Transformation control

January 9-10, 1947.

P9. Inoc YB - Y53, ~~Y57~~. Y40

10A10. Inoc ($\frac{100}{50}$ ml NSB) Y53, Y40 (A)

1P10 Inoc YB - Y40 (B)

4-5P10. Wash (A) cells.

1. Mix Y53-Y40 cells. ✓

8P10. Resuspend Y53 (A) cells in T-minimal. Incubate 3 shaking 3½.
Sediment (C,D) and mix with washed Y40 (B). 2. plate 0.

3. ~~Mix~~ supernatant of C,D + mix + washed Y40 (B) 3. plate 0.

1. 5×10^2 prototrophs.
2. C, D $> 10^2$ " ? turbid for count.
3. filtrate:

C - 1 prototroph ??
1 ??
} supernatant was not entirely freed of cells by the centrifugation. Repeat + controls on influence of dilution of 1 cell type on prototroph yield.

Recombination types

January 10, 1947.

Y40 + Y53 on T(0), T(B,) agar

Pick colonies to EMB lactose. 1/12/47 1-15. 8, 13 + others are −

Streak out densely on (A) BMTL-lactose (B) BMTL lactose + glucose.

Compare the B_1^- types & $\bar{E}$ types isolable from these plates.

Colony-Plate:	Colony: 0 = B_1^- orig.	A	B	C	D	E	F	G
Loc 1	$-S^{(+R)}$	$-S$	$-S$	$-S$	$-S$.			
Loc 2	−							
* Loc 3	$-R$	$+S^{(+R)}$	$-S^{(+R)}$					
* Loc 4	$-S$	$+S^{(+R)}$	$-S^{(+R)}$	$\pm S^{(+R)}$	$\pm S^{(+R)}$			
Loc 5	$-S^{(\pm R)}$	$-S$	$-S$	$+R^{(-R)}$				
Loc 6	$-R^{(\pm R)}$	$-R$	$-R$	$-R$	$-R$	$-R$		
6 8	* $-S$	$-S$	$-S$	$-S$	$-S$	$-S$	$-S$	$+R$
6 11	$-R$	$-R$	$-R$	$-R$	$-R$	$-R$	$-R$	$-R$
6 12	$-S$	$-S$	$-S$	$-S$	$-S$	$-S$		all mixed + R
6 13	$+R$	$+R$	$+R$	$+R$	$+R$	$+R$	$+R$	
* 6 15	$-R$	$-S$	$-S$	$-S$	$-S$.			
	↓ B_1^-	↙ B_1^-	↘ BM+R		↙ B_1^-	↘ BM+R		

BM + R
TLB$_1^-$ S

Segregation of T_1^K

January 11, 1947.

Y64 x 58-161.

TLB, Lac- T_1^R x BM Lac+ T_1^S

good material.

a. prototrophs

	T_1^R Lac- R	+ S	- S	+
	42	1	53	23

R = 36% 64%

Lac- = 80%

b. B_1 plates. Much more numerous colonies (10 x) (not well readable). (colonies impure!).

8	0	5	10

January 11, 1947

a. Y67 (Y53M) × 58-161 (Try × Y40.)

shunon lac+! Muc lac- Sm lac- Muc lac+ Sm lac+

P) 17 P) 9 Lac- = .66

~~Y67 (Y53 3,11, M) × 58-161 (Try × Y40)~~

c Y68 (58-161M) × Y53. (Try × Y64)

segregation: ~~M L- M L+ m L- m L+~~

6 P 22 P 12 7

M_{68} linked to lac⁻

Interaction of expression of Lac- + Muc+ on EMB lac medium?

note variation in shunon. Genetic character?

attempted Transformations.

January 11, 1946.

P10 from 100 ml / 125 fl. YB – Y53.

N11. Centrifuge 250 ml (step 25 – 1. Suspend cells in 15 ml .9% NaCl. Add lysozyme + incubate for autolysis on shaker at 25°. (12:45 PM – 3 P.M.) Centrifuge "free falls" and

Mix 5 ml ē 1 ml Y40 suspension + plate 3 x 2 ml samples into T(0) agar.

P14. [illegible]

ca 10 large $10^{2.5}$ small ē halos. v. clear plates. sign?

See 394

January 11, 1948.

Plate Y55⁻ (+1... Lac-) into Lactose-minimal at various dilutions: (Assay. = 10^9)

est. cells	
10^8	Discretely crowded.
10^6	about 10^4 visible colonies
10^4	about 200 large colonies, with haloes of small ones. Small cols. much smaller than below.
10^2 about 5 x.	10^3 small colonies; 6 typical colonies (probably Lac+). Diffuse tint

1) The reversion frequency, ascertained from EMB plates is very high (ca 10^{-4} to 10^{-3}/generation??)

2) At best on this medium, Lac- is capable of developing to some extent. Since they develop haloes, it is likely that there is a limiting factor in the agar which faintly permits growth.

Test large colonies on EMB:

Segregation of T_7^R 390

Jan 11, 47

Y65⁻ x 58-161 No colonies!! (Repeat!)
not turbid - prob mix error.

Repeat Jan 15.

Loaded! Hydrate ????

Recombination in P12 (cont.)

January 11-12, 1976.

P11 Inoc YB- Y43, Y44.

230P12- Inoc YB: Y43, Y44, Y43+Y44, Y43+Y53.

4P12. Wash + plate.

(Y43) + (Y44)	0, 0	red turbid (exc.)
(Y43+Y44)	0	
(Y43+Y53)	0	
(Y43)+(Y53)	0	

January 12, 1947

Plate Y40 + Y53 (cultures as in 391) in T(0) as initial controls.
9-10 P12. .5 ml. ~~25~~

T.0: – ca 2-300.

a. Keep Y40, Y53 in water (.9% NaCl) 25°. M in 4P13.

b. Keep (Y40 + Y53) in water. Plate 4P13

c. Keep Y40, Y53 in T(0). [Add 1 ml to 10 ml T(0)]. M in plate 4P13

[d.] P12. Plate Y40, Y53 in superficial layers of agar. — 4 colonies.

e fresh Y40 × Y53.

a	$10^{-2.5}$
b	10^{-2}
c	10^{-2} clear plate
d	4.
e.	10^{-2}

Cells will react if kept in water for 24 hours + then mixed.
but not many more are formed if they are kept together. ∴ Recombination takes place in the agar.

Differential Centrifugation of Bacteria
Preliminary Expts.

0. density — in sucrose buffer, centrifuge [illegible] washed YS3 [illegible],
at 10 — 15 mins, etc. Sediment vol.

	1.0	1.04	1.08	1.12	1.16	~~1.20~~	
~~Mark~~ time. Mark	+	+	+	+	+		10 / 10
time Mark	++	++	++	++	++		~~1025~~ 10
n.g.	++	++	++	++	++.		20 / 50

1.16 = 20g sucrose / 100cc water
1:4 bacterial susp. in H_2O. — n.g. for density.

Repeat, using 20g sucr / 20g H_2O as d = 1.25. (actually 1.23)

$\left(\frac{1 ml}{4 ml\ 1.25}\right)$	1.0	1.05	1.10	1.15	1.20	
	++	++	+	±?	—	20m / 50.
	+++	+++	+±	+	±	+ 30m / 50
						+ 1hr.

diffuse in bottom layers c̄ a ring at junction.

1.15 1.20 — do.

Use heavier susp. cells. + ± 1:30

This might achieve some separation.

Tiy K1 Mg30Y

Y53 x Y40 – deletion effect.

1/13/47.

½ ml of various dilutions.

	1ml + 1ml Y53	Y40.		
1.	10^{0}	10^{0}	ca. 100	
2.	10^{-2}	10^{0}	6	
3.	10^{-4}	10^{0}	0	
4.	10^{0}	10^{-2}	8	
5.	10^{0}	10^{-4}	1	
6.	10^{-2}	10^{-2}	0	— 0.
7.	10^{-4}	10^{-2}	0	
8.	10^{-2}	10^{-4}	0	

Y40 - Lac - mutant.

January 15, 1947.

Duplicate Y402nn. Dilute 1 ml/10 YB incubate 18 hours + dilute + plate on EMBlact. 20,000 colonies examined.

3 colorless, but rather small colonies were found. Pick + test further.

1 Lac+ Mucoid colony was found. Pick + streak out to isolate.

all Lac+ Lac+ Muc = Y69

January 17, 1947

See 383 (1-6)

P21. Colonies have taken a blue tenge. Make streaks + compare ē Y53. All show coloration in lytic zone ē T1 vennis.

5, particularly, shows few or no papillae. Y70.

1	very few papillae
2	papillae.
3	papillae.
4	few, but some papillae
5	~~Colo~~ no papillae.
6	few but some.

Y71.

Y70. — Further study suggests that fewer colonies have papillae, & fewer of them than 11-12, but some papillae are formed. Comparison should be made of some prototroph segregants. This allele may work to Y53-Lac-.

coli Woffi — papillates very readily

Attempt at transformation

January 18, 1947.

P17 - P18. 24 hour-cultures Y53 autolyze ē 300 ml
washed cells in NaCl under ~~[illegible]~~ benzene. 3 hours. shaken at 25°.

Sediment cells. Remove superficial toluene by evacuating chamber. Suspend Y40 cells in autolysate - Plate ~~[illegible]~~ 1:1 3 c̄ Y40.
Control - use washed cells of above s̄ autolysis x Y40.

See also 399.

Turbidity of autolysate was ~~more~~ < 1/2 than that of the 1:100 dilution in 399.
Keep. ~~[illegible]~~ sample overnight c̄ ~~heavy~~ heavy layer of benzene and repeat later.
Hold autolysates overnight in cold.

6P19. - remove benzene from sample by evacuation.

A. Y40 + bz-autolysate 0, also on EMB. 0
B. Y40 + autolysate 0,0
C. autolysate s̄ ~~[illegible]~~ Y40. 0,0.

autolysate is sterile; no prototrophs.

3-way cross.

January 20, 1947.

A : Y40, Y10, Y64. — −S not unlike.

BM+R × TLB, F1 +S −R

+R	−R	+S	−S
9	7	1	11

B. 58-161, Y46, Y53 — −R not unlike.

BM+S × TLB, F1 +R −S.

+R	−S	+S	−R
20	34	5	40

Some mistake??

See 4/11 for repeat

3-way cross.

Y10

BM Lac+V_1^R × TLB, Lac+V_1^S → +++

Y40 Y64

Lac+V_1^R	
Lac+V_1^S	
Lac−V_1^R	
Lac−V_1^S	not

Y46 Y53.

BM Lac+V_1^S × TLB, Lac+V_1^R → +++

58-161

Lac+V_1^R	
Lac+V_1^S	
Lac−V_1^S.	
Lac−V_1^R	not.

Controls: BM Lac+V_1^R × TLB, Lac−V_1^S → all types
Lac+V_1^S rare.

BM Lac+V_1^S × TLB, Lac−V_1^R → all types,
Lac+V_1^R rare.

already done!

Dilution Effect 1:10

January 18, 1947.

½ units:

	Y53	Y40	
1.	10^{0}	10^{0}	120
2.	10^{-1}	10^{0}	120
3.	10^{-2}	10^{0}	13
4.	10^{0}	10^{-1}	60
5.	10^{0}	10^{-2}	8
6.	10^{-1}	10^{-1}	23
7.	10^{-1}	10^{-2}	16
8.	10^{-2}	10^{-1}	8
9.	10^{-2}	10^{-2}	1

f(Y40) f(Y53)

Y53: 10^{0} 0 120 −1 60 −2 8

Y40: 0 120, 1 120, 2 13 (10^{0})

10^{-1} 0 120 −1 23 −2 16

10^{-1} 0 60, 1 23, 2 8

10^{-2} 0 13 −1 8 −2 1

10^{-2} 0 8, 1 16, 2 1

Y53+Y40 10^{0} 120 10^{-1} 23 10^{-2} 1

Mucoid segregation 400

January 17, 1947.

Y57 x Y68 (TLB, -Lac - $T^{R}_{1,3,5}$ x BM-Mac)

No prototrophs!

See 404

& 387 for mucoidy!

Y53M

Y67 x 58-161 ~~is~~ ok.

Y68 x Y53 ~~is~~ ok.

~~58~~-161M

Toxicity of benzene and removal.

January 19, 1947.

7P19. Layer 1/2 ml benzene on 1 ml Y40 in water. Keep on dark.
do in H_2O.

N20. Remove water layer; evacuate to remove benzene.

1. Plate to determine killing of Y40. — 0.
2. Add 1 ml fresh Y40 to free aqueous layer + let sit for 24 h. Plate.

January 20, 1947.

Plg. 2roz Y40, Y53 into YB + Tween ; A 20 transfer Librairie ; plate
A. 1%
B. .1%
C. .05%
no growth effect!
on T(0) agar + 1% Tween

Plg 2roz Y40, Y53. into YB. etc.
Plate into T(0) agar +
B.
A .1%
B 1%
} Tween.

all ca 10^2

no particular effect of Tween could be established.

January 20, 1947.

5 1 ml samples 58-161 grown 18h. in YS. Wash + irradiate 2 mins. bro 1:100 in nutr. sal.

1
2 5
3
4
5. 1' survivors

58-161 is evidently more sensitive than YS3. (which has had 1 further X-Ray + u.v. exposure).

~~Y64x68.~~
segregations

January 22, 1947.

1. Y65x58-161 (Y10/1/7) (in 1:100 diff.)
2. Y57 x Y68 (~~Y10~~ Y53/1 x BM Muc)

1. Shows no recombination prototrophs. (Is Y65 unable to recombine??)

See 379, 390

2. 1 plate ~ ca 100 (no success). (Try at See 400

Try Y64 x Y65

BM +R × TLB, -S

See 385.

P21. Streaks out 385-3, 4, 15. Test 1 colony isolates on T1.

		385			
1-2	3- 0	~~-R~~	- R 1 + R 2	-R	(B_1^-)
3-6	A	+S	+ S 5,6 - S 3,4	+R	
7-10	B	-S	- S 7,8 + S 9,10.	-S	
				+S	
1	4- 0	-S	- S 1	-S	(B_1^-)
2-5	A	+S	+ S 2,3 - S 4,5	+S	?
6-9	B	-S	- S 6,7 + S 8,9		
11-14	C	± S	- S 11,12 + S 13,14		
15-18	D	± S	+ S 15,16 - S 17,18		
1-2 15	- 0	-R	- R 1,2		
3-4	A	-S	- S 3 + R 4		
5-6	B	-S	- S 6 + R 5		
7-8	C	-S	- S 7 + R 8		
9-10	D	-S	- S 10 + R 9		

(Replates 3-0 also.)

types -R (B_1^-), -S (B_1^-) and +R are present.

Test samples of above: P: parental

above ##			Nutr	Comment	
3-1	1	-R	B_1 ✓		
3-2	2	~~+R~~	~~BM TL?~~	P (BM)	
3-3	3	-S	B_1		
3-5	4	+S	B_1 ?		
3-7	5	-S	X B_1		
3-9	6	+S.	++		
4-1	11	-S	B_1 ? ✓		
4-2	12	+S	+? ✓		
4-6	13	-S	B_1 ?		
15-1	21	-R	B_1 ?		
15-3	22	-S	B_1 ✓		
15-4	23	+R	BM ✓	P.	
15-9	24	+R	BM ✓	P.	
15-10	25.	-S	B_1 ✓		
3-4	31	-S	B_1		
3-6	32	+S	B_1		
3-8	33	-S	B_1		
3-10	34				

B_1 -R
B_1 -S
[B_1 +S]
[++ +S]. See 408.

— 3 tests BML definitely
1 test BM

of 21.

of 21. $(B_1-R)(B_1-S)$.

Transformation - autolysate.

January 21, 1947.

250 ml eq. 24 hour cells of Y53 harvested from YB + trypsin.
~~cells~~ autolyze 24 h. under blanket at room temp.

P22 add Y40 cells + plate.

P24 - no colonies.

Recombination types.

January 22, 1947.

Plate "B_1^-" colonies into T(0) agar + BMTL. Use plates which relatively few, isolated prototrophic colonies.

0 Y65 x 58-161

1 Y40 x Y53.

Test original colonies for V, R, lac – :

	"0"	"1"
1	+S	–R
2	–S	–S (+R)
3	–S (+R)	–R
4	–S	–R
5	–S	+R (–R)
6	–S	–R
7	+S	–R
8	+S	–S
9	+R	–S
10	–S	–R

Plate colonies into BMTL. Pick + test samples of colonies which arise.

		# colonies		
1	+S	10^5		
2	–R	1000	8 + S	
3	–S; +R	1000	8 + S	
4	–S	200	34 + S	
5	–S	1000		
6	–S	500	~~7 + S~~	7 + S
7	+S	300	7 + S	
8	+S	300		
9	+R	10^6		
10	–S	200.	8 + S	
11	–R	200	10 + R	
12	–S (+R)	500	10 + R	
13	–R	50	2 – R	7 + R.
14	–R	500	8 + R	
15	+R (–R)	300	9 + R	
16	–R	20	1 – R	5 + R.
17	–R	10^6		
18	–S	200	10 + R	
19	–S	500.		
20	–R	200	9 + R.	

(Margin, rows 3–6: –R x +S, TLB1 BM; row 10: labelled "into T(0)"; rows 13–17: –S x +R, BM TLB1)

(Rows 1–10:)
+S = BM type
–R = TLB1 type
+R = lac type.
all +S. [illegible] (BM) Test for a [illegible] requirement.

(Rows 11–20:)
+R = BM type
–S = TLB1 type
+S = lac type
[–R = B1 type]

How explain "10" – Reversion of B_1^-?? [illegible]

Recombination types - se 283

January 25, 1947.

Retest 405-2. (475) in plates. Dil to ca 100/ml + pour plates ~

1. BM 346
2. BMT do
3. MBL do
4. BMTL do.
5. BMTLB, 365