January 25, 1947.

Test types of 407 in BMTB, (-L) of very light inocula of the pure-
purified cultures.

Streak out colonies of 407 on EMB-lactose. Test on:

			BMB.	BMTL.	
1.	407- 2	+S	+	+	P
2.	3	+S	+	+	
4.	4 use a colony in line of prior streak (maybe mutant from S to R)	+S+R	+	+	
5.	6	+S do.	+	+	
6	7.	+S	+	+	
7	10.	+S	+	+	
8.	11	+R	+	±	P
9	12	+R.	+	+	P
10	13	+R	−	−	
11	13	−R	+	+	
12	13.	−R	+	+	
13	13	+R	+	+	P
14	14	+R	+	+	P
15	15	+R	+	+	P
16	16	−R	−	+	+
17	16	+R	+	±	
18	18	+R	+	+	P
19	20	+R	+	+	P.

Transfer 10, 11, 12 to slants + test further.
16, 17

January 27, 1947

1. BM + R Y40 < TLB, −R Y64 → −R, +R
TLB, +S Y10 → +R, +S.

−S	−R	+S	+R	
0	16	7	28.	] 51

Therefore one can assume that an error was made in the previous experiment.

See 368, ~~400~~ 398.

58-161 < Y10/1 Y53 → 38 −S
16 +R
0 +S. (rare!)
0 −R

January 27, 1947

BM + S 58-161.

TLB, −S	Y53	→	−S, +S
TLB, +R	Y46	→	+S, +R.

−S	−R	+S	+R
3	0	0	0.

Tests for >2 strand cross-over.
Tests for phenotypic lag.

January 27, 1947

1. Plate Y40 + Y53 in B, agar.
(Use colonies of 407) stand in coldroom.

a. Streak out on EMB agar. & b. plate remainder of colonies in T(0) agar.
Use well separated colonies

		hrs.	variability in $\times 10^3$		
	1	—	$0/10^3$	0/3	0-turbid
	2	—		0/3	
Y40 +	3				colonies
Y53	4	+		0/3	0
and A	5	+		0/3	0
	6	—		0/3	turbid
	11	—		0/3	turbid
	12	—		0/3	0
Y64 +	13	—		0/3	colonies
SR-161	14	—		0/3	0
and B	15	—		0/3	ca 20 colonies.
	16.	—		0/3	0

b. Streak out 18 other colonies on EMB, looking for variation.

	1	+	$0/10^3$
	2	—	0/3
	3	—	0/3
A	4	—	0/3
	5	—	0/3
	6	±	ca 4/3 ca 1/2 each.
	7	—	0/3
	8	—	0/3
	9	—	0/3
		—	0/3
		—	0/3
		—	0/3
B		+	0/3
		—	0/3
		—	0/3
		—	0/3
		—	0/3
		+	0/3
			0/3

Total: 6 + / 31 ca 80% —
only 1 heterogeneous.

Mucoid segregations. 4/4.

January 27, 1947

1. Y57 × Y68 (check on previous latter) No colonies

2. Y64 × Y68 in B, No colonies!
   Y53/1 × 58-161M

3. Y53 × Y68 (test for recombinability). No colonies

4. Y67 × Y40. in B, very numerous, very elongate colonies.
   Y53M × 58-161/1

5. Y69 × Y53. in B, Fair, v. elong. colonies.
   Y53 × 58-161/1/M

6. Y67 × Y68 in B, No colonies
   alleleins

7. Y67 × Y69. in O rather few (< 10^{-8}) colonies. all deep.
   alleleins.

1	Y53 × 68	n.g.	
2	Y57 × Y68	n.g.	∴ Y68 is n.g.
3	Y64 × Y68	n.g.	
6	Y67 × Y68	n.g.	
4	Y69 × Y53 (Y40M, Y53S)	OK.	
5	Y40 × Y67 (Y40S × Y53M)	OK.	
7	Y67 × Y69	OK, best proof.	

Y67 = Y53 M
Y68 = 58-161 M
Y69 = Y40 M

Single cell isolates of Y55.

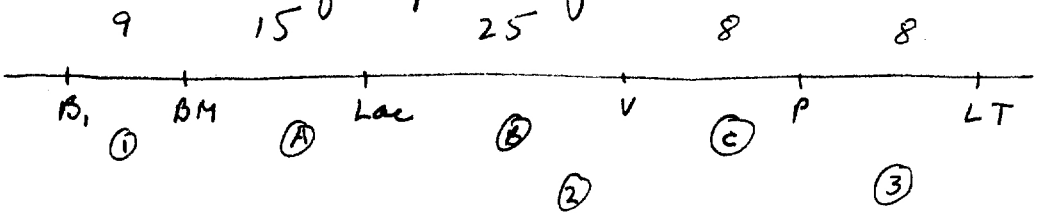
January 30, 1947.

Pedigree:

Received from Dr. Max Zelle, N.I.H., Bethesda, Md.

	bac	V_1
B	–	S
C	–	S
	–	S
	–	S
	–	S
	–	S
D	–	S
	–	S
	–	S
	–	S
	–	S
	–	S
	–	S
	–	S

An basis of map theory:



1. Prototrophs are ①③ types or ①③②$^{2-}$. By the cross: BP × Y64:

−	+	−	R	+	−
+	−	+	S	−	+

① Ⓐ Ⓑ Ⓒ ③

the most frequent prototroph class, by far, should be −R.

The other types all require double crossovers. The relative frequencies of types should be of the order of:

a)

−R	.87
+R	.08
+S	.03
−S	.04

The position of P is inferred from rather complex data. It can be confirmed by showing that LT does not affect the segregation, nor appr. increase the yield. L (Unf. B_1^- may want + it would likewise be difficult be use only B_1 as the marker.

b) $B_1^- > B_1^+$, ∴ influence on segregation.

$B^- > B^+$ ∴ following distribution (ca.)

−R	.30
+R	.50
+S	.15
−S	.05

BLT, B_1P, LT, should be readily recoverable.

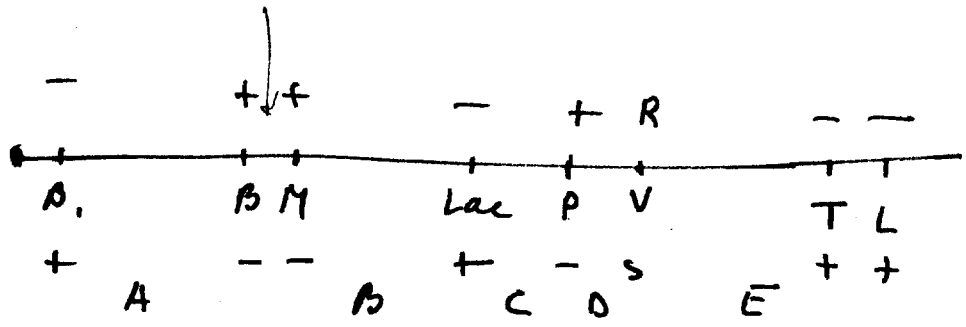
BP1 x TG

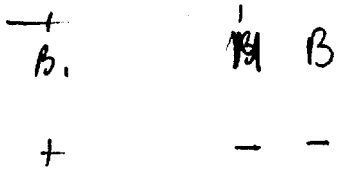
4/6

To demonstrate genetic as well as biochemical distinctness of prolineless (glut -) and prolineless (glut +)

58-5255 x 679-662 Rattus small inoc. ($< 10^8$)

8 colonies found.


−
+ +
−
+
R
− −
B.
B M
Lac
P
V
T L
+
− −
+
−
S
+ +
A
B
C
D
E


B.
B
+
− −

58-5255 x Y64.

1. Plate 5255 in O, Biotin alone for R test. O: no colonies. B- " "

2. Plate into O, B_1; B. P- too turbid } use more dilute inocula
B_1 P.

BLT - very turbid, but several colonies recoverable

B_1 >> O. (5-10 x)

B ca 2-3 x O.

Prototrophs:	−R	−S	+R	+S	Total	Total −
	25	27	0	4	56	
from B_1 plates:	62	41	2	1	106	.93
						.97
from B. plates	74	69	10	12	167	.85

χ^2_3 B/B_1 = 11.1

of which 8.3 is +R, +S classes. ∴ B is actually misdefining the distri-

On basis of map B. B Lac V P TL, the prototroph distribution should have been predominantly −R, while B⁻ should be ½ +R, which is not suggested by these data. Also need data on the frequency of B⁻/B⁺. This distribution suggests the map order:

B_1 B Lac P V TL.

Lac$^{R}_{-}$ x Lac+ ~~Y6~~
Y76 x Y40.

B_1^- 35/35 Lac+

B_1^+ 10/10 Lac+.

Y5 tested all Lac+

Streptomycin Resistance

Recd. 200,000 Units of Streptomycin·HCl, Merck, Lot 277
Potency 250 U/mg unofficially from H. Robinson.

A. Dissolve 100,000 units in 10 ml H_2O for stock solution: 10^4/ml
Dilute serially for stocks of 10^3 & 10^2/ml.

Use 10^4, 10^3, 10^2 u./plate & controls on washed Y53
(standing 24 hours in H_2O prev.).

10^4	—
10^3	—
10^2	ca. 100, very small "resistant" colonies at 18 hours. Incubate further.
0	+++T
0	+++T.

Recotypes - see 407

January 25, 1947.

Test types of 407 in BMTB (-L) on very light streaks of the unpurified cultures.

Streak out colonies of 407 on EMB-lactose. Test on:

		BMB,	BMTL.	
	407 –			
1.	2 +S	+	+	P
2.	3 +S	+	+	
4.	4 +S +R use a colony in line of primary streak (maybe mutant from S to R).	+	+	
5.	6 +S do.	+	+	
6.	7. +S	+	+	
7.	10. +S	+	+	
8.	11 +R	+	±	P

Deoxyribonuclease and recombination. ~~450~~. 420

Dissolve 10 mg DRNase (gift of Avery & McCarty) in 10 ml 2x coli minimal. Sterile filter – filtered well. Store in cold. Presence of denaturation.

Plan: Add .1 ml of DRNase (1 mg/ml) to 1 ml of cell suspensions, separately. ~~Mix~~ Mix cell suspensions + plate ½ ml. Also, hold cells in DRNase, in minimal medium

A.	.1 ml	Total 200 r/4 plates	17, 8, 19, 15	Av. 15 ✓
B	.5 ml ea.	2 mg/4 plates	6, 11, 12, 33	15.5 ✓
C	control		9, 7, 23, 13.	13. ✓

In this expt., DRNase has had no appreciable influence on recombination.

.5 mg ca. 50 r

Acidiate T L ✓

2/10/47

BT/1 × B, L.

i.c. 42 16

B_1	B_2	V	T	L
+	−	S	+	−
−	+	R	−	+

Prot: S > R. ↑ ↑ ↑

T^- R >> S ↑ $T^- >> T^+$

L^- S > R ↑ ↑ $L^- < T^- > L^+$

or.

B_1	B	V	L	T
+	−	S	−	+
−	+	R	+	−

Prot: R > S. ↑ ↑

T^- R > S. ↑ ↑

L^- ↑ ↑

Plate mixture into 0, T, L.

0: 1/4

T 1

L 1.

n.v.g. at recombination.

Three-way cross. Y22

February 10, 1947

Y64 x Y10 x Y40. —

Yield very poor. Do not use ~~it~~ for testing.

cf. other experiments this date !!! — minimal medium ??

Jo February 10, 1947

Repeat part B and controls of 420. Y40 x Y53.

B: ½ml cells + ½ ml DRNase separately + mix cells. Plate into minimal + B, agar. (½ ml of mixture).

B: 0 20
B, ca 150.

C: 0 2
B, 8

controls did not do well here!
(agar base cloudy!)

Test various polimeters - es against Y41, etc. for gene homology.

			No. prot. / 10^9
1.	Feb. 10 '47.	58-3214 × Y41.	0
2.	Feb. 13	6177	0
		3232	0
		6079	0
		6317	0
		5450	0
		5255	0, 0
	679-440 × 5255		0

Test Roepke's mutants for recombination. 425

February 10, 1947.

		24h.	48h.	
1.	Thr × bid	—	5	
2.	meth × arg	~~2000~~	$10^{3.5}$	
3.	lys × leuc	~~trace~~ —	0	
4.	pro × cit-ura	~~trace~~ —	0.	
5.	[redacted] meth × Y64.	—, —, —, —		later 3/4 plates!
6.	thr	—	0	558-228
7.	ind	—	5	1250-228
8.	meth	—	2	532-171
9.	arg	~~2000~~	$10^{3.5}$	572-228
10.	lys	—	40	R152-171
11.	leuc	—	1	(probably contam!). 45
12.	pro	—	100	209-301
13.	cit-ur.	~~trace~~ —	0 (tubid)	823-304

no evidence of recombination.
thr-en + leuc seem to be most stable types in this series.

Recessives in Y53.

Febr. 10, 1947.

Treat cell suspension of Y53, Ȳ40 .01% HN_2 (bis-β-chloroethyl methylamine-HCl) in phosphate-citrate buffer, pH 6.0 for ½ hour at room temperature. Terminate treatment by diluting with broth + centrifuge + wash into fresh YB broth. Incubate overnight.

A. Streak out EMB agar after 4-hour incubation.
Y53 has proliferated considerably; Y40 has not!
Take isolated colonies to YB liq. | do. below.
Y53: 21-40 → Y40-1-20.

B. Streak out cultures after 20 hour incubation.

Also, take slants from entire population.

P13. Cross 10 cultures from A, each, Ȳ Y40 + Y76 resp. in O + B_1 agar.

H15.

	O	B_1		O	B_1
1	10	100	11		
2	14	100	12		
3	26	800	13		
4	15	100	14		
5	20	100	15		
6	0	60.	16		
7	1	100	17		
8	2	100	18		
9	7	30T.	19		
10.	3	50	20		
21	20	100	31		
22	10	100	32		
23	60	300	33		
24	20	500	34		
25	8+	100	35		
26	14	50	36		
27	20	100	37		
28	40	500	38		
29	50	500	39		
30	10	500	40		

all OK.

[Repeat 6, 7, 8 + compare Ȳ appr. controls.]

467 O

Febr. 13, 1947.

Y76 x 58-161. in T(0) + T(B.)

B_1- 44 lac+

B_1+ 9 lac+

53 lac+ add to 418: 45 tests.

= 98 tests. This tests for only 49 recombinations, since ½ would be E+ lac+.

Resistance. 428

Febr. 13, 1947

1/2 ml eq. 426. per plate: NSA.

Streptomycin —

10 u. turbid plate.

50 u. As below!

100 u. ca 10^2 small resistant colonies. (did not mix adequately in agar).

~~1000 u.~~

med > 5 u/ml.

Brilliant Green (1:1000)

1 ml —

.5 ml — no resistants found!

~~2 ml~~

.1 ml turbid.

$HgCl_2$

10 mg —

1 mg —

.1 mg not well diffused; evidence of resistance in some regions.

Tyrocidin (in alcohol)

500 γ turbid (ca 10^7 colonies) = 50 γ/cc no inhibition.
200 γ do.
100 γ do.

∴ Tyrocidin mg.

B.G. OK at ca +1:100,000
Hg OK at ca 5 γ/ml.
Streptomycin OK at 5-10 u/ml.

Fermentation tests.

February 17, 1947.

Repeat — [Used mustard treated cultures].
streak out Y40, Y53 on sugar-EMB media.

	Y40	Y53
Lactose *	+++	—
Maltose	+++	+++
Mannitol	variable.	variable, predominantly —. later - pred +
Glycerol	— (1+).	variable late +!
Alcohol	±	
Sucrose	— to ±	— to ± (faint blue colonies not a + reaction).
Citrate	pH too low	

* some — colonies ? - pick + retest.

Vote diff. Y53 + Y40.

Note 3/18: Xylose: K12 is ++

Maltose is definitely +.

Sucrose seems distinctly — - select for + mutants ??
Mannitol + glycerol may be too variable to be useful.

Sucrose — = E. coli communis.

Inversions in Y40.

February 18, 1947.

Cross 426-6, 7, 8 $\bar{c}$ Y53; Y76 $\bar{c}+\bar{s}$ B$_1$.

		A × Y76 O	A × Y76 B$_1$	B × Y53 O	B × Y53 B$_1$
1	426-6.	40, 50	ca 200, ✓	50, ✓	200T, ✓
2	426-7	ca 50.	400,	50	500
3	426-8	150,	++T;	150,	++T;
4	~~#~~ 58-161! (no Y40m).	100T	++T;	200,	++T

~~#.~~

Also inversions

Inhibitors

February 18, 1947.

per "20" ml plate NSA.

Y64 Hg 100γ "1 resistant colony. not too well diffused
Y46 do. clear. — "secondary colonies!!

Y64 Streptomycin 50 u turbid
100 u. clear in parts. Not wholesale.

Y7*

58-161. 50 u. turbid
100 u. as above.

Y64. Brilliant Green .5 ml 1:1000 clear! (1:40,000). pick dye resistant
" Malachite Green 1:500 1 ml clear! (1:10,000). ca 2 large; 40 sm.
.5 ml " 1:20000
.1 ml (1:100,000). poorly diffused.
clear when dye is visible

Y46. Sodium Azide 10γ turbid
100γ turbid
1 mg. irregular clearing.

Y46 AG from fluorescence

Y64 "Spergon" (1/20) .1%. no inh.

Y64 tri-iodobenzoic ac. (1/20) 1% " .

58-161 } Hg Cl2, 1 mg. autoclaved with 100 ml N.A. — not inhib.
Y64 }

" Bi. Cl2. 2 mg/100 ml. N.A. — ~~subside~~ only faintly inhib.
= 1:50,000. (need more!)

" Streptomycin 5 u/ml. OK.

Y64 NaN_3 2 ml/20 .1% inh. — later grew uniformly.

Y64, 58-161. Mal. Cl2. 2 mg/100 NA — not enough!

Sex in L15 mutants?

February 17, 1947.

Grow separately; plate together.

Y5	2
"them.	0
Y5 x them	0.
Y5 x Y44	0
Y5 x 58-161.	0

no evidence for sexuality under these conditions. Try growing together!

Y40 x Y53. 432

~~Recombination~~

2/21/47

Mix Y40+Y53 in water, add to agar, mixing. Add 5 ml aliquots to various suppl. plates. ≐ ca. ½ mln. (see 433 for note on medium).

5 ml. 0 12
10
10
3
11
10.

B 11
10
4
15
10

B_1 >25 T.
" T.
" T.
" T.

This expt. illustrates influence of conditions on detection of recombinants.

10 ml - 5 sublayer.
0 —
0 —
0 —

c sublayer. 0 5
0 4

B_1 T. B. 12.

Tests for inversion in B_1-B_{region}.

2/21/47.

Cross mustard treated (476) isolates of Y40, Y53 ♀ Y53 × Y40 resp. on O, B_1 medium resp. Plate in medium lacking NH_4NO_3 (inadvertent omission).

		O	B_1
Y40 T.	11	++	T ++
	12	++	T ++
	13	++	T ++
	14	++	T ++
	15	T +	T ++
	16	++	T ++
	17		
	18	++	T ++
	19	++	T ++
	20	++	T ++
Y53T.	31	++	T ++
	32	++	T
	33		
	34	++	T ++
	35		
	36	++	T
	37	++	T
	38	++	T ++
	39	++	T ++
	40	++	T

No evidence of inversion in any of these isolates — 17+20 = 37 tests.

numbers of prototrophs on this minimal seem quite unusually high.

In Fisher test tubes, Y40 × Y53 ♂ spp. inhibited by anaerobic conditions