Hemizygosity tests: HZ42

May 14 ff 1952.

HZ42 is a culture giving weak lac+ (v?) colonies which may segregate very infrequently. In previous tests, Gal+ or Mal+ revertants were usually pure lac−.

3 Mal+ } from EMS. Apparently pure +. No sign of
4 Gal+ } segregation on lac. Gal+ are strongly lac+. Held in incubator.
No sign of segregation.

Lactase Efficiency

4370 units split $\frac{17.45 - 5.91}{2.18}$ mg in 20 mins.

1 unit = 6×10^{-5} mg. per minute / ml

~~= .06 r/min. of 45 r/minute each.~~

1 unit = .6 r/minute

Lactase assay on lactose (Barford) 752

May 18, 1950.

K12 harvested from 10 plates (= ca 400 ml) BNZ .3% Lactose, washed and resuspended in 15 ml H_2O. Store equal aliquots in water (A) and in water under benzene (B) (shake 3-4 hours). (overnight in refrigerator. Assay with onpg

		Di	Donpg	Acor	Assay stock susp:	mg/ml
1/10 dilutions from stock.	.1 ml A	(176)	301	123	123 u/ml	28.3
	.1 ml B.	110	++++		>	
	.01 ml.	011		437	4370 u/ml.	

LACTOSE ASSAY SYSTEM:

1 ml stock suspensions + 2 ml 10% lactose + 1 ml M/5 NaP buffer + 5 ml 10% $CaCO_3$ + 2 ml H_2O. Incubate 20 mins. Boil 2 minutes. Sediment and assay supernatants (1 ml)

	"Cells"		Assay
1.	A	no lactose	0
2.	A		4.84
3.	B	no lactose	0
4.	B		17.45
5	Novello lac		5.91
6		Glucose 1 mg.	2.18

The boiling with $CaCO_3$ appears to cause appreciable lactose hydrolysis, but the increased hydrolysis of benzene-treated cells is quite apparent

←

Lactase activity measured on lactose

Preliminary

Substrate = 2% lactose. All experiments in M/100 NaP buffer. 7.5

a). 1 ml 744 extract (= 880 units/ml) + .6 ml 10% lactose + .15 ml NaP M/5 + 2.55 ml H_2O @ 37° 3 38 – 8 45

744 extract 880 u/ml

1 ml extract + .6 ml 10% lac + .2 ml NaP M/5 + 1.2 ml H_2O.

Ca. 5 hours incubation.

Add 4 ml Benford's Cu reagent to 1 and to .1 ml.

	N/100 ml KMnO4
Glucose 1 mg	2.47
lactose 10 mg	<< 1 drop
Assay .1 ml	5.67
Assay 1 ml	36.0

This is the equivalent of complete hydrolysis. Repeat with lower unitage; shorter time.

5/18/50. System as above: .1 ml extract .6 ml 10% lactose .2 ml NaP 2.1 ml H_2O to 3 ml volume.

Sample .1 ml from time to time into 5 ml Cu reagent.

9 25 0

1 15 .5 ml sample 3.65

Glucose 1 mg 2.43 Take 2.4 as standard.

" " (1 ml boost H_2O) 2.13

∴ sample contained $2 \times \frac{3.65}{2.4}$ mg "glucose"/ml = 3.04 mg/ml.

∴ 88 units enzyme · 230 minutes split $3 \times 3.04 \times 10^3 / 360$ μ moles lactose

1 unit = $\frac{9{,}120}{88 \times 230 \times 360}$ μM/min. = 1.25×10^{-9} moles/minute.

= .45 γ/minute

Trial Run.

Calibration of method.

(1)	1 mg glucose	3.35 − .63	2.72
(2)	10 mg glucose	23/35 × 20.10	33.3
(3)	10 mg lactose	" − 1.86	1.86
(4)	100 mg lactose	" 14.72	11.86

later: titrate with Setaphonic C, no phosphoric acid

Constitutive – Lac, Recombinations from Heterozygotes

May 22, 1950.

748 B1 and B2. Each, 8 Lac v colonies, streaks out on EMB Lac.
Note Lac+ colonies c + s sheen. ~~Brush on~~ Brush on DN2 Glu, test on onpg spot plates. npg tests not correlated with sheen, but some were ~~constitutive~~ constitutive; others not. (Possibly a negative correlation (sheen + npg – . sheen – npg +).)

		sheen	npg	Nutrition
B1-1		+	–	
		+	+	
	(1)	–	+	M–
		–	+	
2		+	+	
	(2)	+	+	TLB,
		+	+	
3		+	+	
		+	+	
	(3)	–	+	++
		–	–	
5		+	–	
		–	+	
6	(4)	–	+	++
		+	+	
		+	–	
7		+	+	
		+	–	
		+	–	
		+	–	
8		+	–	
		–	+	
		–	+	
	(5)	–	+	M–

		sheen	npg
B2-1		+	–
		+	–
		+	–
		–	+
		–	+
		–	+
2		+	–
		–	+
3		+	–
		+	–
		–	+
		–	+
4		–	+
		–	–
5		+	–
		+	–
6		+	–
		+	+
7		+	–
		+	–
		–	+
		–	+
8		+	–
		+	+
		–	+
		–	+

	sheen	npg:	+	–
B1	+		7	7
	–		8	1
B2	+		2	12
	–		11	1

Use 1, 2, 5 in crosses. Recover

753-1 × Y10
753-2 × 58-161
753-3 × Y10

} on EMS Lac

Ca 250 prototrophs from each. All Lac+.

∴ None of these is Lac, - Cst+

Brush Lac - segregants on EMB Lac.

Replica and separate -, + reversions and pick to ~~E~~ DN2 Sln. Lac- components are all npg-.

B1: + comp.

	npg
1	-
2	-
3	+ 1
4	+ 2
5	-
6	+ 3
7	+ 4
8	-
9	-
10	+ 5

1319
1320

B2

	npg
1	±
2	+ 6
3	+ 7
4	+ 8
5	±
6	+ 9
7	-
8	+ 10
9	+ 11
10	-
11	+ 12

Restrick Lac- which give on pg+ reversions.

Retest

1	-
2	+
3	+
4	+
5	-
6	+
7	~~+~~ -
8	-
9	-
10	-
11	+
12	-

Classification from brief growth on DN2 probably incorrect. In retests (aerated DN2)

W1301 ++++
W1318 -
1319 -
1320 ±

Kickuck molecular reaction.

Irradiation of H226

754-0

May 22, 1950.
Fresh D(Lac). Dilute 10^{-6}. A: control B: UV 20 secs.
0.1ml per plate.

P22

A. EMB Lac

V	−
170	18
162	19
332	37

EMB Mal

+	V	−
33	139	7
10	122	4
43	261	11

B EMB Mal

+	V	−
40	42	16
24	26	13
11	18	6
40	80	15
26	25	7
38	24	10
43	94	14
222	219	81
32	31	12

Many of the Mal + in A may be V

B, the Mal V were typically much
Mal − than in A.

EMS ~~Mal~~ Lac

+	−
150	0
150	0

A. EMS Mal

+	−
118	1
39	2

May 23, 1950.

B EMB Lac
36-40 hours.

	+	−
	25	33
	29	52
	27	52
	38	58
	23	72
	35	73
6	187	340
	31	57 /68

EMS Mal

	+	−	S
	28	3	6
	24	20	16
	29	8	6
	26	12	9
	32	18	10
5	139	73	47 /
	28	15	9 / 52

Notice very high proportion of prototrophs in yield! Test by picking at random from EMB Lac.

5/24/50.

a) Pick Mal− from B and ~~streak~~ brush on ~~EMB~~ (EMB Mal) Lac ; Xyl; EMS Lac

b) Pick Lac v from EMB Lac (B) to water; spot on EMS Lac; EMB Mal for partial segregants.

c) Pick apparently pure Lac − from EMB Lac to water. Spot on EMS Lac. After 4 days, none of 40 spots was lac+. 6 Lac − prototrophs. These colonies may be presumed to be pure

A25. a) ①. Pure Mal−. Spot from water scrap. 35 tests: EMB Mal, Lac; EMS Lac

1 Mal− Lac+ ; remainder are Lac−. Hold EMS plate. Streak out Mal−Lac+ on EMB Lac as 754a1

① Brushes from Mal− and Mal +/−. (From middle, no suspension). [LEFTHAND IS MAL− COMP. I

✓=growth
+=lac+

Xyl EMB	Lac EMB	Lac EMS
−	−	
−	−	
−	−	
−	−	
−	−	
−	−	
+	−	
−	−	
−	✓	✓
+	+	−
−		−
+		−
+	+	−
+		−
−	+	−
−	+	−
−	+	−
−	✓	✓
+		−
−		−
−	+✓	−
−	+	−
−		−

X		L B		L S	
−	✓	−	✓		+
−	—	−	−		
−	+	−	−	≠	
−	+	−	−+	✓—	✓—
−	−	−	−	✓—	✓—
−	+	−	✓		+−
−	✓	−	−		
−	+	−	+	#	+
+	+	−	−	—	—
−	✓	−	−		
−	+✓	−	+	✓	+
−		−			✓
−		−			✓
+		−			
+		−			✓
−		−			
−		−			
−		−			
+		−			
−		−			
−		−			
+		−			
−		−			
−		−			
+		−			

754a2.

Subtotal: 47 tests: 1 Mal− Lac v

Mal− : 35 Xyl− : 12 Xyl+

Total 2/82.

	Lac	Mal	Xyl	EMS lac	Nutr
a1	✓	—	—	ng	M−
a2	✓	—	—	ng	M−

May 25, 1950

b) 120 Lac v picked to Lac EMS; Mal EMB.
All Mal+(or v) except #'s: 8, 10, 17, 23, 59, 66, 67, 73, 82, 84, 88, 117 [12/120]
which are Mal−, predominantly. Save suspensions.
All formed colonies on EMS by 24 hours except:
~~5~~, ~~6~~ 19, 11, 14, 20, 21, 22, 24, 30 32, 35, 36, 38, 40 43, 48, 49, 50 54, 55, 56, 60
61, 66, 68, 69, 70, 81, 82, 83, 87, 88 ~~92~~ 91, 93, 100 106-110, 113, 114, 116, 119, 120
Hold additional 24 hours.

Restreak Mal− suspensions on EMB Lac; Mal; EMS Lac.

c) EMS Lac: hold EMB Mal: 29 app. pure +
6 app. pure −
6 clearly mixed.

more gave EMS Lac+

d) As a) but from A

e) As b) but from A
f) 3 Mal− from A, EMS Mal. to EMB Lac, Mal, S Lac } See 756.
g) Mal− prototrophs from B↑. Brush to EMS Lac

c754

May 26, 1950.

b)

	Lac	Mal	EMS	Mutation
~~8~~	✓	–	+	
10	✓	–	+	
17	✓	–	ng	L–
23	✓	–	+	
59	✓	–	ng	TL–
66	✓	–	ng	TLB$_1$–
67	✓	–	+	
73	✓	–	+	
82	✓	–	–	
84	✓	–	–	
88	✓	–	ng +	M–
117	✓	–	–	

Thus about 10% of the surviving Lac✓ colonies are Mal– Lac✓.

From a) one infers that 2/82 = ca 3% of the surviving Mal– colonies are Lac✓.

Cf. d, e, f.

see 754b

See 755.

c) Held

d) Mal– from control plates. Also sectored colonies.

7 pure Mal–: 6 Lac– 1 Lac✓ (prototroph.) Restreak as 754d1. ✓Mal– Lac✓ prototroph

14 sectored colonies. Mal– fraction is Lac–.

e). Lac✓ from control plates: to EMB Mal, EMS Lac 89 tested.

3 are Mal–, prototroph. Restreak. 1 Mal+ (or ✓) non-prototroph

754e1-3 1 Mal– Lac✓ 3 Mal✓ Lac✓ | 754e4 Lac– ✓Mal+,–,✓
– Mal– Lac✓ ✓prototrophs. non-prototroph.
≡ T– L–

f: Each of 3 is Mal– Lac✓ prototroph. Save 754f1.

g) ~~f~~. Replate H226 ~~on Lac~~ on EMB Mal: 6 plates. Pick pure Mal– to Lac

g) 42 Mal– prototrophs from B. 19 are Lac+.

29 sectored pairs. Mal– component of 11 pairs is Lac+.

To be verified. Pick possible exepts, and their sibs, to water and spot on EMB Mal, ~~EMS~~ EMS Lac.

May 27, 1950.

h) 13 pure Mal− colonies : 2 Lac ± ; 13 Lac− Retest the +.
2 pairs Mal−/+ tested Lac of the recorded Mal−/ were Lac v, but also scored Mal+, and presumably Thal v.

g.

June 1, 1950

25 Lac+ av cultures reisolated as autotrophs. Streak out on various sugars to determine further characteristics

	Lac	Mal	Xyl	Mtl		
1	v	v	—	—		
2	v	v	v	—		
3	v	v	—	v+ ?		
4	v	+ v?	—	—		
5	v	v	v	v	M	call it B12
6	~~—~~ v	+	—	v		
7	~~—~~ v	v	+	v	FL	B13
8	v	—	v	v		
9	v	v	+	v		
10	+?	v	v+	v	M	
11	v ~~+?~~	v	v	—		
12	v	v	v+	v	M	
13	v	v+	+	+		
14	v	—	v	v		
15	+?	v	+	v+		
16	v+? ~~—~~	v	v	v	M	
17	—v	+	+	v		
18	o	o	o ~~—~~	o		
19	-v	+	~~—~~ v	—	L	
20	++ +?	—	+	+		
21	v	+	+	+v	M	B14
22	v	—	v	—		
23	v	v	v	v	M	
24	v	—	—	v		
25	v	+	v+	+		

XX

See 756

Restreak v as possible use for outcrossing. Determine mutations.

June 2, 1950

Separate 7 Mal^{sec} colonies of which Mal− component is lac^{v}.

	EMBLac	EMBMal	EMSMal	EMSlac	
1 −	v	−		+	
+	v	v	++	+	
2 −	v	−		+	ξ unstable − throws off Mal−lac^{v} prototrophs.
+	v	v	++ −	+	
3 −	v	−		+	
+	v ⊙	++	++	+	
4 −	v v	−	−	+	
+	− −	v	++	−	
5 −	v	−		+	
+	v ⊙	v	− ξ?	+	
6 −	v	−		+	
+	v	v+	+, −	+	
7 −	−v	−		+	
+	−v	v+	++	+	

2, 6 Mal+ (EMS) may be segregating Mal− prototrophs (lac^{v}?)
Verify from single Mal+ colonies (EMS).
3 may illustrate a segregation from Mal^{v} into Mal+ and Mal− lac^{v}.
Verify from EMS Lac.

4 seems to suggest another type of separation. Lac Mal^{v} ⇄ lac^{v} Mal− / Lac− Mal^{v}

5 may be confused: Mal− presumably was streaked on EMSMal, for Mal+.
Verify from EMS lac. ✓

6/3/50 ✓ 3 → Mal+ lac^{v} and Mal− lac^{v}

4 → Mal^{v} lac− and Mal− lac^{v}

6/4/50 5 → pure Mal+

6 → each of 4 tests pure Mal+ prototrophs

2 → 1−10% Mal− prototrophs in each of 8 tests. Restreak; slant as 754g2; test Mal− on EMB Lac 4|10 were lac^{v}.

A. Lac v ≐ ~~total~~ population ≐ prototrophs.

Auxotrophs = 1%

Mal- lac v = 2%

Mal- prototrophs = 2% (all lac v)

B. Lac v = 35.5% Prototrophs = ca 2/3? p Kill = ca 88/180 = 50%

	/Total	/ lac v	/prototrophs	/original total //	
Auxotroph lac v	7.4	21		3.7	
Mal- lac v	3.5	10		1.8	
Mal-	15.5			8	3.5
lac -	64.5			33	10
Mal- prot. lac v			12.7		

The predominant effect is to make haploids pure for Mal, lac. The residual lac v have a high proportion of perturbations. (should be checked on other characters).

754

B u.v. Mal- (EMB) 81/522 = 15.5%

Lac- (EMB) 340/527 = 64.5% Lac v = 35.5%

Mal- (EMS) 73/259 = 28.2%

a) Mal- (EMB): 2/56 = 3.57% Mal- Lac v.

Total fraction = 3.57 x .155 = .55% intact Mal- Lac v.

b Lac v. 25/120 are auxotrophic: $\frac{25}{120} \times 35.5 = 7.4\%$ auxotroph

12/120 are Mal- Lac v = 3.5% Mal- Lac v.

c. Mal- (EMS). 19/42 are Mal- Lac v = $\frac{19}{42} \times 28.2 = 12.7\%$ of prototrophs are Mal- Lac v.

754

A. Control: Mal− = 11/315 = 3.5% EMB Mal

Lac − = 37/369 = 10.0% EMB Lac

EMS: Mal − = 3/157 = 1.9%

d 7 Mal− from EMB Mal : 1 Mal− Lac v.

∴ H226 suspension is ca .5% Mal− Lac v.

e. Lac v from EMB Lac /89 : 2 Mal− Lac v / Mal v Lac v non prototroph

This gives estimate of 2/89 = 2.2% Mal− Lac v.

1/89 = 1% auxotroph Lac v.

f. Mal− prototroph 3/3 is Mal− lac v. This gives estimate of ≅ 1.9% Mal− Lac v (prototroph).

Summary: controls. Take 2% as Mal− Lac v (prototroph)
1% as Lac v (auxotroph)

$$3080\ u/ml = V_{max}\ of\ \frac{1.25 \times 3080}{27} / mg.$$

Lactose activation re lactose

R-12 harvested from 6 x 40 ml DN2 .3% lactose agar. Wash and resuspend in + 10 ml H_2O. (A). 3 ml aliquot of (A) shaken with benzene 2 hours. Stored overnight ~~at~~ in refrigerator.

a) onpg assay (before storage). in NaPM/50 7.5

	ml cells or extract	9 ml Di	20 ml Donpg	Δ cor	Assay
RA/.69.6 7.7 u/mg	A .01	129	221	090	A = 90 u./ml
	B .01	076	>900 6 mins.	corrected:	
RA 23.9 142 u/mg	B .001	010	332	308	B = 3,080 u/ml.
	—	-004	011		

b) Manometric assay. Dilute 1.5 ml (A) with bicarbonate to 10 ml M/20. 2 ml cells; 0.1 ml 10% substrate in sidearm.

Flask	Subst.	3^{20} ↓	3^{25}	3^{32}	3^{40}	3^{50}	4^{00}	4^{15}	4^{27}	4^{37}
2A	Glu	26'	40	62'	91	124	157'	213	258	294'
3B	Lac	34'	41'	60'	87	118	147'	197'	239	270
6B	—	31'	36'	39	37	39	37	40	40	41
7A	ThBar. —	24	29	29'	29'	30	27	29	32	31

Increments:

	T	TB-	A	B
		0	0	0
3^{32}	7	2'	20	16'
3^{40}	15	0'	50'	45
3^{50}	25	2'	81'	74
[illegible]	35	0'	117	105
4^{15}	50	3'	169'	152
[illegible]	62	3'	214'	[illegible]
[illegible]	72	4'	250	224

A: 215 mm/hour k = 1.84 (glucose) 396

B: 190 mm/hour. k = 1.78 (lactose) 338

∴ A ≐ 17.7 μM CO_2/hr ≐ 1.27 mg glucose/hr.

B ≐ 15.1 μM CO_2/hr. ≐ 1.09 mg glucose/hr.

(Assuming 1 μM glucose ≐ 2.5 μM CO_2 - Stokes - J. Bact. Feb. 1949)

$Q_{glu}^{CO_2}$ = .128

$Q_{lac}^{CO_2}$ = .110

Original suspension A) 33 ~~u/mg~~ mg/ml

33 x .3 = 9.9 mg / Warburg flask.

27 mg/ml original suspension

c) Lactose hydrolysis. System: 37° 10 minutes.

2% lactose 2.5 ml
Cells 1.0 ml
NaP 4/5 7.5 .5 ml.

Terminate Reaction by adding .5 ml 5% $ZnSO_4$. Clarify with .5 ml .15N $Ba(OH)_2$. Sediment and Decant clear supernatant. Dilute 1:10 in water, and take 1 ml for assay, after Caputto, Leloir and Trucco, Enzymologia, 12:350 (1948). To read optical density, read the reduced Mo reagent at 1:10 dilution at 520 mμ in spectrophotometer. All readings of same single cuvette.

6.6 mg/ml

		Density
1.	A. No lactose	004
2.	B. No lactose	000
3.	A. lactose	002
4.	B. lactose	110
5.	— lactose	003 ~~110~~
6.	Glucose (1 ml 2% = 4 mg/ml).	107
7	Blank. + reagents.	003

Thus, 1 ml B in 5 ml liberated 4 mg glucose / ml in 10 minutes.

∴ 1 unit ≐ 20 / 3080 × 10 mg / min.
= .65 γ / min.

No accumulation of monose by A could be detected. It can be assumed to have hydrolyzed ~~at the rate of 1.09/6 × 10/1.5 × 1/2 →~~ 121 γ / ml ($\frac{33\ \gamma}{5\ ml} \times \frac{10}{60} \times 110\ \gamma/mg$), ignoring temperature coefficients. Activation of 4/.12 = 33 × re lactose. Activation re enzyme was 3080/90 = 34 ×

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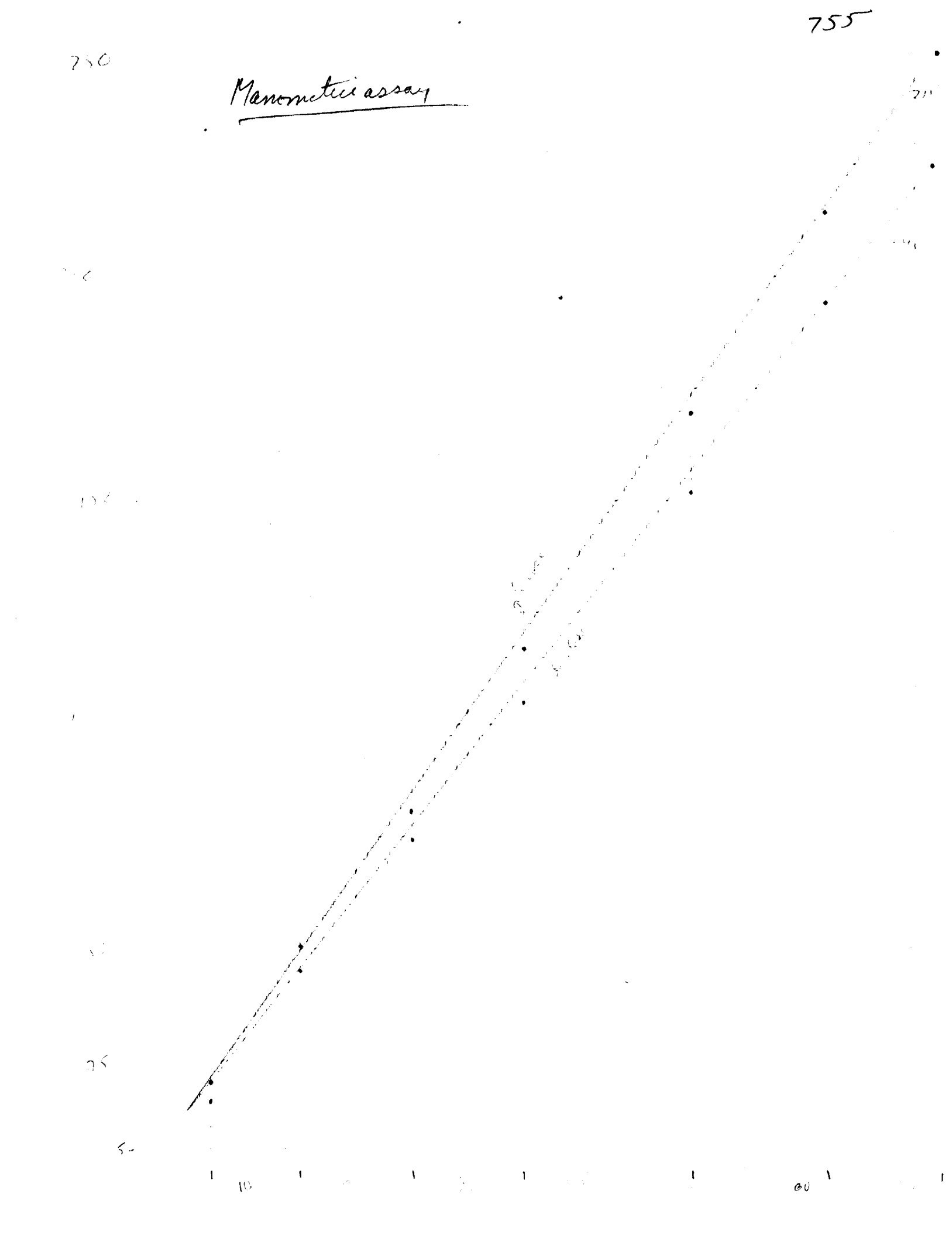
Manometric assay



Auxotrophic partial segregants.
FROM H226

May 29, 1950.

	#		Nutr.	Mal	Xyl	M4l	Agent
	H244	M	~~B ~~	–	V	+	uv
T54	A1		M	–	–	–	uv
	A2		M	–	–	+	uv
	B3		L	–	V	V	uv
	B5		TL	–	+	–	uv
	B6		TL	–	V	–	uv
	B11		TLB,	–	–	–	uv
H245	E4		~~M~~ TL	V	V	V	spont
~~H246~~	F1		~~H~~	–	–	–	spont.
	H244M+		M	V	V	+	

Attempt crosses of H245, H246.

~~B11~~					
B12	M	V	V	V	uv
B13	TL	V	+	V	uv
B14	M	+	+	+	uv

Irradiation of H226

4 single colony isolations of H226 (A-D) used to start D(lac) cultures.
A-D are 10^{-7} controls
A_x-D_x are UV 20 secs. at same dilutions. UV and EMS at 40 hrs. Others at 24.

A.

EMB Lac	V	−	EMB Mal	+V	−
	264	17		280	4
	291	24		258	2
	317	15			
	872	56		538	6
		928			

~~EMS Lac~~

EMS Mal

+	−
322	2
185	0!
184	0
691	2

EMB Lac	V	−	Mal	+V	−	EMS Mal	+	−
B.	311	12		215	2		321	0
	334	12		276	4		254	1
					4			
	645	24		491	6		575	1

C.

	217	8		260	5		248	1
ca ⅓ pl.	62	3		228	4		178	1
	202	17		274	2			
	481	28		762	13		426	2

D.

	1	smeared or crowded - uncount.	(.2)	487	4		419	0
		236	23	283	2		131	0
		395	25	260	2			
		635.48						

Essentially homogeneous.

	EMBlac		EMBMal			EMSMal		
A	V	—	~~(2)~~	V +	—	sec.	~~+~~ +	—
	88	76		201	29			
	143	97	(omit summarized part.)	114	12	17	135	30
	80	93		260	25	24	161	26
	311	266						
B	1+ 70	70		199	14	8	101	23
	94	98						
	135	87						
	299	255						

Cx
Dx ditto.

June 2, 1950.

Pick Lac v sinuosis from A_x – D_x to water suspensions. Spot 120 on EMS ~~Lac~~ Mal, Xyl, Mtl and EMS Lac.

	Slac	Mal	X	Mtl		Slac	Mal	Xyl	Mtl								
1	+	V	+	V	11	+	+	+	V	21	+	V	+	V	31	+	V	+	V
2	+	(−)	+	V		+	+	+	V		(0)	+	+	(−) V		+	V	V	V
3	+	V	V	V		+	V	+	V		+	+	+	V		+	V	+	V
4	+	V	V	V		+	+	+	V		+	+	+	V		+	V	+	V
5	+	V	(−)	V		+	+	+	V		(0)	+	(−)	V		+	V	+	V
6	+	+	+	V		+	V	+	V		+	+	+	(−)		+	V	+	V
7	+	V	V	V		+	V	+	V		(0)	V	+	V		+	V	+	V
8	+	V	+	V		+	V	(−)	V		+	V	+	V		+	V	+	V
9	+	+	+	V		(0)	V	V	V		+	V	+	V		+	V	V	V
10	+	+	+	V	20	(0)	+	V	V	30	+	V	+	V	40	+	V	V	V

41	+	V	+	V	51	0' +	+	+	V	61	+	V	+	V	71	+	V	+	V
2	0	(−)	+	+		+	V	+	V		+	V	+	V		+	V	+	V
3	+	+	+	V		+	V	+	V		(0)	V	+	+		+	V	+	V
4	+	+	+	+		+	V	+	V		+	V	V	V		−	+	+	V
5	+	+	(−) V	V		(0)	V	+	V		−	+	V	V		−	V	V	V
6	(0)	+	V	V		(0)	V	V	V		+	V	V	V		(0)	+	V	V
7	+	+	V	V		+	V	V	V		+	V	+	V		+	V	V	V
8	+	+	V	V		(0)	V	+	V		+	+	+	V		+	+	+	V
9	+	V	V	V		(0')	+	+	V		+	V	+	V		+	V	V	V
50	+	V	V	V	60	(0')	V	+	V	70	+	+	+	V	80	+	V	(−)	V

81	+	+	+	V	91	+	+	V	V	101	+	+	+	V	111	+	+	V	V
2	+	V	+	V		(0)	V	+	V		+	V	+	V		+	V	+	V
3	+	+	+	V		(0)	+	+	V		+	+	+	V		+	+	+	V
4	+	+	+	V		+	V	+	V		+	+	+	V		−	V	+	V
5	+	V	+	V		(0) +	+	+	V		+	+	+	V		+	+	+	V
6	+	V	+	+		(0)	V	(−)	V		+	(−) V	+	V		+	+	+	V
7	(0)	V	+	V		(0)	V	V	V		+	V	+	V		+	V	V	V
8	+	(−)	+	+		+	+	+	V		+	+	+	V		−	+	V	V
9	+	V	+	V		+	+	+	V		+	+	+	V		+	+		V
90	+	V	+	V	100	+	+	+	V	110	−	V	+	V	120	+	V		V

11 possible fermentative deviations.

19 additional auxotrophs. ~~check on EMS Lac −.~~

30

See 759.

Aimed — [illegible] still hetero [illegible]

[illegible]

Some sum hetero [illegible] are stabilised →

lethal mutations.

compare balanced lethals vs unbalanced

50%

[illegible]

10%

Zelle 6/5

M	Blac	Small	Large	Bud
7	—	+	+↓	+↓
8	—	+	+	+
9	—	+	+	+
10	—	+	+	+
23	—	0	+	+
24	—	0	+	+
25	—	0	+	+
26	—	0	+	+
27	—	0	+	+
28	—	0	+	+
29	—	0	+	+
30	—	0	+	+

L				
23	+↓	+↓	+↓	+↓
26				
27				
30				
49				
50				
51				
52				
57				
58				
59				
60				

E				
6	+↓	+↓	+↓	+↓
11				
12				
15				
16				
17				
18				
19				
20				
21				
22				

G				
1	+↓	+↓	+↓	+↓
5				
6				
7				
8				
9				
10				

Note. sterile H_2O added to all empties

788

Zelle 6/5/50

C	Blac	Smal	Bryo	BMH
7	− ?	+	+	+ ↓
9	+	+		
11	+	+		
14	+	+		
21	+	+		
35	+	+		
36	+	+		
37	+	+		
38	−	−	prot −	++ ?
45	+	+	+	
46	+	+		
51	+	+		
52	+	+		
53	+	+		
54	+	+		
56	+	+		
58	+	+		
111	+	+		
112	+	+		+ ↑

B	+ ↓	+ ↓	+ ↓	+ ↓
7				
17				
18				
19				
21				
22				
23				
24				
25				
26				
59				
60				
61				

Zelle 6/5

D	Blac	SMal	Kyl	Mel
7	+↓	+↓	+↓	+↓
9				
11				
17				
21				
27				
28				
29				
30				
37				
38				
46				
51				
52				
53				
54				

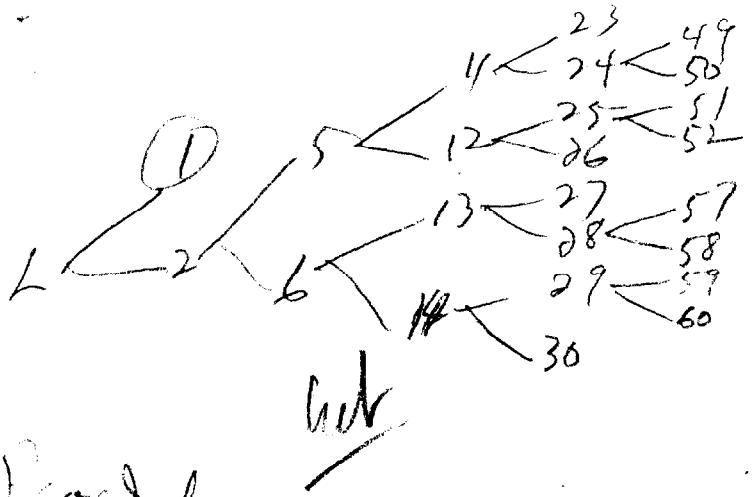
A	+↓	↓+	+↓	+↓
10				
15				
Empty 16				
18				
19				
20				
23				
24				
25				
26				
27				
28				
29				
empty 30				
35				
36				

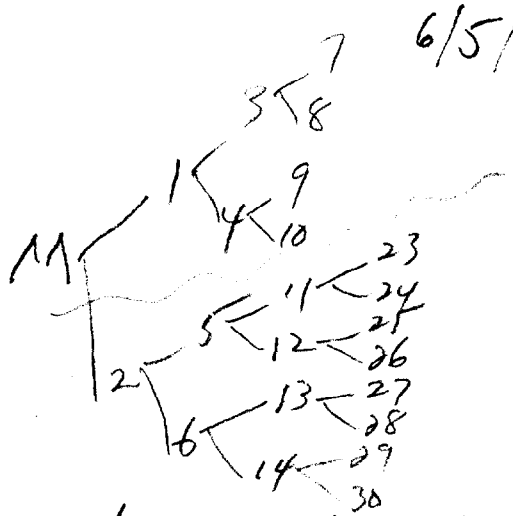
H			+↓	+↓
7	+	+↓		
8	+	+		
12	—	o		
23	+	+		
24	+	+		
27	+	+		
28	+	+		
29	+	+		
30	+	+		

Zelle 6/5

F	EMBLac	EMSMal	EMBXyl	EMBAra
11	++	+↓	++	+↓
15				
16				
19				
20				
21				
22				
25				
28				
29				
30				
35				
36				
37				
38				
53				
54				
55				
J	++	+↓	+↓	++
7				
17				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				
29				
30				
37				
38				
K	+↓	+↓	+↓	++
21				
23				
24				
25 empty				
26				
27				
28				
30				
45				
46				
59				
60				

6/5/50





Dear Josh:

Just to prove I haven't entirely forgotten about the stuff, I'm sending the above cultures. This will be all for another two weeks as I'm off on another rover mission. I haven't had any time to think, let alone write but I'll try to get at it when I get back to town.

If I haven't already given you it, my new home address is:

30 Decatur St
Kensington, Maryland.

I hope the cultures don't all turn out to be haploids. I'm sorry about so many uncertain groups of 4 cells but they grew too fast for me, probably due to the horribly warm humid weather we've been having.

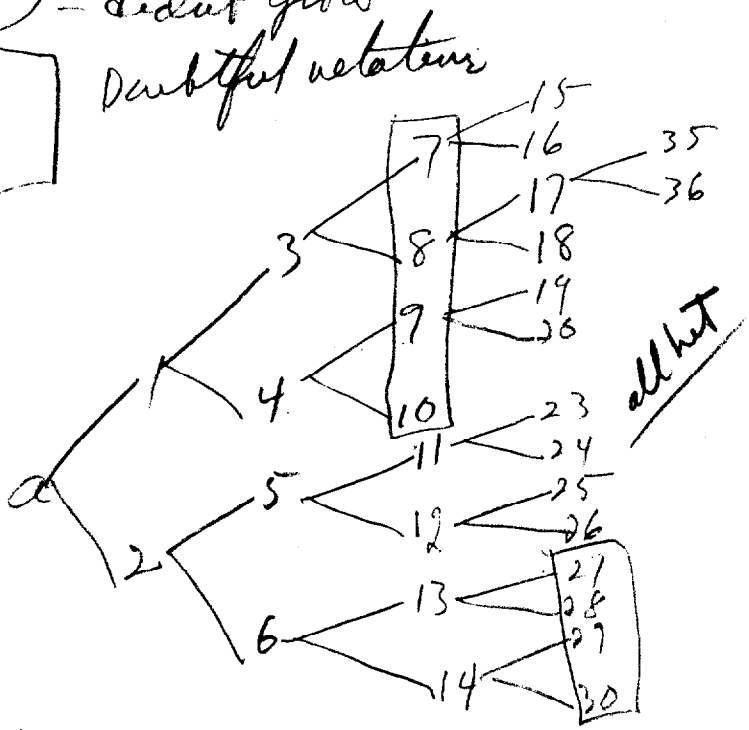
As ever

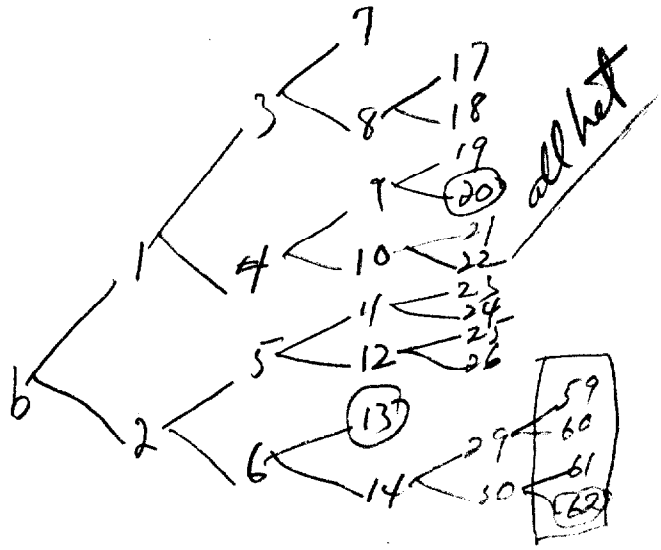
Max

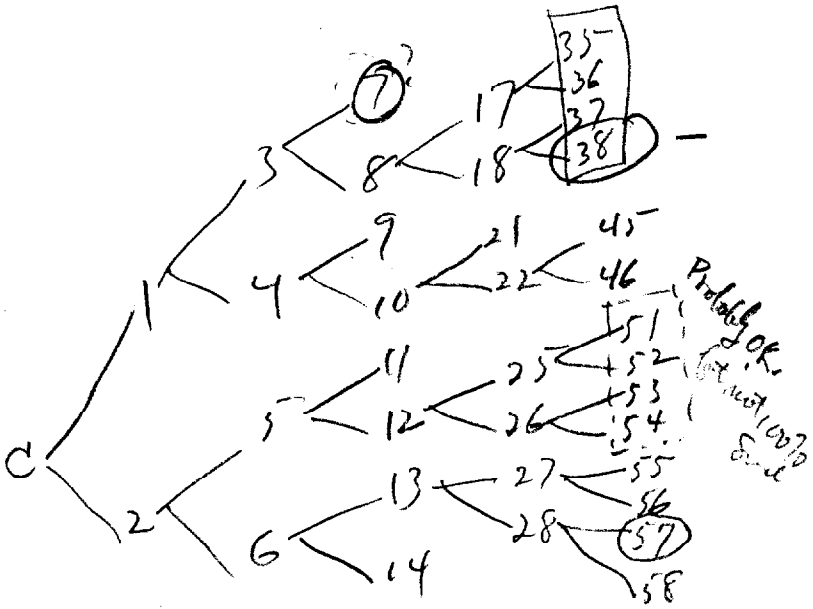
Cultures of 6-5-50.
Source H-226 - EMS colony in Davis synthetic media.

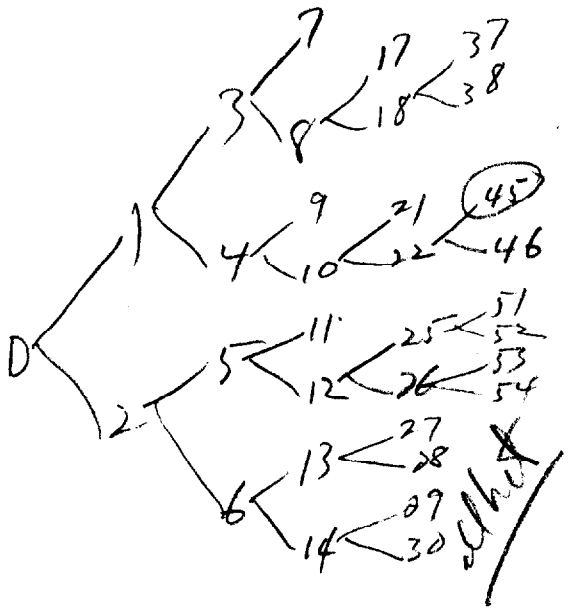
O = didn't grow

Doubtful notations



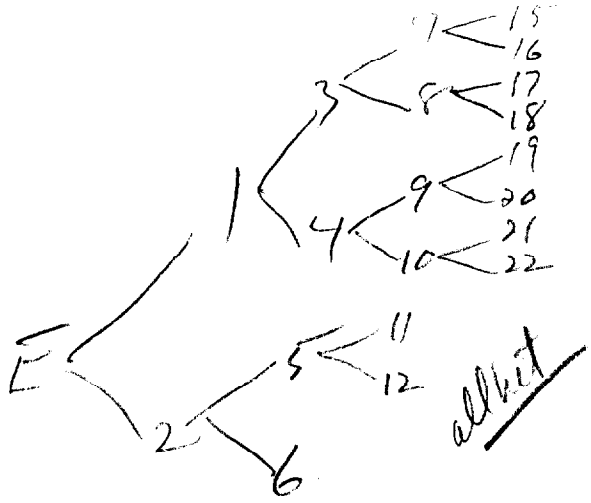


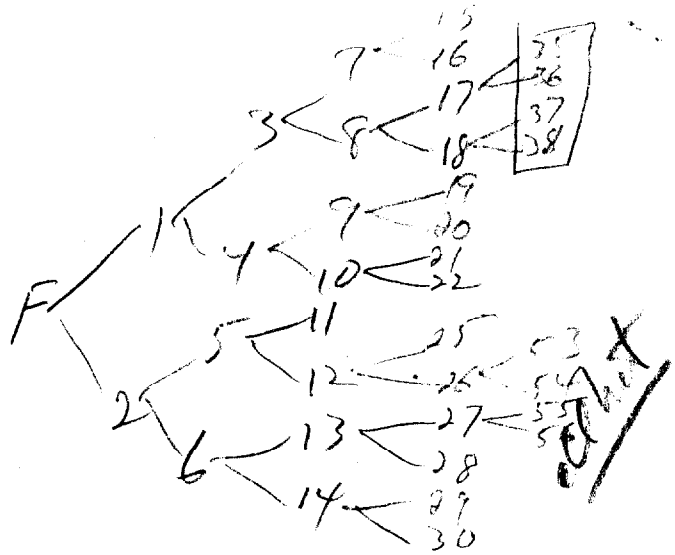




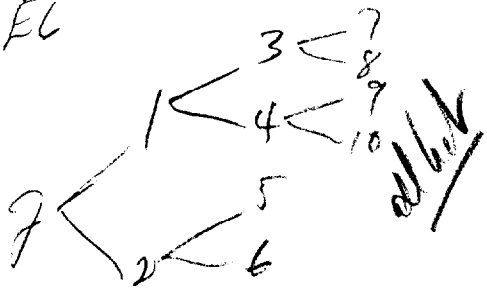
Pious
3 equal cells

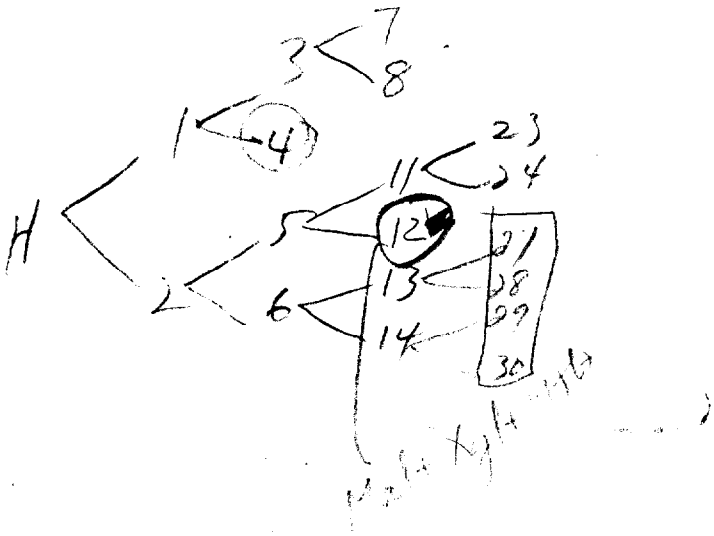
/ = 21
/ = 45
/ = 46



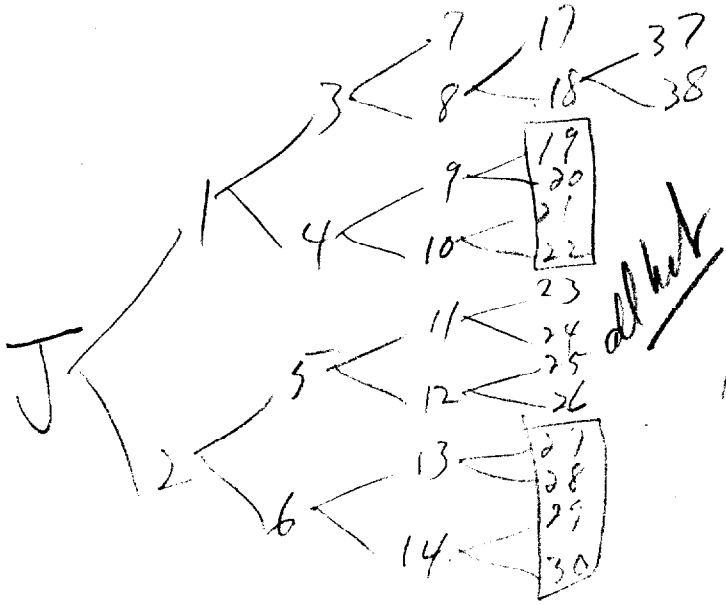


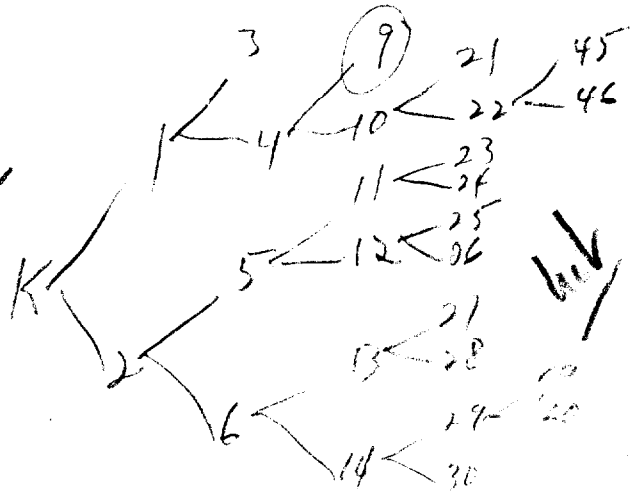
E6 [illegible] E13, E14 was [illegible] by the needle, I left E13 as E6





F series were very slow moving filamentous cells, i.e. larger than usual.





	A	B
Q_{CO_2}	40.0 ul/hour mg^{-1}	34.2 /hour mg^{-1}
=	1.78 uM^{CO_2}/hr/mg	1.52 /hr/mg
=	.71 uM^{glucose}/hr/mg	.61 uM^{glu}/hr/mg
=		

Original suspension (A) had $\frac{1}{.01} \times \frac{129}{.9} \times .23$ mg/ml bacteria.

$= 33$ mg/ml.

Each vessel therefore contains $33 \times 2 \times .15 = 9.9$ mg.

$\therefore$ ~~Q_{CO_2}~~ $Q^{lactose} = 1.09$ mg / 9.9 mg / hour

$= .11$ mg / mg / hour.

		glucose	lactose
$Q_{CO_2}^{CO_2}$	= +	396 ul	338
$q_{CO_2}^{CO_2}$	=	40 ul/hr	34 ul/hr
	=	mM 17.8/hr	15.2/hr.
	=	7.1 mM glucose/hr.	6.1 mM glu(lac)/hr
	=	1.27 mg glucose/mg/hour	

MR Zelle: single cell isolations H226

Summary of exceptions:

		Sib
H12	Lac− auxotr.	23-2✓
C38	~~Lac-Mal-Xyl-Mtl−~~ prototroph.	35-37. ✓c7
M 1	prototroph } Lac−	—
M 2	auxotroph } Lac−	

Check samples of each pedigree for heterozygosity

		Lac	Mal	Xyl	Mtl
A 1	A 10	✓	✓	✓	✓
2	B 7	✓	✓	✓	✓
3	D 7	✓	✓	✓+	✓
4	E 6	✓	✓	✓	✓
B 1	F [redacted] 11	✓	✓	✓	✓
2	G 5	✓	✓	✓	✓
3	J 7	✓	✓	✓	✓
4	K 3 28	✓	✓	✓+	✓
5	L 23	−✓	+✓?	✓	✓

M	7	—	✓	✓	✓	} prototroph. Restricts on EMS Mal.
	9	—	✓	✓	✓	
	23	—	++	++	++	
	30	—	++	++	++	
	35	✓	✓	✓	✓	
	36	✓	✓	✓	✓	
C	37	−✓	−✓	−✓	−✓	
	38	✓	✓	✓	✓	
	7	−−✓	✓	✓	✓	
H	12	−	+	+	+	
	23	✓	✓	✓	✓	
	24	−,✓	+ (=)	+,(−)	+(−)✓	

∴ H12 is haploid segregant.

C: none heterozygous!

M 1 prototroph partial segregant — No information!

M 2 auxotroph segregant.

Irradiation of H226

June 12, 1950.

Exp. 757 interrupted owing to "Virus Seminar".

Repeat. A = H226 10^{-7} B = H226 10^{-7} UV 20 sec. Plate on EMB lac, and pick Lac v. Spot on EMS lac EMB Mal EMB Xyl

A). 100 suspensions.

nogr. on # 2, 29, 43. Remaining 97 are all Mal+ Xyl+ (v presumed).
" " on EMS: #49 Residuals: Nogrowth

B). 99 ~~100~~ suspensions.

nogr: 36, 44, 56, 91

Xyl − 28, 31, 37, 83

Mal − 37, 39?, 43 83, 89.

~~Auxotr: 37, 39?, 4~~ 6, 9, 10, 13, 17, 18, 25, 26, 27, 29, 32, 34, 35, 39, 43, ~~##~~ 46, 48, 49
Lac-pr.: 5, 12, 30, 45, 57, 63, 72, 74 51, 52, 53, 59, 61, 62, 65, 66, 67, 69, 70, 75, 78, 79, 80, 81, 84, 86, 87, 89, 91, 95, 96, ~~100~~

Exceptions: 7 Fermentative (Mal- or Xyl-)

Residuals on EMB Lac. Examine for non-segregating + colonies.

Lac − 95 None found.
ng ~~100~~ 49 52

Replate lac v for confirmation of EMS character.

This test takes into account only those cases in which no Mal+(e.g.) is produced by the colony. A closer test would involve the fractions of Mal− and Mal ~~B~~ which are partial segregants

Retest single lacV colonies

Mal: − : 6, 16,17 22 24 37 40 42 43 51 52 53 56
63 64 71 83 89 90 92 98

Xyl − 4 7 14 18 19 25 28 31 37 38 42 43 58
63 64 68 75 83 92 97

MR − 4 7 14 18 20 24 25 30 36 37 42 43 51 52 58
63 64 68 72 80 83 84

DISCREPANT TYPES. lacV:

Mal	MR	Xyl	
−	−	−	~~37~~ ~~24 42~~ 42, 43, 63, 64, 83
* −	+	+	16, 17, 22, 40, 53, 56, 71, 89, 90, 98
+	−	−	4, 7, 14, 18, 25, 58, 68,
+	−	+	72, 80
+	+	−	~~#~~19, 28, 31, 38
−	−	+	~~24, 43~~, ~~37~~ 24, ~~*~~37, 51, 52,
−	+	−	92

Prototrophs	1	11	21	31	41	51	61	71	81	91
1	·	+	+ /	+	+ /	·	· /	+	(+)	·
2	+	· /	·	· /	+ /	·	+ /	· +	+ /	+
3	·	·	+	+ /	· /	· /	·	(+) /	·	+
4	+ /	+ /	·	−	·	+ /	+	* · /	−	(+)
5	+	+ /	−	·	−+	·	* · /	(+)	+	·
6	/ + /	·	− /	+	· /	+	* ·	+	−+	·
7	/ · /	·	· /	+	+	+	+	+	· /	+
8	· /	· /	+ /	+	+ /	· /	+	+	· /	+
9	+ /	+	+	· +	+	·	* ·	+ /	·	·
10	· /	+ /	(+) /	·	+	+	· · /	· /	+	

lacV

40

	Mal	Xyl	Mtl	Nutrition
4	+	−	−	+
6	−	+	+	+
7	+	−	−	−
14	+	−	−	+
16	−	+	+	−
17	−	+	+	−
18	+	−	−	−
19	+	−	+	+
20	+	+	−	+
22	−	+	+	−
24	−	+	−	−
25	+	−	−	+ (±)
28	+	−	+	+
30	+	+	−	(+)
31	+	−	+	+
36	+	+	−	+
37	−	−	−	+
38	+	−	+	+
40	−	+	+	−
42	−	−	−	+
43	−	−	−	−
51	−	+	−	−
52	−	+	−	−
53	−	+	+	−
56	−	+	+	+
58	+	−	−	−
63	−	−	−	−
64	−	−	−	+
68	+	−	−	+
71	−	+	+	+
72	+	+	−	...
75	+	−	+	+
80	+	+	−	−
83	−	−	−	−
84	+	+	−	...
89	−	+	+	−
90	−	+	+	+
92	−	−	+	+
97	+	−	+	+
98	−	+	+	+

18 −

22 +

Total aberrants (no overlaps)
are 40 fermentative (18 aux, 22 prot)
+ 19 aux (sum +);
59% detected changes!

S^R x Prototroph Method

September 21, 1950.

Inoculate 8 PM 9/20 from young aerated cultures in Penassay. Grow overnight & wash.

Aerated.

1 K-12
2 K-12 + W-1177
~~3 K-12 + W-1246~~
~~4~~ W-1246
5 W-1177

Unaer. (less dense)
6 K-12
7 K-12 + W-1177
8 W-1177
~~9 W-1246 + W-1177~~

Results:

9/22 1, 5: 3 plates each. No colonies

2: ca 1000/plate

9/23: 1 colony on 5. (K-12 S^R mutant, check unselected markers.

See 763

9/23 6; 8 2 plates each. No colonies

7 ca 400/plate.

Test single colonies on EMB lac

760-2 Either found, on EMB lac

	+	−	?
	42	46	88
	57	41	98
	109	87	196

$\chi^2 =$

Conclusion: (11/12/50): $S^R X^+$ selection is a reliable method for detecting recombinations.

Pseudomonas fluorescens

Preliminary and penicillin sens.

Sept. 21, 1950.

P. fluorescens. A3.12 received from R. Stanier.

Grow at 30 ±. Aerated cultures gave heavy growth overnight in Penassay or in D(glucose). However D— also supported growth, presumably due to citrate utilization.

a) Test "PF" base: per liter with addn. of 0.1% substrates.

K_2HPO_4	4
KH_2PO_4	4
$MgSO_4$	.5
NH_4NO_3	2

n.g. with benzoate or glucose.

Throw out!

b Dilute cells from dense Penassay culture 1:20. Add varying units penicillin and aerate. 230 PM.

Pen.	4 30	6 30
0	++++	++++
50/ml	"	++++
100	"	+++
500	"(+++?)	++ lysed
1000	++ lysed? (granular deposit).	

∴ 500 – 1000 units/ml will lyse Pseudomonas fluorescens.

9/22/50. [20-40-60 sec. doses. Dilute in Penassay 1145]

Wash 48 hr. aerated D(Glu) culture and resuspend in $M/_{15}$.

UV at 50cm 10ml samples in Petri dish. Dil. ½ml 1/10 and dilute from this as 10^n for viable counts

UV sec.	Count
0	5.3×10^7; 4.8×10^7 – 5×10^7
20	5.6×10^6
40	5.5×10^6
60	10^7

See over for conclusions

Auxanograms – P. fluorescens

Culture	Responses	(old symbols)	new symbols · TEST	
1	Yx, HC, A1(?), A5 (illegible)	A5	A4	HISTIDINE (±)
2	Yx HC A3	A3	A2	A2. (comprg.)
3	Yx HC		A2	
4	Yx. HC very slight		A2.	
5	A3	A3	A2	A2
6	Yx Vits?	Vit?	V	not VITS.
7	Yx HC A4 A5	A4 A5	A3 A4	ϕalan. HIST (±)
8	A3	A3	A2	A2
10	A4	A4	A3	TRYPT. HC ng
11	Yx HC		A4	
12	A4	A4	A3	TRYPT. HC ng

Further tests

				PF
1			HISTIDINE	1
2	IV	✓		2
3	IV	✓		
4	IV	✓		
5	IV	✓		
6	Reculture	9/30 A2		
7			ϕalanine histidine	3
8				
9	IV	✓		
10			TRYPT.	4
11	A4	TRY HIST.		
12			TRYPT.	

10/1 Throw out all but PF – x stocks.

Notes:

PF-1. Growth on histidine is slow and limited compared to A4.

761-8. After 48 hours, growth on glutamate exceeded histidine. Try hist + glut and hist, glut + vits; + yna.

PF-2 Growth on A2 ~~slightly~~ considerably faster, than on isoleucine-valine. (balance?)

PF-3 and PF-4 probably preferred as mutants for further work

Pseudomonas – penicillinum

Sept. 23, 1950.

P.F. A3.12 irradiated 60 sec. Grown overnight in aerated Penassay. Reinoculate into Penassay 9⁰⁰ AM for young growth.

2 PM wash and resuspend in D(Glu). Rm. temperature

2²⁰ aerate A) .5/10 dilution

B) .05 × .2 = .01/10 dilution

2⁴⁰ add 1000 u penicillin per tube. (1000 u/ml)

6¹⁵. A strongly lysed cf. non glu – non pen. control

Plate A, B, in D(0); NSA.

9/24 1PM. Comparison of D(0) with NSA impractical owing to failure of subsurface colonies.

A2 → ca 30 surface colonies

B1 → ca 3-4.

Pick from each to water. Test on NSA; D(glu)0 agar.

A: 30 tests. 11 did not grow on D(0).

B: 20 tests 2 did not grow.

lower dilution into penicillin is not more effective. Possibly a longer interval should be used.

Pick presumed mutants to nutrient agar for ~~pass~~ preservation.

Further colonies tested

A 20/27 + 13/19 = 33/46 Store on NSA plate.

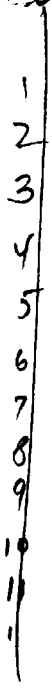
B 4/10

Cryptanograms.

Positions 1-10 on periphery
A - D in center

A	Yx	1	A12
B	HC	2	A3
C	YNA	3	A4
D	Vit.	4	A5
		5	A6

Culture	Responses
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
1	



Test remaining stocks of mutants on D-agar + ϕal, tupt, ~~#3~~ IV, hist + glut. to screen hitherto undetected mutants. hold only those which show 0 response.

		D -- agar
PF	0	++
	1	++
	2	+
	3	++
	4	++

Keep 14/36 as distinctive mutants. Some ± growth not retained.

Test on plates c̄ random supplements. All grow on EMB.

			HC	V,+	YNA	A1	A2	A3	A4	A5	FINAL TEST IN LIQUID
	PF 0		+	++	++	+	+	+	+	+	
	1		+	−	−	−	−	−	+	−	
	2		+	−	−	−	+	−	−	−	
	3		+	−	−	−	−	+	−	−	
ERROR? CROSSFEED?	4		+	++	++	+	+	+	+	+	
	PF #6	1 A2	+	−	−	−	+	−	−	−	LEUC
	~~2~~	~~XX~~	~~+~~	~~±~~ +	~~++~~	~~±~~	~~+~~	~~±~~ +	~~+~~	~~+~~	
	3	A2	+	−	−	−	+	−	−	−	LEUC
	7	4 YNA	−	−	+	−	−	−	−	−	GUAN OR AD±
4 YNA		5 HC	+	−	−	−	−	−	−	−	AA
	8	6 A1	±	−	−	+	−	−	−	−	ARG
2 A2		7 YNA	−	−	+	−	−	−	−	−	AD, GU OR wk±
5 A1											
	9	8 A1	+	−	−	+	−	−	−	−	METH ~~GUAN OR~~
1 XX		9 A1	+	−	−	+	−	−	−	−	
1 HC		10 A1	+	−	−	+	−	−	−	−	
		11 A1	+	−	−	+	−	−	−	−	
		12 YNA	−	−	+	−	−	−	−	−	PUR
		13 YNA	−	−	+	−	−	−	−	−	PUR
		14 ??	−	−	−	−	−	−	−	−	

(see over)

PF5 = PF3 S^R (surface plating - ca 100u/ml effective).

Still to check:

5: AA OK Single add. allo. Single oss: -3, -2 } no growth

9 } A1 Meth
10 } Meth.
11 } — Meth

14 Yx. → YNA + V gavegs.

PF 1

Hist + A1235	→	+++
− Hist (AA)	→	−
Hist + V		+
Hist		+
" + Isolut		+
" + YNA		+±

other AA stim.

PF 4 now prototrophic. T. O.

5 ϕal + A2 (L, Isol., IV n.g.)

14 YNA + B12 !! Purinix n.g. for YNA

H-1 F2 self. 762

September 23, 1950.

Lac+ Mal+ Lac,- Mal,-
W478 × W1178.

~~ca 1- : 100+~~
2- : 100+

on EMS Lac.

9/25 Pick 100 Lac+ colonies and streak on EMB lactose.
All Lac++

9/26. 28 addnl. Lac+ 2: Lac v. (762-1, 2).

9/29 EMS Lac → Restreak 4 single Lac+ from EMS to EMS, EMB Lac + Mal.

1. All Mal++, Lac v. ~~Rep on EMS Lac.~~
2. " " "

Conclusions:
This cross yields too many Mal+ (Lac+ and Lac v) to be very efficient in testing for Mal-Lac v hemizygosity. Renew with other combinations of markers. See W1325 × W588 (767)

E. coli outcrosses

September 23, 1950.

See 760. (W1177 × K-12).

Inoc. from overnight cultures W 1177 + various prototrophic E. coli isolated by S. Shapiro from chickens. into Permassay 9AM

Wash + plate mixtures on D(SM) agar.

	#
1	803
2	115
3	110
~~4~~	~~111~~
5	109
6	105

no X+ S^R noted

~~3~~ plates each.

K^+ = prototroph
X^- = auxotroph

" 7, " 8 " nogr.

Continue, using 2 drops from mixed Permassay culture, overnight, as inoculum, without washing, on D(SM) plates.

W-1177 + Culture #

K-12 3 plates: ca 50 S^R prototrophs/plate

Other cultures, 1 plate only.

	Ag. Bact. #
7	866
8	114
9	7
11	10
12	23
13	834
14	841
15	865
16	863
17	
18	828
19	
20	833
21	827
22	
23	
24	2 plates 830
25	825
26	
27	839
28	
29	818
30	

31	111
32	820
33	114
34	
35	
36	816
37	106
38	845
39	838
40	120
111	

No X+ S^R found in any of these test crosses

E. coli outcrosses.

9/27/50. Plate mixtures of indicated strains + W1176 on DSM without washing:

W 1115
1113
1114
1117
W1045
W1176
} 2 plates (2-3 drops culture)

W1258 5 plates. No X+ S^R.

No X+ S^R colonies except for

1) 1176 → pure lac−

1) 1115. → pure lac−

Pick and streak out on EMB Lac.

9/29/50. Concentrate mixed cultures above from 10 ml YE to ca. 1 ml H_2O and plate .2 ml per plate. W1113, W1114, W1117 gave pellets not readily redispersed. Disperse these as far as possible P30.

B.

(CONCENTRATED SUSPENSIONS)

10/1/50. B: K-12 x gave 10^3 X+ S^R per plate

1115	~~0~~ 1	(2 plates)
1176	2	(2 plates)
1045	ca 50 /plate	(2 plates)
1258	0	

10/2/50

1113	0
1114	0
1117	0

10/3/50. Plate 12 plates, conc. W1117 — no S^R X+.

Stock is also pure − in lac, Mal EMB.

760-5 is mixed on EMB Mal: must be a mixed culture, or somewhere. Not a W1177 X+ lac+ Mal+ !!

Marker tests on various prototrophs

Test on EMB Lac, Mal, etc:

	Culture	Lac	Mal	Xyl	Gal	Sucr.
1	W 1177	−	−	−	−	−
2	W 1176	+	+	+	+	+
3	W 1115	+	+	+	+	+
4	W 1045	+	+	+	+	+
5	760-5	+	− +	−	−	−
6	K-12	+	+	+	+	−
7	K-12 × 1177	+	−	−	−	
8	"	+	+	+	−	
9	"	+	−	−	−	
10	"	+ −	−	−	−	
11	1115 × PUR.	−	−	−	−	−
2	1176 × PUR.	−	−	−	−	−
3	1176 ×	+	+	+	+	+
4	1176 ×	+	+	+	+	+
5	1045 ×	+	+	+	+	
6	"	+	+	+	+	
7	"	+	+	+	+	
8	"	+	+	+	+	
9	"	+	+	+	+	
20	"	+	+	+	+	
1a		−				−
21-30	1045 ×	all +	all +	all	+	all +
31-42	direct from colonies in suspension.					++

! Repeat. See 760

Re: K-12 [illegible]!

1045× very likely S^R mutants. W1115×, 1176× may be either recombinants or very peculiar types like 760-5 which demands explanation.

Inoculate Y2 with stock W-1177. Inoculate heavily into DSM.

H248 : 3 papillae on Mal EMS. (hemizyg. test)
all hae - segr. pure Mal+ pat in test.

E coli outcrosses

10/8/50 + prev.

W1177 ~~plate~~ cultured with

1 W1176
2 W1115
3 W1258 in ½ broth 24 hours. Wash + plate very heavily (ca 10^9/plate DSM).

Controls: W1177, etc. alone: no colonies

1. ca 200/plate
2. ca 1000/plate
3. 0 colonies. (10 plates)

These colonies on test proved to resemble their prototroph parent: Lac+ Sucr± and Streptomycin-sensitive!!

10/~~10~~9/50. Repeat with fresh cultures:

Sh24x 5 plates ca ~~4~~/
Sh24c 2 plates ca ~~15~~/

W1045x 3 plates 1
c 2 plates 0

Brush ~~Stroke~~ on EMB Lac, Mal. Result very peculiar:
all slow on Mal. Sh24x; 1045x are Lac+, -
Sh24c Lac+.

Sh24c Lac+ Chief prototrophy! — none prototrophic!
Sh24x (1-8): Lac- S^R
W1045x Lac- S^R

probably S^D mutations.

W1177 x

1 Gauntt +

2 W1281 (Coli Lisbonne - Carrier)

3. ML (Monod - Lwoff mutabile)

4. W1113

5 W1115

6 W1176

1.	5 ⊗ ; 2 control plates	0	
2.	5 ⊗ 2 " "	0.	
3.	5 ⊗	16	#1-16
	2 c	0	
4	2 ⊗	0	
5	2 ⊗	1	#17
6	2 ⊗	15	#18-31
	1 c	3	#32-34.

Pick all colonies to water. Spot on DSM for preservation.

Streak

1-16 (ML x 1177) : all Lac - mutable; Mal+ (~~[illegible]~~ S^R mutations??)

Peculiar sectoring noted in ~~the~~ colonies on EMB Mal

17 uncertain reaction on EMB Lac. mottling of thick streak.

SD ? { 18-31 weak growth on EMB Lac Lac -
32 mg. on EMB Lac

DSM plate host.

#1-17 show peculiar mottling of colonies on EMB Mal.
Resemble (from #3), and ML itself. However, ML is definitely a weak MH+; 763-1-16 are all strong MH+. (Maybe effect of S^R mutation)

Clues:

#3 resembles ML in variegation on EMB Mal, from strong to weak Mal+. Not unique.

#1-16 also resemble ML in Lac^{-mut}; Mal+; Xyl+, (V_1^R) and must be regarded as S^R mutations in absence of evidence for recombination of any other nucleus.

763f- #17 appears to be Lac+ Xyl- MH- V_1^R. v. Poor growth on EMB xylose!
From W1115 x W1177.

Compare 1. W1115
2. W1177
3. #17
4. 1+2
on various media.

EMB:	Glu	Lac	Suc	Mal	MH
1	+	±	+	+	+
2	+	−	+ −	−	−
3	wk. +	wk+	+	wk+	+ wk
4	+	+, −	+, −	+ −	+, −

#17 is a weak fermenter, but may be merely an S^D or S^R type mutant from W1115. It does not provide evidence for recombination.

S^R/S^S heterozygotes

Sept. 26, 1950

9/29 W67 x W1177 heavily plated on EMS lac 7 plates x ca 200/. = 1400 2 (lac+) prototrophs. Restreak on EMB lac, EMS lac, EMB Mal, DSM.

#1. Mal−; gives a few papillae on D(SM). lac v. grows poorly on EMS lac.

#2. Mal−; grows well on EMS lac. large colonies on ~~D~~ DSM. Streak out several colonies of each on EMB lac for segregants for S^S/S^R test.

6 EMS Lac+ colonies to ~~EMS~~ EMS Lac (SM)

3 " lac ± . (noted on streak of 764-2)

all S^R

ca 30 Xyl− S^R (taken from EMB lac segregants).

This culture H248 is evidently pure S^R Mal− Xyl− (coupling of S^R to Mal− is not unexpected). It may be useful in physical comparisons with K-12.

B Repeat W67 x W1177, v. heavy parental inocula, on EMS lac. After 3 hrs., ca 1700 prototrophs. 10 possible lac+ picked for test: streak on EMB lac; spot on EMS lac; streak v. streptomycin on EMS Mal

	lac	EMS	Mal	S				
1	−			R				
2	~~+~~ ++			R				
3	−			S				
4	−			R				
* 5	v			R	1	Pure Mal+	Xyl+	S^R
6	−			R				
* 7	v			R	2	"	−	
8	++			R				
* 9	v			R	3	"	−	
* 10	v			R	4	Pure	−	Xyl− S^R

None of these is segregating S^R/S^S. Repeat cross again (to look for Mal v)

→

S^R dominance?

W67 x W1177

7642

10/11/50.

Cross 67x1177, 10/9/50 EMS Lac.

C Colonies examined A12. 14 plates ca 100/ = 1400.

3 colonies picked (1 very doubtful). Test on EMB Mal
#1 + 2 Lac v Mal −; Mal + (2) S^R. EMS Lac (SM).
#3 Lac − Mal + S^S

D1 ① W67 + W1177 + 1 added volume Y2 9:30 to

② separate until washed.
concentrate each from 20 ml combined volume to ca. 1.5.
(Spread .1 ml each on EMS Lac (± camphor))
Yields in 1 and 2 ca same. (① may be not more than 2 x ②).
2?? Lac+ in 19 x 50 prototrophs
1 (Acenapthene) Lac − Mal + S^S
2 (Camphor). Lac + (pure) Mal − S^R

2da → Neither is Lac v.

3da. P15. 2 additional possible Lac+
(1 - camphor ② Lac v Mal −
3. - acenapth. ① Lac − Mal +) Gal v Mtl − Xyl −. Gives many Lac − prototrophs (partial segregation?)

P17: P18 (camphor; colchicine; controls)

(E) very poor yield.
20 plates x ca. 25/plate = 500. No Lac v.

(F) 10/19 10 plates. Parental inocula derived from cultures exposed to camphor on EMB agar for 60 hours.] Ca 150/plate. 12 Lac+ seen. (ca 1%!) (Reincubate for more)
Is this due to character of parents?
4 additional Lac+ 10/22. (14-17)

No suitable S^R/S^S heterozygotes recovered

10/22/50.

F's streaked out on EMBlac (poor batch!), EMSlac; EMSMal.

	Mal	lac	
1	+ ✓	~~+~~	~~v~~ ✓
2	—	~~+~~	v?
3	—		v?
4	—		v?
5	—		v?
6	+ ✓		~~+~~ ✓
7	—		V
8	—		V
9	(✓ mucoid)		V mucoid
10	—		V
11	—		V
12	+ ?		V ✓
13	—		V
14	—		V
15	—		V
16	+		V
17	—		V

Recheck 1, 6, 9, 12 for Mal, lac v.

Save F1 as example of Mal+ lac v.

all Mal − lac v were strongly mucoid on EMSlac Mal+ were not.

See 771 D

Mal- mutant hunts: W478, W466

9/26/50 ff.

A) W478 2 plates × 300. (600) 1 Mal- (sectorial). Primfy and slant as W-1323 uv 7 secs.

W466 2 " 0.

10/1/50 W466 14 plates 150 (2000) 1 sectorial → 1 Mal- } 1324
4 -? → 1 Mal- } 1325
→ 1 Mal slow 1326

10/3/50. W1326 Appears + in thick streak portions. May be highly unstable.

765-4 Many "-" colonies have + wedges. Restreak these

All Glu ++.

-+ sectors give a profusion of Mal+ colonies.

Restreak apparent pure "-" and "+" + These remain pure.

However, from original plating*, a Mal v line was established.

Hold in refrigerator.

10/6/50. After 3 days, Mal- colonies show + papillae and sectors. Picks to test for segregation.

10/7 → all streaks show: Mal v (D) and Mal-; a few Mal+ ? E.

? W466 → − − → − + → + + ; − − → − + mut → − +

→ W1327

Hypothesis

A * Streak on EMB Mal as 765-4A. Put on NSA slant (gross mixture).

B + Re " " " 765-4B. Pure Mal+. This turns out pure -

C = W1327

D = W1329

E = W1328

Unstable Mal+ ⇄ Mal-
↓
Stable Mal+

Pseudomonas: double mutant penicillin res.

Sept 30, 1952

PF-3 = ϕ alanine
PF-4 = tryptophane

Aerate overnight at room temperature. Harvest and resuspend in saline. Irradiate 9 ml samples, in Petri dish, 40 secs.
Reinoculate 1 ml samples into Yz, aerate 10 AM —

n.g. Agglutinated too heavily in saline

10/4. Repeat, washing in H_2O with PF 3.
Irradiate 40 secs. Inoc. in Yz, grow overnight
Regrow 1:1 inoculum 4 hours. Wash, treat with 1000 u pen. for 4 hours in D (ϕ-trypt). Aeration.
Plate out on EMB lac. Platings of 10^{-3} unpickable.

about 60 clones of PF 3 / tested: no mutants. (Poor lysis)

No useful result

~~9~~ 10/4/50.

1.	W1323 × W1177	Mal EMS.	Mal×
2.	W1324 × W1177	"	Mal, ??
3	W1325 × W1177	"	Mal, ✓
a 4.	W1324 × W588	Lac EMS	
b 5.	W1325 × W588	Lac EMS.	

All crosses gave good yield. Parents checked for purity: o×.

1. gave Mal+, ca 2%. 16+/433 total one plate
5/260 total. " "

2. gave Mal− only. o+/ca 1000 (2 plates) Mal,
Later: 1+ noted. Confirmed on EMS Mal. Crossover or Recomb??

3. gave Mal− only o+/ca 1200 (3 plates) Mal,

4.
30−: 18+
31−: 8+
39−: 22+
100−: 48+ / 148.

In standard cross, excess lac + expected.

5.
119−: 30+
91−: 32+
210−: 62+ / 272

Pick lac+ for test as lac ✓. Spot to EMS Mal. Select "lighter" Lac+
4: 1-11
5: 1-12

Test these crossing in EMS Mal

Met mucosae

767a

10/7/50

#4	Lac	EMB Mal	EMS
1	V	—	—
2	V	—	—
3	+	+	+
4	+	+	+ v?
5	+	+	+
6	+	+	+ v?
7	v ?	—	—
8	v ?	+	+
9	V	—	—
10	V	—	—
11	+ ?	+	+
12			

13–44: Lac v
Lac + : 22, 35, 34?
?? :

All Mal+ on EMS except 14, 22, 31, 35, 46, 47

Restreak likely Lac v on EMS Lac for purification

			767-
	1	Mal −	1
	2	+	
	3	−	2
	4	−	3
	5	+	
a	6	−	4
	7	+	
	8	−	5
	9	−	6
	2 = H254		7
	3		8 → Mal + Lac v
	4		9 Mal + Lac v
b	5		10 Mal + Lac −, no test.
	6		11

Reversions:
2: (3) pure Mal+ Lac v (2); + (1)
3: (1) pure Mal+ Lac+ no test

Mal Δ/− → Mal Δ/+.

Conclude: 767-9 (2 tests) and 767-2 are clearly Mal-/Δ (heterozygous)
767-8

Het microsses.

767b

10/7/52

#5	Lac	B Mal	S
1	V	—	
2	V	—	
3	V	+v?	
4	V	+v?	
5?		+	
6?		+	
7?		—	
8?		+	
9		—v?	
10		+v?	
11		+	
12		+	

13-36 Lac: V 13 17 19 24 36

\+

? 14 21

Mal −: 21 22 24 29 30 35

Restreak likely lac V on EMS lac for purification; to Mal EMS for Mal+ rev.

H253	1	Mal+
H254	2	—
	3	—
	4	—
	5	—
	6	—
	7	Mal+
	8	Mal+

[1 M+L+ (rocket)].

4 Mal+ → 3 Lac V; Mal+. ; 1 Lac − Mal+. 3 kolt: Mal−/Δ

Het mosses → Mal−/Δ

PF crosses.

10/9/50.

Cross streak, incubate 48 hours EMB lac. Pick growth from intersection and spread with loop on D(0). Record #cols. 48 hrs.

PF ×	1	3	6	7	8	9
1	(0)	0	0	0	0	0
3	0	(0)	0	2	0	0
6	5	0	(0)	0	4	2
7	0	1 mm	15 ±	(0) mm	0	0
8	0	0	1	1	(0) ×	3
9	1	±	0	0	3	(0)

Note that parental controls are all negative. 6 × 7 is the most promising. (also 8 × 9)

10/13/50. Inoculate Y2 from EMB Brushes. Grow still 24 hrs; aerate 24 hours. Wash + conc. ca 5×. Plate. 1 ml per plate D(0).

PF	6	1-2/pl.
	7	100/plate
	8	Diffuse growth.
	9	1-2/pl.
	6×7	2-3/plate
	8×9	diffuse or 10^3/plate

No evidence of crossing! Put addtl. markers into PF 6, 9 and attempt these. (e.g. SR)

Inconclusive. Used additional markers.

10/9/50.

Streak W1327 and 1328 on EMB Lac for reversions. Pick papillae for examination as lac V.

W1327

	Lac	− Mal
1		↓
2		
3		
4		
5		
6		
7		
8		

all about same × no obvious variation

W1328/9

1	little.	−, V
2		V, −
3		V, −
4		−, V
5		V, −
6		V −
7		V −
8		V, −

This suggests a mutable Mal allel rather than segregation.

For 1327, Select a lac+ and streak out to allow lac analysis; Mal reversions.

B) 1329: Pick a Mal V from each and restreak on Lac; Mal.

c) Pick any possible lac V from reversion plates and restreak.

"W1328" No pure + obtained! Study other reversions of W1327

c1 1 possible lac ±. Restreak single colony → pure lac+.

B: #3, 5, 8 are pure Lac+, Mal V. Others happen to be Lac − Mal V

No pure Mal+ seen. Repropagated lac+ Mal V as W-

D. Papillae from W1327. Streak out individually: are there any Mal++?

W1327 reversions. 769D

8 Mal+ reversions from single Mal− colonies tested for purity. Same − companions

	Mal
1	+ −
2	++
3	++
4	+ −
5	✓
6	V
7	++
8	++

Restreak − and + components of 2, 3, 7, 8.
Confirm: stable + ✓.

10/19 Repick associated − and + to determine whether a single − type gives both Mal+ and Mal+^{m}. Transfer + to slants as 7 D 8 etc.
D7 is distinctly more powerful Mal+ than the others.
D2− is most papillate

Reversions from 769 D:

2	almost all variegated.	
3	occ. Mal v.	Some Mal++, Mal+?
7	mostly Mal v	" " " ?
8	occ. Mal v.	

∴ all Mal− give Mal v reversions; some Mal+??

P 10/21 Restreak single Mal+ or v colonies from above.

2 varying degrees of instability
3 2 stable +; 1 stable ± *; 1 variegated +
7 2 stable +; 1 unstable ++ *; 1 variegated + *
8 1 unstable very weak + * 3 variegated ++

Repick interesting types *

10/21. Cross D7+ with Y10 for "suppressor" to 11. 1 possible − / ca 1000 protoprotrophs (8 plates)
→ streak out: mucoid Mal+ (not Mal+)

∴ W1327 probably differs in only one gene from K12

PF - Mandelate mutants 770

10/7/50.

Irradiate PFO. (30 secs.) ~~Ae~~ Aerate in D(O) glucose.

A 8: Moc. into T(mand) to preadapt 3-4 hours (heavy inoculum 1:1)

Wash and reinoculate 1:10 into T(mand). After 5 mins add penicillin to 1000 u/ml. After 5 hrs & overnight spread on EMB Lac. Test on EMB Lac; T(mand) agar

P10 55 tests: no mutants

Addnl tests

P11 (Phyllis Fried). 50 tests " ". No mutants.

ca 50 addnl. tests: " ",

Query: Are methods suitable for C-source: 1. Penicillin selection
2. Agargrowth tests.

Check 1 by reconstruction with Mand-strains
2. by T(m) agar (s mandelate).

EMB Lac used solely for convenience & for identification.

W1303 - 4 crosses. 77~~0~~

10/18/50

a. W1303 x W1178
b. W1303 x W1177

10/20 c W1304 x W1303

10/21 d W1304 x W67
e W1304 x W478

10/21 a. very infertile: ca 5-10 small colonies/plate. Reincubate. 8 plates
Meanwhile 2 lac+ seen. Pick to EMS lac, streak on EMB lac, Mal. (lac v? +? Mal −)
b 11 plates ca 40/plate (first crop). New small colonies appearing: reinc.
No lac+ seen.

10/22 a. About 2% lac+. Ca 100/pl. 13 lac+ picked.
(b.) ca 200/plate. No lac+. Striking colony dimorphism O.o
2000/0 apparently both lac −.
Pick to EMB lac to verify lac − of large colonies.
(c) ca 150/pl., small colonies. 14 pl. No+ Reincubate No+. 2000/0
(d) 12 plates, ca. 20/pl. No+. Reinc. 1 unlikely +. No+ 250/0
e Good yield.

+	−
56	21
48	19
3	3
11	7
28	32
148	82
+	−

Repeat:

10/23 b 10 x 50/ 1 mucoid lac?. An Mal EMS, ca 10% Mal+. pure lac+ Mal+ 500
c 10 x 150 1 lac+: ~~[illegible]~~ " 2+ : 8− 1500
Mal− lac v 771-C1 See over:
d 11 x 50 0+. 550
5 x 0 − kept at 42° 12 hours after plating. Then 37.

771 C → see C1

Repeat W1303 - 1304 crosses. 771 -

10/27. large scale

b‡	1303 × W1177	2 × 30ml → 5ml	.1/plate	Shake mixtures at 37
c‡	303 × W1304.	2 × 20ml → 5ml	.1/plate.	11:10 — 2:10

b1 repeat 40° 2:30 — 6:30 (A) → ||A >B (B)

↓ b2 37° At 6:45 PM, expose to ~~(seconds)~~ UV 50cm distance

10/30. b. Very low yield. 768/36 plates. Many small colonies.

b1A ca. 10-15/plate (3 plates)
B 0. 1 plates

b2. 2sec 109 (small colo.)
5 " 146 var. size
10 " 47 " "
15 " 181 " "
20 " 98 " " many small.

1-4 from b; 5 - b1A; 6 = b2-2 7,8 : b2-5 9 b2-10
10,11,12 b2-15
13,14 b2-20.

c. 37 plates very low yield c: 15 — 38

[28 — 38 from one plate. Restricted feature only a possible excess accumulation of MB.]

None except: 2, 6, 7, 8, 10, 11, 12.

Relabel 1-7, 771B

Results of W1303x crosses

771 A

10/23/50.
W1303x W1178.

a.

	EMBlac	Mal	EMS Lac —	
1	V	—	+	
~~2~~	~~—~~	~~—~~	~~—~~	
3	V	—	+	
4	V	—, + ?	v. poor growth — + ?	✓ Lac v + ~~mottled~~ Mal —
5	+, v?	—, +	v p.g.	
6	V	—, +	v. p.g	
~~7~~	~~—~~	~~—~~	~~v. p.g. mucoid~~	
8	V	—	v. p.g.	
9	V	+ mottled	+ single cols.	Lac v Mal + mottled *
10	V	+ —	— + → reisolate	Lac v Mal —
11	V	+ —	— + → reisolate	Lac v Mal —

aa1 ~~1~~ 2
aa2 ~~1~~ 7

verification needed from EMS Lac.

Restrick where possible.

* Rechecks. Pure Mal + but mottled (modifiers?)

✓ Reisolated cultures No Mal v.

	Lac	Mal (seen for [illegible])
1	V	—
2	V	—
3	V	—
4	V	—
5	+	—
6	V	—
7	V	—
8	V	—
9	V	+
10	V	—
11	V	—

	Lac	Mal	SM EMS	SM EMB
1	+	—	R	R
2	v ?	—	R	R
3	+ ?	—	R	R
4	v	—	R	R
5	v	v	S ... (S R)	
6	v	—	R	R
7	+ ?	+	R	R

Xgl 1HR ...

Segregating S^R/S^S

Streak Mal v colonies from 5.

Many Mal − and Mal v. Few or No Mal +. Repick possible +.

20 Mal − : S^R

Reisolated cultures, after purification

	Lac	Mal
1	+	—
2	v	—
3	+	—
4	v + ?	—
H257 5	v	v
6	v	—
7	+	+

Check #4 ✓ Lac v. But very stable.

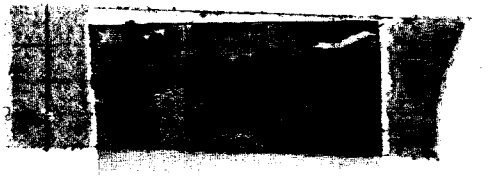
771C

cl see 771. Mal++ Lac$\underline{v}$ extreme "bullseye" type ⊙

Restreak from center of ⊙

95% lac−

Pure Mal+ Sal+ Xyl − Mtl −



EMS lac 48h: colonies are variegated on EMS Lac.

Restreak on EMS Lac; EMB Lac. Streak on EMS Mal.

− Picks from ⊙ center → almost all lac − (some pink, some blue). Very few ⊙

→ ca 10% lac+ on EMS! Extremely unstable Store on # D Lac

Attempt to find Mal+ reversants still lac$\underline{v}$.

771C1-1 : all Mtl lac −.

2-5 ditto.

This enterprise is very difficult owing to the extreme instability of 771C1.

10/25/50.

e. α 100 Lac+ streaked out on EMBLac. Restreak 12 on EMSLac likely Lac v.
β Additional 24 (smaller cols.). Take 4 as likely Lac v.

	Mal		Lac	Mal.
1	−		√ √	
2	−	no gr.	...	
3	−		√ √	
4	++		√ √	
5	−		+	
6	++		+	
7	−		+	
8	+ −		√ √	−
9	−		√ √	
10	− +		√ √	−
11	−		√ √	
12	+ −		+	−
13	−		√ √	
14	++	no. growth.	...	
15	++		+	
16	−		+	

10/28. Repick EMSLac+ colonies to EMSLac; EMSMal (if Mal− or mixed) and EMBL

10/19/50.

.0001 ml 5% Na tellurite in ~~tube~~ phage plate W67 culture added.
$= 50 \times 10^{-4} / 25 = 2 \times 10^{-4}$ mg/ml = .2 r/ml
inhibited W67, with colonial survivors. Festucoid survivors:
When cross-streaked, showed no greater resistance than W67 per se.
2 r/ml plates and higher remain sterile.
Prepare DNG with
.2
.5
1 r / ml Tellurite.
W-1177 poured with ± 1 r/ml tellurite shows considerable turbidity, no definite resistants in 24 h.

Streaking W67 and "W67/Te" (from above) on surface of .2, .5, 1 r plates:

Te		W67	~~67/Te~~	67/Te
	.2	Heavy background. freq. outgrowths		Lighter background. large coal colonies
	.5	light background. a few res. outgr.		Heavier bckg. many large outgrowths
	1 r	v. light background; a few papillae		light bkg many large colonies * festucoid

10/26. Streak W67 and W67 Te, on DNGB + 1, 5, 10 r Te/ml.

24 h.

	67±	Te,
1	—	+++
5	—	+ declining
10	—	±

Medium grossly contaminated throw out experiments

W1034 × W1177

October 25, 1950.

W1034 x W1177 EMS Lac.

Yields (one. mor.) ca 30/plate. Ca. 98% Lac+

Picks 50 Lac+ and streaks on EMB lac.

49 → pure lac+ streakings 1 → lac+ colonies / colony Restreak.

Repeat 11/5 —

+	−
4	6
2	3
3	4
5	9.

No unusual appearance this time. Note low ratio, however!

f. 590