Reisolation of M168 from Zello.

7/8/49

16 cultures tested, streaked on Xyl EMB.
All streaks are Xyl +, − mixture. Occasional ± seen. Pick for propagation on Lac EMB.

Xyl ± recovered. Streak out on MH EMB for material to test Xyl loss.

(320) MH ± colonies touched on Xyl EMB; Lac EMS.

3 Xyl − colonies noted. Grew very poorly on EMS lac, probably segregated, but streak out on MH EMB.

7/17/41

Does buffer activation increase enzyme?

cells harvested from 100 cmc Y2 Lac (36h.) to 5 ml water.

A) 2.5 ml cells + 2.5 ml water

B) " " NaP M/5. Incubate 12 20 PM — 215.

Assay ___ samples. Wash treated cells with water, save supernatants, and dry sediments over P_2O_5.

		Di		
Super .5 ml	A		016 (20 mins.)	No activity is extracted by water. Ca 8-10 fold activation by buffer, and ca. 1/3 activity is extracted!
	B		420 (5 mins.)	
cells .05 ml	A	080	111 271 (20 mins)	
	B	056	490 (5 mins)	

∴ Total sediment of B) should have activity of $4.5 \times \frac{1}{.05} \times \frac{450}{100} \times \frac{20}{5}$

= 1,60,000 u.

and A) should have $4.5 \times \frac{1}{.05} \times \frac{200}{100}$ = 18,000 u

Recover dry cells.

A 7.5 mg.
B. 11.0 mg.

B formed a much more compact pellet than A, of which ca 1/3 was estimated lost in preparing for drying and scraping from slide.

Calculate on predicted basis of 12 mg. cells in each.

Triturate each in 5 ml H_2O to prepare for assay.

Predicted activity, ca. 15000 u/mg for A and 13,000 u/mg for B.

Note / These Lac cells also gave brown purple color ± iodine.

Lysogenicity: conclusions.

Two kinds of phage apparent:

φ_A carried by 7, 13, 15, 18, 24, 32, 34, 36. type: ω1029.

φ_B 17, 19, 23, 31

	φA^+	φB^+	φO^+
A^s	—	—	6, (14), 27, 29,
B^s	7, 15, 18, (24), 32, 34, 36	—	(10), 16, 21
O^s	(13),	(17), 19, 23, 31.	++

Of 40 cultures, 8 carried A, 4 B = 12/40 = 30%

Hence A^s; 7, B^s = 11/40.

The strongest phage action was that of #17.

#24 responded moderately to λ—

11/29/30: Throw out all except 10, 13, 14, 17, 24

Lysogenicity tests on Shapiro's E. coli cultures. 580

7/16/49.

Test by seeding E each strain as indicated, then spotting each strain on surface. Active combinations indicated:

Host			
1			
2	sparse growth		
3	"	"	
4	"	"	
5			
6	#7, 13, 15, 18		
7		17±	19±
8	[illegible]		
9	s.g.		
10		17±	19±.
11	s.g.		
12	s.g.		
13			
14	7, 13, 15, 18		
15	17, 19	++	
16	17, 19		
17			
18	17, 19		
19			
20			

Following groups noted.

6, 14 sensitive to 7, 13, 15, 18

7, 10, 15, 16, 18 sensitive to 17, 19.

Note: 19 → 18 → 14.
6

$(7,15,18)\ P_a^+ P_b^s$

$(6,14)\ P_a^s$

$(17,19)\ P_D^s - P_b$

$(10,16)\ P_a$

Key:

Shapiro			
835	ω 1028	#6	sensitive to ρ7
866	ω 1029	#7	" ρ17, carries ρ7
848	ω 1030	#10	" ;
116	ω 1031	#17	carries ρ17

Should include λ-λ^s; λ-λ^R and λ+ in these tests.

λ tests, continuation

7/19/49.

Check, intense, #21-40.

21	v.sth. 23, 31
22	—
23	—
24	23, 31
25	—
26	—
27	24, 32, 34 (all weak), 36.
28	—
29	24, 32, 34 (v. wk), 36.
30	—
31	—
32	23, 31 v. strong.
33	—
34	23, 31 wk.
35	—
36	23, 31 ++
37	—
38	—
39	—
40	—

21, 24, 32, 34, 36 ← 23, 31

27, 29 ← 24, 32, 34, 36

N.B. 24, 36 → 29 2 or 3 λ's indicated.

↑ ↗

31

Test 21-40, λ, W1029 and W1031 on:

27	24, 32, 34, 36, 1029	
32	no lysis.	λ?? exceedingly weak if at all.
1028	24, 32, 34, 36, 1029	
1029	no lysis.	
1030	no lysis	
518	no lysis	(even λ did not show up).

7/15,17/49.

C) 1. W478 × W1022. 635 Lac+ : 1 / 14 Lac −.
200 Lac+ tested for Lac v. None found.
100 additional tested. Select those that appear weakest Lac+
5 suspicious colonies reincubated. 3 are Lac v.
Picks 1) Lac' Lac v and 2) broad streak to Lac EMS, Lac EMB, Mal EMB.

what are data?

A) (2)(1) W67 × W1022 1 Lac+ / 3000 prototrophs.
Lac v Mal+ Xyl+ Mtl v?

B) 8. Y40/6 × W1022. 10 plates Lac EMS. To determine whether the prototrophs from this cross were diploid. Test 20 Lac+, 12 Lac− as T(o) with T6.
Lac+ : 19 R 1 S
Lac− : 6 S 4 mucoid, unscreenable. 2 too dilute.
Indicates that Lac+ are not Lac+ U_6^R/U_6^S, and therefore that they are haploid.

A)

W1033, 1034 received from Cavalli 7/21/49.
Both are ~~F+~~ Lac+. 1033 is (B)M 1034 is BMB, ? as labelled.
Cross ♀ W677, cf. 58-161
Harvest and concentrate ca 5:1 as usual. Call concentrated cells 1ml = .1, and plate various dilutions of 1033 on EMS Lac or T(0) plate smeared with 677 (= 1ml).

Count: 10^{-6} ml 1033 on EMB Lac gave 580 colonies.
Count = 5.8×10^8.

No prototrophs appeared at 10^{-7} or 10^{-6}.
10^{-5} (on T(0)) gave 1 10^{-4} gave 30.
Talac rate ca $\frac{30 \times 10^4}{580 \times 10^6}$ = ca .05% = 5/10,000.

but 58-161 at this dilution gave 34 colonies !! (mixup?)
on EMS, No colonies were seen at 10^{-4} from 1033 x 677.
10^{-3} gave 18 (5+ 13-)
However streak of 1033 on 677 background on EMS Lac B, gave very much higher yield than did 58-161. The effect must be largely based upon microcolony formation. Efficacy of 58-161 should be checked!

Mfr.

5833 13.

7/24/49

Inoculate 677 and 1033 heavily together in Y2 broth (1:5 ea.) After 24h. streak out on lac EMB and test single +, − colonies on Xyl.

21 Lac− were Xyl−
of 8 Lac+, 6 were Xyl+, 2 were Xyl−.

Rechecked these 2 (583B1, B2) on Lac, Xyl, and mutation.

JUL 25 1949

B1 and B2 are both Lac+ Xyl− Mal− LB1−. Stored as W1048, 1049

Additional tests of same plating:

13 Xyl− :→ 10 Lac−, 2 Lac+, 1 mixed.
36 Xyl− :→ 29 Lac− 5 Lac+, 2 mixed

49 Xyl−	:	39 Lac− 7 Lac+	3 mixed.
5 Xyl+	:	5 Lac+	
14 Lac−	:	14 Xyl−	
24 Lac+	:	18 Xyl+. 5 Xyl−	1 mixed.

There are exceptional Lac+ Xyl− present. 1033 is pure, but 677 needs re-verification. Streak out on lac EMB to check! OK!

7/25.

Cross check plated mixture of supra. Xyl and Mal.

39 Xyl+. 36 Mal+ 3 Mal−. } 1-5
5 Xyl+ 3 + 2 − }
35 Xyl− 34 Mal− 1 Mal+ 6.

6 Xyl-Mal recomb.
79 keto

		Lac	Mal	Xyl	Gal	Mtl	V	Nutr.	W
	1	+	−	+	−?	−	R	L	1060
	2	+	−	+	−?	+	S	MT	1061
	3	−	−	+	−	+	R	TLB$_1$	1062
	4	−	−	+	−	−	S	B$_1$	1063
	5	−	−	+	+	+	R	MTL	1064
	6	−	+	−	−	−	R	TLB$_1$	1065
	677	−	−	−	−	−	R	TLB$_1$	1066
"Hz" =	1033	+	+	+	+	+	S	M	1067

25 Xyl− 1 Mal+
15 Mal+ 1 Xyl−
20 Mal− All Xyl−

~~#7 1066~~
~~#8 1067~~

7	−	+	−	+	−	R	TLB$_1$	1066
8	+	+	−	−	−	R	ML	1067

JUL 23 1949

A	1014 x 1015	T(0)	
B	1014 x ~~88~~	233+ : 110 −	Lac EMS.
C	1033 x 477		

A. Yield quite low, ca 5-10/plate instead of > 100.
Streaked out on EMB Mal. 31− : 7+ No Mal v.
Note: BM Mal − x TLB, Mal+ should be excess of Mal +.
Cf. W1022.

B. 100 tests. Emphasis on streak + prototrophs.
4 distinct, 5 uncertain Lac v. → (7) likely Lac v.
Streaked a) isolated Lac v and b) broad barrels on Lac EMB, Lac EMS, Mal EMB.

52 addnl:

C. 100 tests.
1 distinct Lac v. ~~1,3~~ ?? Lac v. all Lac v.

B)

	Lac EMB	Mal EMB.	
1	v	+	
	v	+	
2	v	+	
	v	+	
3	v	+	
	v	+	
4	v	+	
	v	+	
5	v	+	
	v	+	
6	v	+	
	v	+	
~~7~~			
8	v	+	1 v+ colony?
	v	+	
11	v	+	
	v	+	
12	v	+	
	v	+	
13	v	+	
	v	+	
14	v	+	
	v	+	
15	v	+	
	v	+	
16	v	+	
	v	+	

None Mal v. All Mal+

C)

1	v	
2	v+	
3	v+?	v+?

Crossean double-sugar
Mfr.

JUL 25 1949

(A) (B) (C)
W1048, or W1049 mixed with Y87/6 and inoculated
1:10 into Y2 3PM

~~3PM~~. Streaked out ~~A, C~~ A, B, C on Lac, Mal and ~~Lac Mal~~ Malac

Plate out AC and BC on EMB Malac

Repeat P25.

A, B were pure Lac+ Mal- ; C was Lac- Mal+ All (A, B, C) were pure + on Malac.

BC. 4 plates ca 150 scorable. 1 Malac - colony noted.
AC. 5 plates ca 250 (= 1250) 4 Malac - colonies? [might be a contaminant.] Isolate to analysis.

Repeat:
AC': (2 × 100) No -.
BC' (3 × 150) No -.

These might ~~be~~ not be Mfr.

585a

See 589

24 hr heavy mixed cultures plated on EMB Malac

A	Y87 +	W814	0
B	1056	"	✓
C	1088	"	(✓)
D	1059	"	✓✓

Y87 + W814 pure lac−, lact resp.

4 plates each.

A). 1600 cols. No Malac −

B) 4 Malac − 2 sectorial. 1 sectorial not isolable.

C) 3 − and 2 sect ??

D). 7 − and 6 sectorial, not all isolable

Lac EMB. (2 plates).

D). 2 sectorials

B. 1 " + 2 unisolable

C. 1

A. 0 (4 plate).

JUL 2X 1949

		Origin			
Sh 1		(5)	HC, YE, (A5)	YNA + A6 ±	W1045. Proline
Sh 2		(5)	"		"
Sh 3		(5)	"		
Sh 4	42	K-12	+ all AA		
Sh 5	45	"	± all AA A12, A4++		
Sh 6	46	"	+ in 48hrs. + all AA.		
Sh 7	47	"	± all amino acids. A4, A12 ++		
Sh 8	50	"	like 11		Tyr
Sh 9	51	"	+ or ± all amino acids		Tyr
Sh 10	53	"	± in minimal 36h. do. 11		Tyr. (Φ ±
Sh 11	54	"	24h: HC, Yx, (YNA), A4, (A12) ± others		Tyr.

→ All grow promptly on tyrosine, later adapt.

Sh1-3 were 3 muts/20 tests.

47 additional colonies tested. 6 mutants found: # 4, 22, 26, 35, 40, 43.

Each also showed up very well on minimal agar.

4, 22, 35 were prolineless. T.O.

Random remaining 3:

		W
A:	A12	1050
B:	cystine	1051
C:	histidine	1052

of Sh 4–11.

Summary. 9/67 from treatment and penicillin run on the wild type, W1045, were mutants. Of these 9, 6 were prolineless. Only one was preserved as W1046

The others were W1050–1052, respectively, :

1050
1051
1052

A series of 8 "mutants" were isolated from a K-12 run. All proved to be rather revertible tyrosineless. Keep # Sh 11 as W1053

"Cross" single mutants from W1045. 586

JUL 25 1949

W1050 = A

1051 = B

1052 = C.

Plate separately and in combination on T(0) plates.

Also inoculate into Y2.

At 36 hours, A-C were blank., AB and AC had numerous prototrophs. BC had a few.

A27.(48h.) A revertants noted. Not so numerous as in AB and AC. ?? Reversions or recombinants ??

Check 1050 with various 4 strokes. Cf. 1033. In Vegas.

	T/	2/	3k	4	5	6	7
1050	R	R	R	R	R	R	R.
1033	S	S	R	S	S	S	S

↑
N.G.

Need double mutants for conclusive result

New penicillin mixes gave low yields of double mutants

#1 from 1050 = W1069
lys + methionine tryptophane

#3 from 1051 W1070

Cystine. Gave growth responses to A12 also on Vits.

16 hrs: +++ on methionine or lysine

Also give smaller responses on:

Vitamin mix
B_1
Pant
Inositol
K
B_{12}

} Probably reversions or tube contamination

24 hours — growth on T10). Just a "slow" mutant.

Hfr : Lac- mutation um.

Crosses of Lac- mutants.

7/24/49

Irradiate 1033 on 18 LacEMBplates. Survival high (Ny^R!)
6 mutants recovered. Ca 6000 colonies.

	Lac	Mal	Glu	Gal	
1	−	−	−	÷	W1054
2	−	+	+	+	1055 – 1059.
3	−	+	+	+	
4	−	+	+	+	
5	−	+	+	+	
6	−	+	+	+	"5 – 9"

Cross 5,7,8,9 c̄ W677 for Lac allelism, on EMBLac and in Y2, then to EMBLac.

5 x	All Lac −	None very suitable as recombination markers.
6 x	2-3% Lac+	
7 x	All Lac −	
8 x	2% Lac+	
9 x	All Lac −	
Parents	All −	

EMBLac. (Mixtures plated out after 24 h.)

5:	0/400 +.
6:	0/500
7:	0 /50
8	0 /800
9:	0 /25.

Lac, x Lac$_x$ recombination unfeasible.

Comparison of 1033 & 58-161 HSt.

7/27/49.

As supra. 677 background, on T(B,).

1 ml ≐ 10^9 cells.

Dilution	58-161	1033
0	>1000	
3	11	>1000
4	2	200
5	4	20+
6		4

[Prototype J.

3/21/50: Conclusion is ca 100-fold augmentation in HSt.

Analysis of 585a.

7/29/49

Streak out sectorial colonies on EMB Lac. (see 585a)

1. D Lac
2. D Lac
3. B Lac
4. C Lac
5. D Malac
6. B Malac
7. C ? Malac
8. C? Malac x
9. D Malac
10. D Malac
11. D Malac
12. D? Malac
13. D Malac

Also, 4- from B, 4- from C and 6- from D

		Lac	Mal	Sal	Xyl	Mtl	V_1
B	1	—	—	+	—	—	R
	2	—	—	+	—	—	R
	3	—	—	+	—	—	S
	4	—	—	+	—	—	R
C	1	—	—	—	—	—	S
	2	—	—	—	—	—	R
	3	—	—	—	—	—	R
	4	—	—	—	—	—	R
D	1	—	—	—	—	—	R
	2	—	—	—	—	—	S
	3	—	—	—	—	—	R
	4	—	—	—	—	—	S
	5	—	—	—	—	—	R
	6	—	—	—	—	—	S
Parents:		+	—	—	—	—	R
		—	+	+	+	+	S

10- from 589a D.

3 Lac - Mal - Sal - Xyl - Mtl - V_1^R #3,4,5

6 " " V_1^S

1 Lac - Mal - Sal - Xyl + Mtl - V_1^S #9

A-D plated at 12h to EMB Lac. Pick +, − to thin sugars for random test.

A). 36 Lac+ : 34 Mal-Xyl- " 2 Mal+Xyl+" Recheck!
Both were Lac-
1 Lac- 1 "

40 Lac- : 40 Mal+Xyl+

B). ~~10~~ Lac- : 10 Mal+Xyl+
~~40~~ Lac+ : 40 Mal-Xyl-

D) 40 Lac+ : Mal-Xyl-
20 Lac+ : Mal-Xyl-

60

16 Lac- : Mal+Xyl+

C) 36 Lac+ Mal-Xyl-
20 Lac- Mal+Xyl+

∴ no "recombinants" except in A. 12 hours too short?
Recheck:

589 Analyses.

	Tests.								
1. Lac−	13	All V_1^S :	5 Mal−Xyl−	8 Mal+ Xyl+					
			1, ~~4, 5, 6, 7,~~						
			9, ~~10, 12~~						
			2, 3, 8, 11, 13						
Lac+	22	All V_1^R	All Xyl− Mal−					!! No Recom. Mal+	
2. Lac−	16	V_1^S ± 25 V_1^R	All Xyl− Mal−						
Lac+	14	All V_1^R	All Xyl− Mal−					No Mal+!	
3. #10 artifact									
Lac+	14	V_1^R	Mal+ − Xyl−					(No) recombination here.	
Lac−	6	V_1^S	Mal+ Xyl+						
	29	V_1^R	Mal−	Xyl−	} 37				
4. Lac+	8	V_1^R	Mal−	Xyl−				No Mal+	
Lac−	4	V_1^R	Mal−	Xyl−					
5. Lac+	8	V_1^S	Mal−	Xyl−			2 Recombinants		
Lac−	2	V_1^R	Mal−	Xyl−					

6.

Lac+	13.	V_1^R	Mal-	Xyl-
Lac-	8	V_1^S	Mal-	Xyl-

8.

Lac+	25	V_1^R	Mal-	Xyl-
Lac-	11 indistinct		Mal+	Xyl+?

No - colonies on Malac! - Not segregating

9.

Lac-	10	V_1^R	Mal-	Xyl-
Lac+	11	V_1^R	Mal-	Xyl-

10.

Lac-	12	V_1^R	Mal-	Xyl-
Lac+	23	V_1^R	Mal-	Xyl-

Summary: Parents are [Lac, Mal, V_1]: + - R / - + S

1	- - S	- + S	+ - R	Selected as Lac v.
2	- - S - - R		+ - R	
3		- + S	+ - R	
4	- - R		+ - R	
5	+ - S - - R			
6	- - S		+ - R	
9	- - R		+ - R	
10	- - R		+ - R	

7/30/49

Replate 589D, after 24 hrs. additional at room temperature, on EMB Lac and Malac.

10 Lac plates Lac+ > Lac−. ca 500 colonies.

2 ? Lac ±

20 Malac plates. ca 1000 colonies.

10 Malac −
2 Malac v (+/−)
2 ? Malac v (may be +/±)

14 1.4%

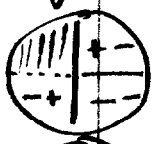
14 D Lac ? Pure Lac+ ! Mucoid margins.
15 D Lac + Test +, − on Mal. Pure Mal−! 2 L−M− 11 L−M+ ; 10 L+M−
16 D Malac
17 D Malac
18 D Malac? (+, −)
19 D Malac? (+, −).

(may be +, −, ±..)

[4 o Lac+ tested. All Mal−].

Lac segregation seems to be a good criterion of recombination.

17. 5 Lac+ : Mal−
35 Lac− : 22 Mal+ ; 17 Mal−

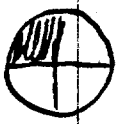


No Lac+ Mal+ !

18. 20 Lac+ Mal−
20 Mal+ Lac−

Looks for recombination of other factors — all parental types + U, R

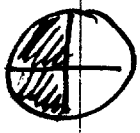
19. 30 Lac+ Mal−
3 Lac− Mal−
7 Lac− Mal+



14. 8 Lac− : 3 Mal−
5 Mal+

33 Lac+ : all Mal−.

16: 4 o Lac− : Mal−
4 o Lac+ : Mal−



7/29/49

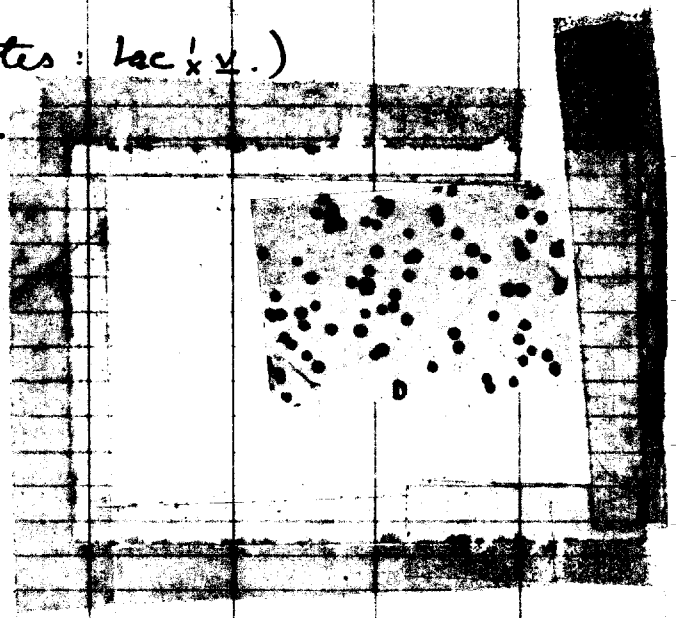
W1034 x 677

100 lac+ tested for lac−. All lac+

Many prototrophs were sectored on EMS Lac!

January 31, 1949.

A) W1058 + 477 (for persistent heterozygotes: lac + x v.)
B) W1058 + 814 24hmi. 72h. Roomtemp.
C) W1056 + " " " "
D) W1059 + " " " "
E) W1022 + 1033 (Reverse symmetry)
8/1 F) 487 + 814. [like B-D + 24h.]



A: W10x200. No lac+ noted.
B-D: high proportion of lac- Mal-
Select segregating colonies from lac EMB:
But frequency of Mal-lac- makes it difficult to decide what parents are!

F. 8/2. Ca 300 colonies: No Malac-. Antac, +, - seen =
E' p. 8/2 1 lac v colony. Also Xgl, Mal v. Cross-feat.

G. 8/2. 1033 + 595 24h.
E" p2. 8x100. lac EMB. Almost all +. No v noted.
B: ca 200/plate 10 lac: - record. Contamination unavoidable
Several weak + colonies noted (?) may be Mal- lac+. 1-6 lac v.

2 Xgl	1 v	#7
2 Mal	1 v ?	#8
5 Mal	2 v ?	9-10
1 Xgl Mal	1 v ⊕	11

E: ~~75~~ 300 lac+ Mal+ 18 lac- Mal-. No recombinations! (Do 1022 anti Hfr?)

August 4-6, 1949

89. (W1033+ W595).

selection

	Lac	Mal	Gal	Xyl	Mtl	T1 (Lac)		
1	+−	++	++	++	+	SR	4L+ : MG+	NoL−!
2	+−	+−	++	++	+	SR	8L+ : 6M+G+ (2MG−)	
3	+−	+−	++	++	+	SR	10L+ : MG+	8L− : MG−
4	+−	+−	++	++	+	SR	10L+ : M+G+	10L− : M−G− (1) M±
5	+−	+−	++	++	+	SR	10 "	7 "
6	+−	+−	++	++	+	+S−R	10L+ → M+G+	6L− → M−G−
7	+−	++M	−+	−+	+(−)			
8	−	++	−	−	−	−R	10M+ → L−	10M− → L−
9	+−	+−	−+	−+	+(−)	SR	10L+ : M+G+	10L− : M−G−
10	+−	+−	−+	−+	"	SR	10L+ : M+G+	10L− : 6M−G−; 5M+G−
11	+−	= +	−+	−+	"	SR.		

Lac
Xyl v
Mal v
Gal v
Xyl Mal

#1 → 10 Xyl+ → LMG+ 10 Xyl− → 9 LMG− 1 LG−M±?

#8 is Mal v all else −R!

#7 → 10 Xyl+ → LMG+ 10 Xyl− → 10 L+G−M− sic?

Summary:

#1 was "pure" Lac+M+G+ (parental). − not 32 complete but were there!

#3,5,6,9,11 parentals only (re Lac Mal Gal)

2: Lac+M+G+ (parental) and (Lac+ M−G−) (recomb.)

4: Both parents and (?) L−M+G−?

7: L+M+G+ X+ (par.) and (L+G−M−X−) (recom.)

8: LMGX− (par) and (M+LGX−)

10 LMG+ (par) ; LMG− (par). and (M+L−G−)

~~11. LMGX+ (par) and LMGX− (par.)~~

Only 4 likely recombinants. Exhaustively check all of these. (2,7,8,10)

8/7/49

Maltemated!

#7. 10 Lac+ (Mal−) Xyl+ Gal+ Mtl+ V_1^S] M Complementary? Recombinants
10 Lac− Mal+ Xyl− Gal− Mtl− V_1^R] TLB₁

Mal scoring questionable. Also some inconsistency. Colonies which originally scored (Xyl+) Lac+ and (Xyl−)(Lac+) → Lac−! Recheck! and mutation

#10 (A) 10 Lac+ Gal+ Xyl+ Mtl+ (Mal−?) V_1^S Recomb?
(B) 5 Lac− Gal− Xyl− Mtl− Mal+ V_1^R ~~Recomb.~~ Recomb.
(C) 5 Lac− Gal− Xyl− Mtl− (Mal−) V_1^R Parental

These appear quite difficulty. a may be Mal+. Is there an interaction between Lac or other factors on Mal expression?

#8 10 Mal+ Lac Gal Xyl Mtl− V_1^R TLB₁ Recom.
10 Mal− Lac Gal Xyl Mtl− V_1^R TLB₁ Par.

#2. 6 Lac+ Mal+(?) Xyl+ Mtl+ Gal+ V_1^S
2 Lac+ Mal− Xyl− Mtl− Gal− V_1^S

Mal+ was mucoid when examined.

8/10 Confirmations: 1) Lac+ is weak Mal+
Lac− is strong Mal+] Both Mal+!
2) Gal+ is Mal+ (slow)
Gal− " −

10) A Mal slow
B Mal+
C Mal−

Interpretation of Mal slow??

P4. Replate 6.

20 Lac. ca 150/ Lac- reisol. 2 v (difficult to ascertain)

15 Mal 3 v Many not isolable or certain

5 Xyl 4 v Clear cut plates.

5 Gal No v

Note —: + served higher on Xyl and Lac than on Mal! (i.e., exc Mal+ Lac-?)

	Selection	Lac	T1	Mal	Xyl	Gal	steaks
1	Lac	+S	-R	+-	+	+	Lac+ -
2	Lac	+S	-R	"	+	+	Lac+(-)
3	Mal	+S	-R	"	—	—	Mal+ —
4	Mal	+S	-R	"	+-	+	Mal+ —
5	Mal	+S	-R	"	"	"	Mal+ -
6	Xyl	+S	-R	"	"	"	Xyl+ -
7	Xyl	+S	-R	"	"	"	Xyl+ -
8	Xyl	+S	-R	"	"	"	Xyl+ -
11 5926X	Xyl	—S		"	+	"	Xyl+, — All Lac-S; 3 Xyl-Mal+; 7 Xyl+Mal+
12 5926M1		—S		"	+	"	Mal++ Not recomb. (T.O.)
13 5926M2		++SR		"	+	"	Mal+ -

Steaks on selection sugar. Brushall on Lac, Mal, Gal, Xyl, T1

Patent Recomb: #3: Mal+- : Lac-Xyl-Gal- (R?)

Lac↓

11 (592B Xyl±)		Xyl+	Lac-	Mal+ V_1^S	Parental
		Xyl-	Lac-	Mal+ V_1^S	Recomb.
13 Mal±	10	Mal+	Lac-	V_1^S Xyl+	ζ Parentals only.
	10	Mal-	Lac+	V_1^R Xyl-	

P4. Plate again from "B" on various: ca 100/plate

20 Lac	16 v.	[2BL]	
15 Mal (joint selection on EMB)	2 v	MI-2	} see 591b for analysis
5 Xyl	1 v	X	
5 Malac	4 –	No v.	

of 1-16, #4,7,8,11 were pure Mal– but Lac+,–
Analyze others for recombinants. (4?)

a) Verify 4,7,8,11 as having Lac+ Mal– and Lac– Mal–

b) Sample ca 10 each side

1	Lac+ Mal– and	Lac– Mal+
2	"	"
3	"	"
5	"	"
6	"	"
9	"	"
10	"	"
12	"	"
13	"	"
14	"	"
15	"	"
16	"	"

} Parentals only. ie Lac; Mal

c) Reanalyze 4,7,8,11.

4	Lac+	Mal –	Sal + ↓	Xyl –	MR –	V,	R	TLB,	
	Lac–	–	–	–	–		R	TLB,	Rec
7	Lac+	–	+	–	–		R	TLB,	
	Lac–	–	–	–	–		R	TLB,	Rec
8	Lac+	–	+	–	–		R	TLB,	
	Lac–	–	–	–	–		R	TLB,	Rec
11	Lac+	–	+	–	–		S	TLB,	Rec
	Lac–	–	–	–	–		R	TLB, v	Rec
Parents	+	–	(–)	–	–		R	TLB,	
	–	+	(+)	+	+		S	M	

Include Sal chart!

August 3, 1949

A) Mix 4 ml ea. 24h. grown cultures of 814 and 1059
B) Mix 1 ml ea. in 10 ml YZ.

Plate after 12h.

A) Malac 2×50. No –
Lac ca 150. No Lac v.

B). Malac 1×100 No –
Lac 2×100. several Lac v.

Streak out and brush on Lac, Mal, Xyl etc.

	Lac	Mal	Xyl	Mtl	T1. (Mal)	
1	+ –	–	–	–	R	Lac-Mal+ 27; 16 + –
2	+ –	– +	– +	– +	+S – R	All Mal –
3	+ –	–	–	–	R	
4	+ –	–	–	–	R	
5	+, –	– +	– +	– +	+S – R	10 L-M+ 25 L+M–
6	+, –	– +	– +	– +	+S – R	15 L-M+ 13 L+M–

∴ 1, 3, 4 are pure Mal-Xyl-Mtl-Lac V₁ R.
2, 5, 6 are mixed, but show parental combinations only (No Mal+Lac+)

P2. Plate B after 36 hours for further segregation material.
14 Lac EMB. 3 Mal EMB 2 Mal Xyl EMB. Ca 150 ea. plate
3 Mal v. Only 2 isolable (7, 8) No Mal Xyl v
On Lac v, some were distinctive v, others were 9, 10 ⊙, and some were either v or conjunctions.

11–19 were distinctive Lac v. 20–24 simply appeared + and – c̄ a common radius. #12 may have a fortuitous Lac –; #13 a Lac +

Analyses of 7-24.

August 4-6, 1949

streak

	Lac	Mal	Ryl	Mtl	T1		
Mal						(MR)	
+ −							
+ −							
7	= +	+(−)	++	+(−)	+S −R	10L+ : M+ X+ ;	2L+ M−X−
8	= +	"	+	"	"	10L− "	8L+ M− X−
9	+ −	"	+(−)	"	"	10L− M+	10L+ M−
10	+ −	"	"	"	"	"	"
11	++ −	"	"	"	"	L−M+	L+M−
12	+ −	"	"	"	"	L−M+	L+M−
13	++, −	"	"	"	"	L−M+	L+M−
14	+ −	"	"	"	"	L−M+	L+M−
15	+ −	"	"	"	"	L−M+	L+M−
16	+ −	"	"	"	"	L−M+	L+M−
17	+ −	"	"	"	"	L−M+ ;	L+M−
18	++ too mild	"	"	"	"	10L+M−	6L−M+
19	+ −	"	"	"	"	L−M+	L+M−
20	+ −	"	"	"	"	L−M+	L+M−
21	+ −	"	"	"	"	"	"
22	+ −	"	"	"	"	"	"
23	+ −	"	"	"	"	L−M+	L+M−
24	+ −	"	"	"	"	"	"

None of these (7-22) were recombinations of Lac with Mal!

X-	Lac+Mal-	Lac-Mal-X+
3A	W814	+ W1062
3B	"	W1063
3C	"	W1064

Plate on EMB Lac and Xyl. 24h. 37°
B, C had one Lac v each. (ca 5 x 100 cols)

B' had several Lac v colonies. One of these streaked out and cross-tested Lac ... Xyl. All parentals: B1: 14 Lac-Xyl+
12 Lac+Xyl-
C1: 20; 23 resp.

A-C' set plated after 48 hours.

1 from Lac EMB 2,3 4 ⊙ from MalXyl EMB.

3A-C. Ca 300 cols. ca. No Lac-Xyl-. Probably not Hfr!
unless Xyl+ is favored in these combinations.
1 Lac v seen on 3A Streak and analyse as 593-5.

no recombination

593:1-5. Cross test.

	Lac	Xyl
1	+ -	+ -
2	++ -	+ -
3	++ -	+ -
4	++ -	=, +

All Lac- were Xyl+
Lac+ were Xyl-

No Recombination apparent

W1062-1064 apparently not Hfr

Conditions of Hfr recomb.

August 4, 1949

GPM. Mix overheavily 814 + 1059 (20X)

A) In water (saline)

B) in = vol. T(0)

C) in = vol Y2.

Final suspensions ca 5X.

A6: dilute and plate out on Malac and Lac EMB (36 hours). (3 plates ea.)

A. Malac: ca 100 cols. total No –
Lac +, – = No u.

B: No colonies

C: Numerous Malac – ! 15 – / 40 +
Numerous Lac u. ca 4/50

∴ Recombination occurs in heavy Y2 suspensions but not in saline

Cross of Ψ1045 derivs.

August 8, 1949.

A = 1069 (LyMeth + tryp)
B = 1077 (hist + iso-val)
C = 1078 (cyst + isoval)
D = 1081 (cyst + Lymeth)
K = 1033 (BM Htr)

Mor. separately P7, and also AB, AC, BD, and BK

	~~24h.~~ 48h.
A	—
B	—
C	—
D	—
K	—
AB	3-4 diffuse colonies.*
AC	—
CD	—
BK	—
A + B	—
A + C	—
A + K	—
B + K	—
B + D	—
C + K	—
D + K	—

Pick + streak on T(0)

Hfr crosses

~~W1073 x~~

W1073 x W909

August 8, 1949

W1073 W909

BM Hfr Lac-Mal- x Y10 Gal- plated after 36 hrs. in Y2 Lac

ca 100/plate

16 Lac ~~[illegible]~~ possible Lac v. 1-19 fairly distinctive. 20-32 ①

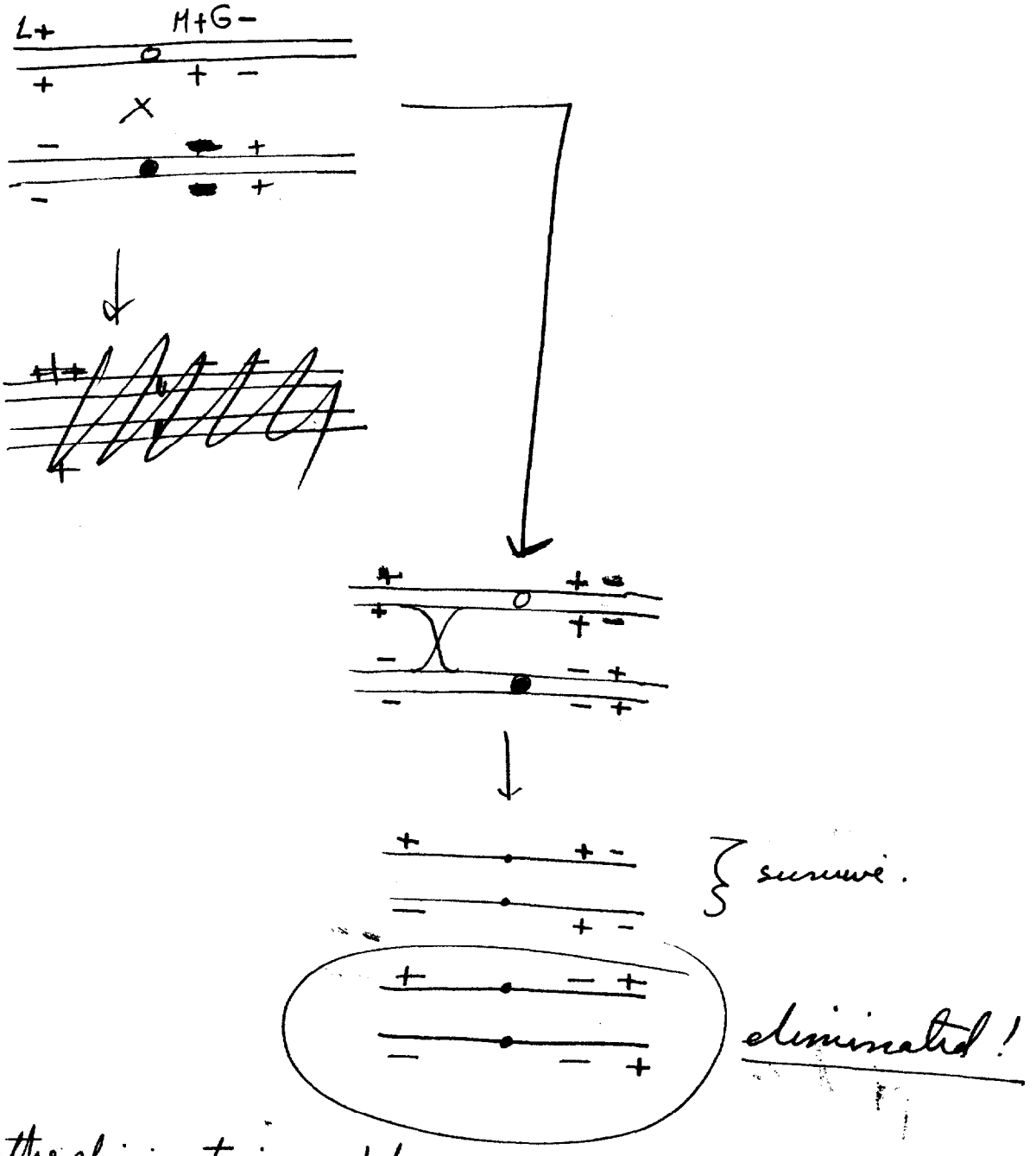
ca 10 tests each ($L^{10}-$, $L^{10}+$)

5 Mal 2 Mal v? 33-34

5 Gal 1?? Gal v. 35 | Reincubate.

	Lac	Mal (brush)	Gal				
1	All L+	+	−	L+M+G− ;			
2			−	−+− ;	++−		
3			−	−+− ;	++−		
4			−	−+−	++−		
5			−	−+−	++−		
6			−	−+−	++−		
7			−	−+−	++−		
8			−	−+−	++−		
9			−	−+−	++−		
10			−	−+−	++−		
11			−	−+−	++−		
12			−	−+−	++−		
13			−	−+−	++−		
14			−	−+−	++−		
15			−	−+−	++−		
16			−	−+−	++−		
17			−	−+−	++−		
18			−	−+−	++−		
19			+−	−−+	++−	*	
20			−				
21	only + are papillae	−+	+	−+−	(++−)		−−+?
22		+	−	[illegible]	[illegible]	++−	−+−
23			−	−+−	++−		
24			−	−+−	++−		
25			−	"	"		
26			−	"	"		
27			−	"	"		
28			−	"	"		
29			−	"	"		
30			−	"	"		
31			−	"	"		
32			−	"	"		
33				++−	sl +−−		
34			+?−	−+−	++−	−−+	
35			+?−				

P9: ca 5% Galac−, or Galac v



But the elimination must be due to deficiency, as deficient chromosomes can be kept in heterozygotes!

596B
D.G.

8/17/49

Recheck of results of 596. One + and one − picked from each of 1-34 and rechecked on lac, mal, gal.

	LMG	LMG	
1	~~+ + −~~	+ + −	(no 1 −)
2	− + −	+ + −	
3	− + −	+ + −	
4	− + −	+ + −	
5	− + −	+ + −	
6	− + −	+ + −	
7	− + −	+ + −	
8	− + −	+ + −	
9	− + −	+ + −	
10	− + −	+ + −	
11	− + −	+ + −	
12	− + −	+ + −	
13	− + −	+ + −	
14	− + −	+ + −	
15	− + −	+ + −	
16	− + −	+ + −	
17	− + −	+ + −	
18	− + −	+ + −	
19	− − +	+ + −	
20	− + −	+ + −	
21	− − +	~~+ + −~~	+ + − (no 21 +)
22	− + −	+ + −	
23	− + −	+ + −	
24	− + −	+ + −	
25	− + −	+ + −	
26	− + −	+ + −	
27	− + −	+ + −	
28	− + −	+ + −	
29	− + −	+ + −	
30	− + −	+ + −	
31	− + −	+ + −	
32	− + −	+ + −	
33	− − −	+ + −	
34	− − +	+ + −	− + −
W909	+ + −		Vector characteristic of W909 weak.
W1073	− − +		

W1084 x 1059.

August 9, 1949.

Plate 24h. cultures on Lac, Mal, Malac. (M-L+ x M+L-).

ca150/

N10:

6 Malac : No – found.

8 Lac : No v found.

8 Mal : 2 v, unisolable.

Replate 3P10.

ca200/

5 Malac No –

10 Lac

10 Mal

Replate 1A14:

No Malac –

No Lac v

Replate from 8/14 on 8/20: (Malac)

DG
598a

August 15ff. 1942

Mutant yields not recorded.

W		Sh 3		
1097	A.	histidine		
1098	C.	"		
1104	D.	aromatic?	mutant!	Any 2 of (tyro; Val; esp.) tryp
1102	E.	threonine		
	F.			
~~1099~~	G.	histidine		
1101	H			
	K	Valine		
1105	L	Thramine or	any pair from	Arg Lys Meth Cyst.
1100	M	histidine		

Arg+Lys	Cyst+M.	1K	A 2K	L	C	M

		Sh 4	
1106	B	histidine	
1107	D	histidine	
1109	E	histidine	Val 200
1108	G	histidine	Val 200.
1110	K		

8/23/49

Nutritional tests of 596 B

	+				+			
	M	T	L	B$_1$	+	M	T	L	B$_1$	+
1										
2	+	−	−	−	+	+	−	−	−	+
3	+	−	−	−	+	+	−	−	−	+
4	−	−	+	−	+	−	−	−	−	±
5	+	−	+	−	+	−	−	−	−	+
6	+	+	+	−	+	+	−	−	−	+

Hfr in and outcross

598 b

August 20, 1949.

9P20. Moc heavily in Penmassay:

D. ~~[illegible]~~ W 1084 (L+M−) + W 1059 (L−M+) (Hfr × Hfr)

E. W 1084 + ~~[illegible]~~ W 842 (Hfr × +)

1) plated P22. (ca 40 hours).

D Malac 10 plates 100/ No − (all on ~~one~~ two plates!)

E 30/ No −.

2) Plated P23. Yield very low 0-2-16/plate

No −.

Plated P24 10^{-6} ml 5 plates Malac each.

3 D No Malac − // 400 cols.

E 1? or No Malac − / 600 cols. (ratio Lac − : Mal − ??)

August 16 ff. 1949

W1073 ×	Mal: +	Mal: −	Plates (sample)	Lac: +	Lac: −	
A. W1033	29	24	0	15	25	1
B. 58-161	28	61	4	45	77	9
C. K12.	22	26	1	12	27	3

Incubated together 48 hours in ~~+2 of~~ Prunassay. Plated on 10 plates each of Lac and Mal EMB. Possible sectored colonies streaked out on homologous medium.

[sectored colonies in 10 plates in table above ↑]. Ca 10 tests each −, +.

A: Lac v. L+M+; L−M−.

B. 9 Lac v All: L+M+; L−M− 4 Mal v. 4: L+M+ L−M−

C. 3 Lac v All L+M+ L−M− 1 Mal v ~~#~~ L+M+ L−M−; L+M− L−M−

No Recombination!

Plate 122. 10 Lac each. Cultures are badly changed.

A). Ca 25 ea. + > − 3 Lac v

B) Ca 2 ea.

C) Ca 150 ea. 5 Lac v.

10 colonies on EMS: all +.

#3 all Lac+Mal+

Plated P23. Yields v. low
10 Lac, 10^{-5} Mal ea.

2. A). ca 20/. 3 Lac v. High variance of +/− points to clumping but Lac+ >> −

B) ca 5/
No v.

C) ca 2/

Colony yield too low for any conclusions

---

3 Plate P24 ca 10^{-6} ml./plate.
10 Lac 10 Mal ea. ca 200/. for A, B ca 150/ C.

A 3 Lac v 4 Mal v

B 3 Lac v 11 Mal v

C 3 Lac v

All parentals only

Mtr crosses.

August 25, 1949.

In NB mix hourly as follows: Plate 10^{-7} ml: 8/27.

A	1033 × 677	Lac 15	Mal 10	
B	1059 × 814	Lac 10	Malac 5	
C	1059 × 1084	Lac 10	Malac 15	~~Malac~~ Mal 10
D	1073 × 1033	Lac 15	~~Malac 1~~	
E	1073 × 8161	Lac 15		
F	1073 × ~~0~~ K12	Lac 15	EMS Lac 5 (2 drops)	

80

A ca 80/ Lac+ >>> − (20:1)

B ca 30/ No Malac −

C ca 20-50/ No Malac − Mal − = Lac −

D ~~Ca 200~~/ Lac+ >> Lac −

E ~~Ca 150~~/

F Ca 100/

A. 1 Mal v 0 Lac v / 800; 1200 resp. [Note reversal of + > −].

B. 4 Lac v / 500. 0 Malac / 150

C. 2 Lac v / 400 0 Malac − / 600.

D 3 Lac v ? / 2000

E. 8 Lac v / 2200

F. 2 Lac v / 1500 EMS: No −

		L+	L-
D	3.	4 M+	10 L-?
	1	10 M+	
	2		
E	1	10 M+	10 M-
	2	7 M+	5 M-
	3	10 M+	10 M-
	4	10 M+	10 M-
	5	10 M+	10 M-
	6	9 M+	6 M-
	7	5 M+	10 M-
	8	10 M+	10 M-
F	1	7 M+	10 M-
	2	10 M+	5 M-

No Recombination