October 24, 1950.

A. 58-161 x W~~1177~~ 677 on EMS Lac.

Pick -, + and streak out on EMB Lac (for use in backcrosses)

	Lac	Mal	Xyl	Mtl	Gal
1	−	−	−	−	
2	−	+	−	−	?
3	−	+	−	−	
4	−	+	−	−	
5	−	−	−	−	
6	−	−	−	−	mucoid
7	−	−	−	−	
8	−	−	−	−	
9	−	−	−	−	
10	−	−	−	−	mucoid
11	−	+	−	−	
12	+	−	−	−	
13	+	−	−	−	
14	+	+	−	−	[illegible]
15	+	−	−	−	?
16	+		−	−	

Cross Lac+ to W1177
Lac− to ~~W1177 Lac+ (W 1372)~~ W 1394 - 4/10/S.

12x : 47+ : 37−
14x : 8+ : 39−
1x 110+ : 31−

B. Also ~~58-161~~ W478 x W1177 ; 1-3: ~~[illegible]~~ Mal− Lacv ; 4-12 — #3 is Lac+ others Lacv — 3/20 Lac EMS, 9/28 Mtl EMS

C. 58-161 x W1022

D. 478 x 677

#9 Mal+ all others Mal−

#6, 10 Lac− all others Lacv

B: 26 Mal+ tested on S. 25 S^s 1 S^R. (linkage pattern)

Check above: 6 Mal− Mtl? Lac v?
10 " Mtl v has Lac+ (Rev??)

Repurify! H268

11/30/50.

Repeat W478 x [redacted]177 mEMS MH. Isolate possible MH$\underline{v}$ and check. (Plates have ca 30%+ → 26/80.).

a. 12/2 80 tests : Reisolate 22 EMS MH+ from gross streaks on EMB MH.
12/3 26 — 6 ... 1

+ = streaks from gross streak. on EMS

	MH	lac	Xyl	Mal	lac	
1	v	v	v	—	+	
2	+	—	v	—	—	—
3	v	v	v	+	+	
4	v	—	v	—	—	-
5	+	+	—	+	+	
6	v	—	v	— +sat.	—	-
7	v	v	v	—	+	
8	v	v	v	—	+, —	—
9	v	+v? (—)	v	—	+	
10	v	—	v	—	—	
11	v	—	v	—	— +	v
12	v	—	v	—	— +	v
13	v	v	v	—	— +	—
14	v	—	v	—	— +	—
15	v	v	v	—	+	
16	v +	v (—)	+ v?	—	+	
17	v	v	v	—	+ —	
18	v	—	v	—	— +	v
19	v	—	v	—	—	
20	v	— v v	v	—	+	
21	v	v v	v	—	+	
22	v	— + —	v	—	—	

worthy mixable by 6/21/52 see 951.

∴ of 20 diploids MH$\underline{v}$, allare Xyl$\underline{v}$. 9 are lac —
1 lac +
10 Lac $\underline{v}$.

23	v	v or +	v	—	v
24	v	—	v	—	—
25	v	—	v	—	—
26	v	—	v	—	—
27	v	v	v	+	v
28.	v	—	+	—	—

See 799

Lac+,− components: 11, 12, 18: on EMB Lac, these papillae show lac$\underline{v}$. On EMS Lac:

11: + colonies obtained → lac$\underline{v}$ Xyl$\underline{v}$ MH$\underline{v}$

12: − and $\underline{v}$ on EMS lac. → Lac$\underline{v}$.

18: EMSlac +. Lac$\underline{v}$. Xyl+ MH$\underline{v}$

8 Lac −, (+)

13 Lac −, (+)

~~EMSlac: pure+!~~ (Error in picking?) or recording.

H268 Revisions 777E

11/29/ff/50.

(8) Reisolated from single Mtl v colonies streaks on EMS Lac; Mal.

12/1/50. #2 shows several papillae on both Lac, Mal. Purify.

#6, 1 isol. pap. on Mal. "

→ 2: All Mtl+, Lac+; Mal+ ...

On EMS Lac, H268 slowly forms very dark (v. slow Lac+ ??)

3 (1) Mtl − Mal+ } no test

4 (2) " Mal+ }

6 (1) Mtl v Mal+ ✓

7 (2) Mtl v Mal+ ✓

Lac – homozygotes

October 26, 1950

10/26 A W466 × W1177 BM Lac+ Xyl+ lact × lac– Mal– Xyl– on EMS Xyl

B " × W814 " × lac+ Mal– Xyl– "

10/28. B: EMS Lac

+	–
25	14
40	19
65	33

No yield on EMS Xyl.

10/29. 1 colony A. Streaks on EMB, EMS Xyl.
ca 10/plate B.

B. 20 picked: 4 Xyl+ 16 Xyl– (sic!) No ↓.

Repeat on EMS MH.

A) 50 MH+ streaked on EMB MH, Xyl. No Xyl, ..↓.
Repick further colonies

11/7 52 picked, streaked on MH: 3 very likely MHv 1-3
3 possible 4-6

	MH	Xyl	Mal	S	Gal
1	V	V	—	R	—
2	+ on V	+	—	R	—
3	+	+	+	S	+*
4	+	+	+	S	+
5	+	+	+	S	—
6	+	+	+	R	—

H258
H261

778-2: MH↓ verified from EMS → EMB MH

H258 8 distinct REVERSION TESTS: H258–, 261 on EMS Lac, Mal
each lac↓ MH↓ Test segregants from #1: 6 lac+ → MH–
1: Mal+ MH↓ 10 lac– → MH–8; +2
not suitable for linkage phase study

H261 8 " " each Lac↓ MH↓
2 – Mal+ Mal+ (no test).

Lac–/–: Mal–/a

Met outcross (histidineless)

October 26, 1950.

A	W1325	x	W826	Mist; ser/gly	EMS Lac, Mal
B	"	x	W828	Mist; glut	
C	"	x	W836	Melys; hist	

Mal	−	+
A	81	26
C	80	38

Lac	−	+
A	143	8
B	69	27
C	174	25

Pick +, − to EMS Lac for [illegible].

		Lac −	Lac +
A	Mal −	25	3
	Mal +	19	4
C	Mal −	20	2
	Mal +	18	4

no linkage ~~to~~ Mal to Lac

Ca 70 tests each. Retest likely lac v: (1'etc. from their streak not single lac EMS col.)

A.	1	Lac +	
	1'	Lac +, −	
B	1	Lac +	Mal −
	1'	Lac +	"
	2	Lac +	"
	2'	Lac +	"
C	1	L +	−
	1'	+	−
	2	+	+
	2'	+	+ −
	3	+	−
	3'	+	−
	4	+	=
	4'	+	

None are heterozygous!

H might be linked to Δ.

UV Effect on recombination

November 1, 1950.

W67 x W1177. Mix suspensions (20 ml → 1.5)

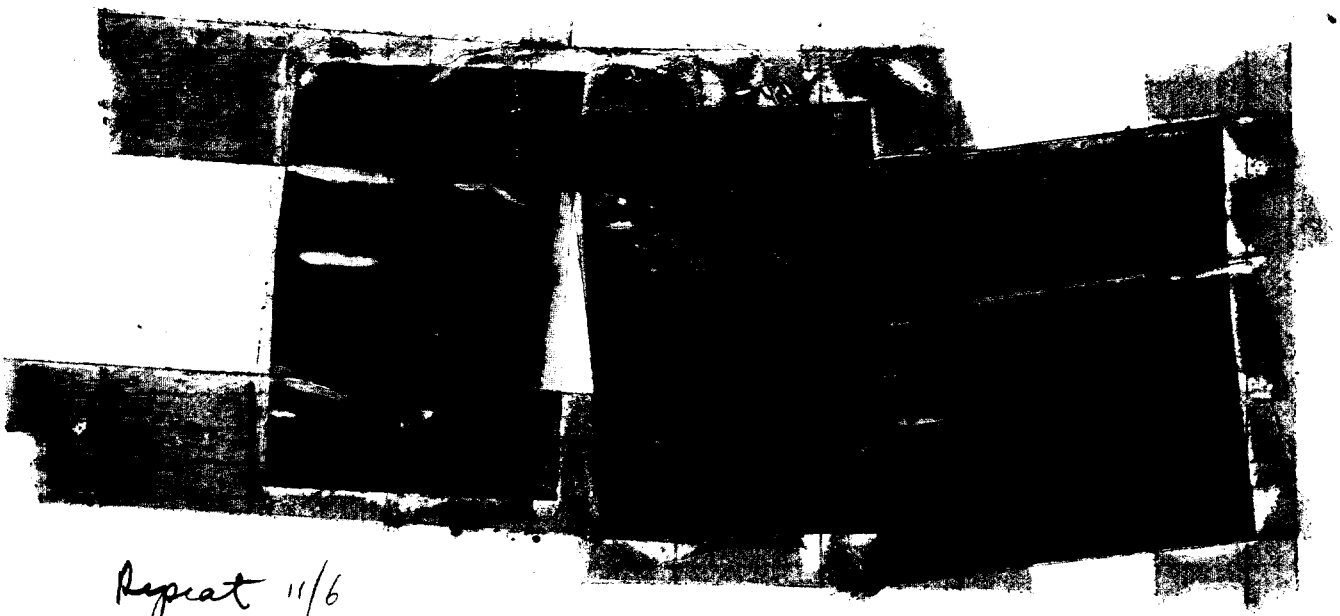
Plate .1 ml / EMS 1 ac.

a = no treatment

b = 10 secs UV 50 cm.

	~~EMB~~ lac−	lac+
a	24	
	29	
	31	
	37	
	25	
	58	
	69	
	38	
ca	40 /	0

	−	+
b.	17	
	146	1 → Pure +.
	61	mostly tiny
	57	
	66	
	66	
	87	many tiny
several hundred	large, very many small	?
ca	100 /	3?



Repeat 11/6

Spread mixture (in saline together ca. 2 hrs.) at 3:15 PM.

Irradiate 10 secs. at intervals:

11/1-10. 3^{15} Control: no irrad. 10 plates
3^{15} 10 secs UV 3 "
5^{10} " 3 "
8^{10} " 3 "

No marked effect of irradiation at any time: see prints.
Probably more small colonies in UV series.

In ca. 6000 colonies, 3 likely Lac+.

		Lac	Mal	S (EMS)		
#1	at	V	−, +	S	Mal− is pure S^s	Mal+ S^s probably cont. but is pure Lac−.
#2	3^{15}	+	−	R		
#3	3^{15}	~~B~~ V	V	? (s)	S^R/S^s	3 Mal+ reversions pure M+ Lac v. ∴ Mal−/A

Restreak 1, 3 from EMS Lac to EMB Lac
EMB Mal
Broth EMS Lac
Test S^R.

Also streak B on EMS Mal for Mal+ component.

1a-d Lac v (rel. stable) Mal− S^s

3 a-8. Lac v. Mal v very sensitive to sm. (entire streak selected or destroyed exc. for segregants).

Restreak segregants for $S^R//S^s$. For 1, use sec. growth of s test.

Plate H267 on EMB Lac, Mal ± SM. (5x10^-8)

Initiate H267 in Summary

	v	−	+
Lac	46 ~~37~~	~~4~~ 3	~~1~~
Lac SM	~~37~~ 0	~~0~~ 0	0
Mal	37	0	4
Mal SM.	0	1	0

Mal+ S^s may be pen. segregant

E. coli outcrosses
Verifications

× W1177 on DSM

Mal- 1. W1362 a No yeild.
2. W1362 b ca. 20-30 colonies/plate all Lac+ on EMSLacSM. *
{ 3. W1373 ca 5-10 EMSLacSM plate. Mostly Lac+ (- maybe slow to grow: Recessive.
{ 4. ~~776-26~~ W1374
5 W1377 = 23a.

* Check colonies on EMSMal. cf. original streak on EMB Mal: uniform Mal+

2: 100 Lac+ tested on Mal: 99+ 1-.. Restreak + check:

Streak out mixture as plated on DSM; EMSLacSM: ca 99% Lac+ 1% Lac-.

5: Pick Lac- for test on S. cf. Lac- from 776-23 original cross plate, streaked.

Lac- from mixture 2: S^R
from plate 776-23 {9: S^S }?! (also Lac, Xyl, or Mal -)
{3 S^R }

5 colonies were Lac±. Streak out and compare with Lac- and W1377.

---

2 Pick from streaks on EMSMal (unpur.) and spot on various sugars, phages

	Mal	Lac	Xyl	Mtl	T4	T5	T6	T7	λ
1	+	+	+	+	S	S	S	R	−
2	−	−	−	−	S	R	S	S	+
3	−	−	−	−	S	R	S	S	+
4	+	± +	−	+	S	S	S	R	−
5	+	+	±	+	S	S	S	R	−
6	+	+		+	S	S	S	R	−
7	+	+	−	+	S	S	S	R	−
8	+	+	−	+	S	S	S	R	−
Remaining 14.	+	+ or +	#6, 8, 9, 10 − others +	+ #10 ±					

15 purified 781-2. 13 L− M− TS^R $T7^S$ X−
2 L+ M+ TS^S $T7^R$ X+
check on Xyl; T7; prototrophy; ~~TS~~ app. Parental! ~~only~~ non-prototrophs

Restreak on EMBLac. ✓ char of W1362 a & b.

3: 22 streaked on EMBlac 3 Lac− } Brush to Mal: all Mal−
+8 " " 6 Lac− } Mal − hold! " "

---

4 16 " " 15 L− } Mal−
1 L+ }

-23 clones from 116-23 plate

11/2/50.

58-161 x W-1177.

20ml → 3ml susp. ca .1ml/plate.

EMS Mal
(EMS Lac)
(DSM)

EMS Mal, (Lac)
+, − differentiation very poor.

ca. 200-300/plate. No sectorials noted ↑. Too crowded.
~~On DSM, 3 colonies were observed. Streakout on EMB Lac~~

[Check on parents.]

	Mal	Lac	(SM)
1	−+	−	S
2	+	−	R
3	−	+	S

Repeat 11/6.

11/8/50: 12 plates EMS Mal 1112 prototrophs examined under binoc micr. Pick any colony that might be Mal+/− . Mal+ not always readily scored (thick plates). Where scoreable:

+	−	s	Σ
17	80	1	98
9	64	2	75
33 ?	80	0	113
17	68	2	87
76	292	5	375

or 20% + probably an overestimate.

Hold x-plates mixing for more sample

conc. inocula in EMS Lac SM. (ca 5x) ca 10 colonies/plate.
20: Test for S^R: all S^R, λ+

11/10 of 15 possible Mal s streaked out on EMS Mal, 6 were Mal+/− (4+ 5−).
#1 also had a sectored colony. Restreak as 782A1.
Test paired segregants for Lac, Mal, Xyl, Mtl, Gal, V_1 and S^R

	Mal⁺		S	S	Lac		MH		Xyl		V_1		Gal	
1	+	−	R	R	−	−	−	−	−	−	S	S	+	+
2	+	−	S	R	−	−	+	−	+	−	R	R	+	−
3	+	−	S	R	−	−	−	−	−	−	S	S	−	−
4	+	−	S	R	−	+	+	−	+	−	R	S	+	−
5	+	−	S	R	−	−	−	−	−	−	R	R	−	+
6	+	−	S	R	+	+	−	−	−	−	S	S	−	−
1a	±				−		−		−		S		+	

Correlation is best for Lac, V_1 (#4 only exception).

C 4/19/50. 58-161 × W1177 m EMS Lac; B_1. Lac, Mal B, were turbid.

15 plates ca 30/plate. No Lac ±.

→ +.

Lac ± no true regular occurrence!

Duplex prototrophs

11/11/50. Repeat 58-16 1 x W1177. EMS Mal

30 plates. ca 100/. Mal+/- scoring: optimal

Total Mal+ (incl. ±) 277.

11/13. Ratio of Mal+ : - (sample plates).

+		−	
19		101	
8		47	Pick these; reincubate all plates. 11/14: 7 additional possible Mals.
9		47	
10		42	
4	(15)	42	
10	(15)	53	Also pick non-selected Mal+ and - to EMS Lac.
60		332	

∴ $60 > \frac{277}{50}$.

Among ca 5 x 50 colonies on EMS Lac, 1 Lac± noted. Purify as 782L.

Non selected colonies. (to EMS Lac; Mal for-) Hold for later analysis

	Lac+	Lac−
Mal+ :	33	34
Mal−	25+2	44+.

	Mal				Lac		Xyl		Mtl		Gal		V_1	
	+	−	S	R	+	−	+	−	+	−	+	−	S	R
1	+	−	R	R	−	−	−	−	−	−	−	−	S	S
2	+	−	R	S	+	+	−	−	−	−	−	+	S	S
3	+	−	S	R	−	−	+	−	−	−	−	−	R	R
4	+	−	R	S	−	−	−	−	−	−	−	−	S	R
5	+	−	S	S	−	−	+	−	−	−	−	−	S	S
6	+	−	S	R	−	−	−	−	−	−	−	−	R	R
7	+	−	R	S	−	−	+	−	−	−	+	−	S	S
8	+	−	S	R	−	−	−	+	−	−	−	−	S	S
9	+	−	R	R	−	+	−	−	−	−	+	+	S	S
10	+	−	S	R	−	−	+	−	−	−	−	−	S	S
11	+	−	S	R	−	−	+	−	−	−	+	−	S	S
12	+	−	R	R	−	−	−	−	−	−	−	−	S	S
13	+	−	S	R	+	+	+	−	−	−	−	−	S	S
14	+	−	S	R	+	+	−	−	−	−	−	−	S	S
15	+	−	S	R	+	−	−	−	−	+	−	−	S	R
16	+	−	S	R	+	−	+	−	−	+	−	−	S	R
17	+	−	R	R	−	−	−	−	−	−	−	−	S	S
18	−	−	S	S	+	−	−	−	−	+	+	+	R	S

Punct mutuals

of 17 tests, Mal+/− and $S^{R/S}$ accorded in 12

Lac	concorded	14	
Xyl		10	Both − −
Mtl		15	" " − −
Gal		13	
V_1		14	

Lac; V_1 concorded 13 .

#18 was Lac$\underline{s}$. In view of concordance of Mal-S^R probably not an artifact.

~~Patient 78265~~

11/6/50.

Grow P6. into D(Lac). Grow 36 hours aeration.
Plate out at 10^{-7} on EMS Lac, EMB Lac { ± SM, EMB Mal   12 N 7
Repeat 11/9:

11/8. EMB Lac:

	v	—			v	—
	174	79	245			
	129	62	191			

	v	—	Σ		v	—
EMB Lac SM	0	51	51	1000u SM	0	7
	0	57	57	Al ?	1	4
	0	54	54		1	8
	* 3	60	63			

	v	—	+	Σ	v	—	+
EMB Mal	193	52	4	249	170	12	
	206	64	3	273	194	11	1+

Presumably, all diploid cells are killed by sm, with 3* exceptions.
Test these for Lac, Mal, S heterozygosity.
M257' segregants tested for S on EMB Xyl.
~~8~~ 8 Mal− : Xyl− S^R
4 Mal+ : Xyl+ < 2 S^R, 2 S^S

?

Δ S Mal Xyl

Continue only be verified?

* Exceptions:

	Mal	Lac
1	—	v
2	—	v
3	v	v

Streak out 3 for Mal+.   204 1; 210 2

	Lac+	Lac−	
EMS Lac	196 + (49)	2	49 small cols. Lac+?

EMS Lac SM 0, 1, 1, 0, 55 66, 47, 44. Total: 2+/212 − 0, 4 (tiny)

	Mal+	Mal−	Mal v! (sic)
EMS Mal	172	55	21

M257

783A, D

See previous page
S^R Lac ± exceptions.

	Mal	lac
1	—	v
2	—	v
3	v	v
4	v	
5	v	
6	+	?
7	—	

or E.

4 Mal+ segregated : S^R v.

D. Inoc M257 1/100 Permassay. Grow overnight and plate out.

11/16 EMB Lac

v	—
85	26
75	15 + sprinkling −
73	14
81	37

average − : $\frac{92}{4} = 23$

= M257'

5.

EMB Mal

v	—	+
75	11	6
89	14	4

EMB Lac SM+

8
3

Phenotypic lag?
Test Lac− segregants for S^R. — Rather uncertain tests:

	R	S
	25 (2 Mal+ 23 Mal−)	11 Mal−

SM .5u/ml

36
36

EMB Mal SM +

5	1
11	0
10	0
11	1

.5/ml

29	0
33	2

Note: Colonies on EMB + SM .5u may represent late segregation products of Lac± cells and may not reflect phenotypic lag. However, comparison of EMB Lac with Lac + SM (100u) may reflect phenotypic delay. Repeat plating. Also test Lac+ from EMB Lac for SR.

See over

M257'

EMS lac SM

V	—
1	0
2	2 v. sm.
2	1
2	1

Transfer to EMS lac; test on EMB Mal

H257

183B

(EMS) Mal v. Almost all colonies of H257 plating show some signs of Mal aggregation

Streak out 8 Mal v colonies from EMS Mal to same.

Pick Mal+, − patch streaks separately to EMB Lac:

6: Mal+ lac+ / Mal- Lac − 1: Mal+ lac− / Mal- lac −

1: Mal+ lac+ / (Mal- lac+). Restreak on EMB, EMS lac as 183B1 ~ Lac v.

BB. H257' (~~Pcf~~ Y2 1:100 . 24h. 37° 48h. Room T.)

EMB lac	v	−	EMB lac SM 100u/	v	−	
	123	93		4 (3... incl.)	82 *	smeared
	129	100		1	71 ±	smeared
	116	94		0	60	
	125	107		0	83	smeared.
	127	111		1	56	not smeared.
	620	505		6	349	
m	132	101		1+	70.	

* These counts are likely overcompensated for smearing, i.e. overestimates. Repeat plating, also with H267.

Test Lac− from H257' for S^R/Mal. (Also, see F)

Mal-S^R	22	20	42	42:12 S^R/S^S
Mal-S^S	1	2	3	
Mal+S^R	0	0		
Mal+S^S	IIII	~~IIII~~	9	

(EMS) Mal –.

Test 20 Mal – colonies on EMS Mal:

a. Brush on EMS Lac /SM
b. " " EMB Mal /SM } all S^R. Lac – Haploid prototrophs? (~~Ephemeral?~~)
c. Streak (few) on EMB Lac. cf. Mal+ colonies.

EMS Lac –

Pick ~~5~~ 3 "small colonies" and streak out on EMB Lac, Mal. ~~Lac EMS Lac~~, ESM.
cf. 1 standard. ✓: Lac – Mal – control Lac ✓ Mal ✓

11/16/50: From 783D

CC. Segregant colonies from H257' on EMB Mal SM. (i.e. S^R only).

		Xyl	Mtl	Xyl	Mtl
Mal+	~~5~~ 4	+	+		
Mal–		–	–	–	–
		–	–	–	–
		–	–	–	–
		–	–	–	–
		–	–	*	–
		–	– +	–	–
		–	– +	– +	– +
		+	+	–	–
		– +	– +	–	– +
		–	–	–	– +
		–	–	–	–
		–	–	–	–
		–	–	–	–
		–	–	–	–
				–	–
				–	–
				–	–
				– +	–

From EMB Mal.

Mal+ ~~5~~ 4 all S^S; 1 Mtl – Xyl –
4 Mtl + Xyl +

Mal – 17 all S^R
15 Mtl – Xyl –
1 Mtl + Xyl +
[1 Mtl – Xyl – +]

31 shown in detail?

Consistent with order:

$$\frac{R\ \ -\ \ -\ \ -}{S\ \ \text{Mal}\ \ \text{Xyl}\ \ \text{Mtl}} \quad \frac{}{S\ \ +\ \ +\ \ +}$$

+ – Δ

Limiting conc. SM. H257.

783DP

Plate H257 on EMB ± SM (.5u/; 100u/ml).

	v and +	—
EMB Lac	149	8
	150	16
EMB Mal	185 (+ mucoid)	10
EMB Lac SM.5	127	19
	44	16
	18	24
EMB Lac SM100	0	3
	1	1
EMB Mal SM.5	63	27
	30	27
	58	18
SM 100	0	2

} Lac v in this series have very luminished faded + var. centers.

very faint

At this concentration of streptomycin, diploid S^S/S^R are not regularly killed but are strongly selected against in favor of S^R segregants. This conc. cannot therefore be used for phenotypic log as it will produce artificial S^R from S^R/S^S.

M257 / Gradd conc sm 783D

Plate M257 11/10 sm indicated under. Read at 40 hours

	+ = v	−	v	E
EMBLac	169	14		183
	184	11		195
EMBMal	+	−	v	
	2	10	149	162
EMSMal	209	4		213
EMBMalSM. (100u)	0	13	0	
	2	5	0	
	1	4	0	
	1	4	0	
	1	3	1	
	5	29	0	
EMSMalSM (100u)	0	4		
EMSLacSM (100u)	1	0		
	0	0		
	0	0		
	0	2		
EMBLacSM	+	−	v	
(st.) (100)	0	9	0	
	~~0~~ 0	~~1~~ 7	2 1	
1000		4	0	
100		17	3	
50		9	2	
10		9	0	
5		5	0	
2		9	1	
1		18	1	
.5 *		24	45	
.2		13	177	
.1				

Test Mal+ S^R and Mal− S^R on EMB Xyl:

	Xyl+	Xyl−
Mal+	5 (5+ 0−)	0
−	3 (3+ 0−)	25 (2+ .23+)

Consistent with:

	R	−	−	−
− Δ	Sm	Mal	Xyl	MH
	S	+	+	+

↑ M+X+ ↑ M−X+

0X = M−X−

Test MH: Distribution of +, − and above.

* sm app. unevenly distributed as entire plate is virtually sterile. Many v colonies have very faint + component 2 Lac v have very little −. Rest are possible crossovers.

M257 part seq. Mal Revertants. 78BE

11/18/50 ...

	Mal	Lac EMB	Lac EMS
1	—	V	
2	—	V	
3	V	V	
4	V	V	
5	V	V	
6	+ ?	V	
7	—	V	
8	—		+
9	—		+
10	—		+
11	V		+
12	—		+
13	—		+
14	V +		+
15	—		+
16	—		+
17	— +		+
18	+		+
19	—	— ?	—
20	—	—	—
21	—	*	+
22	—		—
23	—		—
24	—		—

From EMB lac SM M257

From EMS lac SM M257'

8 Mal+: 1, 5, 8 are Mal v. Lac V
2, 3, 6, 7 Mal + Lac −
V #4 → Mal +, (−) (Mal v+ ?)
Lac v.

E7 #4 → Reisolated from single colonies
Mal v (+ predominant) Lac v.

783 F Phenotypic log. 783F

November 20, 1950.

Plate H257', H267' on EMB Lac; ± SM 100. Cf. with °/s R.

11/19/50: H257.

	Lac		Lac + SM.	
V	—		V	—
92	143	src	0	125
78	98		2	99
52	130		0	130
82	132		0	94
84	135		1	126
399	637		3	574
$\bar{m}$ =	80	127	<1	115

Fraction s's, from streak tests on Lac−:

	Mal		Mal SM	
V	+	—	V	+ —
102	10	109	1	3 92

H267

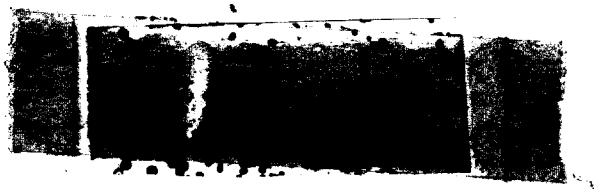
	Lac		Lac SM	
V		—	V	—
43		84	0	6
25		78	0	4
28		73	0	6
34		69	0	5
26		97	0	5
156		401		
$\bar{m}$ =	31	80		

	Mal		Mal SM		
V	+	—	V	+	—
28	54	18	0	0	2

Threshold sus: # S^R/S^1 heterozygotes. 183

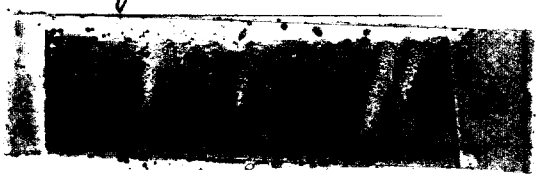
11/20/50.
Plate each of following strains, grown as D(Lac) (except W1177-42) at ca. 10^{-7} on indicated EMB. 8 45 PM. Read at 4: 45 PM 11/21. = 20 hrs.

K12. # Lac
Lac SM 1 — sl. inh.
Mal SM .5 — sl. inh. turn.
Lac SM 100 — No colonies



0 .5 1

W1177 No differences.

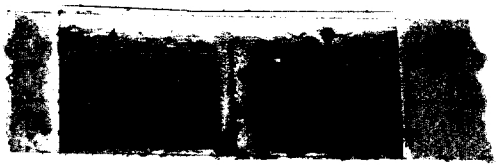


0 .5 1 100

H266 ($S^S/-$) 40 hours.

Lac		Mal
.5	typical mosaic colonies ca 400 reduced count; smaller Lac−	do.
1	19 colonies	All Lac − Full ct.
100	No colonies.	21 Lac − colonies No colonies.

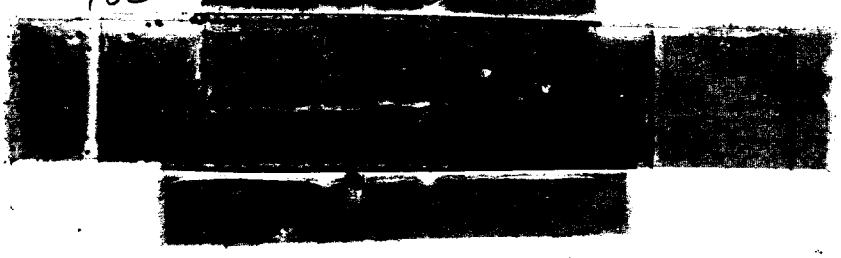
(Segregating partial resistance)



.5 0

H257 Lac typ. (somewhat small) Lac v. ca 400

.5 reduced Lac − Mal − (hint of +)
1 cols.; some normal.
100



0 .5 Mal .5 100 1
Lac

H267

Lac	typ lacV ca 300	
Mal.5	5 large 1-3 v. small cols	—
Lac.5	10 med 1-2 " "	—
Lac 1	2 large cols. Lac −	
Lac 100	5 large cols. Lac −	

H267 may be more resistant than H267, or give SR survivors more readily.

Linkage comparisons : S^R minimal

11/7/60.

W1368 × w677 standard A
w478 inv B
w1022 inv C
w1015 inv D

		−	+	
A	+ TLB,	12	45	(some ~~[illegible]~~ doubtful mucoids recorded as +)
		18	40	

		Lac−	+
B	SM+ TLB,	4	72
C		1?	12
D		5	15
~~SM-0.~~		~~0~~	~~0~~

Need repetition

11/18...

	+	−
A	39 ; 33	14 ; 13
B	37 42	0
C	6 15	5! 0
D	20 16	0 0

Mutant Run 1373

10/7/50.

See 786.

A. Sterile.

B. (1): ca 10 colonies. Same tac - ?

Pick to water; spot on EMB, D(o).

B: 11 tests: nanogram on D(o) in 24h.

Light

		A1	A2	A3	AY	A5	YEx	YNA	MC	
785B	1	(A1) 1421	++			+	++	−	+	Cyst
	2	(A3) 1425		±		-	+	−	+	Tyro.
	3	(A3) 1426		+		-	±	−	+	Tryptg
	4	(A2) 1423	+			-	++		+	IV
	5	A3		±		-	±		+	
	6	(A1) 1427				-	±		±	
	7	(A4) 1428			+	-	++		+	No A4
	8	A1 +				-	+		±	resp
	9	(A3) 1427		±		-	±		+	Trp or ø not tyr
	10		+			-	++		+	
	11	A3		+	−	-	+		−	
	12	++	+			±	++	+	+	
	13	(A4) 1429			+		++		+	
	14	(A2) 1424	+				++		+	Hist
	15	+	+	+	+		++		+	IV
	16	A3		+			+		+	
	17	A1 +					+		+	
785B	0				+		++		+	−
786B	0				+		++		+	
	1	(A4) 1432			+		++		+	− A4 −
	2			+			+		+	
	3			+			±		+	
	4			+			±		+	
	5			+			±		+	
	6			+			±		+	
	7			+			±		+	
	8			+			±		+	
	9			+			±		+	
	10			+			±		+	
	11			+			±		+	−
	12			+			±		+	
	13			+			±		+	
	14	A4		+	+		++		+	
	15			+			±		+	
	16	A4 1433		±	+		±		+	A4 −
	17	(A3) 1431		++			++		+	A3 −
	18			±			±		+	
	19	(A2) 1430	+				++		+	Leucine
	20			+			±		+	

All − unless indicated otherwise

(38-40 = 1431-33)

Mutant Run: W1374

10/7/[illegible].

UV - 30sec - medium. Penicillin overnight.

A. 1:20 300u P/ml 29 tests: all x+

B 1:1000 100u/ml 40 tests: 2 x+ (24h.)
38 x-.

See 785

			A1	A2	A3	A4	A5	HC	YC	
	21	A3?	−	−	±	−	−	+	+	No response to A3.
	22	A2	−	++	−	−		+	+	
	23	A4	−	−	−	+		+	+	PROL to.
	4	A1	+	−	−	−		+	+	D(0) +
	5		−	−	−	−		+	+	
1421	6	A4	−	−	−	+		+	±	PROL
	7		−	−	−	−		+	+	
	8	A4	−	−	−	±		±	−	
	9	A2	−	+	−	−		+	+	
	30	A2	−	+	−	−		+	+	
	1	A4	−	−	−	+		+	+	
	2	A4	−	−	−	+		+	+	
		A4?	−	−	± ✓	+ ✓		+	+	
	3		−	−	−	−		+	−	
	4		−	−	−	−		+	−	
	5		−	−	−	−		+	+	
	6		−	−	−	−		+	+	
	7	A4	−	−	−	+		+	+	
1431	8	A2	−	+		−		+	+	No resp. #2 IV; 0; +.
1432	9		−	−	−	−	↓	+	−	
1433	40	A2	−	+	−	−		+	+	as 1431
	0	++	+	+	+	+	+	+	+	

None on YNA

A1 : 24.
A2 : 22, 29, 30 ; 1431; 1433
A3 : 21 ?
A4 : 23, 26, 31, 32, 37.

Throw out non W −.

Double mutants:

1421	1
1423	5
1429	3
1430	2

11/25/50.

A W 1377
B W1395
C W1396
D W1397
[E W1441]

A-D grown in ~~[illegible]~~ D(0) E. D(prol).. Irradiate directly (30 sec. UV 50 cm) and incubate 1:10 in Penassay "A25. Wash 8P. (C shows very little growth - unusually sensitive to UV ?) Dil. ca 1:500 in D(0) + penicillin [+ prol. for W1441].

A-D give erratic tests on minimal agar as they themselves grow erratically on D(0). Restreak parents on D(0).

		sucrose	colicin /578 -
nuc.	70	+	
	71	−	+
	72	−	−
	73	−	±
	74	+	−
S	75	+	−
S	76	~~+~~ −	−
all prol +	77	++	−
	78	[illegible] −	++
	79	++	−
	80	~~[illegible]~~ +	−
	81	lac −	±
	82	[illegible] −	+
	~~83~~		
	~~84~~		
	~~8~~		

[illegible] from 2d plating of C.

W1377 .... 97 "A" isolated as repeated selections on D(0) agar until homogeneous, uniform good-size colonies are obtained.

1st Penicillin Run: [W1396 is extremely sensitive to UV.] In 6 hr. run, 100 u/ml., C gave 1/10 mutants ; A, B, D 0/10. ~~[illegible]~~ Overnight, A, B, D were overgrown. 1/20 additional mutants from 1st plating of C (= 2/30). Test 50 cols.

12/12 Repeat penicillin runs. using 300 u/ml., 6 & 24 h. platings C did not grow sufficiently after UV.

12/21 Repeat with A, B, D. Each is resistant to 1000 u/ml penicillin and therefore unamenable to the penicillin method!
4 mutants obtained from C above. 3 A2; 1 A1.

Outcrosses : nutritional

K12 - W1373 - W1374 - 776.44

			24h	48h.	
1	785	B1	Many ++		x
2		B2	-	0	
3		B3	+		x
4	786	B1	+	50+	x
5		B2	-		0
6		B3	-	0	
7	776-44 = W1416		-	0,0	
8	W-1177			0	
9	1+8		++	200+	x
10	2+8		-	(0)	2+ → Mal − lac − + Mal+ lac+ (small colo.)
11	3+8		+	20+	x
12	4+8		+	10+	x
13	5+8		-	(0)	0
14	6+8			(0)	0
15	7+8		-	(0)	0,0
16	1+2		Many ++		x
17	1+3		++		x
18	2+3		+	20+	x
19	1+4		++		x
20	1+5		++		x
21	1+6		++		x
22	4+5		+	40+	x
(23	4+6		+	20+	x
24	5+6		-	(2+)	●

Repeat 10, 13, 14, 15, using growth together + sep. 48hrs.

31	785-2	0	0	
32	786-2	0	0 0	
33	786-3	0	0	
34	W1416		0	
35	W1177	0	0	
36	31+35	5+	8+8+	+, − ++−
37	32+35	+	1+	1+ 1− 3−
38	33+35	2+ 2−	1+	2+ 2−
39	31+32	1+	1+	1+ 0
40	31+33	0	0	1±? 0
41	32+33	1+	0 0	0 0
42	34+35	0	0 0 0	

36' etc. mixed with washing

36'		0 0 7+ =	40'		
37'		1+	41'	1+	
38'		0	42'	0 0	
39'	0 several v. small	0			

W1416 uncrossable
W1374,75 mutants
remarkably infertile
of $s^R x^+$ crosses.

Infection of W518 with λ

11/14/50.

To 5ml washed W518 suspension in saline add
1ml broth lysate of λ (11/7). 2:38 PM Held at R.T.
Centrifuge 15 mins at 2:58. Resuspend in saline
Resediment, completed 3:43.
Plate 2×10^{-7} dilutions of each on EMB Lac, and on W518

A. washed cells
B supernatant 1
C supernatant 2
D original λ, titrate.
E original 518

A. Ca 300 colonies. No plaqued colonies or nibbling. Test sample for lysogenicity. 100 tested: all λ- ! (slow absorption)

E 18; 6 plaques on W518
B 19; 11 " " "
C 20; 18 " " "
D 10^{-5} 45 " "
-6 47
-7 28
-8 64

Nov. 20, 1980.

A W836 x W1177
B x W1178
C x W1406

2 MHs. colonies on EMS MH. A: <+ − Mal + S^s
∴ true duplex colonies. B <+ − Mal − S^R, Mal − S^R !

A. Plate on EMS Lac; MH. Lac: 60+ = 104− MH: 57− 47+ 2s (2 plates)

Test linkage of Lac, Mal.

	Mal +	−		Mal+	Mal −
Lac +	+	+	MH+	++	±
−	+	+	−	±	++

B. Lac: 137+ : 29−   C.

C. Lac: 33+ : 60−   MH: 70+ : 32−
34+ : 57−.

B. 24 tested: 4 possible v → not v, but maybe segregating modifiers!
C. 20 " No v.

50 addnl. B + C. →
a few Lac −; all others Lac++ no v!
C1 − Mal+.

B 1-4 } apparently pure Lac + { Mal − B / Mal + C
(C1)
→ self-plaqued (λ). Recheck B3 which gives some V^R, on EMB.

Check parents: [redacted] to be λ^s. W1406 is λ^+ (Rev)

790 B3 is verified Lac v, but very stable. Test also for λ.
Apparently λ −. H

Dianzini strains: coli "transformations"
(also miscellaneous phage tests).

✓ strains various sugars + phages.

		Strain	T1	T2	T4	T5	T6	T7	λ	518	Lac	Mtl	Xyl	Mal	Suc	
776-66	1	1442 I	R	↑	R	R	P	R	R	−	+	+	+	+	−	
67 (x W1127)	2	1443-6	R		R	R	R	R ·	R	−	+	+	±	# ↓ ÷	−	
68 (x W1127)	3	1444 UG	R		R	R	R	R	R	−	+	+	+	+	++	
69 (x W1127)	4	1445 5516	R		≠R	R	R	R	R	−	+	+	±	# ÷	−	
	5	~~1373~~ 1374	R		R±	±	R	R	R	−	+	+	±	+	−	~~colicin~~?
	6	~~1375~~	R		R	R	R	R	R	−	+	+	+	+	−	col?
	7	1377	R		R	R	R	R	K	−	+	+	+	+	−	
	8	1395	R		R	R	R	R	R	−	+	+	+	+	+	
	9	1396	R		R	R	S R	R	R	−	+	+	+	+	−	colicin?
	10	1397	R		R	R	R	R	R	−	+	+	+	+	+	
	11	~~W1177~~ K	S	R	S	S	S	S	R	λ+	+	+	+	+	−	

Allare P^R ever K12 P^P

Dianzini, M.G. (1950) Bolletino I.S.M. 29: 161-172. Mutazioni indotte dagli acidi nucleinici batterici.

He claims that 1443-5 are sucrose-positive but deals inadequately with problem of adaptation. Character of growth - agar not clear in his paper.

783: Partial segregants: tests for Mal heterozygosity

11/20/44. '50.

	783E−+	EMB Mal	trans EMB Mal / EMB Lac	
1		++	−	
2	8 −		−	
3	−		−	
4		++	−	
5	9	++	−	
6		++	−	
7		V	V	✓ Mal+ Lac+
8		−+	−	
9	10	++	−	
10		++	−	
11		++	−	
12		++	−	
13		V	V	✓ Mal+ Lac+
14		++	−	
15		++	−	
16	12 −		?	Mal− lac+ (~~weaker~~??)
17		++	−	
18		++	−	
19		++	−	
20		++	−	
21	13	++ −	V	Mal+ Lac+
22		++	−	
23		++	−	
24	—	n.gr.	−	
25		++	−	
26		++	−	
27		n.gr.	−	
28		++,(−)	−	
29	16	++	−	
30		++	−	
31		++	−	

Reds: 7, 13, 16, 21 on EMB Lac; Mal.

~~K12 x K13, K14~~

WG-1 x WG 3, 4

A W1446 x W1435 (WG4 x WG1 Het) → M269
B W1446 x W1177
C W1449 x W1435 WG3 → M270
D W1449 x W1177 (WG3 x WG1)
E W1447 x W1177 WG4
F W1448 x W1177 WG3

G 1451 x 1435 WG3. L−: 2 M+ / 1 M− L+: 30 M+ / 1 M−

A.

Lac +	−
1	25
1	8
0	6
0	16
0	7

Lac- predominates!
Streaks not Lac+. Brush Lac− to MalEMS.

B. Rather low yield (3-5/pl.) all Lac −

C. Mostly Lac+

4	5
7	1
2	3
12	1
8	2
33	12

very variable colony morphology.
Pick 52. and streak out on EMB Lac

D. No prototrophs (4 plates) [Allelic exclusion ???]
E " " " " ? (But cf. B)
F " " " "
G V. Numerous prototrophs. Mostly Lac+. Cf. on EMS Mal.
Ca 1% Lac −.

L+ 1. Lac v. (clearly). Restreak a group of single lac v colonies and restreak on EMS lac; EMB Lac, Mal, MacConkey.

L+ 2. Lac ++. Restreak as above.

Lac − : on EMS Mal + 10 − 20 Not easily scored.

Restreak some Mal+.

M269' segregants

Lac − : 25 all Acriflavine R.

Lac + : 15 2 ? S 1 ? ? 12 R.

Restreak representatives for each.

32 lac+ streaked on EMB lac.

#6 Lac v. All others Lac++. Reisolate.

Of others, all are Mal+ except #7. Pick single colonies to EMB-O

Restreak #6. ⟶ Pure lac+! Mal?

Lac−: 4 Mal+ 4 Mal−

M270

Choose weak lac+ for possible lacx.

W1452 x W1262

For M[redacted].

W ~~138~~ 1452 x W1262 on EMS Mal.

Pick Mal+ and brush against S/4 on Lac EMS.

only 16+ among 8 plates (ca 50/plate).

4 Mal± noted. Restreak on EMS ~~Lac~~ Mal, EMB Mal; Lac.

795:1-3 of 16 tested in first selection, 3 are Mal+ S[s] on EMS. ~~Repick~~

Streak on EMB Mal for ± test. 10 weak Mal- on EMS..

After reincubation, additional Mal+ appear Test those as above 8/19 tested 4-11

1 pure mal+
2 ?
3 Mal+.

Retest: Mal+ Lac±.

Held in abeyance.

12/16- 1950s

				Yield mEMS lac. /plate	
A	1482 x 1451	4-3	5-10	+, -?	
B	1482 x 1435	4-1	20	-	
C	478 1482	1-4	20	- (+)	
D	410 1482	1-4	10-15	-	
E	1455 1451	4-3	10		(contaminants ?)
F	1435 1455	1-4	0, 1	Lac -	
G	1451 1435	3-1	3-4	+, -	
H	1482 1455	4-4	0		

W1455 highly infertile!

C: 20 colonies streaked out: 16 - 4+ Lac. No v.

E: 4 Lac+

G 8: 6 Lac+ (#2-7) 1 Lac- (#1) 1 Lac v. Reisolate.

C: 16: Lac-Mal- #3 Lac+ Mal- 1 Lac+ Mal+.

12/25 E 4: Lac+ Mal+ (no sign.) #1 and 4 are R #2,3 S. Recheck ✓✓

G 1 Lac- Mal- 5 Lac+ Mal+ 1 Lac+ Mal-.

G1 Lac v, Mal v, Mal -

6 Lac+ } 9 Lac- } sep. tested all Acr R.

E: ✓ n Acriflavine : 2S 2R ok.

colonies very similar on Tryptone agar.

12/26. Cf. Ck reactions

C' all Acriflavine R. (as parents).

G. 1 S

2a R b S
3 R
4 R
5 R
6a R b S
7a R b S.

2 morphological types noted upon streaking out. Restreak duplex components on N.A.

see over!

Compare various prototrophs of 796 G.

	T7	Acr.	λ	Morph.
1	S	R	+ ?	″ R
3	S	R ✓	R	R
2a	R	R ✓	R	R
2b	R	S ✓	R	S
6a	S	R ✓	R	R
6b	R	S ✓	A	S
7a	S	R ✓	R	R
7b	R	S ✓	R	"S's more opaque" green diffraction / pink diffraction

#1 gives an undoubted Acrif. R reaction, but redisperses very readily to resemble S or RS.
Morphological differentiation probably better on EMB.

M271 — 273.

W1435 x W112 on EMS MH.

Pick MH +, purify on EMS MH. Test for discordant V_6 reaction on EMB, EMS (MH).

Out of ca. 30 such tests, 3 likely cultures segregating V_6^R.

M271 – 273.

M271 is verified as segregating $V_6^{R/S}$. V_6^S predominate.

M272 – Lac V?

$V_6^S \rightarrow$ Lac - stable $V_6^R \rightarrow$ Lac – mutable on EMB Lac.

Streaks (MH)

cf EML 60 11/28/50. Nonallelism of Lac_{1a} – 6.

12/23/50.
See 777B.

Ca 1/2 MtlV isolates are ~~#~~ Lac − 1/2 LacV.

Some Lac − EMS may have come from duplex, whence Lac + might be isolated. All original 1-22 are on D(Mtl) or D(Lac) resp.

Lac+ isolated: 11, 12, 18. All appear to be stable Lac +.

11/23 In course of isolation 8, 13.

To be isolated 14, 17.

#8+ is ~~[illegible]~~ Lac +, apparently pure, but restreak of EMS Lac → both Lac + and −. Restreak + to verify, and to provide Lac − for further testing.

#13 → both + and − colonies. Restreak Lac +.

#14 → pure + EMS Lac. (mislabeling ?). Isolate to slant. ~~[illegible]~~ EMB of "14 −"

#17 + and −

Note: since Lac V components of #8, 13, and 17 have already been isolated, attend to Mtl V character of Lac − "segregants".

12/27. "14+" is pure Lac + Mtl −   14 − : pure Lac − Mtl + (? Rev).

~~#8~~+ LacV OK.

17 − : 3 Mtl + 1 Mtl − No V.

8 − 4 Mtl ~~+~~ − no V.

13 − 4 Mtl − no V.

Tentative conclusion: These cultures which give Lac − prototrophs from LacV isolates are throwing prototroph segregants, not partial segregants. Recheck from original slants. This does not explain 11, 12, 18 which are apparently duplex.

Comparison of Lac,- homozygotes diploids and parents

12/24.../50.

Lac (EMB) 364..

1	H271	Bright red centers (confluent papillae ?)
2	H258	type - papillae in streak
3	H268	type - no " "
4	H273	as 1
5	H261	as 2
6	799-11	as 3
7	W1435	— pap.
8	466	— pap.
9	112	— stable
10	1177	— stable!!!

H271 and 273 may show very slight + reaction; more likely frequent crossovers lead to lac+ segregants.

W-1177 appears to have become lac- stable. Therefore lac- types such as H268 are unsuitable for homozygosity analysis. Review strains for lac- mutability. Resuscitate W660 for new set of diploids carrying mutable Lac,-.

see 803