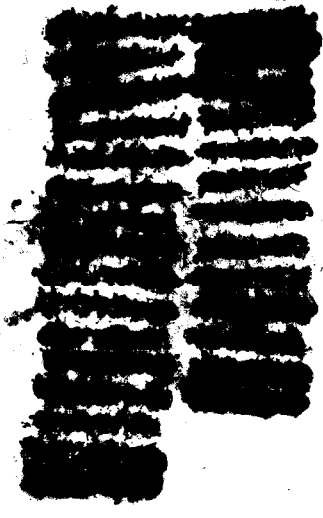
Selective marker : Leu - Meth.

10'

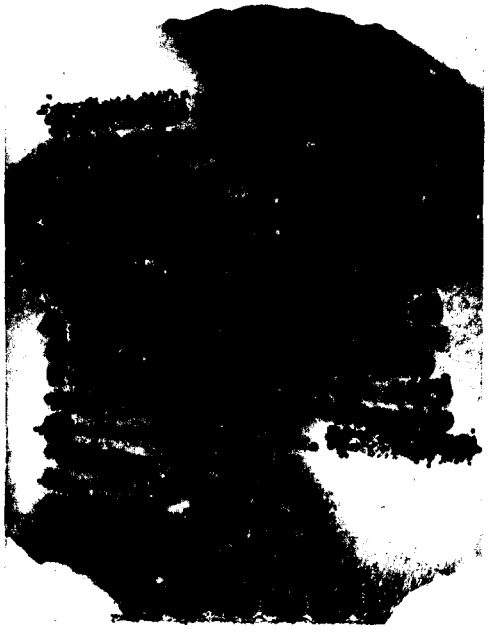
15'



15'



20'



20'

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12~~0~~5a

# Time experiment of Lac – $F_B$ transfer.

25/VII ; 1959 REF: 125b.

- $F_B$ ♂: 3747 ; ♀ 93:85 ; Cultural age : 2hrs on rotator at 37°C
  0.5 ml overnight culture / 10 ml Penassy.
  Penassy grown
  : 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 : 70 ; 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 | 40 |
| | | 0 | 0 | 0 | 0 | 1 | 5 | 41 |
| M Glut Sm. ($10^{-3}$ ml) | Pur. | 0 | 0 | 0 | 1 | 8 | 43 | 154 |
| | | 0 | 0 | 0 | 1 | 9 | 40 | 182 |
| EMB-Lac. Σ ($10^{-6}$ ml) | | 149 | 163 | 124 | 152 | 125 | 177 | 196 |
| | | 141 | 149 | 130 | 113 | 121 | 187 | 180 |

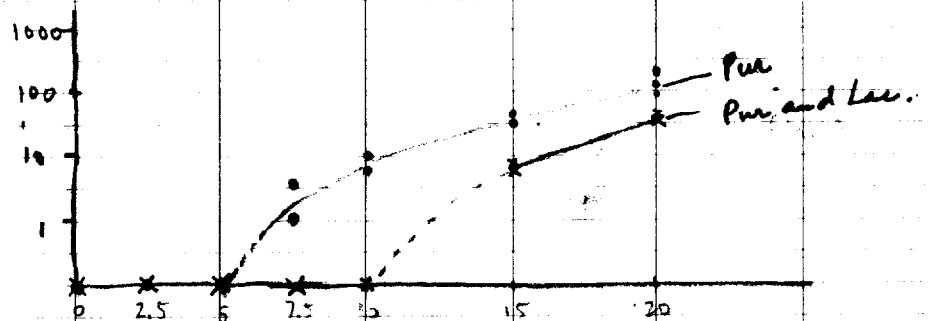
# of recombinants per plate.

Blank (befor blending) ($10^{-6}$ ml) 143, 127, 121

① # of cells used ♂ : Ca. $1.5 \times 10^8$
♀ : Ca. $1 \times 10^7$

② Frequency of $Pur^+$ transfer per ♀ within 20 min.
ca. $10^{-2}$
1 %.

Conclusion : ① Purine looks first, and Lac is next.
② Test Lac, Pur, and Ab. of these recombinants.
I have no marker to test $F_{B_1}$ cilly.



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markers

~~125~~ 126

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

30/VIII ,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.

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

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

Which side of the fragment is it attaching?

Result:

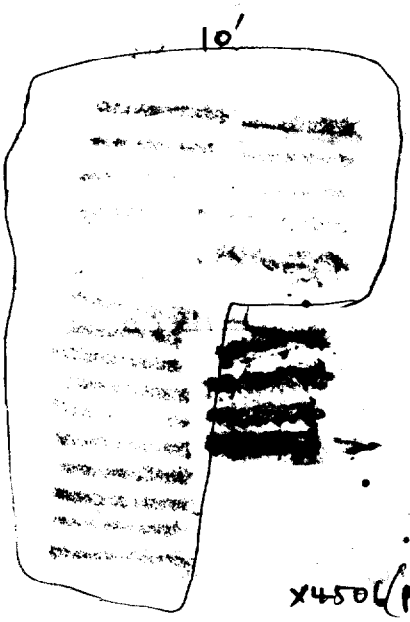
1. purine is first : Pur – Lac – $F_{13}$
2. $F_{13}$ is latter than Lac.

Conclusion:

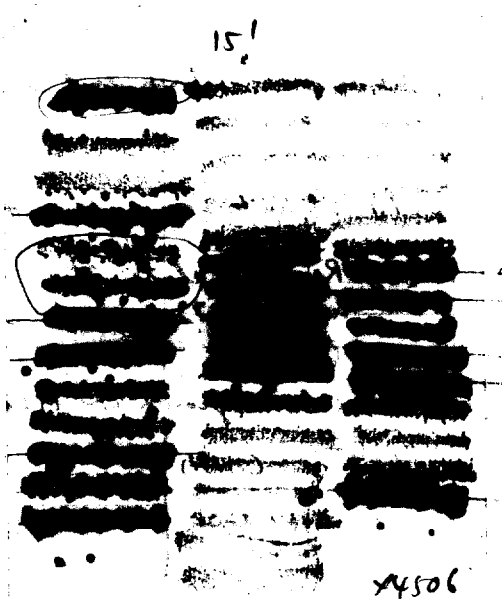
– –

$Pur^+$ $Lac^+$ $F_{13}$

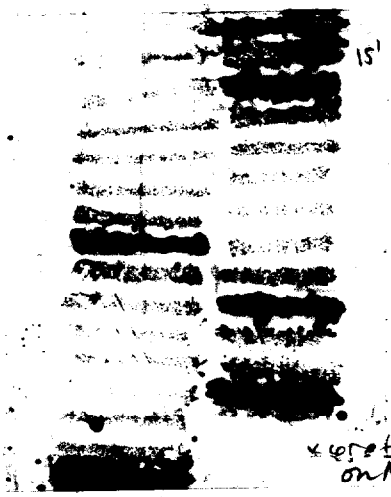
Selected on Lac - Pur transfer




X4506 (pur F⁻)
on Mlac

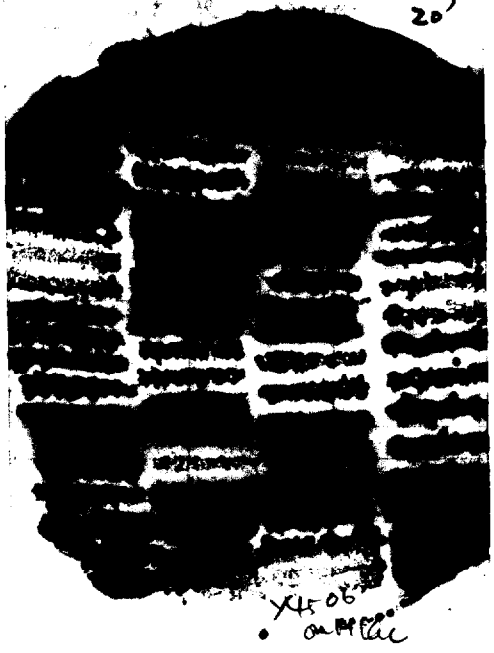



X4506
on Mlac




X4506
on Mlac

20'



X4506
on Mlac