

October 24, 1950.

A. 58-161 x W1177 ⁶⁷⁷ mEMS lac.

	Pick	-	+	and streak out	mEMS lac	(for use in backcrosses)
	lac	Mal	Xge	MH	Sal	
1	-	-	-	-	-	
2	-	+	-	-	-	?
3	-	+	-	-	-	?
4	-	+	-	-	-	.
5	-	-	-	-	-	
6	-	-	-	-	-	mucoid
7	-	-	-	-	-	
8	-	-	-	-	-	
9	-	-	-	-	-	
10	-	-	-	-	-	mucoid
11	-	+	-	-	-	
12	+	-	-	-	-	
13	+	-	-	-	-	
14	+	+	-	-	-	^{2/16} ?
15	+	-	-	-	-	?
16	+	-	-	-	-	

Cross lac+ to W1177
 lac- to ~~W1177 lac+~~ (W 1372) W 1394-410/S.

12x : 47+ : 37-

14x : 8+ : 39-

1x 110+ : 31-

- B. Also ~~58-161~~ ^{W478} x W1177 : 1-3: ~~Mal-lac~~ ^{#3 is lac+ of this lac} 3/20 lac EMS
 4-12 : 9/20 MH EMS
 - C. 58-161 x W1022 : #9 Mal+ all others Mal-
 - D. 478 x 677 : #6, 10 lac- all of these lac
- B: 26 Mal+ tested on S.
 25 S^s 1 SR. (lineage fatum)

Check above: 6 Mal - MH? lac v?
 10 " MH ✓ hestact (Rev??) Reconfirm! H268

11/30/50.

Repeat W 478 x 1177 mEMS MH. Isolate possible MHv and check. (Plates have ca 30%+ to 26/80.)

a. 12/2 80 tests: Reisolate ²² EMS MH+ from gross streaks on EMB MH.

12/3 26 - 6 ...

A+ = streaks from gross streak. m EMS lac

	MH	lac	Xyl	Mal		lac	
1	✓	✓	✓	✓	-	+	-
2	✓	✓	✓	✓	-	+	-
3	✓	✓	✓	✓	-	+	-
4	✓	✓	✓	✓	-	+	-
5	✓	✓	✓	✓	-	+	-
6	✓	✓	✓	✓	-	+	-
7	✓	✓	✓	✓	-	+	-
8	✓	✓	✓	✓	-	+	-
9	✓	✓	✓	✓	-	+	-
10	✓	✓	✓	✓	-	+	-
11	✓	✓	✓	✓	-	+	-
12	✓	✓	✓	✓	-	+	-
13	✓	✓	✓	✓	-	+	-
14	✓	✓	✓	✓	-	+	-
15	✓	✓	✓	✓	-	+	-
16	✓	✓	✓	✓	-	+	-
17	✓	✓	✓	✓	-	+	-
18	✓	✓	✓	✓	-	+	-
19	✓	✓	✓	✓	-	+	-
20	✓	✓	✓	✓	-	+	-
21	✓	✓	✓	✓	-	+	-
22	✓	✓	✓	✓	-	+	-

worthy isolate by 6/24/52 see 951.

of 20 diploids MHv, all are Xylv.

9 are lac -
10 are lac +
lac v.

23	✓	✓	✓	✓	-	✓	
24	✓	✓	✓	✓	-	✓	
25	✓	✓	✓	✓	-	✓	
26	✓	✓	✓	✓	-	✓	
27	✓	✓	✓	✓	-	✓	
28	✓	✓	✓	✓	-	✓	

lac⁺, - components: 11, 12, 18: on EMS lac, these papillae show lac⁺. On EMS lac:

11: + colonies obtained

→ lac⁺ Xyl⁺ MH⁺

12: - and ⁺ on EMS lac.

→ lac⁺.

18: EMS lac +.

lac⁺. Xyl⁺ MH⁺

8 lac⁻, (+)

13 lac⁻, (+)

~~EMS lac: pure! (Error in picking?)
or recording.~~

11/29/ff/50.

- (8) Reisolated from single MHL^v colonies streaked on EMS Lac; Mal.
 12/2/50. #2 shows several papillae on both Lac, Mal. Purify.
 (#6, 7) isol. pap. on Mal. "
 → 2: All MHL⁺, Lac⁺; Malt⁺ ...

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

- | | | | |
|-------|------------------|-------------------|------------|
| 3 (1) | MHL ⁻ | Malt ⁺ | } not test |
| 4 (2) | " | Malt ⁺ | |
| 6 (1) | MHL ^v | Malt ⁺ | ✓ |
| 7 (2) | MHL ^v | Malt ⁺ | ✓ |

lac - homozygotes

778

October 26, 1950

10/26 A W466 x W1177 ^{Xyl+} BMlac⁺ het x lac - Mal - Xyl - on EMS Xyl
 B " x W814 " x lac⁺ Mal - Xyl - "

10/28. B: EMSlac

+	-
25	14
40	19
65	33

No yield on EMS Xyl.

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

B. 20 picked: 4 Xyl+ 16 Xyl- (sic!) No X.

Repeat on EMS MH.

A) 50 MH+ streaked on EMB MH; Xyl. No Xyl, ... v.

Repick further colonies

10/7 52 picked, streaked on MH:

	MH	Xyl	Mal	S	Sal	
1	v	v	-	R	-	H258
2	+ out?	+	-	R	-	H261
3	+	+	+	S	*+	
4	+	+	+	S	+	
5	+	+	+	S	-	
6	+	+	+	R	-	

3 very likely MHv 1-3
 3 possible 4-6

778-2: MHv verified from EMS → EMB MH

258 REVERSION TESTS: H258, 261 on EMS lac, Mal
 8 distinct reversions lac⁺ each lac⁻ MHv
 1: Mal + MHv
 H261 8 " " each lac⁻ MHv
 2 - Mal + Mal + (no test).
 Test organisms from #1: 5 lac⁺ → MH-
 10 lac⁻ → MH-8, +2
 not suitable for linkage phase study.

lac - / - : Mal - / -

October 26, 1950.

A W1325 x W826
 B " x W828
 C " x W836

Hist; sup/ly
 Mist; glet
 Met^{lys}; hist

EMS Lac, Mal

	Mal	-	+
A		81	26
C		80	38

Pick +, - to EMS Lac for linkage test.

		lac -	lac +
A	Mal -	25	3
	Mal +	19	4

	Lac	-	+
A		143	8
B		69	27
C		174	25

	Mal -	20	2
C	Mal +	18	4

Ca 70 tests each. Retest likely lac^v:

no linkage to Mal to lac
 (1 etc. from their trials
 not single lac EMS
 test.)

A. 1' lac+
 1' Lac+, -

B. 1' lac+ Mal-
 1' lac+ "
 2' lac+ "
 2' lac+ "

C. 1' + -
 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 (20ml → 1.5)

plate .1 ml / EMS lac.

a = no treatment

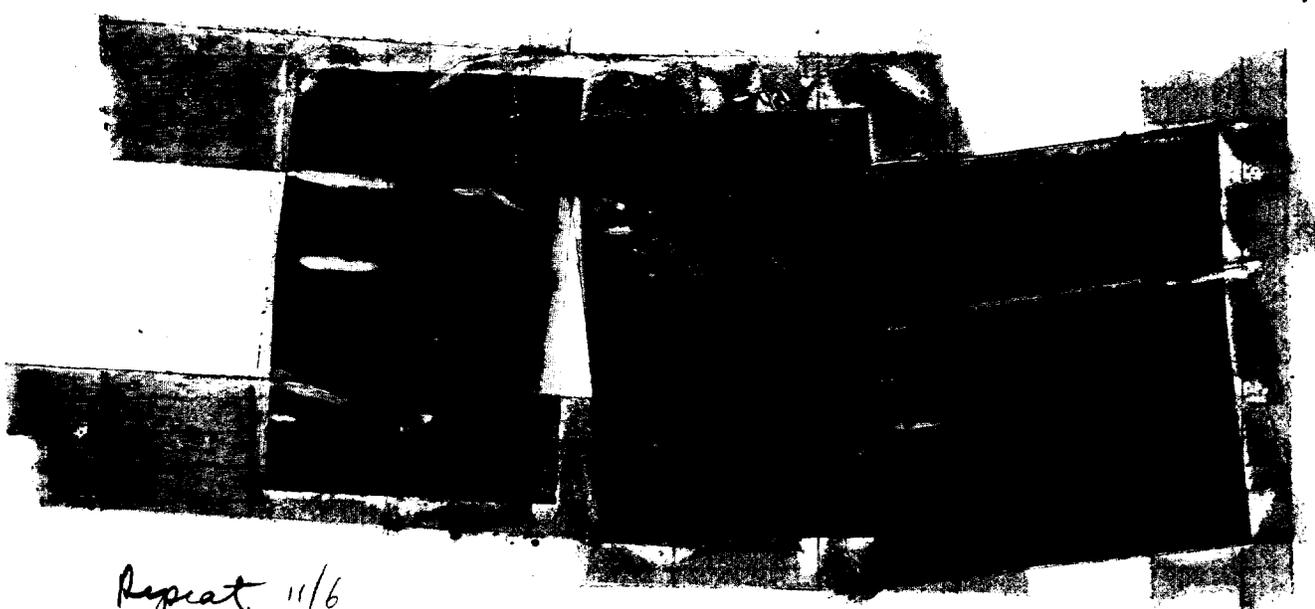
b = 10 secs UV 50cm.

	EMB	
	lac-	lac+
a	24	
	29	
	31	
	37	
	23	
	58	
	69	
	38	

ca 40/ 0

b.	-	+	
	17		
	146		→ Pure +.
	61		mostly tiny
	57		
	66		
	66		
	87		many tiny
			several hundred
			large, very many 2?
			small

ca 100/ 3?



Repeat 11/6
 Spread mixture (in saline together ca. 2 hrs.) at 3:15 PM.
 Irradiate 10 secs. at intervals:

11/6-10. ³¹⁵ Control: no rad. 10 plates
³¹⁵ 10 sec 4V 3 "
⁵¹⁰ " " 3 "
⁸¹⁰ " " 3 "

No marked effect of irradiation at any time: see points
 Probably more small colonies in 40 series.

In ca. 6000 colonies, 3 likely lac+.

	lac	Mal	S (EMS)	
#1 at	✓	-, +	S	Mal- is pure S ^S
#2 315	+	-	R	3 Malt recessions pure M+ Lac ^v . ∴ Mal-1A
#3 315	✓	✓	? (S)	

Restreak 1, 3 from EMS lac to EMS lac
 EMS Mal
 Bush EMS lac
 Test SR.

Also streak B on EMS Mal for Mal+ component.

1a-d lac^v (rel. stable) Mal- S^S

3 a-g. Lac^v. Mal^v very sensitive to sun. (entire streaks selected or destroyed ex. for segregants).

Recheck segregants for SR/S^S. For 1, use no. growth of S test.

Plate H267 in EMS lac, Mal ± SM.
 (5x10⁻⁸)

	✓	-	+
lac	46 37	3	3
lac SM	37 0	0	0
Mal	37	0	4
Mal SM.	0	1	0

Restreak H267 in Pannacoy
 Malt S^S may be per. segregant

-23 colonies from 116-23 plate.

Duplex Prototrophs

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. Strained out on EMBlac~~

[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 kinetic micr.

Reck 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 are overestimated.

Hold x-plates in ref. for micr

sample conc. inocula in EMSlac SM. ca 10 colonies /plate.

20: Test for SR: all SR, λ+

11/10 of 15 possible Mal_± streaked out on EMS Mal, 6 were Mal+/- (4+ 5-).

#1 also had a sectorial colony. Restreaked as 782A1.
Test paired segregants for lac, Mal, Xyl, Hfr, Gal, V₁ and SR

	Mal ^T	-	S	S	lac		Mal	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, V₁ (#4 only exception).

C 4/19/50. 58-161 x W1177 in EMS lac; B₁.
 15 plates ca 30 / plate. No lac±.
 -> +.

lac, Mal B₁ were
trivial.

lac± are not a regular occurrence!

Duplex prototrophs

782b.

11/11/50. Repeat 58-16 \ x W1177. EMS Mal
30 plates. ca 100%. Mal+/- scoring: optional
Total Mal+ (incl. \pm) 277.

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

+	-
19	101
8	47
9	47
10	42
4 (15)	42
10 (15)	53
60	332

defect inspection, 13 possible Mal±
Pick these; reincubate all plates. 11/14: Additional possible Mal±.

Also pick non-sectored Mal+ and - to EMS Lac.

f. $60 > \frac{277}{50}$.

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

Non sectored colonies. (to EMS lac; Mal for) Hold for later analysis

	lac+	lac-
Mal+	33	34
Mal-	25 _{±2}	44 _{±1}

Punct 7
mistake

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

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

Lac	concorded	14	Butall --
Xyl		10	" " --
MHE		15	
Gal		13	
V ₁		14	

Lac; V₁ concorded 13

#18 was Lac_s. In view of concordance of Mal-S^R probably not an artifact.

~~reference 78265~~

11/6/50.

Incubate P5 into D(Lac). Grow 36 hours aerobically.

Plate out at 10⁻⁷ m EMS Lac } ± SM 12 N7
 EMB Lac }
 EMB Mal

11/8. EMB Lac:

v	-	
174	79	245
129	62	291

Repeat 11/9: v

EMB Lac SM

0	51	51	1000u SM	0	7
0	57	57		1	4
0	54	54	Alt?	1	8
* 3	60	63			

EMB Mal

v	-	+	Σ		
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.

28 Mal- : Xyl- S^R
 4 Mal+ : Xyl+ S^S

A S Mal Xyl

* Exceptions:

	Mal	Lac
1	-	v
2	-	v
3	v	v

Streak out 3 for Mal+.

204 1
210 2

EMS Lac

Lac+ (196+49) 2 Lac- 49 small eds. Lac+?

EMS Lac SM

0, 1, 1, 0, 55 66, 47, 44. Total: 2+ / 212 - 0, 4 (tiny)

EMS Mal

Mal+ Mal- Mal v! (sic)
 172 55 21

See previous page
SR lac⁺ exceptions.

	Mal	lac
1	-	✓
2	-	✓
3	✓	✓
4	✓	
5	✓	
6	+	?
7	-	

see E.

4 Mal⁺ separated: SR ✓.

D. Inc H257 1/100 Penassay. Grow overnight and plate out.

11/16 = H257'

EMB lac	✓	-	
	85	26	
	75	15	+ sprinkling -
	73	14	
	81	37	

average - : $\frac{92}{4} = 23$

EMB Mal	✓	-	+
	75	11	6
	89	14	4

EMB lac SM+	8	3
SM .5u/ml	36	36
EMB Mal SM+	5	1
	11	0
	10	0
	11	1
.5/ml	29	0
	33	2

Phenotypic lag?
Test lac⁺ segregants
for SR. - Rather uncertain
facts: R S
25 23 Mal⁺ 11 Mal⁻

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 EMS Mal

(EMS) Mal v.

Almost all colonies of H257 plating show some signs of Malbraugeten.

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

Pick Mal+, - prototrophs separately to EMB Lac:

6: Mal+ Lac+ 1: Mal+ Lac-
 Mal- Lac- Mal- Lac-

1: Mal+ Lac+
 (Mal- Lac-) Restreak on EMB, EMS Lac as 183B1 ✓ Lacv.

BB. H257' (~~per~~ Y2 1:100, 24h, 37° 48h. RmT.)

EMB Lac	v	-	EMB Lac	v	-	
	123	43	SM 100u/	4 (3... incl)	82*	smear
	129	100		1	71 ±	smear
	116	94		0	60	"
	125	107		0	83	smear.
	127	111		1	56	not smear.
	<u>620</u>	<u>505</u>		<u>6</u>	<u>349</u>	
m	132	101		1+	70.	

* These counts are likely overcompensated for smearing, i.e., overestimated. 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				9	

Limiting conc. SM. H257.

783DP

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

EMB Lac	^{v and +} 149	8
	150	16

EMB Mal	185 (incl)	10
---------	------------	----

EMB Lac SM.5	127	19
	44	16
	18	24

} Lacu in this series have very diminished faded + var. centers.

EMB Lac SM100	0	3
	1	1

EMB Mal SM.5	63	27
	30	27
	58	18

very faint

SM 100	0	L
--------	---	---

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 phenotyping as it will produce artificial S^R from S^R/S^S .

Plate H257 11/10 on indicated media. Read at 40 hours

EMBlac	+ = v 169 184	- 14 11	E 183 195
EMBMal	+ 2	- 10	v 149
EMS Mal	209	4	213
EMBMal SM (100u)	0 2 1 1 1	13 5 4 4 3	0 0 0 0 1
	5	29	0

EMS Mal SM
(100u)

EMS Lac SM
(100u)

EMBlac SM
(st.)
(100)

1000
100
50
10
5
2
1
.5 *
.2
.1

+	-	v
0 0 0 0 0 0	0 0 2 9 1 7	0 0 200 100 100 100
4	17	200
9	9	100
9	5	100
180	9	100
24	1	45
13		177

Test Mal+^{S^R} and Mal-S^R on
EMBlac Xyl:
Xyl+ Xyl-
Mal+ 5 0
- 3 25²⁺

Consistent with:

	R	-	-	-
- A	Sm Mal	Xyl	MHL	
	S	+	+	+
	↑	↑		
	M-X+	M-X+		
	OX = M-X-			

Test MHL: Distribution of
+, - and above.

* sm app. unevenly distributed as center of plate is virtually sterile. Many v colonies have very faint test component 2 lac v have very little -. Restrict as possible crossovers.

M257 part seq.

Mal Reversion

783E

11/18/50

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

8 Mal+: 1, 5, 8 are Mal^v. lac^v
 2, 3, 6, 7 Mal + lac -
 #4 → Mal⁺, lac^v (Mal^v?)

E7#4 → Rechecked from single colonies
Mal^v (+ predominant) lac^v.

From
EMS
M257

From
EMS
M257

November 20, 1950.

Plate H257¹; H267¹ on EHB lac; ± SM 100. Cf. with % S^R.

11/19/50: H257.

V	Lac
92	143
78	97 ^{etc}
82	130
82	132
84	135
<hr/>	
399	637

$\bar{m} = 80 \quad 127$

V	Lac + SM...
0	125
0	99
0	130
0	94
1	126
<hr/>	
3	574
<1	115

Fraction S^S, from streak tests on Lac -

V	Mal	-
102	10	109

V	Mal	SM
1	3	92

H267

V	Lac
43	84
25	78
28	73
34	69
26	97
<hr/>	
156	401

$\bar{m} = 31 \quad 80$

V	Lac	SM
0	6	
0	4	
0	6	
0	5	
0	5	
<hr/>		

V	Mal	-
28	54	18

V	Mal	SM
0	0	2

Threshold sm: # S^R/S^S heterozygotes.

1835

11/20/50.

Plate each of following strains grown on 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	st. unk. +
lac SM 1	sl. unk.
Mal SM.5	sl. unk. form.
lac SM 100	No colonies



0 15 1

W1177 No differences.



0 15 1 100

H266 (S^S/-)

40 hours.

lac	typical mosaic colonies ca 400
15	reduced count; smaller lac-, Mal-
1	19 colonies
100	No colonies.

do.
All lac - Full et.
21 lac- colonies
No colonies.

(Suggesting partial resistance)



15 0

H257 lac typ. (somewhat small) lac^v. ca 400

15	reduced Lac - Mal - (hint of +)
1	cols. () some normal.
100	



0 15 Mal 15 lac 100 1

H267

lac	typ	lacV	ca 300	
Md. 5	5 large	1-3 v. small	cols	—
lac. 5	10 md	1-2 "	"	—
lac 1	2 large	cols.	lac -	
lac 100	5 large	cols.	lac -	

H267 may be more resistant than H269, or give SR survivors more readily.

Linkage comparison: S^R minimal

784

11/7/50.

W1368 x

W677	standard	A
W#78	1mV	B
W 1022	1mV	C
W 1015	mV	D

A	+ TLB,	-	+
		12	45
		18	40

(same ~~unreliable~~ doubtful
mucoids recorded as +)

B	SM + TLB,	Lac-	+
C		4	72
D		5	12
			15

SM-0.

0 0

Need repetition

11/18...

	+	-	
A	39; 53	14; 13	
B	37 42	0	
C	6 15	5; 0	
D	20 16	0 0	

10/7/50.

See 786.

A. Steile.

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

Pick to water; spot on EMB, D(0).

B: 11 tests: uneg on D(0) in 24h.

Light

		A1	A2	A3	A4	A5	YE _x	YNA	MC	
785B	1	(A1) 1421	++				++	-	+	
	2	(A3) 1425		±			+	-	+	
	3	(A3) 1426		+			±	-	+	
	4	(A2) 1423	+				++		+	
	5	A3		±			±		+	
	6	(A1) 1427					±		±	
	7	(A4) 1428			+		++		+	
	8	A1					+		±	
	9	(A3) 1427		±			±		+	
	10		+				++		+	
	11	A3		+	-		+		+	
	12		+				++	+	+	
	13	(A4) 1429			+		++		+	
	14	(A2) 1424	+				++		+	
	15		+	+	+		++		+	
	16	A3		+			+		+	
	17	A1	+				+		+	
785B	0				+		++		+	
786B	0				+		++		+	
	1	(A4) 1432			+		++		+	
	2			+			+		+	
	3			+			±		+	
	4			+			±		+	
	5			+			±		+	
	6			+			±		+	
	7			+			±		+	
	8			+			±		+	
	9			+			±		+	
	10			+			±		+	
	11			+			±		+	
	12			+			±		+	
	13			+			±		+	
	14	A4		+	+		++		+	
	15			+			±		+	
	16	A4 1433		±	+		±		+	
	17	(A3) 1431		++			++		+	
	18			±			±		+	
	19	(A2) 1430	+				++		+	
	20			+			±		+	

Cyst Tyro. Tryptoph IV

No A4 resp. Trypt. or hist. not tryr.

Hist IV

A4-

A4- A3- Leucine

All - unless indicated otherwise

(38-40 = 1431-33)

10/7/8

UV-30sec - irradiated 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	Y ₂	
21	A3?	-	-	±	-	-	+	+	No response to A3.
22	A2	-	#+	#-	-	-	+	+	
23	A4	-	-	-	+	-	+	+	PROL TO D(0) +
4	A1	+	-	-	-	-	+	+	
5		-	-	-	-	-	+	+	
1441	A4	-	-	-	+	-	+	±	PROL
6	A4	-	-	-	-	-	+	+	
7	A4	-	-	-	+	-	±	-	
8	A2	-	+	-	-	-	+	+	
30	A2	-	+	-	-	-	+	+	
1	A4	-	-	-	+	-	+	+	
2	A4	-	-	-	+	+	+	+	
3	A4?	-	-	±	+	+	+	+	
4	-	-	-	-	-	-	+	-	
5	-	-	-	-	-	-	+	-	
6	-	-	-	-	-	-	+	+	
7	-	-	-	-	+	-	+	+	
1431	A4	-	-	-	+	-	+	+	No resp #2
1432	A2	-	+	#	-	-	+	+	W; 0; +.
9	-	-	-	-	-	-	+	-	
1433	A2	-	+	#-	-	-	+	+	as 1431
0	A4 ++	+	+	#+	+	+	+	+	

None to YNA

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

Throw out non W -

Double mutants:

1421 1
 1423 5
 1429 3
 1430 2

11/25/50.

- A W1377
- B W1395
- C W1396
- D W1397
- [E W1441]

A-D grows in ^{D(0)} ~~Penicillin~~, E. D (prod).. Inactivate directly (30 sec. UV 50 cm) and inoculate 1:10 in Penicillin 11A25. Wash 8P. (C shows very little growth - unusually sensitive to UV?) broc. ca 1:500 in D(0) + penicillin [+ prod. for W1441].

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

U/ml.	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85
	+	-	-	-	+	+	-	++	-	++	+	+	-	-	-	-
	+	-	-	±	-	-	-	-	±	±	-	±	±	-	-	-

all 5
subcultured

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

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

from 2d plating of C.

12/12 Repeat penicillin runs. using 300 u/ml., 6 & 24 h. platings & 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	Mary ++		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+ (smaller cols.)
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	Mary ++		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	
32 786-2	0 0	
33 786-3	0 0	
34 W1416	0	
35 W1177	0	
36 31+35	5+ 8+8+	+, - ++
37 32+35	1+	1- 3-
38 33+35	2+ 2-	1+ 2+ 2-
39 31+32	1+	1+ 0
40 31+33	0	1+? 0
41 32+33	1+	0 0
42 34+35	0	0 0

36 etc
 mix after washing

36'	0 0 + =	40'	
37'	1+	41'	1+
38'	0	42'	0 0
39'	0		

W1416 uncrossable
 W1374, 75 mutants
 remarkably infertile
 if SR x+ crosses.

Infection of W578 with λ

11/14/50.

To 5ml washed W578 suspension in saline add
 1ml broth lysate of λ (11/7). 2:38 PM ^{7/10} 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 W578

- A. washed cells
- B supernatant 1
- C supernatant 2
- D original λ , titrate.
- E original W578

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

~~E~~ 18; 6 plaques on W578

~~B~~

B 19; 11 " " "

C 20; 18 " " "

D 10⁵ 45 4 7
 -8 47
 -7 28
 -8 64

Nov. 20, 1980.

A W836 x W1177
 B x W1178
 C x W1406

2 MHR 2. colonies on EMS MHR.

~~A: Malt+ S^S~~ A: <+ Malt+ S^S
~~Malt+ S^S~~

∴ true duplex colonies. B <+ Malt- S^R
 Malt- S^R

A. Plate MEMS lac; MHR.

lac: 60+ = 104- MHR: 57- 47+ 25 (2 plates)

Test linkage of lac, Mal. lac + Mal + } MHR+ ++ ±
 - + } - ± ++

B. lac: 137+ : 29-

C.

C. lac: 33+ : 60-
 34+ : 57-

MHR: 70+ : 32-

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

50 added. B + C. → a few lac-; all others lac++ nov!
 C1 - Malt+.

B 1-4 } apparently pure lac+ { Mal- B
 C1 } Mal+ C

→ self-ploid (λ).

Reshells B3 which gives some V₁^R MEMB.

check parents: ~~W836~~ parents to be X^S. W1406 is λ⁺ (Rev)

790 B3 is verified lac v, but very stable. Test also for λ.

Apparently λ-. H

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

Various various sugars & phages.

		TI	72	T4	T5	T6	T7	λ	518	Lac	MAL	Syl	MAL	Suc
776-46 67 68 69	1	1442 ^S A	R	R	R	P	R	R	-	+	+	+	+	-
	2	1443 ⁴⁶ R	R	R	R	R	R	R	-	+	+	±	# ↓	+
	3	1444 ^{u₆₇} R	R	R	R	R	R	R	-	+	+	+	+	++
	4	1445 ^{S₁₀} R	R	R	R	R	R	R	-	+	+	±	# ↓	+
	5	1374 R	R	R	±	R	R	R	-	+	+	±	+	-
	6	1375 R	R	R	R	R	R	R	-	+	+	+	+	-
	7	1377 R	R	R	R	R	R	R	-	+	+	+	+	-
	8	1395 R	R	R	R	R	R	R	-	+	+	+	+	+
	9	1396 R	R	R	R	S	R	R	-	+	+	+	+	-
	10	1397 R	R	R	R	R	R	R	-	+	+	+	+	+
	11	1397 K S	R	S	S	S	S	R	+	+	+	+	+	-

~~colicini?~~
col?

colicini?

All are P^R w/ K12 P^P

Diazini M. U. (1950) Bollettino I.S.M. 29: 161-172. Mutazioni indotte dagli acidi nucleici batterici.

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

11/20/44. '50.

	783E-+	EMB Mal	trans EMS Mal	EMB Lac	
1	8	++	-	-	
2		-	-	-	
3		-	-	-	
4	9	++	-	-	
5		++	-	-	
6		++	-	-	
7		v	v	v	Mal v lac v
8	10	++	-	-	
9		++	-	-	
10		++	-	-	
11	12	++	-	-	
12		++	-	-	
13		v	v	v	Mal v lac v
14		++	-	-	
15		++	-	-	
16		-	?	?	Mal- lac+ (reversion??)
17	13	++	-	-	
18		++	-	-	
19		++	-	-	
20		++	-	-	
21	++ -	v	v	Mal v lac v	
22	16	++	-	-	
23		++	-	-	
24		hgr	-	-	
25		++	-	-	
26	16	++	-	-	
27		h.gr.	-	-	
28		++(-)	-	-	
29		++	-	-	
30		++	-	-	
31		++	-	-	

Reverts 7, 13, 16, 21 in EMB Lac; Mal.

~~K12 x K13, K14~~

WG-1 x WG 3, 4

- A W1446 x W1435 (WG4 x WG1 Het) → H269
- B W1446 x W1177
- C W1449 x W1435 WG3 → H270
- 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+ L+: 30 M+
 1 M- 1 M-

	lac +	-
A.	1	25
	1	8
	0	6
	0	16
	0	7

lac- predominates!
 streak out lac+. Bunch lac- to Mal EMS.

B. rather low yield (3-5/pl.) all lac -

C. Mostly lac+

4	5
7	1
2	3
12	1
8	2
<hr/>	<hr/>
33	12

very variable colony morphology.
 Pick¹⁰² and streak out on EMS lac

- D. No prototrophs (4 plates) [Allelic cures??]
- E " " " " ? (Part of B)
- F " " " "
- G. v. Numerous prototrophs. Mostly lac+. Cf. on EMS Mal.
 Ca 1% lac -.

32 lac^+ streaked on EMB lac .

#6 Lac^- . All others lac^{++} . Reisolate.

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

Reisolate #6. \longrightarrow Pure lac^+ ! Lac^- ?

lac^- : 4 Mal^+ 4 Mal^-

M270

Choose weaks lac^+ for possible lac^x .

W1452 x W1262

795

For ~~Malt~~

W ~~452~~ 1452 x W1262 m EMS Mal.

Pick Malt and bush against 5/19 m Lac EMS.

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

4 Malt₂ noted. Reacts on EMS ~~to~~ Mal; EMS Mal; Lac.

795:1-3 of 16 tested in first selection, 3 are Malt S³ on EMS. Repeat
Streak on EMS Mal for v test. 10 react Mal- on EMS..

After re-incubation, additional Malt appear. Test these as above
8/19 tested 4-11

1 pure malt

2 ?

3 Malt.

Retent: Malt + Lac.

Hold in abeyance.

12/16-1955

			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	(carotennants?)
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. Nov.

E: 4 Lac+

G: 8: 6 Lac+ #2-7 1 Lac- #1 1 Lac. Resolute

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

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

G: 1 Lac- Mal- 5 Lac+ Mal+ 1 Lac+ Mal+ #1 Lac ✓
MHV

E: ✓ m Aciflavine : 2 S 2 R OK.

colonies very similar on Tryptone agar.

6 Lac+ } sup. tested
9 Lac- } all Acif. R.

12/26.

Cf. characteristics

C' all Aciflavine R. (as parents).

G: 1 S
2 a R b S
3 R
4 R
5 R
6 a R b S
7 a R b S.

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

see over:

Compare various photomicrographs of 796 G.

	T7	Aer.	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" macrophage " grain diffraction pink diffraction

#1 gives an undoubted Acif R reaction, but

redispersed very readily to resemble S or RS.

Morphological differentiation probably better on EMB.

W1435 x W112 in EMS M^H.

Pick M^H + , purify in EMS M^H. Test for discordant V_6 reaction in EMS, ~~EMS~~ MS (M^H).

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

M271-273.

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

M272 - Lac ↓ ?

$V_6^S \rightarrow$ Lac - stable $V_6^R \rightarrow$ Lac - unstable in EMB Lac.
 strains ~~D~~ (M^H)

cf EML60 11/28/50. Nonallelics of Lac^{lac} - b.

12/23/50.

See 777B.

Ca 1/2 MHE isolates are ~~lac~~ lac - 1/2 lac^v.

Some lac- EMS may have come from duplex, whence lac⁺ might be isolated. All original 1-22 are on D(MHE) or D(lac) ~~media~~
 lac⁺ isolated: 11, 12, 18. All appear to be stable lac⁺.

11/23 In course of isolations: 8, 13.

To be isolated: 14, 17.

#8⁺ is ~~purely~~ lac⁺, apparently pure, but unstable.

in 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. ^{vs} _{ET45.}

#17 + and - of "14-"

Note: since lac^v components of #8, 13, and 17 have already been isolated, attend to MHE character of lac- "segregants".

12/27. "14+" is pure lac⁺ MHE - 14- : pure lac- MHE + (? Rev).

#8+ lac^v OK.

17- : 3 MHE + 1 MHE - NOV.

8- 4 MHE + NOV.

13- 4 MHE - NOV.

Tentative conclusion: These cultures which give lac- prototrophs from lac^v isolations are throwing prototroph segregants, not partial segregants. Recheck from original slants. This does not explain 11, 12, 18 which are apparently duplex.

Comparison of lac₁- homozygous diploids
and parents

12/24.../50.

lac (EM13) 36h..

1	H271	Bright red centers (confluent papillae?)
2	H258	type - papillae in brush
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 studies for lac- mutability. Reconstitute W660 for new set of diploids carrying mutable lac₁-.