November 21, 1947.

1... Streak out presumed + and − derived from 58-161 on EMB-βΦgal. etc

	βΦgal	galac.	galac+ΦOH	
a)	++	++	no growth	WY52
b)	−	++	no growth	WY53. (58-161 purified re βΦgal +)

↳ Note: this is failure to ferment, not growth inhibition.

2... derived from Y10.

segregation of 3φ, Lac.
W52 × Y53.

November 21, 1947.

Stocks which are 3φgal + Lac+ and 3φgal-Lac- are available. If there are three alleles:
Lac$^{\phi}$+, Lac+ and Lac-, only the parentals should be recoverable. If there are two loci, the type Lac+ 3φ- should be found in this cross. It can be controlled by testing the cross Lac+ 3φ- × Lac- 3φ- which should not segregate for 3φ. For additional segregating characters, M+ may be used.

P21 Broc cultures into 25 ml/125 YP broth. Incubate overnight at 37°. A22. Transfer 5 ml ea. to new 25 ml YP. Incubate 9AM- . Wash, etc., mix in T(0) and T(B,) plates.

A. W52 × Y53

B. W53 × Y53

A24. Suspend in 1 ml. H_2O and streak out on Lac EMB to obtain single colonies of Lac+ and Lac- segregants.

November 24, 1947.

A. WS2 × YS3 (Lac+ ϕ+ × Lac− ϕ−)

B. WS3 × YS3. (Lac+ ϕ− × Lac− ϕ−).

Bϕ+ Bϕ−

Streak out prototrophs on EMBlac agar and separate Lac− from Lac+ in pure form. Test them on ϕgal.

A (0) 9 : −

A (B1). 20 Lac− } all appear to secrete Bϕ+ on mass streaks.
7 Lac+. } Streak out 3+ and 3− on a Bϕg plate.

B (B1). 16 Lac−
1 Lac+

incl. parentals.

	Bϕ	Lac	
WS3	+	+	
YS3	+	−	!
Lac− segr.	+	−	
"	+	−	
Lac+ segr.	+	+	
"	+	+	

B 0) 1 +
2 −

If is possible that ϕgal− is not adept on Bϕgalactoside but that lactase splits the glycoside! N. B.

Assumption that YS3 is Bϕ+ Lac− is incorrect. All parentals must be retested.

Complementary genotypes.

Nov. 25, 1947.

Y87 Y10

Plan. Cross $B-M-T+L+B_1+Lac-V_1^R \times B+M+T-L-B_1-Lac+V_1^S$

and recover B_1-Lac- segregants. Plate these colonies into BMTL lactose agar to suppress the parental and major recombinant type. The only types which could survive are B_1+Lac+ which includes the complementary genotype ~~B+M+~~ $B-M-T-L-B_1+Lac+V^?$ and also possible reversions of $B+M+T-L-B_1-Lac+V^S$ in re B_1-. This procedure affords at least some chance, however, of recovering the complementary type by selective means. 20 colonies plated.

#11, 13, 14, 15, 17. are Lac− (i.e. 5/20). Throw out other plates.

Streak these out.

	TL	
1	T	
2	T	
3	T	
4	T	
5	T	
6	separate colonies	
7	separate colonies.	
8	T	
9	T	
10	T	
− 11	S.C.	1 colony.
12	T	
− 13		1 colony.
− 14	S.C	1 colony. Pick.
− 15	S.C.	>10 colonies. Pick 1-5.
16	T	
− 17		3 colonies. Pick 1-3.
18		>10 colonies. Pick 1-5.
19	T	
20	T	

	TL	BM	TLB$_1$	BMTLB$_1$
1	11-1	−	+	+
2	13-1	+	−	+
3	14-1	+	−	+
4	~~15-1~~	−	+	+
5	15-2	+	+	+
6	15-3	+	+	+
7	15-4	+	+	+
8	15-5	+	+	+
9	17-1	+	−	+
10	17-2	+	−	+
11	17-3	+	−	+
12	18-1	−	+	+
13	18-2	−	+	+
14	18-3	−	+	+
15	18-4	+	+	+
16	18-5.	+	+	+

B−M− probably are reversions of Lac; TLB$_1$+ maybe B$_1$− reversions of the TLB$_1$ parent. Use maltose instead which does not seem to allow reversion!

Streak out and test single Lac+ colonies for nutrition and phage. Compare by segregants.

No complements found.

Comparison of various grades of sugars for EMB test.

November 20, 1947.

EMB +1%:	Mal+	Mal-	Lac+	Lac-
Lactose c.p.		[+++]	-	alls+.
Lactose U.S.P.			+++ ±	alls-
Maltose, c.p. (Paragon)	+++	-	alls++	
Maltose, c.p. (E+A)	+++	-	alls+	
Maltose, purified (Merck)	+++	-	alls+	
Maltose, technical (E+A)	+++	±	alls±	

Larger colonies than c.p. lactose. Probably minimal amounts of monosaccharide.

24hr. readings.

+++ denotes good sized colonies with deep, uniform purple-blue coloration and a green metallic sheen. ± is faint pink coloration, suitable for scoring.

- denotes pale or translucent colonies. alls refers to development of blue coloration

Technical grade sugars therefore, seem to be suitable for preparation of EMB plates. Hereafter unless otherwise specified, EMB plates for mutant detection will be made up from Lactose USP (milk sugar) Mallinckrodt and Maltose (Malt Sugar) Technical, E+A.

Lucis, program, appar. follow:

	(c.p.)	(Merck USP.)
Maltose	.03	.002
Lactose	.002	.001

Adaptation Expts: Prelim.

Nov. 18, 1947.

Cells grown in lactose, β-φ galactoside + glucose are sedimented and washed. Resuspension ca 10^{9-10} cells/ml. Cells diluted to comparable concentrations. Add 1 ml cells to 1 ml 4% sugar + .01 ml M/5 phosphate buffer pH 7.0. Add .03 ml BCP .15% pH tube as indicator.

Made up 11.15 AM. Acid production as a + ... +++ scale.

		11:30	11:45	1:30	A 19.
Glu /	glucose	−	−	−	+++
	lactose	−	−	−	+++
	β-φgal	−	−	−	−
Lac /	lactose	++	+++	✓	+++
	βφgal	−	−	−	−
βφ /	lactose	−	−	−	~~+++~~ ++
	βφgal.	−	−	−	−

Urease in coli.

3/

Nov. 20, 1947.

Prepare media with peptone 1% agar 1.5%, Phenolphthalein .01% ± glucose .2%, ± urea 2%.
After autoclaving, phenolphthalein turned slightly. This subsequently disappeared.

	A21	A22	A24
–	growth, no color	~	turning pink
Glucose	" "	~	~
Urea	Growth inhibited	growth, no color	~
Urea + Glucose.	Growth inhibited.	" "	~

This does not seem to be a satisfactory method for demonstrating urease.

Nov. 26, 1947.

Make up media containing ½% Na Formate, 1% peptone, 1½% agar. and various indicators, ± glucose .3%

1. EMB. glucose a) [illegible] 24h. 36 do.
   glucose + formate b) [illegible] light lavender.

2. Phenolphthalein .01%
   formate a) [illegible]; growth inhibited somewhat.
   — b) [illegible], good growth.

3. Bromcresol purple. Add AcOH to medium until turned acid.
   glucose a)
   glucose + formate b)
   formate c).
   } no growth (pH?)

EMB seems to be the most suitable, esp. glucose + formate.

Methyl Green sulfate - lactose:

Lac + colonies green, diffusing into agar
Lac − colonies transient & light blue
n. satisf. because of diffusion

EMB + sugar 1%: Inoc. streaked - out 58 161.

gentiobiose −
β methylglucoside —
& phenyl glucoside + uniformly.

Colony formation on synthetic agar.

~~32~~
52

Nov. 25, 1947

T(m) agar + various concentrations of sugars. Add BATLB.

	24h. 58-161.	36h.	24h. Y87.	36h.
Lactose:				
.1%	as below.	2 mm.	microscopic pinpoint; papillae.	
.05%	small, dfinite.	1-2 mm.	microscopic pinpoint (.1 mm)	
.01%	pinpoint.	1 mm.	no visible colonies; mici.	

.1% is a satisfactory level of carbon supplementation.

later, Y87 shows continually forming papillae on all plates.

On .1%, Y87 forms distinct colonies certain proportions of which contain revertents. .01% is also suitable.

Bϕ segregation; mal segregation.

November 25, 1947.

Cross W52 × W-1 on O, B, agar.

B- M- T+ L+ B_1+ Lac + Bϕg + Mal + × B+ M+ T- L- B_1- Bϕg - Lac - Mal- .

Came up very slowly and in small numbers. Segregants not used in view of 27b.

~~Use for maltose segregation~~:

Nov. 26, 1947.

Streak out 58-161 on EMB agar: .3% N.D. Amm. B, 1.2% galactose A 26.

A Definite colony dimorphism as previously described. : S R.
about 1:1

B. Streak out components and mixture on galactose EMB. A 26.
W-28 + W29.

Reversion? of C-2 mutants.

Nov. 29, 1947.

Plate 24hr. YP cultures into agar supplemented as indic. 10^9 per plate

Y138: T(0). No colonies.
Y138: Arginine: 1 colony?
Lysine: No colonies.
Arg + lys. No colonies. Not turbid!!!

Y142. T(0). >30 colonies.
+ isol + val. "
+ arginine + isol-val. >100 cols. Only sl. turbid.
+ arg. turbid.

Y138 + Y142 ... 0 >30 cols.
A. turbid. colonies form.

Check the requirements of these strains!!

Check C_2 mutants.

11/29/47.

	Y	T(0)	T:	T:	T:	T:	
1.	114.	0: −	iso −	val −	i+v. +	+++36	48 hrs. OK!
2.	117	0: −	±36 arg. +++			+36	adapted.
3.	120	0: −	✓ val + +++36			OK.	OK! Try crossing with 138 or 139, or make mutants from this strain.
4.	121	0: −	+++36 cyst +++			adapts.	
5.	132	0, −	arg. −	gly −	arg. −	no growth 36h. ✓	AB Both A+AG +++ Recheck Req.
6.	133	0, ±	arg ±+++36	lys ±+++36.	arg +++	adapts.	
7.	134	0:	arg	thre	arg.		
8.	137	0:	arg	tryp	arg		
9.	138	0: −	arg −	leuc −	arg +++ OK.		all OK.
10.	139	0: −	arg −	hist −	arg +++ OK.		T(0) OK others adapted.
11.	142	0, −±36	i+v −+++36	arg +++.	i+v	+arg. +++.	Requires arginine only! all +++. adapts on minimal +arg!

First readings at 24h., 2d at 36, 3d at 48. Inc. at 37°.

Y142 is very adaptable. Y138 + Y139 are fairly stable, especially Y138. do. Y120. and Y114.

Utilization of starches.

Dec 2, 1947

.05% in T(m) (BM) and 1% in EMB.

A Amylose (Clinton – from K.P.L.)

B Amylopectin (do.)

C Waxy Starch, soluble, from Brink.

D. Glucose.

P 11. Continued, slow utilization of amylopectin noted. to "++" compared to +++ for glucose.

v. slight utilization of ~~B~~ W, noted.

P16. Continued increase in turbidity. Density ≅ ca. ~~.1%~~ .01% glucose

P24 Utilization apparently complete. Rate measurements were exceedingly crude. Waxy starch was not utilized to nearly the extent that amylopectin was. This should be repeated for confirmation. Save flasks of amylopectin culture.

Exp. terminated this date.

Jan. 7, 1948. Compare molds from B with V55 mould on T(m)/BM + following:

		A10	A17	Johns #17 color
Ap. .05%				
Amylose .05%	α	±	+	faint red-violet.
	β	—	±	blue
Waxy starch.	α	++	+++	No color
	β	±	±	~~blue~~ blue
	α	+	+	Light Red
	β.	—	—	~~blue~~ Unstained.

SC 86.

all starch utilizations are correlated thus. Possibility of adaptation rather than mutative utilization not excluded. Compare c inulin on EMB!

Synthetic EMB Medium.

Nov. 29, 1947.

Medium,	per l.	/200 ml.
$Na\ Succ.6H_2O$.	5	1
Lactose	10	2
$(NH_4)_2SO_4$	5	1
$NaCl$	5	1
$MgSO_4$	1	.2

EMB; Agar

(Phosphate is in EMB mixture).

OK! Compare K-12 +++

B_1-lac- -- (+ B_1 added).

Dec 1, 1947.

W-1 x W-S3. T-L-B$_1$-Lac-Mal-βφ+ x B-M-Lac+Mal+βφ-

a) T(o) plates.

	Mal+	M-
Lac+	2	15
L-	2	44

b) T(B$_1$) plates.

	Mal+	Mal-
Lac+	1	10
Lac-	2	47

Total:

Lac+	3	25
Lac-	4	91.

/ 123.

In %

	M+	M-
L+	2.4	19.7
L-	3.1	71.6

Total Lac+ / Lac- = (23%)

	M+	-
L+	2.4	20.3
-	3.2	74.0

Lac+ = 22.7
Lac- = 77.2

Mal+ = 7.6
Mal- = 94.3.

∴ Mal is v. closely linked to B-M. Evidently not to B$_1$ in view of homogeneity of distributions.

probably between B and Lac. This leads to an excess of the triple type, M+L+. check on each purported example here of M+. Checked. Scores correct.

Dec. 3, 1947.

From same cross plates as 411, streak colonies on maltose agar + count, pick out M+ for lac characterization. (TB, plates).

M+	M−
1	38
0	47
1	50
1	27
3	36
4	43
3	31
13	272 / 285.

Mal + = 15/285 = 4.6%.

Test all Malt + on lactose:

	Lac +	Lac −
M+	10	5

Summary of Lac distribution among Mal +:

+	−
3	4
10	5
13	9 / 22

Total distribution:

	M−	M+
Lac +	272	15
Lac −	116	7
	388	22 / 400.
	94.5%	5.5%
Lac −	74.1%	2.2%
Lac +	20.4%	3.3%

From same plates as 41, segregate Lac+ and Lac- and streak an isolated colony on βφgal agar, EMB.
at 24 hours:

	Lac+	Lac-
βφ+	20	36 +1
βφ-	0	~~1~~ 0

20. 37

The parents were compared by streaking from YP broth and, unfortunately are not comparable. Neither W-1 nor W-53 was readable at 24h.

~~Isolate all segregants to small plants~~

Parents are also both βφgal+ and cannot be distinguished. A modifier may enhance βφgal-ase in ~~Y53~~ W-52.

To summarize, all available Lac+ are βφgal+

The "Lac-" of Y53+der., Y87+der., W30, W40 and W42 are βφgal+; The "Lac-" of W35;36, W43, W45, W48, W49

Maltose segregation.

41d.

Cell suspension stored 2 days in H_2O at room temperature was plated on T(0) and T(B.) as well as EMB.

On EMB, None of 171 was Maltose +.

On 3 comparable plates only 2 possible Malt.

On T(0). None of 139, streaked to Maltose, was Lac +.

(Check for B_1 interaction again.

On EMB, Lac segregation was: Plate rather crowded.

+	−	
40	66	Some colonies were noticed to be sectored!, as
16	37	if complementary or supplementary types were
56	103 /159	present.

In this sample, therefore, only 2/>600 was Mal+. Compare with above!

12/ /47

Lac- Bϕ- Lac+ Bϕ+

W45 x Y10.

T(0)

T(B,). Streaks to synth Lac(B,). →

Lac+	Lac-
68	9
58	3
126	12. / 138.

Lac- 8.6%!

Few or no Lac- noted on EMS-lactose crossing plates [Spiegelman phenomenon?].

This is a much lower proportion of Lac- than hitherto found.

Streak Lac- and Lac+ to Bϕgal

	Lac-	Lac+
Bϕ +	0	40
Bϕ -	12	0

[Check for alleleism with Y53.]

Suggests identity of Bϕ and Lac loci.

Cf.

Maltose segregation:

A. Y+O x W-1 Lac+ Mal+ Lac- Mal-

~~B. W-20 x Y64. Lac+ Mal- x Lac- Mal+~~

all plates too crowded.

On EMB nearly all Mal-. < 1:100 Mal+. These can be picked out more readily than in the mixed cross. However, the plates are too crowded to be very useful. Use T(O) etc. plates to confirm ratios.

Y120 × Y133.

12/4/47.

See 43 for cells.

(1) Base into minimal only (plates).

Y120 10^{-7} colonies

Y138 No colonies — two plates

Y120 + Y138. As above

~~Add 1 loopful to 4 plates for further incubation~~.

Y120 is too unreliable for sex tests

12/4/47.

Inoc. Y120 into YP. Irradiate 3ml. suspension in quartz flask. PS.

A6. Inoc. 10^{-7} dilution into T(Val) plates (detection).

A8. Layer 1% Y.Ext., 1% NZCase, 1% Agar on plates.

15 plates. Sample counts:

58
72
51
54
70
54
60

7 | 419 = 60 average

(3) small colonies recovered.

- -1 Not mutant, though inhibited by isoleucine
- -2 Contaminant
- -(3.) see ff.

Test Jan. 6. 1948:

		Valine + 3do.
1.	–	–
2.	NA	–
3.	EA	–
4.	N+EA	–
5.	HC (GB1)	±
6.	NZCase	+
7.	Y.Ext.	+++

Acc. 5, 6 also neg. on ints.

Allelism of Y45 Lac − and Y53 Lac −

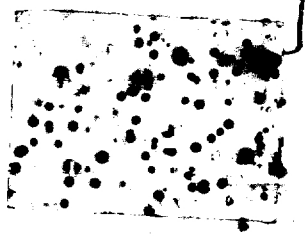
December 4, 1947.

Y45 × Y53. On T(B,) and EMS Lac(B,)

On EMB. Ca. 1:6 Lac+ : Lac− !!

[This suggests faulty identification of Y45 as Lac −
1) May be Lac+
2) May be $Lac_2 - Lac_1 +$]

~~Reddish~~. + −
ca. 16. 40

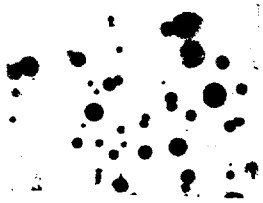


Yields seem to be higher on EMB. Come up with varying lag.

From T(B,).

−	+
25	4
19	7
31	8
22	8
97	27

/124



W45 x Y53.

Repeat 46. Check parents: Both −. W45 Allealigenic.
Lac+ parent in error! Of 41 isolates from T(0), 8+ 33 −

Streaked from T(B,) on Lac EMB agar to purify. Also, 29− 4+

62− : 12+ / 74

ca 5 : 1

On EMS Lac, most plates too heavy.

3 Thanim,

+	−
3	11
6	11
6	12
3	3
18.	37 / 55

The EMS procedure seems to be biased for lac+ compared to T(0) plating. It should possibly be improved.

1 Thanim.

9	20
14	31
12	35
34	56
69	142 / 211

Read from paper impression slips.

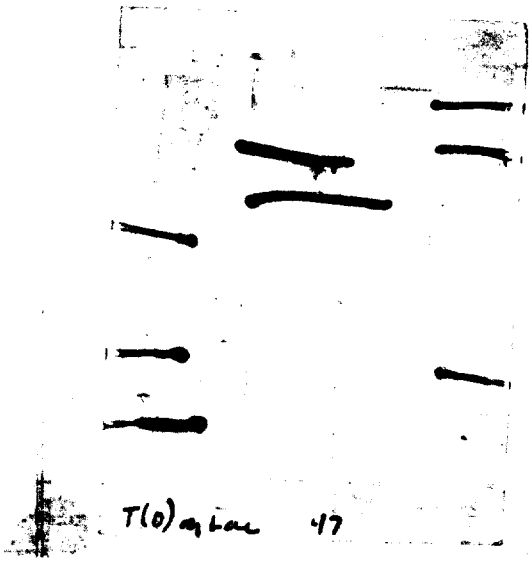
Gave same average ratio, however

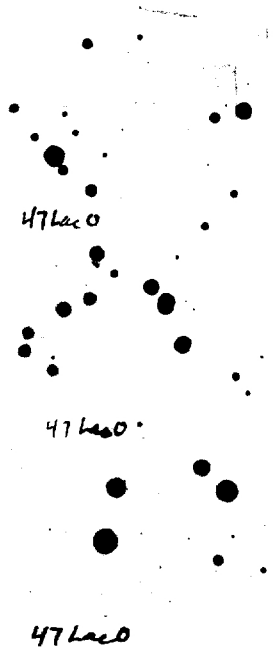
of 67% Lac +, whereas the random isolations give 80% Lac −.

χ^2 for difference is, approximately, at the 5% level.

8	33	41
14	28	

$\chi^2 = \text{ca. } \frac{36}{14} + \frac{36}{28} = 2.5.$





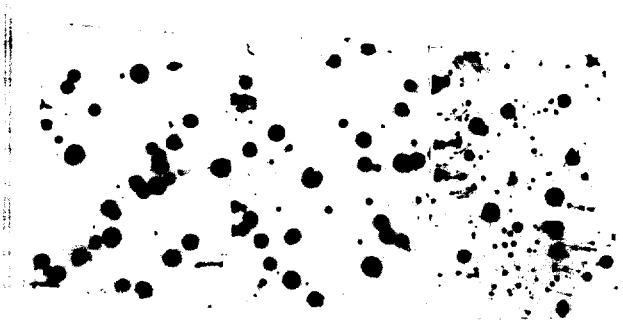
47 Lac O

Maltose Segregation

W-21 × Y64 B-M-T+L+B+ Lac+ Mal-V_1^S × B+M+T-L B_1 lac- Mal+V_1^R

An EMB, nearly all Mal+. << 1:100 Mal-

Cf. 43, where cross where Malt+ is very rare.



Melibiose, Raffinose, Salicin utilization. 44

Dec. 8, 1947.

a). Raffinose 3%, Melibiose 1% + Salicin 1% EMB. Streaked 58-161. Dec. 7.

		A9.
R	− to ±	
Sal.	−	~~++~~
Meli	++	

Colicin therefore split glucose α-galactoside but not sucrose α-galactoside!

b). Same sugars, .05% in T(m)+BM. 48 hours reading.

		A9.
R	±	
Sal	±	++)
Meli	+++	
Glucose	+++.	

↳ Streak to Salicin EMB and inoculate second tube of T(m)+Salicin. + and − colonies seen. Selection for Sal+ has therefore been successful. Sal+ is W-55

Test 453, W-45 on melibiose: both +++.

E-M-S- Modifications.

EMS: old formula, + : , streak K-12. Read at 48-72 hours.
(succinate)

		Growth	Color.
K Gluconate	.1%	±	—
	.2%	+	±
	.5%	++	+
	1%.	++	++
Glucose	.05%	+++	+++
	.1%		—
	.2%		±
	.5%.		+
			+++

Glucose .05% May be useful. Try with Na formate equimolar, or perhaps with K-saccharate.