The following is a true copy of my laboratory notes, Volume 1, covering the period October 5, 1951 to July 7, 1953, pages 1-218a, with a number of unnumbered pages included.

There are two additional volumes of my notes, one of which is labelled "Summaries".

In this first volume there are a number of unnumbered pages at the front which represent an index, but it should be noted that at page 110 there is a statement "not indexed beyond here".

The chief interest in these notes will be for the specialized transduction produced by the bacteriophage lambda and its discovery. However, many of the notes deal with other matters. Each of us in the Lederberg laboratory had some side assignments and some of these, such as isolation of mutants in E. coli strains believed fertile with K-12, radiation resistance as a function of ploidy and lysogenicity for phage, phage induction by UV, and others were my assigments.

The discovery of the special transduction by lambda was not planned. It came shortly after the discovery of generalized transduction by phage PLT22 in Salmonella by Zinder and Lederberg, and was followed several years later by the generalized transduction in E. coli by phage P1 by Lennox.

As I was producing large amounts of phage lambda is some radiation experiments I was curious as to whether lambda transduced any genetic material. I tried my first transduction (for methionine independence) on March 26,1952 (page 47) and of course it didn't work.I tried the same experiment again a few days later (pages 48,50).

It is my recollection that Norton Zinder and I were alone in the lab while the Lederbergs were at a meeting at Rutgers University in March or April of 1952, when Norton and discussed the possibility of lambda transducing genetic material. I recall that Norton said he thought that Esther Lederberg had tried some experiments with lambda but he did not know what they were. Since I had a lot of lambda preparations I suggested that we try and so we took all the selctive medium plates available, and appropriate recipient strains and mixed lambda and cells and plated them out.

I have always thought that chance played a big part in Norton Zinder's failure to discover special transduction. Norton worked with Salmonella which do not metabolize lactose and therefore he had no EMB lactose plates, on which the first lambda tranductions were observed.

It was my good fortune to have had the EMB lactose plates which provided the selective environment for gal^+ clones.

However, it was still baffling in that the papillae on EMBlac produced by lambda and quantitatively related to the amount of lambda used - proved to be lac^- on further examination. I spent much of April 1952 trying to resolve this confusion. It was at this point that Esther Lederberg suggested, on the basis of her prior knowledge and experience with lac-gal interactions and the strains that I was using, that I should look at lambda transduction in terms of selection of gal^+ clones, So on May 5, 1952 (page 71) I began studying lambda transduction of gal genes on EMB galactose, and it all began to come out. Much more was needed to be done: lambda as vector had to be established;the heterogenotic character of the transductants elucidated; the identification of the alleles involved in the segregants when the donor and the recipient were gal-; quantitative relationships established; the discovery of high frequency (HFT) transducing lysates; that the phage in some of the transductions was a defective phage, and other things.

It was an exciting time and it was an exciting experience and the stimulation by the people in the crowded lab helped - the Lederbergs, Zinder, Alec Bernstein, Tom Nelson, Bob Wright, Luca Cavalli-Sforza, Gaylen Bradley, Tetsuo Iino, Dorothy Gosting, and probably others I overlook.

Prior to writing this introduction I made an audio tape that discusses the rest of my notes in Volume 1, and perhaps when it is transcribed it can added to this copy.

M. L. Morse

July 23, 1986.

Research Notes Vol. I

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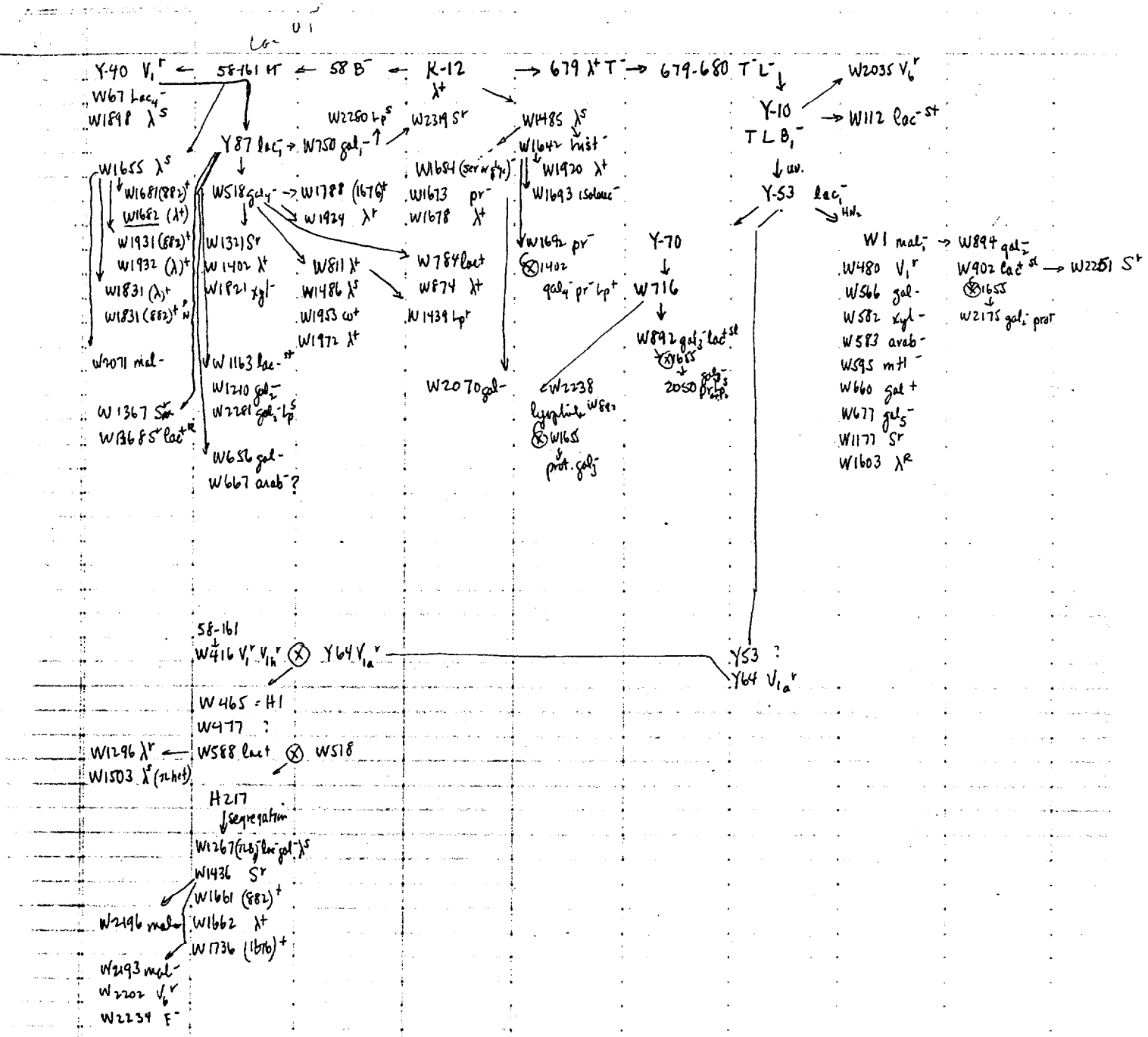
Research Notes



Cultures on Hand 8/20/53

- ✓ K-12
- ✓ 58-161
- ✓ W67
- ✓ W578
- ✓ W588
- ✓ W607
- ✓ W750
- ✓ W811
- ✓ W874
- ✓ W892
- ✓ W902
- ✓ W1177
- ✓ W1210
- ✓ W1368
- ✓ W1402
- ✓ W1436
- ✓ W1439
- ✓ W1485
- ✓ W1503
- ✓ W1655
- ✓ W1673
- ✓ W1678
- ✓ W1692
- ✓ W1808 (W4 31)
- ✓ W1821
- ✓ W1824
- ✓ W2035
- ✓ W2050
- ✓ W2062
- ✓ W2070
- ✓ W2071
- ✓ W2175
- ✓ W2196
- ✓ W2202
- ✓ W2251
- ✓ W2280
- ✓ W2281
- ✓ W2297
- ✓ W2319

Subject	Pages
Gal directions	
58-161	47, 82
W518 gal$_4$-	54, 92(gal), 93, 95(2), 97(stabcal) (518 ELC-12 λ_1) 99(stabcal)
W1655	48, 49, 50, 51
W1736 gal$_4$-	59, 61, 62, 65(gal), 71(gal) 72($\frac{\text{Lac}}{\text{gal}}$), 74($\frac{\text{Lac}}{\text{gal}}$), 75(Lac), 76($\frac{\text{Lac}}{\text{Lac}}$), 77, 78(Lac), 80(gal), 81(Lac), 82, 83(Lactin), 86, 87, 88($\frac{\text{Lac}}{\text{gal}}$) 95(stabcal) 96(stabcal) 99 (λ_1, λ) (stabcal),
W112	71, 83(quinj), 85, 94(derived $\lambda_{p.s.}$)
W1678	74, 76
W1662	80, 82, 84
W811 gal$_4$-	81, 82, 83(BM), 84, 86, 87, 88(81), 89(811 gal$_4$ + λ / 778 gal$_4$ λ), 92(802 λ), 93, 94, 96(811 ELC-12 stabcal), 99(4 gal) (stabcal), 103(stabcal) — 811 ELC-750
W1439 gal$_4$-Lac	82
W1821 gal$_4$-	83(xyl), 85(gal, xyl, BM), 87
W902 gal$_2$-	88, 100(derived $\lambda_{p.s.}$) 110(derived gal + stabcal),
W750 gal$_1$-	88, 91, 93(several λ) 94(750 λ), 96(stabcal 2)(750 ‰), 98, 99(4 gal)(stabcal), 105(stabcal), 107(stabcal)(other λ), 109(stabcal) — 750 ELC-182
W1692	96
W1920	96
W2050 gal$_3$-	99 (ELC-12 λ_{750}), 106, 107 (stabcal)
W1578 gal$_4$- F	99
W2062	100, 101, 104, 105, 106,
W1924	102, 103, stabcal, 104, 106, 107 (stabcal), 109 (stab).
Adsorption Exp	89

Subject	Pages
Transductions	
58-161 (hy K-12)	47, 82
W518 (hy K-12)	54,
W1655 (hy K-12)	48, 49, 50, 51
W1736 (hy K-12)	59, 61, 62, 69 (gal) 71 (gal), 72 (leu gal), 74 (leu gal), 75 (leucine), 76 (leu cysteine), 77, 78 (leu
W112 (hy K-12)	71, 85 (purification), 85 (leu)
W1678 (hy K-12)	74, 76
W1682 "	80, 82, 84
W871 "	81, 82, 83 (BH), 89, PC, 87
W1439 "	82
W1821 "	85 (UV), 85 (gal, sg BH), 87
	86, 87
	85a (aeration report)
	82
	81 (leu)
	80 (gal)
Reconstruction exp.	82
Crosses	
58-161 X Wg 14 pr- tryptophan-	50, 52
W112 leu X W1655 [λ⁺ 718, lac⁺ strain x λ⁵ BH plate]	85a, 88, 97
W902 X W1655	95, 96, 98, 100 (leu gal)
811 tlc-12 X 1436	100, 101, 102 (4), 103 (2), 104, 105 (repeat), 106, 107 (streptomycin gal+), 108, 109
518 tlc-12 X 1436	100, 101, 102 (4), 103 (2), 105 (repeat), 106, 109
780 X 1803	109
1177 X 1655	109

Subject	Pages
Lwoff effect with λ (for other phages see particular strain)	
determined by gross examination	
K-12	26, 30, 32, 33, 36, 38, 43, 51, 61, 90
SB-161	36, 38, 39, 43, 92
W67	57,
W1177	57,
W1661	59,
W1662	59,
W1736	59, 67, 90(gal+)
W1682 (no effect)	63,
H267	58
90v	92
W1821	90
W811	90, 90(811 gal+)
750	94
W1939	98
by plaque count (see also λ prep.)	
K-12	22*, 23*, 53*(agar), 68*(syn. cult) 69*
K-12A, K-12B	34, 35
W1177	13
W1678	58
W1932	62*
H267	60*, 68*
W1682 (no effect)	63
W1954 (Ωg3)	69*
W1977 (λ*Ω*)	69*, 78*
W1998 (Ωg28*)	78*
effected by post incubation	
K-12	24, 25, 26
W1177	13
W1603	13
W1736 gal+	88
W811 gal+	88
SB-12	102
SB-K-12	102

= cell divided
= cell does not divide

Subject	Pages
Ultraviolet effects –	
Survival	
K-12	12, 17*, 19*, 20*, 22*, 23*, 25*, 26*, 53(agar) 68*(Sym cult) 69*, 71(cult from [illegible]), 73*(Sym)
58-161	6, 8, 9*, 10*, 11*, 15, 18*
W 518 λ[s]	2*, 30*
W-811	1*, 90
W-1177	13
W-1985 λ[s]	12, 17*, 19*, 20*, 22*, 23* 64
W-1603	13
W-1655 λ[s]	6 7 8, 11*, 15, 18*, 20* 70*
W-1655(882) = 1931	46*
W-1673	12
W-1678	58*
W-1681	6, 8, 15, 53
W-1682	6, 8, 15, 63*
W-1831	18*, 55, 64
W-1831(882)(W)(P)	29*, 45*, 55*
W-1931	54*
D[A] [58-161(882)]	15
W-1932	62*
H267	57*, 59*, 60*, 64*, 66*, 68*, 73* 75(Sym gain)
W1736	66
W1959(Wg 31*)	69*
W1972(λ*52)	69*, 78*
W1898(λ[s])	70*
W1503(λ[s])	69a
W1998(Wg 28 λ*)	78*
W1936(λ[s])	81
λ	80, 81
W1953(Wt)	81
W1661	80
Survival curves	
K-12	3,
H267	3,
Misc W1832 for J.L.	76
W1931 for E.L.	76
Pages from λ in W11c	77
W1736 gelt	80
Lysates streaked out	104, 106

λ Phage Stocks – lysates

#	Source	titer	
1.	750 (gal_1-)	$>2.4\times10^{10}$	
2.	902 (gal_2-)	4.9×10^{10} ←?	
3.	K-12	1.9×10^{10}	← discarded
4.	58-161	1.8×10^{9}	
5	811 (sp. galt)	4.0×10^{10}	(contam?)
6.	1821 (gal_4-)	1.0×10^{10}	(contam?)
7	K-12	2.3×10^{10}	heated
8	1485 (galhate)	-	
9	750 t 1821	6.5×10^{9} ←?	(contam?) 0.1 ml / EMB gal gave no col 10/9
10	1439 (gal_4-)	1.1×10^{10}	
11	811 (gal_9-)	1.7×10^{10}	
12	811 (gal_9-)	?	
13	1736 (sp. galt)	?	
14	892 (gal_3-)	3.8×10^{9} ←?	
15	K-12 (HEATED Δ)	-	
16	1954	$>140\times10^{6}$	
~~17~~	2046	2.1×10^{10}	

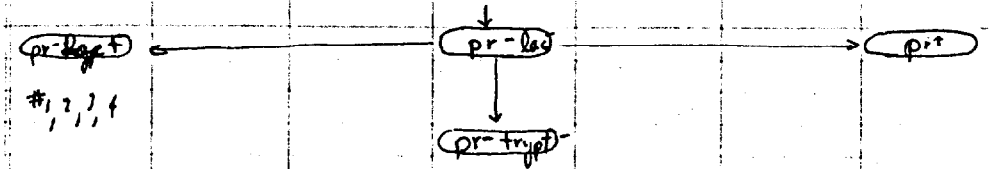
Subject	Pages
Wq-14	27, 28, 32, 34, 35, 36, 38, 39, 40, 41, 42, 56, 57, 59, 60, 61, 66, 67, 68, 85a
pr⁻ verified	35
pr+	36
pr⁻ trypt⁻ " " crosses	35, 44, 46, 78
lac⁻ verified	50, 52
lac+ (*1, *2, *3, *4)	45
pr⁻ trypt⁻ lac⁻	60, 61
	75
Wq-16	27, 28, 36, 37, 38, 39, 40, 41, 42, 55, 66, 67, 68, 75
pr⁻	42, 47, 48, 49, 52
pr⁻ x⁻ (1)	53
pr⁻ x⁻ (2)	56 - not so on retesting
Phage sensitivity	
1682/1681 = λ	
888/1503	

W1584

Wq 14

pr⁻ F⁺ lac⁻ Mal⁺ Suc⁺⁺ Gal⁺ cells⁻ rhamn⁺ Acrif.^{R} Indole⁺ V_1^R, V_2^S V_{3-7}^R λ^R ρ^R λ^{370R}

stock

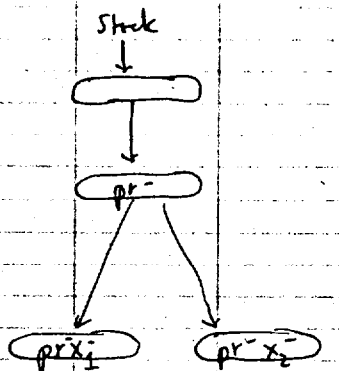


#1, 2, 3, 4

W1716

F⁺ lac⁺ suc⁻ gal⁺ cells⁻ rhamn⁺ acrif^S indole⁺ V_{1-7}^R λ^R ρ^R λ^{370R} lys-for K-12

Wq 16



More

U.V. 10/5/51 Culture R (811) Exp I (1)

Diluted culture from — hour unaerated culture. 0.C. aerated — hours at 37C. Prob. titer 1.5×10^8. Diluted 1:10.0 with Penassy broth blank. 11 ml vol. Shelter set at 30 -

Dose (Sec.)	Dilutions	Plates	Approx Col. Count	Titer	Survival
✓ 0	100, 10 →	R-0-4	300	3×10^7	1.0
✓ 20	100 →	R-20-3			
	↘ 10 →	R-20-4	Over 400	4×10^6	0.133
✓ 40	100 →	R-40-3			
	↘ 10 →	R-40-4	400	4×10^6	0.133
✓ 60	100 →	R-60-3			
	↘ 10 →	R-60-4	30 *	3×10^6	0.10
✓ 80	100 →	R-80-3			
	↘ 10 →	R-80-3			
	↘ 10 →	R-80-4	180 *	1.8×10^6	0.06
✓ 100	10 →	R-100-2			
	↘ 10 →	R-100-3			
	↘ 10 →	R-100-4	10 *	1.0×10^6	0.03

* one lysed colony -

More (2)

U.V. 10/5/51 Culture S (518) Expt I

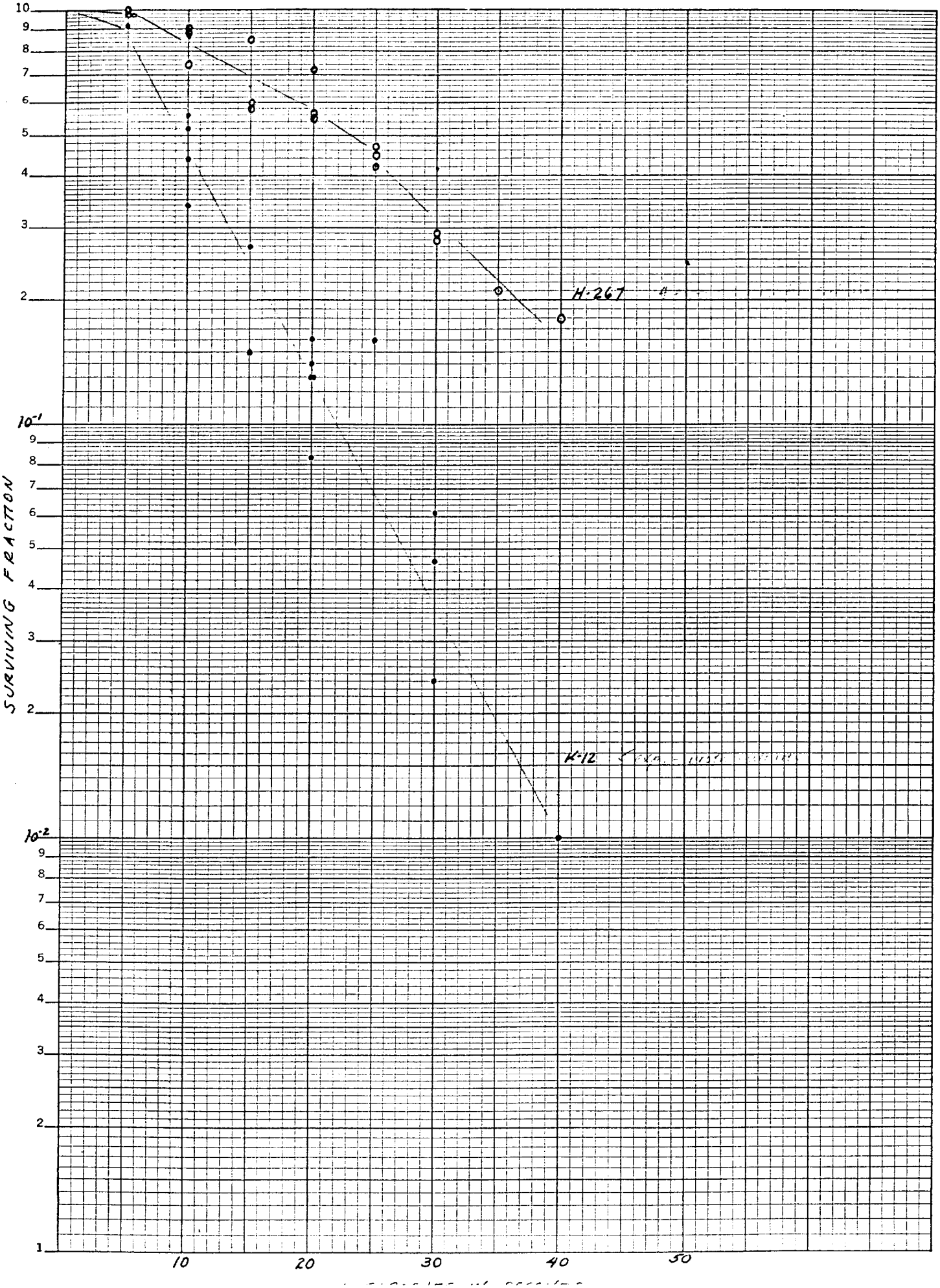
Diurnal culture from ____ hour macerated culture. 1.C aerated ____ hours at 37°C. Prod. Titer 1.5×10⁸. Diluted 1+100 with Penassay Broth - final vol. 11 ml volume shaken @ 30

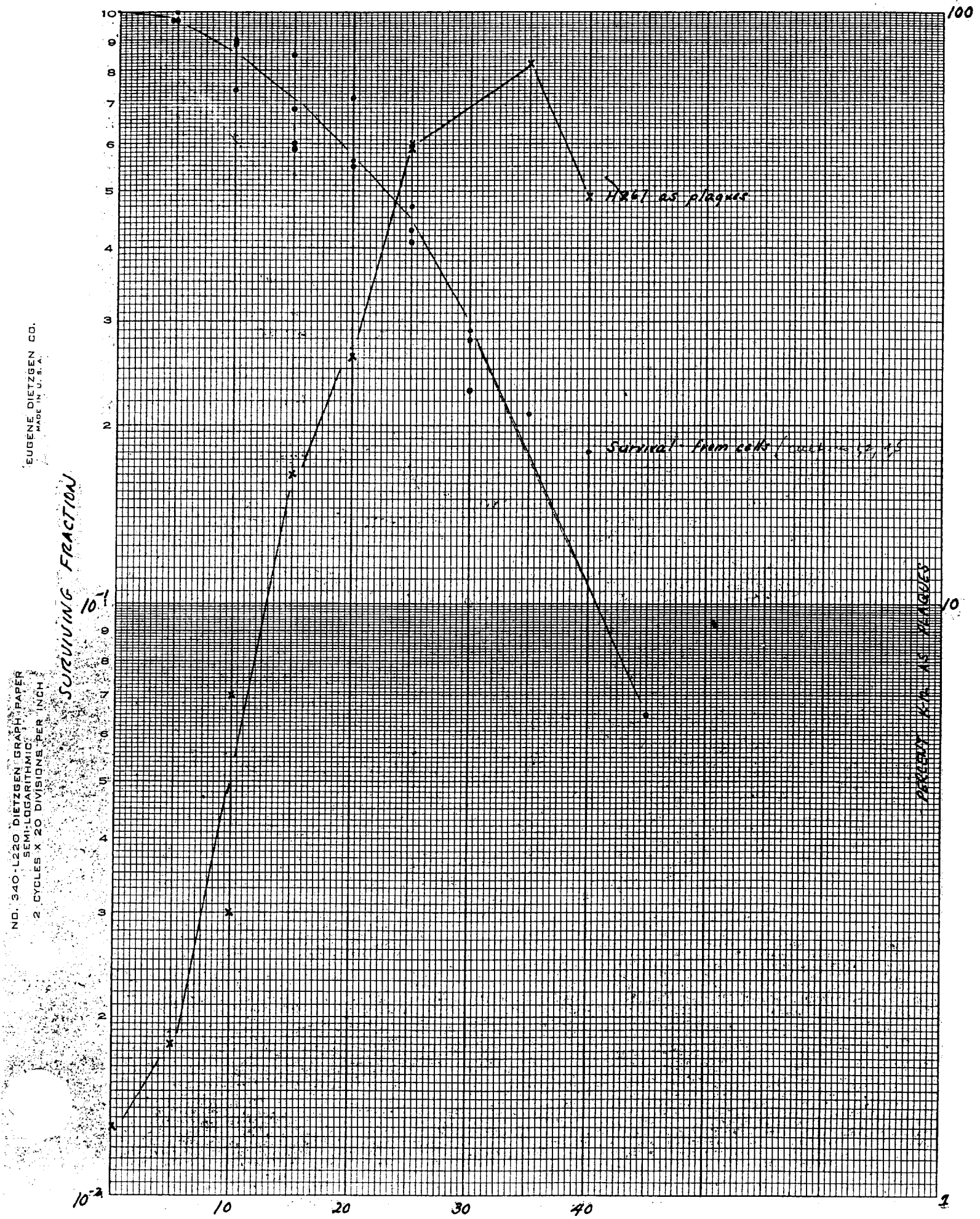
Dose (in Seconds)	Dilutions	Plates	Approx. Col. Count	Titer	Survival
1×10⁶ √0	100, 10	→ S-0-4*	500	5×10⁶	1.0
√20	100	→ S-20-3	~~400~~		
	↘ 10	→ S-20-4	400	4×10⁶	0.8
√40	100	→ S-40-3			
	↘ 10	→ S-40-4	900	9×10⁶	0.8
√60	10	→ S-60-2			
	↘ 10	→ S-60-3	300	3×10⁶	0.6
√80	10	→ S-80-2			
	↘ 10	→ S-80-3	2500	2.5×10⁶	0.5
√100	10	→ S-100-2			
	10	→ S-100-3	200	2.0×10⁶	0.4

* dilution = 10^4

S-20-4

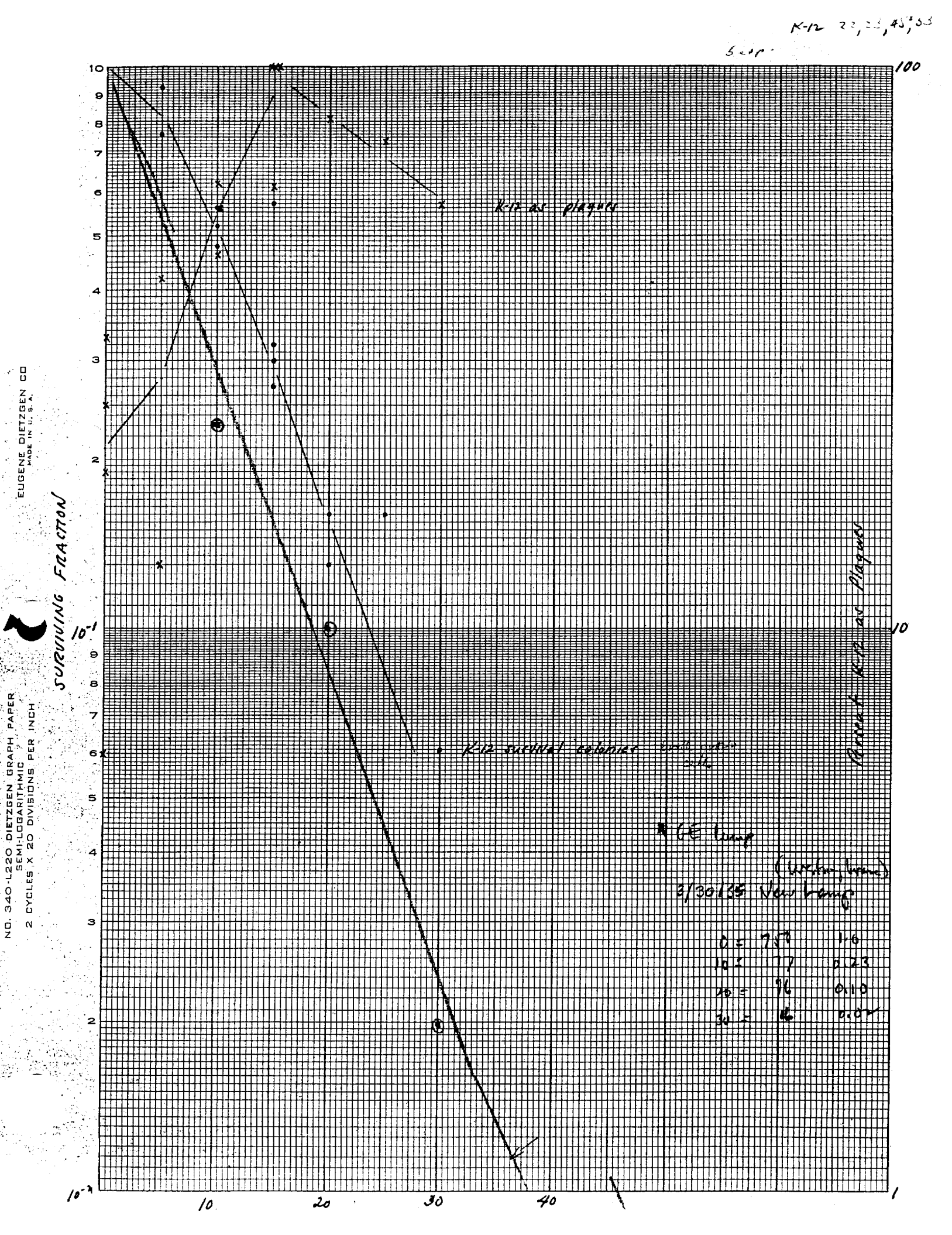
S = sensitive; 20 = exposure seconds; 4 = dilution

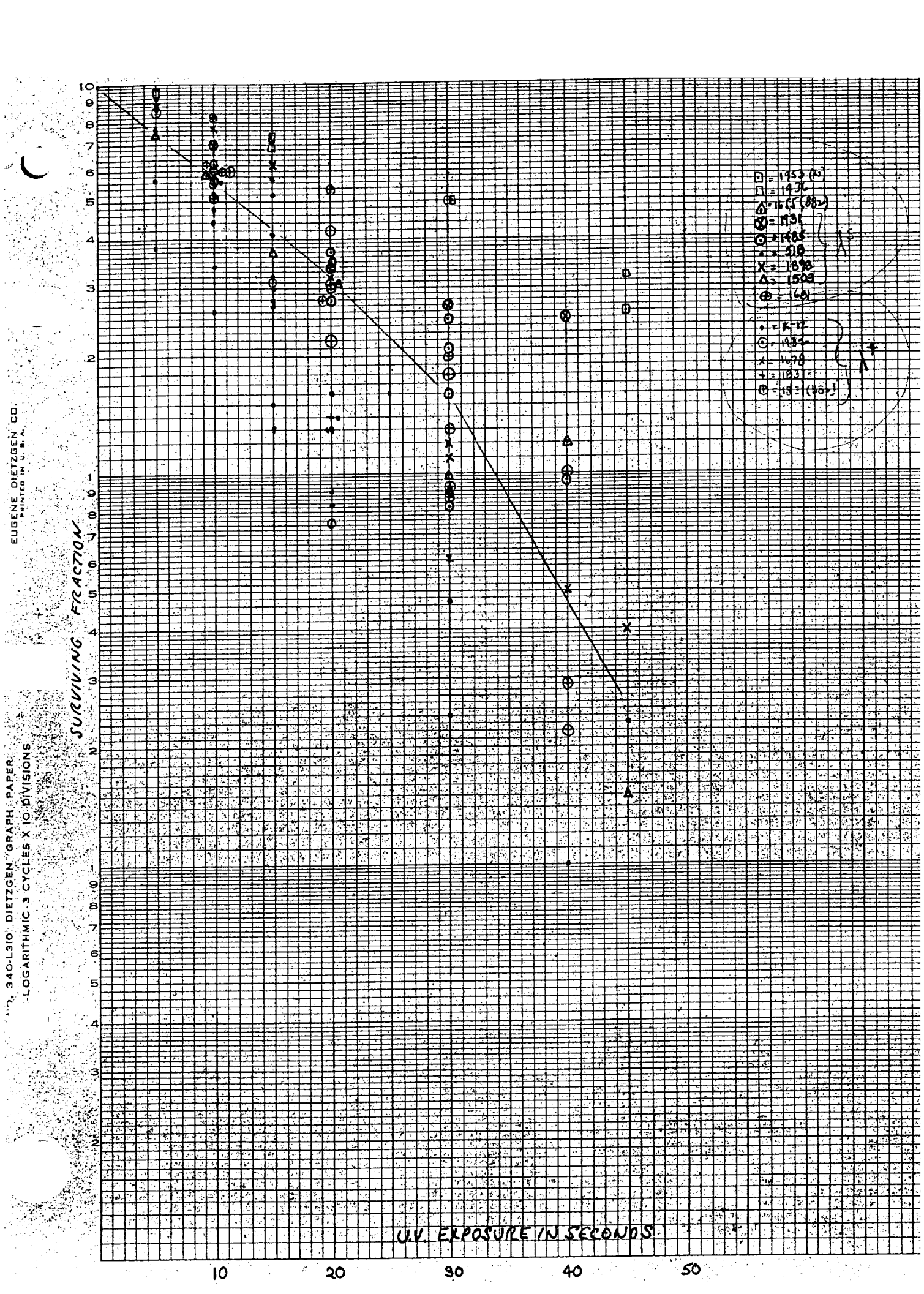




K-12 23, 25, 43, 53

Surv.





Irradiation - U.V - Exp II

(6) Morse 12/26/51

10 ml fresh culture (2 hours from overnight unaerated) diluted 1-10 in W-D buffer after centrifugation and resuspension in 10 ml W-D buffer.

Culture	Dose (sec)	Dilutions (0.1 ml plated)	Count (on EMB-Lac)	D.F.	Irrad. titer	Surviving Fraction (titer ÷ diluted 1-10 before irradiation)
A (W-1655)	0	10^{-6}	174	10^{-7}	1.74×10^8	
B (58-161)	0	10^{-6}	174	10^{-7}	1.74×10^8	
C (W-1681	0	10^{-6}	92	10^{-7}	9.2×10^7	
D (W-1682)	0	10^{-6}	102	10^{-7}	10.2×10^8	
A	60	10^{-4}	995 (2 EMB plates)	10^{-5}	9.95×10^7	5.7×10^{-1}
B	60	10^{-3}	57 (5 EMB-0)	10^{-4}	5.7×10^5	3.2×10^{-3}
C	60	10^{-3}	1937	10^{-4}	1.937×10^7	0.21×10^0 ± 2.1×10^{-1}
D	60	10^{-3}	1081	10^{-4}	1.081×10^7	1.0×10^{-1}

(7) More

Exp III 12/27/51

Stock suspension from Exp II used.
Diluted 1-10 ē $PO_4^{=}$ W-B before Irrad.

Culture	Dose	Dilutions	D.F. (almost plated)	Counts (all on EMB-lac-)	Titer	Irrad Titer (1-11)	S.F.
A	0	1st dilution with 1 drop Stock suspension 2, 4, (6)	10^7	202	2.0×10^9	2.0×10^8	
B	0	2, 4, (6)	10^7	304	3.0×10^9	—	
C	0	2, 4, (6)	10^7	77	7.7×10^8	—	
D	0	2, 4, (6)	10^7	69	6.9×10^8	—	
A	100 secs.	2, (3), (5) 6W	10^6	3	3.3×10^6		$\frac{3.3 \times 10^6}{2 \times 10^8} = 1.6 \times 10^{-2}$ (0.704)
B	100	1, (3), (3) 2W	10^5	93			
C	100	1, (2), (4) 4W	10^3	—			
D	100	1, (2), (4) 8W	10^4	—			
			10^6	—			
			10^4	—			
			10^5	—			
			10^4	—			

no colonies

Exp II (8) Mice
12/20/51

Stock suspension from Exp II used
Diluted with PO$_4$ = W-D 1-10 before inoc.

Culture	Pos		Dilutions	(0.1ml/plate) D.F.	EMB Lac Count	Titer	Tyrod. titer (-10)	S.F.
A W-1685	0	Stock susp 1st dilution into tryptone broth	2, 4, (6)	10^7	111	1.1×10^9	1.1×10^8	
B 58-1684	0		2, 4, (6)	10^7	198	1.9×10^9	1.9×10^8	
C W-1681	0		2, 4, (6)	10^7	69	6.9×10^8	6.9×10^7	
D W-1687	0		2, 4, (6)	10^7	62	6.2×10^8	6.2×10^7	
A	80 sec.		2, (4)(5)	10^5	81	8.1×10^6	—	7.3×10^{-2}
B	80		(1)(2)(3)	10^2	142	1.4×10^4	—	7.4×10^{-6}
C	80		(2)(3)(4)	10^5	7.3	7.3×10^6	>	1.1×10^{-1}
D	80		(2)(3), (4)	10^5	52	5.2×10^6	—	8.4×10^{-2}

Lac
Colonies picked and streaked EMB lac
cultures purified and coded
in stocks 180.5
180.6
180.7

58-161

Exp V (4) [illegible]

Dose	Phage Titer/ml $\times 10^6$	Viable bacteria/ml	Phage / viable cell	Phage / killed cell
0	66	1.8×10^8	$\frac{6.6 \times 10^7}{1.8 \times 10^8} = 3.1 \times 10^{-1}$	—
20	61	3.5×10^7	$\frac{6.1 \times 10^7}{3.5 \times 10^7} =$	—
40	109	4.6×10^6	$\frac{10.9 \times 10^7}{4.6 \times 10^6} =$	4.5×10^7
60	73	3.6×10^5	$\frac{7.3 \times 10^7}{3.6 \times 10^5} =$	
80	46	1.0×10^4	$\frac{4.6 \times 10^7}{1.0 \times 10^4} =$	
100	6	1.2×10^3	$\frac{6 \times 10^6}{1.2 \times 10^3} =$	

(10) More

12/29/31 Exp II

→ 58-161

Survival of B- Suspensions of Exp II

Diluted with PO4 = W-D. 1-10 before Irrad.

Dose	Dilutions	0.1 diluted D.F.	EMB Plate Counts	Irrad. titer	S.F.
0	2, 4, (6)	10^7	176	1.8×10^8	—
~~10~~					
20	2, (4), (5), (6)	10^6	83	3.5×10^7	2.0×10^{-1}
~~30~~					
40	(2), (3), (4), (5)	10^5	46	4.6×10^6	2.6×10^{-2}
~~50~~					
60	(3), (3), (4)	10^4	3.6	3.6×10^5	2.0×10^{-3}
~~70~~					
80	(1), (2), (3)	10^2	101	1.0×10^4	6.1×10^{-5}
~~90~~					
100	4, (1)	10^{11}	253		
		10^2	12	1.2×10^3	6.6×10^{-6}

(A) W-1655 λ (B) 58-161 λ and Luroff Effect (II) Virions

Exp VI

New stock slop. prepared 1/2/52 from overnight unaerated y.e. broth culture by resusp. in W-D PO4 ≡ - Before Irrad. dil 1-10 ≡ W-D PO4 ≡

	Dose	Dilutions	Counts EMB loc 0.1ml	Titer	S.F.
	0	1, 2, (4) →	~~7~~	4X10⁵	1
(B) 58-161	20	1, 2, (3) →	208	2.1X10⁶	1.3X10¹
	40	1, (2) →	349	3.5X10⁵	2.5X10⁻¹
	60	(1) →	435	4.4X10⁴	3.1X10⁻²
	80	(1) →	167	1.6X10⁹	1.1X10⁻²

(1) dilutions made into TSB - incubated 40 minutes then diluted and plated - Keeping 0 dose

(A) W-1655	0	(1) 2, (4) →	4	4X10⁵	1.0
	20	1, 2, (4) →	28	2.8X10⁶	
	40	1, 2, (3) →	~~164~~	1.6X10⁶	1.0
	60	1, 2, (3) →	122	1.2X10⁶	6.6X10⁻¹
	80	1, 2, (3) →	~~20~~ 141	~~2.0X10⁵~~ 1.4X10⁵	1.0X10⁻¹

4/2/52 (12) Mimm
Exp VIII

Radiation Sensitivity of $\lambda^{+/-}$

Fresh suspensions - Made from overnight un aerated broth cultures resuspended in W-0 PO4 - Plated 1-1H before irradiation with W-0 PO4

Pair			Count EMB-Lac (0.1ml)	T.Ter	S.F.
E W-1485 λ^s F K-12 λ^+	E -0	2, (4) →	122	1.2×10^7	
	E -80	(2) (3) →	20	2.0×10^5	1.1×10^{-2}
		→	77	7.7×10^4	$>1.4\times10^5$
V	F -0	2, 3 (4) →	261	2.7×10^7	
	F-80	un (3) →	6	6.0×10^1	2.2×10^{-6}
G W1673 H W-1678 λ^+	G-0	2, (4) →	82	8.2×10^6	
	G-80	2, (2) →	74	7.4×10^4	9.0×10^{-3}
	H-0	2, 3 (4) →			
	H-80	un, 1 →			

no colonies - cells kept out? too few?

J-W-1177 λ^+ K-W-1603 λ^-	J-0	2, (4) →	not done insufficient time
	J-80	un, 1 →	
	K-0	2, (4) →	
	K-80	2, (1) →	

Exp VIII-A (13) more 1/3/52

Radiation Sensitivity and Turn Off Effect in W-1177 λ+ (J) and W-1603 λ− (resistant) (K)

Stock suspension from Exp VIII diluted 1-100 c W-0 PO4 ≡

Strain				Titer/ml	Corrected Titer	S.F.
J	J-0	in TSB 1, 3, 4 →	68	6.8×10^6	6.8×10^6	1−
W-1177 λ+		→ ca 500				
	J-60 immed. plat.	und, 1, 2 →	39	3.9×10^4	3.9×10^4	5.7×10^{-3}
	1.0 ml + 10 ml Pan + YX Broth - Incub. 40 min @ 37 in WB	und, 1, 2 →	27	2.7×10^3	2.7×10^4	3.9×10^{-3}
		→ dil 10^3		plaques 142	titer/ml 1.4×10^5	from 6.8×10^6 titer/cell
		10^4		16	1.6×10^5	2.7×10^4 viable cell
K	K-0	in TSB 1, 3, 4 →	73	7.3×10^6	7.3×10^6	~~8.7×10^{-2}~~
W-1603 λ−		→				
	K-60 immed. plating	1, 3 →	64	6.9×10^5	6.4×10^5	8.7×10^{-2}
	K-60 1.0 ml + 10 ml Pan + YX broth Incubation 40 m @ 37 WB	2, 3 →	79	7.9×10^4	7.9×10^5	1.1×10^{-1}

Repeat of Exp. II (6) Mouse 1 (51 52) (15)

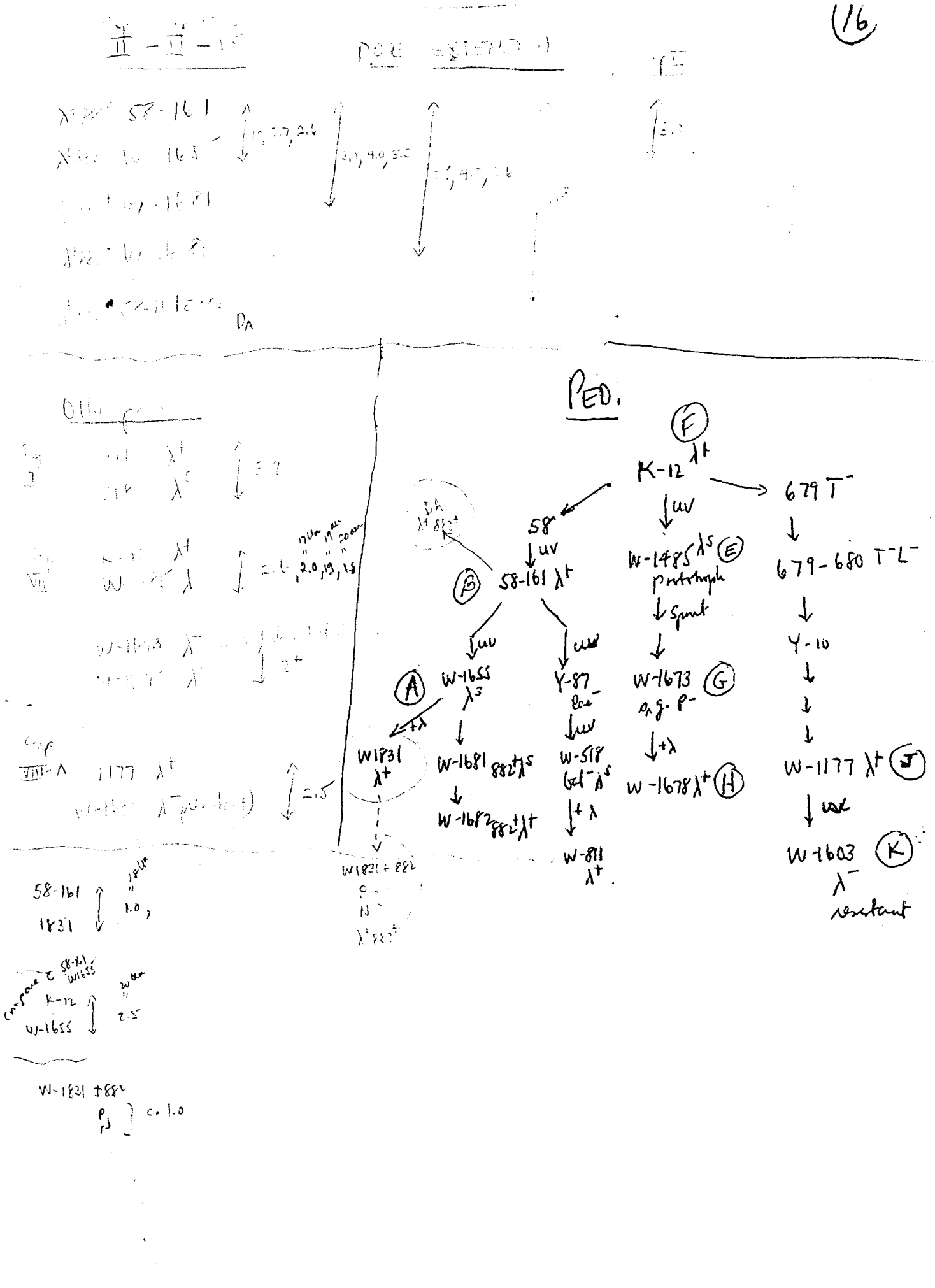
10 ml culture of each (A, B, C, D, D_A) centrifuged and resusp. in W-O PO$_4$ = Diluted 1-100 = W-O PO$_4$ = before irrad. (ca 10^6 cells/ml)

Diluted 1.0 ml + 10 ml Pen + FX - Irr. 40 minutes @ 37C

Culture	Dose	Dilution	0.1 ml on EMB	Titer	S.F.	
A (W-1555)	0	1, (3) →	620	6.2×10^6	—	
B (58-161)	0	1, (3) →	768	7.7×10^6	—	
C (W-1681)	0	1, (3) →	821	8.2×10^6	—	
D (W-1682)	0	1, (3) →	919	9.1×10^6	—	
D_A (58-161 c 82)	0	1, (3) → ↑ TSB	486	4.9×10^6	—	
		These diluted 1-10 further than above because of nitrosation				
A	~~40~~	(1) → (2) (3) →	147	1.5×10^6 / 1.1×10^6 1.3	$\frac{1.3 \times 10^6}{6.2 \times 10^6}$	$= 2.9 \times 10^{-1}$
			11			
B	~~40~~	(1) (2) →	48	1.8×10^5	$\frac{4.8 \times 10^5}{7.7 \times 10^6}$	6.2×10^{-2}
C	~~40~~	2 (3) →	35	3.5×10^6	$\frac{3.6 \times 10^6}{8.2 \times 10^6}$	4.4×10^{-1} —
D	~~40~~	(2) (3) →	28	2.8×10^6 / 2.5×10^6 2.7	$\frac{2.7 \times 10^6}{9.1 \times 10^6}$	2.9×10^{-1}
		→	246			
D_A	40	(1) (2) (3) →	6	6×10^5 / 5×10^5 5.5	$\frac{5.5 \times 10^5}{4.9 \times 10^6}$	1.1×10^{-1}
		→	50			

dilution for growth but considered in S.F.

10^6/ml → 1, 3 →


PED.
K-12 λ+
679 T⁻
679-680 T⁻L⁻
58
58-161 λ+
W-1485 λs (E)
protrophs
spmt
W-1673 (G)
W-1678 λ+ (H)
Y-10
W-1177 λ+ (J)
W-1603 (K)
λ⁻
resistant
(A)
W-1655
λs
W1831
λ+
W-1681
W-1682
Y-87
W-518
λ+
W-811
λ+
(B)
uv
+λ
spmt

(F) = (E) = 2/5/51 (17)

Comparison of K-12 1st and 1485 1s

Unaerated broth cultures - centrifuge and resuspend in W-0 buffer. Dilute 1-100 with W-0 Buffer before inoculation - Make last dilution into P-X broth-inoculate this dilution tube for 40 minutes sample.

Expected Survival of each

Dose	K-12 Inc	K-12 Unirac	1485 Inc	1485 Unirac
0	10^8	10^7	10^8	10^7
20	10^5	10^6	5×10^6	5×10^6
40	10^3	10^4	4^5	10^6
60	10^2	10^3	5×10^4	5×10^5
80	0	10	10^4	10^5

K-12	Dose	Charact	Dilutions	Plate	Count	
	0	unincubated	2, 4 → 0.1 ml	F-0-4	80	$\times10^5$
✓		incubated	2, 4 "	F-0-4I	123	
	20	unincubated	2, 3 "	F-20-3	154	
✓		incubated	3 "	F-20-3I	206	$\times10^4$
	40	unincubated	2 "	F-40-2	141	
✓		incubated	2 "	F-40-2I	367	$\times10^3$
	60	unincubated	1 undil	F-60-1	781	$\times10^1$
✓		incubated	1 →	F-60-1I	58	$\times10^2$
	80	unincubated	und. →	F-80-1	41	$\times10^1$
		incubated	Δ →	F-80-1I	0	$\times10^2$

Surviving Fractions

K-12 Dose	Incubated S.F.	Unincubated S.F.	
0	1.0	1.0	✓
20	1.7×10^{-1}	1.9×10^{-1}	
40	3.1×10^{-2}	1.8×10^{-2}	
60	4.7×10^{-4}	9.6×10^{-4}	
80	0	5.1×10^{-5}	

W-1485

	0	unincubated	2, 4 →	E-0-4	160 $\times10^5$
		incubated	4 →	E-0-4I	229
	20	unincubated	2, 4 →	E-20-4	75 $\times10^5$
		incubated	4 →	E-20-4I	110
	40	unincubated	2, 3 →	E-40-3	290 $\times10^4$
✓		incubated	3 →	E-40-3I	353
	60	unincubated	2, 3 →	E-60-3	*193/2 = 97 $\times10^4$
		incubated	3 →	E-60-3I	140
	80	unincubated	2, 2 →	E-80-2	73 $\times10^3$
✓		incubated	2 →	E-80-2I	147

W-1485 Dose	Incubated S.F.	Unincubated S.F.
0	1.0	~~1~~
20	4.8×10^{-1}	4.7×10^{-1}
40	1.5×10^{-1}	1.8×10^{-1}
60	6.1×10^{-2}	6.1×10^{-2}
80	6.9×10^{-3}	4.6×10^{-3}

Many lysed cells + sectors of K-12, expect 40: 1 col = lot 1872

* 0.2 ml Plated

2/7/52

(18)

Comparison of U.V. resistance of 58-161 = A ○
W-1655 = B ○
W 1831 = C ×

24 hour unaerated cultures used - centrifuged and resuspended in W-10 buffer - diluted 1-10 with W-0 buffer before irradiation

Expected			Experimental			EHB Plate	Counts	Titr	S.F.
Cult.	Dose	Survival	Cult	Dose	Dilutions*				
A	0	$1.0 = 10^7$ cells/ml	A	0	2, 4	A-0-4	201	2.0×10^7	1.0
	20	$0.1 - 10^6$ cells/ml		20	2, 3	A-20-3	579	5.8×10^6	2.9×10^{-1}
	40	$0.01 - 10^5$ cells/ml		40	2	A-40-2	56	5.6×10^4	2.7×10^{-3}
B	0	$1.0 - 10^7$ cells/ml	B	0	2, 4	B-0-4	142	1.4×10^7	1.0
	20	$0.5 - 5\times10^6$ cells/ml		20	2, 4	B-20-4	35	3.5×10^6	2.5×10^{-1}
	40	$0.1 - 0.5 = 10^6$ cells/ml		40	2, 3	B-40-3	237	2.4×10^6	1.7×10^{-1}
C	0	$1.0 = 10^7$ cells/ml	C	0	2, 4	C-0-4	102	1.0×10^7	1.0
	20	$0.1 - 10^6$ cells/ml		20	2, 3, 4	C-20-3	182	1.8×10^6	1.8×10^{-1}
						C-20-4	27		
	40	$0.1 - 10^6$ cells/ml							
		$0.01 - 10^5$ cells/ml		40	2, 3	C-40-3	22		
						C-40-2	255	2.5×10^5	2.5×10^{-2}

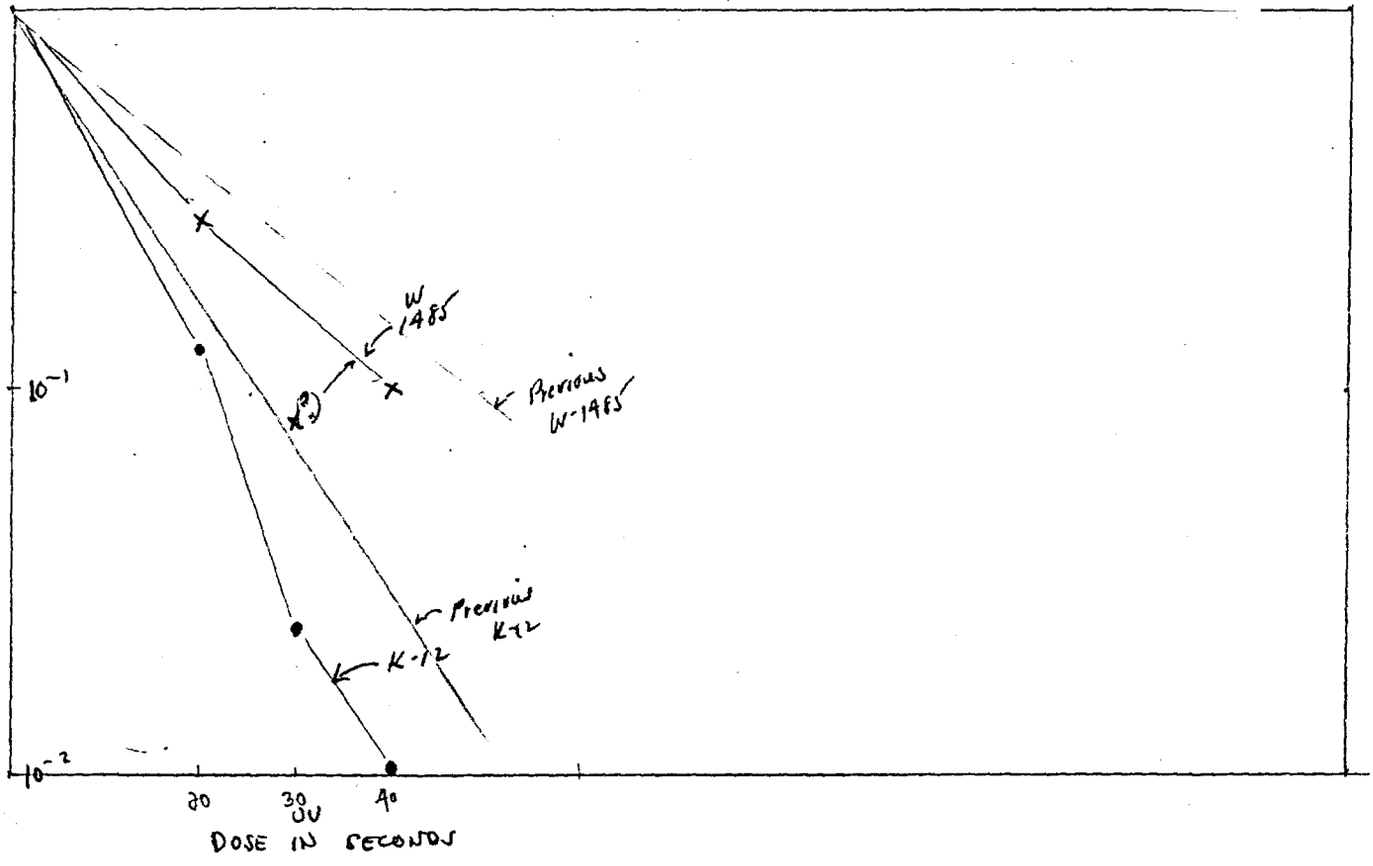
*water

Comparison of K-12 and W1485 —

Aerated cultures = 0.1 ml 24 hour unaerated culture + 10 ml PX broth incubated in air 37C 4½ hours — Centrifuged and resuspended in W-0-buffer-diluted 1-100 before irradiation

K_{12} Dose	Expected Titer	Dilutions	EMB Plates	Counts	Titer	S.F.	
0	1×10^7	2, ④ →	A-0-4	229	2.3×10^7	1.0	4/229
20	1×10^6	2 ③ →	A-20-3	30	3.0×10^6	1.3×10^{-1}	92/229
30	5×10^5	2, ③ →	A-30-2	~	~		
			A-30-3	56	5.6×10^5	2.4×10^{-2}	data obtained from counts prepared in parallel for the two points
40	1×10^5	2 ③ →	A-40-2	238	2.4×10^5	1.0×10^{-2}	83,17/229
			A-40-3	21	—		71/229

W1485						
0	1×10^7	2 ④ →	B-0-4	277	2.8×10^7	1.0
20	5×10^6	2, ④ →	B-20-3	~		
			B-20-4	80	8.0×10^6	2.8×10^{-1}
30	2×10^6	2 ③ →	B-30-3	247	2.5×10^6	8.2×10^{-2}
40	1×10^6	2, ③ →	B-40-3	267	2.7×10^6	9.6×10^{-2}



K W 2/10/52.

Comparison of K-12 and 1485

Cultures from overnight unaerated PX broth by 0.1 + 10.0 and aerated 5 hours. Centrifuge and resuspended in W-D buffer. Dilute 10^6 = ca 10^3 cells/ml. Sampled immediately for O

2, 4, C

K-12	Dose	Plate	Count/plate	Count/ml	Phages	S.F
	0	K0 -	446	4.5×10^8	0	1.0
	10	K-10 -	195	2.0×10^8		4.4×10^{-1}
	20	K-20 -	62	6.2×10^7	No	1.4×10^{-1}
	30	K 30 -	21	2.1×10^7	phage	4.7×10^{-2}

Only indicator

W 1485	Dose	Plate	Count/plate	Count/ml		
	0	W-0	626	6.3×10^8	Contaminated	1.0
	10	W-10	392	3.9×10^8	and	6.2×10^{-1}
	20	W-20	234	2.3×10^8	Carrying	3.7×10^{-1}
	30	W-30	129	1.3×10^8	phage	2.1×10^{-1}

Little lysis. Clear usual

Examination of W-1655 = C

Unaerated culture - 30 hours centrifuge resuspend in W-D - dilute 10^6 - ca 10^3 cells/ml

Dose	Plate	Counts/plate	Count/ml	
0	C-0	175	1.8×10^8	1.0
5 ~~C-5~~	~~mutt~~			
10	C-10	138	1.4×10^8	7.8×10^{-1}
20	C-20	92	9.2×10^7	5.1×10^{-1}
30	C-30	64	6.4×10^7	3.6×10^{-1}
40	C-40	56	5.6×10^7	3.1×10^{-1}

Use dilutions of W-1661 in W-1662 at 9.5, etc

(21)

Examination of W-1655

Uncreated culture - 28 hours PX - Centrifuge, resusp in W-0. buffer - dilute 10^6 - Inoculate

Dose	Plate	Counts
0	W-0 -	?
~~35~~	W-5 -	77
10	W-10	70
15	W-15	60
20	W-20	43
30	W-30	58
40	W-40	-
50	W-50	-

dead exp
fresh plates repeat

1831 + 882 -

Four ~~plaques~~ picked (central growth) and streaked

3 grew -

1482
1837 } to date - single colonies in original EMB last picked - two sets 40 in 1st
16 in 2nd

one possible diploid in 1st 40, more in 2nd

Possible streaked on EMS and EMB - indicated dip.

Three colonies of EMS dip picked to EMB, inoculated in DM+ lactose (1:0 ml/10ml 10%)

aerated 10:15 2/19/52

just probable

Comparison of K-12 (K) with 1485 (W)

2/13/52

4 hour aerated culture from overnight incubated - dilute to contain 10^3 cells/ml - Incubate - Plating for phage on S18

K-12 as Plaque	K-12	Dose	Count/plate	Aff.	Count/ml	Phage/ml	S.F.
0.25		0	278		2.8×10^3	70	40
0.80		10	156	122	1.6×10^3	$\frac{890}{2} = 1560$	5.6×10^{-1}
0.8		20	37	241	3.7×10^2	2240	1.3×10^{-1}
0.57		30	17	261	1.7×10^2	1570	6.1×10^{-2}

1485	0	187	1.9×10^3	S.F. 1.0
	10	106	1.1×10^3	5.7×10^{-1}
	20	66	6.6×10^2	3.5×10^{-1}
	30	30	3.0×10^2	1.6×10^{-1}

Ratio cells/ml / plaque forming — Dose

$\frac{122}{156} = 0.79$ — 0-10

$\frac{241}{224} = 1.1$ — 0-20

$\frac{261}{157} = 1.7$ — 0-30

Culture started PX broth

WB-1] project X → 0.25 + 10 ml PX(1-16) 9:15

W1-14, W1-16] for Penicillin Run → 0.25 + 10 ml PX 9:15

K-12, 1485] possible retake on above exp.

2/14/52 (23)

Comparison of K-12 and W-1485

Lawoff Effect — 4 hr aerated cultures in PX from overnight unaerated cultures

K-12

Dose	Plate	Colonies/plate	diff.
0	K-0	250	18
5	K-5	232	120
10	K-10	130	182
15	K-15	68	210
20	K-20	40	210
25	K-25	41	

/ml		Plaque/plate		diff	S.F.	K-12 (no plaques)
2.5×10^3	0	-	<50		0 = 1.0	
2.3×10^3	5	105	319/3 = 105	105 - <55	5 = 9.2×10^{-1}	<25
1.3×10^3	10	155	155	105	10 = 5.2×10^{-1}	.42
6.8×10^2	15	230	360	205	15 = 2.7×10^{-1}	.62
4.0×10^2	20			134	20 = 1.6×10^{-1}	1.07
4.1×10^2	25	184	184		25 = 1.6×10^{-1}	0.74 ?

Ratio: Cell loss diff. / Plaque gain diff.	Dose	
	0-5	$\frac{18}{<55} = >0.32$
	0-10	$\frac{120}{105} = 1.1$
	0-15	$\frac{182}{205} = 0.89$
	0-25	$\frac{210}{134} = 1.6$

W-1985

Dose	Plate	Colonies/plate	Count/ml	S.F.
0	W-0	251	2.5×10^3	1.0
10	W-10	153	1.5×10^3	6.0×10^{-1}
20	W-20	75	7.5×10^2	3.0×10^{-1}
30	W-30	62	6.2×10^2	2.5×10^{-1}

Cultures

- K-12
- W-1485
- Wq-19
- Wq-16
- WB-1

} are last-tipped ones in incubator

Cultures made - 2/15/52

PX { K-12 - repeat ē incubation
1831 - examine 1831 + FP2 for lysogenicity ē FP2
WB-1 - pyocin X.

Sat. 2/16/52 IC-12 aerated culture started 7:50 out at 9:50 0.5+10 $\frac{1}{20}$ [illegible]
WB-1 " " - 7:55 - no lysis 11:10
refrigerat until 2/18/

Effect of Post Incubation

K-12 - Centrifuge - resusp in W-D - Dilute to 10^4 viable cells/ml - Post incub. incub. 40 min at 37C

Area	Plate	Counts/plate
0	K-0 -	58
	K-0 - Inc	140
15 sec	K-15	25
	K-15 - Inc	20

2/17/52

Lysed WB-1 tube plated at - TSA

10^2 -

10^4 -

10^6 -

Culture started 2/17/52

IC-12

1831

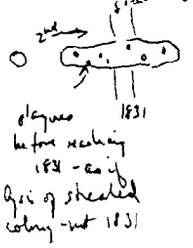
WB-1

WB-1 culture continued at 37C

in 10:00 AM

out 11:05 AM lysis apparently beginning.

20 colonies of 1831 + 882 (from plaques) streaked across 1831 for lysogenicity ē 882 made 2/16/52 examined - no clear cut examples of sensitivity of 1831 to any of streaks noted One streak contained small plaques



Cultures 2/18/52
Started

K-12
1831
1485

2/19/52

Aerated cultures started K-12, 1485 - at 8:15 - 0.25 + 10 cc PX
aerated overnight
not 12:15

Results 2/20/52

1831 + 882

Z = 1831
W = 1485
P = plaque forming - see bottom of L4
N = non-plaque forming ⊙

N gave plaques on at resp.
lysate 1485 = $N^{\lambda+}$
lysate 1831 = N^{882+}

P gave no plaques on resp.
lysate 1485 = $P^{\lambda+}$
lysate 1831 = P^{882+}

K-12 - Effect of post-incubation on Survival

Incubate - aerated 4:30 hours in PX - Centrifuge - resuspend in WO* dilute ~ W-O to contain 10^8 cells/ml - After incub. 1/10 dilution with PX broth - Incubated at 37C ~ air 40 min

Dose	Plate	Count/Plate		S.F.
0	K-0	281	-276	1.0
	K-0-I	270		
10	K-10	84		5.2×10^{-1}
	K-10-I	185		6.7×10^{-1}
15	K-15	42		1.5×10^{-1}
	K-15-I	117		4.2×10^{-1}
20	K-20	23		8.3×10^{-2}
	K-20-I	58		2.1×10^{-1}

Suggest that post incubation in aeration deactivates lysogenic effect.

I = post Incubated counts.

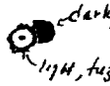
* remainder of this resp. was incubated 25 sec, ca 8 ml PX added and incubated ~ aeration
2:00 PM - 4:00 PM - slight lysis apparent.
left on bench overnight - nearly clear next morning - centrifuged and assayed plaque / 455 -
titer: 10^6 - 43 = ca $8.6 \times 10^5 \times 10 = 8.6 \times 10^8$

Cultures started 2/18/52
K-12
1485
1831

K-12 aerated culture (0.5 + 10 ml PX) began 10:45 -

Main culture of K-12 inoculated 2/19/52 centrifuged 20 min.
supernatant retained and assayed for λ - See previous page yield = ca 10^9

1831 + 832 of previous page -
With N and P picked to water - streaked out on EMB-0 for colony isolation - o.K.

Colony inhibition? 

forms

Series of each picked to PX - incubated - ~~5 hrs~~ ~~5 hrs~~ $5 \cdot 10^7$/ml dilute each 2, 4 → A + B
→ A - ca 300 colonies ± 10 type B
→ B - ca 300-900 colonies ± 5·10 type A.

K-12 Post irradiation effect

Culture aerated 2 hours - Centrifuged - resusp. in W-10 -
Dilute to 10^8/ml - Irrad. dilute both in PX - Incubate ± aeration 40 min.

Dose	Plate	Counts
0	K-0	- 26
	K-0-I	- 98
10	K-10	- 35
	K-10-I	- 54
15	K-15	- 17
	K-15-I	- 28 ++
20	K-20	- 7
	K-20-I	- 18

survival ~~high~~ high ca 2.5×10^{-1} at 20 sec should be ca 10^{-1}
Indicates no effect of post incubation on survival - colony survival

mc 1:25

I = incubated

Cells remaining from above (ca 10^9/ml) irradiated 20 sec
10 ml PX added to 10 ml cells - incubated with aer
in : 1:30
out : 4:00 - partially cleared

Cultures started 2/20/52
K-12
W-1988

2/21

10 colonies from N clones and P clones of 1831 + 882 picked and cultures streaked with 1831 to obs. lys due to 882 — All appeared to give 1831 single colonies picked and restreaked 2/22

Cultures started
K-12
1481
1831

K-12 Forms {A, B} - 6 colonies picked of each - streaked for examination of purity. Culture in PX of each started. — Appeared to be pure on 2/22

2/22 — The day of the stock value —

Cultures started K-12, 1831, H05

2/23 Cultures of Wg 14 and Wg 16 started in PX from culture of 2/14 - 0.5 ml + 10 ml PX at 8:45 - out at 10:45 - centrifuged - resuspended in saline - centrifuged and resuspended in saline - Wg-14 - dil 1-10 add 1.0 ml to DO + 0.3 ml P-n + 0.1 ml Pr; DO + 0.1 ml Pr; DO

Wg-16 - W - 1-100 (mixed cult.) add 1.0 ml to DO + 0.3 ml Pn.; DO

	Growth 2/24
DO + 0.3 ml P-n + 0.1 ml Pr	—
DO + 0.1 ml Pr	+
DO	+
DO + 0.3 ml Pn.	—
DO	+

GROWTH HERE MAY MEAN Wg-14 HAS REVERTED

1831 + 882 - 10 colonies of P + N of restreaking 2/22 - on 2/24 A colony of each of the strains picked to broth - Streaks of 2/23 indicate all 10 clones sensitive

Diplorid 1432 · 1482 DM cultures streaked on EMS and EMB - 2/25 - segregation - EMB,

Yeast parasite

2/24 Cultures started
K-12, 1831, 1831 + 882-P, 1831 + 882-N

2/24 - Wq 14 and Wq 16 - See previous page

Penicillin tubes

Dilute - 0, 2, 4 - spread ~~control~~ Loop on ½ TSA plates

				No.	growth +T 24 hrs	growth added 2/27 2/28
Wq-14	0	2	colonies picked to DC	5	+	~
	2	0		6	+	~
	4	0				
Wq-16	0	3	" " "	1	0	0
	2	1		2	0	+
	4	0		3	0	0
				4	0	0

Penicillin tubes refrigerated

K-12 A Plates of growth tubes made 2/23
B

B → A in colonial form and coloring - slowly -

A — appears stable - contains a few B forms - original inoculum of growth tube contained 2-3% B forms

2/25/52

Penicillin survivors (?) transferred to D(0) - see previous page

K-12 plated out to isolate $\begin{pmatrix} A \\ B \end{pmatrix}$ forms - dilute 10^6 - Plate 1. 343 ± 81A = 0.24A
2. 279 ± 54A = 0.19A
3. 290 ± 53A = 0.18A

Cultures started for 4/26/52

[1681 + 882] N
[1681 + 882] P

K-12
1485

2/26/52 Aerated culture of P, N, K-12 started 7:55

Aerated culture of K-12 - $\frac{A}{B}$ started 8:15

Irradiation of P, N - Cultures - Centrifuge, washed W-D ca. dilute to contain $2 \cdot 10^7$ cells/cc

	Dose	Plates	Col/Plate	S.F.
P	0	P-0	224	1.0
	10	P-10	138	6.2×10^{-1}
	20	P-20	62	2.8×10^{-1}
	30	P-30	21	9.4×10^{-2}
	40	P-40	5	2.2×10^{-2}
N	0	N-0	382	1.0
	10	N-10	229	6.0×10^{-1}
	20	N-20	84	2.2×10^{-1}
	30	N-30	33	8.7×10^{-2}
	40	N-40	11	2.9×10^{-2}

dose extensions of about 1.0
From inspection of family tree the 1681 line may be considered and not representative & other isolates from 1655 — or since DA came from 5T-161 and also has Dw ect. 1.0, the order of establishing is important.

λ^{S882}
↙ ↓
λ^{S882^+} λ^{+882^S}
↓ ↓
λ^{+882^+} λ^{+882^+}
One ect. Rare one ect. 1.0

2/26/52

K-12 A + B forms - centrifuged and resuspended in M-9 Dilute to 10^8 cells/ml - Irradiated 15 sec -

Mix 1.0 ml + 1.0 ml 1960 spread 0.1 ml on TSA plate

A.	time	Plate	Cells/Plate	Phage/plate
	0	A-0	127 (SB/11A)	—
	15	A-15	53 (9B/11A) ✓	136 X 2 = 272

41
127 | 53.0
50.8
220

Mixture A and B applicated when resuspended in ca 9% NaCl

Indicates 200% yield - 2 plaques/1 cell - may possible be due to contam. of irradiation suspension with free phage - doesn't seem likely from standpoint that cells were sedimented and resuspended should be equivalent to 90% efficiency in plaque

B	time	Plate		
	0	B-0	200	—
	15	B-15	104	235 X 2 = 470 ✓

0.5 ml
200 | 1000
1035
1.0

K-12 Lwoff for high titered phage stock.

Irradiate conc. suspension - ca 10^9

35 seconds - Incubate 1 hr after adding 5.0 ml nadt 5.0 ml PX broth

in 11:45
cl. clearing 1:15
out at 3:30

titer $\neq 10^6 \cdot \frac{0.1}{} = 10^7$ X count.

no plaques > 3,000

titer $\neq 3.0 \times 10^{10}$

similarly, this would indicate that the original culture had a titer of about $\frac{272}{470} \times 10^7 = ca\ 3 \times 10^{22}$

Cultures started

K-12
1485
WB-1
WB-4

2/27/52

K-12 aerated cultures started - 11:15': (2 15ml)

K-12 A - } single colonies^(see 2/26) picked to PX to observe rate of A ⇄ B
B - } " " "

Cultures started
W-1655 → to begin $\delta\delta z^+ \to \lambda^+$
K-12
Wg-14
Wg-16

2/28 Wg-16 (If see last page) → see previous page
Of 4 worms surviving penicillin treatment.
one grew in synthetic - After 2 days
purine added - #2 grew - transferred to agar slant.

Aerated Cultures started K-12, Wg14, Wg16 - 8:30 AM
Wg14, Wg16 ↓ penicillinum

K-12A, K-12B } PX cultures of 2/27 plated out for examination of purity.
↳ 0.1 ml added to 10 ml PX = K-12A-2, K-12B-2 } serial transfer to observe rate of change
75B/250
3A/56

K-12 Lwoff - Viscous material. 11:55
Wash with Invad. 35 cc - add 10 ml PX - incubate with aer - noticed.
Segard in WD - 10⁸ cells

1. PX broth not viscous immediately after incubation without Antifoam
2. PX broth not viscous with Antifoam either

lysis - 1:15 - not as viscous as usual

Viscous material removed and tested with and without lysozyme (Hel) with Benedicts' - negative
tested with Stumpf DNA reaction - negative

			Plaques	
Titration of Lwoff-2	2, 9, 6, 7	0.1 →	337	= 3.4 × 10¹⁰
Lwoff-3 (resistant)	"	→	38	= 3.8 × 10⁹

Cultures Started
K-12
1485

3/29 K-12 transff #7 - Aerated cult. 2 hours (ca 10^9/ml) 2 cultures
Centrifuge - resusp'd in MD (15ml) - Inoculate 45 sec (more dense than usual) dilute cult 2 portions 1 in PX
1 in D(0)

Incubate 2-3 hours - Some clearing in both.

3/29 1655 + 1672 - plaques not large or centered with growth - growth not good on EMB-0. Incubation continued discarded as of no value

---

3/2 Titer of transff #.

Sym $10^6 \xrightarrow{0.1}$ 1 plaque = 1×10^7

PX $10^6 \xrightarrow{0.1}$ $177 \times 8 = 1416 \times 10^7 = 1.4 \times 10^{10}$

Jean

3/3 Aerated cultures of 1485, Wg-14, WB-1, WB-4 } Started 8:30

1655 + 882 on TSA - 0.1 ml pour (Stock labelled Agarose A) + 0.1 ml 1655

K-12 plated out for A + B for use

Aerated culture of 1485 11:45 AM - about 10^9 cells/ml
2.0 ml of A stock 3.4×10^{10} phage/ml added : $\frac{1}{17} \times 3.4 = 6 \times 10^8$ phage/ml
Dilute 10^6 plate 0.1 ml TSA - 313 = 3.1×10^9
0.1 ml + 1485 - 340 = 3.4×10^9

Wg-14 - Aerated until ca 10^8 cells/ml - Centrifuged resuspd in sal tubes added to D(A) incubated @ air 12:55 - out at 2:15 - Dilute 1-10 add 0.1 ml
P(0) + 0.3 Penn Sal — plated undil, 1-10 3/4
D(0) Sal — plated in Complete agar — 34 colonies
abs. 1.0 ml for resuspn of pptt —

WB-1 + WB-4
Turbid cultures at 12:45 Centrifuged and cells resuspended in D(A) - Incubation until aeration to steady degree

Closed at 2:00 PM
3/4

Cultures started -
K-12
1485
Wg 16
W 1655
~~WB-1~~
~~WB-4~~

3/5 Primary cultures of Wg 14 (originally mixed up with Wg 16) (55)

All cultures grew tryptophane - all are pro⁻ tryp⁻

all colonies from all labeled Wg16 are pro⁻ - 10 picked from each, 3 are in A_3 group - require added β alanine, tryptophane or tyrosine - all 3 required

Wg-14-1
Wg-14-3
Wg-14-4

Culture originally considered Wg16 pro⁻ was used as Wg14 stock culture and is proven pro⁻ 10 mic D(0) cultures of 3/3 plated on D(0) for reversion & pro⁺ large no colonies >5000 - large (pro⁺) and small (pro⁻) - large col picked to bind.

Aerated cultures of K-12 1915 started 10:30

1655 + 787 -

2 phage/cell mixtures picked and streaked on EMB Lac

K-12 A = 22/192
B = 118/192
} 45 colonies of each picked to BX - mic & ani 11:15 PM

Aerated culture of 1485 - 10 ml broth + (3 x 10^7) added from 1:30 PM culture - placed at room temp & ani

never cleared -

Don't understand

K-12A - centrifuged 2:30 resuspended in MD -

A dilute 1/5 - plate in mind for col EMP —— A-0 - 230 See earlier log

plate in mind 0.1 & 0.1 1485 for phage —— A-0 - 1

used 1Sec plate for phage " —— A-15 - 265

" " col EMB A-15 - 209

Suggestion that cells are clumped

B Same dilute as cultures used. —— B-0 - 270

phage before mixed. —— B-0 - 3

col EMB used —— - >1000

- 228

3/6

Colony from Wg-14 streaked on D(0) agar for reversion picked in broth.
D(0)

3/8 - single colony picked in broth

1655 + 882 - 1st picking → 2 streakings
↓
6 colonies picked from each and streaked on EMB -

Aerated cultures of K-12, 1488, Wg14, WB-1 (D(0)) at 9:00 ← clear on 3/7 3/8

Plating from Pen-2 of Wg14 replica to D(0) agar -

K-12 knife 35 sec.
Sue at 11:45
slight clearing of 1:15 - and 2:00 becoming turbid

Wg14 - washed - Aerated in D(0) 1:15

Dil 1-10
1 ml + 10 ml D(0) + 0.3 ml Pen -
1 ml + 10 ml D(0) -

3/7 SR-161 - 3 hours aerated culture - centrifuged and resuspd in WD
knife 35 sec. partial clearing after 2 hours -

3/F Rec tubes of W-16 plated TJA -
1-10 -
med -

58-161 thru
dil 10^6 0.1 → $900 \times 10^7 = (9 \times 10^9)$

Cultures
W-1655
W-19 prt

3/10

Streaks of W7-19 ~~[illegible]~~ Per mm² on TSA replica'd to D(0) + Pr for the purpose of detecting 2nd step mut. —— (10 failed to grow on D(0)+Pr replica)

PX

Stab culture W7 19 Prt from broth growth tube - PX ← D(0) agar ← D(0) broth ← heavy inoculated D(0)

W7-16 Per 1st mm -
undiluted col count = 18
1-10 dil = ca 250

1. undiluted replica'd to D(0)
2. 30 colonies of 1-10 dil picked, spotted on TSA

Aerated culture of ~~W~~ 5K-161 (W3110 ΔtrpA) and K-12 (W3110 ΔtrpA) started 1:30 PM
(1.0 ml + 10 ml PX)

Each 4:15 Centrifuged and resuspended 5 ml WD -
Read 35° sec - 5 ml PX added → aerated - 4:25 PM

Cleaning 6:00 PM
out 6:45

Cultures started -
K-12
5K-161
W1655
W1485

3/11

Wq-19 Pr⁻ — auxotrophs - picked from TSA plate to 2nd streaking on TSA for Replica -

---

Lwoff 58-161 L2 centrifuged
IC-12 CJ⁻
- dil 10^6 → plaques on 1485⁻ → $600 \times 10^7 = 6 \times 10^9$
dil 10^9 → colonies on EMB low → $15 \times 10^5 = 1.5 \times 10^6$
dil 10^6 → plaques on 1485 → $400 \times 10^7 = 4 \times 10^9$
dil 10^9 → ~~plaques~~ colonies on EMB low → 0×10^5

Wq-16 - Colonies picked from Pur survivor plate
5 failed to carry over to TSA spotting
- Plate replicated to D(6)

W1655 + 882 — Prep of 882 on 1661 lysate.

W1655 } Aerated culture 4 hours - Centrifuged and resusp'd in LwD
58-161 } mod SD see. Added 1.6×10^{10} λ = 1.6×10^9 λ/ml
and PX - Incubate 1:15PM -
no clearing 3:15.
clearing 4:15

3/14

1655+882 – 12 plugs picked and streaked on EMD(0).
from streakings 12 colonies picked streaked on EMD(0)

Wq-16 Pen run 2
15 colonies survived – EMB – Picked to [redacted] Host and streaked on D(0) – 1 failed to grow – Picked to slant.

Wq-19 Pen survivors – replicated to A_1, A_2, A_3, A_4, A_5
growth only on A_3 – (phenol, typ. or trypt)

Wq16 Pen survivors 1
30 additional col. picked to TSA – replicated to D(0) – all grew.

3/15 Wg-14
Pen colonies + trypt ↑ pro tube mix with
pen survivors determined ↑ A_3

1655 + 882
40 colonies picked from 1st purification
3 plaque pickings cross streaked on 1655 for lyso. stain.
One showed plaque on 1655
3/17

Wg 16 - Pen 1 survivors -
two additional colonies picked to NA and streaked

Wg 16 Pen 2 mutant.
Inoculated in primary medium

Tube	1	2	3	4	5	6	7	PEN
Contents	A_1	A_2	A_3	A_4	A_5	YNA	Vit.	
	-	-	-	+	-	-	-	

3/16 Wq 16.

No streaks on NA. replica'd to EMS-box (no D(0) avail) All grow 3/17 (42)

Wq 14 Ten A3 mutants on D(0) + proline + trypt

Tube	1	2	3	4	5	6	7	8	9	10
3/16 24 hrs	+	+	+	−	−	+	−	−	−	−
3/17 48 hrs	+	+	+	−	+	+	−	−	−	−
3/18 minor.	———			+	———	+	+	+	+	← discarded

Wq-16 A4 break down

ent	~~pro~~	asp	thre	glut
−	+	−	−	−

Wq16 pro-

3/18 Alg 14 pm - trypt - aerated culture from overnight culture.
started 8:00 - Centrifuged 12:15, resuspended in sol, centrifuged
resuspended in WD buffer - Aerated - 1:20
dilute 1-10 with sol - add 0.1 ml to 10 ml DB + pr + trypt + pen
" " 10 ml (0) " " —

K-12, 58-161 } Aerated cultures 8:00 AM - centrifuged 12:15
Pen.
Resuspended in W-D - mod 35 sec. + 10 ml Pen

Inc 12:55

58-161 Part. Clear 3:30
K-12 " " 3:20 } cleared 4:00

58-161 L3 Pen

K-12 L6 Pen

3/19

Wg 14 pm - trypt -

Penicillin run - control grew

plated out undil. 16 colonies

EMB-lac 1-10 1 colony

1655 + 882 - Purification of steak on 1655 showing plaques (3/15) 20 colonies picked and cross streaked on 1655

K-12 L6 Pen } Centrifuged and plated in tubes (20 ml each)

SB-161 L3 Pen }

Assay

SB-161 $10^2 - 10^4 - 10^6 - 10^7 \xrightarrow{0.1}$ plaques =

$\xrightarrow{0.1}$ cells =

K-12 $10^2 - 10^4 - 10^6 - 10^7 \xrightarrow{0.1}$ plaques =

$\xrightarrow{0.1}$ colonies =

were done

K-12 Plated out for repeat of A - Runoff

B - Effluent

W-1655 - culture started in (1.0+10 ml) Pen + 0.3% agar

1.0 ml (3×10^{10}) phage added after turbidity of about 5×10^8

Immediate loss of flow time and partial clearing.

(discarded)

3/20

Wg 14 pre- trypt -

Pen Streaked on EMB for prin to replicating
Streptococci
Observed that Wg 14 is lac⁻ -

K-12A (rough col) - 2 colonies picked to Pen and aerated P:30 } out 10:45
K-12B (smooth col) - 3 colonies " " " " " P:30 }

EMB { 1st plate: 2+A / 19(A+B)
2nd plate: 3+A / 16(A+B)

Aerated cult. of (1831 + 882) N in Pen began 8:45

K-12A Centrifuged, resusp in sal- dilute 2, 4, 5.5 — phosphate
Shake 2 min on shaker. 10 × 2 × 10⁶ = 2 × 10⁷

			Cell Survival	K-12 as phage
A-0	0.1 ml	61	1.0	0.33
A-0	0.5 ml + 0.5 ml 1M5 → 0.1 ml	10 × 2 = 20		
A-15	0.1 ml	35	0.57	1.0
A-15	0.5 ml + 0.5 ml 1M5	41 × 2 = 82 / 62		

K-12B Centrifuged and as above

B-0		65	1.0	1.0
B-0	0.5 ml + 0.5 ml 1M5	6 × 2 = 12		
B-15		21	0.32	0.32
B-15	0.5 ml + 0.5 ml 1M5	42 × 2 = 84 / 72		

Assay K-12 as indicator. Yields 100% yield of large plaques. 1 small plaque per cell even at MOI about 30-50% survival

---

(1831 + 882) N aerated cult. ca 5 × 10⁸ — diluted $10^2, 10^4, 10^5$ /2

Dose	Survival
0 - 485	8.2 × 10⁻¹
10 - 398	8.2 × 10⁻¹
20 - 202	1.2 × 10⁻¹
30 - 87	1.8 × 10⁻¹
40 - 49	1.0 × 10⁻¹

3/24 Monday -

Wp 19 pro - trypt -

16 survivors on EMB

Picked to D(0) + trypt + pro on 3/21

3/22 all grew except #6 + #8

Both grew 3/25

on 3/24 inoculated #6, #8 into fresh trypt + pro + D(0) for re check

---

1655 + 882 -

1.0 ml + 10 ml Pen - aerated 90 min.

dilute to ca 10^3 cells/ml - fixed in sal.

Dose	Plate Count	SF
0	211	1.0
10	125	5.9×10^{-1}
20	66	3.1×10^{-1}
30	21 (Low)	9.9×10^{-2}
40	26	1.2×10^{-1}

Dose extension in between 2. - 3.0

Very few colonies due to a too high dilution. Plated in duplicate. Count discarded in error.

Tuesday 3/26

Wg 16 Pr⁻ aerated culture started 9:30 – out 12:30 – Wash
resuspend in D(0) + Pr + Pen – 20 colonies survived
D(0) + Pr –

K-12 B – Effect of agar growth, etc in Tris-Cl.

Colonies removed from K-12 plate (EMB) to Tris-Cl
Spread on EMB – incubated ____ hrs – Washed off, centrifuged and resuspend in sol – dilute to 10^7 cells/ml – irradiate

Not done

K-12 L6 – in Pen – Tris-Cl Extended zone – recovered ca 12

dil 10^6 → 1.0 ml + 1.0 ml 1% PS⁻ → 0.1 ml TSA > 1000
count > 10^9

$10^6 \cdot 10 \cdot 2 = 2 \times 10^7 \times 1000 = > 2 \times 10^{10}$

K-12 Transduction of 58-161

K-12 L6 (above) + inactivated culture of 58-161

L6	1.0 ml	–
58-161 cult	1.0 ml	1.0 ml
Broth	–	1.0 ml

no colonies 2/26 – residual growth
no colonies 2/27 – dil carried

Centrifuge – add 8 ml Sol – Centrifuge

	phage	broth
Plate 0.1 ml → D(0)		
Dil 1-10 → D(2) (not broth)		–

W1655 + λ + 0.7 agar – partial clearing at 2 hours – still partially clear 3/2

3/26

1655+882 plated out from overnight culture – ca 1000 plaques – indicate either change in virus or resistance of 1655+882 cells – Lysogeny

(1655+882) + λ discarded because of above

2/27 Mix up concerning Hfr tubes.

is ~~tube~~ suspected of being contam. spotted on EMB low – all neg
EMB med – all pos.
D(0) – all failed to grow

streak out of another batch of tubes showing heavy growth on EMB

Wq 16 pr- per survivors
20 colonies picked and streaked on TSA

1655 + λ + 0.3% agar – partial clearing in 3 hours

1655 + λ – plated out on D(0) after 20 min to (a1ml)
observe transduction effect.

minute colonies 2/27 <1000
" " 2/29
" " 2/30

Cells and cultures

3/29

K-12 - Lac⁻ K-12 observed(?) - picked and streaked on EMB lac

slight green reaction on EMB lac
& streaked again 2/30 on EMB-0

Wg 16 Pr- - Pen selection survivors (19 colonies) replica'd to DG) + Pr EMB lac.

all grew -

2/30 ~~W~~ winner of 1655 + 882 on EMB lac picked for purification - Streaked on EMB-0 for a small colony format(?) 2/30

Cultures of W, 19 pr- trypt-
58-161 made for crossing

Small colony from 1655 + λ transduction picked to broth - In case transduction requires a 2 step process - one for each requirement (that is BM really exists)

3/31 1655 + 8-2

5 colonies streaked on EMB

Picked 5 from each and restreaked

poor streaking — questionable results - repeat

58-161

Wq 14 pr⁻ trypt -

Centrifuged, resuspend in Saline, centrifuge, resuspend saline

tube	1	2	3
Wq 14	1.0 ml	1.0 ml	—
58-161	—	1.0 ml	1.0 ml
broth	1.0 ml	—	1.0 ml

plate out 0.1 ml on 3 EMB - doubtful (EMB ± lact)

no colonies 4/1

no colonies 4/2 discarded

W1655 λ (transduced one step?)

overnight culture aerated - 0.1 ml λ prep added -

Centrifuged and resuspended in sal.

plate out 0.1 ml on 0/0)

no colonies 4/1

no colonies 4/2 discarded

4/1 Received cultures of (8:30 AM)

Wg/16 pr⁻ – for pen. mun

K-12 – λ strep.

1655 – for transduction trial

Culture of K-12 for NZ phage expmt (Boyd)

K-12 LL?	Tubes.	1	2	3	4
	λ dil	1-10	1-100	1-1000	1-10000
ml	K-12 overnite culture	1.0	1.0	1.0	1.0
	Pen.	10	10	10	10

In bath at 8:45

at	10:15	granular appear	granular appear	turbid	turbid	over all turbidity the same
	11:30	"	"	"	"	"
	2:10	"	"	"	"	"
	3:00	"	"	"	"	"

discarded

1655 + Pg2

16 colonies picked from streaking of 2/20 – tested against 1655

all appear to be phage free

4/2

1655 – Transduction

tube	1	2
(11:30 AM) 1655 cult.	5.0 ml	5.0 ml
λ Lb	0.5 ml	–

Centrifuged – resuspend in Sol'n (5.0 ml each) ~~+ [illegible]~~

~~+ [illegible]~~ Buffer sol'n. (PF stock dil. 0.1 +10 ml)

Plate 0.1 ml on D(0)

Due to exposure to bright

4/2 – plate with phage has heavier background than without.

4/3 – " " " " " "

discarded

K-12 L7

incub. culture 1:15

centrifuged resusp in Sol – mixed 15 sec

Incubation at 1:45

cleared at 4:00

Eliminate requirement for M-9 buffer in resusp. in Sol'n.

4/2

(52)

Wg16 Pr- Pen mm

Plates 1-10 from EMB(0) - Colonies

and [-

Wg14 pr- trypt- x SB-161

grown overnight in Pen in mixed culture.

plated out - D-0 (two plates)

4 colonies on 1 plate

5 colonies on other -

K-12 on agar -

K-12 cells scraped off EMB(0) to Pen
Counted 8:15 out at ———
Centrifuge resuspend in saline - incubate after dil. to 10^8 cells/ml

1655 0.5ml + 10ml 1:5

Dose	Count/ml	Phage/ml	Survival	K-12 on plates
0	256×10 = 2560	5×3×10 = 150	1.0	0.06
5	195×10 = 1950	11×3×10 = 330	0.76	0.13
10	123×10 = 1230	39×3×10 = 1170	0.48	0.46
15	76×10 = 760	52(?)×3×10 1560	0.30	0.61

1681 - Overnight culture - selected for rad. resistance and lung.
Culture aerated (1.0 + 10ml Pen) 8:15 - Centrifuged
Pellet to

1681 cross streaked on 1655 - no plaques observed

Dose	Count/ml	Phage/ml	Survival
0	35	0	1.0
10	21	0	0.60
20	19	0	0.54
30	7	0	0.20
40	8	0	0.23 est

0.54
35 | 19.0
17.5
15.0
11.0
7.00

Is this culture lysogenic for P82?

Wg 18 pr⁻ Pen survivors -
replicated to D(1) and EMD(0)
+Pr

4/7 18 40± colonies picked and replicated
1 failed to grow on D(0) + pr
- Wg 18 pr⁻ transferred to broth and D(0) + pr + trypt.
4 F' deletional survivors picked

4/8

Aerated culture of W1767 in D(0) + Loc [0.1 ml (10%) + 10 ml D(0)]

1.0 ml overnight aerated D(0) Loc culture + fresh D(0) + Loc (8:30 AM)

Centrifuge - resuspend saline · dilute to ca. 10^3 cells/ml

Dose	Counts EMB Loc
0	1.
	2.
	3.
10	1.
20	1.
30	1.
40	1.

discarded apparently overgrown with Loc + prototroph

W518 λ^s

Aerated culture from overnight culture in Pen (8:20 AM) (1.0 ml + 10 ml)

Add 0.1 ml λ (ca 10^9 particles) to aer. cult (ca 1.5×10^8 cells/ml) Phage/cell ≠ calc 1.0

Incubate 15 minutes. and. — Plate on EMB Loc

Dilute 1-10 — many colonies - all Loc, some pinkish showing lysis of cells

1-100 — " " " " " " " "

1-1000 — few " " " " " " "

W1931 $^{88z+}$ $^{\lambda s}$ - Quad

Aerated culture from overnight culture. 1.0 + 10 ml Pen (8:30 AM)

Centrifuge resuspend in sol. dil to ca 10^3 cells/ml

Dose	Count EMB Loc	Survival
0	252	1.0
10	176	0.70
20	86	0.34
30	68	0.27
40	64	0.25

(more resistant than K-12)

4/9 Wed.

Induction of lysis in K-12 by cold shock?

Aerated culture 0.5 + 10 ml — 10:30AM → 1:30

Culture ca 5×10^8 cells/ml

Pre-shock

Cell titer $10^2 - 10^3 - 10^4$ → plate 0.1 ml at 300 = 3×10^9

Phage titer → 0.0ml + 0.0ml 518 → 0.1 → plate 2 = 2×10^7

Post shock

cell titer $10^2 - 10^3 - 10^4$ → plate 0.1 ml at 3000? 3×10^8 (?)

Phage titer $10^2 - 10^3 - 10^4$ → 0.0ml + 1.0ml 518 → 0.1 → plate 4 = 4×10^6

Results not conclusive either way.

1831 10:30AM → 1:30 Centrifuged aerated cult. resusp in saline

Dilute to ca 10^3

Time		Survival
0	242	1.0
10	138	0.57
20	33	0.136
30	15	0.062

U.V. resistance similar to that of K-12

1831 + 8Fz as 1831

Time		Survival
0	1105	0.80
10	589	0.51
20	353	0.317
30	149	0.13

U.V. resistance appears higher than K-12

1831 + 8Fz cross streaked on 1831 no reversion

H267 - Original culture.

Streaked on EMB to recover diploid

EMS

discarded

Short regimen

W916 pr revertants replicated to O(0) 4h

none of course — at on O(0) + pr

4/10

Wq 16 pr survivors (see previous attempt) 48 in run
replicate to D(0) + pr –
1 failed to grow - transferred to stock
for later identification - Wq16 pr⁻ ②

K-12 Repeat of induction of lysis in K-12 with cold.
Vigorously growing culture (two transfer in aerated Pen)
Samples removed and placed in ice bath & aeration 10 min
Samples 10 min - returned to 37°C

Innocul. Cell count – 86
" Plaque " – 3

Post treat cell count – 116
" " plaque count – 1

no evidence of cold shock
uncertainty to cold shock
no evidence of induced
lysis.
no genuine lysis
not considered
further

Wq 14 (Pr⁻) Aerated culture – washed twice in Saline
Ca 10^5 cells added to:

D(0) + pr + trypt + pen (0.2 ml)	– plates not	undil. = 14
D(0) + pr + trypt	– growth	1-10 = 0

4/12

Wq 19 (Pr⁻) Survivors of 1 hr run of above
replicate to D(0) + Pr
2 of 14 colonies possibly diauxotrophs
picked and streaked for further replication

4/14

Aerated cultures W67 and 1177 started 8:15

H267 Radiation - culture in D(0) + lactose 36 hours -
dilute to 10^3 cells/ml

Dose	Count EMB		Phage TSA 1650 (0.5ml + 0.5ml H$_2$O)	Survival
0	391 316 365	cell lobes/plate	0	1.0
10	307	10 lobes -	12 · 2 = 24 (7%)	0.90
20	187		—	0.55
30	98		—	0.29
40	60		84 · 2 = 168 (?)	0.18

W 14 pt - Pen survivors - 2 possible diauxic - 10 colonies picked from streakings of each - streaked on EMB-D

replicated 4/15 to D(0)+Pr, EMB blue.

4/16 - streaks same as before - apparent reversion in replica ∴ ● - Discarded
D(0) EMB

---

2 ml of W67 + 1177
Cells centrifuged - resuspended in H$_2$O (10 ml)
Irrad. 35 sec
10 ml + 10 ml Pen
Incubation with air 1:30
cleaning at 4:00
partially clear following morning

4/15

(58)

Aerated cultures of Wg 14 pr⁻ for plan runs
W1628 for serum "
W1682 " "
} 10:00 AM

W1628 titrop and survival

aerated culture above - dil to 10^3 cells/ml

Dose	Counts/plate	Plaque/plate (0.5ml + 0.5ml 1611)	Survival
0	338	0	1.0
10	259	—	0.77
20	107 (235)	193·2 : 286	0.32
30	40		0.12
40	17	—	0.05

Wg 14 Pr⁻ Plan runs -

aerated culture - centrifuged - resuspend in Saline - centrifuged, resuspend in saline.

add 0.1 ml to:

TC01 + Pr + trypt + 0.8 plan -

" " " ——— -

H267 - culture in broth (from H267-I) 2.0ml + 5.0ml Pa (11)

Incubate 6h 37°C.

Centrifuge, resusp in H2O - mod. 35 sec - add 5ml Pa

Incubate - 2:45 PM

partially clearing 4:00

" cleared next morning.

W1628 Pr⁻ Plan runs -

4/16

Aerated cultures started

1661
1661
1736
} 8:15 AM

Wq 14 Pen Survivors

Plated to EHB loc. – undiluted

pools 1 – >1000
2 – "
3 – "

– 10 colonies picked, tested – all grew 4/19, 4/20

H267 Culture #2

Dilute to ca 10^7/ml

Pen	+	Count/plate	Pure Loc	Sig.	Survival
0		306	12		1.0
5		317			1.04
10		271			0.89
15		259			0.85
20		221			0.72
25		144			0.47
30		86			0.28

~~A-titers~~ EHB-01 titer

~~A-K-BALT~~ lab

H267 — 10^6 → 0.5+0.5 1661 → 0.1 — 2 — 4×10^7

W 1177 same >134 $> 1.3 \times 10^9$

W 67 " 0 : $< 2 \times 10^7$

Incub. of cultures – centrif. pooled in sol – incub 35 sec. add 3.0 ml + 100 Pen – incubated

after 2 hours → W1664 – plated on 1661 – no plaques by daisy ~~Incubated~~ + no plaques on 1661 ~~4/20~~ incub. of 1661 turbid

cleaning – W1662 – $40-50 \times 10^7$

cleaning – W1736 – $50-(?) \times 10^7$

4/17

1736 – } 2 plates 4/20/ 4/4 4/20

1736 + λ (0.1 ml) Lb K-12 } incubated 2 hrs 37° results no⁽⁻⁾ colonies = ca 50 papillae(-)

no⁺ colonies = ca 500 papillae (+)?

4/20 H267 - Prep #2 Plated on EMB-maltose

dilute to ca 10^3 cells/ml - incubate, count cells, plaque

Seq nr (-)	Mol %	Dose	Count/plate	cells killed	Plaque/plate (0.5 wt + 0.5 wt 1455) → 0.1 ml	Survival
	23 (6)	0	461	0	3 · 2 : 6 (1.3%)	1.00
Δ 39	62 (14)	5	445	15	4 · 2 : 8 (1.8%)	0.97
49	72 (21)	10	342	119	7 · 2 : 14 (3.3%)	0.74
	65 (24)	15	274	187	38 · 2 : 76 (16.5%)	0.60
	66 (25)	20	260	201	60 · 2 : 120 (26%)	0.56
	85 (43)	25	196	265	140 · 2 : 270 (57%)	0.43

4/21 Wg14 pr- Pen Survivors - (see previous)

(no background among 40 colonies picked)

on 1 plate a pink colony - picked and restreaked on EMB lac (END lac)

(a lac+ Wg14?)

50 additional colonies picked on each of the 3 survivor plates - 3-4 more opaque, raised colonies - picked and examined.

4/22 H267 alone -

5 mal - colonies picked from each med. ut. and streaked on EMB lac to find change.

0 5/5 lac . 1/5 appears mixed upon basis of opacity.

5 5/5 lac . 0/5 mixed

10 5/5 lac . 1/5 mixed in opacity of colonies

15 5/5 lac . 1/5 mixed in opacity of colonies

20 5/5 lac . 1/5 mixed in opacity of colonies

25 5/5 lac , 1/5 mixed in opacity of colonies

9/22

K-12 L8 Two 10ml Penicillase (100ml + 10ml Pen)
aerated ~~2½ hours~~.
Washed & suspd. in saline. Spun 25 sec.
Added 10ml Pen. incubate with air -
Inc. 2:30 PM. out ca 5:00 cleaning.
Centrifuged on following morning - one appears viscous - K-12 L8 (viscous) 1
K-12 L8 (non visc) 2

Continuation of Transduction of W1736 by L6 of K-12 4/23

	1.	2.	4/25 1.	2.	Ave
Plates with 0.1 ml Pen	83 colonies* (14 lac+)	91 colonies (4 lac+)	110 lac+	132 lac+	123 lac+
Plate with 0.1 ml L6 of K-12	90 colonies 17 (lac+)	70 colonies (28 lac+)	152 lac+	151 lac+	152 lac+

*really papillae on heavy background growth
incubation continued -

Wg 14 pr⁻ second step -
1. All 40 colonies picked after Penicillin selection grew - discarded.
2. Of the lac+ Wg 14 pr⁻ two colonies picked and streaked EMB lac⁻
on 4/22 - 1st day doubtfully lac+
on 4/23 - 2nd day strongly lac+
#1 smooth, glistening raised
#2 rough, dry, raised, wrinkled appearance ● - segregation of lac⁻? delayed pigmentation?

lac+ #1
lac+ #2
lac+ #3
lac+ #4

Both transferred to agar slants from single typical colony.

3. Inoculating and lac+ colonies taken in O(o) + pr + trypt } ten colonies in all
O(o)
- all grew in supplemented and not in O(o)
- on the original streak plate - 3 colonies lac+ - appeared less + on second day after refrigeration - 2 appear to be the same (solid light purple) one marked.
one of each type transferred to agar slants
-> lac+ #3 solid
-> lac+ #4 wrinkled

Spurious mutant(s) forgotten -

Cultures obtained
7 EC - transduction agar
1485 - culture
1229 - [illegible]

4/23 Wednesday -

Titration of K-12 λ8-1 and K-12 λ8-2

dilute 10^7 —0.5ml→ + 0.5ml 1485 —0.1ml→

	plaques/plate	dil.	titer
K-12 λ8-1	105	$2 \cdot 10^8$	2.1×10^{10}
K-12 λ8-2	152	$2 \cdot 10^8$	3.0×10^{10}

- Aerated culture of 1932 started 9:50 - 1.0ml + 10ml Pen. out at 1:05 for titer and survival. - dil to 10^3 cells/ml. used. (0.5ml + 0.5ml 1485) TSA

Time	Count/plate	Plaq/plate			Survival	
0	107	0	0	0/107	1.0	
5	92	0	0	0/107	0.86	
10	60	11x2 = 22		22/107	0.56	U.V. Resistance similar to K-12
15	(65) p...s/plate	24x2 = 48		48/107	-(0.61)	
20	33	53x2 = 106		106/107	0.31	
25	8	64x2 = 128		128/107	0.075	

mix up here - probably sampled twice at 10 - therefore 15, 20, 25 titers are really 10, 15, 20

- 1736 transduction - in EMB lac

1736 cells from unaerated culture -

λ from K-12 λ8 Plate (EMBlac)	λ'	1736 cells	4/24	4/25	4/28		
1	0	0.1	no papillae	papillae appearing	30+ papillae	0	
2	0	0.1	"	"	26+ "	0	gal+
3	0.05	0.1	"	"	189+ "	186	25×10^3 gal+/ml = $\frac{1}{187}\lambda$
4	0.1	0.1	"	"	274+ "	271	$720 \times 10^3 \lambda$/ml
5	0.2	0.1	"	"	372+ "	361	

- Sporulation Sur off 1932 - aerated culture 3 hours - dilute to 10^6 cells/ml with Salts.

Time	Counted EMBlac	Plaq/ml (0.5ml + 0.5ml 1485)	Surv
0			
5			
10			
15			
20			
25			

W1821 } aerated cult w/ at T=15
W1682 } T=0 / T=15 plus

Thursday
4/29

(1.0 ml + 10 ml Pa)

Aerated cultures of 1682 and 1831 started 9:15 AM

- Irradiation survival and Lysis of 1682 ($\lambda^+ 882^+$)
  Aerated cultures.
  Dilute to 10^3 cells/ml

Dose	Col/Plate (Exp 108 Loc)	Plaques on 1485 (=$\lambda^+ 882^+$)	Plaques on 1831 (=882)	Survival	
		((Plaque = 0.5 ml + 0.5 ml sens. cells) → 0.1 ml)			
0	134	0	0	1.0	Slightly more resistant than K-12 1/3
5	125	—	—	0.93	No lysis effect observed in the gross (see below) or by plate for either λ or 882 - is λ really present?
10	83	—	—	0.62	
15	50	0	0	0.37	
20	33	0	0	0.25	
25	16	0	0	0.12	

- 1682 for gross lysis obs. - cells remaining from above centrifuged, resusp in Sol. - mod. 25 seconds. add 10 ml Pen-aerate.
  in - 1:10 PM
  no clearing 4:00
  no clearing following morning.

- 1682 ($\lambda^+ 882^+$) cross streaked on 1831 (λ^+) for the purpose of determining presence of 882 - Plaques and lysis of 1831 obs. indicating 882 present.

4/25 Friday

- Aerated cultures of 1485 and 1831 started 8:30

1- Survival of 1485 $\frac{1}{5}$ — culture diluted to ca 10^7 cells/ml & saline (out at 10:45)

Dose	Count/plate EMBlac
0	20
5	46
10	50
15	2
20	15
25	17
30	7

This exp. 2 or previously value - Increased due to the fraction of variability of EMBlac plates used - they have varied 4/24 - avoid in future -

- Survival and Dev. of 1831 $\frac{1}{4}$ - aerated cult. diluted to 10^3 cells/ml

Dose	Count/plate EMBlac	Plaques on 1485 (0.5 ml of the 1:5)
0	63	1 x 2 = 2
5	41	–
10	27	–
15	5	40 x 2 = 80
20	3	24 x 2 = 48
25	5	28 x 2 = 56

not a good exp. can be said that 1831 undergoes transfer -

- Streak - 1682 colonies across 1681 number to determine if [redacted] is present -

5 colonies picked from 0 plate 2 4/24 used.
none showed evidence of ability to lyse 1681

† 4/26 - Culture #3 dilute to 10^3 cells/ml - used for survival - also to account isolation preinstituted exp.

Dose	Counts/EMBlac/plate	S.F. 1.0
0	192	
5	134	0.70
10	27	0.14
15	19	0.10
20	4	0.02
25	2	0.01

Unusual survival curve - sensitivity

more resistant than 4-17

Tuesday 4/25

λ transduction of 1736 of previous pages
papillae increased in original page.
10 papillae (+) from central, packed, streaked EMB-loc
17 " " " " plate " " " "

		Replicad
4/29	4/30	gal+ 5/1
7/7 loci	7/7 loci	6/7 gal+
17/17 loci	17/17 loci	16/17 gal+

↑ exceptions may be due to heavy culture with background cell of original plate.

4/26/67
5 (+) centers from previous streaked packed and streaked on EMB loc, EMS loc
streakings are lost on EMS loc
either mixed or segregating on EMB loc

Cultures
1415
1736
W19 pr. typ

Tuesday 4/29

Wg 14 pr⁻ trypt⁻ diluted and plated out for Lac⁺ reversion –

10^6 → plates CM B #3 – 3/1
#1 ca 150 (–) → ∞ 4/30
#2 "
#3 "

– W1736 – read – Lucy and Hammarton of plaques for presence of phage other than λ
– aerated culture. dil to ca 10^7 cells/ml

Dose	Colonies/CM B plate	Phage (0.5 ml + 0.5 ml 1485)
0	18	2
5	20	–
10	6	2
15	5	3
20	7	8
25	3	7
30	5	–

insufficient col count – appears old in addition

– H267 Repeat on survival of prep #3

Dose	Colonies/CM B plate (exc plate)	SF.		exc-col.	ag. col.
0	296	1.0	odd?	8	48 (?)
5	168	0.57	appears to check previous run with this prep re over growth	46	74
10	106	0.36		32	46
15	92	0.31			
20	22	0.07			
25	4	0.01			
30	6	0.02			
35	2	0.007			

– Penicillin runs

Wg 14 pr⁻ trypt⁻ → Pen aerated culture → wash → O(o) + pr + trypt aerated cult → add 10^6 cells/tube

tube 1 – pr + trypt + O(o) + Pen (0.2 ml) – 4/3. no growth apparent
2 " " " – showed no growth – why?

Wg 16 → Pen aerated culture → wash → O(o) aerated cult → add 10^6 cell/tube

tube 1 O(o) + Pen (0.2 ml) 4/3. no growth apparent
2 O(o) growth.

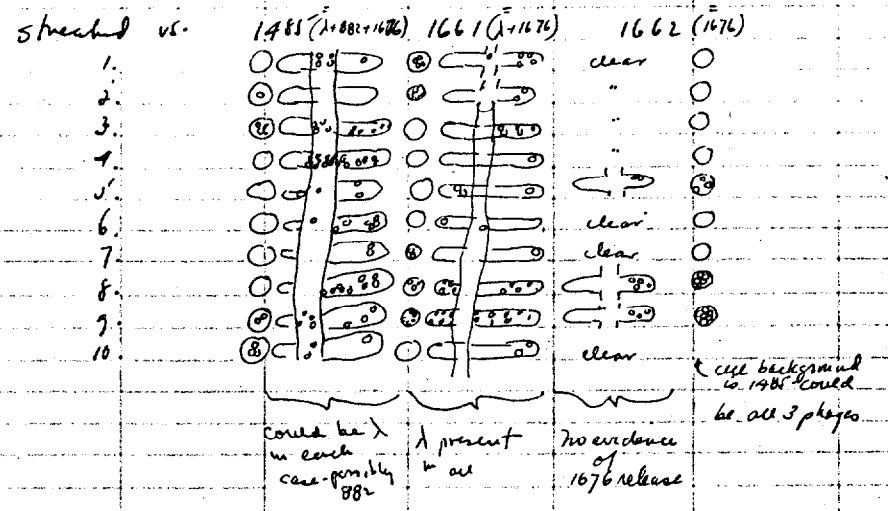
U1661
U1662
1485

Wednesday 1/30

- Loc+ papillations from the lost attempted transduction of 1736 replicated to gal EMB.
- Plate out

		NSA
Wg 14 pi- trypt-	undiluted	>
	1-10	>
	1-100	150-200 col (was something looking in bur tube? - control pulled to grow
Wg 16	undil.	16
	1-10	2
	1-100	–

- Plaques following 1736 mod.
10 pulled to pen broth (0.2ml) - numbered



Thursday 5/1

- titer of K-12 λ LT

  dilute 10^7 plate 0.1 ml $10^{8.2}$ 1485 → 78 = 1.6×10^{10}

- replica Wg 16 pen streaks to O(o) on 12/14 failed to grow } indicating
  on 1/10 2/2 failed to grow } autotrophy

- streak Wg 14 pr-trypt - until O(o) + supplements can be made -

- λ from K-12 LT2 + LP pooled and filtered - giving around 50-60 ml
  after volume
  dilutions 3, 4, 6, (7) → 427
  titer = 4.3×10^{10}
  (or if dilution error 4.3×10^9)

- K-12 - day old aerated culture in O(M) + Leu (0.1^{10}%/ml) - diluted to 10^3 cells/ml - incub.

Dose (sec)	Col/EMB Methyl plate	Plaques 1485 (0.5 ml + 0.5 ml 1485)		Survival	K-12 as plaques = %
0	373	–		1.0	–
5	282	–		0.76	–
10	234	–	total	0.63	–
15	197	89·2 = 178	375	0.53	0.48
20	149	–		0.40	–
25	108	138·2 = 276	384	0.29	0.74
30	86	–		0.23	–

- H-267 as K-12 above.

Dose	Col/EMB-malt plate	Plaques 1485 (0.5 + 0.5 ml 1485)		Survival	K-12 as plaques %
0	285	–		1.0	
5	277	–		0.97	
15	168	–	total	0.59	
25	116	86·2 = 172	288	0.41	0.60
35	59	116·2 = 232	291	0.21	0.82

Cultures.
776 - 58r (58r)
1485
H267 from H267 #1 O(M) + Leu
K-12 in O(M) + Leu

Tuesday
~~Saturday~~ 5/6

- Aerated cultures 8:00 1503 λ°
  1806 Wg 31
  1959 Wg 31 λ+
  K-12

- K-12 Phage plating on 518 - For possible recovery of poss phage and irradiated

Dose	Count/EMB Lac Plate	Phage/518 plate (0.5+0.5 ml 518)		Survival	cell K-12 as plaques
0	206	—	total	1.0	—
5	79	—	plaque forms	0.38	—
10	53	58·2 = 116	169	0.26	0.56
15	27	55·2 = 110	137	0.13	0.53
20	18	68·2 = 136	154	0.09	0.66

- 1954 = Wg 31(λ+) - Observation to see if λ is curable in different strain

Dose	Counts/EMB lac Plate	Phage/518 plate (0.5+0.5 ml 518)		S.F.	1954 as plaques
0	216	—	total plaque forms	1.0	—
5	163	—		0.76	—
10	102	52·2 = 104	(206)	0.47	0.48
15	8	120·2 = 240	(248)	0.046	1.0
20	10	107·2 = 214	(224)		1.0

- 1972 = λ+Ω+ Examination for burst and recombination of λ and Ω

Dose	~~Plaques~~ on EMB lac	Plaques on 518 (0.5+0.5 ml 518)	Plaques on 133
0	8	—	—
5	8	—	—
10	2	10·2 = 20	—
15	0	18·2 = 36	—
20	0	11·2 = 22	0

No apparent reason for low cell no. - apparently large amount of free phage (= λ) in cult. turbidity was approx. 1/2 that of

- Transduction of 1736 - Gal
  Unaerated overnight cult of 1736 in Ven
  + λ on Gal and Lac plates = 0.05 ml 1736 added/plate.

#	EMB lac Plate	λ added	EMB gal plate	λ added	518 Papulae
1	1	0	1	0	
2	2	0	2	0	
3	3	0.05	3	0.05	
4	4	0.1	4	0.1	
5	5	0.2	5	0.2	
6	6	λ alone 0.1	6	λ alone 0.1	

Last done

- 2 Plaques 882/1495 picked to lightly unc. aerated cult of 1503 - incubated overnight - turbid following day

- Cultures 518
  1898 } rad. sens.
  1655 }
  K-12
  11267 (from #1) 5yr

5yr

Tuesday 5/6

– Survival of 1503 - λ^s - aerated cult from overnight cult. diluted to 10^3/ml

Dose	Cfu/EMB low plate	Survival
0	311	1.0
5	231	0.74
15	114	0.37
30	28	0.09
45	3	0.0097

Wednesday 5/7

- W3110 λ[s] Examination of u.v. resistance - Aerated culture - dil. to 10^7 cells/ml

Dose	Cd/EMB Blue Plate	Survival
0	327	1.0
5	186	0.57
15	91	1.28
30	40	0.12
45	8	0.024

- W1895 λ[s] Examination u.v. resistance - same as above -

Dose	Cd/EMB Blue Plate	Survival
0	100	1.0
5	88	0.88
15	62	0.62
30	11	0.11
45	4	0.04

- W1655 λ[r] Examination of u.v. resistance - as above

Dose	Cd/EMB Blue Plate	Survival
0	74	1.0
5	48	0.65
15	47	0.64
30	21	0.28
45	24	0.32

don't understand

- K-12 survivors?) from Tuesday continued — D(0) plates

1485 u.v. sec
1736 hours
1177 hours

Thursday 5/8

- Transduction of W112 Lac⁻ λ from K-12

EMB Lac⁻ plate	λ from K-12	λ from K-12 boiled 10 min	W112 cells* unaerated overnight cult.	# papillae 5/11
1.	—	0.1 ml	0.1 ml	ca. 200
2.	—	0.1 ml	0.1 ml	ca. 250
3	0.1 ml	—	0.1 ml	ca. 300+ *
4	0.15 ml	—	0.1 ml	ca. 250+ *
5	0.2 ml	—	0.1 ml	ca 300+ *
6	λ from W1177 4 0.1	—	0.1 ml	ca 30+

* ~~white~~ plaques (50+) also

Original cult. may be cent.-papillae → on these plates-
not really (+) Some pink with zone
as of contaminated E plates and lysis 5/11

- Transduction of W1736 gal⁻ EMB gal⁻ plate (unaerated cult.)

	λ from K-12	λ from K-12 boiled 10 min	No papillae (+)	Gal+
1.	—	~~0.1 ml~~	17	
2	—	0.1 ml	22	
3	0.1 ml	—	324	$\frac{3.0\times10^{3}}{>2.0\times10^{10}}$ t/ml / l/ml
4	0.1 ml	—	345 (2.1/10^{-7})	
5.	λ from W1177 0.2 ml	—	Trans. plated dried up	

λ from LL preparation

- λ in 1736, 1177 } aerated cult. centrifuged - resusp in 10 ml cal.
mod. 40 sec. ~~Boiled~~ 10 min. Ice - incubate with air 2:20 PM

- K-12 survivors in D(0) from previous - titration of phage + cells survive
mod. of K-12?
centers of 101 plaques picked and streaked on D(0) agar
on 4/8 1 streaking shows good growth - incubate overnight

4/9 1 .. ..
2 .. shows single colonies

5/9 Friday -

- Transduction of W1736 on gal with λ from K-12 page 69

Plate	λ added	no gal+ papillae
1	0	38
2	0	12
3	0.05 ml	153
4	0.1 ml	302
5	0.2 ml	620

$\frac{2.75 \times 10^3 \text{ t/ml}}{220 \times 10^6 \text{ λ/ve}} = \frac{1}{1.3 \times 10^{-7}}$

Gal 4 -

why the diff.?

Transduction W1736 on Gal+ with λ from K-12 page 69

Plate	λ added	
1	0	201
2	0	171
3	0.05 ml	262
4	0.1 ml	344
5	0.2 ml	600

Should be noted that papillae counts are lower than real value since counting is difficult and many small papillae are plugged -

- Plate 1 of 1736 Transd on Lac - 26 papillae picked to gal 26 grew - 24/26 were gal -

- Plate 5 of 1736 transd. on gal - 98 papillae picked to Lac - 22 grew 22/22 were Lac -

→ this finding suggest that papillae on EMB lac contain are different from papillae on EMB gal - that the gal transduction effect did not show appear as a result of integrative inhibition - against gal plus cells

Lac plates were discarded unfortunately -

Monday 5/12

- K-12 Synthetic culture #2 - dil to 10^8 cell/ml

Dose	Count/EMB Co plate	Survival	
0	330	1.0	
5	220	0.67	
15	211	0.64	same resistant than parent
30	155	0.47	
45	83	0.25	

- H267 Synthetic culture #4 dil. to 10^7 cell/ml

Dose	Count/EMB Plate	Survival	
0	215	1.0	
5	222	1.0	
15	148	0.69	
30	50	0.23	
45	14	0.065	

- Titration of K-12 λ L7 + L8

dil	0.05 + 0.5 ml 1415	
10^8	67	$67 \times 2 \times 10^8 = 1.3 \times 10^{10}$
10^8	68	

-

Tuesday 5/13

- Titer of 882/1485 on 1485

die. $10^2 \rightarrow 0.5 + 0.5$ 1485 → no plaques
$10^4 \rightarrow 0.5 + 0.5$ 1485 → "
$10^6 \rightarrow 0.5 + 0.5$ 1485 → "

- Transduction of Sonnei a Glycine on 1678

	D(0) plate	1678	heated λ	λ	5/14	5/15 contam.?
aer + pr	1	0.1	–	–	colonies arrived spot of deposit of pr	3 col
	2	0.1	0.1	–		
	3	0.1	–	0.1		3 col

* unaerated culture

- Titration of L2 of 1736 - from usual exp. - contr. rapid sol, usual 35 etc. incubated 6 air overnight. nearly clear lysis

del $10^8 \rightarrow 0.5 + 0.5$ 1485 → no plaques

- Transduction of 1736 on Lac and Gal (done on Monday 5/12)

Gal EMB plate	1736 Aerated cult ca 5×10^8 cells/plate est. λ from K-12 C7H6T	heat λ from L6	1736 Unaerated cult ca 10^8 cells/plate est. λ from K-12 C7H6T	heated λ from L6
1	0	0.1	0	0.1
2	0.1	0	0.1	0

Papillae on Gal 1 unaerated 5/15 - 25
Gal 2 unaerated 5/15 - 36
what happen?

Gal/aerated 5/15 ~ 29
Gal 2 aerated 5/15 : 425 <

9.3×10^{-7} — 29
2.2×10^{-6} — 425
$\frac{1}{3} \times 10^{-6}$

Lac EMB plate	Unaerated 1736 heated λ (K-12 L6)	unheated λ (K-12 C7H6T)	Papillae (-) 24 hrs (+) 5/15
1	0.1		121
2	0.1		150
3	0	0.1	182
4	0	0.2	218

Little or no effect of λ on unaerated cultures of 1736 - fairly large effect on aerated cultures. Meaning not clear. Incubation of Lac plates continued to increase size of papillae to make picking more successful.

Thursday 5/6

- transduction of W1736 Leu⁻ - aerated culture (1.0+10.0 ml Pen) from a day incubated culture - Cells centrifuged resusp in saline

O/N	Plates TB$_1$	1736 cells	Heated λ (1716)	λ	5/17 colonies
	1	0.1 ml	—	—	0
	2	0.1 ml	0.1	—	50 (contam?)
	3	0.1 ml	0.1	—	12
	4	0.1 ml	—	0.1	0
	5	0.1 ml	—	0.2	1 col
NSA plate 6		—	—	0.1	0

-

H267 #4 Dilute to 10^4 saline — used 5 seconds - Add 1.0 ml + 10 ml Phage broth for incubation with air at time 0

	cl. Time	Plate on EMB Mal	Pure Mal+ col	total colonies per plate	Dark Mal+ colonies
Thick plates reactive to air →	Pre-mal 0'	1	10	168	5
	Post-mal 0'	2	8	162	17
11:00	60'	3	29	256	14
11:30	90'	4	22	268	10
12:00	120'	5	23	302	16
12:30	~~180'~~	6	27	513	36

- Ice box cleaned up.

- Culture in vial mix. of 1832, Pen^r received from J.L. } 1 to PG, 1 to J.L. 5/17
  to ~~inoculate~~ reisolated - Pen broth incubated - }

- Wg 16 auxotrophs from page 68 picked to EMB plate, preliminary to determ requirements

- Gal papillae of 1736 Transduction of page 74 - from aerated cult + λ
  35 picked to EMB base for reidentification

5/17 cul grew - slowly - cul appear lac⁻, most spots are outlined light and dark (opacity) - some appear to be giving pink papillae.

Unstability of W1736

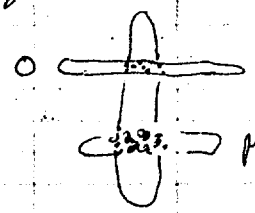
- Wg 14 pr⁻ trypt - lac⁺(?) cul - streaked out
  5/16 slight papillae
  5/17 dark red - probably slow lac⁺
  Slant made from single col -
  Wg 14 pr⁻ trypt - lac⁺.

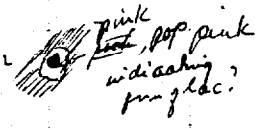
Friday 5/16

- Transduction 1678 (ser ~ glyc) SEE BELOW
  K-pr lysate ~ D(0)
  3 colonies on 1 plate transferred to EMB blue for check
  1(?) col. on control plate transferred

- Slants made of 1731 (2) for DG
  " " of 1732 (1) for JL

- Streak of plaque on W112 on 1485 from pg 75





- Papillae on 1736 blue transduction - with heated 1
  pick to loc - incubate and then replica

  Pick 66 papillae 5/17 - 63/66 growing slowly - all appear Lac⁻ - 5/19 Lac⁻? (red)

  5/18 replicad to gal
  5/19 9/63 gal+

  Lac slow? + Gal+ is glu+

- 1678 above - Transduction

  Control colonies streaking - all dark red, Lac+
  transduced (?) streaking -
  1. Lac⁻, with pale blue centers
  2. Lac⁻ with pale blue centers
  3 mixed - pale colonies small Lac⁻
  - Lac⁻ col, with purple centers (coli)

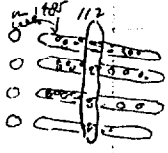
  } probably contamination - pick 2 col from each cross streak on 1485 to see if 1 present

  only control colony showed presence of 1 - remainder must be contaminants.

5/17 Saturday

- W1736 transduction(?)
  35 gal + pop (from page 75) picked to EMB lac. - all lac⁻ (& slight).
  replica to EMB gal to check gal reaction. -
  no plate 5/17
  replica 5/18 - 34 gal + / 35 replica'd

- W112 phage(?)
  cross streak 7 plaques on 1485' picked and cross streaked
  on W112



- Plaques 7 #2 on 1485' picked to H_2O - replated on 1485' h
  increase titer - by plaque growth if nothing else -
  streaked this way
  5/18 failure & only occasional plaques observed.

- H267 mad cult. 7 pg K - incubated 1 day with air ½ time (aerator outside door)
  one five day with air - stored refrig ∴ (H267 - mad)"
  in D(m) + lac

5/20 ~~Monday~~ Tuesday -

- Aerated culture 1786 for retry w transduction of Lecithinase - 8:45AM
Aerated not out at 12:00 - set incub 2:30 - Centrif, resusp in saline 10X

TBg plate	Cells	λ	heated λ	5/21	5/22	5/23
1	0.1	—		no col	1 col	↓
2	0.1	—	0.1	"	1 col	↓ Same discard.
3	0.1	—	0.1	"	2 col contam	
4	0.1	0.1	—	"	1 col	↓
5	0.1	0.2	—	"	0 col	↓

- Aerated culture of 1998 (1j + 2λ) for survival and Lecithinase - 1:00PM

dil to 10^3 cells/ml sol

Dose	col/ml Blue plate	Phage/plate (1930) (0.1 ml + 1930 in phage agar)		S.F.	%1998 as Phage Plaques
0	316	0	~~cells~~ plaques	1.0	0.0
5	309	—	316	0.98	—
10	300	4	301	0.95	2.8
15	242	42	284	0.77	13.3
20	277	50	327	0.88	15.8
25	268	—	—	0.85	—
~~30~~					

Survival high - due to length of incubation?

- Aerated culture of 1972 (1j + 2λ) for survival and Lecithinase - (plate in 1485)

dil. to 10^3 cells/ml 1:00PM

Dose	Cells/ml Blue plate	Phage 1485 (0.1 ml mixed)	Cells/plate	S.F.
0	428	0	428	1.0
5	306	—		0.72
10	283	107	390	0.66
15	223	(0.2 = 333) 167	390	0.52
20	199	174	373	0.46
25	148	—		0.35

5 large plaques, 5 med. plaques, 5 small plaques } picked and streaked against 1732 - no evidence of lysis. only 1 in field

here also 5/21

- Aerated culture of 1988 (5j + 16λ) for survival and Lecithinase (plate in 1930)

dil to 10^3

Dose	Cells/ml Blue plate	Phage (1485)
0		—
20		

out at 2:45 -

tag lit not turned on

Out

5/21
- Wednesday -
W#19 pr-trypt - from cult in P(0) + pr + trypt to
P(0) + pr + trypt + 0.2 penicillin }
O(0) " " }

Thursday 5/22

- Aerated culture of 1788 = 518(1676) · 8:30 ✓ no parent culture contamination bacilles?

-Wg 14 pr-trypt- Perm. new. plated out on NSA

1	undil
2	1-5
3	1-10
4	1-100

replicated to D(0)t Pr-trypt — no triples

- 1426? - #2 culture used - contains about 5×10^9 cell with 5% Mal - dilute to give about 50 cell/0.1 ml $= \frac{5 \cdot 10^9}{5 \cdot 10^2} : 10^7$

Inoculate 5 (RM) tubes - incubate with aeration.

↓ ↓ → plate out 0.1 ml on EM13 mal

Incubate — hours · plate on EM13 mal for segregants -

failed! too small no ½ cells

Tuesday 5/27

H267 – isolate "natural" segregants } use cultures
– isolate ~~re-mixed~~ segregants }

✓ • irradiate 1736 } do make Gal+ Lac+ λ preparations
1177 }

out • irradiate 1788 to see if significant effect is possible with (1676) culture contaminated

✓ • irradiate λ from K-12 to obtain survival data – assay for segregants
– use in "1:10 reducing" 1736

✓ try ~~repeat~~ gal effect with 1662

~~make~~

– Cultures at 8:30 } 1788, 1736, 1177, 1662 (above –)

1736

✓ Irradiation } λ prep – 10 ml of L7+ LF of K-12 – irradiate – remove 1ml samples

Dose	0.1ml λ	0.1ml 1736 cells	papillae	S/sp
0	+	T	+	275
60	+	+	++	c. 903
120	+	+	+++	¼=46 1987
180	+	+	++++	¼=361 295 ↓
240	+	+	+++++	¼=791 3/88 ↓

2.75×10⁸ f : 2/10⁻⁷
1.9×10¹⁰ λ

↓ – ~~Repreparations~~ in W1177 and W1736 –
cultures incubated ō on rot 16:20 – centrifuged resuspended in Sal
W1177 in 10ml saline
W1736 in 2ml ↓ – then to 10ml
use in exp below as mod λ.
} mod 40 sec – incubate ō on
add 5ml Pen
incubated 11:50
1736 cleared 3:00 – centrifuged, 2 disp. chloroform added
1177 – failed to clear –

↓ – Mod. λ – as a transducer see above

↓ Transduction in 1662 – Gal

			papillae	S/sp
aerated cult.	Plate 1	0.1ml mod λ	c. 1-3	19
	2	0.1ml – λ	c. 50	311

} Gal effect in 1662 similar to that in 1736 also similar efficiency

$\frac{2.9\times10^{8}}{1.4\times10^{10}} = 2/10^{-7}$

↓ H267 – ~~cust~~ from cust #2 – dilute 5·10⁶ → ~~three~~ two 0.1 into 10ml dil(4) tubes –

"Normal segregants"

Plate 1	very small (1-5)	5/28
2	number of colonies	

1:10

0.1 mod λ	18
0.05 λ	40
0.1 λ	80
0.2 λ	207

1953 Wednesday 5/28

√ – 8:15 Aerated cultures 1953, 811, 1436, 776-8Rs, 1736

– Transduction with λ of W811 – Aerated cult. 4 hrs – centrifuged 10 ml pipet in sol.

EMB gal plate	heated λ	λ	λ on 794 Ami	811 cells	5/29	5/30 counted
1	0.1	–	–	0.1	c.25 (plaques?)	c.50 plaques (66)
2	0.1	–	–	0.1		
3	–	0.1	–	0.1	c.75 (plaques?)	c.300 (plaques (535)
4		0.1		0.1		
5	–	–	0.1	0.1	>1000 (plaques?)	>1000 (too many plaques)

two few plates

$\frac{5.35 \times 10^3}{1.4 \times 10^{10}} = 3/10^7 \lambda$

– Transduction of 1736 with lysate of 1736 – Titration of 1736 lysate

Sonicated

EMB gal plate	1736 cells	K-12 heated λ	1736 λ	5/29	5/30 counted
1.	0.1	0.1	–	0	17 (14)
2.	0.1	–	0.1	0	13 (16)
3.	–	–	0.1	ca. 1000 colonies – NOT STERILE	

1736 lysate diluted $10^6 \rightarrow 0.5$ ml + 0.5 1485 → 0.1 ml $22 \times 2 \times 10 \times 10^6 = 1.4 \times 10^8$ plaques/ml

$10^8 \rightarrow 0.5$ ml + 0.5 1485 → 0.1 ml $= 0$

√ – Irradiation of 1436 λ – Plating on EMB-lac – Aerated cult., dil 10^3 cells/ml, etc.

Dose	Col/EMB-lac plate	Survival
0	295	1.0
5	282	0.96
15	204	0.69
30	148	0.50
45	93	0.32

√ – Irradiation of 1953 wt – Lysate – Plating on EMB lac NSH – Aerated cult. dil. to 10^3 cells/ml etc

Dose	Col/EMB plate	Plaques on 1485 0.5+0.5	Survival
0	197	0	1.0
5	211	–	1.07
15	144	0	0.73
30	99	0	0.50
45	51	–	0.26

No evidence of host cell reactivation

√ – Titration of uninad. λ

Dose	Dilution	Plaques 1485 0.5+0.5
0	10^8	c. 500 · 2 × 10 × 10^6 → 1.0×10^{10} fairly good agreement with previous assay = 1.4×10^{10}
240	10^8	$22 \times 2 \times 10 \times 10^8 = 4.4 \times 10^9$
	10^8	–

at dose 240 ca 30-40% survival of λ

Continue Tues

Aerated cult. 8:20 SF-161, 1736 galt, 1736, 1439, 811

Thursday 5/29

✓ — SF-161 Transduction with irradiated λ.

D(0) plate	heated λ	UV 240 λ	SF-161 cells	6/1	6/5
1	0.1	—	0.1	no cul.	no cul discard
2	—	0.1	0.1	no cul.	" "

✓ — 1736 galt — "transduction" for loc -

EMB loc plate	1736 galt cells	heated λ	K-12 C7-8 λ	UV 240 λ		6/1
1	0.1	0.1	—	—	c. [illegible] (½ = 226)	
2	0.1	—	0.1	—	c. [illegible] (½ = 213)	ca
3	0.1	—	—	0.1	> [illegible] (½ = 225)	the
4	0.1	—	—	—	c. [illegible] (½ = 266)	same
					(1=2<3<4)	discard

1>4=2>3

✓ — Reconstruction exp. Detection of galt in presence of large number of Gal-

1. dil. galt 1736 to obtain ca 2000 cells/ml — $\frac{10^9}{2\cdot10^3} = \frac{10^6}{2} \times 10^{-3}$ → plate 0.1 EMB gal (= ca 22)
1a. add 0.5 ml + 0.5 ml 10^{10}/ml 1736 galt → plate 0.1

EMB gal plate	ml of following 1736 cell mix	heated λ	K-12 C7 λ	UV 240 λ
1	0.1	—	—	—
2.	0.1	0.1	—	—
3.	0.1	—	0.05	—
4.	0.1	—	0	—
5.	0.1	—	—	0.1
6.	—	—	—	—

	Est 5/30 (Cauchy)	5/31	galt discard	gal+	gal-
ca 80 ppp (103)	119	71	38	—	
ca 70 ppp (81)	111	71	38	—	
ca 70 ppp (162)	217	71	38	98	
ca 70 ppp (205)	326	71	38	207	
> 200 ppp (16-fold)	[illegible]	71	38	3305	
ca 150 (154)	152				

219 / 16 / 203 / 2 / 3 + 2

✓ — W811 — λ prep. aerated cult. Appd in gal — mixed 35 sec c incubated c aer 11:30

first — discarded by accident

W-1439 transduction

EMB gal plate	heated λ	K-12 C7 λ	UV 240 λ	1439 cells	5/30	6/5
1.	0.1	—	—	0.1	1 and numerous	no papilla
2.	—	0.1	—	0.1	1 and few	no papilla
3.	—	—	0.1	0.1	" "	no papilla
4.	—	—	—	0.1	" "	no papilla

apparently plate was too wet.

✓ — W-1736 gal "transduction" — 48 + papillae picked from untreated λ plate (0 dose of 5/27 c 275 resp.) to EMB loc — 5/30 to replicate to minimal + aer ig auxotrophs

5/29

W-1662 gal+ "transduction" — 48 + papillae picked from 1662 + λ plate (page 80) to EMB loc to replicate

5/30 aer loc 97/98 grew

Friday 5/30

BM transduction // Hyl transduction

- Annealed with 811, 1821

✓ - Gal + prop. 811 - picked and streaked - attempt lac transduct in 811

✓ - W112 - principal - plated, streaked on EMB lac

✓ - 1821 - Hyl transduction

EMB Hyl plate	1821 cells	Boiled λ	w240 λ	5/30	6/1	6/2	6/5
1	0.1	0.1	–	w prop	w prop	w prop	$t_{1/2}$ = >161 prop (Small)
2	0.1	–	0.1	"	w prop	>100 prop	$t_{1/2}$ = >310 pp (Larger)

✓ - 811 - BM transduction

D(+) plate	811 cells	Boiled λ	w 240 λ	(1 drop) + MeOH	5/31	6/1
1	0.1	0.1	–	–	w prop	w prop
2	0.1	0.1	–	~~0.1~~	"	w prop
3	0.1	–	0.1	–	"	w prop
4	0.1	–	0.1	+	"	w prop

Call:

811 - for lactose prop

see 1821, 1821 for lactose, count of prop plated for 1/62, 1/76

Saturday 5/31

✓ - Aerated overnight for lysate of 811 - 7:00 pm
out at 7:50
centrifuge, rapid in sol - mod 35 sec - ~~centrifuge~~ rapid in Pen. incubate with air.

continued pg 85 →

✓ - Papilla of 1736 gal "transductant" picked to EMB lac 6/1 48 in all - all lac ~~depleted~~ populating
Replica to NSA + 1662 = 1676 no reaction
D(0) = autotrophy no growth
D(0)+TB$_1$ = leucine no growth
NSA + 811 = 882 no reaction
Gal = gal+ all +

✓ - Papilla of 1662 gal "transductant" on EMB lac — 6/1 all lac- populating
Replica to NSA + 811 = 882 no reaction
47 papilla D(0) = autotrophy no growth
45 gal+/2 gal- D(0)+TB$_1$ = leucine no growth
gal - gal

Sunday 6/1

✓ - Centrifuged 811 ~~lysate~~ lysate -

titer - 10^8 → 0.5 + 0.5 ml 1485 → $10 \times 2 \times 10 \times 10^8 = 20 \times 10^9 = 2 \times 10^{10}$
10^6 → 0.5 + 0.5 ml 1485 → $339 \times 2 \times 10 \times 10^6 = 678 \times 10^7 = 6.8 \times 10^9$

Cultures - continued til Tuesday -
1655 - cross K 112
112 - cross K 1655, also "transduct"
1736 - ✓ aerating, detail -, lysate
1821 - ✓ for gal ~~transduct~~ transduct" -
1485 -
Blyfield -

Tuesday - 6/3 try for gal transductant
Aerated cultures - 112, 1736, 1821, 1485 - ~~absorption~~ of λ

$\frac{5.5 \times 10^3}{1.9 \times 10^6} = 4/10^3 \lambda$

✓ - 1821 - Aerated cultures - centrifuge, resuspend in 1/10 volume (2 ml)

	EMB gal -	boiled λ	K-12 LE λ	811 λ	1821 cells	broth	6/4	6/5	6/7	aux no pop/ pellet	gray gal	~~gal~~ ryl (2)	
✓	1.	0.1	-	-	0.1	0.1	no pop	no pop	30	14	13	10	16
✓	2.	-	0.1	-	0.1	0.1	no pop	>30 pop?	581	67	63	59	57
✓	3.	-	-	0.2	0.1	-	no pop	no pop	36				

							6/4	6/5		
✓	2. EMB ryl -									
	1.	0.1	-	-	0.1	0.1	no pop.	pop?	46	(>1000 minute pop)
	2.	-	0.1	-	0.1	0.1	"	>5 pop?	25	(>1000 " ")
	3.	-	-	0.2	0.1	-	"	pop?	53	(>1000 " ")

✓ 3. BM - 0.5 ml cells + 0.5 ml λ (boiled) } incubate
- 0.5 ml cells + 0.5 ml λ (K-12 LE) } ~~incubate~~ 10 min - ~~add Gal broth~~ ~~incubate~~ - ~~each type with diluent~~
and plate 0.1 ml each of 4 dilutions plates
/ λ prep. -

dilution plate	boiled λ	K-12 λ	6/4	6/5	6/7
1	+	-	no cul.	no cul	no cul
2	+	+	-	-	-
3	-	+	no cul.	no cul	no cul
4	-	-	-	-	-

↓
add 0.1 ml from each to
each of two Pen tubes
incubate overnight -

✓ - W112 Aerated culture - centrifuged + resusp in 1.0 ml Gal

	OMR loop plate	titer	number/l	λ K-12 LE	m280 λ	6/4	6/5
	1	0.1	0.1	-	-	no pop	>445
	2	0.1	-	0.1	-	"	1/2 >216
	3	0.1	-	-	0.1	"	1/4 >217

Tuesday 6/3 continued

✓ – 1736 Aeration effect – aerated and unaerated cultures adjusted to same turbidity at birth – each diluted 10^6 (0.1) → ml EMBlac

Expt'l Lac 6/1	6/4	EMB gal	plate	Aerated 1736 cells	Unaerated 1736 cells	Diluted λ (K-12 LF)	λ K-12 LF
13	1		1.	0.1	–	0.1	–
410	>30 (plaques)		2.	0.1	–	–	0.1
12	0		3	–	0.1	0.1	–
282	<20		4.	–	0.1	–	0.1

Tot. Unaerated cell – $183 \times 10^7 = 1.8 \times 10^9$

Tot. Aerated cell – $91 \times 10^7 = 9.1 \times 10^8$

✓ – 1821 – lysate – aerated cult – ca 20 ml – centrifuged, resusp'd in sal and 35 sec – centrifuged, and resusp'd in 1 ml – aerated 1:20 HM

titered 6/4 dil $2 \cdot 10^8$ – 64 plaques (HFI) – titer – 1.3×10^{10} p/ml

✓ – Titer of 811 lysate (Gilbert)

dil 10^7 0.1 ml → 0.1 ml in plate + 0.1 ml 10X 1485 – $112 \times 10 \times 10^7 = 1.1 \times 10^{10}$

✓ W112 X W1655 cells (centrifuged & resusp'd twice, in sal)

EMB-lac	112	1655	6/5
1	0.1	–	1 col
2	0.1	0.1	>50 col (+) & (–)
3	0.1	0.1	>50 col.
4	–	0.1	no col.

✓ Replicate of Tan survivors of W1 14 from serial work agt (pg 68)

to B(1) + pr + trypt — to see aux. present –

Adsorption of λ by 1736 cells (aerated) – 0.5 ml 10X cells + 1.0 ml phage (K-12 LF)

Cell lines

K-12 λ prop

1821

1736 adsorption

811 ?

1485 indicator, plates, top

1736 $\frac{2.6\times10^{3}}{1.9\times10^{10}} = 2/10^{7}$ λ

811 $\frac{3.5\times10^{5}}{1.4\times10^{12}}$ λ

$1/4\times10^{6}$

(86)

Thursday - 6/5

- W811 - Aerated cult - for gal- λ effect.

EMB gal	boiled λ (K-12)	K-12 λ (K-12)	811 λ	1821 λ
1.	0.1	—	—	—
2.	—	0.1	—	—
3.	—	—	0.1	—
4.	—	—	—	0.1

811 cells	6/6	6/7	no. pop. picked	no. pop. growing	gal +	gal -	ark
0.1	c.10	47	16	16	14	14	14
0.1	c.150	394	56	28	25	28	28
0.1	c.20	50			↑		
0.1	c.20	51			all pure		

Papillae ✓✓

Papillae ✓✓

- W1736 Aerated culture - for gal- λ effect.

EMB gal	boiled λ (K-12)	K-12 λ (K-12)	811 λ	1821 λ
1.	0.1	—	—	—
2.	—	0.1	—	—
3.	—	—	0.1	—
4.	—	—	—	0.1

1736 cells	6/6	6/7	no. pop. picked	no. pop. growing	gal +	gal -	ark
0.1	c.5	12	8	7	5	5	5
0.1	c.60	276	56	52	47	52	52
0.1	c.5	27					
0.1	c.5	13					

about 30 sectored

all sectored

W1736 - attempt to adsorb agent from λ prep -

1. add 1/10 vol λ (K-12) to 1.0 ml c.10⁸ cells/ml - incubate 15 minutes - centrifuge - remove 0.2 ml, resuspd. remainder.

EMB gal
1. 0.2 ml from adsorbate + 0.1 ml fresh 1736 cells
2. 0.1 ml

1. resuspd. 1736 cells in 2 ml λ (K-12) - adsorb 15 min - centrifuge - remove 0.1 ml to make 1. resuspd. adsorbent.

EMB gal
1. 0.1 ml adsorbent + 0.1 ml fresh 1736 cells
2. 0.1 ml resuspend + 0.1 ml BHI

Monday

6/9 ~~Cobas~~ Papillae picked from "transductions" replicated.

1821 pg 85
replica to Lac, Xyl, D(0)

See page 85

	no pap. picked	# growing	1 gal-xyl-lac- aux: # gal+	# xyl-	# lac-	# aux
λ treated	63	~~59~~	58	58	58	58
untreated	16	13	10	10	10	10

1736 pg 86
replica'd to Lac, D(0)

see pg

	no. pap. picked	grow	gal-	lac-	aux
λ treated	56	52	47	52	52
control	8	7	5	5	5

811 pg 86
replica'd to Lac (D(0)) -

	no picked	grow	gal-	lac-	aux
λ treated	56	28	28	28	28
control	16	16	14	14	14

see pg 86

Control plate
replicated to Lac, Xyl, D(0) -
see individual cultures - 91

Tuesday 6/10

F - 1736 gal + - λ lysate - (8:30 → 11:00) (aerated) - centrifuge, repel sol, incub 30 sec., add 10 ml Pen. inc @ 11:45

I - 84 gal + - λ lysate - incubated 12:00

H - 902 try gal transduction - 8:30 → 11:00 (aerated)

EMB gal	bridal λ	K-12 λ LB	902 10X adb	1 pipelines 6/11	6/12
1	0.1	–	0.1	0	13
2	–	0.1	0.1	0	11

N - 84 - try Sr transduction - 8:30 → 11:00 (aerated)

NSA plate	bridal λ (K-12)	[redacted] λ	84 10X adb
1	0.1	–	0.1
2	–	0.1	0.1

} incubate 2 hrs - resuspend with 0.15 ml step - 11:15

plate	6/11 colonies	6/12
1.	0	1
2	0	0

} discard - unit of Sr too high? parent (84) for degree in Sr

– 1405 - try filtrate of 1477 for transduction – centrifuge, repel decant, filter – (8:30 → 11:00)

N - 750 - try gal transduction

EMB gal	bridal λ	K-12 LB λ	750 10X adb	1 pipelines 6/11	6/12
1	0.1	–	0.1	0	0
2		0.1	0.1	230	409

$\frac{4.09 \times 10^{7}}{1.9 \times 10^{10}} = 29/1^{7}$ λ

Gal

– 1736 try general adsorpt. with λ for transduction aerated cells 8:30 : 11:45

1.0 ml λ K-12 LB added – inc @ room temp 30 min → 0.1 ml XO+AT

↓ 0.1 ml to 10 ml Pen

↓ 6/11 no colonies

N - W112 X W1655 - 10 loc - colonies picked and streaked on EMB Gal -

6/11 - 9 streakings - grew

1736
$\frac{3.2\times10^{3}}{1.9\times10^{10}}$ - 2+/3 λ

6/12 Thursday.

— W1736 - try 1485 filtrate for gal effect

EMB gal	1736 10X cells	λ lysate	bacteria λ	1485 filtrate	811 gal λ	1736 gal λ	6/13	6/14
1	0.1	—	0.1	—	—	—	3	12
2	0.1	0.1	—	—	—	—	c. 20	321
3	0.1	—	—	0.1	—	—	1-2?	6
4	0.1	—	—	—	0.1	—	c. 50	191
5	0.1	—	—	—	—	0.1	[illegible]	19

— 811 - try effect of λ from 1736 gal + conc 811 gal

EMB gal	811 10X cells	λ (bacteria)	1736 gal λ	811 gal λ	6/13	6/14
1	0.1	0.1	—	—	c. 13	29
2	0.1	—	0.1	—	c. 50 per plate	20 (not as well developed as #1)
3	0.1	—	—	0.1	c. 15	31 (" " " ")

Confirmed titer of 1736 = 1676
by absorb = 88.1

— Below with both 811 and 1736 using 1(-12) λ

Results

6/14 after 2 days

811 absorb 15 min - 341 - 29 = 312
811 supernat. - 69 - 29 = 40 Controls shown ↑
total spotted = 394

1736 absorb . 281 - 12 = 269 276
1736 supernat. 33 - 12 = 21

10 ml cells
↓
spin
↓
resp in 1.0 ml

0.1 ml
1.0 ml K-12 λ
mix 15 min

0.5 ml

centrifuge

centrifuge - discard

supernat

0.1 ml

cells resp in 1.5 ml buff

plate
= Absorb

plate
supernat

results indicate that 70 - 90% of activity is taken up by the cells in 1st 15 min of contact

Friday 6/13

Phage titrations - agar layer method - all preps filtered.

I - 1736 gal+ (λ + 882 + 1676) /1485

10^8 = 0
10^2 = c. 150 layer plaques } this prep (see pg. 88) gave ca. 5000 small
c. 10^4 small " , probably λ } plaques on 811

I - K-12 C9 /1485
10^8 : 233 = 2.3×10^{10} λ/ml

I - 1821 λ /1481
10^8 : 41 = 4.1×10^9

I - 811 λ /1485
10^8 = 169 = 1.7×10^{10}

I - 811 gal+ λ /1485
10^8 = 396 = 4.0×10^{10}

I - 811 u.v. radiation resistance -

Dose	Ø Bloc count	
0	319 (wet plate)	1.0
5	238	0.75
10	wet plate	—
15	121	0.38
30	38	0.12
45	22	0.07

I - 1661 u.v. rad. resistance

Dose	ETMB loc count	
0	206	1.0
5	157	0.76
10	134	0.65
15	wet plate	—
30	37	0.18
45	16	0.08

Monday June 16

- 750 gal "transduction" - 2 pg .87 EMB
  64 pepsidase picked to gal for check -
  64 green
  64 gal (2 mixed)

- conn 2 W112 X 165 J — EMB
  2 ↗ lac- col picked and streaked - light sufficiently streaked out to pick single - col.
  Tested on EMB(L) vs 1-2

  8 of 1 sensitive to 1-2 indicating 4p2[R]

811 $\frac{3.0\times10^{9}}{1.9\times10^{10}} = \frac{1}{6.6\times10^{}}$ transduction poor efficiency

518 $\frac{2.1\times10^{9}}{1.9\times10^{10}} = \frac{2}{10}$ also incubated with 902 λ

Tuesday 17.

– 518 λ^{s} – "Transduction" for gal – aerated cells –

	EMBGal	518 10x cells	heated λ^{K12}	K-12 LYS+ λ	902 λ	6/17	6/19
I	1.	0.1	0.1	–	–	10	4 4
	2.	0.1	–	0.1	–	>50?	1/16=132 2112
	3	0.1	–	–	0.1	>50?	1/8=139 1112

recorded

7 fold

– 811 λ^{t} – transduction effect with 902 gal$_2$ –

	EMBGal	811 10x cells	heated λ^{K12}	K-12 LYS+ λ	902 λ	6/17	6/19
I	1.	0.1	0.1	–	–	c.45	89
	2.	0.1	–	0.1	–	c.100	296 ± 74 wet plate - count should may be much higher - smear in one quad
	3.	0.1	–	–	0.1	c.50	202 5+ fold

296 − 89 = 207

$\frac{1.13\times10^{9}}{4.9\times10^{10}} = \frac{1}{4.3\times10^{6}}$

– 902 λ – titer

poor plates – too wet!

I $10^{7} \rightarrow 0.1$ ml + 0.1 518 cells (10x) → >50 – means titer around 10^{10}

I 58-161 λ – titer

poor plate also – wet

$10^{7} \rightarrow 0.1 + 0.1$ 518 cells → >30 – titer around 6×10^{9} –

Thursday June 19

- 750 from papillae elimination of pg 87-88
  1 picking streaked on EMB gal to purify - for back cross - (impossible w 750, gal⁻)
- W811 - stock streaked out - to pick single colony - lower background of (gal+) if possible -
- W811 gal+ "transductant" of previous page -
  1 picked and streaked on EMB gal to purify - backgrow
- W750 gal+ transductant
  64 pcp on gal - replicâd to lac, D(c)
  64 gal+
  64 lac-
  64 failed to grow on D(c)

$\frac{5.4\times10^{3}}{1.4\times10^{10}} = \frac{1}{3.9\times10^{6}}$

- 750 gal trans effect.

	EMB gal	gal⁻ 150-1	0 Mut λ	gal+ 1.4×10¹⁰ K-12λ-L7+	gal⁻ 1.7×10¹⁰ 811 λ	gal⁻ 1.0×10¹⁰ 821 λ	gal⁻ 4.9×10¹⁰ 902 λ	Spontaneous Reversion gal⁻ 4×10¹⁰ 811 galt λ	gal+ 0 1485 Filtrab
	1	0.1	0.1	–	–	–	–	–	–
	2		–	0.1	–	–	–	–	–
	3		–	–	0.1	–	–	–	–
	4		–	–	–	0.2	–	–	–
	5		–	–	–	–	0.1	–	–
	6		–	–	–	–	–	0.1	–
	7		–	–	–	–	–	–	0.1
6/20			2	200	15	3	150	100	–
6/21			2	542	43	31	176	144	3
			Plates 1 dilut/λ	3.9×10⁶	3.5×10⁷	3.5×10⁷	3.6×10⁷	3.6×10⁷	

- 811 - transduction by 902 λ -
  from 902 transduction - 71 pcp picked to EMB gal -
  71 gal+, 72 lac⁻, 72 failed to grow D(c)
- 518 - transduction on gal
  49 pcp picked from K-12 λ - plate -, 49 gal+ 49, lac⁻, 49 failed to grow D(c)
  56 pcp " " 902 λ - plate -, 56 gal+, 56 lac⁻, 56 failed to grow D(c)

58-161 λ
del 10^{-7} → 0.1 ml pooled/1485 = 18 = 1.8×10^{9}

902 λ
del 10^{-7} → 0.1 ml pooled/1485 = 49 = 4.9×10^{9}

18H λ
del 10^{-7} → 0.1 ml pooled/1485 = 101 = 1.0×10^{10}

Monday June 23

- 1655 X 112 - Lp2R - Attempt low transduction

EMBlue	10X cells (uncult)	broth	heated K_{12}^{434}	K-12 λ	811 λ	papillae 6/26
1	0.1	0.1	—	—	—	1/4 = 105 : 420
2	0.1	—	0.1	—	—	1/4 = 186 : 744
3	0.1	—	—	0.1	—	1/4 = 75 : 300
4	0.1	—	—	—	0.1	1/4 = 120 : 480

presumably no effect either on Lp2R or on Lp2S - Background too high —!

- 1655X112 - Lp2S - as above

EMBlue	10X cells (uncult)	broth	heated K_{12}^{434}	K-12 λ	811 λ	
1	0.1	0.1	—	—	—	1/4 = 248 : 992
2	↓	—	0.1	—	—	1/4 = 191 : 764
3		—	—	0.1	—	1/4 = 179 : 716
4	0.1	—	—	—	0.1	1/4 = 143 : 572

- Titration 750 lysate

dilute 2, 4, 6-7 → 0.5 mL + 0.5 mL 1485 → 0.1 mL, 37° 20 min → 1 mL (wet plate) → 2.4×10^{10} (8 × 10⁸)

2. >>74

- 811 - Background examination - thin culture from single-colony-isolated stock

EMBgal	811 10X cells	broth	heated K_{12}^{434}	K-12 λ	750 λ	papillae 6/25
1	0.1	0.1	—	—	—	50
2	↓	—	0.1	—	—	417 ←
3		—	—	0.1	—	85
						↑ 3.5×10^2
						$> 2.9 \times 10^{10}$ = 1 / 6.6×10^7

$\frac{9.6 \times 10^3}{1.4 \times 10^{10}}$ = 3/X

1/3.8×10^6 λ

Gal q -

and 750 effect -

- 750 Examination of 750 λ for activity -

EMBgal	750 cells	broth	heated λ	K-12 λ	750 λ	papillae 6/15
1	0.1	0.1	—	—	—	2
2	:	—	0.1	—	405 ←	
3	..	—	—	0.1	2	

$\frac{9.1 \times 10^3}{1.4 \times 10^{10}}$ = 3/X

1/3.3×10^6

Gal l -

Cultures:
518 - Bri Novobiocin, Sm
1485 - ind.
902 - conj ♀ 655
655 - conj ♀ 902

548 Gal-
$\frac{2.9 \times 10^4}{1.4 \times 10^{10}} = 2/10^6 \lambda$ (95)

Tuesday June 29

- W518 - Gal deletion - using 811 λ as control on degree of lysis - selection of preexisting gal + eliminated?

	EMS gal	10X aerobic on 1 cells	boiled K-12 λ	K-12 λ	811 λ
μ	1	0.1	0.1	—	—
	2	↓	—	0.1	—
	3		—	—	0.1

proplica 5/26 higher than previous
163 K
1/11 = 178 = 2848
86 K lower because of killing due to 811 λ

178 IC
1068
78
2848

- W518 S+ -

	NSA plate	1 λ 10X tracked cells	1821 boiled λ	1821 λ	no. col 6/22
	1	0.1	0.1	—	6
	2	↓	0.1	—	11
	3		—	0.1	3
μ	4		—	0.1	6

} apparently no S+ deletion again

- W902 x W1655

	EMS-gal	902 2X cells	1655 2X cells
	1	0.1	—
	2	0.1	0.1
	3	0.1	0.1
μ	4	—	0.1

- Wednesday

736 Transduction - for the purpose of comparing the stability of spontaneous and galduced - expt as previous.

	EMS gal	heated K-12 λ	K-12 λ	6/27 no. of plaques
μ	1	0.1	—	34
	2	—	0.1	253

Gal-
$\frac{2.5 \times 10^3}{1.9 \times 10^{10}} = 2/10^7 \lambda$

Tuesday 7/1/52 - Catching up

- From the cross - to find gal$_2$ - 4p$_2$S

902 X 1655 - 25 gal- picked, streaked once on EMB gal, single colonies streaked against λ-2 - all resist = 4p$_2$R
Continue to pick

- From the papillae picked from
750 - (page 93)
transduced by 902 - segregating - single colonies purified & streaked
811 gal+
811 gal-
1021
still segregating - stocks made -
of 750t 902 both gal+ and gal- stock

750t 902 gal-
spread on EMB gal - after 2 days 3 papillae (after 3 = 9 pap)
apparently no more stable than 750 gal+

- 1692 attempt production on EMB(o) + heat + trypt.

D EMB(o)	heated K-12 λ	K-12 λ	unadsorbed 1692	cul after 3 days	% picked to EMB loc
1	0.1	—	0.1	c. 15	
2	—	0.1	0.1	c. 500	10 = all failed to grow - indicating contamination

- 1920 G$_0$ 1692

1.	0.1	—	0.1	c. 0
2.	—	0.1	0.1	8

- 750t K-12
10 gal+ pap. picked to EMB gal
1st streaking - all mixed +, - cross.
2nd " - 8 show - cul., 6 show ①
3rd " 9 " " " too many col to note mosaic
4th " 7 " " "
5th 4 mixed picked and streaked ② mixed still

- 811t K-12
10 gal+ pap. picked to EMB gal
1st streaking all mixed +, - cul 4th streaking 6 mixed
2nd " 8 " " " 5th " " 4 picked from mixed - all still mixed
3rd " 6 " " "

now → t 1692
- 1736 (S) K-12 gal+ pap.
10 picked
1st streaking all mixed +, - cul. 4th streaking 7 mixed
2nd " 6 " " " 5th " 4 4 picked from mixed - all still mixed
3rd " 7 " " "

error reversed

- recheck - 1736 (EMB) K-12 gal+ pap.
2 2nd streak
photo shows 9 successful streaking
or spont. plate
10 picked, 9 grew
1st streaking all mixed +, - cul.
2nd " " appear pure + but not strong.
3rd " " " "
4th " " " pure

Tuesday 7/1 - continued -

- 518t K-12 salt
  10 aqueduct picked
  1st streaking - all mixed +, - col
  2nd " - 6 mixed +, - col.
- 518t spontaneous salt
  10 aqueduct picked
  1st streaking all mixed +, - col.
  2nd " " pure + col.
  3rd " " pure + col
  4th " " "

2nd streaking - 6 mixed, +, - col
~~streaked 4th time~~
~~streaking~~
3rd streaking 6 mixed,

recorded

Wednesday 7/2

- 518t K-12
  10 gal + col streaked against 1 - all resistant.
  4 gal - from transduced streaked against 1 - all resistant

Friday - Sat July 4-5

New lysates:

- 1439 aerated culture.
  dil. 10^8 = 108 plaques = 1.1×10^{10}
- 872 aerated culture.
  dil. 10^8 = 3 = 3×10^8
- 750 t 1821.
  dil 10^8 = 65 = 6.5×10^9

Titers degrade presumably contaminated

750 galdemiin

EMB gal	Mod	K+L7-8	872 3.9×10^9 λ/ml	750 t 1821 λ
1	+	—	—	—
2	—	0.1	—	—
3	—	—	0.1	—
4	—	—	—	0.1

Capillaries

20. 1 day — Col 1

1 ← — | $\frac{4.3 \times 10^3}{1.4 \times 10^{10}} = \frac{3}{10}$ λ

426 ← | $\frac{1}{3.3 \times 10^6}$ λ

35

388

$\frac{3.9 \times 10^2}{3.9 \times 10^9} = \times 10^6$

Summary of phenotypes picked from cross 902 X 1655

Picking	tp_2R	tp_2S
9	9	
16	16	—
37	30	7
62	55	7

tp_2^r gal - X tp_2^s galt → ½ tp_2^s gal -

7 tp_2S picked EMB gal for purification & tp_2R picked likewise

Thursday - July 10 - Catching up.

Gal 2 - $\lambda p2^5$ - phototroph - from 892 X 1655

- 2050 - Streaked cultures -

EMB gal	2×10^{10} cells	K12 λ ^{LG} = 2.3×10^{10}	892 λ	no papillae after 2 days
1	0.1	–	0.1	½ = 227 = 454
2	0.1	0.1	–	1/16 = 123 = 1968

$\frac{1.414 \times 10^9}{2.3 \times 10^{10}} = \frac{1}{1.6 \times 10^6}$

Reexamine to obtain better estimate of background - 892 λ appears susp. high - some activity.

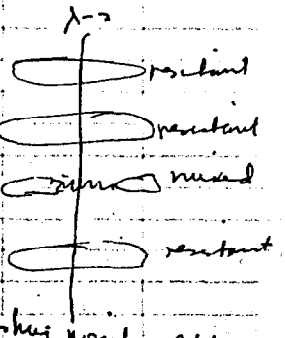
- 750 } Make $\lambda p2R$ and examine for transduction.
- 811 }
- 518 } cross streaked with λ-2 - single colonies picked
- 1736 } and streaked, re streaked - restreaked.

518 appears to have 2 types of variants one very opaque, one only slightly opaque

Making $\lambda p2R$

	EMB gal	10^8 cells added	additions	no papillae (2 days)
	1	750	–	1
	2	750	K-12 λ	2
	3	811	–	50
	4	811	K-12 λ	57
	5	518	–	69 } not $\lambda p2R$ - still mixed
	6	518	K-12 λ	–
	7	1736	–	7
	8	1736	K-12 λ	15

Cells disappearing from gal during spot tests - streaking of gal reexamine results. 892 $\lambda p2R$



interesting point - although this culture is gal- the $\lambda p2R$ from (opaque) is able to grow from gal - papillae of polycell. - poor

Streaking of gal papillae from gal duction - 5th streaking

1736 tK-12 - 4 still unstable +, - col on EMB gal

750 tK-12 - 3/4 still unstable +, - col on EMB gal

811 tK-12 - 4/4 still " " " "

518 tK-12 - 5/5 " " " "

1578 gal 4 - F-518 (?) check.

EMB gal	additions tested	no papillae
1	0.1 ml λ	10 (custom. abs)
2 LG	0.1 ml K-12 λ	1/16 = 190 = 3020
3	0.1 ml 811 λ	18

$\frac{3 \times 10^9}{2.3 \times 10^{10}} = \frac{3}{23} \times 10^{-1}$

Gal 4 -

← 5 picked streaked on EMB gal - appear mixed

Saturday 7/12 Still catching

- 811tK-12 X 1436 (after 3 days) on EMS gal
  control streaking of 1436 } - gal -
  of 811tK-12 } - mixed, predominately gal +

Protoplasts

1. 33 gal +
   8 gal -
2. 40 gal +
   6 gal -

3. control plating of 1436, 811tK-12 showed no growth

carried to next page

73 = 5/14 = 1

- 518t K-12 X 1436 (after 3 days) on EMS gal - 518t11-12 mixed, predominately gal +

1. 38 gal +
   c. 60 x 16 = 960 gal -
2. 48 gal +
   c. 55 x 16 = 880 gal -

3. control plating of 1436, 518tK-12 showed no growth

carried to next page

1840/86 = 21/1

(received)

- 1655 X 902 - Selection and purification of gal$_2$-Lp$_2$R and gal$_2$-Lp$_2$S

Lp$_2$R

EMB gal	10X cells	washed K-12 λ	K-12 λ	902 λ	no lap 3 days
1	0.1	0.1	–	–	14 (contam?)
2	↓	–	0.1	–	19 ← suggestive of plagues
3		–	–	0.1	8 ← in all - clear small pinpoint ppt.

tested at this time & used and found a reverter

Lp^{2}S

EMB gal	10X cells	washed K-12 λ	K-12 λ	902 λ	no lap 3 days
1	0.1	0.1	–	–	20
2	↓	–	0.1	–	4:89 = 356 ← suggestive plagues like obs
3		–	–	0.1	10

$\frac{3.4\times10^3}{1.4\times10^6} = \frac{1}{4.2\times10^6}$

Received from EMC - 1692 X 1402 - to obtain gal - pro - bact +
to pick and streak 3 ~~colonies~~ portions marked (yellow circle)
this is on EMB bac and portions are bac - bac +

2/3 grew

maybe live dilpt and looked on plates

Sunday 7/13 1692tK1402 restreaked on EMB gal -

2/3 grew

Tuesday 7/15

Crosses
518 – make 4p2R ok – streaked agar 1-2
811 – make autotrophs, ~~4p2R~~
1655 – look for gal –
1485 – natural 1, assay.
750 – gal deleted as false chr } cell no's
518tK-12 – stability check ok (streaked on EMB gal from overnight cult.)
lysate
811tK-12, stability check ok (streaked cult. figured)
lysate ok

On the cross 811tK-12 X 1436 page 100
all colonies on plates picked. – 83/87 = 95% grew on first streaking
judging by streakings EMB gal

	picked
10 gal –	14
73 gal +	73

of the gal + streakings
20 show evidence of mosaic colonies – (27%) some streakings with several mosaic colonies / streakings.

Purification 1

Restreaked –

Of the cross 518tK-12 X 1436
sectors of gal plates:
20 gal – picked and streaked } – 20 grew } all from one cross plate
20 gal + picked " " } – 20 grew } other plate saved.
– of the gal + streakings
5 showed mosaic colonies – (25%) (per plate)

1692 X 1402
Purification – restreaked 3rd time EMB gal – 2 representative of 3 originally received.
2nd

Wednesday July 16 - 52

See previous page

- 518tK-12 X 1436
  20 gal - } from primary streaking - restreaked -
  20 gal + } unstable, with mosaics, picked first in order - then apparent stable. ✓

- 811tK-12 x 1436
  10 gal - picked from 1st streaking and restreaked.
  Out of EMB gal - store gal + until 7/17.

- 518tK-12 } lysate - cleared after 4 hours. ✓
- 811tK-12 }

Thursday 7/17/52 - 1924 — from two days previous Monday 7/14

EMB gal	1924 cell not aerated, 10^8/ml	1924 + K-12	K-12 λ	no Lap
1	0.1	0.1	—	29
2	0.1	—	0.1	129

← check for lysogenicity -

Gal + - $\frac{1.3 \times 10^3}{1.4 \times 10^{10}} = 1/10^7 \lambda$

Thurs. -
518tKX1436 - ABOVE - 2nd streaking
20 gal - streaked - 19 grew (1 dirt) all pure gal - ✓
20 gal + - streaked - 20 grew — all mixed ± col. less mosaics (3) Than in 1st streaking
1 of 5 1st streaks showing mosaics - one showed mosaic

811tK-12X1436 ABOVE.
12 gal - picked + streaked pure gal - 2
of 12 / 11 pure gal - , - 1 mixed -

518/λ-2 λ-2 resistant col apparent.

518t K-12 } - from stock → overnight cult. streaked out on EMB gal
811t K-12 } both show mixed ± col - 10 gal +
1 gal -

Thursday 7/17/52

- 518 tK-12 x 1436
  19 gal- from Purification 2 - as streaked (6/plate) ✓
  20 gal+ " " " " (no particular order) ↓
- 811 tK-12 x 1436
  17 gal- from Purif. 2 - as streaked ↓
- 518 tK-12
- 811 tK-12 from stock cultures
  1. streaking ~~from~~ from first with culture dish mixed ↓
  2. single colony picked, as streaked. ↓
- 1924 - gal derived previous page.
  all control plate papillae picked streaked EMB gal = 29 pap.
  equivalent no - " " gal derived picked. = 29 pap. ↓
- 811 tK-12
  gal+ passed up yesterday
  2 dilutions, remainder streaked (P-2) as EMB gal. ↓

~~~~~

Friday 7/18/52

Report as above

518 tK-12 x 1436
  19 gal- all gal- ✓
  20 gal+ 19 appear mixed, no gal+ pure-

P-2
811 tK-12 x 1436
  17 gal- all gal-
  64 gal+ (1 plate with lost from original 72) 62 suitable, mixed,

- 1924 tK-12
  29 untreated plate pap. streaked - 28 grew - all ranges of +, from dark to light
  15 appear mixed, no mosaic clumps noted.
  29 from K-12 d plate streaked - 21 grew - all ranges of +
  10 appear mixed, maybe a mosaic colony.
- 518 tK-12
  811 tK-12 } strains streaked on EMB gal
  still mixed ✓
~~~~~

Friday - 7/18

- 1924 - treated and untreated papillae restreaked - P2
  28/24 treated papillae streaked against 1485
- 811 EK-12 x 1436 -
  papillae picked from P.2, streaked overnight in refrigerator.
  streaked P3 Saturday 7/19

Saturday.

7/19 -

1924 above - of streaking P2

Spontaneous 28 gal +, are pure gal +, varying degrees of gal - -ness
K-12 gal blend 12 +, 3/12 mixed, also varying degrees of gal + -ness = suggestive of ? Streak
≈ plate count 8. dropped (contained few if any unstable) stability?

streaking against 1485
28/21 gal + from K-12 cluster - none showed evidence of phage action in 1915

1924 above restreaked.

Sunday - Monday 7/20 - 7/21

- 2062 Attempted proline reversion -

isolate 1.	EMB gal	D(-)	washed λ^12	K-12 λ	no. days 2 drop	
—	+	−	0.1	−	9	
	+	−	−	0.1	271	
	−	+	0.1	−		} solid growth - prototrophic?
	−	+	−	0.1		
isolate 2						
	+	−	0.1	−	2	
	+	−	−	0.1	180	
	−	+	0.1	−	no growth	
	−	+	−	0.1	solid growth —	

Gal + : $\frac{2.7 \times 10^7}{1.9 \times 10^{11}} = \frac{2}{10^7}$ λ

- 7/21
  1924 EK-12

  28/12 P-2 papillae on EMB gal - only one appears mixed - discard and repeat streaking for better background.

  28 P-2 spontaneous papillae - all pure gal + - discard -

7/21 Monday ~~Friday~~ (Cont)

- lysate streaked out on NSA

- 750 Check on stable gal+ transductn

EMB gal	Nothing	K-12 λ	no pap. 2 days
1/2	0.1 —	0.1	ca. 254 —

$\frac{2.54 \times 10^3}{1.9 \times 10^{10}} = \frac{2}{7.5 \times 10^6}$

- 2062-1, 2062-2

	EMS loc	neutral λ	K-12 λ	
-1	1/2	0.1 —	0.1	no growth again (see pg 106)
-2	1/2	0.1 —	0.1	again shows prototrophy - discarded

7/22 Tuesday

- Repeat crosses -
  - 811 EK-12 X 1436
  - 918 EK-12 X 1436

- Repeat 1924

- Repeat 2050

See next pg

Thursday July 24 -

1924

- Gal duction repeat -

EMB gal	Addition	No of colonies/plate
1	—	40
2	a 1 ml K12 λ	161
3		

L9

$\frac{1.2 \times 10^{3}}{2.3 \times 10^{11}} = \frac{1}{10^{8}}$ (gal 1 -)

- 2062

Attempted protein ductions -

EMB gal	Addition	Observation after 2 days
1	—	no growth
2	a 1 ml K12 λ	no growth

L9

- 2050 gal 3 -

EMB gal	Addition	No. of plaques/plate
1	—	11
2	0.05 K12 λ	1/4 = 172
3	0.1 K12 λ	1/4 = 223

11

688

892

$\frac{8.8 \times 10^{}}{2.3 \times 10^{11}} = \frac{1}{2.6 \times 10^{6}}$ λ Gal 3 -

$\frac{1.86 \times 10^{9}}{2.3 \times 10^{11}} = \frac{1}{1.7 \times 10^{6}}$ λ

- 518 EK-12 X 1436 repeat on cross

- uncentrated cell suspension used = 10^{9}/ml 0.1 ml plated -

518 EK-12 suspension from streaking on EMB gal agar / mixed (10 gal+ ca. / 1 gal- ?)

EMB gal	Addition	No. colonies	No gal+	No gal-
1	0.1 ml 518 EK-12 cells	0	0	0
2	0.1 ml 518 EK-12 + 0.1 ml 1436 cells	35	0	35
3	" " " "	30	0	30
4	0.1 ml 1436 cells	0	0	0

- 811 EK-12 X 1436 repeat cross

- uncentrated cell suspension as in above cross -

811 EK-12 suspension from streaking on EMB gal agar / mixed (>20 gal+ ca. / 1 gal- ?)

EMB gal	Addition	No. colonies	No gal+	No gal-
1	0.1 ml 811 EK-12 cells	0	0	0
2	0.1 ml 811 EK-12 + 0.1 ml 1436	12	11	1
3	" " " "	17	16	1
4	0.1 ml 1436 cells	0	0	0

approximate results obtained previously -

- Phage lysates examined for sterility - streaked on NSA

Source	
1821	- no growth
heated K-12	- no growth
892	- no growth
750 t 1824	- 1 small colony -
1437	- no growth
811 (gal+)	- no growth
K-12	- no growth
811-2	- no growth
811	- no growth
750	- no growth
902	- no growth
1736 gal+	- no growth

Incub. 37°

Invad. K-12 lysate

Time	
60	- no growth
120	- no growth
180	- no growth
240	- no growth

Orders of stability 750 > 2050 > 1924 (K-12) > 811, 518

gal, [illegible]

Saturday 7/26

- 750 from pg 105 - Check on stability of galactose from treated plate to peptone plate.

1st streaking - 12 appeared unmixed - ~~streaked~~ rep from below non-mixed
28 appeared mixed - 28/29 mixed

7/27
2nd streak

- 2050 gal - pg 106 - stability check on galactose
5 spirit picked - all mixed 1st streaking
- all pure 2nd streaking

10 K-12 gal-induced picks - all mixed 1st streaking
- 9/10 appear pure 2nd streaking
10/10 appear pure 3rd "

- 1924 + K-12 (K-12 λ)
24 pop picked from treated plate for stability check.
1st streaking 15 appeared mixed - restreaked -
5/24 appeared mixed - 2nd streaking
8/24 " " - 3rd streaking

Gal 1 -
1.9×10^{-3} : 1/15
1.9×10^{-5} : $1/7.6 \times 10^{6}$ λ

- 750 - Stability and nature of mixed λ galactose-ness from overnight unaerated culture.

Exp'l gal	Addition (0.1)	no. of pop 2 days	
1	—	1	
2	K-12 λ	186	186
3	m 60 K-12 λ	1/6 = 107	856
4	m 120 K-12 λ	1/6 = 208	1664 — Pop picked from here for stability check.
5	m 180 K-12 λ	1/6 = 161	2576
6	m 240 K-12 λ	1/6 = 183	2928
7	λ-2	0	— (constant throughout columns)

24 pop picked from m 120 λ and streaked.
22 grew -
20/22 appear mixed 2nd streaking

discarded - attempt with 93 to see if possible to pick up gal T⁶

- 811 + K-12 X 1436 -
carried through 8 single colony isolations - after purification - still segregating gal - after
discarded -

Sunday 7/27

test not very satisfactory

gal+
811EK-12 X 1436 - check on purified prototrophs

	lac	gal	D(0)		λ	λ-2		No. D(0)	λ	λ-2
1.	-		G	1.	λ	λ-2	31		failed to grow	
2.			G	2.			32	G mostly D(0)	λR	λR
3.			G	3.			33			
4.			G	4.			34			λ-2S
5.			G	5.	λR	λ-2S	35			
6.			N.G				36			
7.			G	6			37			
8.				7.			38			
9.				8	(9)		39			
10.				9.			40	all lac-		
11.			G	10.	failed to grow		41			
12.			~~N.G~~		λR	λ-2S	42	gal+	failed to grow λR	λ-2S
13.			G	11	failed to grow λR	λ-2S	43			
14.				11			44			
15.				12			45			
16.				13	failed to grow		46			
17.				14	λR (12)	λ-2S	47			
18.	all lac-	all gal+ or mutable	~~N.G~~				48			
19.			G				49			
20.			~~N.G~~				50			
21.			~~N.G~~		λR	λ-2S	51		λR	λ-2S
22.					failed to grow λR	λ-2S	52			
23.							53			
24.							54			
25.							55			
26.							56		(43)	
27.							~~57~~			
28.							~~58~~			
29.					(19)		~~59~~			
30.					failed to grow		60			

-Tuesday 7/29

518 HC-12 X 1436 = I

purified "prototrophs"
20 gal+ examined, 19 prototrophic
all loc-
all lysogenic when replicated on 1485 ✓

20 gal- examined, 18 prototrophic
all loc-
all non lysogenic when replicated on 1485

811 HC-12 X 1436
purified "prototrophs."
12 gal- examined 9 prototrophic, all loc- all λ-2 sensitive
5 lysogenic when streaked against 1485 } ones lysogenic found λ^R from before
4 sensitive when " " X } ones found sensitive, found non lysogenic, above

from page 108: 56 gal+ picked, all loc-, 6 failed to grow on D(-)
of those growing on D(-)
43 took successfully against λ - all λ^{-2}
" " λ-2- " λ^R.
56 gal replicated against 1485 - all lysogenic.

750 X 1503 on EMB gal

1177 X 1655 on EMB gal

750 HC-12
gal+ 6 stables, 6 unstables } from pg 107. picked and restreaked - further ✓ instability and progress unstable → stable
EMB gal
6 unstables - unstable after 3 transfers - mixed gal+, gal-
6 appear stable, with possibility of being mixture.

1924 HC-12
of 24 guidances - pg 107.
8 unstables on EMB gal - streaked against 1485 - no evidence of lysis - all presumed non lysogenic
8 apparently remain unstable
↳ combined streaking of 8 = streaking #4
streaking not found 8 unstables - (gal+, gal-)

Wednesday July 30 -

- 1655 X 902 Lp2^S for V vs stability of gal + - begun 7/28

EMB gal	addition	no pgs. 2 days
1	0	9
2	0.1 ml K12 / ↑ assume 1.9×10^{10}	158

- 8 streaked out for stabil. ✓
- 2 streaked out " "

$1.5 / 1.9 = 1/9.3 \times 61$

- Thursday 7/31

1655 X 902 Lp2^S above

8 spontaneous streaked (1) — all mixed } see pg 111

24 EK-12 " — all mixed

Cross-streak page

750 X 1503 to get TLB gal - EMB gal

	centre colonies	periphery
mixed plates	1.	2 gal +, 13 gal -
	2.	2 gal +, 9 gal -

pick gal + streaks on EMB gal -

1177 X 1655 to get mal - - - for gal deletion rev -

	centre plates	barren
mixed plate	1.	c. 1000 colonies, (8-9 gal + w. 1 gal -)
	2.	c. 1000 " " "

- 518 with mal. lambda to get gal deletion with and lysogenicity

EMB gal plate	add. ml	no. pgs. 2 days
1	0	19
2.	0.1 ml 1/100 mitgo	61

pick 2g - exams for 1. stability 2. λ^S see pg 111

← pick single colonies to 1. see segregation of stable - 2g vs spontaneous mutations 2. test lysogenic, stable, periphery 3. spont. deletes 4. 2g lysogens, cross plate, prophage

NOT INDEXED
BEYOND HERE

Thursday 7/31
Friday 8/1

- 518 t m 240 K-12 λ - 29 papulae picked in attempt to find λ^S gal^- unstable?
1st streaking 7 streakings showed mixed colonies ⊛ - these streaked against ~~HFr~~, λ (all λ^R) discarded
all 29 appeared mixed
all restreaked.

- 902 X 1655 Lp_2^S
✓ on stability & gal^- success -
of 30 papulae picked
2nd streaking - 5 unstable ~~still~~ on gal^- used - 4 unstable 3rd — restreaked - } discard
1 unstable in 8 spontaneous - not unstable 3rd streaking. }

- 750 X 1502 - isolation of diploid for purpose of assessing TCB, Gal, -
only suspicious colony from 1st cross found stable EMS gal^- - discard

- 1177 X 1655 -
- 1st 32 ~~gal^-~~ mac - prototrophs picked streaked are EMS mal^- picked and streaked against λ-2 - all λ_c^R discard.
- ~~14~~ additional picked and streaked - tested against λ-2 -
- these 14 also λ-2 resistant - pick more Mal^- -

8/2

- 518 K-12 m 240 above ↑
8 unstables noted 2nd streaking - streak against λ
1 proved to be sensitive and probably unstable — picked restreaked and tested again λ^- probably sensitive, doubtfully unstable - recheck ↑ found λ^S but stable - repeat entire step -

- 902 X 1655 Lp_2^S - Gal^- deduction done in EMS gal^-

EMS gal^-	Additions	no. days	
1	—	8	1.5×10^6
2	0.1 K-12 λ (dilution 1:4 x 10^5)	2	4 x 10^5
		4	1.4 x 10^8
		8	295

~ from the cross of gal^- success X 1436 - check gal^- segregants from the prototrophs obtained by the cross for λ^S

811 EK-12 X 1436 -
7 gal^- segregants picked and tested - all λ^R

518 EK-12 X 1436
5 gal^- seg. picked and tested - all λ^R ✓

- Monday 8/4

- In the cross of the unstable, non lysogenic galdu cross, of 1924 (tp^R) 8 unstable forms obtained.

Cross of 1924tK-12 (-1) X 1436 on EMS gal

EMS gal plate	addition	Reading after 2 days
1	0.1 ml 10X 1924tK-12 (-1) abs	barren
2	0.1 ml of "1436," "1924tK-12	11 gal+, c. 384 gal-
3	" "	5 gal+, (1/4 = 142 gal-) = 568
4	0.1 ml 10X 1436	barren

16 gal+/ c. 952

Cross of 1924tK-12 (-2) X 1436 as above:

poor medium may actually be larger number of +

1		7 gal-
2	as above	7 gal+, (1/4 = 115 gal-) 460
3		4 gal+, (1/4 = 133 gal-) 532
4		barren

11/992

~~~~

- Tuesday 8/5

From the cross 1177X1655 to get Mal$^-$ tp.^{S}

30 additional gal- putative tryptes picked and streaked out (see pg 111) + tested against 1-2 all 12^R - total to date picked 60 - all tp.^{R}

From the cross 1924tK-12 X 1436 (#1)

26/30 gal- putative tryptes successfully tested against 518 for lysog. - all showed no evidence of lysis / possible ?? plaque

15/20 gal+ putative tryptes successfully tested against 518 for lysog. - all showed no evidence of lysis

30 putative tryptes (#2) picked at random - streaked/518 - all showed no evidence of lysis
~~~~

Thursday - sick

- Friday 8/8

14 36 Gal ducken - Tst 1 1954 and 2046 λ for fluccui ductai -

	EMB gal	additn	no papillae 2 days	
1		-0-	5	(halos about 1 pq.)
2		-0.1 K12 λ	¼ = 324 = 1296	(some pq ō halos, some without - no diff apparent)
3		0.1 ½ 1954 λ	77	(no halos)
4		0.1 1954 λ	¼ = 279 = 1116	(some with and some without halos)
5		0.1 2046 λ *	38	(no halos about pq.)

	D(c)HB,T		no. col.
1		-0-	
2	~~2~~	2046 λ *	1
3		1954 λ	2

- New lysate - from 8/7

K-12 mal	30 sec	$> 46 \times 10^8 = 4.6 \times 10^9$	
"	35 sec	$> 11 \times 10^9 = 1.1 \times 10^{10}$	
"	40 sec	$> 3 \times 10^9 = 3.0 \times 10^9$	
1954		$> 140 \times 10^8 = 1.4 \times 10^{10}$	
2046		20' } 20 } $\times 10^8 = 2.1 \times 10^{10}$	

8/11 23 picked streaked and single colonies taken for 1° - all were resistant to λ and preserved by sequence

- 13 picked and streaked, after 2nd streaking 3/13 mixed -

- 2 spontaneous picked and streaked, 2/2 pure gal+ 2nd streaking

- From the cross 1177 X 1655 to obtain Mal- Lp_2^S

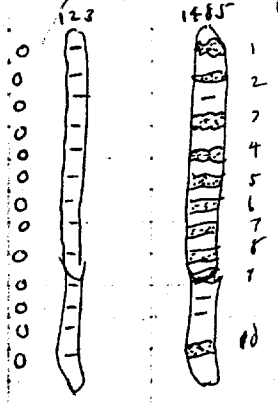
40 odd mal- picked, streaked, picked and streaked against λ-

all rp_2^L total to date 80
40
120

Wednesday 8/13

- 1924 t K-12
  - 6 unstable gal-deccas from pg 112 and before (these 6 are the 6 not yet crossed with 1486)
  are cross streaked against 1485 on EMB loc - reaction slightly interfered with but still showed no evidence of lysogeneity

- 1486 t 2046 of pg. 113
  - the 13 gal deccas picked and streaked - against NTCC 123 and 1485



none lysogenic on 123

10/13 lysogenic on 1485

- 1673 gal- ~~= 2070~~ made by exposing ca. 10^8 ~~cells~~ to 6 sec exposure to (Hanovia) u.v lamp. picked and purified 3 times. -

NO GROWTH	EMB gal	Additions	no pig. 2 days
	3	Δ K12 λ 0.1	0
	4	811 λ = 0.1	0
	7	K-12 λ = 0.1	1 (small)

sensitivity of this culture to λ not tried

probably in error but not gal- but gal slow

- From the cross 1177 X 1655
  - 24 additional Mal- picked, streaked, picked and tested against λ-s all hyR
  total to date 144 - Re-cross and try again!

Friday 8/15

- 1767 gal reduction

EMB gal	Addition	hr exp. 2 days
1		23
2	0.1K-12 λ	1/4 = 367. 1468

- 1487 gal reduction λ^S S^r [illegible] by S^r

EMB gal	addition	hr exp 2 days
1		30
2	0.1 Kpl λ	1/4 = 221. 884

- 518 gal reduction with mal. λ

EMB gal	Addition	hr exp 2 days
1		41 (+ many minutes)
2	0.1 mc 1-50 in 240 K-12 λ	41 (+ ns minutes)

↙ what happened?

41 × 50 = 2005 per ml

doesn't work out using approx equivalent to final with mal. λ

- W/1673 gal -

1673 gal I × 750 gal - 750 barren on EMS gal

EMS gal-1	3+, 7-	3/24 gal + = 1/8 = 12.5%	
EMS gal-2	14-		

1673 gal - × 902 gal 2 - 902 gal 2 - barren on EMS gal

EMS-1	12+, 143-	25/403 gal +	= 6.2%
EMS-2	13+, 235-		

1673 gal - × 892 gal 3 - 892 gal plate shows 9+ col

EMS-1	2+, 57-	6/85	= 9.0%
EMS-2	4+, 22		

1673 gal - × 811 gal 4 -

EMS-1	1+, 19-	= 1/55	=	1.8%
EMS-2	—, 35-			

Agrees 1673 gal plus

Wednesday - 9/3

- 1655 mal- - continue purification to obtain low galt background and reexamine for mal-duction -

- 1177x 1655, second crosses to obtain a mal- $4p_2^s$ for the purpose of attempting mal-duction -

Thursday 9/4-

- Adsorption characteristics of 811
  - washed cells, resuspended in saline - conc. of 16 hr culture in Luria.
  - phage titer = dil 10^7 → 0.1 ml + 0.1 ml 1485 cells →
    cell titer - dil 10^6 → 0.1 ml plated on EMB loc. →
    1 ml cells mixed 1 ml phage -
    at time 1 min - 0.1 ml mix added to 10 ml broth - centrifuged 15 min - (all sample at once)
    2 min - 0.1 ml as 1 min
    5 min - 0.1 ml as above
    10 min - 0.1 ml as above

Time sample	dilution
1	10^7
2	↓
5	
10	

Monday 9/22

EMC's assoc. of 518 X 1956 - for mal- typ's in EMS mal

plate	Malt+	Mal-	totals
1	9	43	52
2	6	29	35
3	14	68	82
4	8	64	72
5	7	28	35
	44		276

276 | 44.0 = 0.158 = 15.9% Malt+

276
1640
1380
2600
2208

EMC's assoc. of 518 X 1817 - for Mal- typ's in EMS mal

	Ft		
1.	24	5	29
2.	35	0	35
3.	38	2	40
		7	104

92.3% Malt

104 | 7.00 = 0.067 = 6.7% Mal-

624
760
728

---

incidental stos. in EMB mal -

1655 mal- obtained from J.L. - purified through 3 single colony isolations

2071 Entered in Stock book — No. 2071

Wednesday 9/24/52

— Characterization of Arg 16 mutants saved up from p. 75-68

cultures revitalized by fresh growth, single auxotrophic colonies of each picked and streaked on EMB(-) - Replicated to O(-) +
A1 - 1 grew } same
A2 - 1 grew }
A3 - none
A4 - 10 of 11 remaining grew for certain (thin plate) - rechecked.
~~A5~~ - Can't find must have forgotten or lost - rechecked if A4 grew unidentified
Vit. - all failed to grow
HC - all grew

- rechecked

811 galductase by 811 galtt λ -

811 galt lambda presumably contaminated - checked below

EM13 gal	addition	no. papillae 2 days
1	none - cells 811	39
2	0.1 ml 811 galtt λ (no cells added)	64 (colonies)
3	0.1 ml 811 galtt λ + 0.1 811 cells	78

apparently no activity of galt + 811 λ or 811 suggesting that this galt is not a true reversion but a suppressor mutation. Cross 811 galt × 1436 galt - if gal- occur further confirmation of suppressor -

— Summary of Mal- typ[s] from 1177 to date.

Since p. 114 about another 60 mal- picked from 1655 × 1177 cross

total	p. 119	144
since		c. 60
total		204

From the cross plates EML p. 119 - this streaked on EMS and replicated against λ-2

picked and - 56

260

on this lot one possible λ-2 sensitive.

Thursday 9/25/52

- 811 - Effect of heat on lambda - or galactose effect

- K-12 λ lot labelled #7, titer approximately 2.3×10^{10}
- Immersed in water bath at 56C for 30 minutes. no postincubation

EMB gal	Addition	no. resp. after 2 days	
		37	increase
1	→ none		
2	0.1 ml K-12 λ	(56)	19
3	0.1 ml. K-12 λ heated as above	~~56~~ (256)	≥19

$\frac{2 \times 10^7 \text{ c.f.t / ml}}{2 \times 10^{12} \lambda / \text{ml}} = \frac{1}{9.5 \times 10^6}$

Apparently the activity in lambda 1 gals is destroyed by this heat treatment. It remains to be seen what has happened to plaque forming lambda.

titer = $2 \cdot 10^9$ dil = 1 = 2×10^9 λ/ml = $\frac{10^{+1}}{10^{-2}}$ reduction in plaque titer

$2 \cdot 10^8$ dil = 12 : 2.4×10^8

- 811 Spontaneous gal+ above - picked, streaked on EMB gal to isolate a few gal+ of both suppressor and true back mutation type - make lysates of both types for galactose (by recrossing?)

- Wg 16 mutants collected - see 119

1. Mutant growing on both A_1 + A_2 -

grew in both — On breakdown of these two groups - the mutant methionine / leucine better in methionine judging by turbidity -

2. Remaining 11 mutants judging by recheck on D(i) + A_4 replication are all A_4 -

Examination in liquid med Q(i) + pr, has 1 + 7 gives indicating proline dependence - discard - evidently these two appear different on A_4 plate - more progress -

remaining 9 are list - (from remainder) have been saved to stock.

(methionine - leucine - see list -)

- 1655 X 1177

Neal - sp.5 search continues.

32 addition meal - picked - now tested against λ-2

Friday 9/26

- 811 salt sp papillae from 121 salt streaked onto EMB
  salt sp picked into water - streaked on flow - discarded
  16 in all

- 2070 = 1673 gal-
  EMB suspicious that is her 2070 bc it is not gal - but gal's
  a something - ✓ of 1673 gal - on gal -
  picked from slant into flat streaked on gal - not gal but slow

- Vg 16 pr- X_1 - } picked from slant and streaked out on EMB blue.
  Vg 16 pr- X_2 - }

Sat 9/27

- Cross

811 salt X T-10 - washed cells - 10ml each susp in 2 ml saline

811 salt #	EMB salt plate	Addition	# gal +	# gal -
	1	T-10	0	
	2	811 salt	0	
	3	both	18	0
	4	"	13	0
	5	"	21	0
	6	"	21	0
			73	0

start of gal sl?

- Vg 16 pr X_1 - replicated to D(-) + pr } X_1 - failed to grow on replication to D(-) + pr
  Vg 16 pr X_2 - " " " - pr } X_2 not diauxotrophic

Sunday 9/28

- 1177 x 1655 - to obtain Mal$^-$ Lp$_2^S$
  - 24 additional tested - all Lp$_2^R$ -

  total to date 260
  32
  24
  316

- Wash pr$^-$ X$_1$ on EMB loc - a single loc^S or loc$^-$ picked and restreaked - loc$^-$ odd appearance - some colonies with no dark centers must loc^S but with sectoring ◐ ○ ◔ opaque sectors etc. discarded

- Wash with - penicillin run —
  control grew —
  plate out EMB loc und. , 1-10, 1-100

- Comparison of K-12 lysates.

λ titers (lysate)	81 gal - EMS gal	Additions	No. of Plaquillas
	1	none	42
2.8 X 10^9	2	0.1 ml X λ	125
1.1 X 10^{10}	3	0.1 ml 30 λ	186
2.8 X 10^9	4	0.1 ml 35 λ	164
2.9 X 10^9	5	0.1 ml 40 λ	131 ← contaminated.

$\frac{1.44 \times 10^3}{2.8 \times 10^9} = \frac{1}{2 \times 10^6}$

Phage
Cells restreaked 2 days later to obtain to be gal adsorbed
33
72

- 2nd Cross

81 gal+ X Y-10 on EMS gal- control plate barren —
washed cells, rapid - saline 3 ml, 0.1 ml/plate

EMS gal	gal+	gal-
1	215	0
2	161	0
3	142	0
4	112	0
	630	

	+	-
Cross 1	77	0
Cross 2	630	0
	703	0

Sat. 10/4

- Comparison of m λ on 811 and 518
  use m180 λ this time

EMB gel	811	518	m180 λ	m. pq. 2 days
1	0.1	–	–	5
2	0.1	–	0.1	2808
3	–	0.1	–	56
4	–	0.1	0.1	932

not much clarity added by this expt.
see previous expts

– Wednesday 10/8

Wyl's mutagenesis – three – previously isolated –
assigned #2097 – culture to D. Shaw.

– Phage titers

lysate from 11-12 del. count (0.1) ml

u.v. dose	X	10^7	~~count (0.1)~~ 33 83 =	2.8×10^9 λ/ml
	Y (2 probed)		43, 67 =	5.5×10^9
	30-1		116, 98 =	1.1×10^{10} λ/ml
do	35-1		15, 40 =	2.8×10^9 λ/ml
do	40-1		(0.1)26, (0.3)94 =	2.9×10^9 λ/ml
osmo tested in Pen found stable	15		0, 6 =	3×10^8 λ/ml
	25		176, 141, =	1.6×10^{10} λ/ml
	35		189, 78 =	1.3×10^{10} λ/ml
also 811 ER-12 λ	811 ER-12	10^7	5, 13,	9×10^8 λ/ml

this stock from J. Mil

Thursday 10/9

- Friday prep -

Spot taken -

811/gal+ (1) - isolated from lysate used - broth culture for X, Y-10
" (2) - stock labeled #2 - previously X's x Y-10
make broth cultures for lysate prep
" (3) new isolate - purified 3X & broth culture for X, Y-10
" (4) " " " " " " "
" (5) " " " " " " "
" (6) " " " " " " "
" (7) " " " " " " "
" (8) " " " " " " "
" (9) " " " " " " "
" (10) " " " " " " "

- Wg16 meth - A_2 -

1 is apparently vaccine - (forms pellicle - contam?)
1 is [illegible] -

stocks made

both added to remaining 3 in the hope of resurrection since original plate lost.

- Wg16 meth "Thre" x Wg16 pr-X_1 -

EMS loc addition

1
2
3
4 no //
5

- 811 gal deletions with addition 0.1 ml of ravin lambda prop in equil as 2 days prop Comment (p 129) retic gal/l

1	—	53			
2	15' K-12 1	58	$\frac{9.2\times10^2}{1.6\times10^{10}} = X_1.7\times10^{-7}$	3×10^8 1/ml lysate	
3	25' K-12 1	145		1.6×10^{10} 1/ml	0.9×10^{-7}
4	35' K-12 1	146	$\frac{9.3\times10^2}{1.3\times10^{10}} = X_1.9\times10^{-7}$	1.3×10^{10} 1/ml	
5	750 E 1821 1	247		c. 6.5×10^9 1/ml according to prev. assay	

- 518 os 811 alone

1	—	20
2	15' K-12 1	78
3	25' K-12 1	691+
4	35' K-12 1	587+
5	750 E 1821 1	963+

$\frac{6.7\times10^3}{1.6\times10^{10}} = \frac{4}{10^7} = \frac{1}{2.5\times10^6}$

- 750t 1821 lysate - 0.1 ml plated EMS gal - no col.

Friday 10/10

- Vg/6 with A_2 - that failed to grow on individual A_2's both added to result of streaked out CHBloc -

- 811 t (750t1821) ~~Transduced~~ 8 papillae picked and streaked for stability check
  IS TRANSDUCTION PRODUCED BY TRANSDUCED STRAIN STABLE? y — — — y 129

- 578 t (750t1821) 11 as 811 above.

- 811 gut R- 2 lysate prepared
  filtered
  sterility checked (0.9 ml) in broth - no growth

Sunday. 10/12

S10 absorption Expt - plated on EMB bac-, all colonies lac- as expected

(1.) cell stock assay = $\times 10^{8}$, — 67, 74 = 7.1×10^{8} cells/ml

(2.) phage stock assay = $\times 10^{8}$ — 531, 453 = 4.9×10^{9} phage part/ml
(a 2+ tube broth dil. of phage stock IC-12 25λ)

INPUT RATIO PHAGE/BACTERIUM $\frac{49}{7.1} = 7/1$

(3.) phage stock gal-dnt/0.1ml = 177, 176 = 1.77×10^{3}/ml - spontaneous

(4.) Spontaneous gal+/0.1ml cells plated = - 15

1.62×10^{3} gal-dnt/ml λ susp.

gal-/ml × dil = original titer

$1.62 \times 10^{3} \times 6 = 7.72 \times 10^{3}$/ml original stock

previous assay 125 = $6.91^{+} \times 10^{3}$
higher titer likely probably due to increased accuracy of longer dil. of lysate

Gal- Ratio $\frac{\text{gal-}}{} = \frac{9.72 \times 10^{3}}{4.9 \times 10^{9}} = \frac{2}{10^{6}} = 1/5 \times 10^{5}$ λ

adsorpt = 5 min. bench
10 min. centrifuge

2ml cell susp + 2ml dil. λ

(5.) Supernat. 1st adsorption λ titer = $\times 10^{7}$ — 72, 126 = 9.9×10^{8}

added = $\frac{\text{stock titer}}{2} = \frac{4.9 \times 10^{9}}{2} = 2.5 \times 10^{9} = 25 \times 10^{8}$

unabsorbed = $9.9 \times 10^{8} = 39\%$ of input

λ taken up/cell $= \frac{25 \times 10^{8} - 9.9 \times 10^{8}}{3.6 \times 10^{8}} = \boxed{4.1 \text{ λ/cell}}$

1st

(6.) Sediment 1st adsorption - no gal- detected

0.1ml plated = 124, 138 . 131

gal-ed = 131 - bkgrd. = 131 - 15 = 116

expected from above stock assay = 81 ← indicates more than 100% adsorpt

(7.) supernat 1st adsorpt.

0.1 ml plated = 33, 33 = 33

gal-ed. present = total pop. - sp = 33 - 15 = 17

(8.) supernat 2nd adsorpt λ titer = $\times 11^{7}$ — 144, 103 = 1.24×10^{9}

put in = 2.5×10^{8}
unabsorbed = $1.2 \times 10^{9} = 50\%$

absorbed/cell = $\frac{25 - 12}{3.6} = \frac{13}{3.6} = \boxed{3.6 \text{ λ/cell}}$

total = 7.7 λ/cell

1st adsorption - amount of killing by λ

cell assay = $\times 10^{7}$ = 139, 132 = 1.56×10^{9} cells/ml
which appears impossible

continued

Sunday 10/12

(9) 2nd adsorption ~~Sed~~ no of gold.

0.1 ml plated = 169 149 = 159

gold added = total - sp = 159 - 15 = 145

expected max if 100% adsorpt.
on basis initial assay = $\frac{16^2}{2} + \frac{16^2}{2} = 162$

expected on basis of adsorpt. of 1st & 2nd = 81 (.61) + 81 (0.56) = c. 90

(10) 2nd adsorption supernat. no of gold.

0.1 ml = 62, 64 = 63 - 15 = 48

gold. added /ml = 81 81

gold. supernat = 63 48 } ↑↓ comp.

gold ads = ~~145~~ - 116 = 29 } total 92.

(11) 3rd adsorpt. ll titer supernat. = $\times 10^7$ = 226, 198 = 2.1×10^9

input = 2.5×10^9/ml
ads abs = 2.1×10^9/ml = 84%

adsorbed $\frac{0.4 \times 10^9}{3.6 \times 10^8}$ = 1.1 /cell.
λ/cell

This value may be off due to the time elapse between the beginning of this expt and this assay - Time is probably in excess of 1 hr and therefore possibly longer than latent period.

total = 7.7
1.1
8.8 λ/ml

(12) 3rd adsorpt. sed. no of gold.

0.1 ml = 179, 214 = 197
increase over 2nd 197 - 145 = 52.

(13) 3rd adsorpt. sup. no of gold.

0.1 ml = 53, 65

gold = total - sp = 59 - 15 = 44

total gold = 52 / 44 (96)

expted (81)

81 / 44 / 37

Thursday 10/16/52

- 518 Second absorption Exp. -

518 blocked with regard to previous assay 41

- Initial phage stock titer - a 1-2 dil. of parent 25[1]
  10^8 dil → 80,74 = 7.7×10^9 λ/ml (parent assay 1.6×10^{10})
- Initial cell assay - stock susp. from overnight cult. resusp. in saline
  10^7 dil → 115,108 = 1.1×10^9 cells/ml

Expt. performed by adding 2ml volumes of λ + cell together. Shake 5 min at room temp. Centr. 10 min. Decant sup, resusp. cells in 2ml volume - Remove samples, i.e., 0.2, 0.1 ml - making vol. either 1.8 or 1.9 ml. For next absorpt add 1.8 or 1.9 ml of λ sup., etc.

1st absorpt.

Supernat λ titer -
10^7 dil → 164,205 = 1.86×10^9
% not absorbed = $\frac{3.9 \times 10^9}{7.7 \times 10^9}$ = 48% absorbed = 52%

Cell survival (of 2nd resuspension)
10^7 dil 134,89 = 1.12×10^9 = 1.1×10^9 cells = no killing

Supernat gold.
0.1 ml = 197,166 = 182 − 41 = 141 % gold not absorbed = $\frac{141}{410} \times 2 = \frac{282}{410}$ = 67.5%

Sed. gold.
0.1 ml = 268,317 = 293 − 41 = 252

Vol. at end = 4.0 ml − 0.1 − 0.1 − 0.1 = 3.7

2nd absorpt

Supernat λ titer.
10^7 dil → 232,329 = 2.8×10^9
% not abs. = $\frac{5.6 \times 10^9}{7.7 \times 10^9}$ = 73 − absorbed = 27%

Supernat gold.
0.1 ml = 309,281 = 295 − 41 = 254 − 354 / 333

Sed gold.
$\frac{0.1}{5}$ = 107,79 = 93 − 41 = 52

Vol. at end = 3.7 − 0.1 − 0.1 = 3.5 ml

3rd absorpt

Supernat λ titer
10^7 dil → 215,342 = 2.79×10^9
% not abs. $\frac{5.6 \times 10^9}{7.7 \times 10^9}$ = 73 − absorbed = 27%

Supernat gold.
0.1 ml = 368,345 = 347 − 41 = 306

Sed. gold
$\frac{0.1}{5}$ = 129,85 = 107 − 41 = 66 × 5 = 330

4th absorpt

Supernat λ titer
10^7 dil → 253,293 = 2.7×10^9
% not absorbed = $\frac{5.4 \times 10^9}{7.7 \times 10^9}$ = 70 absorbed = 30%

Supernat gold
0.1 = 283,246 = 265 − 41 = 224

Sed. gold
$\frac{0.1}{5}$ = 43.49

Thursday 10/11/52

5th adsorpt

Supernat titer
10^7 dil → 552, 527 = 5.4×10^9 = ~~4.2X10~~

% not adsorbed = $\frac{1.1 \times 10^{10}}{7.7 \times 10^1}$ = impossible - either no adsorpt or lysis obscuring effect.

Supernat gold.

Sed. gold.

Sed pt. assay
10^7 dil → 44, 51 = 9.6×10^8

~~~~

- 1924 Examination for presence of λ
14 across streaks of overnight per culture streaked against 1465
9 showed presence of phage - weak.

- 811 - gel + - 2 λ neg.
10^5 dil = 29, 23 = 2.6×10^9 λ/ml

- 1103 [illegible]

← 1.6×10^{11} λ/ml

- 513 [illegible]

Boiled

- 2000 [illegible]
~~~~

Friday 1/11/[illegible]

- 515 (Lyp[illegible]) X 12 - [illegible]
  [illegible] gel +
  [illegible] [illegible] - [illegible]

- 519 + ([illegible]) [illegible]
  [illegible] s/g mixed
  [illegible] s/g mixed

- 811 ([illegible])
  [illegible] s/g mixed
  [illegible] s/g mixed

- W1655 X W702 - Lp[illegible] gel - transmissible gel
  tested on mice - is mice +

- Research on Hypogenicity of 1924
  - 12 cross streaks on 1485 - no evidence of plague
    on SAP gel -
    on EMB Lac, lac -

- 1924.
- Examination of 1924 for hypogenicity of transinduced cells. -

Isol. #	Streaking # ①	②	③	Streaked against 1485	Isol. #	①	②	③	1485	Isol. #	①	②	③	1485
1	m	m	m ⊗	non-lyp.	16	m	+	+	non-lyp.	31	m	+	+	non-lyp
2	m	+	+	"	17	m	+	+	"	32	m	m	m	"
3	+	+	+	"	18	+	+	+	"	33	+	+	+	"
4	m	+	+	"	19	m	+	+	"	34	m	+	+	"
5	⊗ m	m	m ⊗	one plague	20	m	m	+ ?	"	35	+	+	+	-
6	⊗ m	+	+	non-lyp.	21	+	+	+	"	36	+	+	+	"
7	m	+	+	"	22	+	+	+	"					
8	m	+	+	"	23	m	+	+	"					
9	+	+	+	"	24	m	+	+	"					
10	m	+	+	"	25	m	m	m	"					
11	+	+	+	"	26	m	+	+ ?	"					
12	m	M	m	"	27	m	m	m	"					
13	+	+	+	"	28	m	+	m	"					
14	+	+	+	"	29	m	m	m	"					
15	+	+	m	"	30	m	m	m	"					

M = mixed
+ = gel
- = -
⊗ = mixed

Sunday Oct. 19

- 1821 - Reexamination of xylose transduction possibilities

EMB gal	Additions	no gal 2 days	
1	—	174 +	8 picked and streaked -
2	0.1 K gal (25')	229 +	16 picked and streaked -

Examination for instability of "gal deficient" -

- Plan to gal deficient 1821 and examine unstable gal+ for gal stability -

- 1924 H 1242 see p. 129

- 2050 gal$_3$ - mutants - streaked on EMB base for replication on mucinol + supplements

- out of 12 suspected auxotrophs - 1. proto trophs
1. A_2
1. ($A_1 A_2 A_3$) - really proto! failed on A_5 } not tested A_4 - presumably same as A_2

SIP

- Check on background on EMB gal.
each from overnight culture - uninoculated

EMB gal	Additions	no gal 2 days
1	uninoc. susp	53
2	1-10 susp	37
3	1-100 susp	43

(probably)

811-gal+

- Crosses - X 1295 - Results partly unsat. due to large no. of mucoid col. - many mucoid apparently (-)

811-gal+ 2 - (no galactokin in 811)

EMB gal	+	susp -	checked -
1	33	3	
2	39	2	
3	29	0	
4	46	0	
5	34	0	
6	37	0	
7	48	1	
8	23	0	
	289	6	6/6 gal slow

811 gal+ 1

1	956	1
2	71	2
3	70	0
4	62	0
5	67	0
6	64	3
7	66	2
8	78	1
9	84	2
10	66	1

- Wednesday -

1821 - Xyldirection? —

EMB xyl	Addition	No. pap 3 days (papillae appear arrested in growth - failed to develop further after 2 days)
1	—	7
2	0.1 KH2A 25'	> 85

16 papillae on 1 plate picked and streaked xyl - ~~see xyl~~ 10/27 check to see if gal + - 10/29 see gal - ?

- 1924E12-12 #35 - this streaking = #4 from galdirection is mixed - no reisolation
Attempt to establish a stable + from unstable +.
12 well isolated + col. picked and streaked on EMB gal. Replicate 1

	1	mixed
	2	"
	3	"
	4	"
	5	"
	6	"
	7	"
	8	"
	9	"
	10	"
1 single colonies to here →	11	"
	12	pure + → picked

- 1924E12-12 - One plague type / 1485 - see 129
picked and streaked out EMB gal -

- 811 gal + reversions checked for their papillation behavior - on EMB base -

		Indication	
1307	5-10 pap/colony	=	
811	no. papillae		
811 gal + -1	< 1 pap/colony	mimic	does not galden
2	< 1 pap/colony	"	" " "
3	< 1 pap/colony	"	
4	c. 5 pap./colony	true reversion	
5	5-10 pap./colony	" "	galdens! 811, 918
6	< 1 pap/colony	mimic	
7	c. 1 pap./colony	"	
8	5-10 pap/colony	true reversion	galdens! 811, 918
9	c. 1 pap/colony	mimic	
10	< 1 pap/colony	mimic	

Thursday 10/23/52

1924EK-12 #25 - ~~from~~ initial streaking for purpose of isolating stable + 12 additional colonies streaked on EMB gel - 4 streakings from pg.

all mixed - many mutants

Friday 10/24/52

1924EK-12 - the one plague type #5 of testing streaked out EMB gel 10/22 -

~~a [illegible] + streaked out again. examine individual colonies for lactose [illegible]~~

1924EK-12 - #12 of pg (131) for stability isolation
#25 papillae colony

1st streaking from end of purification - pure
mass 10/23 2nd streaking .. .. c. 150 colonies - all pure
10/24 3rd streaking .. .. c. 500 colonies - all pure
10/25 4th streaking .. .. c. 500 colonies - 1 (+) colony - contam.?

SEE PG 133

picked and examined - see subsequent pg.

Saturday 10/25/52

1924EK-12 (above) 1 plague Type - transferred to agar slant (+ #1)
10 + and 10 (-) colonies picked from streaking and tested against 1405

+ colony	reaction	- colony	reaction
1	non lyt	1	swgr plague? → re-examined pg 134
2	"	2	"
3	"	3	"
4	"	4	"
5	"	5	"
6	"	6	"
7	"	7	"
8	"	8	"
9	"	9	"
10	"	10	"

Sunday 10/26

- 811 galt #5 lysate (from) - (this is papillating strain (on lawn)) - (0.5 ml of this lysate in Pa = sterile)

10^8 dil. = 44, 59 = 5.2×10^9 /1/ml

in 518 gives about 600 rp/0.1 ml = 6×10^3/ml $\frac{6 \times 10^3}{5.2 \times 10^9} = 1/10^6$

- 518 - Galdeletion

DNAase no effect

EMB gal	Addition	no pfgs 2 days (30)	
	none		
2	0.1 K12 (25') untreated *	960	some o plaques here?
3	0.1 K12 (25') treated **	998	
4	0.1 811 galt #5 1	4 = 883	← examine for stability - see p 134

gal+ reversion ⇒ activity on gal-

* untreated = 0.1 ml broth added to 0.5 ml lysate - incubated at room temp. 5 min

** treated = 0.1 ml DNAase added to 0.5 ml " - " " " "

"(1.1 γ DNAase)

See also p. 135

DNAase treated greatly decreased in viscosity

Broth

— 1929 tK12 #25 papillae col #12 - stable galt

over night culture from diluted plated EMB gal -

EMB Plate	no colonies	no. gal-
1	565	0
2	440	0
3	774	0
4	622	0
5	609	0
6	559	0
7	598	0
8	523	0
9	560	0
10	573	0
	5824	0

To here — Lustig

3 streaks mixed gal+, gal-

4 streaks gal+

1 plating of 5821 colonies no gal-

$\frac{2.1\times10^{3}}{5.2\times10^{9}} \cdot 0.5\times10^{-6}$

Tuesday 10/28

1821 gal- xyl-dulcitol

	EMB	Additions	No papillae after 2 days
8	1	none	0
	2	0.1 K-12 λ (25')	4
	3	0.1 K-12 λ (25')	7
	4	0.1 K-12 λ (25')	8

cultures for typ

- EMB xyl none
  - 1 ↙ 4
  - 2 ↙ 0
  - 3 ↙ 7 } faint background of many minute papillae (c.10⁴)

- 518 t 811 gal + #5 (p. 135)

8 papillae picked and streaked - 4 papillae in the transduced of the type, colonies, (1) (2) pigmented - papillae of some size (This is not unusual, reason for diff. not known) 4 red purple } streaked on EMB gal.
4 colonies

1st streaking - all mixed +, - } no distinction as to above
2nd " - 1/8 mixed +, - mentioned above
3rd " - 1/8 mixed +, - ← others giving off something intermediate

probably not correct

glucose fixed - this transduced site appears to be stable more quickly than 518 cells transduced by K-12 λ — this also appears to be the case for this lysate and 811 gal+ as below

- 1924 EC-12 One plaque type.
  - presumptive of lysogenicity of one plaque gal- type (p. 135)
  - 4 gal- colonies from streaking examined - all non-lysogenic

- K-12 λ (X) 0.5 ml + 1 drop Pen Tested for sterility - sterile

- Wednesday 10/29/52

- 811 - gal- dulcitol

EMB	Additions	No. pap. after 2 days
1	none	39
2	0.1 K-12 λ (25')	196
3	0.1 811 gal+ #5 (1st lysate (viscous))	204

$\frac{1.57\times10^{3}}{1.6\times10^{10}} = \frac{1}{1.0\times10^{7}}$

← stability of 12 of these examined

1st streaking	7/11	mixed +, -
2nd streaking	1/11	mixed +, -
3rd streaking	1/11	mixed +, -

apparently → the situation is as with 518 t 811 gal + #5 above

This is probably the case with 811 t 811 gal+ #8 on (p. 135a)

Thursday 10/30

1924tK-12S
Cross ♂ X Y-10

1. purity of 1924tK-12 culture -- well plated on EMB gel

EMB gel	no. ♂+ col	no. ♂(-) col.
1	275	0
2	249	0
3	268	0
	792	

this are also additive ∴ data of p. 135 - make $\frac{5821}{792}$ / 6613

2. Cross.

EMS gal	no. (+)	no. (-)
1	175 (1 mosaic)	0
2	116	1
3	152	0
4	141	0
5	165	—
	749	1

The 1 (-) and (1) mosaic colonies streaked out on EMB gal - both gave gal+ on gal gal

λ titrations.
grown on 1485
dil 10^6 = 0, 0, titer $< 10^6$

K-12 λ(X) in 240 seconds
dil 10^6 = 172, 262 = 217 = 2.2×10^9 ← previous assay says titer here originally was 2.8×10^9. Better recheck to observe if this post incubation assay is correct.

DNA no effect on plaque formation

811 gal+ #5 λ
untreated dil 10^8 = 37, 85 . 6.1×10^9 (previous p. 133 5.2×10^9)
DNAse treated dil 10^8 = 52, 68 = 6.0×10^9
} indicates DNA ase has little effect if any on either gal reduction or plaque formation

efficacy of 811 gal+ #5 λ = (for 5) $\frac{8.8 \times 10^3 \text{ gal/ml}}{6.1 \times 10^9 \lambda/\text{ml}} = 1.3 \times 10^{-6} = 1/7 \times 10^{-5}$

(for 811) $\frac{2.0 \times 10^3}{6.1 \times 10^9} = 0.3 \times 10^{-6} = 1/3.3 \times 10^{-6}$

811 gal reductions

	EMB gal	Addition		Papillae after 2 days
	1.	none		25
	2.	0.1 ml 811 gal+ #5 1	untreated	201
	3.	0.1 ml 811 gal+ #5 1	DNA ase treated	296
sterility of theta (0.5) ascertained	4.	0.1 ml 811 gal+ #5 2	—	289 + ← plate contaminated?
	5.	0.1 ml 811 gal+ #8 1	—	291 ←
	6.	0.1 ml K-12(λ) in 240		142

811 gal+ #8 gal reduces!

518 gal reductions (control omitted)

EMB	Addition	Papillae after 2 days
1	0.1 ml K-12(λ) in 240 λ	540
2	0.1 ($\frac{1}{10}$) "	15 (dry plate) ← 10 examined / and 1 K all gave faint mat / 1485
3	0.1 ($\frac{1}{100}$) "	9

(portrait)

Thursday 10/30

- 1402 galdeletion -

EMB gal	Addition	no plaq after 2 days
1	none	5
2	0.1 ml K-12 (25')/1	43

} appears as a deletion in the absolute sense of the effect. Similar to result ~ 182/ of 134. to this culture partially typ. R

811 gal + #1 X Y-10

- Cross for the purpose of determ. 811 gal + #1 suppressor or no deletion

EMB gal	no. gal (+)	no gal (-)
1	333	0
(cumulative count) → 2	462	4
3	541	2
4	671	2
5	781	1
	781	9 <- retest in EMB gal -

Summary table: (+) (-)
X1 @ 130 - 96
X2 @ 135a - 11
107

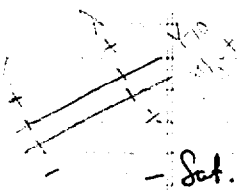
- 811 gal + #2 X Y-10

- Cross for purpose of determ if this 811 cultures is suppressor mut. or no.

EMS gal	no gal (+)	no gal (-)
1	157	0
cumulative count 2	326	2
3	456	1
4	603	0
5	866	2
	866	5

} no gal slow -

Summary table: + -
X1 @ 122 - 0
X2 @ 123 -
X3 @ 130 275 0
X4 @ 135a 871 0
1879 0



- Sat. 11/1

811 + 811 gal + #8 stability ✓
1st streaking - 8/8 mixed +, -
2nd streaking - 0/8 mixed +, - (all +)
3rd streaking - 0/8 mixed +, - (all +)

- 2102 lysate for EML -
mal. 35 sec - incubated 2 hours - centrifuged
dil 10^7 0.1 → against 2096 = no plaques - galdeletion on 518 according to EML?

- 1673 mal for gal - on EMB gal
3 good gal minus obtained - set for galdeletion

1673 gal - #1 #2 #3

- 1692 mal. for gal - unsuccessful attempt

Sunday 11/2

- 1655 X 1177 to obtain xyl- for xyl distinct attempt
  old suspension used - small number of prototrophs
  c. 6 mult from c 100 prototrophs.
  picked and streaked on EMB xyl. — 4/6 xyl minus
  pick and see if λ^s - streak on other med. —

- 1924tK-12 #12 (unstable) X 1436 - to see if λ^+ obtained — but the dmn. to λ^+ cross skewed plus λ^+ if this is so since λ^r recessive to λ^+. Examine unstable gal+ prototrophs to see λ^+ was since $\frac{gal\ \lambda}{+\ +}$ should go together

1924tK-12 parent culture - Analysis - streaks of washed susp. made / 1485 - no evidence of λ

Gal+	Plate	#(+)	#(-)
	1	$\frac{1}{4}$ = 200 (800)	$\frac{1}{4}$ = 11 (44)
	2	$\frac{1}{4}$ = 161 (644)	$\frac{1}{4}$ = 9 (36)
	3	$\frac{1}{4}$ = 173 (692)	$\frac{1}{4}$ = 8 (32)
		2136	112

} purity of 1924tK-12 parent

Cross	Plate	#(+)	#(-)
	1	42	383
	2	38	354
	3	—	—
	4	..	..
	5	68	365
		148	1102

about the same as first two but difficult to count due to agar seed background

28+ picked and streaked against 1485 - all non lysogenic

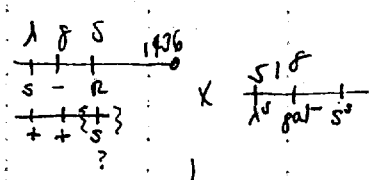
Thursday 11/6

- 1673 gal- fluctuation

		EMB gal	Additions	No. gal+ after 2 days
#1 (no 1 and 2 gal+ plates inferred)	1	0	none	7
	2	0.1 ml 1K-12 λ (25')		$\frac{1}{8}$ = 195 = 1560
#2	1		none	11
	2	0.1 ml 1K-12 λ (25')		$\frac{1}{8}$ = 163 = 1304
#3	1		none	19
	2	0.1 ml 1K-12 λ (25')		$\frac{1}{8}$ = 161 = 1288

10/6 Thursday

– 1436. galduction to Rifani unstable + which is λ^+ gal$^+$ S^R
to cross with 578 to observe if lysogeneity of galduced is due to dominance of λ^+ in transduced fragment → examine to see if unstable i.e. S^a – possibility of transducing S^s which is dominant



what kind of prototrophs?
gal$^+$ $\lambda^?$ $S^?$
gal– $\lambda^?$ $S^?$
design cross
test examine whose problem

1. galduction of 1436

	EMB gal	Additions	Papillae after 2 days
	1	none	34
	2	.01 ml K+12λ (25')	1/8 = 136 = 1248

(1) ← 1st Pure. streak 7/8 gal– ! what ? SEE 112
(2) try again 1st Pure. streak 7/8 gal+
(3) try again 1st pure. streak 15/18 gal– of this lot 9 streaked against 1445 all lysogenic

Pap #	Streak 1	Streak 2
1	mixed	pure +
2	mixed	"
3	mixed	"
4	gal –	—
5	mixed	pure +
6	mixed	"
7	mixed	"
8	mixed	"

207° = 673 gal– –1. Stability check –

Sputum	1st Streak	2nd Streak	3rd Streak
1	mix	pure +	pure +
2	pure +	"	"
3	pure +	"	"
4	mixed	"	"
5	pure +	"	"
6	pure +	"	"
7	pure +	"	"

207° ✓

Single Papillae gal-induced pop picked

Galduced	1st Streak	2nd Streak	
1	mix	mix ⊗	mix ⊗
2	mix ⊗	mix ⊗⊗⊗	" ⊗
3	mix	mix ⊗	" ⊗
4	mix ⊗⊗⊗	mix ⊗	"
5	mix ⊗⊗⊗	mix ⊗	"
6	mix	pure +	pure +(?)
7	mix ⊗⊗⊗	pure +	" +

673 gal– –2

1	mix	pure +	pure +
2	mix	mix	"
3	pure +	pure +	"
4	mix	"	"
5	mix	"	"
6	pure +	"	"
7	mix	"	"
8	mix	"	"

1	mix	mix ⊗	pure +
2	pure +	mix ⊗	pure +
3	mix	mix(?)	pure +
4	mix	mix	mix ⊗
5	pure +	pure +	pure +
6	pure +	mix ⊗	pure +
7	mix	pure +	pure +
8	mix	mix ⊗	mix ⊗

673 gal– –3

1	mix	pure +	pure +
2	mix	"	pure –
3	mix	"	pure +
4	mix	"	"
5	mix	"	"
6	mix	"	"
7	mix	"	"
8	mix	"	"

1	mix	pure +	mix ⊗
2	mix ⊗	pure +	mix ⊗
3	mix	pure +	pure +
4	mix ⊗	mix	mix ⊗
5	pure +	pure +	pure +(!)
6	mix	pure +	mix ⊗
7	mix	pure +	pure +
8	mix ⊗	mixed ⊗	" +

1/4

Sunday-Monday 18/9/19/10

- ~~VHB6 gel electrophoresis~~

- 1177X 1655 xyl- #1

EMB gel	Additions	No pap 2 days
1	none	0
2	0.1 ml K12 λ (1')	0

Attempted xyl reversion of 1177 xyl-

#2		
1	none	0
2	0.1 ml K12 λ (2')	0
#3		
1	"	0
2	"	0

811 transduction ~ 750 λ

EMB gel	Additions	No pap. 2days
1	none	45
2	0.1 ml K12 λ (2')	55

small increase - xyl+? pick a few - look for mitability

slightly large [illegible] passed thru gel - [illegible]

what to do?

518 transduction ~ 750

EMB gel	Additions	No pap 2d
1	none 750 λ	45
2	0.1 ml K12 λ (1')	40

518 lp2R = W1412

EMB gel	Additions	# pap. 2d
1	none	33
2	0.1 ml K12 λ (15')	35

518 lp.R not galactose

Sunday 11/16

1673 gal- #2 × Y-10 – as usual – no control –

EMB gal	Lac+	Lac−
1	324	12
2	166	7
3	261	10
	751	29

(+) slightly purple – some (−) mosaic

← 26 picked EMB gal, 22 (−) streaked /1485/λ SEE BELOW. – 7/22 Sequestrum

≈ 30 picked EMB gal – streaked against /1485, λ 30/30 lysogenic on 1485

1673 gal #3 × Y-10 as above

EMS gal		
1	174	16
2	186	13
3	418	20

(−) slightly purple – some (+) mosaic

518 gal mixed ē 892 λ Gal3- mixture on Gal4- 1S

6 picked streaked EMB gal

	strk 1	strk 2	strk 3
1.	mix	mix	mix – STOCK 518t892-1
2.	pure+(?)	pure+	—
3.	pure+	pure+	—
4.	pure+	pure+	—
5.	pure+	pure+	—
6.	mix	mix	mix – STOCK 518t892-2

518t892

EMB gal	Addition	No lysp. 2 days
1	none	41
2	0.1 892 λ	247

Product

811 gal mixed ē 892 λ mixture Gal3- on Gal4- λt

6 picked pure streaked EMB gal

	strk 1	strk 2	strk 3
1	mix	pure+	·
2	"	mix	mix
3	"	mix	mix
4	"	mix	mix
5	"	pure+	—
6	"	pure+	—

811t892

1	none	39
2	0.1 892 λ	85

$\frac{4.6\times10^{2}}{3.9\times10^{9}} = \frac{1}{8.5\times10^{6}}$

STOCKS 811t892-1, 811t892-2, 811t892-3

Tuesday 11/18

1673 gal-#2 XY-10 above gal- prototrophs tested –

		1485	λ			1485	λ
Lp+	1 ○	lys.	resist.	Lp+	12 ○	lys	resist
Lp+	2 ○	"	"	Lp+	13 ○	"	"
Lp−	3 ○	non lys	sensitive	Lp+	14 ○	"	"
Lp+	4 ◉	lysogenic	sensitive	Lp−	15 ◉	Splequa	[redacted] seno.
Lp+	5 ◉	lysogenic	sensitive	Lp+	16 ○	lys.	resist
Lp−	6 ○	non lys	[redacted] Sen.	Lp+	17 ○	"	"
Lp+	7 ○	lys	resist	Lp+	18 ○	lys	resist
Lp−	8 ◉	non lys	sens	Lp+	19 ○	lys	resist
Lp+	9 ○	lys	resist.	Lp−	20 ○	non lys	sensitive
Lp+	10 ○	lys	resist	Lp−	21 ○	non lys.	[redacted]
Lp	11 ◉	ongle plaque	sensitive	Lp+	22 ○	lys	resist

gal- Lp+ 15
gal- Lp− 7

replica to D(5)

Lp+ and prototrophs

all prototrophs

Wednesday - 11/19

- S1Bt transduction by 902λ - obtain stocks

EMBgal	additions	no gap. 2 days
1	none	39
2	0.1 902λ	1/4 = 288 1152

- 811 transduction by 902λ

EMBgal	additions	no. gap 2 days
1	none	44
2	0.1 902λ	147

$\frac{1.03 \times 10^3}{4.9 \times 10^{10}} = \frac{1}{4.9 \times 10^6}$

- 1436 (purified) Recheck on gal- streaking - q deduction

EMBgal	additions	no. gap 2 days
1	none	6
2	0.1 IC-12 λ (25')	1/4 = 294 1176

← sixteen (16) picked and streaked - all gal-, all non-protoxophic

Thursday 11/20

- S1BtK-12 X Y-10 - for the purpose of uncovering gal bp^S?

	EMBgal	no +	no -	no. mixed
S1Bt K-12 purity ✓	1	384	72	8
	2	406	60	9

EMSgal		
1	184	—
2	172	—

2, 3 ← 4 examined are λ^R and lysogenic on 1485

Booked

- S1BtK-12 X 1673 - for the purpose of uncovering (gal- bp^+)
  X 1678

	EMBgal	no(+)	no(-)	no mixed
S1BtK-12 purity ✓	1	551	43	8
	2	633	37	4

	EMSgal			
X1673	1	73	5	—
	2	91	2	—

7 examined - all λ^R and lysogenic on 1485

	EMSgal			
X1678	1	101 contam.	2	
	✓	56	4	

5 examined all λ^R and lysogenic on 1485

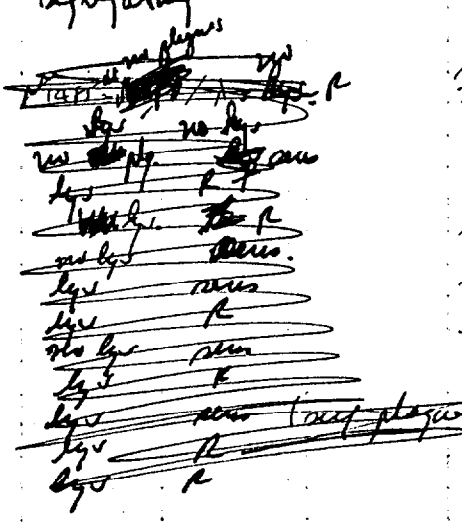
Thursday 11/20

Friday 11/21

- 1673-gal- -3 X Y- 10 - pg 139 - lysate aggregating

gal- Checked on W1485 λ-state of protohaploids

		λ
1	lys	R
2	lys	R
3	lys	R
4	lys	R
5	lys	R
6	lys	R
7	lys	R
8	lys	R
9	lys	R
10	lys	R
11	lysed	R
12	plaques	S
13	lys	R
14		
15		
16		
17		
18		
19		
20		
21		
22		
23		
24		
25		
26		



1485	λ
no plaques	R?
lys	R
no lys	sens
lys	R
lys	sens ?
lys	R
no	sens ?
lys	R
no lys	sens
lys	R
lys	R
lys	R
lys	R

10 (-)

gal+ 22 picked all lys on 1405, all λ resist

Salmonella for JL.

- lysates attempted in 914, 915, 922

lysates in 914-1 some suggestion of lysis?
913 — discarded

lysate in 914-2
915
922

Results

914-1 / 913 → cross streaks on 913 - ① - activity in undiluted
914-2 / ② " " undiluted
known suppressed (JL) " " "

915 / 912 → cross streaks on 912 ① (915) - activity undil., some act. in 10^6 dil? hopeful

② (922) - activity in undil, none in 10^6 -

(142) 152/16 = 9 17/32 = 2432

Tuesday 11/26

– New 1 preparation –

518 (examination for ~~allogenic~~ gal+ as donors of gal+)

EMB gal	Addition	No papillae 2 days
1.	none	28
2.	0.1 518tC892 1-1	3
3.	0.1 811tC892 1-1	0
4.	0.1 K-12 1 (11/23)	1/16 = 152 = 2432

} already observed by microscopic examination

borrowed

– Other gal- mutations – examination for transduction –

stock mode of this

	EMB gal	Addition	No papillae 2 days
566 (tp2 1)	1	none	9
	2	0.1 K-12 1 (Y)	9
583	1	none	6
	2	0.1 K-12 1 (Y)	340

streaking

	1st	2nd	3rd
1.	mixed	mixed	–
2.	"	"	"
3.	"	"	"
picked 4.	"	"	"
5.	"	"	"
6.	"	"	"
7.	"	"	"
8.	"	"	"

– 2050 gal3 – Examination for allogenic transduction (lysates) for activity on gal3

EMB gal	Addition	No pap. 2 days	
1.	none	29	
2.	0.1 518tC892-1 1	solid smear	(3 papillae?) – this lysate not sterile. Retested
3.	0.1 811tC892-1 1	solid smear	(3 papillae?) this lysate tested sterile
4.	0.1 811 1	450	

2050tC811 stock →

$\frac{1.7\times10^{10}}{4.3\times10^{3}} = \frac{1}{4.0\times10^{6}}$

see 144

– 518tK-12 – isolation of several gal- segregants to see if
1. give no gal+ ε X 1436
2. all lysogenic
3. X 1673 to see if all gal- tp+ (linkage intact)

a. streak out overnight culture 518tK-12

– 518tK-12 – crosses with 1678 gal+tp+tp5

gal+ tp5 / gal- tp+ –

borrowed

	EMB gal	Condition	(+) Prototrophs	(–)
	1	0.1 ml of each overnight cultures	6	8
	2	" " " "	11	6 (+) B
518tK-F–	3	Aerated 518tK-12, unaerated 1678	0	0
	4	" " "	0	0
	5	" " "	1	0
	6	" " "	0	0
	7	" " "	heavily crosses	0 (–)
1678 F–	8	Aerated 1678, unaerated 518tK-12	78	2/16
	9	" " " "	70	30
	10	" " " "	72	18
	11	" " " "	76	26
	12	" " " "	83	25

what happen?

Some gal+ appear to be mixed

of the 115 – 110 streaked single colony picked and tested against 1 – all 1R – replica'd against 1485 – all lysogenic

(+) tested sterile – A

Saturday 11/29

— Reexamination of the conjugability of aerated 518tK12 cultures -

1. 518(redacted) X 1678, 1673 — (all cultures resuspended in 1X Saline)

EMB gal	Addition	(+)	Petri dish (-)	
1	aerated 518, unaerated 1678	0	18	F+1673/1678 F-518/F+K12
2	" " 1678	0/0	53	
3	aerated 518, unaerated 1673	0	10	F+1678 X F-518 = 0+/71
4	" " " "	1/1	18/28	F-1678 X F+518 3+/574
5	unaerated 518, aerated 1678	3	591	F+1673 X F-518 17/28
6	" " " 1673	16	411	F-1673 X F+518 16/427

(booked)

2. 518tK12 X 1678, 1673

7	aerated 518tK12, unaerated 1678	0	1	
8	" " " "	1	0	
9	aerated 518tK12, unaerated 1673	0	0	
10	" " " "	0	0	
11	unaerated 518tK12, aerated 1678	135	28	← (of these 28 I examined - all λ^R all lys /1485)
12	" " " 1673	146	32	

(booked)

— Sterility checks

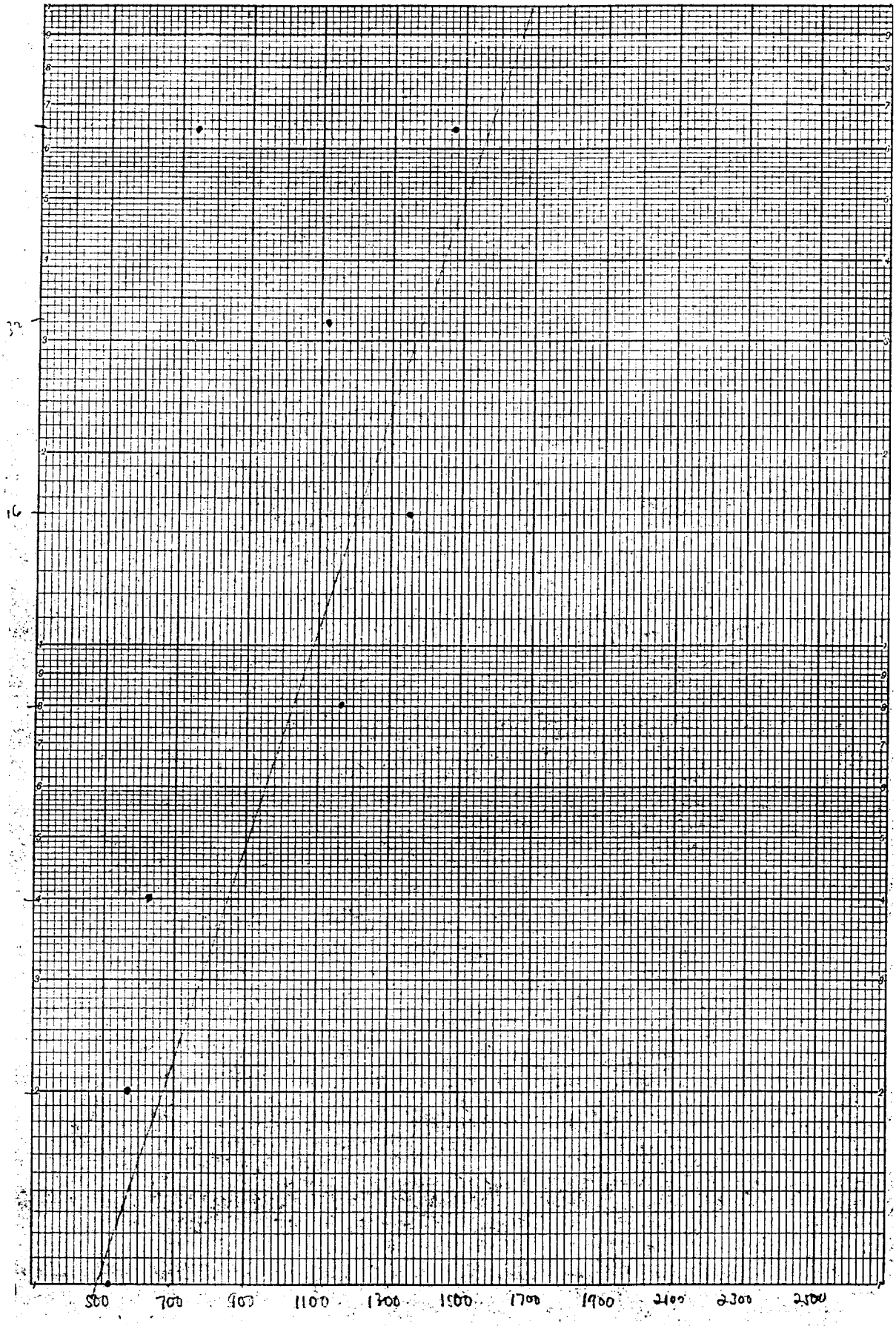
518t892-2	1.0 ml
518t892-1	1.0 ml
811t892-1	1.0 ml
811t892-2	1.0 ml

in Penassay - o/n.

-Sunday 11/30

2050 Reexamination of putative C 2050 in ☐ 142

EMB gal	Addition	In exp 2 days
1	none	½ = 131 (what happened?) (all small)
2	0.1 518t892-1	solid growth exp (mostly +?) c. 5000?
3	0.1 518t892-2 (undiluted)	solid growth exp (mostly +?) c. 5000?
4.	0.1 518t892-2 (dil (1-10))	solid growth exp (mostly +) $\frac{1}{64}$ = c. 100 = 7000
5.	0.1 811t892-1 (und)	solid growth exp (mostly +) c.
6.	0.1 811t892-1 (1-10)	solid growth exp (mostly +)
X	0.1 811t892-2 (und)	½ = 94 (all small
8.	0.1 K-12 (11/13) λ	½ = c. 100 = 800



No papillae →

Al wo = wo and 573 gal
Al K-12 = 0.1 K-12 λ
Al 891 = 0.1 892 λ

Monday-Tuesday 12/1 12/2

573 - No of cells plated - influence on number of transductions observed (papulon)

Stock suspension dil - 2×10^7 — 648,547 - $6.97\times2\times10^7$ = 139.4×10^7 = 1.4×10^{10} cells/ml
dil. stock plated in constant, 0.1 ml K-12 λ (11/23)

titer 2.7×10^{10} (estimated)

# cells		mg/ml K-12 λ	titer with at K-12 λ
1.4×10^4	lawn	1/4=626 (2504)	—
7×10^8	1-2	1/4=445 (1780)	35
3.5×10^8	1-4	1/4=369 (1472)	29
1.75×10^8	1-8	1/4=280 (1120)	10 *
8.75×10^7	1-16	1/2=662 (1324)	33
4.4×10^7	1-32	1/2=581 (1062)	49
2.2×10^7	1-64	1/2=281 (562)	39
1.1×10^7	1-128	535	32
5.5×10^6	1-256	509	23

(plotted)

* heavily contaminated ε spore plaques

1436 transduced by 750 - Distinction between gal$_1$- and gal$_4$-

EMB gal	Addition	No pop. 2 days
1	none	24
2	0.1 750 λ	145 ← many of these papillae have haloes about them; spontaneous do not.

16 papillae picked from EMB gal 1 streaked on gal

	1st alc (-)	2nd alc (-)	3rd
1	..	..	
2	..	..	
3	..	..	
4	..	..	
5	..	..	
6	..	..	
7	..	..	
8	..	..	
9	..	..	
10	..	..	discarded
11	..	..	
12	..	..	
13	..	..	
14	-	-	
15	-	-	
16	..	..	

2050 ± 811 pfu (142) stability

8 picked	1st mixed	2nd mixed pure (+)*	3rd mixed? (+ and ±)
1.	.	..	..
2.	.	..	..
3.	.	..	..
4.	.	..	..
5.	.	..	..
6.	.	..	..
7.	.	..	..
8.	.	..	..

← Stock made of this

* + reaction not as strong as usual-

Sunday 12/7/52

5784-12 · Examination 3 gal- segregants for their allelic state · (–) derived from sectored gal+ colonies · 6 streaked out once and single (–) colony inoculated into Pen. Overnight cultures ~~[illegible]~~ as follows:

Strain/gal(–) /1485		No add	K-12 (λ)	811-2 λ	902 λ-1	Conclusion
	1	(contaminated ε (+) colonies) →				
lgp	2	23	130 (repeat 224)	14	262	gal+
Gal–	3	(contaminated ε (+) colonies) →				—
segregants	4	(contaminated ε (+) colonies) →				
from	5	23	98 (repeat 225)	24	231	gal+
	6	(contaminated ε (+) colonies) →				—
5784-12	7	29	226 (repeat 273)	28	302	gal+
~~mutant~~	8	(contaminated ε (+) colonies) →				
lgp	9	31	146 (repeat 216)	30	248	gal+
lgp	10	48	186 (repeat 225)	80	299	gal+ (?)
lgp	11	37	154	22	284	gal+
lgp	12	40	164	53	245	gal+
	13	(contaminated ε (+) colonies) →				—
lgp	14	32	103	22	263	gal+
lgp	15	41	84	23	345	gal+
lgp	16	28	138	20	271	gal+
lgp	17	30	87	27	287	gal+
lgp	18	20	83+	24	231	gal+
lgp	19	28	67	28	239	gal+
lgp	20	28	107	34	206	gal+
lgp	21	31	97	17	245	gal+

This series = A

?

✓ thru 811 on ~~thru~~ lysate

EMB	Added	Pop 2 days
1.	811 cells - no λ	48
2.	" (811 λ)-2	50
3.	902 x 1658 lysate - no λ	13
4.	" - (811 λ)-2	105
5.	2050 - no λ	42
6.	" - (811 λ)-2	289

Exam. 811 plate stabs

	1	2	3
1.	mixed	mixed	
2.	"	"	
3.	"	"	
4.	"	"	
5.	"	"	
6.	"	"	
7.	"	"	
8.	"	"	
9.	"	"	
10.	"	"	
11.	"	"	
12.	mixed	mixed	
13.	"	"	
14.	"	"	
15.	"	"	
16.	"	"	

Strain note. h-10 (unstable, reisolated) EMB gal+ no add = 74 / 2 (0.811 λ)-2 = 59 — apparently 1st assay in error - contam ε spreader

4 failures / 17 successful tests

magnitude of 902-deletion similar to that of 902 on 811 which with this prep was (147±202) - c 40

16/17 say (–) segregants are gal–

1/17 says (–) segregant is gal+ - with possible change in at.

now 17/17 say gal–

2070 = (1673 gal- 1) examined for its relationship to gal 1-4 - by means of lysate activity.

EMB gal	Addition	No. prop. 2 days
1	none	10
2	0.1 750 λ	54
3	0.1 902 λ	¼ = 314 1256
4	0.1 892 λ	331
5	0.1 811 λ	175

2070 t 750

	1st	2nd	2nd
1	mixed	mixed	mixed
2	+	+	+
3	+	+	+
4	+	+	+
5	+	+	+
6	+	+	+
7	+	+	+
8	mixed	mixed	mixed

2/4

2070 t 892

	1	2	3
1	mixed	+	+
2	+	+	+
3	+	+	+
4	+	+	+
5	+	+	+
6	mixed	mixed	mixed
7	mixed	mixed	mixed
8	mixed	mixed	mixed

2/4

2070 t 902

	1	2	3
1	mixed	mixed	mixed
2	+	+	+
3	mixed	mixed	mixed
4	mixed	mixed	mixed
5	mixed	mixed	mixed
6	+	+	+
7	+	+	+
8	mixed	mixed	mixed

5/8

2070 t 811

	1	2	3
1	+	+	+
2	+	+	+
3	+	+	+
4	mixed	+	+
5	+	+	+
6	mixed	+	+
7	+	+	+
8	+	+	+

0/8

transferred to ε plate for

518+902 gal-
neg
= B transduce ε 811
902

12/9/52
Tuesday

146

518+902 Gal- segregants - Examination - Isolated from single segregating colonies streaked on EMB plates - tested a Bacteriophage disease

	1/485	no. spots			gal+
1.	lysogenic	235	18	50	
2.	"	223+	29	16	4-
3.	"	102+	22	15	4-
4.	"	contam.	29	24	4- ?
5.	-	137+	18	14	4-
6.	"	143+	25	23	4-
7.	"	10	507	9	2- ← }
8.	-	10	474	7	2- }
9.	"	325+	191	214	4-
10.	-	151+	16	31	4-
11.	-	160	20	44	4-
12.	"	232+	44	52	4-
13.	"	143+	16	32	4-
14.	"	136+	18	36	4-
15.	-	46	32	38	? }
16.	"	116+	29	21	4-
17.	-	214	31	31	4-
18.	"	99+	30	33	4-
19.	"	139+	41	41	4-
21.	-	contaminated ε gal+			
21.	"	22+	21	23	4-
	streaked	0.1 ml	0.1 ml	no add	
	/1485	902 λ	(811 λ)-2		
					17 4-

17 gal4-
2 gal2-
1 double?

The B Cross

Isolated

Saturday 12/13

C. -578 & 892 Examination of gal- segregants

gal- isolate	1485 lysogenic	0.1 (892 II)-1	0.2 (811 A)-2	[redacted]	probable locus
1.		92	22	24	gal 4 -
2.	"	78	38	36	gal 4 -
3.	"	99	27	25	gal 4 -
4.	"	353	304	20	*
5.	"	84	26	18	gal 4 -
6.	"	68	29	19	gal 4 -
7.	"	contaminated c + cells →			
8.	"	376	392	29	*
9.	"	442	324	45	*
10.	"	453	410	21	*
11.	"	337	364	30	*
12.	"	99	25	31	gal 4 -
13.	"	427	294	46	*
14.	"	387	416	23	*
15	"	78	36	32	gal 4 -
16	"	contaminated c + cells →			
17.	"	78	30	11 (mid plate)	gal 4 -
18.	"	309	271	22	*
19.	"	296	257	3	*
20.	"	88	25	35	gal 4 -
21.	"	73	29	38	gal 4 -
22.	"	67	33	25	gal 4 -
23.	"	278	190	21	*
24.	"	309	207 (thin plate)	18	*

booking

NS

892 a

mut

11 gal 4 -
* 11 Unknown locus -

streaks

Stability examination of * mutants locus -

	C-4-811			C-4-892			C-4-811			C-4-892		
	①	②	③	①	②	③	①	②	③	①	②	③
1.	mixed	mixed	mixed	mixed	mixed	mixed	mixed	+	mixed	mixed	mixed	mixed
2.	mixed	mixed	-	-	-	-	-	mixed	-	-	-	-
3.	mixed	mixed	-	-	-	-	-	-	-	-	-	-
4.	+	+	-	-	-	-	-	-	-	-	-	+
5.	+	-	-	-	-	-	-	-	-	-	-	mixed
6	mixed	-	-	-	-	-	-	-	-	-	-	-
7.	mixed	-	-	-	-	-	-	+	-	-	-	-
8	mixed	-	-	-	-	-	-	mixed	-	-	-	-

Worked

All galR exconj transferred to a plate for stock purposes

Saturday 12/13

S18[K-12 - √ on stability of gal-segregant after a second transduction - See pg 145 repeat exp

gal-pplate	1st streak	2nd streak	3rd streak
A-2 pp 1.	+	+	mixed
2.	mixed?	mixed	mixed
3.	+	+	+
4.	+ slow growth?	mixed	+
5.	+	+	mixed
6.	+	+	mixed
7.	+ slow growth?	+	+
8.	+ 1/8	+ 2/8	+ 2/8
A-5 pp 1.	mixed	mixed	+
2.	+	mixed	mixed
3.	+	+	+
4.	mixed	+	mixed
5.	mixed	mixed	+
6.	+	mixed	+
7.	+	mixed?	+
8.	mixed 4/8	+ 6/8	+ 7/8
A-7 pp 1.	mixed	mixed	mixed
2.	"	mixed	mixed
3.	"	mixed	mixed
4.	"	+	+
5.	"	+	+
6.	+	mixed	mixed
7.	+	+	mixed
8.	+ 1/8	mixed 5/8	mixed 6/8
A-9 pp 1.	mixed	mixed	mixed
2.	+	mixed	mixed
3.	mixed	+	+
4.	+	mixed	mixed
5.	+	+	mixed
6.	mixed	+	+
7.	+	mixed	mixed
8.	mixed 4/8	mixed 5/8	mixed 6/8
A-11 pp 1.	+	+	mixed
2.	mixed	mixed	mixed
3.	mixed	mixed	mixed
4.	mixed	+	+
5.	+	+	+
6.	+	mixed	mixed
7.	mixed	mixed	+
8.	+ 4/8	mixed 5/8	mixed 5/8

Bottles

previously (p.96) → 4 transfers = 60% mixed

here 23/40 → 3 transfers = 58% mixed

0.57 = 58

40 | 23.0

200

300

280

200

Monday 12/14

Crosses.

811 XY-10 to obtain gal- prototroph to examine the ability of 811 g+-1 and 811-g+-2 to transduce in minimal - Do the weak gal+ of these reversants another allele not having sufficient power to allow for selection in EMB gal ?

		prototroph. (+)	(-)	
EMB gal 1	both Y-10 × 811	237	8	
2.	" "	226	9	
		463	17	17/480

811 g+-6 XY-10 to observe if this is true reversion

		(+)	(-)	
EMB gal 1.	both.	277	0	
2.	"	357	0	
3.		341	1	← in checks on EMB gal this colony +
		975	1	

811 g+-7 X Y-10

1.	both	145	0
2.	"	215	1?
3	"	254	0
		614	1? in ← This col also + on ✓

811 g+-9 X Y-10

1.	both	235	0
2.	"	245	1?
3.	"	280	1?
		760	2? in ← both + on ✓

811 g+-10 X Y-10

1.	both	205	0
2.	"	153	0
3	"	205	0
		563	0

2062
Natural λ

EMB gal	no. pos 3 days
1 no add'd	4
2 a) 1485 λ-1	12
3 0.1 K12 λ (11/23)	219
0(a) 1. no add	0
2. a) 1485 λ	0

Lysate sterility

0.1 of 902, 892, 750 λ into Pen - all sterile -

Tuesday 12/18/52

518 transduced gal- segregants.

B = 518 t 902

B7 = on prelim. tests gal$_2$ -

EMBgal		after 2 days
1.	no add	56
2.	0.1 (650 λ) -1	611
3	0.1 (902 λ) +1	58
4.	0.1 (892 λ) -1	342
5.	0.1 (811 λ) -3	147

← this prep contam

12/23

	Kept'd dark
	63
	463
	65
	387
← this prep contam	205 ← (811 λ) -4

Cross. (+) (-)

B7 x 902 -	4	390
"	7	277
	13	667 + 225 = 892

↑ not releasable 902 contam. E +

B-8 = gal$_2$ - on prelim exam.

EMBgal		
1.	no add	59
2.	0.1 750 λ -1	705
3.	0.1 902 λ -1	77
4.	0.1 892 λ -1	513
5.	0.1 (811 λ) -3	173

← this prep contam.

despite this it apparently has some activity - other tests contam. not usual shows up in B-15 as

Cross.

B8 x 902	1	137
	3	176
	1	145
	5	458

This cross repeated giving about 12 ± 2% ± this called by accident involved about 10 plates with c. 200 prototrophs/plate

B-15 = a double? gal$_2$ - gal$_4$ - ?

EMBgal		
1	no add	30 (many small)
2.	0.1 (650 λ) -1	35 (" ")
3	0.1 (902 λ) -1	36 " "
4	0.1 892 λ -1	27 " "
5.	0.1 (811 λ) -3	28 " "

← this prep contam.

either hp_1 ?

Cross

B15 x 902	3	116
2.	3	176
	6	294
B15 x 892	1 ?	5
	8	4
	1 ?	9
B-15 x 1476	0	322

cross check ? from 1-2 cell not set

C = 518 t 892

C4 = transduced by 811 and 892 - a new allele?

EMBgal		
1.	no add	46
2.	0.1 (750 λ) -1	286 (wet plate)
3.	0.1 (902 λ) -1	27
4.	0.1 (892 λ) -1	190 (wet plate)
5.	0.1 (811 λ) -3	83

this hpate contam.

probably caused by contam in 902 culture

Cross

C-4 x 902	2?	174
	3	160
	5.	334
C4 x 892	1	10
	1	6
	2	16
C4 x 1476	0	155
	0	129
	0	284

C9 = as C4

EMBgal		
1.	no add	72
2.	0.1 (750 λ) -1	586
3	0.1 (902 λ) -1	91
4.	0.1 (892 λ) -1	357
5	0.1 (811 λ) -3	190

← this hp. contam.

Cross

C-9 x 902	1	165
	0	132
	1	297
C-9 x 892	0	0
	1	5
	0	5
C9 x 1476	0	108
	0	117

Tuesday 12/23/52

- 902 hp_2^S - transduction of old culture - Repeat using younger cell.

EMB gal	additions	no. per 3 days	
1	no add	14	
2	0.1 750 λ - 1	52	38
3	0.1 902 λ - 1	11	—
4	0.1 892 λ - 1	51	37
5	0.1 811 λ - 4	43	29

$\frac{3.8\times10^9}{2.4\times10^{10}} = \frac{1}{6.3\times10^0} = \frac{1}{6.3\times10^6}$

values low because of cell age? Repeat.

- 578

EMB gal	additions	no per 2 days	
1	no add	72	high?
2.	0.1 1485 λ	131	
3	0.1 K-12 λ 12/20	$\frac{1}{16} = 120 = 1920$	

- ✓ λ titer of this appears about as expected since titer is about 1/10 that of K-12 λ 12/30

(2/2) 1485 λ = 1.1×10^9 59/1.11

K-12 (12/20) λ = 2.3×10^{10} 1848/23

$59\times\frac{23}{1.11} = 1233$

"nat." λ = 1233

hugg λ = 1848

booked

Thursday 12/26/52

811 prototrophic gals - - To test the hyp that gal+ h of 811 not transducing 811, 578 are reverting to an allele with not sufficient selective advantage in EMB gal to allow detection of transduction.

EMB gal ↓	additions	no per 2 days	
1.	none	23	
2.	(811 λ) - 4	18	
3.	(811 gal+ -1) - 1	15	This gal+ doesn't work in EMB
4.	(811 gal+ -2) - 1	22	" " " " " "
5.	(811 gal+ 5) - 1 untreated (CONTROL)	214	all work in EMB
6	(811 gal+ 5) - 2	465	" " "
7.	(811 gal+ -8) - 1	309	" " "
8	(K-12 λ) 30-1	164	" " "

No screen - presumably gal+ -1, gal+ -2 are since maintains closely linked to gal+

2050 gals - gal deletion - Repeat - has high background - Make purified

EMB gal	2050 - additions	no per. 2 days	
1	none	296	
2	750 λ - 1	$\frac{1}{4} = 255 = 1020$	these counts all low - large nos of small pfg -
3	902 λ - 1	$\frac{1}{4} = 476 = 1904$	
4	892 - 1	288	
5	811 - 4	$\frac{1}{4} = 208 = 832$	

$>2.9\times10^{10}$

4.9×10^{10}

3.8×10^9

$\frac{7.74\times10^3}{2.4\times10^{10}} = \frac{1}{3.3\times10^6}$

$\frac{16.08\times10^4}{4.9\times10^{10}} = \frac{1}{3.1\times10^6}$

Sunday 12/28

874 gal deduction = another Lysogenic out of 518

EMB gal	Addition	no pap 48 days
1	none	17
2	0.1 750 λ -1	77
3	0.1 902 λ -1	518
4	0.1 892 λ -1	167
5	0.1 811 λ -4	23
6	0.1 K-12 λ 30-1	1067

believe as 811 with greater pow ducability by K-12 ?

D- 518 & 892 ← (-) from single transductions

gal - segregants - examination for stable by transducability test - ~~transduced~~ challenged by 811 and 892 → purified thru 3 single col. isolations

12/29 - 1402

ductions to see if 1821 deficiency is also 1402 dif. - apparently is -

EMB gal	Addition	no pap 3 days
1.	none	17
2.	0.1 750 λ -1	28
3.	0.1 902 λ - 1	61
4.	0.1 892 λ - 1	31
5.	0.1 811 λ - 4	22
6.	0.1 K-12 30-1	85

purification begun - streak survival single colony isolation / 12

892 - 2 λ not sterile discarded

902 - 2 λ sterile -

892 - 1 λ - recovered - lost 0.1 ml

$10^8 - 15$

8

$\overline{23} = 2.3 \times 10^9$

Tuesday 12/30/52

Exp D = Repeat examination of segs. from 518 & 892 - Segs purified thru 3 pickings -

Page 2 daily

	Gal-bal	Model	811λ-9	892λ-2	Probable gal locus	1985	From Strain
strk all	1	40	142	253	(?)	Segs	Recheckers - unstable
892	2	53	35	69	4	"	Rechecks
811	3	22	40	87	4	"	not t/811λ
all	4	8 (few small)	58 (all small)	173 all small	(?)	"	Rechecks - unstable
	5	37 >5	32	71	4	"	
811	6	32 >5	40 >5	74 >5	4	"	not t/811λ
	7	28 >small	40	99 >5	4	"	
	8	40	36	90	4	"	
	9	29 >small	37 >small	88 >5	4	"	
892	10	43 few small	46 few small	79 few small	4	"	Rechecks
	11	35	34	71	4	"	
892	12	52 >5	59 >5	81	4	"	Rechecks
892	13	47 >small	31 >small	82	4	"	Rechecks
	14	lost - but both all small low no pop.		73		"	
	15	21 small	121	275	(?)	"	Recks - mistakes
	16	30 custom	33	68	4	"	
892	17	39 >small	35 >small	60 >5	4	"	Rechecks
	18	35 >small	44	75 >5	4	"	
	19	27 (all small)	95 (all small)	256 (all small)	(?)	"	Rechecks - unstable
	20	27 many small	101 most small	324 all small	(?)	"	" "
	21	5	40	146	(?)	"	" "
892	22	45	35	61	4	"	Rechecks 892
892	23	58	36	87	4	"	" "

5/23 probably new type!

5/23 transduced by bac 811λ 892λ

Checks

Pop #	D1 t 811	D1 t 892	D2 t 892	D3 t 811	D4 t 811	D4 t 892	D6 t 811	D10 t 892	D12 t 892
	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3
1.	mix mix mix	mix mix mix	mix + +	+ + +	mix mix mix	mix mix mix	mix + +	mix + +	mix + +
2.	" " +	" " +	" + +	+ + +	" " "	" " "	" + +	" + +	" mix mix
3.	" " mix	" " +	" mix mix	+ + +	" " "	" " "	" + +	+ + +	" + +
4. single colony isolation	" " mix	" " +	" + +	mix + +	" + +	" " "	" + +	mix mix mix	" mix mix
5.	" + +	" " +	+ + +	+ + +	" mix mix	" " "	" + +	" mix "	" mix mix
6.	" mix mix	" " +	mix + +	" + +	" + +	" + +	+ + +	" mix "	" + +
7.	" " mix	" " +	" + +	" + +	" mix mix	" mix mix	+ + +	+ + +	+ + +
8.	" " +	" " +	" + +	" + +	" + +	" " "	mix + +	mix mix mix	+ mix mix

	D13 t 892	D15 t 811	D15 t 892	D17 t 892	D19 t 811	D19 t 892	D20 t 811	D20 t 892	D21 t 811
1.	mix + +	mix + +	mix mix mix	mix + +	mix mix mix	mix mix mix	mix mix mix	mix mix mix	mix mix mix
2.	" mix mix	" + +	" " "	" + +	" " "	" + +	" " "	" " "	" + +
3.	+ mix +	" mix mix	" " "	" + +	" " "	" mix mix	" " "	" " "	" mix mix
4.	mix + +	" " "	" mix mix	" mix mix	" " "	" " "	" " "	" " "	" + +
5.	" mix mix	" + +	" mix mix	" + +	" " "	" " "	" " "	" " "	" + mix
6.	+ + +	" mix mix	" " "	" mix mix	" " "	" " "	" " "	" " "	" mix +
7.	mix + +	" " "	" + +	" mix mix	" " "	" " "	" " "	" " "	" + +
8.	" + +	" " "	" + +	" + +	" " "	" + +	" " "	" " "	" " mix

Check on Exp D = 578892 Second run

Pap

	D21E892			D22E892			D23E892		
	1	2	3	1	2	3	1	2	3
1.	mix	mix	mix	+	+	+	mix	+	+
2.	"	+	+	mix	+	+	+	+	+
3.	"	mix	mix	+	+	+	mix	mix	mix
4.	"	+	-	mix	+	+	"	+	+
5.	"	mix	"	"	mix	mix	"	mix	mix
6.	"	+	"	"	+	+	"	+	mix
7.	"	+	"	"	+	+	"	mix	mix
8.	"	mix	"	+	+	+	+	+	+

1/6/53 Tuesday.

Recheck on D4, D15

1/11/53

D15

EMB gal	Addition	Inc. last 2 days
1.	none	14
2.	750λ-1	$\frac{1}{2}$=151, 302
3.	902λ-1	22
4.	2050λ-1	40
5.	811λ-4	73

1/12/52 >70

D4

EMB gal		
1.	none	12
2.	750λ-1	$\frac{1}{2}$=180 360
3.	902λ-1	12
4	2050λ-1	50
5.	892λ-1	365
6.	811λ-4	90

D4X902	(+)	(-)			
1.	0	88			
2.	2	96			
3.	2	130			
4.	3	109			
	7	923	7/930	1.6%+	

maybe be suspect - 902 culture probably contained (+) cells

second cross D1X902 P8 166 → 4.2%

0.042 / 18.0 / 12.8 / 12

241 / 177 / 422

Tuesday 12/30

2175 = 902 lp_2^S - Repeat duction ē aerated cultures

		EMB gal	Addiks	Pap. 2 days			
		1	mm	S	(gm plate)		
2.4×10^{10}	2		750 A-1	71	57	66	$\frac{1.6\times10^3}{2.4\times10^{10}} = \frac{1}{2.6\times10^6}$
	3		902 A-1	14	"		
3.2×10^9	4		892 A-1	102	51	97	$\frac{9.70\times10^3}{3.2\times10^9} = \frac{1}{2.6\times10^5}$
2×10^{10}	5		811 A	51	43	46	$\frac{9.6\times10^3}{2\times10^{10}} = e.\frac{1}{2.2\times10^6}$
	6				pieces?		

1402 Examination of parent strain for possible presence of lp_2^R
6 isolated colonies tested against 1-2 - all sensitive

902 lp_2^S = 2175 ABOVE - Stability

	902 + 750			902/902 A			902 + 892			902 + 811		
	1	2	3	1	2	3	1	2	3	1	2	3
1.	mix	+	+	mix	+	+	mix	+	+	mix	mix	mix]
2.	"	mix	mix]	"	+	+	"	mix	+	"	mix	"
3.	"	mix	mix]	"	+	+	"	+	+	"	mix	+
4.	"	+	+	"	+	+	"	+	+	"	+	+
5.	"	mix	mix	"	+	+	"	+	+	"	mix	mix]
6.	"	+	+	"	+	+	"	+	+	"	+	+
7.	"	+	+	"	+	+	"	mix	+	"	+	+
8.	"	+	+	"	+	+	"	mix	mix]	"	+	+

[Stock: make]

STOCK OF 1655 X 902 → gal_2 - lp_2^S
made - Entered in Stock book = 2175

Sat. 1/3/53

-2050 gal 3- Re examination of galactose using a single colony isolate from streak to lower of background-

GM Bgal	Addition	No. exp 8 days	
1.	none	32	
>2.9 X 10^10 2.	750 d -1	104+ many small	$\frac{7.3\times10^{2}}{2.9\times10^{10}} = \frac{1}{3.3\times10^{7}}$
4.9 X 10^10 3.	902 d -1	1/3 = 132 = 1056 " "	$\frac{1.0\times10^{3}}{2.9\times10^{10}} = \frac{1}{4.9\times10^{6}}$
4.	892 d -1	130 " "	a large no? See examination below
5.	811 d- 4	1/4 - 55 = 210 " "	

- Sunday 1/4/53

2050 above - stability examination

Pipeline #	2050 + 750 1	2	3	2050 + 902 1	2	3	2050 / 892 1	2	3	2050 + 811 1	2	3
1	mixed	+	+	mixed	+	+	mixed	+	+	+	+	+
2	"	+	+	"	+	+	+	mixed	mixed	mixed	+	+
3	"	+	+	"	+	+	mixed	"	mixed	"	+	+
4	"	+	+	"	+	+	+	+	+	"	+	+
5	"	+	+	"	+	+	+	+	+	+	+	+
6	"	+	+	"	+	+	+	+	+	mixed	+	+
7	"	+	+	"	+	+	+	mixed	mixed	"	+	mixed?
8	pure	mixture		"	+	+	+	+	+	"	+	" (?)

1/7/53
Wednesday (Held over from 1/6/52)
Eq E = 2050+81 - gal- segregants

Segregant #	Wood.	81A-4	2050A-1	1985	Probable age (years)	
1	* 61	1/8 = 87 456	1/8 = 102 204	Eyes	(?)	
2	* 75	1/8 = 76 608	* 111	"	3 (?)	
3	15	1/8 = 33 264	14	"	3	
4	c. 30	c. 200	c. 10	"	3	
5	c. 20	c. 200	c. 20	"	3	
6	17	c. 300	18	"	3	
7	12	1/8 = 39 136	22	"	3	
8	69	1/8 = 83 116	48	"	3	
9	* 116	1/8 = 197 588	* 182 *	"	3 (?)	on 1/12/52 this plate 2050A = c. 360
10	c. 10	c 200	c. 20	"	3	
11	* 20	1/2 = 78 156	20	"	3	
12	* 28	1/4 = 96 384	89	"	(?)	
13	18	1/2 = 41 82	11	"	3	
14	1/2 = 147 294	1/4 = 154 (many small) 616	1/4 = 148 (many small) 592	"	(?)	
15	50 > 31 >	c. 400	c. 200	"	(?)	
16	c. 10	c. 80	c. 10	"	3	
17	c. 100	c. 150	c. 10	"	3?	
18	c. 200	c. 100	c. 10	"	?	
19	17	1/8 = 80 640	1/8 = 62 496	"	?	11 (3)
20.	c. 10	c. 150	c. 20	"	3	9 (?)

Disregard → 2050

* = Nearly
All plates have large numbers of small papillae making counting and interpretation difficult. - In general results suggest the same conclusion suggested by the 81A-4 results.
892

Sunday 1/11/53

- B-7 X 902 - Repeat cross B7 = 578t902 → ←1 sig transduced by anal 1 but 902 1

		(+)	(-)		
EMS	1.	3	222		
gal	2.	7	276		
	3.	9	315		
	4.	7	239		
		26	1052	26/1078	= 2.5% ←

These results suspect since 902 cultures probably contaminated (+)

- B-8X902 B8 same as B7

EMS	1.	3	105		
gal	2.	4	94		
	3.	3	98		
	4.	4	88		
		14	385	14/399	3.5% ←

811 X892

		(+)	(-)		
EMS	1.	0	132		
gal	2.	0	156		
	3.	0	123		
	4.	0	184		
		0	595	0/595	

gal4 X gal3

811 X 902

		(+)	(-)		
EMS	1.	1	143		
gal	2.	0	230		
	3.	0	129		
		1	602	1/603 =	0.16%

gal4 X gal2

811 cultures probably contaminated upon rerun results.

2050 · Galactose

EMB-gal		Day 2 days
1.	no add	22
2.	K-12 (304)	32 what happened?
3.	1486tK-12-1	227
4.	D1 (see pg 153	solid smear of pop 5000?
5.	578t892-1	solid smear of pop 5000?

D1 ← 578t892 → (-) by characteristics by gald as 902, unknown by crossing data?

D1 ← 578t892 results as previous.

8/20/53 Tuesday after xy-

	no ABC 2 days 518	874
518 abt 874 {2 ml cell susp. ⟶ no add assay 0.1 ml {1 ml K-12 (39-1) ⟶ adsorb 5 min	12	19
{centrifuged susp'd 2.0 ml ⟶ assay 0.1 ml = K12 ductin	c.87	593
↓ {leaves 1.8 ml {add 0.9 ml 81-4 λ - adsorb 5 min		
↓ {centrifuge + susp. 1.7 ml ⟶ assay 0.1 = 1st 81 effect	c.59	233
↓ {add 0.8 ml to 1.7 ml remain - adsorb 5 min {centrifuge - susp in 1.7 ml ⟶ assay 0.1 - 2nd 81 effect	c.44	169

these plates rod-growth does not look like 518. sp. ly small after 2 days

Exposure to virus λ seems to reduce sap. - Better rockets

518 vs 518t592

10X cell dil	
1.0 ml	0
1.0 :	1-10 1 ml
1.0 :	1-100 1 ml
1.0 :	1-1000 1 ml

all diluted 1/2

adsorb 5 min, centrifuge, susp. in 1.7 ml, assay 0.1 ml

Lysis

Lysis

this plate shows almost confluent lysis

Titer these 518t592 811t592 preps used as ly oddity observed is due to low titer - rechecked on 2050 - Is this phage lytic? - different from λ, λ-2 ?

SW927 Rugh

1st lysates show 1 plaque / 950 SW in dil 106 - titer c. 109

Wg 16
Wg 19 } Auxotrophs catalogued
date as ~ 120-130.

1/23/6

Lysate titers

811-4
10^8 - 113, 67 = $90 \times 10^8 = 9 \times 10^9$

902-1
10^8 - 93, 90 = $91 \times 10^8 = 9.1 \times 10^9$

902-2
10^8 - 24, 19 $22 \times 10^8 = 2.2 \times 10^9$

902-3
10^8 - 100, 48 7.4×10^9

2050
10^8 - 314, 263 2.9×10^{10}

518E892-1
10^7 - 195, 166 1.8×10^9

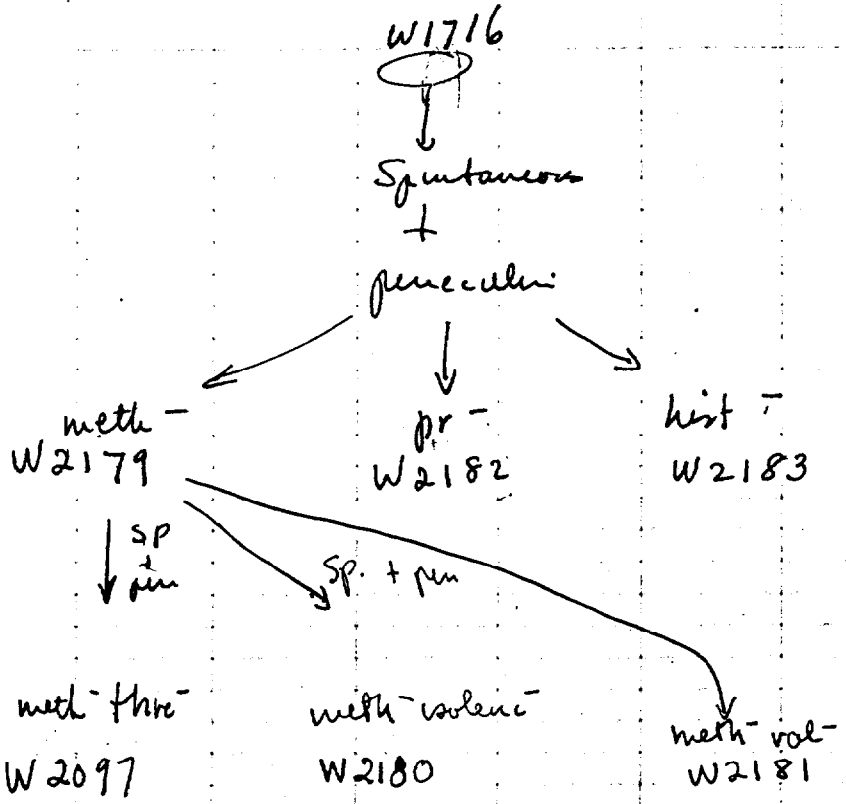
$\frac{2 \times 10^5}{2 \times 10^9} = \frac{1}{10^4}$

01
10^8 10^5 >87 9.6×10^9

K-12 - (12/20)
10^6 = 244, 213 2.3×10^{10}

1485
10^7 = 129, 96 1.1×10^9

Wg 16


W1716
Spontaneous
+
penicillin
meth⁻
W2179
pr⁻
W2182
his⁻
W2183
SP
+
pen
sp. + pen
meth⁻ thre⁻
W2097
meth⁻ isoleu⁻
W2180
meth⁻ val⁻
W2181

1/26/53 Monday -

- Recovering -

518tB92 λ - examination for lytic phage present.

streaked against 1485 = lysis
902 = no lysis } suggests lytic of λ - not λ-2

DI lysate - Examination

		no. pap	
1.	518 alone	– c. 30	
2.	518 + DI lys	– solid smear - 10^4 pap.	suggestive of lysis
3.	902 alone	– smeared – 10^4 pap	lysate added by accident? 902 contaminated C (+)
4.	902 + DI lys	– smeared – " " "	
5.	2050 alone	– c. 10	
6.	2050 + DI lys	– solid smear - 10^4 pap.	

~~7. 518tK12~~

518 for the purpose of examining a larger no. 518tK12 gal- segs.

1.	control alone	c. 30
2.	0.1 K-12 (12/21)	c. 500 (½ days incubation)

Blue contaminant on EMB

EMB lac - blue colonies - indicating ach. need - no use of lac?
lac Su " " " " " " " " " abs. Su
mal - " " " " " - no use of mal?
Ygl - light blue colonies - utilization of ygl?

518 No prod. as function of cell no's.
Adsorption expt.

518 cell samp. → 0.1 X 10 X 100 X 100 X 128 = 6³ 69 ?

$66 \times 10^8 \times 1.28 = 84.5 \times 10^8 = 8.5 \times 10^9$

Tube	Cells added	Saline	K-H (Gal)	Centrifuge/decant	No. reps	No. cells innoc'd
1.0 → 1	1.0 ml	1.0 ml	0.2 ml	rapid	6	4.2×10^9
2	5 (1-2)				2	2.1×10^9
3	5 (1-4)			1.0 ml	8	1.15×10^9
4	5 (1-8)			Assay	5	5.75×10^8
5	5 (1-16)			0.1 ml	5	2.87×10^8
6	5 (1-32)				20 (nitrogen max)	1.73×10^8
7	5 (1-64)				12 bkgrd?	7.1×10^7
8	5 (1-128)				14	

No background assay mode at c 20.

[illegible] titer = 71-(bkgrd) x 10 x

518 X 892

	Exp'mt	Prototrophs (+)	(−)	total
Cross plates	1.	0	4	4
base	2.	2	2	4
cysteine	3	1	1	2
added	4.	0	1	1
	5	0	6	6
	6	1	1	2
	7.	0	1	1
	8.	0	3	3
	9.	0	3	3
	10.	1	3	4
		5	25	30

5+/30 = 0.166 = 16.6%

~~mus~~ B8 lysate
A3 Tum
{519 absorpt
{492 absorpt

mus 519 492 —
2050 mutants

behavi

123
124, 125
126

{519 Hp^5 gal_4^- gal_3^+
{892 Hp^+ gal_4^+ gal_3^-

Make Tus

1. Sens A lysats — A1, ~~A3~~ —
2. Cross (1A36) X A1 (A3) (A11) (A13)
3. ~~Transduce 1, 2, 3, 4~~ A1, A3 A11, A13
4. Make lysate K-12, B8 → str'k out B8 test sub-line
5. 519 absorpt.
6. /

	cold mus	K-12 (B8-1)	Centrifuge decant	
1.	1.0 ml (10X conc)	0.1 ml	//	— plate 0.1 ml
1-2	1.0 ml + 1.0 ml sal	//	//	
1-4	//	//	//	
1-8	//	//	Resusp'd	
1-16	//	//	1.0 ml	
1-32	//	//	Sal	Count X DF // total ml / 1
1-64	//	//	//	
1-128	//	//	//	

1-10^4 (0.1 + 10
1-10^5 (5
1-10^6 → 0.1 ml for count

Expt. A SIBtK-12 → seq (-)

A 3 X 1436 EMS gel

	(+)/total
1.	0/212
2.	0/192
3.	0/194
4.	0/172
5.	0/205
	975

A 11 X 1436

	(+)/total
1.	0/179
2.	0/189
3.	0/273
4.	3/291
5.	0/216
	1048

A 13 X 1436

1.	c. 300 (-)
2.	c 300 (-)
3.	c. 250 (-)
4.	c 200 (-)
5.	0/195
	1250

In all of these suggestion of lysis in some colonies - tendency of some colonies to appear flatter also - pale pink -

SIBtK-12 lysate -

$10^8 = 17,25$

Wednesday 2/4/53

518 hr, 1412 lpr

preliminary adsorption

K-12 λ (12/20) used - 1.0 ml added + 20 ml cmc {518 / 1412} to sed. Cells stored in broth overnight.

518 10^6 = c. 600, 600 = 6.0×10^8 λ/ml ∫ sup.

1412 10^8 = 3,1 = 2×10^8 λ/ml ∫ sup.

initial λ titer = 2.3×10^{10}

Better check this - looks like hyper R adsorbs

750 λ titer (unfiltered prep)

10^8 = 28,94 = $\frac{12.2}{2}$ = 6.1×10^9 lousy assay replates

A3 λ titer (A = 518 CK-12 → (-) seg A3

10^7 = c. 500, 500 = 5×10^9

D1 λ titer (D = 518 CK892 → (-) seg D1

10^7 = 64, 0

0.5 ml D1 + Pen ——— sterile —

Thursday 2/5/53

$Lp^+ gal_4^- gal_3^+ \times Lp^+ gal_4^+ gal_3^-$

518 × 892 on EMB gal · no S added – an aerated culture of 892

	(+)	(–)	total
1.	0	3/18	3/18
2.	0	19	19
3	1	23	24
4.	0	19	19
5.	0	21	21
	(1)	100	101

$101 \overline{)1.000}$ (0.0099, 909, 910) = 0.99 %

see pg 163 there recomb = 16.6%

this (+) confirmed on EMB gal

750 × 892 on EMB gal – no S added

1.	0	3/19	3/19
2.	0	19	19
3	1	25	26
4.	0	27	27
5.	18	22	23
	1 (18)	112	114

$114 \overline{)1.000}$ (0.0087, 912, 880) = ~~0.87 %~~ 0/119 %

these not confirmed on EMB – both (–)

518 transductions.

EMB gal	Additions	Pop. after 2 days
1.	none	40
2.	518 & 892-1 und.	solid smear
3.	518 & 892-1 1-10	$>10^4$
4.	" -1 1-100	1/2 = c. 180 · 2080
Exp. 5.	DI und.	Solid smear
6.	" 1-10	" "
7.	" 1-100	1/2 = c. 200 = 6400
518 & 892 (–) exp. 8	AI und	9 (lysis?)
used for test of (–) exp. 9.	K-12 (14/20) und.	1/16 = c. 120 = 1920

evidence of lysis – plaques around margin

no evidence of lysis – calculated

$2.0 \times 10^3 \times 10 \times 10^2 = \frac{2.1 \times 10^6 \text{ transductants/ml}}{1.8 \times 10^9 \lambda / \text{ml}} = 1\,t / 8.6 \times 10^2 \lambda$

$6.4 \times 10^3 \times 10 \times 10^2 = \frac{6.4 \times 10^6 \text{ transd}}{9.6 \times 10^9 \lambda} = 1\,t / 1.5 \times 10^3 \lambda$

$\frac{1.9 \times 10^4 t}{2.3 \times 10^{10} \lambda} = 1\,t / 1.2 \times 10^6 \lambda$

2/5/53
Thursday

2175 transduction

	EMB gal	Addition		No. Pap. 2 days	1 day plate
	1.	none		13	
	2.	518C892-1	und	$>10^4$	
	3.	"	1-10	$\frac{1}{16} = 85 = 1360$	
	4.	"	1-100	$\frac{1}{2} = 138 = 276$	
DI + 518C892	5.	DI	und	19	
1 day	6.	"	1-10	9	
gal$_2$ - by t test	7.	"	1-100	19	
not gal$_2$ by cm					
	8.	A3	und	29	

$\frac{1.4\times10^3 \times 10 \times 10}{1.9\times10^9\ \lambda} t = \frac{1.4\times10^5}{1.9\times10^9} = \frac{1\epsilon}{1.3\times10^4\lambda}$

$\frac{2.76\times10^3\times10\times10^2}{1.9\times10^9\ \lambda} t = \frac{2.76\times10^5}{1.9\times10^9} = \frac{1\epsilon}{7\times10^3}$

apparently no effect — if DI + like A3 and the other 1 this ✓ with ability to be transd.

DI ~~not~~ tr. by 902 λ

Indicates DI is gal$_2$
1/19/53

2050 transduction

	EMB gal	Addition		No. Pap 2 days	
	1.	none		22	
	2.	518C892-1	und	$>10^4$	
	3.	"	1-10	$\frac{1}{32} = 121 = 3872$	
	4.	"	1-100	$\frac{1}{16} = 85 = 1360$	
	5.	DI	und	solid smear	
	6.	"	1-10	" "	
	7.	"	1-100	$\frac{1}{16} = 93 = 1488$	

$\frac{3.8\times10^3\times10\times10}{1.9\times10^9\ \lambda} t = \frac{3.8\times10^5}{1.9\times10^9} = \frac{1\epsilon}{5\times10^3}$

$\frac{1.4\times10^3\times10\times10^2}{1.9\times10^9} = \frac{1.4\times10^6}{1.9\times10^9} = \frac{1\epsilon}{1.3\times10^3}$

$\frac{1.5\times10^3\times10\times10^2}{1.6\times10^9} = \frac{1.5\times10^6}{9.6\times10^9} = \frac{1\epsilon}{6.4\times10^3}$

750 transduction

	EMB gal	Addition		No. Pap 2 days	
	1.	none		1	
	2.	518C892-1	und.	solid smear	
	3	"	1-10	$>10^4$	
	4.	"	1-100	$\frac{1}{32} = c.100 = 3200$	
	5.	DI	und	solid smear	
	6.	DI	1-10	$>10^4$	
	7.	DI	1-100	$\frac{1}{32} = c.200$ 6400	

$\frac{3.2\times10^3\times10\times10^2}{1.9\times10^9} = \frac{3.2\times10^6}{1.9\times10^9} = \frac{1\epsilon}{5.9\times10^3}$

$\frac{6.4\times10^3\times10\times10^2}{9.6\times10^9} = \frac{6.4\times10^6}{9.6\times10^9} = \frac{1}{1.5\times10^3}$

Sunday 2/8

Crosses · 518 X 892 — mixed cell-strains — gal$_9$⁻ x gal$_3$⁻

EMS gal.	(+)	(0)	total
1.	0	1	1
2.	1	12	13
3.	0	5	5
4.	0	5	5
5.	0	6	6
6.	0	3	3
	1	32	33

1(+)/33 = 3.8 %(+) recomb.

p. 165, 0.99 %
p. 163, 16.6 %

Crosses 518 X 902 — gal$_9$⁻ ~~x~~ gal$_2$⁻

EMS gal			
1.	13	216	229
2.	8	218	226
3.	8	170	178
4.	19	171	190
5.	19	160	179
	67	935	1002

1002 / 67.00 = 0.0667 = 6.67%

probably not valid - 902 contains (+) cells -

01 = 518 ⊂ 892 → (-) segr. = gal$_2$⁻ by transd.

01 X 902

EMS gal			
1.	11	234	245
2.	7	170	177
	18	404	422

4/4 strains after 3

422 / 18.00 = 0.0426 = 4.26 %

probably not valid 902 contains (+) cells

Confirmed on EMS gal

01 X 892

1.	1	5	6
2.	0	3	3
	1	8	9

9 / 1.0 = 0.111 = 11.1 %

Stability examined

1st streak - mixed + ; one .. - - ; 3rd .. - ..

Confirmed on EMS gal

01 X 1436

1.	0	166	166
2.	0	153	153
3	0	128	128
	0	447	447

0/447 = < 0.22%

518 X 897 — 165 = Arg+. F

Monday 2/9/53

98

518 X 892. prototrophs - all gal (-) except #1 gal (+)

	Str	/1985		λ	Rx	/1985
1.	S	n. lys	14.	r		lys.
2.	S	"	15.	S		n. lys.
3.	S	"	16.	S		n. lys
4.	S	"	17.	r		lys
5.	S	"	18.	r		lys
6.	r	lys	19.	S		n. lys
7.	r	lys	20.	r		lys
8.	S	n. lys	21.	r		lys
9.	r	lys	22.	S		n. lys
10.	r	lys	23.	S		n. lys
11.	r	lys	24.	r		lys
12.	S	n. lys	25.	S		n. lys.
13.	S	n. lys				

← blue on EMB(0) - does this indicate a double (-) ?

14 S 4p^s = 13 4p^s gal-
11 lys 4p^r = 11 4p^r gal-

B8 transduction

EMB gal	Addition	No. pop 3 days
1.	none	75
2.	0.1 2050 λ - 1	106
3.	0.1 750 λ - 1	556

D1 transduction

EMB gal	Addition	No. pop 3 days
1.	none	101
2.	0.1 2050 λ - 1	120
3.	0.1 750 λ - 1	707

D4 transduction

EMB gal.	Addition	No. pop 3 days
1.	none	46
2.	0.1 2050 λ - 1	100
3.	0.1 750 λ - 1	536

518X892 pg 166 Leakage already not possible

(+) proto troples picked all grew 40/40 (−) proto troples picked 8/40 grew — why?

one picked all three

578X892 EXPT F: Examination of (−) proto troples from the cross for their travel behavior

Proto. #	80 adh	811 λ-9	2050 λ-1	Comment	Stockable Locus	/λ-2	Lp Chas. 1967
1	Gal + proto troples —					S	—
2	c. 400 (large small)	c 100 large small	c. 200 large small		?	S	S
3	63 (like 4)	38	68	Lp_2^R ? no	? — 1-	S	S
4	45 (l. 4-)	15	35	Lp_2^R ? no	? — 1-	S	S
5	38 (like 4-)	21	49	Lp_2^R ? no	? — 1-	S	S
6	13 (like 3-)	96	4		3-	S	lys
7	13 (like 3-)	118	15		3-	S	lys
8	29 (like 4-)	11	16	Lp_2^R ? no	? —	S	S
9	28 (rocks like 4-)	29	39	Lp_2^R ?	? —	S	lys —
10	2 (like 3-)	40	2		3	S	lys
11	117 (rocks like 3-)	~~527~~	171		~~3~~ 3	S	lys
12	47 (like 4?)	15	57	Lp_2^R ? no	? — 4-	S	S
13	99 (rocks like 4-)	21 many small	76		? 4-?	S	S
14	3 (like 4-)	11	5	Lp_2^R ?	? —	S	lys —
15	c 300 (large small)	c. 100 L s	c. 100	Lp_2^R ? no	? — 4-	S	S
16	53	14	61		? — 4-	S	S
17	0	36	0		3 —	S	lys
18	0	0	0	minute film 2 tiny Lp.	3 see pg. 1706	S	lys
19	57 (like 4)	25	44	Lp_1 ? no	? —	S	S
20	3 (like 3-)	122	2		3	S	lys
21	1 (like 3-)	202	2		?	S	lys
22	30	21	44		? 4-?	S	S
23	c. 400 large small	c.100 (L:S)	c. 300 (L:S)		? — 4-?	S	S
24	3 (like 4-)	11	5	Lp_2^L ?	? —	no growth	lys —
25	37 (l. 4-)	29	68		? 4-?	S	S

blue blue — 8/8 stable after 2 — 8/8 stable after 2 — blue — 3/8 unstable after 2 — blue blue — 2/8 unstable after 2 — 8/8 stable after 2

4+ col contains stable about appear. of plate - Lp appears.

8 clear cut 3- and Lp^+

gal- λ on gal+ has a tendency to lower the no. of outcrossers pos. On this basis no's 2, 3, 4, 5, 8, 12, 13, 15, 16, 19, 22, 23, 25 called be 4-

8 3-
13 possibly 4-
4 unknown

Wednesday 2/11/53

Transductions - Spot Method

Trial 1 - Using K-12 12/20 λ

	no. pap.
518 - 0 add	0
+ K-12 λ	61
811 - 0 add	0
+ K-12 λ	15

Trial 2

518 - 0 add	0
+ K-12	66
811 - 0 add	1
+ K-12	32

B8 = 518t902 ⟶ (-) seg

14/84 spontaneous papillae stable thru 3 purification streaks -

B8t750 unstable

D1 = 518t892 ⟶ (-) seg grew by tr and dense but not quite by ~~X gal~~ 4hr // possibly grew by conc X 1436

7/10 16/18 spontaneous papillae unstable after 3 pickings

D1t750 unstable

D1X892 (+) prototrophs unstable still after 4

D4 = 518t892 ⟶ (-) seg ~~was~~ a D1

13/13 spontaneous papillae stable thru+ after 3 pickings

D4t750 unstable

Tuesday 2/17

750XY-10 & obtain gal,- prototroph:
c. 2(-)? /300 prototrophs.

Lysates:

750-2 (2 bottles)
10^8 = 312, 296 = 3.04×10^{10}

811-5
10^8 = 49, 49 = 4.9×10^9

B8 - 578 T 902 (+) seg
10^8 = 699, 785 7.43×10^{10}

Expt. G : DI apart. 5th (unstable) (+) segregants to observe their pattern of transduction.
Numbers 1-5

Cr H - 578 tlc 12 (+) seg tested against 2-, 4-

04 T 750
10^7 dil = >1000 titer > 10^{10}

Friday 2/20/53

Expt. G. 01 = 578t 892 → (-) seg. By transducability 6st = 2-
" donor " = 2-
" X 902 ≠ 2 ⇒ the mice 902 cont. (+)

01 spont. gal+ are unstable.

5(-) seg from 01 - from seg segregation.

G-1, 2, 3, 4, 5 - Addition		No. gal 58	days G-2 12	G3 25	G4 75	G5 10
1	none					
2	758A-1	1/4=38 304	1/4=69 552	1/4=69 276	1/4=91 364	1/4=49 392
3	702A-2	59	16	32	32	28
4	2030A-1	108	42	67	62	47
5	811A-5	102	49	65	55	53
Prob. locus		2-	2-	2-	? 2-	? 2-

Streak of middle plate
1. 7/8 mixed (-) | 8/8 mixed | 8/8 mixed, 1 let. (+) | 5/8 mixed (-)
2. 7/7 pure → stable | 6/8 mixed | 8/8 mixed, 6/8 mixed | 5/5 (+) → stable
3. | 6/8 mixed | some of these may be slows

G-2-3 1. 7/8 mix, 1 (-)
2.
3.

G-2-4 1. 7/8 mixed, 1 (-)
2. 4/7 mixed,
3. 7 medicals transduct. by 4-

G-1-4 1. 8/8 mixed
2. 7/7 mixed
3. medicals transd. by 4-

G-5-2 1. 4/8 mixed, (-)
2. 6/6 (+)
3. ↓ medicates no. 2 - transd.

Friday 2/20/53

Exp. H. = 578 tK-12 → (-) seg from 21 separate transductions

No.	Add			Probably lives	
1.	27	43	1/4=36 144	4-	pure(+) on 1st streak -
2.	29	32	1/4=42 168	4-	
3.	35	29	1/4=27 216	4-	
4.	15	22	1/4=61 244	4-	
5.	32	36	1/4=71 284	4-	
6.	32	42	1/4=73 292	4-	
7.	31	30	1/4=56 224	4-	
8.	28	37	1/4=45 180	4-	pure(+) 1st streak -
9.	(+) cell contamination ?			(?)	
10.	17	27	1/4=54 216	4-	
11.	27	31	1/4=42 168	4-	
12.	(+) cell contam ?			(?)	no add plate 1st (apparent)
13.	36	23	1/4=63 252	4-	
14.	30	33	1/4=59 236	4-	
15.	26	31	1/4=55 220	4-	
16.	27	31	1/4=42 168	4-	
17.	(+) cell contam ?		-	(?)	no add plate 1st (apparent)
18.	32	31	1/4=59 236	4-	
19.	31	39	1/4=61 244	4-	
20.	31	24	1/4=64 256	4-	
21.	40	44	1/4= 52 208	4-	

All 4 organisms pure (+) on 1st streak - but not recorded

H-9-0 - 8/8 stable after 2 isolations,
H-17-0 - 5/5 unstable after 2 "
H-12-0 - 6/6 unstable after 2 "

} probably contamination occurs on purification (selecting a (-) colony) and replating - plating out on EMB gal. and picking spot + papillae no unstables found after 2 - No (+) sp. th/pop about 30-40/plate

From the transduction data these (-) seg appear to be 4-

18/18

Test some of them for (+) / for allele test

Friday. 2/20/53

518	Addition	No. pap 2 days
1.	None	67
2.	04 ± 750 $1-10^3$	24
2.	" $1-10^5$	11
4.	" $1-10^7$	80
5.	" $1-10^9$	46

2062 – isolation of double mutant.

colonies in cmd $\left.\begin{matrix}1-10^6\\1-20\end{matrix}\right\}$ suitable for replication to DOI + pr -

510 X 882 prototroph #18 gal- - alkaline rx on EMB gal; B(c) suspicious that it is 3-4-?

After 3 days EMB gal

1	no add	– c. 100 pap mostly small	enclosed flu. is a 3- but
2.	750 +/- 1	– c. 300-400 pap (larger than no add)	slow in response for some reason
3.	912 -/- 2	– c. " " " "	
4.	2050 +/- 1	– c. 75 pap mostly small	
5.	811 +/- 5(?)	– c. 300-400 pap larger than no add	

Thursday 2/16/53

mixed culture

578 X 892 B, added to X plates.

EMS gal	(+)	(−)	tot
1	0	9	9
2	0	12	12
3	1	13	14
4	1	11	12
	2	45	47

Confirmed in EMS gal

SOME OF THESE PROTO- ✓ ON PG 173 FOR 4, L, 2 [illegible]

0.042
47 / 2.0000
188
120
= 4.2 %

		(+)	(−)
ρ. 166	= 3.3	1	32
ρ. 165	= 0.99	1	100
ρ. 162	= 16.6	5	25
This	4.2	2	45
		9	202

211

0.0476
211 / 9.00
844
560
422
1380

Error 4.3%

578 X 902

EMS gal	(+)	(−)	tot
1	2	381	383
2	1	278	279
3	0	341	341
4	2	284	286
	5	1284	1289

0.0038
1289 / 5.000
3867
11330
10312
= 0.38 %

Confirmed in EMS gal - stable after 2 p.c. isol.

811 X 902

EMS gal	(+)	(−)	total
1	0	198	198
2	0	151	151
3	0	184	184
4	0	173	173
	0		706

(Estimated < 0.14 %)

0.0014
707 / 1.0000
707
2930

Friday 2/27/53

2068 pr - A - X 892

EMB gal	(+)	(-)	total
1	3	64	67
2	2	33	35
	5	97	102

$\frac{0.079}{5.00} = 4.9\%$

gal$_1^+$ x gal$_3^-$

NO — indicates 2000 gal$_3^-$ distrib. that gal$_2^-$ what gal$_3^-$

Weakening on crosses

01 X 902 } repeat crosses to see if initial results (contradicting in that 04 X 902 } (01, 04) were gal$_2^-$ by donor + recip. transd. tests) was caused by + cont in 902

01, 04, 902 pure (-) in EMB gal weakening of washed susp.

811 + 902 — to see if 811 is different from 578 as suggested in last cross — apparent recombinate gal$_1^-$ gal$_3^-$

811 pure (-) in EMB gal

2/20/53 Int. — nucleid culture

578 X 892 Prototroph-cross on pg 171.

gal$_1^-$ Lp^s gal$_3^+$

gal$_1^+$ Lp$^+$ gal$_3^-$

	Proto	gal rx	/1	/1-2	/1485	Probable on Gal$_1^+$ gal$_3^-$
one of these possibly unstable	1	+	r	s	lys	
	2	+	r	s	lys	
	3	—	s	s	n. l.	4
	4	—	r	s	lys	
	5	—	s	s	n. l.	4
	6	—	r	s	lys	
	7	—	r	s	lys	
	8	—	r	s	lys	
	9	—	s	s	n. l.	4
	10	—	r	s	lys	
	11	—	s	s	n. l.	4
	12	—	r	r	lys	
	13	—	[illegible]	s	[illegible] lys	
	14	—	s	s	n. lys	4
	15	—	r	s	[illegible] lys	
	16	—	r	s	[illegible] lys	
	17	—	r	s	[illegible] lys	
	18	—	r	s	[illegible] lys	
	19	—	r	s	lys	
	21	—	r	s	lys	
	21	—	r	r	lys	
	22	—	r	s	lys	
	23	—	r	r	n. lys	
	24	—	s	s	n. lys	4
	25	—	s	s	n lys	4
			7 s	23 s	8 n.l.	
			18 r	2 r	17 lys	

D1 X 892 - unstable + prototroph.
stable neg (-) on EMB gal after 5$^-$ p.c. isolation
homogeneous (-) on {EMB / Loc}

578 X 892 - above (+) possible unstable
s.c. 1. mixed (+),(-)
2. mixed (+),(-)

Sunday 3/1/53

1655 X 1177 on EMS gal to obtain gals - typ[illegible]
25 gal - tested against t2 - all typ R

~~~

811 X 902 on EMB gal - purity of parent checked on p. 172

	(+)	(−)	total
1.	0	65	65
2.	0	41	41
3.	0	32	32
4.	0	62	62
	0	200	200

total to here 0+/907 = (< 0.11%)

0.0011
907 / 1.000
907
930

~~~

D1 X 902 on EMS gal - to recheck on D4 since 902 culture could have been contaminated with (+) cells.

	(+)	(−)	total
1.	0	209	209
2.	0	265	265
3.	0	134	134
4.	0	265	265
		873	873

0/873 not in agreement with previous

D4 X 90 as D1

	(+)	(−)	total
1.	0	248	248
2.	0	154	154
3.	0	175	175
4.	0	122	122
		699	699

0/699 = not in agreement with earlier results - probably correct - test D4 for gal - neo

D1 from duchess = mutant ?

Thursday 3/6/53

Crosses with 902 B7, B8, D1, D4, 811 all checked on EMB gal all found (-)

811 X 902

EMB gal	(+)	(-)	total
1.	0	77	77
2.	1?	49	50
3	0	52	52
4	0	105	105
5.	0	75	75
	1?	358	359

1? confirmed on EMB gal

total to here

	(+)	(total)
p. 171	0	706
p. 174	0	200
above	1?	359
		1365

1365 / 1.0000 = 0.00073 = 0.073%
9555
4450

578 5/1289 = 0.38%
6/2654

Recheck on crosses with 902 - 902 pure gal -

D1 X 902

EMS gal	(+)	(-)	total
1.	0	273	273
2.	0	339	339
3.	0	247	247
4.	0	296	296
			1155

0/1155

D4 X 902

1.	0	1/8 = 237	1896
2.	0	1/8 = 249	1992
3	0	1/8 = c. 300	2400
4.	0	1/16 = 109	1744
			7932

0/7932

B7 X 902

1.	0	1/2 = 264	528
2.	0	1/2 = 292	584
3.	0	1/2 = 239	578
4.	0	1/2 = 271	542
			2232

0/2232

B8 X 902

1.	0	1/2 = 203	406
2.	0	1/2 = 176	352
3.	0	1/2 = 191	382
4.	0	1/2 = 227	454
			1594

0/1594

3/6/53 Friday

Exp. J Examination of prototrophs from cross of 578 × 892.

Prototrophs	Parental	881/1-5	892/1-2			
1. } Salt		1st smear	2nd ✓			
2. }						
3.	52	18	S. S smear	4⁻	S	
4.	2	153	S. S smear	?	Lys	J-4-4 8/8 stable after (2)
5.	24*	42*	Solid S smear	4⁻	✓	
6.	72	347	S. S smear	?	Lys	J-6-4 8/8 stable after (2)
7.	44	227	S. S smear	?	Lys	J-7-4 8/8 stable after (2)
8.	1	6	S. S smear	4⁻	Lys	J-8-4 6/6 stable after (1)
9.	40	21	S. S smear	4⁻	S	
10.	41	36	S. S smear	4⁻	Lys	
11.	37	11	S. S smear	4⁻	S	
12.	23	21	29	Lp₂ R	✓	
13.	1	8	S. S smear	4⁻	Lys	J-13-4 8/8 stable after (2)
14.	39	10	S. S smear	4⁻	S	
15.	2	61	S. S smear	?	Lys	
16.	77*	211	S. S smear	?	Lys	J-16-4 8/8 stable after (2)
17.	33	60	S. S smear	4⁻	Lys	J-17-4 8/8 stable after (2)
18.	23	½=208	S. S smear	?	Lys	J-18-4 8/8 stable after (2)
19.	2	11	S. S smear	4⁻	Lys	
20.	1	7	S. S smear	4⁻	Lys	J-20-4 8/8 stable after (2)
21.	4	9	14	Lp₂ R	✓	
22.	1	17	S. S smear	4⁻	Lys	J-22-4 7/7 stable after (2)
23.	30*	35*	46*	Lp₂ R	✓	
24.	87	12	S. S smear	4⁻	S	
25.	30*	18	S. S smear	4⁻	S	

Cured by from spreader

* small in bkgrd.

11	4⁻	6/14 S
6	?	
3	Lp₂ R	

Mixed auxotrophs

(1765)

3/6/53

Transductions

			N. pfu 3 days
811	EMB 5		
	1.	no add	43
	2.	B8	122
	3.	D4t750	42
	4.	A3	47
2050			
	1.	no add	16 (+ some small)
	2.	B8	1/4 = 110
	3.	D4t750	1/2 = 210
	4.	A3	1/4 = 177
750			
	1.	no add	2
	2.	B8	78
	3.	D4t750	2
	4.	A3	18
2175			
	1.	No add	24
	2.	B8	18
	3.	D4t750	12
	4.	A3	64

Tuesday 3/10/53

518 transduction with high activity ~~B~~ phage -

0.1 ml of saline resusp 518 cells (overnight culture)
0.9 ml of 01 lysate - 1 - 1 hr at 37 1.0 ml - dil

10^{-2}, 2×10^{8} 0.1 ml → 10 plates.

	unstable (+)	(-)	total
1	72	1708	1780
2.	83	1/4 = 493 (1972)	1855
3.	77	about the same	
4.	83	" " "	
5.	89	" " "	
6.	95	" " "	

remainder about the same

} 24 of these (+) sheared out - all mixed - many mosaic col.

1708 | 72.00 (0.04) (68 20) (580)

c 4.5 cells transduced

6 plates of 518 untreated dil to contain c. 150 colony/plate showed no plus colonies

1 plate of undiluted 518 also showed no (+).

Extended Examination of prototrophs from 518 x 892 mixed culture. Exp J.

J 4 (-) 1 day 2 days

1. no add	7	
2. 750 λ-2	22	
3. 902 λ-2	1/8 = 66 528	} says it probably gal, - ?
4. 892 λ-2	Solid smear	
5. 811 λ-5	1/8 = 70 = 560	

J 6

1. no add	c. 50	
2. 750 λ-2	1/8 = 73 584	
3. 902 λ-2	1/8 = 87 - 696	} says it a new locus
4. 892 λ-2	Solid smear	
5. 811 λ-5	1/8 = 68 534	

J 7

1. no add	21	
2. 750 λ-2	1/8 = 57 456	
3. 902 λ-2	1/4 = 107 428	} says it a new locus
4. 892 λ-2	Solid smear	
5. 811 λ-5	1/8 = 72 576	

(1775)

3/10/53

1655 (−) gal transduction ~ 892 λ
0.1 ml 1655 saline suspended (aerated overnight) + 0.9 ml 892-2 λ
+ mucin at 37 C 10 min dil 10^{-1}, 10^{-2} → 0.1 ml / 10 plates EMB gal

	tot. no. colonies	no. mosaic col.	
1.	155	5	3.2 % mosaic
2.	294	8	2.7 %
3.	not about the same		

2 plates made to indicate the amt. of phage added to the gal plates indicate about 10^{3}-10^{4} λ / ml

Lysate sterilities

892-2 bottle 1 — sterile 0.5 ml
01-1 0.2 ml sterile
04-2 bottle 1 sterile 0.5 ml
01-2 ~~bottle 1~~ not sterile 0.5 ml. — Re chloroform

1655 1 day 27 mosaic colonies picked and streaked out — no segregg of ± colonies evidently ± deletion not easily accomplished

3/17/53 Tuesday.

Transduction: To identify prototrophs from 578 x 892

		Rep 2 days
750	EMB gal	
	1. no add	4
	2. J4	6
	3. J7	3
2175		
	1. no add	8
	2. J4	26
	3. J7	30
2050		
	1. no add	1/4 = 56 = 224
	2 J4	1/4 = 58 = 232
	3. J7	1/4 = 41 = 164
	4. 892 λ-2 (1-110)	> 2000
	5. D2 (presumed 4-) Seq SH48h	1/4 = 83 332
578		
	1. no add	33
	2. J4	18
	3 J7	23
	4 D2	15

J4, J7 appear to transduce gal+ - only what this means I don't know

J4, J7

probably says D2 is gal+ altho deletion of 2050 is low (hi bkgd?) also this culture is lysogenic for "normal" λ - not hi-act λ

1485 - Attempt to transduce to hi act λ from 892 - Rep 2

0.2 ml of resuspd overnight culture c. 10^9 + 0.5 ml 892 λ-2

5 Control plates EMB gal: c. 400 colonies/plate - some intermediate gal+ & possible mosaic colonies - streaked out. On streaking showed + and slow + forms.

5 Expt Plates EMB gal: c. 80 colonies/plate - represents about 80% killing? c. 24 mosaic colonies - streaked out

$\frac{0.04}{400/35}$ = c. 4% ← of the 24 colonies - 1 segregating (+) on 1st streak

to avoid selection against (+)

5 Expt plates EMB (0): c. 80 colonies/plate - 31 colonies showing evidence of lysis

3/21/53 Sat.

Exp K 811 & 892 (+1 segregant analysis)

On EM13 gal

Seg #	Sw add	892 1-2 (1-100 del.)	811 1-5	Probable locus	
1.	31	c. 2000	33	4	
2.	21	c 3000	54	(?)	1/8 mucid 2 1/8 mucid 3
3.	71	c 2000	68	4	
4.	~~32~~	c 2000	~~33~~ 63	(?)	1/8 mucid 2 1/8 mucid 3
5.	26	c. 2000	28	4	
6.	26	c 2000	29	4	
7.	17	c. 1000	59	(?)	4/8 mucid 2 3/8 mucid 3
8.	37	c. 2000	32	4	
9.	36	c 1000	72	(?)	
10.	27	c. 1500	31	4	
11.	~~32~~	c 1500	48	4	
12.	28	c. 1500	33	4	
13.	24	c 1500	22	4	
14.	24	c. 500	54	(?)	4/8 mucid 2 3/8 mucid 3
15.	12	c. 1000	45	(?)	5/8 mucid 2 5/8 mucid 3
16.	33	c. 1500	19	4	
17.	47	c. 2000	43	4	
switched 18.	28	c. 2000	21	4	

892

1 on the Stability of spontaneous pap – after 2 all appear pure +

Exp D

Instability of DI spontaneous pap. confirmed (again) 3/8

stability of D4 " " " " 8/8

2050 – from EMC – transduced by 892 1-2 to high ~~titer~~ frequency

892 – from EMC – ~~Sw~~ low – – colony) distribution about the same
from) as own 892

– own abs. low – ——— small col. pure + spontaneous colony form

Sun
Mon: 3/22/53 - 3/23/53

518 Perfect stage ~~transduction~~ as a function of multiplicity -

~~2050 Exp~~ -

892 - streaked out EMB line - appears homogeneous lac- mucin - 2 colony types - small round, large spreading
well separated of each picked, lysates made 892-isol 1 / 892-isolate 2 } don't know which is which! Obtain from culture

2050 Exp is these lysates.
518

cell	lysate →	isolate 1	isolate 2
2050 E	c. 3	150 lysis?	lysis
518	c. 30	c. 20 no plaques & mucoid →	c. 20

c. 200 plaques plus gram lysis - transduction difficult to assess - mucoid? Stock and use extent of gal + recs

no transduction bkgd ∈ in 8 different areas

Abscess not examined

Idea: 1-100 dil. c. 10 pfu 1-5 plaques.

Results suggest not 1+2 are gal + ?

518 + 2050 - 3 (purified) stocks made of separate transductions
not a high incidence of transduction 2050 → 518

3/24/53
Expt L stored on EMB gal plate

518 - one plate - transduced ∈ 892 lysate to see if transinduced are M+
replica (slightly) to D(0) - no growth D(0) - no prototrophs

3/16/53
Thursday

892 - all confused - probably complicated by a switch in media.
Apparently 892 mixed with gal+, gal−? possibly (−) of
a couple of types.

578 Examination of 04 to see if activity of A present.

1. to add	=	12
2. 04 A	=	$>10^9$

Summary 518tK-12 See pages 170a

page 1. Examination of a single gal- segregant from ~~[illegible]~~ separate transductions of W518 gal$_4$- by lysates of K-12

		Receptor test No. of segs.	No. transd. by gal$_4$- lysates	No. transd. by gal$_2$- lysates	Probable locus by receptor test
170a	A.	22	0	22	22 gal$_4$-

1. (A3

2 - A1

3 - A4

4 - A3

2. Examination of a number of gal- segregants from a single transduction

		Receptor test No. of segs.	No. transd. by gal$_4$- lysates	No. transd. by gal$_2$- lysates	Probable locus by receptor test
145	A.	15	0	15	15 gal$_4$-

163 B. Cross test (X1426)

	Seg no.	No. prototrophs	No (+)	Probable locus
1.	A3	975	0	gal$_4$-
	A11	1048	0	gal$_4$-
	A13	c. 1250	0	gal$_4$-

C. Donor Test

	Lysate of Seg	Transductants gal$_1$-	gal$_2$-	gal$_3$-	gal$_4$-	Probable locus
165, 1766	A1	+	+	+	no	gal$_4$-
1766 1.	A3	+	+	+	no	gal$_4$-

Summary of 518t902

page

1. Examination of ~~a series of~~ single gal- segregants from different transductions.

2. Examination of a series of gal- segregants from a single transduction

A. Receptor test

No of Seg.	No transd. by gal+ lysate but not by gal-	No transd. by gal- lysate Observed gal-	Probable locus
146 39	2	17	2 gal-, 17 gal+

B. Crossing test

1. Seg. gal- by receptor test (K902)

Seg. no.	No prototrophs	No. (+)	Probable locus
175 B7	c. 2200	0	gal-
B8	c. 1600	0	gal-

2.

C. Donor Test

Lysate of Seg	gal+	Transducers gal-	gal+	gal+	Probable locus
B7					
176B B8	+	no	+	+	gal-

76
16
446
76
1216

Sunday 3/29/53

Analysis of 892 mutants

811

892-1 } = 2 (-) gal colonies from stock of 892 - lysate made
892-2 } of these - both are loc- TLR-

811	Agr 2 days
1. no add	28
2. 892-1	39
3. 892-2	33

} these lysates appear to have at least 2 plaques in them - 1 resembling 1, the other giving plaques on 1+ cultures

2175 for the purpose of ~~segregating~~ reisolation of 518 892

1. no add	-	6
2. K-12λ 30-1	176	← pick 24 for analysis

750 For the purpose of picking transductants for analysis - 8 segregants.

Monday 3/30/53

1. no add		2	
2. K-12λ 30-1	1/8 = 18		342
3. 902λ-3	1/4 = 29		116

518 For the same purpose as 750 above

1. no add		28
2. 750λ-2		79
3. 902λ-3	1/16 = 76	= 1216
4. K-12λ-30-1	1/8 = 65	= 280
5. filtrate of culture of 1485E01		57 ← indicates that 1 from "natural" lysis is not very active - assuming high titers a few plaques resembling 1-2

518

a second mutant to test a lysate of gal + from mixture 892

7 day incub

1. no add	< 20	
2. lysate of 892 gal+ colony 3a from culture of this approved mix	7104	high activity

Monday March 30, 1953

1673X D1 to see if (lt) from this cross have bi-act lambda-
prods of the constituent
D1 prod.-
1677- +, with slow gal present - a plus picked for purif.
and a new

4/2/53 Thursday

— 1485tD1 - the negative transductin - Examination of (-) segregants to determine
their nature.

Exp L.

		no pop 2 day	Locus suggested	
21	no add	4		
	902λ-3	19	2-	
	811λ-6	37		
22	no add	6		Reason for low
	902λ-3	5	2-	transductn
	811λ-6	21		not known.
23	no add	10		Data appear
	902λ-3	9	2-	ok. but better
	811λ-6	22		recheck.
24	no add	5		
	902λ-3	5	2-	
	811λ-6	21		
25	no add	5		
	902λ-3	8		
	811λ-6	13	2-	
26	no add	3		
	902λ-3	7	2-	
	811λ-6	21		
27	no add	3		
	902λ-3	8	2-	
	811λ-6	14		
28	no add	2		
	902λ-3	3	2-	
	811λ-6	22		

all these found 5 hp- and probably

892 bi powered λ - do bi prime DNAse susceptible?

578	no add	16		
δ	892λ untreated (-100)	>10⁴		no
	892λ DNAse treated at 37c 10 min 1-100 x 6/5	>10⁴		

4/2/53

518 gal+- is there a diff. between 578 gal+^{R} and 811 gal+^{R} because 811 was made Lp+ ? Data on 811 reversion as gal+ does not decide because strains not transducing gal$^-$ do not give (-) in crosses with Y-10. Examine 578 gal+ to find strains giving (-) with Y-10, then make lysogenic and examine transducing power.

Check in the assay cultures. Y-10 are (+)
578 g+-1 " "
578 g+-2 " "

578g+-1 X Y-10 - are + prototrophs formed
nos/plate = 65, 48, 75, 114, 67, =

578g+-2 X Y-10 - are + prototrophs formed
nos / ¼ plate = 75, 125, 95, 87, 108

~~~

892 mixed stock - plated against A^2 - large proportion of cells Lp_2^R
Selection for Lp^R pertains to stock.

~~~

2175 ZK-12
24 (-) ray obtained - carried thru (2) purifications

518C750-1 } made from (2) purification
518C750-2 }
750 C902-1 } made from (2) purification
750C902-2 }
750C12-12-2 } made from (2) purify.
750C11-12-1

April 5, 1953

892(mix) preliminary examination of occurrence of transducing phage in bursts from single cells of irradiated 892

1. Growing culture of 892 diluted to contain c. 500 cells/0.1 ml
2. irradiated 15 seconds.
3. plated before and after with 750, 1485, and 5y at ref. ⊥.

1. 892 cells/0.1 ml before irrad. = 585 (gal+ = 101)
2. 892 cells - plaques/0.1 ml on 1485 before = 1.
3. 892 cells - transd/0.1 ml before on 750 = 78 (from 892, 78)

4. 892 cells/0.1 ml after irrad 16.8 (gal+ = 26)
5. 892 cells/0.1 ml plaques on 1485 after irrad. ~~44~~ 247 (total = 415)
6. 892 cells on 750 cells 35 (gal+ from 892 = 31) / no aperture plaques yielded

750 no add = 2

~~~

518 gal+ R #3 X Y-10 controls o.k.

	(+)	(−)
1.	1/4 = 60	240 or more
2.	1/4 = 33	132 "
3.	1/4 = 46	184 "
4.	1/4 = 40	160 "
5.	1/4 = 51	204 "
		930

0(−) / 930 (+)

518 gal+ R #4 X Y-10 controls o.k.

	(+)	(−)
1.	1/4 = 53	212 or more
2.	1/4 = 57	228 "
3.	1/4 = 50	200 "
4.	1/4 = 58	232 "
5.	1/4 = 55	220 "
		1114

0(−) / 1114 (+)

~~~

1485 ED1 — unstable gal+ ⟶ hi lysates ind. λ

save virus to stable + ⟶ low lysates λ

~~~

902 St X 2175 (gal2− X gal2−) on low sm - about 200 recombinants per plate.

(2251)
~~~

Tuesday 4/7/53

- 1821 - w. hi powered 892 λ

On EMBgal.
1. no add - c10
2. 892λ 1/100 - c. 1000? } appears that hi act λ has less act on 1821. - situation similar w. "naive" λ.

On EMBxgl
1. no add - many small, 3-4 large
2. 892λ - 1/100 - " " c10 "

Repeat xgl using undiluted 892.

1485tD1 - Re-examination of the possibility of (-) ductii.

1. Lysate D1d - 2 pmp.
2. Cross printed on 1485 on EMBio).
3. after 1 day lysed area picked w. 1/4 - streaked out several times on EMBgal.
4. a single (-) colony observed
several intermediate gal + col.
a few segregating (what?) colonies.
many gal + colonies.
5. some of above colonies picked and checked.
6. Results - streaks -
a. 2 pure (-) col.
b several mixed?
c. mostly gal + col.

Summary Stock cultures on Plates in Refrigerator

- 1924 t K-12 Unsuitable + 11 in all
- 811 gal + R. 10 in all
- 518 gal + R 8 in all

Expt A 518 t K-12 all exp single transductants
" B 518 t K-12 all exp from single transductants
" C 518 t 902 (mix)
" D 518 t 811 mix
" E 2050 t 811
–
–
–
Exp J
" K
" L
" M 750 t K-12 (-) exp ~~18~~ 17 in all
" N 750 t 902 (-) exp 23 ~~26~~ in all } replaced using adapted gal+ - purified to single mutant ≈ 750 S ✓ ≈ 902 R ✓ = 229
" O 2175 t K-12 (-) exp 21 in all
" P 518 t 750 (-) exp 8 in all
" Q 518 t K-12 (-) exp 13 in all
" R 518 t 902 (-) exp 23 in all
" S 811 t 902 (-) exp 19 in all
T 2175 t 750 (-) exp 8
U 2175 t 811 (-) exp 16 in all
V 811 t K-12 (-) exp 20 in all
W 1924 t 902 (-) exp 18 " "
X 1924 t K-12 (-) exp 24 " "

4/9/53

1485tD1 - Occurrence of hi-act λ in bursts of single cells mod.

Pre mod.

1. 1485tD1 cells count pre mod. = 493 /0.1 ml
2. Spont pop. 750 1
3. No. pg in mix of 1+2. Expected 494
4. Plaque by 1485tD1/1485 Found 494 0

Post mod.

5. No. 1485tD1 cells survs. 123
6. No. " cells giving plaque/1485 140
7. No. " cells giving pop. 750 123
" Sp. pop 750 1
Total 124
8. No. pg mod. 1485tD1 to 750 222

No of surv 1485tD1 cells giving pop 750 222-124 = 98

Fraction of surv 1485tD1 cells giving rise to 1 hi act. λ particle 98/140 = 70%

4/10/53 Friday

750 and D1 Occurrence of hi-act-1 in single bursts of D1

C. 10^3-10^4 cells of D1 - sampled before irrad., then irrad ≅ U.V. sampled immediately and after a hour incub with aerated cult. D1

Dil to 10^3-10^4 → dil 10 sample → for plagues/1485, for pop/750

irrad 5 sec, 10 sec. → dil 10 with broth → sample immed. → cells survival, plagues induced pop 750

→ inc. 2 hours → sample as ↑

Preirrad 0.1 ml

1. Total no. cells = 310
2. plagues/1485 = 1
3. Pop./750 (3) 6

750 spop = 1.

Post Irrad.

5 second exposure.

4. Surviving cells 159
5. plagues induced/1485 40 (40 plagues / 151 surviving cells)
6. pop/750 (3) 6
7. plagues after 2hrs/1485 3344
8. pop/750 after 2 hrs. — (3) >47 (many small) upper limit ≅ c. 600.

Plagues assays against 1485 when streaked out - centr of plague shows evidence of (+) ductin

10 sec. exposure.

9. Surviving cells 84
10. plagues induced/1485 40
11. pop/750 (8 - 15
12. plagues after 2hrs/1485 2720
13. pop/750 after 2hrs (20) >53 (many small) upper limit = c. 200

---

842 mix gal+ (also resistant)
lysate has hi act / 518

1485-tD1 — (-) exp
lysate probably has hi act / 518 but appears quite lysogenic also.

---

518 gal+ R. #5 and #6 tested in crosses × Y-10 for mimic recovery

518 gal+ #5 gal+ prots = 420, 650, 512, 416, 466 (all obtained by gal+ / counting either ¼ or ⅛ plate)

< 0/2304

518 gal+ #6 gal+ prots: 440, 440, 432, 440, 340, 672 (also obtained by counting ¼, ⅛ plate) less (-) prots

< 0/2764

3/10/53
Sat.

Various transductions to secure (-) seg for classification.

		Seg 2 days
217N	1. no add	13
	2. 811λ-4	89
	3. 811λ-7	50

} (-) seg isolations made from both. Discarded

750	1. no add.	2
	2. 811λ-4	10
	3. 811λ-5	14
	4. 811λ-6	33
	5. 811λ-7	25

} discard - transduce gal, prototroph and use 750s as crossing tool

518 1. no add. 29
2. 750λ-2 72

1821 On EMB xyl -

1821 xyl transduction?	No	1. no add	3
		2. 892λ-2 (hi prototroph)	c. 150

no ✓ These are gal+ xyl- Apparently EMB xyl can select for gal- deletion -

3/16/53 Thursday.

750 S^r = 2319 test for validity

1. no add		21
2. 750λ-1		25
3. 902λ-3	1/4707	428

4/16/53

750 pr = gal$_2$ prototroph - Isolation of (-) segregants.

1.	no add	1.
2.	750 λ-1	0
3.	K-12 λ 30-1	143
4.	902 λ-3	84
5.	811-6	4

(check on authenticity)

after 2 single colony isolations 2/4 are crossed - probably indicates that stable transductions result 811 → 750

gal$_2$ -

1655 × 2238 (= loc$_1$ gal$_2$) to isolate tp_2^s from (tp^s also if possible)

Control plating	1.	1655	pure	+
	2.	2238	pure	-
		(4)	total pure	
cross assay	3	30	220	
	4	33	211	
	5	44	433	
		107	864	

$\sqrt{107.0}$ = 0.129

864

1760

1728

3320

± 12.4%

Pickings for tp^s, tp_2^+ × back

1. 5/34 tp_2^+ all tp^+

2. 30/30 tp^+ × tp^+ / λ

750 pr (gal$_2$ prototroph) - Irradiation to obtain tp^s from.

5 pink colonies on EMB low noted. Pick and streak

tp_2^+

1871 purified - by transduction of single colony isolate - stock made of this.

1.	no add	10
2.	K-12 λ (30-1)	84

1485 TD1 - 2 additional segregants tested

Seg #9	1.	no add	10
	2.	750 λ-1	4-15 = 60
	3.	902 λ-3	10
	4.	811 λ-7	29
Seg #10	1.	no add	8
	2.	750 λ-1	1/16 = 54 = 864
	3.	902 λ-3	24
	4.	811 λ-7	1/32 = 55 1760

apparently gal$_2$ -

probably gal$_2$ -

Saturday 4/18/53

2175 - attempt to observe "satellite" phenomenon in 2175 found - check to see if other transductants

give 1
1. no add 17
2. K-141 3-1 248

Examination of some lysates.

518
1. No add 11
2. 892 λ_2^r (unadd) 18
3. 892 M+ (unadd) 11
4. 811-8 11
5. 902-1-4 1/4 = 118 472
6. 015 1/16 = 52 896
7. 844 1/16 = 44 704

(unknown locus 27-1.9 →)

W2346.3

(192) A

4/25/53 Saturday. (tested by spotting 0.05ml of 1 drop in each of 3 sectors of single plate - 1/2 plaque never ab confluent) (1/2 control)

Expt. M 750 prototrophic gal,- transduced by K-12 λ 30-1 - Examination of Segregants (-)

Segregant	No. of Segregants (1/4 plate)	No. prop. 750λ-2 pfu	No. prop. 902-4 pfu	Probable Locus	To be taken by X	Satellite of gal, λ
1.	7	0	6	gal,-		0
2.	1	0	10	"	✓	0
3.	4	0	9	"		0
4.	0	0	11	"		0
5.	0	0	11	"		0
6	7	0	7	"		0
7.	0	0	9	"		0
8.	0	0	6	"		0
9.	0	0	8	"		0
10.	0	1	10	"		0
11.	0	0	13	"		0
12.	2	7	9	"		0
13.	0	0	13	"		0
14.	1	0	8	"	✓	0
15.	0	0	10	"	✓	0
16.	0	0	15	"		+
17.	0	0	13	"	✓	+
						2/17

2/24/54 Stocks of this experiment discarded as not worthy of carrying

Examination of lysates M2, M14, M15, M17 Test cultures X

	750	518	2175
1. no add	1	40	14
2. M2	3 ab ov ✓	29	50
3. M14	5 ab ov	62	70
4. M15	4 ab ov ✓	15	33
5. M17	5 ab ov ✓	28	74

Test crosses of M2, M14, M15, M17 X 750 s[r] - Controls E118 gal[r] 750, M14, M15, M17 ok., M2 no germ

	(+)	(-)	total	2nd cross	total	3rd cross - Controls ok	total
M2	0(?) ab	125, 89, 115	0[+]/332	2, 2, 2, 3 = 11	0[+]/343	21, 16, 14, 13, 19 (83) (4/5) / 426	
						x 2196 gal[r] = 37, 57, 51, 66, (16/45)	
M14	0(?) ab	121, 126, 136	0[+]/383	0, 3, 2, 4 = 9	0[+]/392	24, 38, 37, 36, 27 = 162	0[+]/554
						x 2196 gal[r] = 7, 9, 13, 12, 15	
M15	0(?) ab	210, 180, 171	0[+]/511	9, 6, 2, 1, = 18	0[+]/529	contaminated	
M17	0(?) ab	87, 181	0[+]/268	0, 4, 5, 9 = 21	0[+]/289	13, 21, 29, 33, 27 = 123	0[+]/391
				controls ok on 2nd X		x 2196 gal[r] = 62, 63, 63, 59, 58	

ab = check.

SUMMARY 17 segregants.

by transd. test 17/17 gal,-

by donor test 4/4 gal,-

by cross gal, 4/4 gal,- with following mix. prototrophs (332, 383, 511, 268)

source and number

(1913)

4/25/53 Sat. 750 prototroph t 902 → (-) req Examination - Plated as 750tK12(-)Xg p. 1924

Experiment N 750t 902 → (-) req Examination - Plated as 750tK12(-)Xg p. 1924

Segregant	No. spont. /g (½ plate)	No. prop 750t-2 spot	No. prop 902t-4 spot	Probable Nacc.	Scorable	Test cross ± 25% err
1.	0	0	20	gal1-	0	ok ✓
2.	1.	0	24	1-	0	
3.	4	c. 50	0	2-	- 0	
4.	3	0	32	1-	0	ok ✓
5.	3	48	0	2-	- 0	
6.	0	0	14	1-	++	
7.	0	0	19	1-	0	ok ✓
8.	0	0	25	1-	±	
9.	5	45	7	2-	- 0	OK SEE BELOW
✓ 10.	0 (2)	1 (2) many small	14 17	1-	±	weak ? ok mutab. + ? 1-duction confirmed
✓ 11.	1	0	26	1-	0	ok ✓
12	4	0	26	1-	0	
13.	0 ✗	0 ✗	13 ✗	✗	0	
14.	7 ✗	0 ✗	16 ✗	2< 1-	0	
15.	9	60	0	2-	- 0	
✓ 16.	4 (12)	53 (98)	4 (27)	2-	- ±	weak ? ok unstability ? + confirmed each ducto SEE BELOW
17.	0	7	22	1-	0	
18.	3	1	13	1-	+	
19.	0	0	33	1-	±	
20.	3	0	25	1-	0	
21.	0	0	12	1-	0	
22.	0	1	14	1-	0	
23.	0	0	23	1-	0	

750tr ↑ = ok

5 gal2- 21
18 gal1- 4±
170

( ) = 4/26/53

5/4/53 Segt test of Activity of Segregants of These Segregants (spot 5, 2e about)

					/750	/518	/2175
See well plate / Crosses	gal2- req X 902tr on EMB gal SM						
controls	1. N3 (no + prot. observed)	(-) = 679, 582, 531, 443	= 2135	ok/2135	78	52	0
on EMB gal SM - no growth	2. N5 (")	(-) = 369, 280, 274, 359	= 1283	ok/1283	79	60	1
on EMB gal - all (-)	3. N9 (")	(-) = 363, 477, 419, 540	= 1799	ok/1799	41	46	1
	4. N15 (")	(-) = 454, 529, 479, 466	= 1928	ok/1928	44	43	0
	5. N16 (")	(-) = 638, 530, 535, 655	= 2358	ok/2358	raised vs appear. (well T ~~shadow~~)	raised vs appear. (well c 37 pap.	2
N16 → 2 5 (5/9/53)		Reversed no (-) = (c.1500) 6 = c.9000			control area 1/67 plate = 18	control area c. 16	control area = 1

5/4 N10 streaked out (i.e. picked) → spot tested → regal duced → /750l = 0 /811 = 4 /902l = 16 - control area 1

transductions confirmed here by brk. room

5/9/53 N16 purified (not transduc III) 5/8

N16 " " " " /750l = 0 /811 λ = 3 /902l = 0 ∧ Reactels! double(-)? control area = 0

N16 " " " " /750l = 0 /811 λ = 0 /902l = 0 double (-)? or tpr? ~~trp~~

5/8 Crosses gal- req X 750tr (no plaques observed)

				state of cross tet
1. N1	31, 26, 34, 23 =	ot/114	8, 3, 6, 1, = 18	ot/132
2. N4	21, 28, 11, 28 =	ot/88	7, 4, 1, 3, = 15	ot/103
3. N7	39, 46, 38 35 =	ot/178	5, 3, 7, 8 = 23	ot/201
~~4. N10~~	~~50, 33, 41, 52 = ot/176~~			
5 N11	32, 20, 13, 40 =	ot/105	5, 3, 1, 1; = 10	
6. N16	37, 33, 25, 44 =	ot/139	3, 2, 2, 2 = 11	ot/150 ot/160

controls ok. skip

Activity of gal- segregates 5/9/53

	spotted 750	spotted/2175 solid smear	spotted/811 solid smear	N1, N4, N16, 750tr	gal- 1-100/750	gal 1-100/2175 spot/plate	gal 1-100/811 spot/plate	gal 1-100/gal- spot/plate
N1	0				= bkgnd.	—	—	—
N4	0	15	15			—	—	—
N7	0	solid smear	solid smear	does this indicate gal- ↓ 6 fold?	= bkgnd	1/c.78 : 580	c. 500	c. 300
N11	0	11	11			—	—	—
	control = 0	control = 1	control = 2		control a little high	control 19	control 27	control 19

Expt. N continued.

N16 plate = original plate stock

5/26/53 N16 - single colony isolation - Hfr referred to as N-16 original or pure

lysates (pure = restreaked original)

original		control
/150	0(?)	0
/2175	0	2
/2238	c. 10	c. 10 ← not likely to be same as 2238 Hfr
/891	c. 7	c. 7

N16 original	trans'd	control	← this lysate also fails to transd 1405 → (-)
/750 λ	0	0	1/14/54
/902 λ	0	1	residuum
/2238	0	4	N16 titer now is 1-2-?
/891	0	4	
/K-12	0	4	
/842 gal+	solid smear	4	

N16 "plate" - because s.c. of N16 plate suspension as lysate gave no transd. and was not transd. by anything but 842 gal+ - (This repeated) - Lysate of plate inoculation was made.

N16 "plate" ½ stock

/891	solid smear ← stock of this showed +, gave tan growth 0(0)	control: c. 5	hi rec. reconfirmed.
/2175	0	1	

Additional crosses gal- x 2257

N7	1 plate = 1432 x 4 = c. 4200 (-)	no (+)
N4	1 plate = 844 x 4 = c. 3376 (-)	no (+)
N1	1 plate = 2676 x 4 = c 10,500 (-)	no (+)
N11	1 plate = 1640 x 4 = c. 6560 (-)	no (+)

7/6

Additional crosses gal- x 2251

N3	1 plate = 2660 x 4 = >10640	total >12,775
N5	1 plate = 994 x 4 = >3976	total 5359
N7	1 plate = 1484 x 4 = >5936	total = >7735
N15	1 plate = 968 4 = >3872	total >5900

Gal+ reversions obtained of

N1 - 1 mm HFT (total 811)
2

N7 - 1 mm HFT
2
3

5/16/53 N16 lysates of 30', 40', 60' exposure dont show a great deal of diff. All HFr

Monday 4/27/53

750 prototrophs x 1436 on EMB gal SM

1.
2.
3.
4. } all plates barren - Control streaks on EMB gal show pure (-)

1924 transduced $\bar{C}$ 01 λ - Observation to see if any plaques present - an indication of the new phage in lysates of 892 gal+ a mix

1. no add 18
2. 01λ-1 (10^{-4} dil.) 1/4=64. ~~256~~ 238 × 10 × 10^4. 2.38×10^7 transductants/ml lysate
-1 f

2238 × 1655 - gal_3 - dp_2^s prototrophs.

1. no add 64
2. 750λ-2 199
3. 902λ-4 123
4. 811λ-8 156

2343
750 prototrophic gal,

1. No add 5
2. 811λ-8 8

W 2319
750 s^r =

1. no add 29 a suspensions? - high for gal_1^- - why the change?
2. 811λ-8 128

750

1. no add 3
2. 811λ-8 10

4/24/53 Friday -

Lysate checks: On single plates - 0.05 ml lysate added (spots)

518	1. no add sector	2	
	2. 2238 - 1 spot	4	grow lysate - aeration stopped intermediate - may contain new phage action on 4+
	3. 2175-4 spot	109	
	4. 750 pr "	3	
	5. 750 sr "	3	
750.	1. no add sector	0	
	2. 750 pr spot	1	
	3. 750 sr "	0	
	4. 2175-4 "	148	
	5. 2238 "	2	
2175	1. no add. sector	4	
	2. 2238 spot	7	
	3. 2175 "	0	
	4. 750 pr	10	
	5. 750 sr	16	

~~= W2314~~

Cal 4 - gal 4 - pr+ x 750 sr = on EMS gal SM both F+ in cross?

1. Control resp. - no growth EMS gal SM
   - pr+ (-) on EMB gal

X plate	(+)	(-)	total
1.	5	0	5
2.	12	0	12
3.	21	0	21
4.	16	0	16
			54

0(+)/54

~~= W2314~~

750 pr x 750 sr = (both probably F+ in cross
EMS gal SM

1. Controls - no growth / both EMS gal SM
   - both (-) on EMB gal

2. X plate.

(+)	(-)	total
0	42	42
0	35	35
0	30	30
1?	32	33
		140

0/140 ?

↑

Hm ✓

5/2/53 Saturday

750t 811 - (-) seg 1 Examination

1. no add - 7
2. 750d-2 4
3. 811d-8 10

} no decision possible -

750pr - For the purpose of making transductions of 750 by gal- to observe segregant (-)

1. no add 3
2. 811d-8 12 - pick and look for unstable

811 - For the purpose of making transductions gal- by gal, - to obs. segregant (-)

1. no add 51
2. 750d-2 96 - pick and look for unstable no unstables observed

5/4/53 Monday -

750pr - For the purpose of making transductions of 750 by gal+

Spread plates

1. no add 3
2. 811d-8 3
3. 811d-8 2
4. 811d-8 1

} again / no success

5/4 → 5/7 confusion about gal EMB - due to new mix

2175 transductions test - partly modified by part of medium

17/72 gal seg carried out + on und. streaking of these showed one to be gal -

5/7/53 gal₂-

Expt. 0 2175 tk-12 gal₂ (-) segregants.

Segregant	~~Spotted~~ No. ~~segregants~~ ~~Tk-12 Spot~~	No. prep. 902A Spot	No. prep. 8111-8 Spot.	Probable locus
01	1	0	11	2⁻
02	0	0	4	2⁻
03	contaminated →			—
" 4	10 0	1 1	13 12	2⁻
" 5	6 0	1 0	17 4	gal₂⁻
" 6	1 0	0 0	12 18	2⁻
" 7	½ plate 11 0	0 0	13 14	2⁻
" 8	8 0	1 1	13 6	2⁻
" 9	← not spread →			—
" 10	2	1	12	2⁻
" 11	1	0	13	2⁻
" 12	0	0	6	2⁻
" 13	0	0	15	2⁻
" 14	0	0	4	2⁻
" 15	0	0	9	2⁻
" 16	0	0	10	2⁻
" 17	1	0	9	2⁻
" 18	0	0	10	2⁻
" 19	0	0	11	2⁻
" 20	0	1	7	2⁻
" 21	0	0	13	2⁻
" 22	0	1	10	2⁻
				20/20 2⁻

Tet Crosses (-) Seg X (2251) 902 sr on EMS gal SM- control streak on these EMB gal-OK

	total prots	tracts (+)	2nd cross: tetr prots	(+)	Grams prots	Grams (+)	total
03	162, 154, 89, 95, 130 = 630	0/630	604, 608, 567, 796, 814	0	4070	0	
05	139, 167, 162, 161, 128 = 757	0/757	1248, 1064, 914, 1092, 1016	0	5384	0	
011	64, 78, 67, 47, 77 = 336	0/335	237, 228, 400, 416, 456, 8	0	2072	0	now 0/4235
014	278, 201, 242, 318, 284, 73 = 1323	0/1323	1089, 1328, 1382, 644, 1516	0	6988	0	
					22 677		

011 X 2251 - 2nd cross
569, 861, 733 = 0/1563

Segregants	1811	2175
03	c. 15	0
05	c. 50	0
011	c. 50	0
014	c. 30	0
no add	2	0

loci: ~~Spot~~

2/24/54

Strains of this experiment discarded as not worth saving

5/9/53 Sat.

Various 'ductions for the purpose of obtaining seq. for classification

2175

1. mixed 16, 15
2. 811 + 8 32 — 3/24 unstable after 2 streaking
3. 750/1 - 2 50 — 4/21 unstable after 2

Hfact. 1 - Anti serum test.

Hfact 1 = 01 - dil 1-100 of 01-1 c. 10^{10}/1 /ml

1-100 = 10^8/ml (Pre-heat) → phage assay - dil 10^1, 10^2, 10^2 0.1ml (10^6 dil) = 35,24 = 2.95×10^7

'duction assay - 0.1ml → 1/8 = 272 2176

+ { 0.5 ml; 0.5 ml 1-100 Anti P1; incubate 37C for 10 minutes }

2176 · 10 · 10 · 10^2

2.2×10^9

(post-heat) → phage assay - dil 1/10, 1/5, 10^2 0.1ml (10^5 dil) = 206,254 = 2.30×10^7

'duction assay 0.1ml → 1/4 = 213 852

to equate to pre cmc mult. X2 = 1704

Fract. phage survivors = $\frac{2.30 \times 10^7}{2.95 \times 10^7}$ = 0.78

Fract. phage survivors = $\frac{1704}{2176}$ = 0.78

$\frac{2.2 \times 10^7}{2.95 \times 10^9}$ = $\frac{1}{1.3 \times 10^2}$

Stocks of 2/24/54 this experiment (198) discard.

5/14/53 Source: duction: Pl 191 (w. para shape al...)

Expt. R- 5/8 t 902 (-) seq. tested by spotting ~ 703λ-4, 841-P.

Seq	Control spot area	902 spot	811 spot	Platable factors	
4X R 1.	0	0	13	2-	✓
2.	1	7	1	4-	— L
3.	0	10	0	4-	— L
4.	0	1	9	2-	✓
5.	0	14	0	4-	✓ L
6.	0	4	2	4-? spt.	not gal⁻ see below ↓
7.	contaminated c (+)				
8.	1	10	1	4-	— L
9.	1	11	1	4-	— L
10.	contaminated with (+)				
11.	0	13	0	4-	— L
12.	0	17	1	4-	—
13.	2	11	0	4-	—
14.	0	14	0	4-	—
15.	1	20	1	4-	— L
16.	2	15	2	4-	—
17.	1	0	14	2-	✓
18.	1	8	2	4-	—
19.	0	8	1	4-	
20.	2	15	7	4-	
21.	11	12	1	4-	
22.	0	6	0	4-	
23.	0	18	0	4-	

3. 2-
18 4-

Lysates of Gal₂⁻ R1, R4, R17

	Spotted on 811	/2175
R1	c. 70/2	1/0
R4	c. 60/2	0/0
R17	c. 80/2	0/0

Lysate test. (looking for lic ant. & gal⁻ variety) Crosses. Gal₂⁻ X 2257

R2	18/1	0/4
R3	33/8	26/42
R5	83/8	34/42
R8	67/8	34/42
R9	16/1	0/4
R11	8/1	0/4
R12	17/1	2/4
R13	6/1	1/4
R14	14/1	2/4
R16	11/1	0/4
R18	5/1	2/4
R19	2/1	1/4
R 20	3/0	3/4
R21	7/0	1/4
R22	7/0	2/4
R23	5/0	0/4

In these cases no equal → + lysate pop/control pop

others spot test, no pop. sort / control pt.

7/21 R7 was HFT acquired 2275, 7580

lost in 2nd plate

1. controls R1, R6, R17, 2257 on EMB gal⁻ plates. only 2257 & R6 on EMB lactose
R1 = 104, 99, 117, 39, 110, 126 = 0+/595 } 0+/1188
7/6/53 2nd 368, 458, 348, 247
Ignore not included { R6 = 147, 175 (225) 127, 108, 109 = 14/891 } NOT gal₂⁻
(2nd 152, 138, 318, 444 also normal 1436) c 5000
R17 = 83, 41, 76, 78, 113, 85 } 0+/
7/6/53 2nd 221, 107, 231, 152 } 1187

2. Gal₂⁻ X 1436 — 4/7 λ^S; 8/8 stable on strk
7/6/53 R1 17/1116, 24/873, 24/837, 24/276 = 77/3102 = 0.22

6/8 λ^S; 8/8 stable on strk
R17 - 6/1063, 892, 554, 3+/511, 1/344 = 10+/4364 = 0.228%

3. Gal₂⁻ X 1436 - controls o.k.
R2 - 4 plates one crowded - 696 total c. 0+/2786
R3-5 " " " = 538 " " 0+/2675
R5- " " " 697 " " 0+/3485
R8- " " " 368 " " 0+/1840 } 0+/5952
7/6/53 " " " 1028 " " 0+/4112

4. Gal₂⁻ X 902 - controls
3/3 stable on strk R2 885, 583, 14/740, 14/410, 1+/345 = 3+/3183 = 0.094%
2/2 " " " R3 800, 679, 13/563, 14/625, 804 = 2+/3471 = 0.057%
14/14 + stable on strk R5 6/849, 2/1513, 4/776, 4/1441, 7/905 = 19+/5432 = 0.35%
1/1 stable on strk R8, 188, 269, 369, 585, 14/254 = 1/1668 = 0.06%
20+/12161 = 0.21%

(+) recombinants
12/15 λ^S

5/16/53 Saturday.

Effect of anti λ serum on hi-act λ -

antiserum = anti P1
hi-act λ = D1 dil 1-100

plaque assays against 1485
ductus assays against 750

Experimental

antiserum dil 1-10 → 0.2 ml + 0.2 ml hi-act λ → incubate 10 min at 37°

1-100 dil D1-1 → 0.1 ml → 1 plaque assay dil 1-100, 1-100, 1-10, → 75, 63 plaques ave 69

PRE-ASSAYS

1-100 → ductus assay: 123, 139 ave 131

1-10, 1-5, 1-10 → 0.1 ml: 313, 285 ave 299 plaques.

surviving plaque titer = 2.99×10^7

ductus titer = 0.1 ml : 709, 608 ave 6.58×10^5 ductus/ml

titer 1-10 dil = 6.9×10^7/ml

6.58 duct/ml / 6.9 plaques/ml = 1 ductus / 100 plaques

surviving ductus titer = $131 \times 10^5 \times 2 = 2.62 \times 10^5$

Plaque survival = $\frac{2.99}{6.90}$ = 0.433

ductus survival = $\frac{2.62}{6.58}$ = 0.398

---

750 x 902 to assay anti λ recombinates between parental suspensions 3 hr at 37°

Cross plate.

1.	296 (-) c. 300	4 (+)
2.	c. 300	0
3.	c. 300	2 (+)
4.	"	0
5.	"	0
	c. 1500	2

↖ a minimum no. since there were many small (+) appearing colonies not counted

2/1500 = c. 0.13 %

Recombination between the various gal -

Ref. above		% Recomb	no. particles
p. 171, 174, 175	1. gal$_1^-$ x gal$_2^-$	0.13	c. 1500
	gal$_1^+$ x gal$_2^+$	0.13	c. 1600
	2. gal$_4^-$ x gal$_2^-$		
	578 x "	0.38	1289
	811 x "	0.07	1365
	3. gal$_4^-$ x gal$_1^-$	<1.9	54
	1486 x 750	0.83	4588
	4. gal$_3^-$ x gal$_1^+$		

0.3 - 0.6

5/18/53 Monday.

81 vs lysate of 2277 2278 - cultures of 1485 lysogenic for 892 1

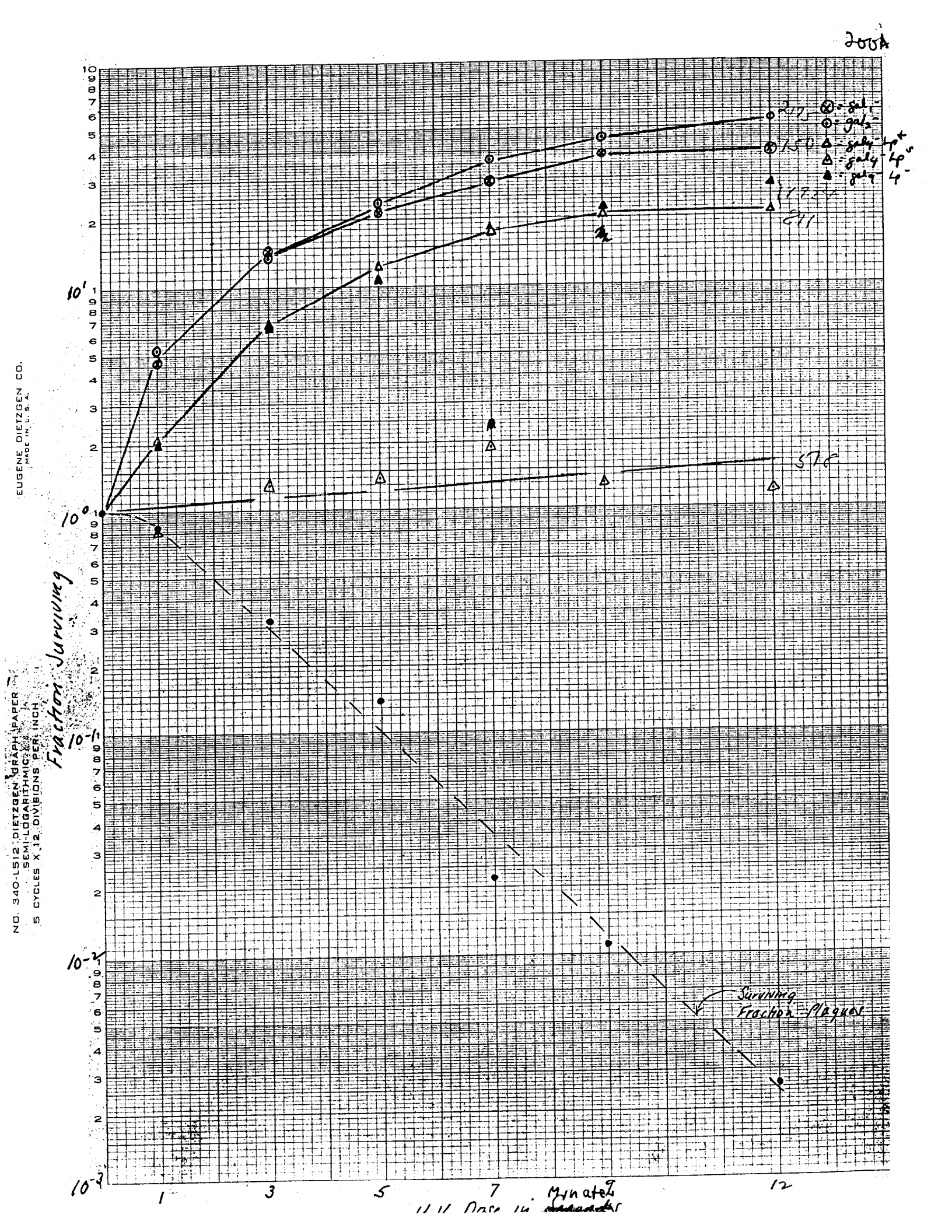
1.	no add	42	
2.	2277	52	plaque c 10^3
3.	2278	6	many plaques c 10^4

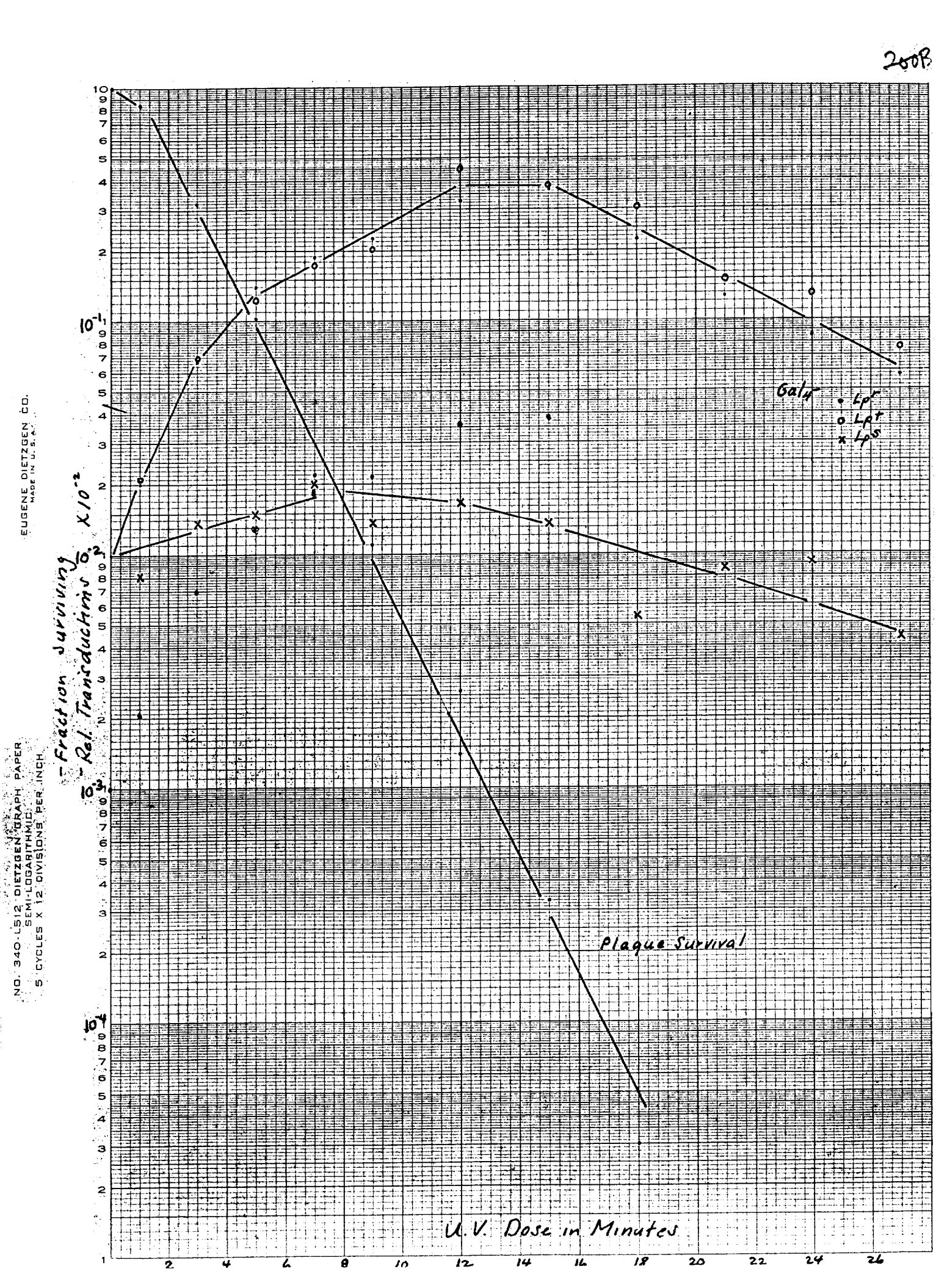
5/25/53 Monday.

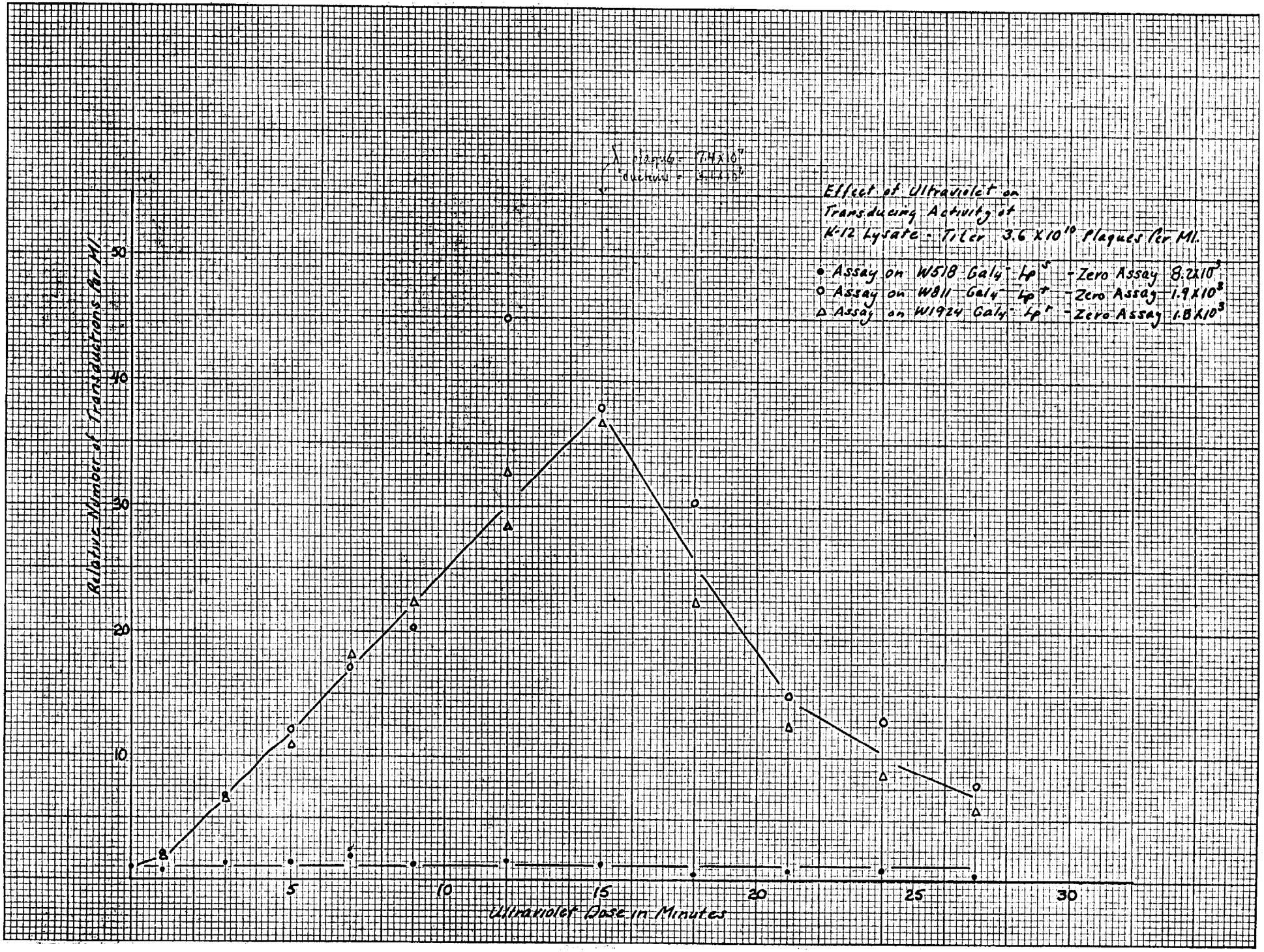
750 X 2238 to obtain double mixed phage. Controls ok.

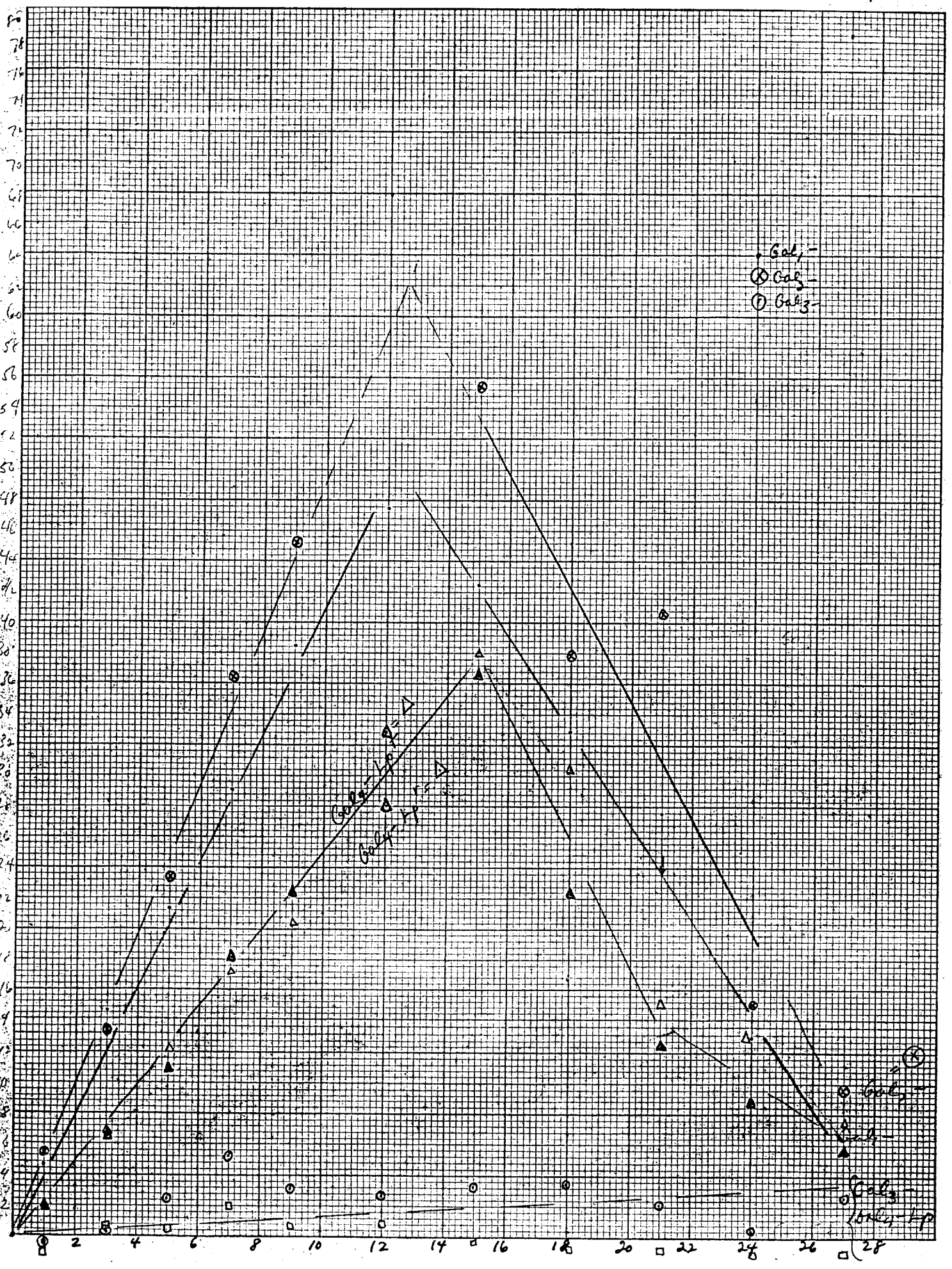
	(+)	titer
1.	2	c. 400
2.	0	c. 400
3.	0	c. 400
4.	0	c. 400
	2	1600

$\frac{2}{1600} = 0.00125$ for 1. = 0.13%









Lysate K12 5/30

No's of papillae/ml lysate

Irradiation of K-12 λ - Effect on Induction

Irradiation	W750 +λ	W750 -bkgrd	W750 ratio	W2175 +λ	W2175 -bkgrd	W2175 ratio	(1-10 die) W2297 +λ	W2297 -bkgrd	W2297 ratio
control-blk	3 (4)	—	—	9 (14)	—	—	6 (12)	—	—
control + 0 dose	89	86	1.0	83	74	1.0	43	370	1.0
1 min	410	407	4.7	401	392	5.3	39	330	0.89
3 "	1257	1254	14.5	1006	997	13.4	49	420	1.3
5 "	2034	2031	21.2	1641	1632	23.4	82	760	2.3
7 "	2512	2509	29.1	2706	2697	36.4	185	1790	5.4
9 "	3306	3303	38.4	3356	3347	45.2	109	1080	3.1
12 "	3420 (1/10 dil = 413)	4100	47.6	405 (1/10 = 60)	6011	81.4	97	910	2.7
15 "	368	3640	42.3	424	4100	55.5	119	1070	3.3
18 " 1/10	277	2730	32.9	294	2800	37.9	131	1190	3.6
21 " dil.	243	2390	27.8 1/10	313	2990	40.5	82	700	2.1
24 "	116	1120	13.0 dil	115	1100	14.9	58	460	1.4
27 "	55	510	6.0	84	700	9.7	102	900	2.7

Irradiation	W518 +λ	W518 -bkgrd	W518 ratio	W811 +λ	W811 -bkgrd	W811 ratio	W1924 +λ	W1924 -bkgrd	W1924 ratio
control blk	19 (41)	—	—	32 (36)	—	—	44 (49)	—	—
control + 0 dose	835	816	1.0	226	194	1.0	223	179	1.0
1 min	686	667	0.82	443	411	2.1	392	358	2.0
3 min	1118	1099	1.35	1369	1337	6.9	1264	1220	6.7
5 " 1/5	1182	1163	1.4	2409	2377	12.2	1996	1952	10.9
7 " 1/5	1680	1661	2.04	3384	3352	17.2	3296	3252	18.1
9 " 1/5	1108	1108	1.36	3977	3945	20.3	4056	4012	22.4
12 " 1/10 dil = 1060 (132)	1370	1150-41=1109	1.6	4036 (1/10 = 878)	8724	45.0	5184 (49)	5140	28.6
15 " 1/5	115	746	0.91	776	7400	38.0	6600	6551	36.6
18 " 1/5	48 (105 plaques)	450-41=939	0.36	626	5900	30.4	4020	3971	22.2
21 " 1/5 dil	74 (1.3×10⁴ plaques)	740-41=699	0.54	328	2920	15.0	2280	2231	12.5
24 " dil	79 "	790-41=749	0.86 1/10	290	2540	13.0	1580	1531	8.6
27 "	40 —	400-41=359	0.44	186	1500	7.7	1080	1031	5.8

$$\frac{1300 \cdot 10 \text{ d/ml}}{1.8\times10^{3}\ \lambda/\text{ml}} = \frac{1.3\times10^{4}}{1.8\times10^{3}} = \frac{13}{1.8}$$

at 12

$$\frac{8724\times10 \text{ d/ml}}{1.8\times10^{3}\ \lambda/\text{ml}} = \frac{8.7\times10^{4}}{1.8} = \frac{87}{1.8}$$

8724
4036
12760 = 6380
2

226 | 6380 (25)
502
1360

226 | 4036 (17)
226
1770
1582

200E

K-12λ 5'30

Irradiation of K-12λ - plaque survival

Dose	dilutions	plaque counts	titer	survival
0	$10^7 \xrightarrow{0.1}$	357, 370	364×10^{10}	1.0
1	$10^7 \rightarrow$	337, 282	3.10×10^{10}	8.5×10^{-1}
3	$10^7 \rightarrow$	122, 114	1.18×10^{10}	3.2×10^{-1}
5	$10^6 \rightarrow$	548, 506	5.27×10^9	1.4×10^{-1}
7	$10^5 \rightarrow$	853, 749	8.00×10^8	2.2×10^{-2}
9	$10^5 \rightarrow$	202, 612	4.07×10^8	1.1×10^{-2}
diluted 1-10 routinely	$10^4 \rightarrow$	1033, 897	9.65×10^7	2.6×10^{-3}
	$10^5 \rightarrow$	69, 36	5.3×10^7	1.4×10^{-3}
15	$10^3 \rightarrow$	103, 140	1.22×10^7	3.3×10^{-4}
18	$10^3 \rightarrow$	18, 3	1.1×10^6	3.0×10^{-5}
21	10^2	6, 6	6.0×10^4	1.4×10^{-6}
24	10^2	0		
27	10^2	0		

3/26/53

— 2238X1655 Lp, $\frac{S}{gal}$ – photograph – entered in stocks as 2297

3/30/53 –

811 dextrin
no add 44
2175t750 (1sq 332
2175t750 (4) solid smear } three from same repropation

NO no add 1
2175t750(1sq 212

1924 no add 28
N16 (1-100) c. 3000

1/14/55
recommend 3×10^6 bacteria/ml

Thursday 6/11/53 — Analysis by the spot method

Expt. S - 811 x 902 — the (−) segregants - 24 picked, 19 (−) received 6 probable (+)

	Segregant	Background	811-8	/N16 (1+00)	Probable locus	Character of bact. culture
lysate ✓	1.	7	0	S. smear	4−	rough
	2.	mixed ē (+)?	6 kgrd.	S. smear	4−	smooth (clear to mat?)
✓	3.	2	0	solid smear	4−	smooth
✓	4.	1	0	S. smear	4−	rough
	5.	<1	>30	<1	2−	smooth
✓	6.	<1	0	S. smear	4−	rough
✓	7.	3	0	S. smear.	4−	smooth
✓	8.	<1	0	S. smear	4−	rough
	9.	<1	>30	1	2−	smooth
✓	10.	<1	0	S. smear.	4−	rough
✓	11.	<1	0	S. smear	4−	rough
✓	12.	2	0	S. smear	4−	~~rough~~ ~~smooth~~
✓	13.	2	0	S. smear	4−	smooth
	14.	<1	0.	S. smear	4−	rough
	15.	1	0	S. smear	4−	rough
	16.	2	>30	2	2−	smooth
	17.	1	0	S. smear	4−	rough
	18.	mix ē (+)?	6 kgrd	S. smear	4−	rough
	19.	1	1	S. smear	4−	rough

6/18/54 Stock 4 tui elpt discarded S16 retained

16 gal$_2$- = 12 rough 4 smooth (1 clear to + contam?)
3 gal$_2$- = 3 smooth

At this time gal$_2$- 902 gave smooth culture
gal$_2$- 811 " rough culture

Lysates	Spot test /811	/2175 (old cult.)	Prob. locus	
S1	1/1	0/0	4	← S2 was HFT / 2175, 750
S3	0/1	2/0	4	
S4	0/1	5/0 (plaques)	4	
S6	0/1	5/0	4	
S7	1/1	2/0	4	
S8	0/0	3/0	4	
S10	1/0	2/0	4	
S11	0/0	1/0	4	
S12	0/0	0/0 (new lysate?)	4?	
S13	0/0	2/0	4	

2/8/54 10 colonies tested for HFT
only one #8, = NFT = 202-16N
#1 HFT = 202-16H

no. lys. spot / no. control spots

Crosses — wanted ole

X 1436 EMS gal

S14	95, 75, 70, 85, 215	= 540	ot/
S15	82, 49, 70, 62, 99	= ot/362	
S17	73, 51, 68, 51, 61	ot/364	
S19	92, 76, 63, 122, 111	ot/364	

II controls ot except S14 - Redo S15

533, 609, 459, 370, 479, 538	= 2988	ot/3528	
1 plate crowded - 452 x 6 plates	= 2712	ot/3074	
" " = 509 x 6 "	= 3054	ot/3358	
" " = c. 2000 x 4 "	= c. 8000	ot/8000	

Crowded Seg

	Total		Total	
III controls ok 2/3/54 X1436		2/3/54 X902 II		
1572 x 8 =	ot/12576		0/650	ot/4339
>332 x 8 =	ot/2656		ot/164	
	ot/5730		0/142, 0/100 = 0/222	
>6048 - 4848 (+) =	ot/12848		ot/171	

Lysate controls

S14	1/1	10/0
S15	1/1	0/0
S17	1/1	5/0
S18	1/1	solid smear
S19	1/1	4/0

HFT gal$_4$- : see clr 210

2/3/54 Gal$_2$- = ot

S5	6 B/1	0/<1	5/0
S9	8/1	0/<1	15/0
S16	solid HFT smear	3/<1	solid smear HFT

2/3/54 controls ok

14 / 1400 x 8 =		ot/11,200	
8404 8 =		ot/6720	
>824 x 8 =		ot/2268	

	II		total
	ot		
	6+/3888		
	3+/2432	(3+)	6+/10,688

2/4/54 controls ok

	I	II	total
	X902		
	0/401	0/226	0/852
	ot/510	0/208	ot/718
	ot/272	0/137	ot/409

6/15 - 6/16 53

1485 Transduction to (+) with N1 (gal, HFT)

Two mixes obtained. Tested against N1 (1-100) N16 (1-109) (both AFT lysate)

	/N16	/N1	locus	tp
1.	solid smear	no effect	1-	+
2.	s.s. lysis	n.s. - lysis	1-	s (?)

1673 Transduction to (+) with N1, N16 (HFT's)

9/3/54 Strain suspected of being mixed with gal(+). Purified and tested - grown in gal + liq 20 hrs tested vs N16 in presence of gal

		obtained	/N1 (1-100)	/N16 (1-109)
1. /N1	no (+)	obtained.		
2. /N16	3 (+)	obtained.		
6/30/53 test ?=N16	1.	0	0	0
	2.	0	c. 50	0
	3.	0	0	0

W2432

only one appears to be gal-
are others tp2 - ?
stock made 6/20/53
(two (1,3) are tp2-)

1673 Induction of gal- ∈ U.V.

1. closer c. 10-12 seconds 12 (+) obtained on 12 plates.

902 - 6 sp gal+ obtained and stored.

→ (N7) 3 spont. + obtained - (appear stable) - stored

(N1) 2 spont + " " " "

Recross

	Nbλ (1-100)	N16(1-109)	7232	811-8λ	probable locus.
1673 ~~1485~~ N16-1	0	0	0	0	(?)
1673 1485 N16-3	0	0	0	0	(?)

1/14/56
This is a 2 plaque on the cross Must 2342λ is a mixture 1-2- and 1+2- as indicated by the 2342λ Must

6/16/53 Tuesday -

811 - Transduction with λ mutants to observe fate of infecting phage -

1. no add	26	→	analyze to obtain parental phage type
2. 1485-1	105	→	" " " " " mutant phage type
3. 1485-6	262	→	" " " " " " "

2175 1. no add 9
2. 1485-1 63 } as 811
3. 1485-6 183

discarded

6/16 N3 Wednesday -

Experiment Q - 578 E/K-12 (-) segregants. Spot test

Segregant	No add	N16 (1-10)	578 A-8	Prob. lysis
Q 1	1	S. smear	1	4-
2	2	S. smear	0	"
3	contaminated c	+		
4	1	solid smear	2	"
5	1	" "	1	"
6	0	" "	1	"
7	1	" "	1	"
8	1	" "	1	"
9	1	" "	2	"
10	0	" "	1	"
11	2	" "	2	"
12	1	" "	0	"
13	0	" "	1	"

Lysates	811	2175	Motile [illegible]	811	2175
Q 4	1/1	8/1	4-		
6	lysis	3/1 partial lysis	4- R ok/2 reassay	2/2	5/1
8	1/1	6/1	4-		
10	3/1	75/1	4-		

Crosses	X 1436		
Q8	control streak	109, 152, 173, 176, 231	= ot/896
Q6	on EMB gal	144, 184, 107, 280, 203	= ot/918
Q4	none on H)	238, 214, 181, 129, 372	= ot/1134
Q10	—	191, 161, 153, 178, 173	= ot/863

3511

6/18/53 Thursday

2175 ductions to obtain more (-) segregations and

				Fraction obtd (+) at ②	③	
1. no add		14				(Σ to obtain 19 (-) segs
2. 750λ-2	(1)	86				
	(2)	76	all plates but one	5/24	6/24	+3 from stock in suspn 7½ (?) picked
	(3)	88				
3. 811λ-8	(1)	77	each set			
	(2)	80	stored .24	10/24	11/23	← Those circled with 1 (+) previously obtained – 2 lost = (16) or (+)
	(3)	77	top picked from each plate			

750 ductions & 8bt. more (-) segregants

1.	no add	4		
2.	811λ-8 (1)	5	all picked (26)	0/26 after ②
3.	(2)	9		
	(3)	10		
		Σ = 26		

1673 gal (-) mutations U.V. induced p. 203 tested

Gal⁻ mut. no.	λNM(1-10)	λN16 (100)	22381λ-1	811λ-8	bkgrd	probable 10 cc	Comment
1.	bkgrd	bkgrd	bkgrd	bkgrd	high	?	appears to be slow - discard
2310 2.	6-10	c. 50	6-10	6-10	6-10	?	transduced only by 2
3.	<bkgrd	>bkgrd	<bkgrd	<bkgrd	c. 20	?	transduced doubtfully by 2 probably slow
2311 4.	<bkgrd	solid smear	<bkgrd	<bkgrd	c. 10	?	transduced only by 2
2314 5.	0	c. 20	0	0	0	?	transduced only by 2
All λ⁻-one spot plus lysis 2313 6.	solid smear	3	2	20	c. 0	2? or new locus?	
2314 7.	solid smear	c. 70	4	c. 30	c. 0	new locus ?	
2315 8.	solid smear	solid smear	0	c. 25	0	3⁻	
9.	c. 25	solid smear	c. 10	c. 10	c. 10 (small)	?	transduced only by 1 & 2
10.	2-3	c. 50	2-3	2-3	2-3	?	
11.	>bkgrd ?	bkgrd	bkgrd	bkgrd	high	?	

6/26 Repeat on above

2.	2	>100	3	4	5	1+? 2nd	1-	1, 2, 3, 4, 9, 11 established by + by 8bt+ possibly by K-12 & + by 8bt+ K-12 λ
4.	5	solid smear	5	2	3	lag double	1-	
hybrid gal⁻s transd. sm(?) 5.	0 *	0 * ✓	0 *	0 *	?	?	1-	rev not 8bt+ transd. K-12 / not + locus ?
6.	>100	10	2	>30		2 new	3- (?)	3-
7.	>100	>50	0 mixed →	>30	0		3-	3-
8.	>100 (250)	>100 (210)	0 (0)	80	>20 sec	0	3- (?) 3- spt.	3-
9.	0	+++(?)	0	>50 small	0	2nd (13)- (?)	double	double
10.	0 *	>30 (small)	0	0 *	c	double (?)	double	new
11.	0	0	0	0	0	(?) / new	new	

SEE PAGE 220

* plaques in bkgrd. of spot

7/2

1673 gal- #5 × 811 - controls 5h.

1. 1+/27
2. 0+/19
3. 0+/24

1+/84

P4 / 1.05 0.012

84/160

1.2% revrts.

2061

12

Needed

Ft. M — 750 t K-12 crosses of M's x 750's

N — 750 t 902 " N gal$_1$- x 750's

O — 2175 t K-12 Complete

P — 518 t 750 (duplicates)

Q — 518 t K-2 lysate test and crosses x 14726 gal$_4$-

R — 518 t 902 crosses of (R gal$_2$- x R gal$_4$-) and lysates of gal$_2$-

S — 811 t 902 crosses of (S gal$_1$- x S gal$_4$-) and " " gal$_2$-

T 2175 t 750 all

U — 2175 t 811 crosses, lysates) in general

V — 811 t K-12 20/24

W — 1924 t 902 19/24

Thursday 6/25/51

Expt. A. 2175 Å Pt (−) Xg vapour. spot tested

	~~Crosses~~	~~Blocks~~	Ni6 (2⁻) (1-in)	811 λ-8	Prob. Lines			∠λ-2	Neutrals	Ni (1-in)	049 pt (1-in)	Ni6 (1-in)
	1	1	2	3	4 (?)	+	1	sens.	1	0	c. 200	c. 100
	2	0	1	> 100	(also λ 5) 2⁻		2	"				
	3	0	0	6	2⁻		3	"				
	4	1	2	11	2⁻		4	"				
	5	0	solid smear	0	4⁻	+	test	"	2			
	6	0	1	12	2⁻		5	"				
	7	0	0	13	2⁻		6	"				
	8	0	1	9	2⁻		7	"				
	9	0	0	9	2⁻		8	"				
−	10	1	0	8	4⁻ (?)	+	9	"	3	0	c. 200	c. 100
	11	1	1	8	2⁻		10	"				
	12	0	solid smear	0	4⁻	+	test	"	4			
−	13	0	1	L	4 (?) L	+	11	"	5	0	c. 200	c. 100
	14	0	solid smear	0	4⁻	+	test	"	6			
	15	1	2	10	2⁻		12	"				
−	16	0	2	1	4⁻ (?)	+	13	"	7	0	c. 200	c. 200

9 2⁻
1 4⁻

Curve 1 —

13

Hypate mode 2/5/54

new #		811	2175	758
U1	U1	50/1	1/0	22/0
U9	U10	70/1	0/0	61/0
U11	U13	20/1	3/0	14/0
U13	U16	30/1	0/0	16/0

Col - one 2⁻ because of conflict with above results. All U strokes. retested against Ni6, 318

2/8/54

		Ni6 λ	518 λ
Remember	1.	0	+
but in	2.	" (4p³)	" (4p⁵)
order	3.	"	"
of	4.	"	"
above	5.	"	"
	6	"	"
	7	"	"
	8	"	"
	9	"	"
	10	"	"
	11	"	"
	12	"	"
	13	"	"
		Acc 2⁻	

In view of the results suggest intensities ought be redone - were the 4⁻ weak so or perhaps 2-4⁻ which later revised to give the above results?

2/24/54 Slant mode of U2 since it is ~~not~~ discarded

Saturday 6/27/53

97
22~~7~~ ductions to obtain segregants.

		cntrd halt	o.i.Sus/immigrant unstg.	
1.	750λ-2	2	88	24 picked from each and streaked - source of
2.	902λ-4(?)	5	34	segregants - (1)
3.	811λ-8	12	52	

750 24/24 stable after 3

902 22/22 · stable after 3 · 2 lost of orig. 24

811 20/23 stable after 3 · 3 questionably unstable, 1 lost.

7/5/53

902 lysate · 902λ - 7/4

Spotted against	2175	811
	0/1	109/2

Transductions for the purpose of obtaining segregants
Spread plates

811 -

			2nd strk	(5) picked
no add	41			
K-12λ 1/20	573	← 24 picked & streaked.	23/26 unstbl	20/20

1924

			2nd strk	←'s picked	2nd base	total
no add	31					
K-12λ 1/10	320	← 24 picked from each & streaked	13/24 unstbl	13/24	14/24	= 29/48
750λ	25	probably doesn't transduce ←				
902	235		19/24 unstbl	19/24		

Sunday
July 5, 1953

Experiment T — 2175 transduced by 750 1 — the (-) segregants. Spot tested against N1 (1-100) and N16 (1-100)

Segregant	Rf gal	N1 (1-100)	N16 (1-100)	Lysable by	Bact. characteristic
T1	0	c. 50	0	2⁻	R
2	0	c. 50	0	2⁻	S
3	0	c. 50	0	2⁻	R
4	0	0	>100	1⁻	S
5	0	>50	0	2⁻	R
6	0	0	>100	1⁻	R
7	0	c. 50	0	2⁻	S
8	0	c. 50	0	2⁻	R
9	0	c. 50	0	2⁻	R
10	0	>50 smear	0	2⁻	R
11	0	>50 smear	0	2⁻	S
12	0	c. 50	0	2⁻	S
13	0	0	>100	1⁻	S
14	0	c. 50	0	2⁻	R
15	1	>50	0	2⁻	R
16	0	c. 20	0	2⁻	S
17	0	c. 20	0	2⁻	S
18	0	0	0	? 4pi⁻	R
19	0	0	0	? 4pi⁻?	S

all small after 2 days

tested: 4pi⁻ (7/5); 4pi⁻ (7/5)

Recheck	K-12 1	8921 (1-100)
bl/gal's	65	20
= 0	41	28

9/2/53 T18 not ↓ by 811

14 gal2⁻
3 gal1⁻
this may be 2⁻ — 2 (?)

gal2⁻ 2R
gal1⁻ 1R
? 1S
1R

9/2/53
2070 to study on segs. taken from 1, 4

Lysate examinations

	750	2175	2297	811	816	1924
T18	0/0	0/1	3/1	12/2	25/4	11/3
T19	2/0	1/1	1/1	5/2 stable	19/4 6/6 stable (1) 6/6 stable (2)	8/3

Failure 5/3

Lysates of T18, T19 on
(1) 811/ET18 → segregant 1 = gal1, gal4⁻ ? not ↓ by HFT 4⁻ 1⁻
(2) 1924/ET18 " 1. = gal2⁻ ? not ↓ by HFT 2⁻
(3) 1924/ET19 " 1 = gal1⁻ ? " " " HFT 1

9/16/53 Second lysates of T18, T19 made — tested against 2297 — segregants isolated for

	2297	isolated
T18-1	67	13
-2	110	16
T19-1	135	26
-2	240	30

24 picked after 2 stab. 3/24 unstable

23 picked — all stable after 2 stab

Lysates (not good) made of	750	2175	Crumble hours
6	0/0	0/1	1⁻
13	0/0	0/1	1⁻

7/24/54 Short Stocks of T18 T19 retained — used as met control of strains

July 6, 1953
Monday

750 X 1436 cross - on EMB gal - controls on a EMB gal

	(+)	total
1.	0	614
2.	0	677
3.	2	854
4.	1	835
5.	2	660
6.	1	958
	6	4588

750 = 1- Lp+ 4+
1436 = 1+ Lp- 4-
Lp closer to 1 than 4

4588 / 6.000 = 0.0013 = 0.13 % recomb.
4588
14120
13764

6/6 recomb. stable on EMB gal
1 (+) recomb appears mixed for Lp - Lp+ / Lp-
5/6 (+) λ⁵

7/7/53
Lysate test

	750	2175	2297	811	1924
control spot	= 0	0	0	2	0
2.238 λ	= 0	2	0	3	0
2.251 λ	= c.40	0	2	c.40	0
S18 (to date) λ	: solid smear	solid smear	solid smear	1	0

Relationship of gal's - Lp.

above gal 4 - gal 1 = 0.13 % +
eqt. A gal 4 - gal 2 = 0.21 % +
p 199 gal 1 - gal 2 = 0.13 % +

Lp 1+ = 0.83 as Lp 4 control.
Lp 2+ = 0.80 as Lp 4 control.

2/12/53

$Lp^s\ S^r$

$F^-TLB,^-Gal_4^-Mal^-V_6^r Lac_1^-$ × $F^+BM^-Lp^+S^sGal_2^-Lac_1 V_6^s Mal^+$

2234 × 1210

EML's 580 B. Cross of Gal_2^- × gal_4^-

8/27/53

Prototroph	$^{2-}_{NTG}$ Gal_2^- HFT	$^{4-}_{SIB}$ gal_4^- HFT	Lp(1)	TL	Strep	Mal	1K-12 d
1	no reaction	no reaction	?	r	s	r	–
2	–	+	2	s	r	r	– (s) weak
3	+	–	4	s	r	r	– weak
4	+	–	4	s	s	s	+ s
5	+	–	4	s	r	s	– s
6	+	–	4	s	r	s	– s
7	–	+	2	s	s	r	– (s) weak
8	+	–	4	s	r	s	– s
9	–	+	2	s	s	r	– (s) weak
10	–	+	2	r	s	s	– r
11	–	+	2	s	r	r	– (s) weak
12	–	+	2	s	r	r	– (s) weak
13	–	+	2	s	s	r	– (s) weak
14	–	+	2	r	s	r	– r
15	no react	no react	?	s	r	+	– s
16	+	–	4	s	r	s	+ s
17	+	–	4	s	r	s	– s
18	+	–	4	s	r	s	– s
19	–	+	2	r	s	s	– r
20	–	+	2	r	s	r	– r
21	+	–	4	s	r	r	– s weak
22	–	+	2	r	s	r	– r
23	+	–	4	s	s	r	– s weak
24	+	–	4	s	s	s	– s
25	+	–	4	s	r	s	– s
26	+	–	4	s	r	s	– s
27	+	–	4	s	r	r	– s
28	+	–	4	s	r	r	+ s
29	–	+	2	s	s	r	– (s) weak
30	no react	no react	?	s	r	r	– s weak
31	+	–	4	s	s	r	– r
32	no react	no react	?	s	r	r	– s weak
33	+	–	4	s	s	s	– s
34	–	+	2	s	r	r	– (s) weak
35	+	–	4	s	r	s	+ s
36	+ some no.	–	4	s	r	r	– s weak
37	+ some no.	–	4	s	r	r	– s
38	+	–	4	s	r	s	– r

gal_4^- = 21 Gal_2^- 13

4 gal_2 gal_4^- ?

Weak 1K 8 gal_2^- 4 gal_4^- 2 gal_2^-

Parental

O = 2234

6/18/54

Record several statements of EML parents of this strain are in doubt

7/18/53 — up to ductwin page 208

Sol$_2$-HFT · Sol$_4$-HFT

Sept. V - 811E16-12 (-) segregants. Spot tested with N16 (1-100), S18 (1-100)

Segregant	/N16 (1-100)	/S18 (1-100)	
1.	+	−	Streaks 3/24/54 as V
2.	+	−	dirt
3.	+	−	"
4.	+	−	"
5.	+	−	
6.	+	−	
7.	+	−	
8.	+	−	
may be contaminated 9.	+	−	dirt
10.	+	−	
11.	+	−	
12.	+	−	
13.	+	−	
14.	+	−	
15.	+	−	
16.	+	−	
17.	+	−	
18.	+	−	
19.	+	−	
20.	+	−	

20 gal$_4$ -

(+) = ca 500 μg./disp (−) = no μg./disp.

Cvm X1436
V2
V10
V12
V18

6/18/54 Streaks, Dais. Opt discarded

Typestested 1/22/53

	Sporulated 811	/2175'
V1	2/100	30/0.1
10	3/100	14/0.1
12	0/100	10/0.1
18	2/100	14/0.1

7/13/53

Expt. W 1924 & 902 ← 1 segregant. Tested ē N16 (1-100) and S1F (1-100)

Segregant	/N16 (100)	/S1F (1-100	Probable locus	Yield/S18
1	—	+	2-	H. lys
2	+	—	4-	"
3	+	—	4-	"
4	—	+	2-	one plaque w. lys.
5	+	—	4-	"
6	+	—	4-	"
7	+	—	4-	"
8	+	—	4-	"
9	+	—	4-	"
10	+	—	4-	"
11	+	—	4-	"
12	+	—	4-	"
13	+	—	4-	lysogenic
14	—	+	2-	w. lys.
15	+	—	4-	"
16	+	—	4-	"
17	+	—	4-	"
18	+	—	4-	"
			15 gal$_4$-	1 lp$^+$
			3 gal$_2$-	17 lp^r

W2893

2/24/54

The stocks of Pan ... slope. Slant made of W1 since it is [illegible] lpr

Reversion of W1 tested spring '55 (see page) One found diploid. Possibly W1 was lp$^+$ Gal$_2$ lpr - Gal$_i$ -

Cross × 902, 1436

1
4
14
17
18

No. segregating
to picked

rotten) duction plate
control plate

Transduction Made

λ Source

	(tr) K-12	(gal_1^-) 750	(gal_2^-) 902	(gal_3^-) 2238	(gal_4^-) 811
(gal_1^-) 750 prototroph					
(gal_2^-) 2175					
(gal_3^-) 2297					
(gal_4-lp^s) 518					
(gal_4-lp^t) 811					
(gal_4-lp^r) 1924					

B. The occurrence of stable transductants.

Exp. = no. of stable (+) expected. Obs. = no. of stable (+) observed.

Comparable (equivalent) no.s considered.

Lambda Source

Lam-s Transduced	K-12 Exp.	K-12 Obs.	gal_1^- Exp.	gal_1^- Obs.	gal_2^- Exp.	gal_2^- Obs.	gal_3^- Exp.	gal_3^- Obs.	gal_4^- Exp.	gal_4^- Obs.
gal_1^-	1/143	42	[illegible]		1/84	3.5 $\chi^2 = 6.75$	not done		[illegible] 128 27 27 *	
gal_2^-	17/248	20.7 $\chi^2 = 0.87$	14/83	61.1	[illegible]		not done		14/79 [illegible]	52.1
gal_3^-	not done		2/88 (are stable)	88	5/34 (are stable)	34	[illegible]		13/58 Gal	48.7
$gal_4 - bp^s$	19/835	383	29/72 (are stable)	72 *	11/472	19.7	not done		[illegible]	
bp^+	41/573	133	51/96 (are stable)	96 *	47/147	30.6 **	not done		[illegible]	
bp^r	31/370	127	ratio deviation plate / control plate = 25/31 *		31/238	49.6 $\chi^2 = 12.7$	not done		[illegible]	

* deviation does not go well apparently

** estimated from two different experiments

$$\text{Expected} = \frac{\text{control (sp. pop.) plate}}{\text{phage plate (= sp + transd.)}} \qquad \text{Obs.} = \frac{\text{no. stable obs.}}{\text{no. sample taken}} \times \frac{\text{no. phage plate (sp + transd.)}}{\text{no. in sample taken}}$$

Key to Experiments

Lambda Source

Recipient Cells	K-12	750 gal$_1$ -	902 gal$_2$ -	2238 gal$_3$ -	811 gal$_4$ -
gal$_1$ - 750 prototroph	Expt. M p. 192A		Expt. N p. 192B	not done	no expt. designation p. 206
gal$_2$ - 2175 (902 prototroph)	Expt. O p. 196	Expt. T p. 209		not done	Expt. U. p. 207
gal$_3$ 2297 (2238 prototroph)	not done	no. expt designated All stable p. 208	no expt. designation All stable p. 208		no. expt. designation All stable p. 208
518 gal$_4$ hp^s	Expt. Q p. 205	no. expt design. all stable p. 189	Expt. R p. 198	not done	
811 " hp^t	Expt. V p. 212	no. expt. design. all stable p. 195	Expt. S p. 208	"	
1924 " hp^r	Expt. X p. 216	no. expt. design. all stable p. 208	Expt. W p. 213	"	

No. segregants	
class. by transduction test	(what they are transduced by)
class by donor test	(what they can transduce ≡) usually 4 seg. taken at random
class by test cross	

Lambda Source

Recipient cells	16-12 ~~gal+~~	gal_1^-	gal_2^-	gal_3^-	gal_4^-
gal_1^-	17 17 gal_1^- 4/4 gal_1^- 4/4 gal_1^- (incomplete)		23 18 gal_1^- 5 gal_2^- 5/5 gal_2^- 4/4 gal_1^- 5/5 gal_2^- 4/4 gal_1^- {incomplete}	not done	Stable (+) ?
gal_2^-	20 20/20 gal_2^- ~~4/4~~ gal_2^- 4/4 gal_2^- (inc.)	19 14 gal_2^- 3 gal_1^- 2 (?) doubles? incomplete appears to transduce gal_4^- 2(?) inc.		not done	16 8 gal_2^- 4 gal_4^- 4 (?) doubles? incomplete
gal_3^-	not done	stable (+)	stable (+)		stable (+)
gal_4^- Lp^s	13 13 gal_4^- 4/4 gal_4^- 4/4 gal_4^- (inc)		21 18 gal_4^- 3 gal_2^- 14/16 gal_4^- (gal_2^- incomplete) 4/4 gal_4^- (complete) 3/3 gal_2^- (incomplete)	not done	
Lp^r	20 20 gal_4^- incomplete incomplete	(+)	19 16 gal_4^- 3 gal_2^- 15/15 gal_4^- gal_2^- (incomplete) 4/4 gal_4^- (complete) gal_2^- incomplete	not done	
Lp^r	29 29 gal_4^- incomplete incomplete	stable	18 15 gal_4^- 3 gal_2^- (4°) incomplete incomplete	† done	

Occurence of HFT Lambda (Among Segregants)

Lambda Source

Recipient cell	K-12	gal_1^-	gal_2^-	gal_3^-	gal_4^-
gal_1^-	0/4		1/5 gal_2^- HFT 2/4 gal_1^- HFT	not done	not done stable (+)
gal_2^- <	0/4	not done one unstbl. + HFT		not done	not done
gal_3^-	not done	stable (+) not done	stable (+) not done		not done
gal_4^- lp^s	0/4	not done stable (+)	0/16 gal_4^- gal_2^- not done	not done	
lp^t	not done	not done stable (+)	1/15 gal_4^- gal_2^- not done	not done	
lp^r	not possible	not do stable (+)	not possible	not done	

Previously unstable (+) from 578 t K-12

750 t 1821 (gal_1^- traced by gal_4^-)

1436 t K-12 (gal_4^- t K-12)

Ignore this - ignore - ate evidence indicate 2281 not gal- but Gal-

(214)

IGNORE

7/16/53 Thursday

2257 - test cross to examine its authenticity as gal_2-

1. X750 on EMS gal

	(G)	Actual
1.	1	285
2.	0	357
3.	3	505
4.	0	453
5.	0	355
	4	1957

2. X811 on EMS gal

1.	0	167
2.	0	148
3.	0	225
4.	0	270
5.	0	229
	0	1039

3. X2281 (in gal_2-4p^1)

1. 1/4 of one plate counts = 342 = 1368/plate x 5 plate = 6840
   - 3(+) colonies observed

1957 | 4.000 | 0.0020 | 3914 | 460

Since both gave (+) and showed something wrong with cross 1? says no!

Rpt 9/10/53
10 plates - control ok

1. 1/4 = 119 x 4 = 476 x 10 = 4760

0.2%

4(+)/6517

6517 | 0.0006 | 4.0000 | 39102

= 0.06%

6840 | 3.0000 | 0.00044 | 27360 | 2640

= 0.044% recomb. between 2 ≡ gal_2

Needs checking

2251 x 2281

Rpt 9/10/53
10 plates - control ok

1. 1/4 = 241 x 4 = 964 x 10 = 9640

10/13/53
1/4 = 78 = 312 x 6 = 1872

1872
11,512

K-12 =
750 = 750 + 2
902 = 902 7/4
811 = 811 - 8

7/19/53 Sunday.

750 cell no.s vs constant K-12 λ

Aerated cultures used - 2 20ml overnight Pen. Growth resuspended in about 1.5 ml. Dilutions made and plated with and without K-12 λ - (0.1ml + 0.1ml K-12 λ)

Sol	Dilution	+K-12 λ	control	Δ	/750 λ	/902 λ	/811	No. of cells/plate
	med.	12	0		3	82	8	3.6×10^9
0.3 +0.3	1-2	plugs omitted	0		1	74	9	1.8×10^9
0.2 +0.6	1-4	16	2		4	117	7	9×10^8
0.1 +0.7	1-8	46	1		3	106	9	4.5×10^8
(1-2) 0.1 +0.9	1-20	plugs omitted	3		1	193	8	1.8×10^8
(1-4) 0.1 +0.9	1-40	43	1		4	plugs omitted	6	9×10^7
(1-8) 0.1 +0.9	1-80	46	2		1	104	6	4.5×10^7
etc	1-200	plugs omitted	1		4	85	6	1.8×10^7
"	1-400	28	3		2	90	7	9×10^6
"	1-800	18	1		1	69	3	4.5×10^6
"	1-2000	19	1		0	61	6	1.8×10^6

$1 - 2 \times 10^3 \times 10^{-2}$

$1 - 2 \times 10 \times 10^2 \times 10^{-1}$

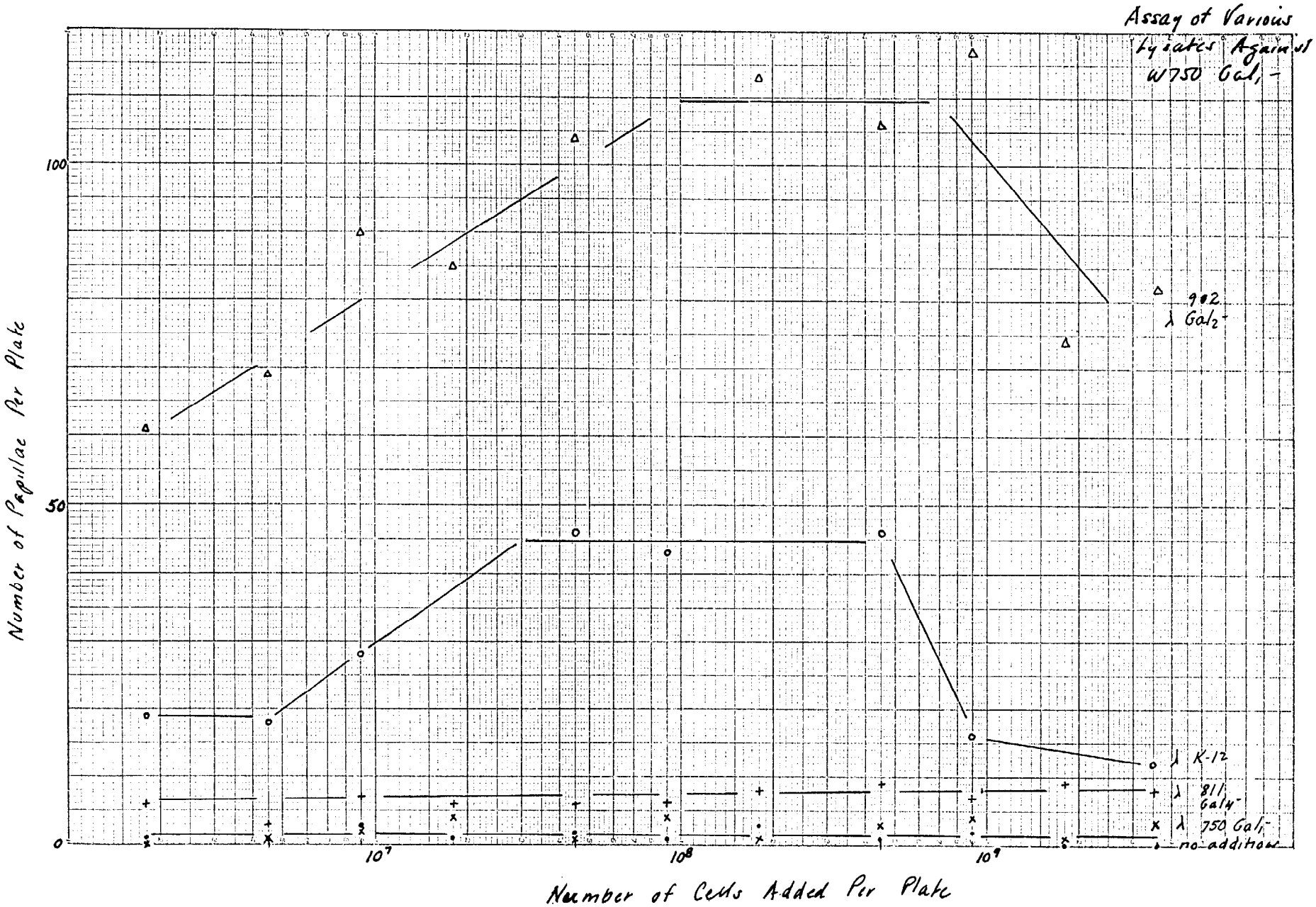
0.1 ml 1. 2003
2. 1679
3. 1850

2×10^7 dil

5532 / 3

$1844 \times 2 \times 10^7$

$3688 \times 10^7 = 3.7 \times 10^{10}$ cells/ml



These segregants tested on two plates by cross brushing against HFT 1

7/20/53 Monday.

HFT gal- HFT gal+

Experiment X - 1924t K-12 - (-) segregants tested /518 N16

Segregant	Ham. for Lys. /518	/N16	/518
X 1	non-lysog.	solid smear	no read
2	"	" "	"
3	"	" "	"
4	"	"	"
5	"	"	"
6	"	"	"
7	"	"	"
8	"	"	"
9	"	"	"
10	"	"	"
11	"	"	"
12	"	"	"
13	"	"	"
14	"	"	"
15	lysogenic	"	"
16	non-lysogenic	"	"
17	"	"	"
18	"	"	"
19	"	"	"
20	"	"	"
21	"	"	"
22	"	"	"
23	"	"	"
24	"	"	"
25	"	"	"
26	"	"	"
27	"	"	"
28	"	"	"
29	"	"	"
	1 lysogenic & 1 p+	29 gal-	

2/24/54

Stock original culture of this experiment has been lost by discard. A new set obtained not tested - discarded. This data is not worth saving.

7/24/53 Friday - Effect of U.V. on HFT λ. N16 gal$_2$ λ used.

N16 lambda diluted 1-10 and irradiated for varying time intervals. 0.5ml removed and added to 10 ml pneumococ. broth - Assayed against various gal- typ. forms.

Irrad.	Trial phage assay	dil 1-10 add.	/2281	ENB gal K	dil 1-10 additional	/518	
0 min.	dil 10^5 → 0.1 ml	2023			197, 170 = 1540		
1 "		4628			164, 102 = 1330		
3 "		2264		.49%	44, 15 = 290		
5 "	dil 10^3 → 0.1 ml	1444		.637	17, 18 = 180		
7 "		853		.591	3, 6 = 50		
9 "		509		.597	35, 17 = 27		
11 "	dil 10^1 → 0.1 ml	331		.650	11, 6 = 8		
13 "		266		.807	0, 6		
15 "		54			0, 0		
17 "	und. → 0.1 ml	95					
19 "		10			1, 0		

2023 X10X10 X20 X2 = 4046 X 10^4 = 4.1X10^7

1540X10X10X10X20 = 3.08X10^5 = 3.1X10^7

Transductions (Approximations)

	750	2280	2175	2281	811	518	1924
0	3648	6464 (6)	6	0 (6000)	4800	50 (6000)	
1	4056	c.9000	6	0 (6000)	(6000)		
3			7				
5			5				
7			—				
9			8			(2700)	
11			6	(300)		(1600)	
13			10				
15			6	1234		960	
17			8	176		72	
19			3	81		31	
no add			6				

7500 X10X20 X10 = 1.5X10^6

1.92 X 10^5 = 1.92X10^7

Not Counted

dil 1-20 → 1-10 → 1-10 = 1-2000

rig-hter 437X10X2000X10 = 874X10^5 = 8.74X10^7

dil = $\frac{1}{20} \times \frac{1}{10} \times$ 0.1 ml

TRANSDUCTIONS - RPT ASSAY 1-10 dil. of above.

2368X10X10X20X10 = 4736 X 10^4 = 4.7X10^7

no add							
0	thin plate	thin plate	mut	910	176 (2368)	thin plate	
1	thin plate	4296		thin plate	5 (3088)	580	
3	2216	3344		3744	128 (2872)	2480	
5							
7							
9							
11							
13							
15							
17							
19							

Rot. Assay 1-10 dil of previous assay.

Irrad.	750	2280	2291	518	811	1924	Plagal Assay 518
0 bkgd	0	2		19	39	28	0
0 (+λ)	~~[illegible]~~ 96	197	Contaminated	36	114	96	437 = 8.74X10⁶/me
1	~~[illegible]~~ 166	~~[illegible]~~ 632		77	219	214	497 = 9.94 X10⁶
3	334	632	with	148	621	438	149 = 2.98X10⁶
5	470	685		337	774	667	117 = 2.34 X10⁶
7	595	569	(+)	385	922	660	33 = 6.6 X10⁵
9	458	346		327	912	519	13 = 2.6 X10⁵
11	~~[illegible]~~ 403	246		258	634	489	2 = 4 X10⁴
13	358	195		254	553	370	0
15	226	98		201	394	321	0
17	191	88		120	278	194	0
19	87	42		107	217	139	0

dil = 10 X 10 X 10 X 20 = 2 X 10⁴

λ assay 518 same dil. 651, 579, 1460 ~~[illegible]~~ 895

λ Assay 2281 Same dil

331, 243 = 574

203, 258 = 461

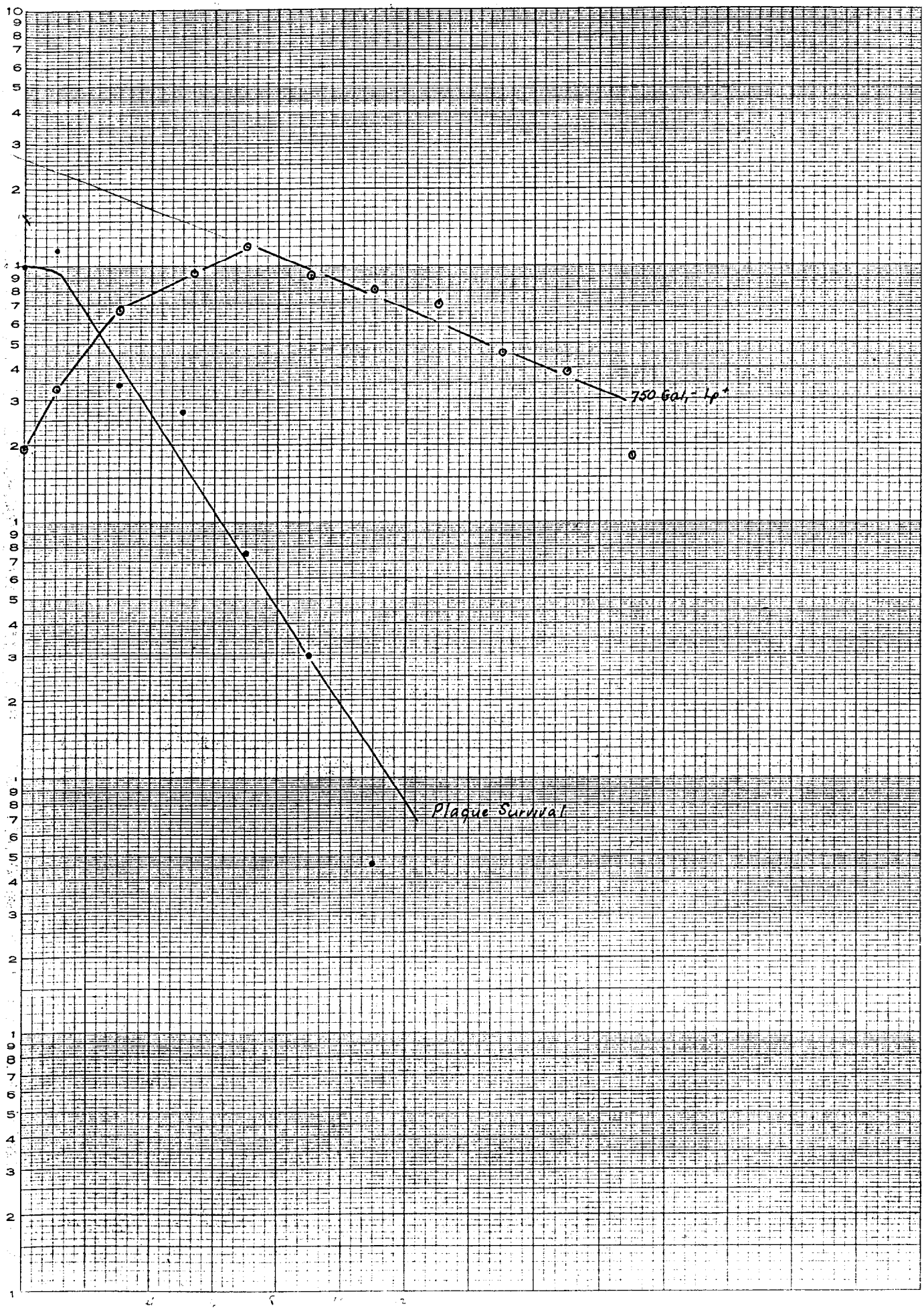
173, 63 = 236

32, 37 = 69

total = 26

Corrected for bkgd.

		dx2		x2			x2				
0	96	192	195	390	17	34	75	150	68	136	119, 124 = 243
1	166	332	~~[illegible]~~ 435	870	58	116	180	360	185	370	30, 31 = 61
3	334	668	630	1260	129	258	582	1164	409	818	
5	470	940	683	366	218 ~~309~~	636	735	1470	638	x76	
7	595	1190	567	1134	366	732	883	1766	631	1262	
9	458	916	344	688	308	616	873	1746	490	980	
11	403	806	244	488	239	478	595	1190	460	920	
13	358	716	193	386	235	470	514	1028	341	682	
15	226	452	96	192	182	364	355	710	292	584	
17	191	382	86	172	101	202	239	478	165	330	
19	87	174	40	80	88	176	178	356	110	220	



Irrad. HFT 1 · N16

	λ Survival as Plaques		Pet. microtiter	
	750		750	2280
	titer	N_s/N_0	— 192 $\times 10^{-7}$	3.9×10^{-8} ↓
0.	8.74×10^6	1.0	1.0 ↓	1.0
1.	9.94×10^6	1.14	1.7 (3.3)	2.2 (5.6)
3.	2.98×10^6	0.341 3.41×10^{-1}	3.5 (6.7)	3.2 (12.5)
5.	2.34×10^6	0.26 2.68×10^{-1}	4.9 (9.4)	3.5 (13.7)
7.	6.6×10^5	0.076 7.6×10^{-2}	6.2 (12)	2.9 (11.3)
9.	2.6×10^5	0.030 3.0×10^{-2}	4.8 (9.2)	1.77 (6.9)
11.	4×10^4	0.0046 4.6×10^{-3}	4.2 (8.1)	1.25 (4.9)
13.			3.7 (7.1)	0.99 (3.9)
15.			2.4 (4.6)	0.49 (1.9)
17.			2.0 (3.8)	0.44 (1.7)
19.			0.91 (1.8)	0.205 (0.80)