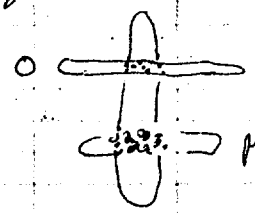
Friday 5/16

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

- Starts made of 1931 (2) for DG
  " " 1832 (?) for JL.

- Streak of plaque on W112 on 1485 from pg 75



papillae of W112 showing lytic area around on 112

pink, pop pink indicating from glac?

plaque on W112

- Papillae on 1736 lac transduction - with heated λ
  pick to lac - 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? is glu + ration

- 1678 above - Transduction

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

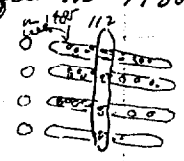
  } probably contamination - pick 1 col from each
  cross streak on 1485 to see if λ present

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

5/17 Saturday

- W1736 transduction(?)
  35 gal + pap (from page 75) picked to EMB lac - all lac⁻ (or slight).
  replica to EMB gal to check gal reaction -
  no gal plates 5/17
  replica 5/18 - 34 gal + / 35 replicated

- W112 phage(?)
  cross streak of 112 plaque on 1485 picked and cross streaked on W112



- Plaques of 882 on 1485 picked to H_2O - replated on 1485 to increase titer - by plaque growth if nothing else -
  spotted this way
  5/18 failure - only occasional plaques observed.

- H267 mad cult. in D(M) + lac of pg 75 - incubated 1 day with air ½ time (aerator broke down)
  one five day with air - stored refrig "(H267 - mad)"

5/20 ~~Monday~~ Tuesday -

- Aerated culture 1786 for retry w transduction of Lecithinase - 8:45AM
  Aerated until cent 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	✓
3	0.1	—	0.1	"	2 col contam	Same discard.
4	0.1	0.1	—	"	1 col	↓
5	0.1	0.2	—	"	0 col	✓

- Aerated culture of 1998 (λ, 28λ't) for survival and Lwoff - 1:00PM

dil to 10^3 cells/ml sol

Dose	col/Complete plate	Phage/plate (1930) (0.1ml + 1930 in phage agar)		S.F.	%1998 as Phage Plaques
0	316	0	cells+phage	1.0	0.0
5	309	—	316	0.98	—
10	300	4	309	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~~					

Survivors high - due to length of incubation?

- Aerated culture of 1972 (λ+52't) for survival and Lwoff - (plate in 1485)

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

Dose	Cells/Complete plate	Phage 1485 (0.1 mixed)	Cells+phage	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 λ in field

here also 5/21

- Aerated culture of 1988 (5't (1671)) for survival and Lwoff (plate in 1930) out 2:45 -

dil to 10^3

Dose	Cells/Complete plate	Phage (1485 in 1930)
0		—
20		

top left not turned in

Out

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

Thursday 5/22

- Aerated culture of 1788 = 518(1676) 8:30 X no parent culture contamin with bacillus?

- Wg 14 pr - trypt - Pen. rem.
  plated out on NSA
  1 undil
  2 1-5
  3 1-10
  4 1-100

replicated to D(O) + pr + trypt — no triples evident

- H267 - #2 culture used - contains about 5×10^9 cell with 5% Mal - dilute to give about 50 cells/0.1 ml $= \frac{5 \cdot 10^9}{5 \cdot 10^2} = 10^7$

  Inoculate 5 (DM) tubes - incubate with aeration.
  ↓ ——— ↓ ——→ plate out 0.1 ml on EMB mal

  Incubate —— hours. plate on EMB mal for segregants -

failed
too small no of cells

Tuesday 5/27

H267 – isolate "natural" segregants } use cultures
– isolate ~~w~~ mixed segregants }

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

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

✓ • irradiate ↓ from K-12 to obtain survival data – assay for effective-
~~?~~ use in "H-O reducing" 1736

✓ ~~try~~ Gal effect with 1662

~~make~~ ↓ ↓

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

– ✓ 1736
✓ Irradiation ↓ prep – 10 ml of L7+ LF of K-12 – irradiate – remove 1ml samples

Dose	0.1 ml ↓	0.1 ml TTC agar	Stg papillae	EMB	5/29
0	↓	T	+		275
60	↓	↓	++		c. 903
120	↓	↓	+++		¼=46 1987
180	↓	↓	++++		¼=361 295 ↓
240	↓	↓	+++++		¼=797 3/88 ↓

~~2.75 X 10⁸ ↓~~ 2/10⁻⁷
1.9 X 10¹⁰ ↓

↓ – ↓preparations in W1177 and W1736 –
cultures incubated ō on rec 16:20 – centrifuged resuspend in Sal
W1177 in 10 ml saline
W1736 in 2 ml ↓ – then to 10 ml
use in exp below as mod ↓. } mod 40 sec – incubate ō on
add 5 ml Pen
incubated 11:50
1736 cleared 300 – centrifuged, 2 disp. chloroform each
1177 – failed to clear –

↓ – Mod. ↓ – as a transducer see above

↓ Transduction in 1662 – Gal

			Stg papillae	5/29
aerated cult.	plate 1	0.1 ml treated ↓	c. 1-3	19
	2	0.1 ml – ↓	c. 50	311

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

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

↓ H267 – ~~cust~~ from #2 – dilute 5·10⁶ → inoc 0.1 into 10 ml broth + ice – ~~three~~ two tubes –

"Normal segregants"

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

+

16 (?)

	stg papillae
0.1 mod ↓	= 10
0.05 ↓	= 40
0.1 ↓	= 80
0.2 ↓	= 200

1953 Wednesday 5/28

√ – 8:15 Aerated cultures 1953, 811, 1436, 776-882, 1736

L7+L8 probably

– Transduction with λ of W 811 – Aerated cult. 4 hrs – centrifuged 10 ml, pspd in sal.

EMB gal plate	heated λ	λ	λ 240 min	811 cells	5/29	5/30 Counted 5/30
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 (plaques)

too few plates

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

261
16
1566
261
4176

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

EMB gal plate	1736 cells	K-12 heated λ	1736 λ	5/29	5/30 Counted 5/10
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 → 0.5 ml + 0.5 1485 } → 0.1 ml 22 × 2 × 10 × 10^6 = 4.4 × 10^8 poor lysate
10^8 → 0.5 ml + 0.5 1485 } → 0.1 ml = 0

smutted

√ – Irradiation of 1436 λs – Platings 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 W+ – Lwoff – Platings on EMB lac NSA – 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 lwoff of W

√ – Titration of irrad. λ

Dose	Dilution	Plaques 1485 0.5 + 0.5
0	10^8	c. 500 × 10 × 10^6 → 1.0 × 10^{10} fairly good agreement with previous assay = 1.4 × 10^{10}
240	10^7	22 × 2 × 10 × 10^7 = 4.4 × 10^9
	10^8	–

actually 10^6 ?

at dose 240 ca 30-40% survival of λ

Confusion here

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 col.	no col discard
2	—	0.1	0.1	no col.	" "

✓ — 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 = 4 > 2 > 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

ml a follows: 1736 cells/ml

EMB gal plate		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 (Counted)	5/31	galt discard	Gal⁺	Mixed
		—	—	—	—
ca 80 [illegible] (103)	119	71	38	—	
ca 70 [illegible] (81)	111	71	38	—	
ca 70 [illegible] (162)	217	71	38	98	
ca 70 [illegible] (205)	326	71	38	207	
> 200 [illegible] (16-fold)	[illegible]	71	38	3305	
ca 150 (158)	152				

219/16 = 13.69; 13.69/2 = 6.8; 6.8 + 2.5

✓ — 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
1.	0.1	—	—	0.1
2.	—	0.1	—	0.1
3.	—	—	0.1	0.1
4.	—	—	—	0.1

5/30: tan, num colonies — no papilla; tan few colonies — no papilla; " " — no papilla; " " — 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 pops) to EMB loc — 5/30 col loc 97/96 to replicate to minimal + aer if auto-hyphae

(5/27 c 275 pops)

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

to replicate

5/30 col loc 97/48 grew

Friday 5/30

- Aerated cult. 811 (BM transduction); 1821 (Kgl transduction)
- ✓ Gal + pap. 811 - picked and streaked - attempt lac transduct in 811
- ✓ W112 - purified - slant picked, streaked on EMB lac
- ✓ 1821. Kgl transduction

EMB Kgl plate	1821 cells	Boiled λ	m240 λ	5/30	6/1	6/2	6/5
1	0.1	0.1	—	no pap	no pap	no pap	1/16 = >161 pap (small)
2	0.1	—	0.1	..	no pap	>100 pap	1/16 = >310 pap (larger than)

- ✓ 811 - BM transduction

D(v) plate	811 cells +	boiled λ	m 240 λ	(1 loop) + Meth	5/31	6/1
1	0.1	0.1	—	—	no col or pap	no pap
2	0.1	0.1	—	~~0.1~~	..	no pap
3	0.1	—	0.1	—	..	no pap
4	0.1	—	0.1	+	..	no pap

Cult.
811 - for lysate pap.
are [illegible] for [illegible] pap. picked for 1662, 1736

Saturday 5/31

√ - Aerated cult for lysate of 811 - 7:00 AM
out at 7:50
centrifuge, resp'd in sol - mod 35 sec - , ~~centrifuge resp'd~~ resp'd in Pen. incubate with air.

continued pg 85 →

√ - Papilla of 1736 gal "transduction" picked to EMB lac 6/1 48 in all - all lac ~~[illegible]~~ papillating
Replica to NSA + 1662 = 1676 no reaction
D(0) = autotrophy no growth
D(0)+TB1 = leucine no growth
NSA + 811 = 882 no reaction
Gal = gal+ all +

√ - Papilla of 1662 gal "transduction" on EMB lac — all 6/1 lac- papillating
Replica to NSA + 811 = 882 no reaction
47 papilla D(0) = autotrophy no growth
45 gal+/2 gal- D(0) + TB1 = 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
1485 -
Blizzard

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

$\frac{5.5 \times 10^3}{1.9 \times 10^6} \cdot 4/10^7 \lambda$

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

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

✓	#	EMB xyl -						6/4	6/5		
	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 Sue buffer~~ ~~incubate again~~ - ~~add xyl+gal reagent~~
and plate 0.1 ml each of 4 D(o) plates
/ λ prep. -

D(o) 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
to each of two Pen tubes
incubate overnight -

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

QAR	loop	plate	titer	number/λ	λ K-12 LE	m280 λ	6/4	6/5
1		0.1	0.1	-	-	no pop	>445	
2		0.1	-	0.1	-	"	½ = >216	
3		0.1	-	-	0.1	"	¼ = >217	

Tuesday 6/3 continued

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

Lapidae 6/ 6/5	6/4	EMB gal plate	Aerated 1736 cells	Unaerated 1736 cells	brilcal 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

No. Unaerated cell – $183 \times 10^7 = 1.8 \times 10^9$

No. Aerated cell – $91 \times 10^7 = 9.1 \times 10^8$

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

titered 6/4 dil $2 \cdot 10^8$ = 64 plaques (1485) – titer = 1.3×10^{10} γ/ml

✓ – Titer of 811 lysate (filtered)

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

✓ W112 X W1655

EMS-lac	Cells (centrifuged & resusp'd twice, in sal) 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	50 col.

✓ Replication of Pen survivors of Wq 14 from several weeks ago (pg 68)

to D(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)~~

Cultures

K-12 λ prep

1821

1736 adsorption

811 gr

1485 make filtrate, try

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

$1/4\times10^{6}$

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. Papillae growing	no. gal +	# Papillae / colonies	dark
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			↑ all pure		
0.1	c.20	51					

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. Pap. growing	gal +	# Papillae / colonies	dark
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) (L) - adsorb 15 min - centrifuge - remove 0.1 ml to make 1. resuspd. adsorbent.

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

Monday

6/9 ~~Colors~~ Papillae picked from "transductions" replicad.

1821 pg 85

replica to lac, xyl, D(0)

See page 85

	no pop. picked	# growing	# gal +	# xyl −	# lac −	# aux
			gal-xyl-lac-aux			
λ treated	63	59	58	58	58	58
untreated	14	13	10	10	10	10

1736 pg 86

replica'd to lac, D(0)

see pg 86

no. pop.	picked	growing	galt	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	galt	lac −	aux
λ treated	56	28	25	28	28
control	16	16	14	14	14

see pg 86

Control plate

replicad to lac, xyl, D(0) -

see individual culture.

Tuesday 6/10

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

I - 841 gal+ - λ lysate - incubated 12:00

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

EMB gal	boiled λ	K-12 λ 28	902 10x cells	popular 6/11	6/12
1	0.1	–	0.1	0	13
2	–	0.1	0.1	0	11

I - 841 - try Sr "transduction" - 8:30 → 11:00 (aerated)

NSA plate	boiled λ (K-12)	[illegible] λ	841 10x cells
1	0.1	–	0.1
2	–	0.1	0.1

} incubate 2 hrs - 11:15 - spread with 0.15 ml strip -

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

} discard - unit of Sr too high? ✓ parent (1821) for degree of Sr

- 1485 - try filtrate of 1485 for "transduction" - centrifuge, ~~rspd~~ decant, filter - (8:30 → 11:00)

-

II - 750 - try gal "transduction"

EMB gal	boiled λ	K-12 L8 λ	750 10x cells	popular 6/11	6/12
1	0.1	–	0.1	0	0
2		0.1	0.1	230	409

$$\frac{4.09 \times 10^3}{1.4 \times 10^{10}} = 29/10^7 \lambda$$

Gal+

- 1736 try growth, adsorpt. with λ for "transduction" aerated cells 8:30 ; 11:45

1.0 ml λ K-12 L8 added - inc a room temp 30 min → 0.1 ml D(O)+BT
↓ 0.1 ml to 10 ml Pen
↓ 6/11 no colonies

λ - W112 x W1655 - 10 lac - colonies picked and streaked on EMB lac -

6/11 - 9 streakings - grew

1736

$\frac{3.2\times10^3}{1.4\times10^{10}}$ - 2+/10^3 λ

(89)

6/12 Thursday.

— W1736 - try 1485 filtrate for gal effect

EMB gal	1736 10x cells	λ lysate	heated λ	1485 filtrate	811 gal + λ	1736 gal + λ	6/12	6/14
1	0.1	—	0.1	—	—	—	0	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

— W811 - try effect of λ from 1736 gal + and 811 gal +

EMB gal	811 10x cells	λ heated	1736 gal + λ	811 gal + λ	6/13	6/14
1	0.1	0.1	—	—	c. 10	29
2	0.1	—	0.1	—	c. 5000 plages	20 (not as well developed as #1)
3	0.1	—	—	0.1	c. 15	31 (" " " " ")

confused by [illegible] = 88 1676

— Below with both 811 and 1736 using K-12λ

Results

	6/14 after 2 days		
811 adsorb - 15 min	341 - 29 = 312		
811 supernat.	69 - 29	40	Controls above ↑
total expected	= 394		
1736 adsorb	281 - 12	269	276
1736 supernat.	33 - 12	21	

10 ml cells
↓
seed
↓
resp'd in 1.0 ml

↙ ↘

mc 15 min { 0.5 ml, 1.0 ml K-12λ } | 0.5 ml

↓ ↓

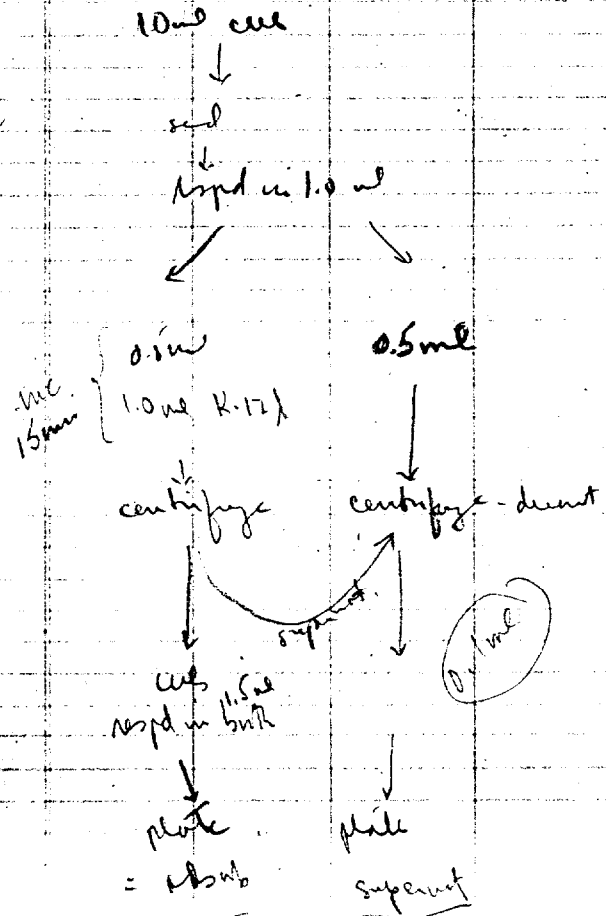
centrifuge | centrifuge - decant

↓ supernat. ↓

cells resp'd in 1.5 ml broth | 0.1 ml

↓ ↓

plate = adsorb | 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 galt (λ + 882 + 1676) / 1485

10^8 = 0

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

I - K-12 L9 / 1485

10^8 = 233 = 2.3×10^{10} λ/ml

I - 1821 λ / 1485

10^8 = 41 = 4.1×10^9

I - 811 λ / 1485

10^8 = 169 = 1.7×10^{10}

I - 811 galt λ / 1485

10^8 = 396 = 4.0×10^{10}

- 811 u.v. radiation resistance -

I -

Dose	EMBlac 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	EMBlac 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" - of pg. 87
  64 papillae pulled to gal EMB for check -
  64 grew
  64 galt (2 mixed)

- cross of W112 X 1655 - proto
  of 9 lac - col pulled and streaked - eight
  sufficiently streaked out to pick single - col -
  Tested on EMB(0) vs f-2

  8 of 7 sensitive to f-2 indicating LP2R

811 $\frac{3.0\times10^{3}}{1.9\times10^{10}} = 2/1.7 = 1/6.6\times10^{6}$

518 $\frac{2.1\times10^{4}+}{1.9\times10^{10}\lambda} = 2/10^{6}$

transduction more effective with λ also indicated with 902 λ

B. = 2.11 ... 7

B. = 1.42

132
16
792
132
2112

92

139
8
1112

Tuesday ~~[illegible]~~ Jun 17.

- 518 λ^s - "Transduction" for gal - aerated cells -

EMB Gal	518 10x cells	heated λ (K-12)	K-12 LTH λ	902 λ	6/17	6/19	
1.	0.1	0.1	–	–	0	4	4
2.	0.1	–	0.1	–	>50?	1/16 = 132	2112
3	0.1	–	–	0.1	>50?	1/8 = 139	1112

I

recorded

7 fold

- 811 λ^+ - Transduction effect with 902 gal₂ -

EMB Gal	811 10x cells	heated λ (K-12)	K-12 LTH λ	902 λ	6/17	6/19
1.	0.1	0.1	–	–	c. 45	89
2.	0.1	–	0.1	–	c. 100	1/4 = 74
3.	0.1	–	–	0.1	c. 50	202

I

296 – 89 = 207

296 wet plate - count solid may be much higher - smear in one quad

5+ fold

$\frac{1.13\times10^{3}}{4.9\times10^{10}} = 1/4.3\times10^{6}$

- 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)

$\frac{5.4\times10^{3}}{1.4\times10^{10}} = \frac{1}{10^{7}}$ $\frac{1}{3.9\times10^{6}}$

- 750 gal transduction

	EMB gal	gal⁻ 750-1 lac	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 1985 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	2
6/21			2	542	43	31	176	144	3
			Plates 1 dilut/λ	3.9×10^{6}	2.5×10^{7}	3.5×10^{7}	3.6×10^{7}	3.6×10^{7}	

- 811 - transduction by 902 λ - from 902 transduction - 72 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 lac transduction

EMBlac	10X cells (aerobic)	broth	heated K-12 λ	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

EMBlac	10X cells (aerobic)	broth	heated K-12 λ	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

- Tchatm 750 lysate

dilute 2, 4, 6-7 → 0.5 ml + 0.5 ml 1485 → 0.1 ml, 8X10^8 > 121 (wet plate) → 2.4X10^10

2. >>74

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

EMBgal	811 10X cells	broth	heated K-12 λ	K-12 λ	750 λ	papillae 6/25
1	0.1	0.1	—	—	—	50
2	↓	—	0.1	—	—	417 ←
3	↓	—	—	0.1	—	85

↑ 3.5X10^2 / >2.9X10^10 = 1/6.6X10^7

Calc - 9.6X10^3 / 1.4X10^10 = 3/3.8X10^7 = 1/1.3X10^7

and 750 effect -

- 750 Examination of 750 λ for activity -

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

Calc - 9.1X10^3 / 1.4X10^10 = 3/X10^7 = 1/3.3X10^6

Centers -
518 - Bri Rancocas, Sr.
1485 - ind.
902 - cross ē 655
655 - cross ē 902

518 Gal-

$\frac{2.9 \times 10^4}{1.4 \times 10^{10}} = 2/10^6 \lambda$

(95)

Tuesday June 24

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

EMB gal	10X accepted 811 cells	boiled K-12 λ	K-12 λ	811 λ
1	0.1	0.1	—	—
2	↓	—	0.1	—
3		—	—	0.1

popular 6/26 163 higher than previous

1/10 = 178 = 2848

86 lower because of killing due to 811 λ?

178
16
1068
78
2848

recorded

- W518 St - λ from 1821 St

NSA plate	10X heated cells	boiled λ	1821 λ	no. cl 6/26
1	0.1	0.1	—	6
2	↓	0.1	—	11
incubated 2 hours 3		—	0.1	3
4		—	0.1	6

apparently no St-duction 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
4	~~2~~	0.1

- Wednesday

1736 Transduction - for the purpose of comparing the stability of spontaneous and galduced - expt as previous.

EMB gal	heated K-12 λ	K-12 λ	6/27 no popular
1	0.1	—	34
2	—	0.1	253

$\frac{2.5 \times 10^3}{1.4 \times 10^{10}} = 2/10^7 \lambda$ Gal-

Tuesday 7/1/52 – Catching up

– From the cross – to find gal_2 – Lp_2S

902 X 1655 – 25 gal – picked, streaked once on EMB gal, single colonies streaked against λ-2 – all resist = Lp_2R
Continue to pick

– From the papillae picked from
750 – (page 93)
transduced by 902 – segregating – single colonies purified 4 streakings
811 gal+
811 gal –
1821
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 = 4 pop)
apparently no more stable than 750 parent.

– 1692 attempt production on D(o) + best + trypt.

D EMB(o)	heated K-12 λ	K-12 λ	1692 unaliquoted cells	col after 3 days	picked to EMB lac
1	0.1	–	0.1	c. 15	
2	–	0.1	0.1	c. 500	10

are failed to grow – indicating contamination

– 1920 bs 1692

1.	0.1	–	0.1	c. 0
2.	–	0.1	0.1	8

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

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

now → t 1692
– 1736 spont. gal+ pap.
10 picked
1st streaking all mixed +, – col. 4th streaking 7 mixed
2nd " 6 " " " 5th " 4 picked from mixed – all still mixed
3rd " 7 " " "

error reversed

now spontaneous
– recheck – 1736 t K-12 gal+ pap.
10 picked, 9 grew
1st streaking all mixed +, – col.
2nd " " appear pure + but not strong.
3rd " " "
4th " " " pure

of 2nd streak plates shows 9 successful streakings on spont. plate

Tuesday 7/1 - continued -

- 518t K-12 galt
  10 papillae picked
  1st streaking - all mixed +, - col
  2nd " - 6 mixed +, - col.
  3rd streaking - 6 mixed, +, or - col
  ~~5 mixed streaked 4th time~~
  4 ~~still segregating~~
  4th streaking 6 mixed,

- 518t spontaneous galt
  10 papillae picked
  1st streaking all mixed +, - col.
  2nd " " pure + col.
  3rd " " pure + col
  4th " " "

recorded

Wednesday 7/2

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

Friday - Sat July ~~4-6~~

New lysates.

- 1439 aerated cult.
  del. 10^8 = 108 plaques = ~~[illegible]~~ 1.1×10^{10}
- 892 aerated cult.
  del. 10^8 = 3 = 3×10^8
- 750 t 1821.
  del 10^8 = 65 = 6.5×10^9

750 galduction

EMB gal	no. ~~[illegible]~~	K-12 L7-8	892 λ (3.9×10^9 λ/ml)	750 t 1821 λ	no. papillae ~~[illegible]~~ 2 days
1	+	–	–	–	1
2	–	0.1	–	–	426
3	–	–	0.1	–	35
4	–	–	–	0.1	388

this lysate possibly contaminated

~~Gal~~

$\frac{4.3 \times 10^3}{1.4 \times 10^{10}} = \cancel{3/10^9}\ \lambda$

$1/3.3 \times 10^6\ \lambda$

$\frac{3.4 \times 10^2}{3.9 \times 10^9} = \times 10^6$

Summary of pactophyllus picked from cross 902 X 1655

Picking	Lp_2R	Lp_2S
9	9	
16	16	–
37	30	7
62	55	7

Lp_2^r gal - X Lp_2^s gal + → 7/62 Lp_2^s gal -

7 Lp_2S streaked EMB gal for purification 4 Lp_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 added	K-12 λ ^{49} = 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

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

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

- 750, 811, 518, 1736 } Make $\lambda p2R$ and examine for transduction.

cross streaked with λ-2 - single colonies picked and streaked, restreaked - restreaked.

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

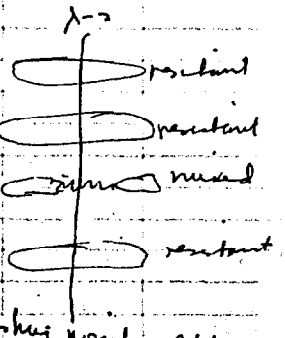
Making $\lambda p2R$

EMB gal	10^{10} cells added	additions	no papillae (20 days)
1	750	—	1
2	750	K-12 λ	2
3	811	—	50
4	811	K-12 λ	57
5	518	—	69
6	518	K-12 λ	—
7	1736	—	7
8	1736	K-12 λ	15

} not $\lambda p2R$ - still mixed

caused disappearance of gal deletion effect

Streaked some of resistant colonies to check purity of $\lambda p2R$



interesting point - although this culture is gal- the $\lambda p2R$ from (opaque) is able to grow large quantities of polysacch. from gal - gives

Streaking of gal+ papillae from gal deletions - 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 " " " "

Gal+

$\frac{3 \times 10^9}{2.9 \times 10^{10}} = \frac{x}{10^7}$

1578 gal+ F-518 (?) check.

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

← 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 -
  811tK-12 } - mixed, predominately gal+

Prototrophs

1. 33 gal+
   8 gal -
2. 40 gal+
   6 gal -
3. control platings of 1436, 811tK-12 showed no growth

41/16/18

carried to next page

73/14 = 5/1

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

1. 38 gal+
   c.60x16 = 960 gal-
2. 48 gal+
   c.55x16 = 880 gal-
3. control platings of 1436, 518tK-12 showed no growth

carried to next page

1840/86 = 21/1

(recorded)

- 1655X902 - Selection and purification of gal_2-Lp_2R and gal_2-Lp_2S

Lp_2R -	EMB gal	10X cells	heated K-12 λ	K-12 λ	902 λ	no pop 3 days
	1	0.1	0.1	-	-	14 (contam?)
	2	↓	-	0.1	-	14
	3	↓	-	-	0.1	8

tested at this time of use and found a reorder

← suggestion of plaques on all - clear, small punched out.

Lp_2S	EMB gal	10X cells	heated K-12 λ	K-12 λ	902 λ	no pop 3 days
	1	0.1	0.1	-	-	20
	2	↓	-	0.1	-	1/4:89 = 356
	3	↓	-	-	0.1	10

← suggestion of plaques here also

$\frac{3.4\times10^3}{1.4\times10^{10}} = \frac{1}{4.2\times10^6}$

~~$\frac{3.6\times10^3}{1.4\times10^{10}}$ = 3/10^7 Gal₂~~

Received from EMC - 1692X1402 - to obtain (gal-pro- lac+)
to pick and streak 3 ~~[illegible]~~ spotting marked (yellow circles)
this is on EMB lac and spotting are lac - how?

2062 ~~2053~~

2/3 grew

maybe lac slight and faded on plates

Sunday 7/13
1690X1402 restreaked on EMB gal -

2065 ~~2053~~ =

2/3 grew