Zelle single cell isolates

~~696~~
702

2/24 – done by Zelle 2/21/50.

		LAC	MAL	× M+L
A.	47	+	+	±
	48	+	+	
	52	+	+	
	55	+	+	
	56	+	±	
	57	+	+	
	58	+	+	
	59	+	+	
	61	+	+	
	62	+	+	
	190	+	+	
	91	+	+	
	92	+	+	
	93	+	+	
	99	+	+	
	103	+	+	
	121	+	+	
	169	+	++	
	170	+	+	
	189	+	(+)	
	201	+	±	
	202	+	+	
	246	+	+	
	491	+	+	
	492	+	+	
C	10	+	++	
	13	+	±	
	16	+	±	
	18	+	±	
	19	+	±	
	20	+	±	
	29	+	±	
	30	+	++ *	
	35	+	±	
	36	+	±	
	48	+	+	
	54	+	+	
	95	+	+	
	96	+	+	
D	17	+	+	
	20	+	+	
	21	+	+	
	22	+	+	
	23	+	+	
	24	+	+	
	26	+	±	
	31	+	+	
	32	+	+	
	33	+	±	

		LAC	Mal × M+L
D (cont.)	34	+	++
	37	+	±
	38	+	− + − +
	39	+	+
	51	+	+
	82	+	+
	105	+*	++
	106	+*	+*
*	D61	−+	±+ ±(+)
E	4	+	±
	~~11~~	+	++ *
	25	+	± ±
	26	+	±
	35	+	±
	55	+	±
	59	+	±
*	[D60]	+	++
	62	+	++
	63	+	++
	64	+	++
	~~66~~	+	++
	68	+	±
	69	+	±
	70	+	±
	74	+	±
	113	+	±
	114	+	±
	115	+	±
	116	+	(++)
	121	+	(++)
±	122	+	±
F	7	+	++
	8	+	++
	9	+	++
	24	+	±
	25	+	±
	26	+	±
	27	+	±
	28	+	±
	30	+	±

no segregants

		LAC	Mal	× M+L
G	16	+	++	
	35	+	±	
	36	+	±	
	37	+	±	
	38	+	±	
	63	+	±	
	64	+	+	
	65	+	±	
	66	+	±	

		LAC	Mal	×
H	7	+	++	±
	17	+	(⊖)	±
	18	+	++	±
	20	+	++	±
	21	+	++	
	22	+	++	
	24	+	±	
	25	+	±	
	27	+	±	
	39	+	+	
	40	+	+	
	47	+	+	
	48	+	+	
	53	+	±	
	54	+	±	
	57	+	±	
	58	+	+	
	60	+	++	

All ± unless indicated

Restreak A164, A189, C30, E116, E121, H17 on EMB Mal

C30, on EMB Xyl.

All still diploid!

Chemicals on H226
$\phi_2 I^+ Cl^-$

March 6, 1950.

A. Diphenyl iodonium chloride 2.7 mg/ml in D(0) 10 ml + 1 ml H226 at 37 C. 10 minutes + plate ~~out~~. dilutions as from treatment tube.

B ~~Acetic~~ anhydride per 692 C. dil. from treatment tube.

C Control. 10^{-7}

D. P1. $\phi_2 ICl$ 28 mg in 10 ml D(0). Add 1 ml H226. Incubate 30 mins at 37°. Plate out.

Results.

A: Plates were ~~[illegible]~~ discarded before counted, but killing was << 50%. Some haploidizing effect?

C: ?? Ca 90% diploid 10^9. [Discarded].

B: slow growth: hold.

48 hours: Dilution 2. 10 Lac− 0 Lac v
Dilution 1 82 Lac− 1? Lac+ Streak out: Lac v

] See 704

hold plates further:
at 72 hours, no further + appeared; no residual + in centers.

Note: Very low proportion of "residual" diploids with high doses of Ac_2O.

March 8, 1950.

Use once washed H226 (3/5/50) in D(0) in this series. 37°

✓ A. $\phi_2 I^+ Ce^-$ 25 mg / 10 ml D(0) + 1 ml H226. 30 min.

– B. $IAcNH_2$ (Doherty – brown sol'n: must contain free I_2) 50 mg in 1 ml + 10 ml D(0) + 1 ml H226. 25 minutes.

✓ C. Benzoyl Chloride: 2% alcoholic. Add .1 ml to 10 ml D(0); 1 ml $CaCO_3$, + 1 ml H226 10 MINS.

✓ D. Dimethyl sulfate 10% alcoholic Add .1 ml to 10 ml D(0) + 1 ml H226 + 1 ml $CaCO_3$ 10% 15 min.

✓ E. Phenyl isocyanate: 2% alcoholic. Add .1 ml to 10 ml D(0) + 1 ml H226. 10 MINS.

✓ F. Ethyl carbamate (urethane). 2.5 ml 20% solution + 1 to 3 (ca 2 hours, 5%). 1 ml H226 + 6.5 ml D(0)

Results:

A 5 ca 200 ca 85% Lac V. Killing too meagre to be decisive.

B Steps at every dilution. Repeat at lower concentrations! ✓

C 6 ca 100 mostly 2n – Killing too meagre

D 1 ca 100 all Lac – Later some delayed Lac V.

E 6 ca 100 mostly 2n – Killing too meagre.

F 2 ca 100 90% Lac V.

Use larger intervals with Benzoyl Chloride; Phenyl Isocyanate

Chemicals on H226

A. H226 3/5 washed. 1ml in D(O) 7ml + Propylene Oxide 2ml 10%
37° 3^{45} to $.8^{30}$! STERILE

B. do. 50mg Ninhydrin in 4.5ml D(O) .5ml H226 37°. **50 mins.**

C. $IAcNH_2$ 1% 1:10 } D(O) + 1ml H226 37°. 10 mins. Excessive killing

D. " 0.1:10 } No killing.

A. Use shorter treatment; more dilute Pr<0

B. Not haploidizing B2: ca. 100; 2n.

C. 2 : ~~4 [illegible]~~ 5 lac+, 1 Lac− ; C1: several hundred; several hundred lac+ center inhibited; periphery lac−.

D. 6 Ca 100 lac+.

Chemicals on H226

March 10, 1950.

A. Benzoyl Chloride } see 704 C + E. 1:20 to 400 PM
B. Phenyl isocyanate } Sterile 48 hours. 4:10 PM

• 2 ml 2% solutions + 1 ml H226 3/9/50 washed + 10 ml D(0)
+ 1 ml 10% $CaCO_3$ for ♦ cocc.

4 hours

C. K_3FeCN_6 M/100 1 ml + 10 ml D(0) + 1 ml H226 10 m.
D Lumine Sulfate 0.1% 1 ml + " " . 5:5
E Iodine M/20 1 ml + 10 ml ... 10 m.
F " 0.1 ml 10 m.
G H_2O_2 final concentration 0.03% 10 m.
H H_2O_2 added to Permanganate to 3%. Incubate 5:2 – 8:30.
Add .1/10 to D(0) and add 1 ml H226 10 m. = 6.
I CH_3COCl 1/10 in EtOH. .01/10 ml D(0) + 1 ml H226 H226.

A. A4 Ca 150 ~~all~~ 90%+ diploid REPEAT!
B Sterile

C6 : ca 100 diploid 90%. No killing!
D 6 ca 100 diploid ca 90% Inadequate killing
E 1-6 Sterile
F Sterile
G 5: 30 Lac – 17 Lac V.
H 6: 6 Lac – 11 Lac V H5: 65 Lac – 36 Lac V. haploidized? ✓
I 6 ca 100 diploid 90%.

(over)

Native peroxide and peroxide-treated broth appear to be equally active as haploidizing agents.

700·3 x Y10

"Diploid ???"

March 10, 1950.

700·3 x Y10 on EMSLac.

Ca 600 prototrophs : all hac+

Chemicals on H226

March 13, 1950.

C. H226 3/9 ~~3/9~~ 1 ml + D(0) 10 ml + $CaCO_3$ 10% 1 ml + CH_3COCl 0.1 ml
Immediate hydrolysis obvious from CO_2 evolution. Keep cold
Compare with tube i same addition, but let stand 4 hours. before
adding bacteria. N.K.

D → N.K.

E Benzoyl Chloride + ~~D(0)~~ $CaCO_3$. + 1 ml H226 Shake at Room
temperature. ~~5~~ min. N.K.
0.1 ml

F. Propylene oxide to 1% 1 ml AD(0) ~~1 ml~~ - 1 ml H226 ~~10 min~~

G. Caffeine citrate to 0.3% N.K.

H. $K_2S_2O_8$ to M/200 N.K.

I K_3FeCN_6 to M/100 N.K.

J. I_2 to M/20,000.

		Dil.	Lac v	Lac −	
CH_3COCl	C	6	25	27	Effective (?) CH_3COCl
Hydrol.	D	6	101	14	$CH_3COOH + HCl$
$\phi COCl$	E	6	49	9	Inconclusive N.K.
I_2 >0	F	6	98	17	Inconclusive N.K.
Caffeine	G	6	99	14	Inconclusive N.K.
Persulfate	H	6	117	7	" "
Ferricyanide	I		149	10	" "
Iodine	J	3	41	7	Does not haploidize: 10^{-3} survivors!
		4	8	4	

March 14, 1950

St (K) Benzoyl Chloride (+ NaOH · $CaCO_3$) Shake at RT 1100 AM to 1PM
H226 3/9 STERILE

L. CH_3CHO to 0.5% H226 3/14 unwr. 20 min. 37 KK

St (M.) ϕCHO .1ml /10 " " 4 hours — Shake at RT.
STERILE

St (N.) HNO_2. Add to 10ml M/10 KNO_2 (= .06 ml) 1mM AcOH. Make up to M/50 in pH 4 Citric – KP buffer. Add /ml H226 w. 3/14 in water for effect of HNO_2. cf. O. STERILE } 30 minutes.

O. No KNO_2 – AcOH; buffer only. } OK.

P. H_2O_2 0.03% Dark 5ml H226 3/14 wash / 10. 10 mins Sediment and resuspend.

Q " " Light 30 mins.

R " " Dark control (cool in dark while Q is illumin.)

PIS: K, M, N — all sterile.

	Lac+	Lac−	
L6:	81	51	Possible effect.
O5:	9	6	Possible effect!
4:	91	78	
P6 (no correction for extra concentration)	158	81	Inadequate killing
Q6	146	47	
R6	106	47	

March 15, 1950

S. MeCHO to 1% H226 3/14 w. 30 m. 37

T Propylene oxide to 2% " " 30 m. 37

U. Ethylene oxide to 1% " " 30 m. 37

W pH4 citrate buffer } H226, washed in water.
X Control, water. } 8:25 – 15 mins.

			Larv	Lac–	
pH4	W	4:	13	27	
		5:	3	4	
		3			too heavy for accurate score, but – clearly >> lac v.
	X	6	75	8	smeared but countable
(control)		6	76	7	
		6	41	3	The increase in proportion of haploids is beyond question.
CH_2>O / CH_2	U	6	38	44	Definite haploidization. Many ♀ are ⊙ types.
CH_3–CH>O / CH_2	T	6	68	35	" " " "
Acetaldehyde	S	6	25	9	Uncertain?
		55			– Some increase in proportion of haploids??

P16. Y H226 (H_2O) in Acetate-Na-buffer, pH4, M/10. 15 mins.
36 hr. rdg.

Larv	Lac–	
4 12	19	} indubitable augmentation of haploids. a few ⊙. More than control?
3 138	83	

(over)

Z. Potassium hydrogen phthalate buffer
pH 4.0 19/20.

15 mins 37°. H226 (H_2O) 1:10

	Lac V	Lac −
Z3:	108	11

Despite 3 decades of killing, no alteration of haploid-diploid ratio is found. The effects of peroxic sections may be ascribed to acetic and citric acids respectively ($pK_{(1)}$ = 4.76; 3.06

But pK (phthalic) is 2.89, 5.41, so at pH 4 there should be as much free phthalic as citric!

Photorecovery of peroxide-treated *E. coli*

March 13, 1950.

H_2O_2 to .05% in D(o) 6 ml + 4ml H226 3/5.

Incubate 10 mins, sediment 10 mins. Divide washed suspension into equal aliquots, dil. 1:1 thio, one to be exposed to visible light 30 minutes. Control left at room temperature.

3°5 - 4°5.

A (light) Stock

B (dark) B1 3 tac - 1 Lac v

Dose too high!

Partial segregation of H168

3/11/50.

Grow H168 from single EMS lac colony in D(Lac). Inoc 1:1000 in Y2 and grow, aer., to saturation. Plate out on EMS Lac, Xyl, Mtl. 10^{-6}: ca 500/plate. Pick – colonies and replify.

EMB: + Lac ~~Mtl~~ Mtl Xyl

A. Lac

B. Xyl

C. Mtl

A. 9 – – –

B. 24 23 Lac+ Mtl- Xyl-

1 Lac- " "

C. 16: all Xyl- Mtl- 3 Lac- 13 Lac+.

No lac v ∴ No partial segregation.

No partial segregants found in H-168

Partial segregation : 17226

March 15, 1950.

Grow from 1:1000 in Y2. Plate out on EMS 10^{-5}.

A. Lac. Considerable Lac− (ca. 30% of count of 3-500/plate).
Pick probable pure Lac− and brush lightly on EMB Mal, Xyl, Mtl, Lac for discernment of Lac− ... v.
All are, indeed, pure Lac−. Scoring of v as against + is possibly uncertain. ✓✓

699-20,21 1 prob. Lac− Mtl v } noted. Recover and retest. Lac − Xyl v Mal v MtlV
1 prob Lac− Mal v } Hold for appearance later: − v v MtlV.

~~All but 2 of~~ of others, 96 Lac− Mal+ Mtl+ Xyl+. 2 − − − −
Of an additional 100, no Lac− Mtl, Mal, or Xyl v noted, subject to deficiencies of the brushing technique.

B) Mtl. <1% of prototrophs possible Mtl− . Of 20 picked, 6 are Mtl−

c) Mal 4 Mal−

D) Xyl 10 X−

B: 6 tests: 5 are Lac− Mal+ Xyl+ 1 is Xyl slow = #3 (distinct from + or −)

C:	Mal	Xyl	Mtl	Lac
1	−	v	v	v
2	−	+	+	−
3	−	−	+	−
4	−	−	−	−

D: 10: all Lac− 5 Mtl+ 5 Mtl− 9 Mal+ 1 Mal− all Xyl−

No Partial segregants except C1
Do not keep.

H168 - Mal- revertants.
for hemizygosity test

March 15 ff 1950.

Reisol H168; grow in O(Lac); culture ca 85% Lac v.

A. Streak on EMS Mal to select revertants.
Papilla picked en masse 48-72 hours. Streak on EMS Mal to purify. N21 Pick "single" + colonies and retest

B. As above

C. Grow mainly 3 tubes of O(Mal) and aerate. Growth after 48 hours. Streak out to purify.

D. Single Mal- colonies from EMS Mal as same for revertants.

A: 3 Mal+ prototrophs. 2 are Mal++ Lac v
1 is Mal++ Lac +.
∴ 2 additional tests for hemizygosity of Mal in H168.

C: 3 Mal+ prototrophs all Mal++ Lac v. " "

B 8 Mal+ " " " " "

Acid effects on diploid coli

714

March 21, 1950.

? See 716

A. Acetate pH 4 M/10 M/26 2/24 15 minutes.

B " pH 4 M/100

C. Phthalate pH 4. M/20

D " " M/100

E Control

This culture apparently contains some lac+. Streak out as 714-A

	lac v	lac -	lac +	
A. 5	620	34	20	very little killing !!
6	2	5	2	??
B 6	115	3	6	
C 3	93	3	7	
D 6	29	5	4	
E 7	121	2	8	
"	133	5	7	

Utilization of neolactose

March 22, 1950

A	58-161	Mal
B	"	Lac
C	W1301	Mal
D	"	Lac

1 cc cells from 4/10 conc. O(O) medium. aerated overnight; twice washed Bicarb buffer 7/20. .2ml 2% substrate

	Flesh	Culture	Substr.
	in Mal.		
1	10B	B	—
2	5B	B	Lac
3	3A	B	Neolac
4	6A	D	—
5	2B	D	Lac
6	4B	D.	Neolac

7/22/50

	THB	1	2	3	4	5	6	L16
230	82+	05+	42	51	29	32+	2+	
235	85	05	40	49	28	32	1	
240	90 83	8+	42+	51+	29	32+	1+	
245	86	−1	32	40	18	21	−6	
255	85	+1	35+	42	21	23	−5 6	
305	81	−4	36	42	19	22+	−5	
410	81	−4	52+	47	16	22	−10	

No fermentation at all!!

M226 segregants for outcrossing

March 22, 1950.

Pick single lac⁻ colonies from 714E7, streak on EMB Mal.

All were pure Mal− ! Very likely suspension of M168 was used in this experiment and in 714. This would account for low killing as M168 is resuspended in buffer.

Appearance of lac⁻ is not unlike M168!

Repeat from M226.

10 Mal+ and 10 Mal− (conjugate) isolated and nutritionl tested

	+		−		lac	Mal		Xyl		Mtl	
W1305 *	1	MTL	11	TLB, W130Y	−	−	+	−	+	−	+	−
	2	TLB,	12	TLB,	−	−	+	−	+	−	+	−
	3	TLB,	13	TL	−	−	+	−	+	−	+	−
	4	TLB,	14	TLB,	−	−	+	−	−	−	−	−
	5	TLB,	15	TLB,	−	−	+	−	+	−	+	−
	6	TLB,	16	TLB,	−	−	+	−	+	−	+	−
	7	TB,	17	TB,	−	−	+	−	+	−	+	−
	8	TLB,	18	TLB,	−	−	+	−	+	−	+	−
W1303 *	9	M	19	TLB,	Blue early	−	+	−	+	−	+	−
	10	TLP,	20	TLB,	−	−	+	−	+	−	+	−

Check fermentative reactions.

∴ #9 can be presumed to be the recovered B-M− parental W67 type.

Cross with #11, and with W1177 lac+.

Hold W130Y for lac+ reversion for crossing.

Alan Pardee

H206 single-cell segregants

5-224 = segregant

5-223 = 2n : resolete

5-86 Lac+Mal+

5-85 2n

20:

Partial segregation:

8 Lac- and 8 Mal- prototrophs from H206 plated each was Lac- Mal-

Studies on single cell segregants
(14226)

Recover from vials sent by MR Zelle and stored in refrigerator.

Series 2/5

A15 * inviable A33 also inviable!

D— some inviable on EMB agar. D15 and D20 are Lac-Mal-Xyl-Mtl- others inviable!

E	215	✓
	216	✓
	~~E~~221	x ?
	222	✓
G	103	✓
	104	x ✓
	105	✓
	106	✓
	107	✓
	108	x ✓
	109	x ✓
	110	x ✓

* Recover from EMB blue agar!

Sib diploids.

G3
G13 Sporable

G9 O/K
G24 O/K

E 219 viable OK
220 n.v.
52 n.v
108 n.v.

[HO] radicals in H226 718

"Fenton's" Reagent.

March 28, 1950

A H_2O_2 .01%
B " + Fe^{++} (NH_4SO_4) M/1000 } + 1ml/10 H226, H_2O.
C Fe^{++} M/1000

	Larv	Lar-
A6	37	22
B6	23	27
C6	112	10

Ferrous by itself has no effect. No very marked potentiation of H_2O_2 observed. Should be repeated under slightly more drastic conditions.

Chemicals on H226

March 31, 1950.

A. ~~Benzoic anhydride 0.4% (2/100 of 20% alc.) [sat'd.]~~
~~in D(0) H226 3/24/.50~~ 445 —

~~B Benzoyl peroxide 0.4% "~~

C. Acetate buffer M/10 pH 4

D. " " M/50 pH 4

E phthalate " M/20 pH 4.

F Control 10^{-7}

		Lac +	Lac −
F	10^{-7}	9	5
		40	4
		16	5
		65	14
C 3:		8	101 ←
D 5:		5	12
D "4"		1	18
D 3	10^{2+}	178	548
E 2		5	10
E 1		131	70

} any effect?

056
862
213111 — 061

New heterozygous diploids

April 1, 1950.

A W67 × W945 1 Lac+/ 12 plates, ca 100 each. (#18)
B W67 × W950 8 " / 14 plates " " "

For recovery of presumptive diploids, reisolate (A - single col.) and (B - thick streak) from EMS lac and streak on EMS lac EMB Lac, Gal, Mal.

		Slac	Blac	BMal	BGal
A18.	A		−, v	+, −?	++
(from EMB)	B		v, −	+	+, −, v?

Reisolate from S lac.

			Xyl	MH	Gal	Mal	Sfl
B 1	A	Lac−					
2	B	n.gr. on EMS lac					
3	A		v	v	v	−	v
	B		v	v	v	−	v
4	A		=	+ −	++	−	+ ++
	B		=	+	++	=	− ++
5	A		[illegible]	−	−	=	= =
	B		+ [illegible]	− +	− +	=	= = −
6	A		v ✓	−	+	=	− ✓ ++ =
	B		v ✓	−	+	=	− ✓ ++ =
7	A		v ✓	−+	−+	—	− − −
	B		*			—	− −
8	A		v v	−	−	v	v? + = =
	B		v ✓	−	−	v	+ = =
A 18			v	−	+	+	v

Keep A18, B3, ~~B5~~, B6, B7, B8 when reisolated!

H		Lac	Mal	Xyl	MH	Sfl	Gal	
233	A18	v	−	v+?	v−		+	
234	B3	v	v	v	v		−	grows poorly
235	B6	v	−	−	−		+	
236	B7	v	−	−	−		−	
237	B8	v	−	v	v?		−	Use for reversion studies

Effect of pH on segregation of H226

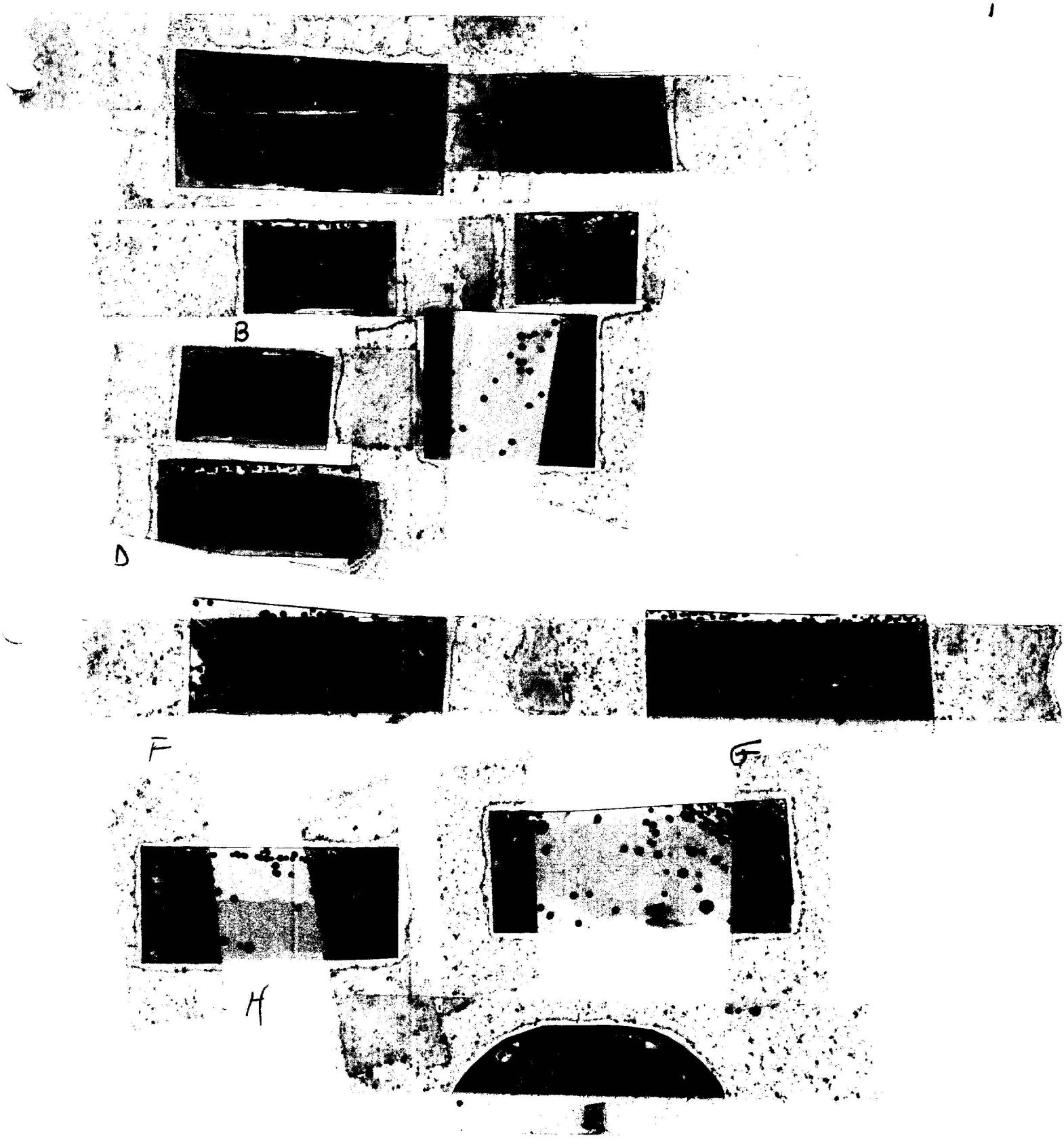
April 2, 1950.

Prepare D(0) + 1% N2Case. Measurement of pH. adjust to various lower pH's.

	Medium:	24h.	48hr.gr.	%V.O
A	D(0) + 1% N2Case	+	+++	70
B	" + .1% glucose	++	++++	50-60
C	+ 1% glucose	+++	++++	<50
D	D(0) +1% N2Case to pH 5.9 with AcOH		+	20
E	" " " 5.0		−	sterile
F	D(0) + .1% glucose		++	>80
G	D(0) + .1% lactose		++	85
H	D(0) + 1% glucose		+++	60
I	D(0) pH 6 0.1% glucose		+	65
J.	D(0) pH 4.9 0.1% glucose.		±	all −

Streak out on EMB var at 48h.

Results indicative but should use smaller inoculum.


B
D
F
G
H

Outcrosses of H226 segregants

April 2, 1950.

"F" W1304 x ~~58~~ 58-161 / EMS Lac.

72 Lac+ picked and streaked on EMB Lac. None were Lac v.

~~[illegible] = [illegible] Lac+ / possibly~~ + > −.

Test on EMS Mal for Mal segregation:

Lac+	Mal+ 11	Mal − 62
Lac −	Mal+ 10	Mal − 58

A W1303 x W1304 13 plates EMS Lac ca 200/plate = 2600

13 ~~[illegible]~~ 5 + colonies at 80 hours. hold plates for delayed +.

Pick these and streak on EMS Lac EMB Lac EMB Mal.

6 plates EMS Mal − dilute suspension: yield low
9 Mal − 2 Mal + Streak + as 6, 7.
Both pure Mal+ Lac −.

	Lac	Mal	√ Reisol.	Lac	Mal	Xyl	Mtl	H
1	v	v		v	v	v	v	240a
2	v	v		v	v	v	v	240b
3	−, v	−, +	v?	− v	−	v	v	241
4	v	v		v	v	v	v	240c
5	v	v		v	v	v	v	240d

Note the prevalence of Mal v here

H241 should be tested for hemi/homo-zygosity of Mal.

44 colonies picked from A as darker after 3 days. Restreak to look for faded Lac v. None were after 4 days incubation.

Outcross H226 segregants

April 10 ff. 1950.

E. W1303 × W945. (? Does Gal become heterozygous?)

P16: ~~100~~ 10 plates EMS Lac. ca 200 scoreable prototrophs per plate.

8 possible lac+ or lac v. Pick and streak out on Lac EMS, Lac EMB, Mal, Gal EMB.

#6 = Lac v. #2 = Lac + others Lac −. Recheck as 722-E1
Remarks "−" on EMB.
Mal − Gal −.

of 10 added plates (ca 1500...), 4 "+" picked for testing. 2−, 1+ (1 v. E2)
E2 is lac v Mal v check Gal?

G. W1304 × W478. ca 1/3 Lac +. Pick 90 Lac + and streak on EMB Lac.

5 possible lac v. Repick to EMB Lac; Mal EMS Lac.

	Lac	Mal
1	v	−
2	v	−
3	++ −?	−
4	v	+
5	++ −? v?	−

Xyl − Mtl −
E1 Lac v (weak) Gal − Mal −
E2 Lac v Gal v Mal v Xyl v Mtl v

Note: ~~no~~ Mal v.

After additional 24 hours, a number of "above" lac + picked from EMS.
of 21 isolations, 16 are apparent lac v. Repick these as above.

1	+	−	
2	+	−	
3	+	−	
4	v	−	
5	v	−	
6	v	+, − v?	Mal −
7	v +	−	
8	+, − v?	+	
9	v	−	
10	v	−	
11	v	−	
12	v	−	
13	+ − v?	v?	Mal v ?
14	v	−	
15	+	+	

No definite Mal v but
Recheck.

April 20, 1950.

H. W1311 × W67 ("A")
I W1311 × W1304 ("B") → on EMS Lac. → no yield Note 1/5/51 both are TUB.

H 100 Lac+ picked and streaked on EMB Lac
Hold possible ½ in refrigerator.

Pick purified lac+'s to DN2 Glu for spot test of constitutive lactase.

28 Lac+ 26 mpg + 2 possible mpg − . (11, 18)
Replate these on DN2 Glu for recheck.

Restreak −1 for ~~al~~ prototrophic ~~constitutive~~ lac+.

12 Lac slow 1 possible

April 3, 1950.

Continued from 699 -.

Type 1 (Lac - Mal v): on EMS lac, may eventually assume + appearance.
Select "papillae" from D(o) agar - lactose.

These cultures grow slowly to rather large colonies on D(lac) which may account for the poor selection!

699-1 No papillae found Colonies remain very small.

A 699-2. 2 papillae: #1 gave - and Lac v colonies on EMB lac
2 gave only Lac -! Recheck.

B 699-20. 8 papillae: #1-7 Lac v. #8 Lac -.
more appeared later!

But all of these cultures are Lac - on EMS lactose !!
See below - eventually gives + center colonies.

C 4/3: Pick single prototrophs from EMS Lac A1.
D " 4 " Lac v colonies from EMB Lac A1
E From EMB Lac B1-7, 1 each.

But after 3 days, colonies with dark centres appear on EMS Lac, probably representing "Lac +". This appearance develops very slowly.

723C #1 and #3 are weak Lac v on EMB Lac and show comparable appearance on EMB Lac. Restreak #1 on EMB Lac. Transfer to D(Lac) slant as 723 A1 ✓: ✓ mostly weak Lac v.

723D More or less typical Lac + on EMS; Lac v on EMB. Streak out to compare with 723 A1. A1 and D1 both give weak Lac + on EMS

F 4 new colonies from 699-20 on D(Lac), to EMS Lac
after 72 hours Lac + centres.

Lac heterozygosity tests

723a.

April 7, 1950.

723: A + C compared:

Both are Lac- after 24 hours; but give Lac V mosaics apparent in 48 hours on EMB Lac. On EMS Lac, colonies taken at 48-72 hours. This holds for all of these series! How many Lac V may be missed? Or, are these not true reversions?

See 727: no comparable Lac V failed isolated from ~~[illegible]~~ W1303 x W1304

Hemizygosity tests
Mal-partial segs.

724

4/2/50 ff.

From 699:

A. 699-11:

2 papillae: lac v Mal v Save single Mal EMS+ colony from each as 724A1, A2 OK ✓ Cf. 699-11R1.

an additional 2 papillae to EMS Mal. Residate from second. Check(1) as A3. Mal v Lac v ✓

1 gave Mal+; the other only papillae (2d) - Restreak on EMS Mal v/c Check as A4: 15 M- X- ∴ ++/-- i.e. "t16" 13 M+ X+

D Grow 699-11 in D(Lac) aer., overnight. Spread on several EMS Mal and D(Mal) plates to obtain additional Mal+ revertants. N10 About 50 cols on 7 plates. 30 picked and streaked rich on EMS Mal. Repick single colonies and streak on EMB Mal, D Lac then EMS Mal; EMB Lac —

#20 = Mal+ Lac- ~~—~~

#25/#26 = Mal+ Lac?

each was Lac —

Others are all Lac v Mal v.

Hold on D Lac plate

B 699-9. 3 papillae from EMS Mal to EMS Mal for purification. Residate pure +, check, and to slants as B1-3 H/6

#2 Lac v Mal v Keep as #1, 3 Lac - Mal + . 724B1 Reject others

C 699-12 2 Mal +, both give Mal v. 724C1, 2 purified and rechecked. To slants H/6 Lac v Mal v ✓

Linkage phases of 699-11 M+ reversions.

4/8/50

Streak out A0 – A4 on EMB Mal.

P7. Restreak Mal v. Pick isolated Mal+, – on the same plates and brush on EMB Mtl:

	Mtl+	Mal+	Mtl-	Mtl+	Mal-	Mtl-	
A0	0	6		17	2		+-/-+
A1	1	5		5	1 (v?)		+-/-+
A2	1	2		5	1 (v?)		+-/-+ ??
A3	0	0		0	1		++/-- ??

P8 8 Mal v from each of above streaked out on EMB Mal to obtain distinct segregants. Pick app. pure tested – from each quadrant to ~~A0~~. Xyl EMB. one +, one –

A0.	Mal+	Mal-	A1 M+	M-	A2. M+	M-
1	8X-	15X+	X-	X+	–	+
2	1X-	6X- 1X+	X-	X+	–	+
3	5X-	5X+	X-	X+	–	+
4	2X-	5X+	X-	X+	-	+
5	3X-	6X+	X-	X+	–	+
6	3X-	6X+	X-	X+	–	+
7	8X-	8X+	X-	X+	–	+
8	8X-	6X+	X-	X+	–	+

Each of these is +-/-+

A3.	M+	M-
1	4+	4-
2	3+ 1-	4-
3		4-
4	5+	4-
5	2+ 2-	4-
6	4-	4-
7	3+	4-
8	8-	1-

This is very likely in the ++/-- phase. Are the segregations accurately complementary ??

Proc 724D: —— into ~~V2~~ Pennassay
plate out on EMB Mal for segregants to test linkage phases

Linkage phases of 699-11 Reversions 724b

April 12 ff. 1950.

A 0 trans
A 1 trans
A 2 trans
A 3 cis
A 4 cis
D cis

} See 724a.

	Mal +		Mal −	
	X+	X−	X+	X−
A 4	10	0	0	1
D 1	10	0	6	0
D 2	10	0	1	3
D 3	10	0	0	0
D 4	10	0	0	4
D 5	10	0	2	2
D 6	10	0	0	4
D 7	10	0	0	1
D 8	0	1	10	0
D 9	9	1	0	2
D 10	10	0	0	3
D 11	10	0	0	2
D 12	10	0	0	2

Contras ++/−−

Recheck.

all but D8 had a preponderance of + segregants

D1-12 appear all to be in the cis phase ++/−−. However, since they were recovered from a single plating they might represent recurrences of the same mutation and should be counted as but a single reversion, viz., D.

Reinitiate the experiment by starting cultures from separate single colonies.

E 22 reversions, independent reversions on Mal, from EMS Mal from single lac+ (EMS) colonies. 15 were lac−, Mal+ pure (segregated!)
5 were lac∪ Mal∪ (#10, 13, 15)(21, 22) (Recheck 22: maybe lac∪ Mal+)

(See over)

F

		Mal +	Mal −	type
E	10	10 − 0+	10+ 0 −	trans
	13	12+ 0 −	10 − 0+	cis
	15	10+ 0 −	10 − 0+	cis.
	21	13+ 7−	18 − 0+	cis
	22	20+ 2−	18 − 0+	cis
F	1	10 − 0+	10 + 0 −	trans (Note: Lac −) (~~Do not cumulate~~)
			F1+ trans	see 724c
	2	10 − 0+	5+ 3 −	trans
	3	6 − 0+	2+ 1−	trans 6,7 0
	4	11+ 0 −	11 − 0+	cis
	5	11− 0+	7+ 2 −	trans

G. From dilute plating of H238 (D Lac) on EMS 14 al.

1-4 OK.

Cumulative score:

TRANS 𝍸 𝍸 |

CIS 𝍸 𝍸 ||

724 protocols

F8 724 F8 Mal v Lac v

~~"49" Mal+ Lac− segregated.~~

Definitive ~~G5 Mal+ Lac−~~

~~6 Mal+ Lac−~~

~~7 Mal+ Lac−~~

~~8 Mal+ Lac−~~

G5 9 Mal v Lac v

~~10 Mal+ Lac−~~

	Mal+	Mal−	
F6	8+ 2−	8−	cis
F7	9+ 0−	10−	cis
F8	10−	10+	trans
G1	10+0−	7−	5 cis!
G2	10+0−	9−	
G3	10+0−	3−	
G4	9+ 1−	6−	
G5	10+	8−	

cumulative score
9T 14C

	G					
G6	a	Lac v Mal v +	10 M− X+	4 M+ X−	3 M+ X+	TRANS
G7	b	Lac v Mal v +	8 M− X+	2 M− X−	10 M+ X−	TRANS
G8	c	lac v Mal v +	10 M+ X+	9 M− ? X+		??
	d	lac − Mal +				

G8 was almost completely M+. Repeat. 11T 16C

5/23 G8 is pure Xyl+ lac v Mal v (partial segregant?)

Associated Mal− should be saved to determine whether there is any correlation of the mutation with partial segregation.

H like G. "1-11" purified. 1, 2, 4 were lac v Mal v; others were lac− Mal+.

	M+	M−
1	7X+ 3−	10−
2	9X+ 1−	10−
4	9+ 1−	8−

#3 had lac v more complement [illegible] from lac HIS. Eliminate designations 5-11.

Not lac v; lac− Mal+.

Rest of H is all lac −

724 F1 (Lac - Mal v) 724c

May 10, 1950.

Apparently, a partial segregation occurred after mutation from Mal = to Mal ±, resulting in a Lac - strain. The Lac + and - components present in the finally isolated Mal + prototroph (a direct from EMS Mal) were separated. Each was Mal v. The Lac + was Lac v. The Lac + component must be ancestral; scored for Mal-Xyl linkage phase with remainder of series. Rep Lac - as a partial segregant. F1 + : 7 Mal + : Xyl + 8 Mal + : Xyl - also trans

F1 - apparently gives somewhat mosaic colonies on EMB Lac after 48 hours, resembling ~~the "Lac +" colonies of~~ H239. (Lac + trans 699-20 Lac -)

Repick single colonies from EMS Lac (4 colonies)

24h. Each is Lac - Mal v.

60-72 hours. On Lac, definite mosaics with dark centers & horns by most colonies. On EMS Lac, some colonies are much darker than others. ✓ these.

The "dark" type gives colonies on EMB Lac mosaic at 24-30 hours

The "light" requires 48-72 hours for Lac v.

5/22/50 Streaks of "light" on EMS Lac acquire "dark" papillae: Restreak these:

See over

Heterozygosity tests

Segregation H229.

4/4/50.

1 Mal+ obtained from D(Mal)

A. Streak on EMSMal (purify); EMB Mal and Lac — mostly Mal+ Lac v.

Verify from single EMSMal+ colonies. ✓ verified Mal+ Lac v from 4 EMS cols.

B. Streak out on EMB Xyl Test pure Xyl− for Lac+.

31 tests. 2 indicated Lac+. Restreak on Lac, Xyl.: Both Lac v Xyl v

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A. 1 additional Mal+. Purify on EMS Mal. check purified colony: again: Lac v Mal+ mottled on Mal EMB, but no Mal− colonies or sectors.