Selective Segregation: ClA^R.

A. Y88 x Y40. B. Y53 x Y91 C. Y40 x Y53.

Plate into T(0) + :

	0	Cla 500	Biotin	Biotin + ClA.
A.	ca 80	26 30.	see 0.	> Cla.
	do.	smallish		ca 70-80

were too turbid. It may provide a source of Cla^R cells.

In A, the difference between 0 and Cla is not clear cut. In B, it is. There are relatively few resistants in X B as compared to A. Clear plates will be needed: use B, suppl.

This experiment is not entirely valid because the plates less turbid.

		ca 40		
B	ca 40 · turbid.	3	see 0.	see Cla 500
	ca 50 · turbid	5		
		12		~~[illegible]~~
		19		~~[illegible]~~
				40
				12
C	12	0		0
P3:	25	1?		24

olonies.

A & B B, have large numbers of isolable c

anies.

Test A to determine % sensitive col

g. demonstration of ts. Use B, plates.

later, more colonies appear in B probably n. mid direct ratio

Cla segregation.

See 509

See 496.

A. Y88 x Y40.

T(o) 17/17 Res.

B_1 18/18 Res.

35/35 Res.

B_1	Bal-Cla	Lac	V	TL
−	+ R	−	S	−
+	− S	+	R	+.

Y53 x Y91.

T(o)

$T(B_1)$ 74/76 Sens.

2(?) Res.

∴ Reverse cross is supported.

Summary.

A. Y91 x Y53

496. 4/56 $T(B_1)$

509 ~~74/76~~. 2/76 $T(B_1)$

6/132

4.5%

Y40 x Y88

486 14/16 < 1/7 T(o), 1/7 $T(B_1)$

35/35 T(o); $T(B_1)$.

49/51

4%.

~~Stra~~ Camphor

May 3, 1947.

Streak Y40, Y87, Y64, Y10 on 10 Camphor./NA.

May 5 - streak out on EMB

May 7 - Isolated colonies. Test by streaking heavily on Cla 2 agar. Following showed no papillae + were recovered:

Recover + streak out on EMB.

Y40 2 / 15

Y87 1/18

Y10 ~~10~~ 9/9

Y64 1/8

Y53 0/8

Phage resistance patterns.

5 11

May 5, 1947.

Spread on EMB lactose plates, Y53 + phages:

T1 many resistants; some smooth; a few mucoid.

T3 do. mostly mucoid.

T4 scattered small plaques. and bitten stocks.

T5 many smooth resistants. 30 tested on T1 all resistant.
parent stock from Demerec's bottle

T7 all mucoid?

T1+T5 as T1; no mucoid.

T3+T4 scattered small plaques! (protection by T4???) Repeat!!!

T3+T7. as T3.

T6 confluent plaques; not continuous lysis except (?) ~~in~~ in center. Streak out to obtain Y53-V_6^R

T1/Y40. no plaques (rough mutants?)

Y53-mutants.

1. Pick colonies from /T3 and /T7 and test reciprocally.

2. Test and compare /T1S and /T1M on T1 + "T5"

6. Purify + cross-test /T6. < of 20 tested ca 3 were sensitive!

7. Repeat: /T1, T3 /T4 /T3, T4.

/3 on T7: Some sensitive. Streak out and compare with Y53/3/7 No.: turned out Y53/3, 7

May 4, 1947.

streak out on NAagar +

B.G. 50 M.G. 100.

Y77 A few isolated colonies — only a small part of the inoculum. Background shows ± growth.

Y79. A few isolated colonies.

streak out Y77/Mg on Mg + BG same as above!

Y79/Bg on BG.

Identification of "T5".

Enumerate stocks.

[wait for indicator stocks from Demerec.]

α: 506 - (5) K-12; Y40 S.,

β "T5 - Batch 2" 506 - (6) Y40 R.,

γ. "small plaques" from 506 (5)

δ "large plaques" from 506 - (5)

1. Streak out stocks with K-12; Y40 for plaque size determination.

2.

	T1	T5	T1+T5	α	γ	δ	γ+δ
Y40	R	R	R	S	R	R	R.

∴ α has another component not yet isolated. Isolate on Y40.

Location of *cla*^{R}.

Plate Y53 × Y91 on B$_1$ Cla agar. Pick ons second day +
TLB$_1$- BM Cla^R

test the resistant recombinants.

With considerable lag, varying from colony to colony, an equal yield ($ca > 10^{-6}$) was obtained ~~for~~ on Cla-B$_1$ and B$_1$ plates. The "~~resistants~~ susceptibles" formed tiny colonies early, & evident frequent ~~adapted~~ mutations! Need higher conc. of Cla??

V_m^R.

May 15, 1947.

1). Streak 58-161 c̄ T1.

2) Streak 58-161 alone.

no mucoids developed!!

~~[illegible]~~ Mucoids sometimes develop on old plates on lactose agar. back into this!

May 16, 1947.

Test isolates from 510 by following crosses. on T(0).

A: Y10/Cam$_m$ x Y87/Cam. T-L-B,-B+M+Lac+V^S x ...

m T(0) 1
2
3
4

B. Y40$_m$/Cam x Y88 B+M+Lac+Cla^{S}V^R

1
2

401). -R 5 -> 2 +R 2 +S. 0 / 9. Cla all R. normal segr.

402)

*	-R	-S	+R	+S.		Cla*
8		2	7	0	17	all R.
	7	5	5	0	17	1)-R 2)-R are Cla^S
	4	4	5	1		
	19.	11	17	1		normal segregation

2/43 Cla^S.

Phage - Resistance Patterns.

May 17, 1947.

	T1	T3	T4	T5	T6	T7	"T5"
K-12	"R"	"R"	S	"R"	S ?	"R"	"R"
Y40	"	"	S	"	S	"	"
Y94 (Y53/6)	"	"	S	"	R ?	"	"
Y95 (Y53/6)	"	"	S	"	R ?	"	"
Y53/3	"	"	R	"	?	"	"
Y53/3,7.	"	"	R	"	?	"	"

These tests are obviously fallacious. Probably phage strains were allowed to "set" too long before adding bacteria

Repeat. P18.

		T1	T3	T4	T5	T6	T7	"T5"
K-12		S	S	S	S	S	S	S
Y40	/1	R	S	S	R	S	S	S ← small plaques only.
Y94	/6	S	S	S	S	R	S	S
Y95	/6	S	S	S	S	R	S	S
Y53/3		S	R	R	S	R	R	R
Y53/3,7	=	S	R.	R	S.	R	R	R.

Resistance to T6 seems to be included in the 3,4,7 pattern.

probably T4.

∴ $V^R_{1,5}$; V^R_6; $V^R_{3,4,6,7}$ are available!

Y10/1cam x Y87/cam.

+R	+S	-R	-S.
3	6	8	0
9		8.	

	Lac+	Lac-	
Y10/3.	15.	1.	
Y10/4.	22	6	
	17	1	
	39.	7	/46. ?
Y10/2.	22.	5	

Is $Y10_4$ aberrant?

Complex-Resistance Patterns

Add mixtures of phages + Y53 and spread on EMB agar.

T3 ca 200 R.

T4 lysis patchy at circumference. scattered resistants.

T5 complete lysis only in center; occasional resistants.

T6. " " ". occasional resistants.

T1+T3.	Complete lysis :	Ca 10-12 R	* lysis in confluent zone; nibbled colonies. Some do hole!
T1, T4.	"	1 surviving colony. _ ?	* Some nibbling! mostly OK.
T1 T6	"	1 surviving colony?	* small colonies.
T3, T4	"	Many R.	
T3, T5	"	O- — R; many tiny	* OR. plaques in regions of confluent growth
T3, T6.	"	Many R!	
T4, T5.	lysis patchy.	Occ. mucoid R.	* somewhat mucoid; no nibbling
T4, T6.	lysis patchy	No resistants.	
T5, T6	Complete lysis.	3 clones???	* v. small colonies. see (1, 6.)

* streak out.

Segregation of V_6^R

H B

Y94, Y95 × Y40. (T-L-B_1-Lac-$V_1^S V_6^R$ × B-M-Lac+$V_1^R V_6^S$)

O, B_1.

Y94-O.

Lac	T_6	T_1
+	R	R
+	S	R
+	R	R
−	R	R
−	R	S
+	S	R
−	R	R
−	R	R
−	R	S
−	R	S
−	R	R
−	R	R
+	S	R
−	R	S
−	S	R
−	R	R
−	R	R

Y94-B_1

Lac	T_6	T_1
−	R	S
−	R	S
−	R	R
−	R	R
−	R	S
−	R	S
−	R	R
+	S	R
+	S	R
−	R	R
−	R	S
−	R	R
+	R	R
−	R	R
−	R	S
−	R	R
−	R	R
−	R	R
+	S	R
−	R	S
+	R	R
−	R	S
+	R	R

	Lac V_1	V_6^R	V_6^S	
V_1^R 43	− R	23	1	41 Lac−
	− S	17	0	
V_1^S 18	+ R	5	14	20 Lac+
	+ S	1	0	
		46.	15	
		V_6^R	V_6^S.	

Cum:

Lac	6R	6S	T1
−	44	2	R
−	28	0	S
+	9	20	R
+	1	2	S

82 74

Sue 528

B 1 V_6 Lac

Y95 x Y40.

T(0)

Lac	T_6	T_1
−	R	S ✓
−	R	R ✓
−	R	R ✓
+	R	S ✓
+	S	R ✓
−	R	R ✓
−	R	R ✓
+	S	R ✓
−	R	S ✓
−	R	R
−	R	S ✓
−	R	S ✓
−	R	R ✓
−	R	R ✓
+	S	R ✓
+	S	R ✓
−	R	S ✓
−	R	S ✓
−	R	R ✓

Lac T_1	V_6^R	V_6^S
− R		
− S		
+ R		
+ S.		

T(·B1)

Lac	T_6	T_1
+	S	R ✓
+	S	R ✓
−	R	S ✓
+	S	R ✓
−	R	S ✓
−	R	S ✓
+	S	R ✓

Phage effect

Y40 x Y53

May 19, 1947.

(T1)

A. Add phage to Y53. Absorb 10 mins. Mix ± Y40 and wash

good yield of colonies!!

B. Mix Y53 + Y40. ~~let stand ... hours~~. Let stand in H_2O overnight.

Add phage before final wash + plate

C. Mix as above, no phage.

(Repeat)

V segregations.

May 21, 1947.

A Y94 x Y40 ($V_6^R \times V_1^R$).

T(a).

Lac	V_6	V_1
−	R	R
−	R	S
+	S	R
+	S	S
+	S	R
−	R	S
−	R	R
−	R	R
−	R	R

T(B_1)

Lac	V_1	V_6
−	S	R
−	R	R
−	R	R
−	R	R
−	S	R
−	R	R
−	R	R
−	S	R
−	R	R
+	R	S
−	S	R
−	R	R
−	R	S
−	R	S
+	S	R

Lac	V_6	V_1
−	R	S
−	R	R
−	R	R
−	R	R
−	R	S
−	R	R
−	R	R
−	R	S
−	R	R
+	S	R
−	S	R
−	R	R
−	R	S
−	R	S
+	S	R
−	R	R
−	R	S
−	R	R
+	S	S
+	S	R
−	R	R
−	R	R
+	S	R
−	R	R

T(B_1)

Lac	V_6	V_1
+	R	R
−	R	R
−	R	S
−	R	S
−	R	R
−	R	R
−	R	R
−	R	S
−	R	R
−	R	R
−	R	R

	V_6^R	V_6^S	
−	4+17	0+1	V_1^R
−	2+9	0+0	V_1^S
+	0+4	2+4	V_1^R
+	0+0	1+1	V_1^S

Total:

	6R	6S	
−	21	1	1R
−	11	0	1S
+	4	6	1R
+	0	2	1S

Cum. total: See 518.

B.) Y96 x Y40. (Y53 - $V_4^R V_6^R$ x Y40 - V_1^R). Test on 1,4,6.

12 strains - all rather ~~smooth~~ mucoid. - all resistant n-q.

Tests on complex mutants.

May 19, 1947.

Y53/	T1	T3	T4	T5	T6.	
1, 3	S	R	R	R?	R	
1, 4	S	R	R	R	R	
1, 6	?	?	?	?	?	Y99.
3, 5	R?	R	R	R	R	
4, 5 MRC.	R	S	R	R	R	
5, 6.	S	S	S	S	S.	!

Do not use these mutants further; their origin as independent mutations is not excluded.

Y53/3 on T6. 19 R. comparable to Y94.
pick at random. 1 good sensitive. Y98.
(3 nibbled throughout.).

check on 16 types.

~~Y53/~~	T1	T3	T4	T5	T6	T7	
Y94 Y53/6.	R	R	S	R	R	R	
Y98. Y53/3,6	R	S	S	S	S	R	
Y99 Y53/1,6	S	R	S	S	R?	S	
Y8[illegible]. Y53/1M.	R	S	S	R	R	R	
Y53/3	R	R	R	R	R	R	
Y53/3	R	R	R	R	R	R	probably contam.

Mix Y53 + phages in a tube + plate on EMB.

16,1 No survivors. See Y99.

16,5 2 v. mucoid colonies; a few tiny ones.

13,5 Numerous mostly mucoid.

13,6

11,4 Very numerous colonies. (test) on 1,4. (V. considerable growth before lysis!) Probably invalid.

14,5 several mucoid colonies; occ. smooth.

11,3 several "mucoid" colonies. Test on T1,3. Mostly very mucoid + nibbled. Isolates apparently $T_1^R T_3^R$. = 521-1

Y53/6. Mostly patchy lysis, but many well-defined resistant colonies. Test for T_3^R.

Y53/1 Test for T_5^R. large colonies: 65/67 = T_5^R. small col. pick Y100. = $T_1^R T_5^S$. 5/18 = T_5^R. Pick both var.

Y40/6 Test for T_3^R. smooths are susceptible; mucoids generally resistant. isolate one as Y101

Y53/1,4. Mostly nibbled. Keep are $T_1^R T_4^R$ as Y102. larger colonies are mucoid; V. watery or nibbled.

Y53/4,5. Test on 1,4. Mucoids are doubly resistant. Do not use.

Tests on Resistant mutants.

Y53/(1,4). on T1, T4.

Y40/6 on T3.

Y53/1 on T5.

		T1	T3	T4	T5	T6	T7	
~~Y53~~	Y40/6	R	R	S	R	R	R	T4S! T3R!
"Y53/(1,6)	Y99	R	S	S	S	S	R!	T7R! stock??
Y53/3 6^{s}!	Y98	S	R	(S)	R	R	R	
Mucoid!	Y86	R	S	S	R	R	R	
Mucoid!	58-161 Cam.	R	R?	R	R	R	R	
Y53/3	Y96	S	R	(R)	R	R	R	

Papillation of the L-Reversion.

May 20, 1947.

To 20 ml plates of T(0) + excess B, and threonine, add varying amts. of leucine. Streak Y53 on these plates to determine suitability for assay of mutation frequency. Anoc. A21.

Leucine, per plate.	24h.	48h.	72h.	84h
0	0	0	✓	
1r	micr. colonies.	→ do.	✓	no change!
2	"	Pin points	✓	
5	"	— " do.	✓	
10	Bare visible pinpoints	v. tiny	(1 colony)	
20	> " "	tiny but visible	(1 colony)	
50	> " "	v. small; fairly uniform (Neurospora cont noted) fair size.		
100	tiny colonies.	small colonies. No papillae.		
1 mg.	v. small colonies. papillae in gross streak?	Good sized (1-2 mm.) Some variations. no obvious papillation.		

range for further tests.

Sincerity Y26 x Y87 523

May 19, 1947.

ϕ-c- B+M+ x ϕ+c+B-M-Lac-V_1^R

a) Y26 grew poorly in YB. [OK c cystine supplement or c "Protease Peptone 1%"

b) Y87 in B gave no M+ colonies. ∴ Probably OK as a single factor.

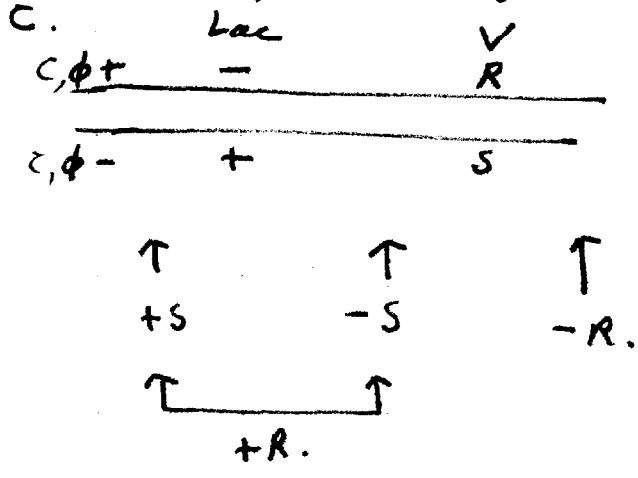
c) in T(0). 5 in 6 plates. (ca 10^{-8}).

d. in T(B) ca 10/plate (ca 10^{-7}).

These testify to the predicted tight linkage of

B - M c ϕ - C.

		-R	-S	+R	+S.
523	B.	9	1	1	1



May 21, 1947

A. Y26 x Y53. B. Y46 x Y87.

B. - No colonies in 9 T(0) plates
2 colonies in 4 T(B) plates !

A T(0). 75. T(B,) 184.

~~B~~ Φ-C- x T-L-B,-Lac- all V,s.

Compare with previous intermediates of 1:1 T(0): T(B,).

T(0) 6/51 Lac +.

T(B,) ~~12~~ 14/43 Lac +.

The 43 tests are divisible into 2 parts:

(9)	14	(34) 29	43
(11)	6	(42) 46	51
	20	75	94

a) 75/184 x 43 = 17.5 ≐ B,+ ≐ 2 Lac - ; 15+Lac+
25.5 ≐ B,- ≐ 12 Lac - ; 13+Lac+

∴ the B,- may have ca. ½ Lac - ½ Lac +.
This may not be precisely true.....
i.e.

$\chi_1^2 = 25(.091 + .11 + .03 + .02)$

$= 25(.09)$.

.11
.03
.02
(.25)

B, — Lac — TL.

$= 6.2$ $p = .01+$

Camphor.

May 16, 1947.

Add 3mg/ml Camphor to NA plates. Streak with Y40, etc. Growth not markedly inhibited, but ca 3-4 day. papillae are noted. On 5th day, streak out. Test colonies by streaking on Cla 1 plates. Reject those that show papillae. Inoculate others in broth for further test.

Y40 "C"	Pap.	No pap.
	8	8 (too heavily streaked)
	14	0.
	12	0
	2	0
Y10 (pure reisolate)	5+5	0
	11	11. (too heavy!
58-161 C.	0	17. (too heavy).

all these eventually gave papillae

May 24, 1947.

A. Y26 × ~~[illegible]~~ ~~[illegible]~~ ~~[illegible]~~
×
Y87

B. Y26 × Y64.

No colonies in 4 minimal plates.

ca. 50 colonies / plate c̄ biotin. Reversion of Y87 to M^+ not checked.

Test c̄ V_1; Lac. ∴ compare Lac | V

ØC +S
×
BM −R

−R	−S	+R	+S.
21	1	1	—
18	2	0	1
18	0	0	0
23	0	0	0
19	1	0	1
15	1	0	0
114.	5	1	2

reversion of BM to B not checked; perhaps suggested by relative frequency of B^- in this series!

~~ØC~~	M^+C^-	+	S
~~ØC~~	M^-C^+	−	R

↑ +S ↑ −S ↑ or none. −R.

B. Plates very turbid; samples poor.

	−R	+R	−S	+S.
T(O).	3	1	8	10
	12	10	1	0
T(B_1)	9	1	3	6

indicators; phage n.g.

Total, reserve Lac segregation compare 524: (n.g.).

T(O) : 29− : 31+ 48% Lac − 45− : 6+ !

T(B_1) : 70− : 15+ 82% Lac − 29− : 14+

Segregation of Lac, V_1, V_{15}

May 24, 1947

Y40 × Y99 [B-M-V_1^R × T-L-B_1-Lac-V_{1T}^R].

T(0) too turbid; auto-inhibited

T(B_1): rather turbid, but colonies can be picked.

T(0). → T(B_1)

Lac	T1	T5	V_{15}	V_{1T}
−	R	R	R	−
−	R	R	R	−
−	R	S	R	R
−	R	R	R	−
+	R	R	R	−
−	R	R	R	−
−	R	R	R	−
+	R	R	R	−
+	R	R	S	R
−	R	R	R	−
+	R	R	R	−
+	R	R	S	R
+	R	R	R	−
+	R	R	R	−
−	R	R	R	−
+	R	R	R	−
+	R	R	R	−
+	R	R	R	−
+	R	R	R	−
−	R	R	R	−
+	R	R	S	−
+	R	S	R	−
−	R	S	S	−
−	R	R	R	−
+	R	S	S	−
−	R	R	R	−
−	R	S	S	R
−	R	R	R	−
+	R	S	R	−
+	S	S	S	−
−	R	R	R	−
+	R	R	R	−
−	R	R	R	−
+	R	R	R	−
+	R	R	R	−
−	S	S	S	S

	T1	T5	V_{15}	V_{1T}
−	R	R	R	−
+	R	S	S	R
+	R	R	R	R
−	R	R	R	R

	V_{15} R.	V_{15} S
Lac-V_{1T}^S	16 P	5 *
Lac-V_{1T}^R		2 P
Lac+V_{1T}^S	18 P	1 *
Lac+V_{1T}^R		3 P

* = visible recombinations between V_{1T}^R and V_1^R.

Segregation of V_1 and Lac is:

−R	−S	+R	+S
16	7	18	4

V_{1T} can be expressed only in V_{15}^S. It is clearly not closely linked to ~~V_{15}~~ V_{15}

Segregation of Lac, V_1, V_6

Y94 x Y40.

T(0)

Lac	V_6	V_1
+	S	R
−	R	S
−	R	R
+	S	S
−	R	S
−	R	S
−	R	R
−	R	R
−	R	R
−	R	S
−	R	R

See 588.

Lac	6R	6S	T1	
−	28	1	R	
−	25	0	S	
+	2	7	R	
+	1	1	S	

Cum.

Lac	6R	6S	T1	
−	72	3	R	75
−	53	0	S	53
+	11	27	R	38
+	2	3	S	5
				171

T(B_1).

Lac	V_6	V_1
+	S	R
−	R	R
+	R	S
−	R	R
−	R	R
+	S	R
−	R	S
−	R	R
−	R	S
+	R	R
−	R	R
−	R	S
−	R	R
−	R	S
−	R	S
−	R	S
−	R	R
−	R	R
−	R	S
−	R	R
−	R	R
+	S	R
−	R	R
−	R	R
−	R	R
−	R	S
−	R R	R
−	R	S
−	R	S
−	R	S
−	R	R
−	R R	S
−	R	S

Lac	V_6	V_1
−	R	S
−	R	R
−	R	R
−	R	S
−	R	S
−	R	R
−	R	S
−	R	R
−	R	R
−	R	S
−	R	S
+	R	R
−	R	R
−	R	R
−	R	S
+	S	R
−	S	R
−	R	S
+	S	R
−	R	S
−	R	R
+	S	R
−	R	R

Effect of hardness of agar on recombination.

Use plates ē underlayer of T(B) + 1½% agar. Add 5 ml of a mixture of Y53 + Y50 to 100 ml of a series of agar concentrations, mix thoroughly, and pour 10 ml quantities. Compare yields. [This should further delimit the mobility of the transforming principle.]

Agar concentrations of ½%, ¾%, 1%, 1.5%, 2% should be tried.

A 27.

½%	Underlyspread: 54+; —	
1%	16, 7	
1.5%	5, 2	
2%	32, 27	seeds-spacing.
2.5%	29?, 10.	¾% is lowest suitable concentration.
¾%	54, 36	

Acenaphthene.

May 17-1947.

Streak NA plates ± Y40; 58-161, and invert over acenaphthene crystals. Incubate 5 days 37°. No marked inhibition noted; no papillation. Streak out on EMB. A23. P24. Marked sugg dimorphism noted.

Test colonies on cla for papillation.

Streak out large + small colonies and Y40 standard:
dimorphism breaks true, but is present in standard stock!
Test biochemically.

	B	M	BM
L.C.	—	—	++
S.C.	—	—	++

This dimorphism must be pursued, as it may be responsible for the heterogeneity in segregation data previously observed.

Colony tests:

	Pap.	No. out pap.	
58-161 Sm. col.	10	3	
	3	9	probably too heavily streaked.
161 L.C.	11	0	
Y40. L.C.	6	0	
	10	0.	
S.C.	7	5	
	6	; 6?	

Some small colony types do not papillate on first test.

Segregation of V_3^R.

May 27, 1947

Y98 x Y40. Test on T6, T3, T1.

T(0); T(B,).

	Lac	T6	T3	T1		Lac	T6	T3	T1
T(0).	−	R	R	S	T(B,).				
	−			R					
	−			R					
	+			R					
	−			R					
	+			S					
	−			R					
	+			R					
	−			R					
	−			R					
	−			R					
	−			R					
	−			R					
	+			S					
	−			R					
	−			R					
	+			S					
	−			R					
	+			S					
	−			R					
	−			R					
	−			R					
	−			R					

all R →

all R →

all R

or R.

probably selective picking of T_3^R T_1^R types.

Y26 x Y87.

532

May 27, 1947

error: ~~[illegible]~~ Y87 x Y64. (the reverse cross to n.g.)

no tests.]

Segregation of V_{1T} S 33

May 27, 1947.

Y100 x Y40

See below for Y100 x ~~Y40L~~.
x 58-161L.

Phages n.g.? — all R.

Batal — tested, erroneously, on T6, T3, T1.