January 28, 1949.

Streak out single colonies from Lac EMS of H 154 - 157.
8 colonies from each on Lac EMB

P30: Each shows Lac+ only! Reason from initial plates: Test 8 colonies from Lac EMS:

None of these show any signs of segregation as Lac EMB

Conclude: H154-7 are not heterozygous.

January 30, 1949.

Y-161 7 secs. in lac EMB UV. 30 plates ca 600 = 18,000

12 rechecked as Lac- mutants (1 slow)

Test segregant from "sp." heterozygote for "H"

1/31/49.

W721×440 100 tested all Lac++. Retest #7+ : Lac+

~~Test for "H" in a spontaneous heterozygote segregant~~ 4/29.
W589 ...

Feb. 1, 1949.

~~W-721 x 440. n lac EMS~~
W477 x W589. n EMS Lac

20% Lac+ colonies streaked out. No clearly Lac v.
Restreak 1-4 on Lac EMS for verification.

1A, B ++
2C, D are Lac v - ; A+B are Lac ++. H173
3. ++
4. ++.

Streak out 4 cols. H173 n Lac EMB; Take 1+ and 1- and test nutrition:

	BMTLB,	BMT, Ad	TLB, T, Ad	Σ.	V1	Lac
1A	+	—	+	+	S	+
1B	+	+	+	+	S	—
2A	+	+	+	+	S	—
2B	+	~~+~~ —	+	+	S	+
3A	+	+	+	+	S	—
3B	+	—	+	+	S	+
4A	+	+	+	+	S	—
4B.	+	—	+	+	S	+

Growth in Σ rather sparse in 1A, 2B, 3B, 4B; Very heavy in others.

Check from Σ tube on T1.

February 2, 1949

W ~~991~~ 251 (Lac_3-S+) x W478 (Lac+ "H") on GluEMS.

Majority are Glu+. High yield. Streaks out on EMB glucose.

100 tested on GluEMB. All Glu+

Chicks on $BaLac_3$ - ?) 431

2/2/49.

Rectuals stock cultures of Glu on EMB Glu. → Lac

1 W249 h.g.
2 386 small cols. v. slow +
3 387 compact
4 388 erythem.
5 389 slow +
6 423 minute cols
7 432 good −
8 433 " −
9 434 "
10 435 many +: − good size.
11 467 good size −.

chick plates of W434 and W435 on EMB Lac show papillae in their stools with lytic clearings around them!! See 437

chicks crossed: 2 plates each. x W108

a. W433: 0+/400.
b. 435: 0+/150
c. See p. 434. W467
d W432 0/300
e. 4434 No prototrophs. a few hundred microcolonies

Stocks for suppressor cross x. 432

Feb. 2, 1949.

~~Spread~~ S.O. W251 ($Lac_3 - Sl_3^{L+}$) on EMB/Glu to select for Lac_3+ revertants. Picks 8 papillae and restreaks to purify + and − colonies noted generally. One colony was noted which looked (*) as if it might be segregating. (Heterozygous?)

Picks from dark center and restreaks as 432-1.:

Picks pure + from ~~str~~ remainder and restreaks, for confirmation; on EMB-Mal.

432-1: mostly +. +few −. None could be identified as segregating and this will be true except for the most stable heterozygotes.

Feb 1 ff. 1949.

Inoculate Y10 into T(m)TLB, Lac and Glu. Thereafter loopful transfers in homologous medium.

B) A4.
C) ~~A5~~
D) P6
E) P8
F) A9
G) A10 → EMB Glu Lac
H) A11
I) A12 ←

etc.

W589 - Inhelia type

February 6, 1949.

A. W589 x W~~5~~466. 92 tested; Retest 6 for Lac v.

B. W589 x 471 100 tested. No Lac v!

A): 1-3 fairly certain Lac v; 4-5 ? 1-3 yield approx. proportions of Lac - prototrophs Cf. 429 where several Lac - tested were prototrophic

1, 3 are Lac v H-174 and 175 (A) (B)

Test nutrition of Lac + segregants:

A 1 + on BM TLB, i.e. Ad T-r +
2 "
3 "
B 1 "

8 additional A 8 Ad T-r + ~~+ T-r -~~?

8 " B. all Ad T-r +

These strains do not seem to be segregating nutr. req.

Suppressor tests

February 6, 1947.

W463

A. R21 w112 (+ Su.) x W461.

B. W108 " .

B. 5 plates, ca 200/plate. No +. ∴ Lac_3^-.

A. Picks + and streaks out on lac EM3

Feb. 5, 1944

Test Σ - segregants of H167 nutritionally to select for further "H" derivatives.

		W
1.	TL	[redacted]
2.	LB,	
3.	TLB,	735
4.	B,	
5	TL	736
6	TL	737
7	TL	738
8	TL	739
9	TL	740
10	TL	

Feb 6, 1949.

Inoculate Y10 on Y2 galactose + Y2 glucose Picks Ga and Gl colonies to

a) .1 ml M/1000 ONPG + .2 ml KP buffer 9/10 pH 7.5

b) .2 ml " ".

After 3 hours incubation, blue cells were — in both series; all but one was + from Gal series, I was rather weak. Streaked ↳ Justifying Method on Lac EMB as #436-1.

Mixture of Lac+ and Lac−!

2p phage.

Feb 7 ff. 1949.

See 435. Stock cultures of W435 and W435^{1} on Lac EMB, showed signs of plaque formation a central papilla.

Pick uncontaminated colonies, and spread as detector, pick papillae and streak out a) on W435^{5} (sensitive indicator) and b) Lac EMB for purification.

A 8.] Plaque formation clearly evident on 435^{5}.

Pick individual colonies from EMB and s.o., testing for phage also.

A9: All 8 cultures carry considerable phage (comparable to number of bacteria in colloid streak.

P9: Repeat

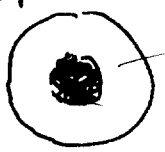
A10. Results in same sense. Conclude that these cultures are lysogenic on W435.

Stock, streaked, does not show this response.
W435

Filtered, heavy suspension of 437 "lysedans". Test on 410, W435:

Feb. 11, 1949.

1. N11: passage lysate in NSB from ~~single~~ plaques of 437-H on W435.
   437 mixture. | 5 further growth.
   No action of control plaque noted on Y10, although active on W435.
2. Continue single colony isolations from 1 and 2 (lac + and − resp.) testing lysogenicity concurrently: 8 single colonies from 1 and 2 and 1 each from 3-8 were all lysogenic!
3. Test nutrition of 437-(1-8).
   1-1 : TLB$_1$ 1-2 : TL(B,?) 2-1 : poor ++ ? 2-2 : do.
4. Typical appearance of a plaque is:



   In heavy regions of overgrowth, occasional clear spots are seen, possibly virus mutants? Picks and ~~cultivate~~ ~~437-1.~~ mass containing such clearings and spread: no clearings noted!

5. Tests of Phage sensitivity.

	T1	T2r	T3	T4	T5	T6	T7	C	
Y10	S	S	R	S	S	S	S	R	
438-1	S	S	R	S	S	S	S	R	
W435	S	S	R	S	S	S	S	R	O.K.

   stocks ng.

   ∴ the lysogenic derivative has same phage reactions as the sensitive and standard strains.

Feb. 12, 1948

look for Lac v among progeny of crosses of W167 segregants.

x 58-161

A. W-736

B. W-737

C. W-738

D. W-740.

A. ~~3~~ 56 tested. No Lac v.

B. 64 " No Lac v

C. 100 " No Lac v.

D. 16 " No Lac v

∴ Segregants of #167 do not carry Het.

Feb. 12, 1949.

W705 × W126. 15 plates. Ca. 50/plate = 750 colonies.

1, (mucoid), lac+ formed. 2.0. on EMB lac All mucoid.

Feb. 12, 1949.

W705 × W126. 15 plates. Ca. 50/plate = 750 colonies.

1, (mucoid), lac+ formed. 2.0. on EMB lac all mucoid.

seperation of λ from W153.

Feb. 12, 1949.

Harvest cells from EMB plate and suspend in 10 ml saline. Sediment and remove supernatant as λ. Filter most of the supernatant to remove bacteria. Keep a portion unfiltered, but substantially bacteria free. Wash sedimented cells with saline and resuspend.

A) Dilute cells 10^{-3} and plate out: a) on EMB Lac b) on W435 for λ determinations.

B). Dilute λ' ~~10^{-2}~~ 10^{-1}, 10^{-3} and 10^{-5} and plate as above 11 plaques

c) Titrate λ on W435.

D) Heat 1:10 dilution of Cells A at 56°C. 1 hour. Titrate cells and phage

A) Ca 50 colonies per plate on EMB Lac. 46, 38, 57

Plaques very difficult to count ~~3~~, 25, 17, 34

B) 10^{-5} 17 plaques; 7 bacterial colonies.

10^{-1} Almost continuous lysis + occasional colonies. Bacteria continuous

~~10^{-3}~~ 10^{-3}. Several hundred colonies; do. plaques.

c) No plaques at any dilutions

E) Test W753 as other coli strains:

34 single rods. 58-161

and 35 of K-12 tested by short stroke + .

pinpoint on plate spread ± W Y 35°. Each caused +

Most readily scored on strokes.

E. W435: Patchy lysis.

Y10	No plaques
Y40	"
ML	"
B/1	"
B/1,5	"
B/4	"

W435 seems to be unusually sensitive

Note: W753 is T-L- Lac ± Glu++.

W754 is (B)-M- Lac_3-. Where W753 could have come from is not clear; possibly the original source of λ.

For further study, use W754 as a marker strain.

Test R+S colonies from (B) above for lysogenicity on W435.

34 colonies tested, all carried λ.

Transfer of λ.

Feb. 13, 1949.

Inoculate W754 in Y2 galactose with a series of other λ-, 2 types to look for transfer of λ.

1. W754
2. 58-161
3. W-108
4. K-12

12 58-161 + W754
13 W108 "
14 K-12 "
15 W477 + "

On plates, test various cultures for sensitivity + content of λ.

Cannei → / Host ↓	W754	K-12	Y10
W435	L	L?	L?
K-12	O	O	O
W108	O	O	O
W467	O	O	O

Conceivably, K-12 cannis λ and W435 is a mutation sensitive to it! See 443.

An important problem now is to devise best methods for scoring λ and obtaining it free from bacteria. (recover)

1. Show that λ is transmissible. Mix K-12 and W435 and streak out. Test Lac- colonies for λ, testing for its transfer.

2. Rapid methods of testing susceptibility + infection:

   a. Try cross streaking

   b. Spray disturbed colonies with suspension of λ^s.

February 15, 1949.

Test K-12, W753 + W754 for λ by streaked on W435 strain + W435 old culture susp, on NSA. Also plate ca 150 K12 or W754 + mix with bacteria.

1) Plate: K-12 : no plaques; W754: 1 large plaque

2) Streakout: K-12: a few plaques noted in both sets of streaks. Rather more are found in 753 + 754. ∴ K-12 is lysogenic.

W435 is a susceptible mutant, and W753, etc. are merely standards carrying λ.

Cross-streak tests for λ. 444.

Feb. 15, 1949

Lay down heavy streaks of W~~5~~754 (for λ) and 435 (for λ^5) Cross streak ē each other; K-12 etc. on EMB Lac

	NSA v. 754	NSA v. 435	EMB Lac v. 754	EMB Lac v. 435
1. ↻	λ +++	λ +++	++	+++
2. K-12	−	λ ++	−	++
3. SP-161	−	λ +	−	++
4. W478	? ±	±	−	++
5. W477	λ ++	++ ?	Patchy. −	+
6. W108	−	++	−	+
7. W595	−	++.	−	+.

Patchy appearance along entire streak

Cross-streaks are very difficult to read. However, lac + λ on lac-S, or EMB lac are not so bad.

(N7) Lysate from penicillin treatment of K-12, 10^7/ml initially, filtrate. 2/17/49. .1ml had no λ, was sterile.

Transfer of λ

2/15/49.

Grow K12 with W435 for 8 hours in Y2 broth. Plate out on EMBlac.

(179+ : 8−) = ca 5% − .

A) Pick − and test for λ on W435 EMBlac plates. Keep in order.

This test was inconclusive. Repick 16 and streak out on 435 film. 13 were apparently ~~sensitive~~ non λ. 3, all of which had a lac+ component, showed λ. Pick + and − from each of these for retest. Pick others to test sensitivity (445a). K-12 controls had λ; W435 did not.

#14.	All+ cols.,	λ+ and prototrophic.	No transfn.
#16.	2− cols.,	λ−; lac+ was λ+.	" .
#11.	2− "	λ−; 2+ " "	" .

14 other − cols which were λ − were tested and all found sensitive to λ

B). W756, λ+ streaked out on W435. λ developed. Pick from confluent area to find lac −. Streak out on EMBlac. Only 1 lac − colony found. Streak out ~~#~~ 2/5 W435 background. (not well isolated)

On lac EMB − Pure lac − ; on W435, lysogenic. Therefore transfer of λ can occur under these conditions. Induced λ strain is W767 Check institution. 4 colonies all H− like W435. See 445a.

c) Sensitivity tests in A were done with λ from K-12. Streak out zones of lysis to find λ+ lac − (for evidence of transfn. Do. ∈ 448).

Transfer of λ.
Retest original λ⁻ strains.

4/5/9.

2/20/49.

B). All 4 s.c.i. of W767 agree in Glu-, M-, and λ+. All are pure from sup⁰ as tests of induced lysogenicity.

C. Many plates were primarily Lac- with patchy lysis, and lysis at intersection of mutant colonies. Picks Lac- colonies to test for λ+.

	λ.	Autolysis
a.	1-3 λ+	1-3 −
	4 λ−	4 +
b.	1 +	1 −
	2 −	2 −
b'	1-2 +	1-2; −
c	2-3 +	1-4; −
	1, 4 −	
d.	1-4; +	−
e	1-2; +	1+; 2−
e'	1-2; +	1-2; −
f	1-2 −	1-2 −
f'	1-2 +	1+ 2−
g	+, +	−, −
g'	+, +	−, +

W	λ	Autol.
432	+	−
433	+	−
434	−	−
435.	−	−

W434+ 435 are, therefore, merely λ⁵−. Their sensitivity was detected presumably as a result of mixture or contam. with W753, possibly related to W108.

1 clear plaque noted. Picks as possible virus mutant and streaked on λ− and λ+

Autolysis probably indicates a sensitive strain which has phage mixed with it. ~~In most cases~~, the autolysis and lysogenicity are comparable, consistent with this picture.

UV - Lac Mutationsum.

446

2/16/49.

1) W760 43pl x ca 50 / 2500. colonies. Very high yield of mutants apparent.

2) W758. 40pl. ca 5% / 2000 cols.

1) 5 -- W768-772
   2 ± W773-774
   3 slow W775-777.

2). 5 - W778-782.

	Lac	Mal	Glu	Ina	Gal	
W 768	−	−	−		−	Hex -
9	−	+	+		+	Lac -
770	−	−	−		±	(108)
1	−	+	+		+	Lac
2	−	+	+		+	Lac
3	±	÷	−		±	(108')
4	±	÷	−		±	(108')
5	±	+	+		+	slow lac
6	±	+	+		+	slow lac
7	±	+	+		+	"
8	−	+	+		+	Lac
9	−	+	+		+	Lac
780	±	+	+		−	Gal - slow lac
1	−	±	±	+	+	(108'?)
2	−	+	+	+	+	Lac

Lac_3 suppressors.

2/18/49.

Streak out W467 on EMB lactose. Restreak, and pick lac+ colonies to EMB Mal + Glu.

27 tested on Glu + Mal. 3 Glu − Lac+. Alternate all ++. Later, 33 tested on Mal. All +.

Purify the Glu - Lac+ on EMB Lac. All were Mal - W764-766.

Inoculate W766 on Glu EMB to recover revertants.

2/21/49. Inoculate W768 on Glu, etc. media to find specific reversions. Maltose: slow +. Lactose full +'s. Nothing on gal., Mtl or glucose

2/23. Collect W677 lac+. Check on other sugars. W814

3/1. Test 1 Mal+, 8 lac+ purified from homozygous plates.

	Glu	Mal	Mtl	Gal	Lac	
1	− −	+ ++	− −	− +	++	W815
2	− −	+ ++	− −	− +	++	
3	− −	+ ++	− −	− +	++	
4	− −	− −	− −	− +	± +	W816
5	− −	+ ++	− −	− +	++	
6	− −	+ ++	− −	− +	++	
7	− −	+ ++	− −	± +	++	W817
8	− −	+ ++	− −	− ++	++	

not spec. lac response! Save 1, 4, 7. as W815-817

3/9/49.

A set of "Hal+" colonies was tested on 4 sugars. Many undoubtedly subs.

Types.	Mal	Lac	Gal	Glu	W
1	+	−	−		856
2	+	++	++		857
3	++				856

3/2/49.

5 Gal+ isolated and tested:

	Gal	Lac	Glu	Mal	Mtl	
1	+	—	—	+?	—	840
2	+	+	—	+	—	
3	+	+	—	+	—	
4	+	+	—	+	—	
5	+	+	—	+	—	841

Fermentation of Gal is sluggish; Mal and Lac slow.
Picks as W839 + 840

Routine tests for λ.

2/18/49.

Preliminary tests have shown λ in K-12 and a number of derivatives. Retest + check by streaking out on EMB 5 sugar, (for autolysis) and on W435.

		λ On W435.	Autolysis.
1.	K-12	+	—
2.	W754	+	—
3.	58-161	+	—
4	Y40	+	—
5	Y87	+	—
6	Y70	+	—
7	W677	+	—
8	W70~~81~~	+	—
9	W45	+	—
10	Y10	+	—
11	~~Y83~~ Y77	+	—
12	~~Y44~~ W	+	—
13	W~~58~~ 125	+	—
14	W680	+	—
15	~~W677~~	+	—
16	W478	+	—
17	W467	+	—
18	W108	+	—
19	W145	+	—
20	W126	+	—
21			

∴ Most standard strains still carry λ and are resistant to it.

λ- sensitivity in W518 "A" and "B" 448

2/18/49.

EML noted that W518 A + B were lysed by Y70. W518 itself, when streaked out was autolytic, suggesting a mixture of λ- and λ+.

1. Streak out W518 on EMB Lac
2. Test A + B for lysis of each other, of W435, and by K-12.

Cann. → / Mat ↓	A	B	435	K12
W435	λ-	λ-	λ-	λ+
A	-	-	-	+
B	-	-	-	+

∴ 518 A + B show same pattern of sensitivity as W435 and are λ^S, λ-

Test for transfer of λ from K-12 to 518 A + B. [Streak out plaques to find λ+ Lac-]. Cis λ/518.

Mostly + colonies. Some - had plaques. Pick clear Lac- colonies + streak out on EMB Lac; EMB W435.

A). ~~1 + 2 + 3 + 4~~ 1-4 λ+ (3 had 1 plaque), 1; 3 are autolytic. Use (2).

B. 1-4 all λ+ no autolysis. 3 has papillae, probably not pure -.
Use #1.

C.)(W518 λ+). /435 /auto.
1 - (1 pl.)
2 -
3 - no growth -
4 ++ - → → W518 λ+

λ− x λ+.

2/18/49.

Y10 x W435

8 colonies found in 15 plates. Very low yield! All lac+

Streak out on Lac EMB. Use 2 isolates colonies per isolate, to gave (A-D)(1-4).

		/435	/auto.
A	1	+	−
	2	+	−
	3	+	−
	4	+	−
B	1	+	−
	2	+	−
	3	+	−
	4	+	−
C			
	1	+	−
	2	+	−
	3	+	−
	4	+	−
D	1	−?	−
	2	+	−
	3	+	−
	4	+	−

Retest D1:

D1/518 No plaques.

Y10/D1 Some questionable plaques.

Y10/518 Numerous plaques ± central papilla

D1.

Retest: was sensitive to λ. Recheck 4/7/49.

Sensitive to λ. λ−

2/23/49. Test 19 segregants from 10 mosaics of Y1/176 (W518 x W538) for λ.

		/518	auto.
A	1	+	−
	2	+	−
	3	+	−
	4	+	−
B		+	−
		+	−
		+	−
		+	−
C		+	−
		+	−
		+	−
		+	−
D	1		
	2	+	−
	3	+	−
	4	+	−
E		+	−
		+	−
		+	−
		+	−

Attempts to obtain free λ

2/19/49.

A. Scrape area of lysis of W756/W435⁻ into H_2O. Sediment and filter supernatant (sintered glass).

B. Extract 100mg dried K-12 ~ 10ml H_2O. Sediment 1:10 dil. and filter supernatant. B' is test on sediment. 9 colonies K12/1ml (noted. Numerous others cont. (from water?))

C. Inoculate Y2 ~ W435 and K-12, young cultures. Shake + incubate ca 2-3 hrs. Sediment and filter.

C' Let grow overnight and filter.

A). .1ml: ~~80~~ plaques on ~~W758~~A.W435⁻ on EM13-s. ✓
A hopeful streaked out was similarly effective.

B. No plaques.

B' 2 plaques in hopeful, probably from K-12.

C. No plaques in .1ml. 1/plate; 2 plaques in another.

Free phage from A) only.

C': ca 500 plaques/.1ml i.e. titer of ca 5000.

Synergisms in Lac tests
Lac_1 .... Lac_7.

450

2/19/49.

1. [redacted] W112 — Cross streaks on EMB Lac.
2. W45
3. W108
4. W126
5. W145
6. W125
7. W133.

	1	2	3	4	5	6	7
1	−	−	−	−	−	+++	−
2	−	−	−	−	−	++	−
3	−	−	−	−	−	−	−
4	−	−	−	−	−	+	−
5	−	−	−	−	−	+	−
6	++	++	++	++	++	±	+++
7	−	−	−	−	−	+++	−

At 24 hours, Lac_6 – reacted regularly. Its isolated response was irregular, sometimes ++, sometimes −! Needs study in liquid medium!

Hold for 48 hr. Rdg! : No change Lac_6 shows most interesting interactions

Segregation of M179. 460

2/26/49.

M179 is W126 x W778. (TLB, Lac$_4$− x IV-M18t Lac$_3$−).

Streak out 4 original var. cols. on Lac EMB. Practically no pure +. Purify 1 - from each. Pick + centres for new segregations Test nutrition of 6 additional - segregants from diffrent Lac v Also streak out 16 additional v colonies.

d −−
D guarm - H only.
c ++ (weak on - T)
a −−
1 −−
2 ++
3 −−
4 −−
5 −−
6 TL

nutrition diff. to establish. Probably IV requirement interferes.