

4/1 Aerial culture of 5:30 AM  
 Wg 16 pr - for pen. run  
 K-12 - 1 prep.  
 1655 - for transduction trial

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

K-12 L6?	Tubes	1	2	3	4
λ dil		1-10	1-100	1-1000	1-10000
ml K-12 culture		1.0	1.0	1.0	1.0
Pen.		10	10	10	10

In bath at 9:45

at	10:15	granular appear	granular appear	turbid	turbid	
11:30	..	"	"	"	"	"
2:10	..	..	..	..	..	..
3:00	..	..	..	..	..	..

over all the tubes the same  
 discarded

1655 + P82  
 16 colonies picked from streakings of 2/30 - tested against 1655  
 all appear to be phage free  
 4/2

1655 - Transduction

Tube	1	2
11:30 AM 1655 cult.	5.0ml	5.0ml
λ L6	0.5ml	-

Centrifuged - resuspended in saline (5.0ml each) + ~~transductant~~  
~~1655 + L6~~ Burt's soln. (PF stock dil. 0.1 + 10ml)

Plate 0.1 ml on D(0)

4/2 - plate with phage has heavier background than without.  
 4/3 - " " " " " "  
 discarded  
 due to exposure to broth

K-12 L7

ant. culture 1:15  
 centrifuged, resusp in sal - used 35 ml  
 incubated at 1:45  
 cleared at 4:00

Eliminates requirement for H<sub>2</sub>O buffer in L<sub>6</sub> - use saline

4/2

52

Wg 16 Pt- Pen run Colonies  
Plated 1-10 (x100) -  
and } -

---

Wg 14 pr-typst - x 58-161  
grown overnight in Pen in mixed culture.  
plated out - D-O (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 component in saline - immediate after del. to 10<sup>8</sup> cells/ml

Time	Count/ml	Phage/ml	Survival	K-12 as plaque
0	256 x 10 = 2560	5 x 3 x 10 = 150	1.0	0.58
5	195 x 10 = 1950	11 x 3 x 10 = 330	0.76	0.13
10	123 x 10 = 1230	39 x 3 x 10 = 1170	0.48	0.46
15	76 x 10 = 760	52 x 3 x 10 = 1560	0.30	0.61

1681 - Overnight culture - selected for red. resistance and turb.

Culture counted (1.0 + 10<sup>8</sup> Pen) 8:15 - centrifuged plate +

Time	Count/ml	Phage/ml
0	35	0
10	21	0
20	19	0
30	7	0
40	8	0

is this culture pyrogenic? for ppt.

Survival
1.0
0.60
0.54
0.20
0.22 ext

1681 cross streaked on 1655 - no plaques observed

35 | 0.54  
19.0  
17.5  
15.0  
12.0  
10.0

W<sub>9</sub> 14 pr<sup>-</sup> Pen survivors -  
replicated to D(0) and EMB(0) + Pr

4/7 of 40± colonies picked and replicated, failed to grow on D(0) + pr - W<sub>9</sub> 14 pr<sup>-</sup> transferred to broth and D(0) + pr + trypt. 48 additional survivors picked





4/9 Wed.

Induction of lysis in K-12 by cold shock?  
 Aerated culture 0.5 + 10 ml - 10:30 AM to 1:30  
 Culture ca  $5 \times 10^8$  cells/ml

Pre-shock  
 Cell titer  $10^2 - 10^9 - 10^{10}$  → plate 0.1  $10^8 \frac{300}{10^8} = 3 \times 10^9$   
 Phage titer → 0.01 + 0.01 578  $\frac{0.1}{10^8}$  → plate 2 =  $2 \times 10^7$

Post shock  
 Cell titer  $10^2 - 10^9 - 10^{10}$  → plate 0.1 ml  $\frac{3000?}{10^8}$   $3 \times 10^8$  (?)  
 Phage titer  $10^2 - 10^9 - 10^5$  → 0.01 + 1.0 ml 578  $\frac{0.1}{10^8}$  → plate 1 =  $9 \times 10^6$   
 Results not conclusive either way.

1831 10:30 AM → 1:20  
 Centrifuged aerated cult. resusp in saline  
 Dilute to ca  $10^7$

Dose	Survival
0	242
10	138
20	33
30	15
	0.062

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

1831 + H2L cross checked  
 1831  
 No recombination

1831 + 8Pr ca 1831

Dose	Survival
0	1105
10	589
20	353
30	149
	0.13

U.V. resistance appears higher than K-12

H267 - Original culture.  
 Strained in EMB to recover diploid  
 EMS

discarded  
 Shut again

W916 pr survivors replated to O(a) 48

new of crosses -  
 at on O(a) + pr

7/10

W916 pr survivors (see previous attempt) 48 in no  
replicated to D(0) + pr -  
1 failed to grow - transferred to start  
for later identification - W916 pr X-2

K-12 Repeat an induction of lysis in K-12 with cell.  
Vigorously growing culture (two transfers in aerated Pen)  
Suspend evenly and placed in ice bath in aerobic 10 min  
Sample 10 min - returned to 37c

- Inmed. cell count - 86
- .. Phage .. - 3
- Post treat cell count - 116
- .. .. phage count - 1

No evidence of  
accumulation to critical amount  
No evidence of induction  
lysis.  
No phage lysis  
not neutralized

W914 (Pr-) Aerated culture - washed twice in saline  
ca 10<sup>5</sup> cells added to:

- D(0) + pr + trypt + pen (0.2ml) - plates out
- D(0) + pr + trypt - phage

undil = 14
1-10 = 0

4/12

W914 (Pr-) Survivors of Pen run of above  
replicated to D(0) + Pr

7 of 14 colonies possibly diautotrophic,  
picked and streaked for further replication

4/14  
Aerated cultures W67 and 477 started 8:15

H267 Rodentic - culture in D(0) + lactose 36 hours -  
dilute to 10<sup>3</sup> cells/ml

Date	Count EMB	Phage TSA 16hr <sup>-</sup> (0.5ml + 0.5ml H <sub>2</sub> O)	Survival
0	341 } 316 } 365 } ca 10000/plate	0	1.0
10	307 10 sec -	12.2 = 29 (7%)	0.90
20	187	—	0.55
30	98	—	0.29
40	60	84.2 = 168 (EM)	0.18

Wg 14 p1 - Pen Smears - 2 possible diase. - 10 colonies picked  
from streakings of each. streaked in ~~EMB~~ EMB-O

replicated 4/15 to D(0) + Pr, EMB blue.  
4/16 - streaked same as before - apparent  
reversion in replica  
D(0) ● - Discarded  
EMB

2 wags of W67 + 1177  
Cells centrifuged. resuspended in H<sub>2</sub>O (10 ml)  
Inoc. 35 sec  
10 ml + 10 ml Pen  
Incubation with air 1:30  
cleaning at 4:00  
partially clear following morning

4/15

Aerated cultures of Wg 14 pr - for pen run  
 W 1628 for sensibank  
 W 1682 " " " " } 10:00 AM

W1628 Drop and Survival

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

Dose	Count/plate	Phage/plate (0.5 ml + 0.5 ml 1661)	Survival
0	338	0	1.0
10	259	-	0.77
20	107 (235)	193.2 ± 286	0.32
30	96	-	0.12
40	17	-	0.05

Wg 14 Pen run - pr-

aerated culture - centrifuged - resuspend in saline -  
 centrifuged, resuspend in saline.  
 add 0.1 ml to:  
 0.1 + pr + hypot + 0.1 pen -  
 " " " " " "

H267 - culture in bottle (from H262-I) 2.0 ml + 5.0 ml pen (22)

Inoculate 60 minutes.  
 Centrifuge, resuspend in H<sub>2</sub>O - mod. 3.5 ml - add 5.0 ml pen  
 incubate - 2:45 PM

partially clearing 4:00  
 " " cleared next morning.

Wg 14 pr Pen run -

4/16

Amended cultures started

1661  
1662  
1736 } 8:45 AM

Wg 14 Pin survivors  
Plated to EMB loc. - incubated

Plate 1 - > 1000  
2 - "  
3 - "

- 40 colonies picked, tested - all green  
4/19, 4/20

H267 Culture #2  
Dilute to ca  $10^7$ /ml

Plate	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-titer  
~~to EMB loc~~

EMB loc

Wg

H267 -  $10^6 \rightarrow 0.5 \pm 0.5$  1661  $\rightarrow 0.1$  2

$4 \times 10^7$

W 1177 same > 134

$> 1.3 \times 10^9$

W 67 " 0 =  $< 1 \times 10^7$

Med. of cultures - centrif. - ranged in soil - mixed 35 sec. add 20ul + 100ul pin - incubated  
after 2 hours turnid  
cleaning - W 1661 - plated on 1661 - no phages by itself  
cleaning - W 1662 - no phages on 1661  
cleaning - W 1736 -

4/17

1736  
1736 + A (0.1 ml)  
L6  
E-12

2 plates each  
4/20/  
w<sup>4</sup> colonies =  
w<sup>4</sup> colonies =

4/20  
CS  
50X  
populated  
C(?)

4/20 H267 - Prop #2 Plated on EMB-maltre

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

Med Syr (C)	Dose	Count/plate		Phage/plate (0.5ml + 0.5ml 1485) → 0.1ml	Survival
			cells killed		
23 (5)	0	461	0	3.2 = 6 (10%)	0.80
39	5	445	15	4.2 = 8 (18%)	0.97
49	10	342	119	7.2 = 14 (30%)	0.74
65 (24)	15	274	187	38.2 = 76 (16.5%)	0.60
66 (25)	20	260	201	60.2 = 120 (25%)	0.56
85 (43)	25	196	265	140.2 = 270 (59%)	0.43

4/21 W914 pr- Phe survivors - (see previous)  
 (no autotrophs among 40 colonies picked)  
 on 1 plate a pick colony - picked and retested on EMB loc  
 (a loc + W914?)

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 med - colonies picked from each med. int.  
 and streaked on EMB loc to find charact.

- 0 5/5 loc, 1/5 appears mixed upon basis of opacity.
- 5 5/5 loc, 0/5 mixed
- 10 5/5 loc, 1/5 mixed on opacity of colonies
- 15 5/5 loc, 1/5 mixed on opacity of colonies
- 20 5/5 loc, 1/5 mixed on opacity of colonies
- 25 5/5 loc, 1/5 mixed on opacity of colonies

9/22

K-12 LB Two 10ml Percutives (10ml + 10ml Pen)  
asated ~~20ml~~

Washed & susp. in saline. Incub 35 sec.

Add 10ml Pen. incubate with air -

Inc. 2:30 PM. out ca 5:00 cleaning.

Centrifuged on following morning - one appears viscous - K-12 LP (viscous) 1  
K-12 LP (non visc) 2

Continuation of Transduction of W1736 by LB of K-12

9/23

Plates with 0.1ml Pen 83 colonies (19 lact) 91 colonies (4 lact)

Plates with 0.1ml LB of K-12 90 colonies (17 lact) 70 colonies (28 lact)

4/25	1	2	123 lact
110 lact	132 lact		
152 lact	151 lact		152 lact

\* really papillae on heavy background growth.  
incubation continued.

W9 14 pr - second step -

1. All 40 colonies picked after Penicillin selection  
grow - discarded.

2. of the loc+ W9 14 pr - two colonies picked and streaked EMB loc-  
on 9/22 - 1st day distinctly loc+  
on 9/23 - 2nd day strongly loc+

#1 smooth, lustening, raised  
#2 rough, dry, odd sitting appearance

• - segregation of loc-?  
delayed fermentation?

Both transferred to agar slants for analysis  
typical colony.

3. Replicating and loc+ colonies taken in P(+) + pr + hapt } ten colonies in all  
O(0)

- all grew in supplemented and not in O(0)

- on the original streak plate - 3 colonies loc+ - appeared less + on second day  
after replication - 2 appear to be the same (solid lit w/ purple) one mixed.

one fresh type transferred to agar slants

lact #3 solid  
lact #4 mixed

opaque mutant(?) for later

Cultures started

786 - transduction repeat

1488 - incubator

1222 - 1000

loc+ #1  
loc+ #2  
loc+ #3  
loc+ #4

->  
->

4/23 Wednesday

Titration of K-12 L8-1 and K-12 L8-2

	delute $10^7 \rightarrow 0.5ml + 0.5ml$ 1985 $\rightarrow 0.1ml$	plagues/plate del.	titer
K-12 L8-1	105	$2 \cdot 10^8$	$2.1 \times 10^{10}$
K-12 L8-2	152	$2 \cdot 10^8$	$3.0 \times 10^{10}$

- Aerated culture of 1932 started 9:50 - 1.0ml + 10ml Pen. out at 1:05  
for twiff and survival - del to  $10^3$  cells/ml with.

not up here - prob. it's sampled from at 10 - 11:00 AM and really 10, 11, 12, 20

Time	EMB log <sub>10</sub> Count/plate	Phage/plate	(0.5ml + 0.5ml 1985) TSA	Survival
0	107	0	0	1.0
5	92	0	0	0.86
10	60	11x2 = 22	22/107	0.56
15	(65) <sup>over</sup> plate	24x2 = 48	48/107	-(0.41)
20	33	53x2 = 106	106/107	0.31
25	8	64x2 = 128	128/107	0.075

U.V. Resistance similar to L8-12

- 1736 transduction - in EMB loc  
1736 cells from un-aerated culture -

Plate (EMB loc)	$\lambda'$	1736 cells	4/24	4/25	4/28	no papillae	papillae approx	30 <sup>+</sup> papillae	0
1	0	0.1	no papillae	papillae approx	30 <sup>+</sup> papillae	0	0	0	0
2	0	0.1	"	"	26 <sup>+</sup>	"	"	0	0
3	0.05	0.1	"	"	189 <sup>+</sup>	"	"	186	186
4	0.1	0.1	"	"	274 <sup>+</sup>	"	"	271	271
5	0.2	0.1	"	"	372 <sup>+</sup>	"	"	361	361

galg -  
 $25 \times 10^3$  salt/ml  
 $220 \times 10^3$  /ml =  $\frac{1}{187} \lambda$

- Irradiation of twiff of 1932 - aerated culture 3 hours - delute to  $10^8$  cells/ml with saline  
Survival

Time
0
5
10
15
20
25

W1621 } aerated culture at 1:15  
W1682 }



Thursday  
4/29

(1.0 ml + 10 ml Pea)

Activated cultures of 1682 and 1831 started 9:15 AM

- Inactivation survival, and lysis of 1682 ( $\lambda^+$  882<sup>+</sup>)  
Activated cultures.

Dilute to  $10^8$  cells/ml

Time Col. Plaque (K<sub>90</sub>)

Time	Col. Plaque (K <sub>90</sub> )
0	134
5	125
10	83
15	50
20	33
25	16

Plaque on 1485 (=  $\lambda^+$  882<sup>+</sup>) (Plaque = 0.5 ml + 0.5 ml sens. cells) → 0.1 ml

on 1831 (= 882)

Survival

Survival
0
0.93
0.62
0.37
0.25
0.12

Slightly more resistant than K<sub>90</sub> 1/3  
No lysis effect observed in the guro (see below) or by plate for either  $\lambda^+$  882 -  
is  $\lambda$  really present?

- 1682 for gross lysis obs. - cells remaining from above centrifuged, resusp. in sol. - read. 3<sup>rd</sup> count. add 10 ml Pea & activate. in - 1:10 PM  
no clearing 4:00  
w clearing following morning.

- 1682<sup>+</sup> cross streaked on 1831<sup>+</sup> for the purpose of determining presence of 882 - Plaque and lysis of 1831 obs. indicating 882 present.

4/25 Friday

- Created cultures of 1485 and 1831 started 8:30  
 1- Survival of 1485 <sup>5</sup> - <sup>out at 10:45</sup> culture diluted to ca  $10^7$  cells/ml & serial

Dose	Count/plate EMBlac
0	20
5	46
10	50
15	2
20	15
25	12
30	7

This exp of no virus - presumably due to the freshness or variability of EMBlac plates used - they have found a 1/4 word in future.

- Survival and L<sub>50</sub> of 1831 <sup>1+</sup> - <sup>0.5 ml of 10<sup>7</sup> cells/ml</sup> diluted to  $10^7$  cells/ml  
 Plaque on 1485

Dose	Count/plate EMBlac	Plaque on 1485
0	63	1x2 = 2
5	41	-
10	27	-
15	5	40x2 = 80
20	3	24x2 = 48
25	5	28x2 = 56

not a 9 ml exp. can be said that under good perf.

- Streak - 1687 colonies across 1688 in order to determine if ~~is~~ present -

5 colonies picked from 0 plate of 1/14 used. none showed evidence of ability to give 1688

+ 4267 - Culture #3 diluted to  $10^7$  cells/ml - used for survival - also to attempt isolation of reconstituted exp.

Dose	Counts/EMBlac plate
0	192
5	134
10	27
15	17
20	4
25	2

universal survival curve - sensitivity

S.F.
1.0
0.70
0.14
0.10
0.02
0.01

none survived was 4-12



Tuesday 4/29

Wg 14 pr - trypt - diluted and plated out for lac+ reversions -  
 10<sup>6</sup> → plates ETMB 10<sup>6</sup> - 3/1  
 #1 ca 150 (+) → 4/30  
 #2 " " " "  
 #3 " " " "

- W1736 - read. Loeff, and diameter of plaques for presence of phage (the thin) -  
 - aerated culture, dil to ca 10<sup>7</sup> cells/ml

Date	Colonies/ETMB plate	Phage (0.5 ml to 0.5 ml 1985)
0	18	2
5	20	-
10	6	2
15	5	3
20	7	8
25	3	7
30	5	-

insufficient col count - appears odd in addition

- H267 Repeat in survival of prep #3

Date	Colonies/ETMB plate	SF.
0	296	1.0
5	168	0.57
10	106	0.36
15	92	0.31
20	22	0.07
25	4	0.01
30	6	0.02
35	2	0.007

odd? appears to check previous run with this prep re. one given - lac+ prototyp?

exc. col.	avg. col.
8	48(?)
46	74
32	46

- Penicillin runs

Wg 14 pr - trypt - → Pen aerated culture → wa. hnd → D(0) + pr + trypt aerated cult → 10<sup>6</sup> cells/tube

tube 1 - Pr + trypt + D(0) + Pen (0.2 u.c.)  
 2 " " " "

no growth apparent showed no growth - why?

Wg 16 → Pen aerated culture → wa. hnd → D(0) aerated cult → 10<sup>6</sup> cells/tube

tube 1 D(0) + Pen (0.2 u.c.)  
 2 D(0)

no growth apparent growth

W1661  
 W1662  
 W1685

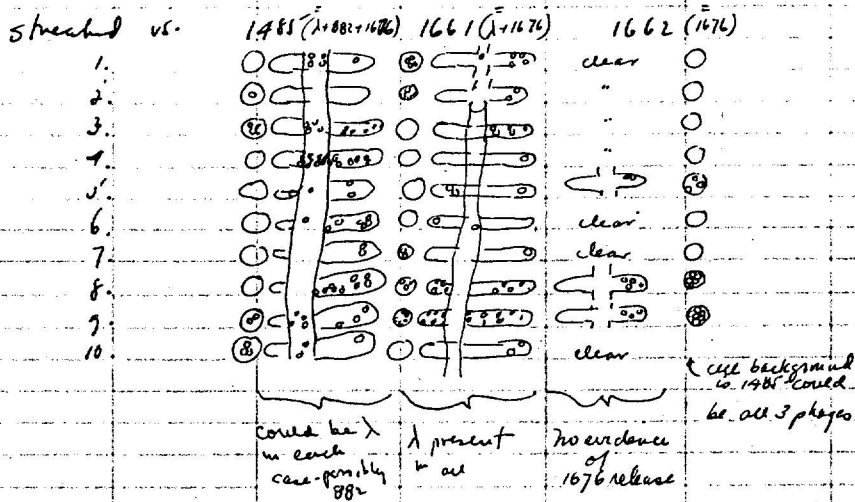
Wednesday 1/30

- Loc + populations from the lost attempted transduction of 1736  
 repeated to gap EMB

- Plate out

Wg 14 pt - trypt -	undiluted	NSA
	1-10	>
	1-100	>
		150-200 col (was something locking in her tube? - control pulled to grow)
Wg 16	undil.	16
	1-10	2
	1-100	-

- Plaques following 1736 read -  
 10 plaques per both (0.2mc) - numbered



Thursday 5/1

- titer of K-12  $\lambda$  L7  
dilute  $10^7$  plate 0.1 ml  $10^{8.2}$   $1965 \rightarrow 78 = 1.6 \times 10^{10}$
- replica Wj 16 per survivors to D(0) and 12/18 failed to grow } indicating autotrophy  
in 1/10 2/2 failed to grow }
- store Wj 17 pr - tupt - until D(0) + Supplements can be made -

- 1 from K-12 L7 + LP ported and filtered - growing around 50-60 ml  
after volume  
dilution 3, 4, 6, 7  $\frac{0.1 \text{ ml}}{7} \rightarrow 927$   
titer =  $4.3 \times 10^{10}$   
or if dilution error  $4.3 \times 10^9$

- K-12 - day del aerated culture in D(10) + lac (0.1% ml) - diluted to  $10^3$  cells/ml - mixed.

Time (hr)	Col/EMB- $\lambda$ plate	Plaque in 10 <sup>15</sup> (0.5 ml + 0.5 ml 1965)	Survival	K-12 as plaque %
0	373	-	1.0	-
5	282	-	0.76	-
10	234	-	0.63	-
15	197	89.2 = 178	0.53	0.48
20	149	-	0.40	-
25	108	138.2 = 276	0.29	0.74
30	86	-	0.23	-

- H-267 as K-12 above.

Time	Col/EMB- $\lambda$ plate	Plaque in 10 <sup>15</sup> (0.5 ml + 0.5 ml 1965)	Survival	K-12 as plaque %
0	285	-	1.0	-
5	277	-	0.97	-
15	168	-	0.59	-
25	116	86.2 = 172	0.41	0.60
35	59	116.2 = 232	0.21	0.82

Cultures  
776-982 (88%)  
1965  
H-267 from H-267 #1 OLM + lac  
K-12 in OLM + lac

Tuesday  
Saturday 5/6

- Aerated cultures 8:00 1503  $\lambda^+$   
1808 Wg 31  
1959 Wg 31  $\lambda^+$   
K-12

- K-12 Phage plating on S18 - For possible recovery of both phage and irradiated

Time	Count / EMB lac Plate	Phage / S18 plate (0.5+0.5 ml S18)	Survival cell	% K-12 as phages
0	206	-	1.0	-
5	79	-	0.38	-
10	53	58.2 = 116	0.26	0.56
15	27	55.2 = 110	0.13	0.53
20	18	68.2 = 136	0.09	0.66

- 1959 = Wg 31 ( $\lambda^+$ ) - Observation to see if  $\lambda$  is lysozyme sensitive in different strains

Time	Count / EMB lac Plate	Phage / S18 plate (0.5+0.5 ml S18)	S.F.	1959 as phages
0	216	-	1.0	-
5	163	-	0.76	-
10	102	52.2 = 104	0.47	0.48
15	8	120.2 = 240	} 0.046	1.0
20	10	107.2 = 214		1.0

- 1972 =  $\lambda^+ \Omega^+$  Examination for lysis and recombination of  $\lambda$  and  $\Omega$

Time	Count / EMB lac Plate	Phage on S18 (0.5+0.5 ml S18)	Phage on 183
0	8	-	-
5	8	-	-
10	2	18.2 = 36	-
15	0	18.2 = 36	-
20	0	11.2 = 22	0

No apparent reason for low cell no. - probably was approx 10<sup>8</sup> cells

- Transduction of 1736 - Gal

Un-aerated overnight cult of 1736 in Pen +  $\lambda$  in Gal and Lac plate = 0.05 ml 1736 added / plate.

#	EMB lac Plate	$\lambda$ added	EMB gal plate	$\lambda$ added	Populac
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	$\lambda$ alone 0.1	6	$\lambda$ alone 0.1	

- a plaque 882/1485 picked to lightly inc. aerated cult of 1503 - incubated overnight - turbid following day

- Cultures 1598 } rad. sens.  
1653 }  
K-12 } Syc  
11267 (faint) } Syc

Tuesday 5/6

- Survival of 1503 -  $10^5$  - diluted cell from original. dil to  $10^3$ /ml

<u>Days</u>	<u>CA/EMBlor plate</u>	<u>Survival</u>
0	311	1.0
5	231	0.74
15	114	0.37
30	28	0.09
45	3	0.0097



Wednesday 5/7

- W1578 1/5 Examination of u.v. resistance - Altered culture - dil. to 10<sup>2</sup> cells/ml

<u>Time</u>	<u>Col / EM18 Plate</u>	<u>Survival</u>
0	327	1.0
5	186	0.57
15	71	0.28
30	40	0.12
45	8	0.024

- W1898 1/5 Examination u.v. resistance - same as above -

<u>Time</u>	<u>Col / EM18 Plate</u>	<u>Survival</u>
0	188	1.0
5	88	0.88
15	62	0.62
30	11	0.11
45	4	0.04

- W1655 1/5 Examination u.v. resistance - as above

<u>Time</u>	<u>Col / EM18 Plate</u>	<u>Survival</u>
0	74	1.0
5	48	0.65
15	47	0.64
30	21	0.28
45	24	0.32

Dist. undisturbed

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

1485 hrs  
1736 hrs  
1177 hrs

Thursday 5/8

- Transduction of W112 loc -

EMBLose plate	1 from K-12	1 from K-12 boiled 10 min	W112 cells overnight cult.	unagitated # 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.	1 from W1177 0.1	-	0.1 ml	ca. 30+

\* ~~the~~ plaque (50+) also

Original cell. may be cont. - papillae in these plates - not really (+) some pink with zone as if contaminated C plaque and lysing

- Transduction of W1736 Gal -

EMB gal plate	1 from K-12	1 from K-12 boiled 10 min	no papillae	Gal 4-
1	-	0.1 ml	17	
2	-	0.1 ml	27	
3	0.1 ml	-	324	$3.0 \times 10^3 / \mu$
4	0.1 ml	-	345	$3.45 \times 10^3 / \mu$
5.	1 from W1177 0.2 ml	-		22.0 x 10 <sup>3</sup> / $\mu$

1 from L6  
mutant

Then plated dried up!

- 1 in 1736 } seeded cell. centrifuged - resusp in 10 ml oal.  
1177 }  
mal. 40 sec. Seed 10 ml. per - incubate with air 2:20 PM

- K-12 survivors in D(0) from previous - turbid de plaque + cell survi  
mal. of K-12?  
culture of 101 plaque picked and streaked on D(0) agar  
n 4/8 1 streaking shows good growth - incubate centrifuged  
f/9 1 .. show single colony  
2 ..

5/9 Friday -

- Transduction of 1736 on gal with  $\lambda$  from K-12 page 69

Plate	$\lambda$ dose	no gal + papillae
1	0	38
2	0	12
3	0.05ml	153
4	0.1ml	302
5	0.2ml	620

$$\frac{2.75 \times 10^3 + \text{gal}}{720 \times 10^6 \lambda/\text{ml}} = 1.3 \times 10^{-7}$$

Goal 4 -

why the diff.?

Transduction w/1736 on lactose with  $\lambda$  from K-12 page 69

Plate	$\lambda$ dose	no gal + papillae
1	0	201
2	0	171
3	0.05ml	262
4	0.1ml	344
5	0.2ml	600

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

- Plate 1 of 1736 Transd on lac - 38 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 suggests that papillae on E13 lac are different from papillae on E13 gal - that the gal transduction effect did not show up as a release of negative inhibition - against gal plus cAMP. Another papillae pick to lac to find larger substrate lac plates were discarded unfortunately -

5/10/69

Monday 5/12

- K-12 Synthetic culture #2 - del to  $10^8$  cells/ml

Time	Count/EMSA Plate	Survived
0	330	1.0
5	220	0.67
15	211	0.64
30	155	0.47
45	83	0.25

more resistant than previous

- H267 Synthetic culture #4 del. to  $10^7$  cells/ml

Time	Count/EMSA Plate	Survived
0	215	1.0
5	222	1.0
15	148	0.69
30	50	0.23
45	14	0.065

different from previous

- Tetracycline of K-12 L7+L8

100	67	} $6.8 \times 10^8$ = $1.3 \times 10^{10}$
100	68	

$0.65 + 0.5 = 1.15$

Tuesday 5/13

- Titer of 882/1485 on 1485

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

- Transduction of *Serratia plymorum* in 1678

all	2(0) plates	1678	heated $\lambda$	$\lambda$	5/14	columns around spot of deposit of ph	5/15
	1	0.1	-	-			contam.?
+pr	2	0.1	0.1	-			3 col
	3	0.1	-	0.1			3 col

\* unactivated cultures

- Titrations of L2 of 1736 - from unactivated exp. - cent. vial, used 35 etc. incubated in air overnight - nearly clear but

dil  $10^8 \rightarrow 0.5 + 0.5$  1485  $\rightarrow$  no plaques

- Transduction of 1736 on Loe and gal (done on Tuesday 5/12)

Gal of EM13 plate	1736	unactivated cult ca $5 \times 10^8$ cells/plate est.	$\lambda$ from K12C76CF	heat $\lambda$ from L6	1736 unactivated cult ca $10^8$ cells/plate est.	$\lambda$ from K12C76CF	heat $\lambda$ from L6
1	0	0	0.1	0	0	0.1	0
2	0.1	0	0	0	0.1	0	0

Papillae on Gal 1 unactivated 5/15 = 25  
 Gal 2 unactivated 5/15 = 36

Wind happens

Gal 1 activated 5/15 = 27  
 Gal 2 activated 5/15 = 425

$9.3 \times 10^8$   
 $2 \times 10^{10}$   
 $\frac{9.3 \times 10^8}{2 \times 10^{10}} = \frac{1}{21.5}$

Loe EM13 plate	unactivated 1736	activated $\lambda$ (K12)	unactivated $\lambda$ (C776F)	Papillae 5/15
1	0.1	0.1	0.1	121
2	0.1	0.1	0.1	150
3	0	0.1	0.1	182
4	0	0.2	0.2	218

Little or no effect of  $\lambda$  on unactivated cultures of 1736 - fairly large effect on activated cultures. Meaning not clear - incubation of Loe plates continued to increase size of papillae to make picking more successful.

Thursday 5/6

- transduction of W1736 Lac<sup>-</sup> - aerated culture (1.0 + 10 ml Pen) from 2 day un-aerated culture - Cells centrifuged respd in saline

(10) Plates TB <sub>2</sub>	1736 cells	head (1714)	$\lambda$	colony count
1	0.1 ml	-	-	0
2	0.1 ml	0.1	-	30 (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 Oculi to <sup>5 sec</sup> - used 5 seconds - Add 1.0 ml + 10 ml Pen for incubate with air at time 0

Time	Plate on EMB Mal	Pure Plate col	total no colonies on plate	Dark Met + colonies
Pre-10' 0'	1	10	168	5
Post-mix 0'	2	8	162	17
11:00 60'	3	29	256	14
11:30 90'	4	22	268	18
12:00 120'	5	23	302	16
12:30 180'	6	27	513	36

*Thick plate results near to air*

- Ice box cleaned up.
- Culture in vial min. of 1832 Pen<sup>r</sup> received from J.L. to inoculate re-aerated - Pen broth inoculated - } 1 h 90, 1 h U.L. 5/17
- Wg 16 autotrophs from page 68 picked to EMB plate, preliminary to determine requirements

- Get papillae of 1736 Transduction of page 74 - from aerated cult +  $\lambda$  35 picked to EMB for identification

5/17 all grew - slowly - all appear lac<sup>+</sup>, most spots are sectored light and dark (opacity) - some appear to be giving pink papillae.

W1736  
stability

- Wg 14 pr<sup>-</sup> hyst<sup>-</sup> lac<sup>+</sup>(?) col. - streaked out
- 5/16 slight pinkish
- 5/17 dark red - probably slow lac<sup>+</sup>
- slant made from single col.
- Wg 14 pr<sup>-</sup> hyst<sup>-</sup> lac<sup>+</sup>