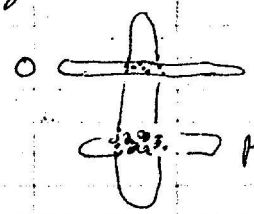
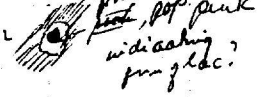
Friday 5/16

- Transduction 1678 (ser a glyc) SEE BELOW
  K-ir lysate on D(0).
  3 colonies on 1 plate transferred to EMB blue for check
  1(?) col. on control plate transferred
- Slants made of 1931 (2) for DG
  " " of 1932 (?) for JL.
- Streak of plaque on W112 on 1485 from pg 75





- Papillae on 1736 lac 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+ in 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)

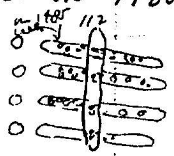
probably contamination - pick 2 col from each cross streak on 1485 to see if 1 present

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

5/17 Saturday

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

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



√ - Plaques of 112 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 1/2 time (aerator broke down) one five day with air - stored as "(H267-mad)"

Tuesday
5/20 ~~Monday~~ -

- Aerated culture 1786 for retry on transduction of Leucine - 8:45AM
  Aerated wet cont at 12:00 - set until 2:30 - Centrif., respd in saline 10X

TB1 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	"	30 col. contam	
4	0.1	0.1	—	"	1 col	
5	0.1	0.2	—	"	0 col	↓

- Aerated culture of "1998" ($λ^+$ ~~[illegible]~~) for survival and Lwoff - 1:00PM

dil to 10^3 cells/ml sol

Dose	col/EMBlac Plate	Phage/plate (1930) (0.1 ml + 1930 in phage agar)	total cells + phage	S.F.	% 1998 as Phage Plaques
0	316	0		1.0	0.0
5	309	—	316	0.98	—
10	300	9	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~~					

Survivals high - due to length of incubation?

- Aerated culture of 1972 ($λ^+ Ω^+$) for survival and Lwoff - (plate on 1485)
  dil. to 10^3 cells/ml — 1:00PM

Dose	Cells/EMBlac plate	Phage 1485 (0.1 paired)	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

here also

5/21
5 large plaques
5 med. plaques
5 small plaques } picked and streaked against 1736 - no evidence of lysis. only λ in yield

- Aerated culture of 1988 (S[illegible] (1675)) for survival and Lwoff (plate on 1930) - out 2:45
  dil to 10^3

Dose	Cells/EMBlac plate	Phage (1485) (pair [illegible])
0		
20		

Out

light left on turned

5/21
- Wednesday -
  W 14 pr - trypt - from cult in D(0) + pr + trypt to
  D(0) + pr + trypt + 0.2 penicillin }
  D(0) " " }

Thursday 5/22

- Aerated culture of 1788 = 578(1676) 8:30 / no parent colton contamin with bacillus?

- Wq 14 pr - trypt - Pen. res.
  plated out on NSA

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

replicad to D(O)+ pr + trypt — no trypts

- H267 - #2 culture used - contains about 5x10^7 cell with 5% Mal^-
  dilute to give about 50 cell /0.1 ml $= \frac{5 \cdot 10^7}{5 \cdot 10^2} = 10^5$

  Inoculate 5 (BM) 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 uv-induced segregants }

✓ • irradiate 1736 } to make high titred λ preparations
1177 }

out • irradiate 1788 to see if any effect in possible with (1676) culture contaminated

✓ • irradiate λ from K-12 to obtain survival data – assay for effective – dose in "transducing" 1736

✓ ~~repeat~~ try gal effect with 1662

~~make a~~

– Cultures at 8:20 of 1788, 1736, 1177, 1662 (above –)

✓ 1736 Irradiation of λ prep – 10 ml of L7+CF of K-12 – Irradiate – remove 1 ml samples

Dose	0.1 ml λ	0.1 ml 1736 cell	5/28 EMB papillae	5/29	
0	+	+	+	275	← $\frac{2.75 \times 10^3}{1.4 \times 10^{10}}$ λ = 2/10⁻⁷
60	+	+	++	c. 903	
120	+	+	+++	½: 446 1987	
180	+	+	++++	½: 361 2952	
240	+	+	+++++	½: 797 3188	

✓ – λ preparations in W1177 and W1736 –
cultures incubated ō air till 11:20 – centrifuged resuspend in sal
W1177 in 10 ml saline
W1736 in 2 ml, – then to 10 ml } irrad 40 sec – incubate ō air
use in exp below an irrad λ. add 10 ml Pen
incubated 11:50
1736 cleared 300 – centrifuged, 2 drops chloroform added
1177 – failed to clear –

✓ – Irrad. λ – as a transducer see above

✓ Transduction in 1662 – Gal

			5/28 papillae	5/29	
aerated cult.	plate 1	0.1 ml water, λ	c. 1–3	19	} gal effect in 1662 similar to that in 1736 also similar efficiency
	2	0.1 ml – λ	c. 50	311	$\frac{2.9 \times 10^3}{1.4 \times 10^{10}}$ = 2/10⁻⁷

✓ H267 – cult from #2 – dilute 5·10⁸ → inoc 0.1 into conc. day these – two tubes –

"normal segregants"

plated 1 very small (1–5)
2 number of colonies

5/28

166(?)		approx.
0.1 heat λ	=	10
0.05 λ	=	40
0.1 λ	=	80
0.2 λ	=	200

1953 Wednesday 5/28

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

(marginal notes: dilute with phage sol W phage resistant act; read sens. λ = phage ppt in plaque with pps = transduction with λ from 1736; L7+L8 probably)

- Transduction with λ of W 811 - aerated cult. 4hrs - centrifuged resusp. in sal. 10ml

EMB gal plate	heated λ	λ	λ uv 240 min	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 (plaques) (to 261)

too few plates

$\frac{5.35 \times 10^3}{1.4 \times 10^{10}}$ (3/10⁷ λ)

- Transduction of 1736 with lysate of 1736 - Titration of 1736 lysate

Smeared

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 $10^6 \rightarrow$ 0.5ml + 0.5 1485 } → 0.1ml $22 \times 2 \times 10 \times 10^6 = 1.4 \times 10^8$ poor lysate
$10^8 \rightarrow$ 0.5ml + 0.5 1485 } → 0.1ml = 0

✓ - Irradiation of 1436 F^- - 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 wt - Lysog. - Plating 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 lysog. of w

✓ - Titration of irrad. λ

Dose	Dilution	Plaques 1485 0.5+0.5
0	10^8 (actually 10^6)	c. 500 · 2 × 10 × 10^6 → 1.0×10^{10}
240	10^8	$22 \times 2 \times 10 \times 10^8 = 4.4 \times 10^9$
	108	—

fairly good agreement with previous assay = 1.4×10^{10}

at dose 240, ca 30-40% survival of λ

confusion here

Aerated cult. 8:20 SB-161, 1736 gal+, 1736, 1439, 811

Thursday 5/29

✓ — SB-161 Transduction with irradiated λ.

D(0) plate	heated λ	w240 λ	SB-161 cells	6/1	6/5
1	0.1	—	0.1	no col.	no col discard
2	—	0.1	0.1	no col.	" "

✓ — 1736 gal+ — "transduction" for lac —

EMB lac plate	1736 gal+ cells	heated λ	K-12 λ	w240 λ	counted 5/30	6/1
1	0.1	0.1	—	—	c. pap (ξ = 228)	
2	0.1	—	0.1	—	c. pap (ξ = 213)	ca
3	0.1	—	—	0.1	> pap (ξ = 225)	the
4	0.1	—	—	—	c pap (ξ = 266)	same
					(1 = 2 = 3 = 4)	discard

1 > 4 = 2 > 3.

✓ — Reconstruction exp. Detection of gal+ in presence of large number of gal−

1. dil. gal+ 1736 to contain ca 2000 cell/ml — $\frac{10^9}{2 \cdot 10^3}$ = 5 × 10^5 → plate 0.1 EMB gal (= ca 200 col)

1a. 0.5 ml 10^10/ml 1736 gal− → plate 0.1

ml a follows

EMB gal plate	1736 cell/ml	heated λ	K-12 λ	w240 λ	Est. 5/30 (counted)	5/31	gal+ desired	5 plat	induced
1	0.1	—	—	—	ca 80 pap (103)	119	71	38	—
2.	0.1	0.1	—	—	ca 70 pap (81)	111	71	38	—
3.	0.1	—	0.05	—	ca 70 pap (164)	217	71	38	98
4.	0.1	—	0.10	—	ca 70 pap (205)	326	71	38	207
5.	0.1	—	—	0.1	300 pap (1/16 = 191)	[illegible]	71	38	3305
6.	—	—	—	—	ca 150 (154)	152			

219 / 16 / 1284 / 219 / 3723

✓ W811 — λ prep. aerated cult. dropped in sol — read 35 sec
incubated c air 11:30
first — discard by accident

W-1439 transduction

EMB gal plate	heated λ	K-12 λ	w240 λ	1439 cells	5/31	6/1
1.	0.1	—	—	0.1	± acid, few [illegible]	no papilla
2.	—	0.1	—	0.1	± acid, few [illegible]	no papilla
3.	—	—	0.1	0.1	" "	no papilla
4.	—	—	—	0.1	"	no papilla

apparently plate was too wet.

✓ W-1736 gal "transduction" — 5/27 48 + pap picked from untreated λ plate (0 dose of 5/27 ≈ 275 pap.) (pg 80)
to EMB lac — & to replicate to minimal to see if auxotrophic
5/30 all lac

irradiated λ expt pg 80

✓ W-1662 gal+ "transduction" — 48 + pap picked from 1662 + λ plate (page 80) to EMB lac
to replicate
5/30 all lac(w) 47/48 grew

Friday 5/30

- Assorted cult. 811, 1821 (// BM transduction; // Hyl transduction)
- ✓ - Gal + pap. 811 - picked and streaked - attempt lac transduct in 811
- ✓ - W112 - purified - clones picked, streaked on EMB lac
- ✓ - 1821. Hyl transduction

EMB Hyl 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	t_c = >161 pap (small)
2	0.1	—	0.1	..	no pap	>100 pap	t_c = >310 pap (larger than)

- ✓ - 811 - BM transduction

D(c) plate	811 cells +	boiled λ	m240 λ	(1 loop) + Meth	5/31	6/1
1	0.1	0.1	—	—	no col or pap	no pap
2	0.1	0.1	—	~~+~~	..	no pap
3	0.1	—	0.1	—	..	no pap
4	0.1	—	0.1	+	..	no pap

Cult.
811 — for lysate prep.
[illegible] of pap picked from 1662, 1776

Saturday 5/31

✓ - Aerated cult for lysate of 81 - 7:00 am
out at 7:50
centrifuge, resp'd in sol - irrad 35 sec - , ~~centrifuge~~ resp'd in Pen. incubate with air.

continued pg 85 →

✓ - Papilla of 1736 gal "transduction" picked to EMB lac, 48 in all - all lac ~~[illegible]~~ papillating 6/1
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 "transduction" on EMB lac — all lac papillating 6/1
Replica to
47 papilla
45 gal+/2 gal-

NSA + 811	=	882	no reaction
D(0)	=	autotrophy	no growth
D(0)+TB$_1$	=	leucine	no growth
gal	-	gal	

Sunday 6/1

✓ - Centrifuged 811 ~~X~~ 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 X 112
- 112 - cross X 1655, also "transduct"
- 1736 - ✓ aeration detail, lysate
- 1821 - ✓ for gal g^+ "transduct" -
- 1485 -
- ~~B M gal~~

$\frac{5.5 \times 10^3}{1.4 \times 10^{10}}$ · 4/10^7 λ

Tuesday - 6/3

Aerated cultures - 112 (xyl lac), 1736 (aeration detail), 1821 (xyl gal, xyl, M), 1485 (adsorption of λ)

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

✓ 1. EMB gal -

	boiled λ	K-12 Lf λ	811 λ	1821 cells	Pen broth	6/4	6/5	6/7	no papillae pulled	gal	gal +	✓ aux	xyl (2)	
1.	0.1	-	-	0.1	0.1	no pap.	no pap.	30	14	13	10	10	10	
2.	-	0.1	-	0.1	0.1	no pap.	>30 pap?	581	67	63	57	57	57	57
3.	-	-	0.2	0.1	-	no pap.	no pap.	36						

✓ 2. EMB xyl -

	boiled λ	K-12 Lf λ	811 λ	1821 cells	Pen broth	6/4	6/5	6/7
1.	0.1	-	-	0.1	0.1	3 pap.	pap?	46 (>1000 minute pap)
2.	-	0.1	-	0.1	0.1	"	>5 pap?	55 (>1000 " ")
3.	-	-	0.2	0.1	-	"	pap?	53 (>1000 " ")

✓ 3. BM - 0.5 ml cells + 0.5 ml λ (boiled) } ~~incubate~~ incubate 10 min - ~~add 5 ml broth and~~
- 0.5 ml cells + 0.5 ml λ (K-12 Lf) } ~~incubate~~ - ~~centrifuge~~

and plate 0.1 on each of 2 D(o) plates / λ prep. ↓ add 0.1 ml from each to each of two Pen tubes incubate overnight -

D(o) plate	boiled λ	K-12 λ	6/4	6/5	6/7
1	+	-	no col.	no col	no col
~~2~~	~~+~~		-	-	
2	-	+	no col.	no col	no col
~~4~~	~~-~~	~~+~~		-	

✓ - W112 Aerated culture - centrifuged + resuspd in 1.0 ml sal

OMR lac plate	cells	boiled λ	λ K-12 Lf	W290 λ	6/4	6/5
1	0.1	0.1	-	-	no pap.	>445
2	0.1	-	0.1	-	"	½ = >216
3	0.1	-	-	0.1	"	½ = >172

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

~~topplac~~ 6/1	6/5 6/4	EMB gal plate	Aerated 1736 cells	Unaerated 1736 cells	boiled 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 cells – $183 \times 10^7 = 1.8 \times 10^9$

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

✓ — 1821 – lysate – aerated cult – ca 20 ml – centrifuged, resuspd in sal
mixed 35 sec – centrifuged and resuspd in [illegible] – aerated 1:20 FM

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

✓ – Titer of 811 lysate (filtered)
dil 10^7 ~~[illegible]~~ → 0.1 ml on plate + 0.1 ml 10X 1485 – $112 \times 10 \times 10^7 = 1.1 \times 10^{10}$

✓ W112 X W1655 cells (centrifuged & resuspd 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.

✓ Replication of Pen survivors of W4 14 from several weeks ago (p 8 68)
to D(1) + gr + 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 [illegible] filtrate, try

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

811 $\frac{3.5\times10^{5}}{1.9\times10^{12}}$ 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. picked	no. growing	no. gal +	# lac-	ans
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-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 +	lac-	ans
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.10⁹ cells/ml – incubate 15 min. – 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 adsorbed λ + 0.1 ml fresh 1736 cells
2. 0.1 ml resuspend + 0.1 ml gal

Monday

6/9 ~~Colon~~ Papillae picked from "transductions" replica'd.

1821 pg 85

replica to lac, xyl, D(0)

See page 85

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

(gal⁻ xyl⁻ lac⁻ aux)

1736 pg 86

replica'd to lac, D(0)

see pg 86

	no. pap. picked	growing	gal⁺	lac⁻	aux
λ treated	56	52	47	52	52
control	8	7	5	5	5

811 pg 86

replica'd to lac, (D0) -

	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 cultures. 91

Tuesday 6/10

I - 1736 gal+ - λ lysate (8:30 → 11:00 aerated) - centrifuge, suspend sed, irrad 30 sec., add 10ml Pen. inc @ 11:45

I - 811 gal+ - λ lysate - incubated 12:00

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

EMB gal	boiled λ	K-12 λ 28	902 10X cells	papillae 6/11	6/12
1	0.1	—	0.1	0	13
2	—	0.1	0.1	0	11

I - 811 - try S^r transduction - 8:30 → 11:00 (aerated)

NSA plate	boiled λ (K-12)	[illegible] λ	811 10X cells
1	0.1	—	0.1
2	—	0.1	0.1

} incubate 2 hrs - 1:15 - respread with 0.15ml strep - colonies

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

discard - out of S^r too high? parent (811) for degree of S^r

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

-

I - 750 - try gal transduction

EMB gal	boiled λ	K-12 28 λ	750 10X cells	papillae 6/11	6/12
1	0.1	—	0.1	0	0
2		0.1	0.1	230	409

Gal+

$\frac{4.09 \times 10^{2}}{1.4 \times 10^{10}} = 3/10^{7}$ λ

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

1.0 ml λ K-12 C8 added - inc a room temp 30 min → 0.1 ml D(O)+AT → 6/11 no colonies

↓ 0.1 ml to 10ml Pen

I - W112 X W1655 - 10 lac- colonies picked and streaked on EMB lac -

6/11 - 9 streakings grew

1736
$\frac{3.2 \times 10^3}{1.4 \times 10^{10}} - 2^+/10^7\ \lambda$

6/12 Thursday.

— W1736 — try 1485 filtrate for gal effect

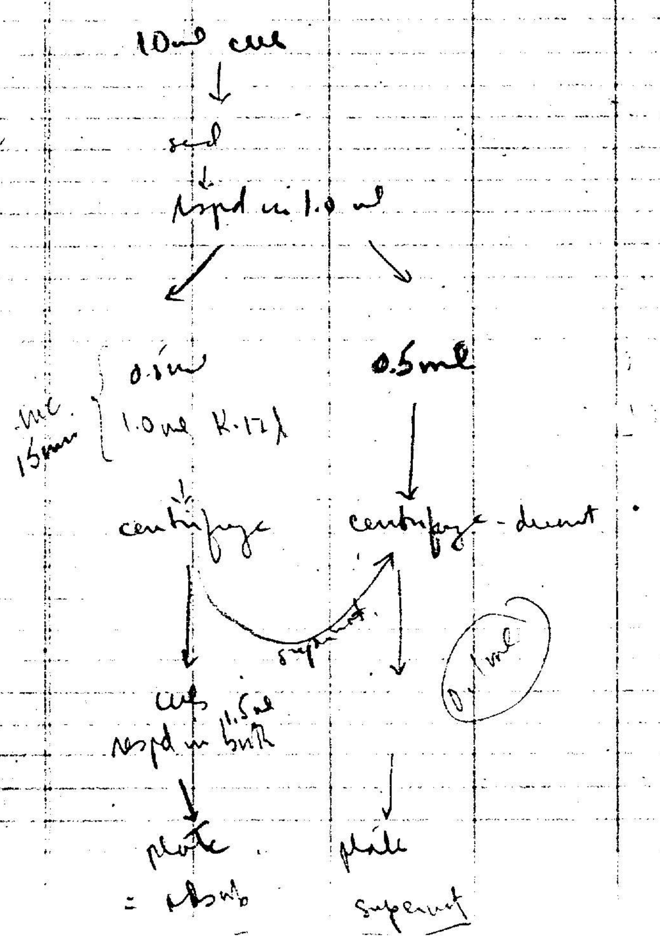
EMB gal	10x 1736 cells	λ hybrid	K-12 λ	1485 filtrate	811 gal + λ	1736 gal + λ	6/13	6/14
1	0.1	—	0.1	—	—	—	0	12
2	0.1	0.1	—	—	—	—	c. 20	321
3	0.1	—	—	0.1	—	—	1-2?	6
4	0.1	—	—	—	0.1	—	c. 50	191
5	0.1	—	—	—	—	0.1	[illegible]	19

— W811 — try effect of λ from 1736 gal + cure 811 gal+

EMB gal	10x 811 cells	λ (K-12)	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 plaques	20 (not as well developed as #1)
3	0.1	—	—	0.1	c. 15	31 (" " " " ")

compared with gal ... = 88 ... 676

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



Result:

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

Controls above ↑

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 large plaques } this prep (see pg. 88) gave ca. 5000 small
c. 10^4 small " , probably λ } plaques on 811

I - K-12 49 /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 gal + λ /1485
10^8 = 396 = 4.0×10^{10}

I - 811 u.v. radiation resistance -

Dose	Effloc 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	Effloc 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 picked to gal EMB for check -
  64 grew
  62 galt (2 mixed)

I

- cross of W112 x 1655 - proto of 9 lac- col picked and streaked - light sufficiently streaked out to pick single - col.
  Tested on EMB(o) vs λ-2

I

  8 of 9 sensitive to λ-2 indicating λ2^R

811: $\frac{3.0 \times 10^3}{1.9 \times 10^{10}} = 1/6.6 \times 10^6$

518: $\frac{2.1 \times 10^4}{1.9 \times 10^{10}}$ λ $= 2/10^6$

transduction more effective with λ also indicated with 902 λ

132 / 16 / 792 / 132 / 2112

(92)

139 / 8 / 1112

Tuesday ~~[illegible]~~ Jun 17.

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

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

7 fold

recorded

I

– 811 λ^+ – "transduction" effect with 902 gal₂ –

EMBGal	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 → 296
3.	0.1	–	–	0.1	c. 50	202

296 wet plate – count small may be much higher – smear in one quad

296 − 89 = 207

291 — 5+ 3 fold

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

I

– 902 λ – titer

I $10^7 \to 0.1$ ml + 0.1 518 cells (13X) → >50 – means titer around 10^{10}

poor plates – too wet!

I 58-161 λ – titer

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

poor plate also – wet

Thursday June 19

- 750 from papillae examination of pg 87-88
  1 picking streaked on EMB gal to purify · for back cross – (impossible w TLB, gal)

- W811 – stock streaked out – to pick single colony – lower background of (gal+) if possible –

- W811 gal+ "transduct" of previous page –
  1 picked and streaked on EMB gal to purify – backcross

- W750 gal+ transduct
  64 pap on gal – replica'd to lac, D(o)
  64 gal+
  64 lac-
  64 failed to grow on D(o)

$\frac{5.4 \times 10^3}{1.4 \times 10^{10}}$ = [illegible] 1/3.9x10⁶

- 750 gal trans. effect

EMB gal	gal₁ 750-10⁹ [illegible]	0 heat λ	gal+ 1.4x10¹⁰ K-12λ-LT+	gal₁- 1.7x10¹⁰ 811 λ	gal₄- 1.0x10¹⁰ 1824 λ	gal₂- 4.9x10¹⁰ 902 λ	Spontaneous Reversion gal₄- 4x10¹⁰ #1 811 gal+ λ	gal+ 0 1485 filtrate
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
		Rates 1 duct/λ	3.9x10⁶	2.5x10⁹	3.5x10⁷	3.6x10⁷	3.6x10⁷	

- 811 – transduction by 902 λ –
  from 902 transduction – 72 pap picked to EMB gal –
  71 gal+, 72 lac⁻, 72 failed to grow D(o)

- 598 – transduction on gal
  49 pap. picked from K-12 λ – plate –, 49 gal+ 49, lac⁻, 49 failed to grow D(o)
  56 pap " " 902 λ – plate –, 56 gal+, 56 lac⁻, 56 failed to grow D(o)

(recorded)

58-161 λ
dil $10^7 \to$ 0.1 ml poured/1485 = 18 = 1.8×10^9

902 λ
dil $10^7 \to$ 0.1 ml poured/1485 = 49 = 4.9×10^9

1824 λ
dil $10^7 \to$ 0.1 ml poured/1485 = 101 = 1.0×10^{10}

Monday June 23

- 1655 X 112 - Lp2R - Attempt lac transduction

EMBloc	10X cells (anaks)	broth	heated K_{λ}^{12}	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 Lp25 — Background too high —!

- 1655X112 - Lp25 - as above

EMBloc	10X cells (anaks)	broth	heated K_{λ}^{12}	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 / 8 x 10^8 > 121 (wet plate) → 2.4 X 10^10

2. >> 74

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

EMBjar	811 10X cells	broth	heated K_{λ}^{12}	750 λ	papillae 6/25
1	0.1	0.1	—	—	50
2	↓	—	0.1	—	417 ←
3	↓	—	—	0.1	85

↑ 3.5 X 10^2

> 2.9 X 10^10 = 1/6.6 X 10^7

$\frac{9.5 \times 10^3}{1.4 \times 10^{10}} = \frac{3}{3.8 \times 10^6}$

Gal q -

and 750 lysate -

- 750 Examination of 750 λ for activity -

EMBjar	750 cells	broth	heated λ	K-12 λ	750 λ	papillae 6/25
1	0.1	0.1	—	—	—	2
2	↓	—	0.1	—	405 ←	
3	↓	—	—	0.1	2	

$\frac{9.1 \times 10^3}{1.4 \times 10^{10}} = \frac{3}{3.3 \times 10^6}$

Gal 1 -

Centers -

518 - BA transduct, Sr-

1485 - ind.

902 - cross ē 655

655 - cross ē 902

(95)

Tuesday June 29

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

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

proplica 6/26 — higher than previous
163 ←
1/11 = 178 = 2848
86 ↖ lower because of killing due to 811 λ

178 / 16 / 1068 / 78 / 2848

Gal+

$\frac{5 \times 10^{8}}{2.9 \times 10^{9}} \times \frac{1}{1.4 \times 10^{10}} = 2/10^{6}$ λ

- W518 S^{r}-

NSA plate	1 λ 10x aerated cells	1821 boiled λ	1821 λ	no. of 6/26
1	0.1	0.1	—	6
2	↓	0.1	—	11
3 (incubated 2 hours)		—	0.1	3
4		—	0.1	6

apparently no S^{r}-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
4	↓	0.1

- Wednesday

T36 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. of plaques
1	0.1	—	34
2	—	0.1	253

Gal+

$\frac{2.5 \times 10^{3}}{1.9 \times 10^{6}} = 2/10^{3}$ λ

Tuesday 7/1/52 - Catching up

- From the cross - to find gal₂ - λp_2 S
  902 X 1655 - 25 gal - picked, streaked once on EMB gal, single colonies streaked against λ-2 - all resist = λp_2^R
  Continue to pick

- From the papillae picked from 750 - (page 93)
  transduced by 902 - segregating - single colonies purified 4 streakings
  811 gal+
  811 gal- still segregating - stocks made -
  1021 of 750t 902 both gal+ and gal- stock

  750 t 902 gal -
  spread on EMB gal - after 2 days 3 papillae (after 3 = 4 pap)
  apparently no more stable than 750 parent.

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

D EMB(o)	heated K-12	K-12 λ	undiluted 1692	col after 3 days	Picked to EMB lac
1	0.1	–	0.1	c. 15	
2	–	0.1	0.1	c. 500	10 all failed to grow - indicating contamination

- 1920 6s 1692

1.	0.1	–	0.1	c. 0
2.	–	0.1	0.1	8

- 750 t K-12
  10 gal+ papillae picked to EMB gal
  1st streaking - all mixed +, - col.
  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

- 811 t 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 (S 1692) gal+ pap.
  10 picked
  1st streaking all mixed +, - col. 4th streaking 7 mixed
  2nd " 6 " " 5th 4 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

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

Tuesday 7/1 - continued -

- 518t K-12 gal+
  10 papillae picked
  1st streaking - all mixed +, - col
  2nd " - 6 mixed +, - col.

2nd streaking - 6 mixed, +, - col
~~5 mixed streaked 4th time~~
4 ~~still segregating~~
4th streaking, 6 mixed,

- 518t spontaneous gal+
  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.
  dil. 10^8 = 108 plaques = ~~III~~ 1.1×10^{10}
- 892 aerated cult.
  dil. 10^8 = 3 = 3×10^8
- 750t1821.
  dil 10^8 = 65 = 6.5×10^9

this lysate possibly contaminated

750 galdurchen

EMB gal	no seed	K12L78	892 λ (3.9×10^9 λ/ml)	750t1821 λ	20. ~~[illegible]~~ papillae 2 days
1	+	—	—	—	.1
2	—	0.1	—	—	426
3	—	—	0.1	—	35
4	—	—	—	0.1	(388)

~~Gal₁~~

$\frac{4.3 \times 10^3}{1.4 \times 10^{10}} = \frac{3}{10^7}$ λ ; $1/3.3 \times 10^6$ λ

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

Summary of prototrophs picked from cross 902 X 1655

Picking	Lp₂R	Lp₂S
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 Lp₂S streaked EMB gal for purification. 4 Lp₂R picked likewise

Thursday - July 10 - Catching up.

Gal2 – Lp2S – motility – from 892 X 1655

– 2050 – Streaked cultures –

EMB gal	10X Cells	K12 λ	892 λ
1	0.1	–	0.1
2	0.1	0.1	–

$\lambda^{LY} = 2.3 \times 10^{13}$

no papillae after 2 days

$\frac{1.414 \times 10^4}{2.3 \times 10^{10}} = \frac{1}{1.6 \times 10^6}$

½ = 227 = 851

1/16 = 123 = 1968

Recheck to obtain better estimate of background – 892 λ appears susp. high – virus activity.

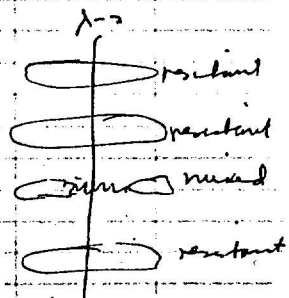
– 750 } Make Lp2R and check for transduction.
– 811 }
– 518 } cross streaked with λ-2 – single colonies picked
– 1736 } and streaked, as treated – retested –

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

Making Lp2R causes disappearance of gal ductin effect – streak made of colonies to be rechecked for Lp2R

EMB gal	10X Cells added	additions	no papillae (colonies)
1	750	–	1
2	850	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 Lp2R – still mixed



interesting point – At this time culture is gal– the Lp2R (opaque) is able to synth. large quantities of polysacch. from gal – guess

Streaking for papillae from gal ductin – 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+

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

$\frac{3 \times 10^4}{2.3 \times 10^{10}} = \frac{x}{10^7}$

← 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+

  Prototrophs

  1. 33 gal +
     8 gal -

  2. 40 gal +
     6 gal -

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

  carried to next page

  (73/14 = 5/1)

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

  carried to next page

  (1840/86 = 21/1)

- 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 pap 3 days
1	0.1	0.1	—	—	14 (contam?)
2	↓	—	0.1	—	14 ← suggestion of plaques
3	↓	—	—	0.1	8 ← in all - clear, small punched out

tested at this time for use and found a reminder → Lp_2S

Lp_2S EMB gal	10X cells	heated K-12 λ	K-12 λ	902 λ	no pap 3 days
1	0.1	0.1	—	—	20 ←
2	↓	—	0.1	—	1/4:89: 356 ← suggesting plaques here also
3	↓	—	—	0.1	10 ←

$\frac{3.4X10^3}{1.4X10^{10}} = \frac{1}{4.2X10^6}$

~~$\frac{3.6X10^3}{1.4X10^{10}}$~~ ~~1/10^7~~ Gal_2 -

Received from EML - 1692X140² - to obtain (gal- pro- lac+)
to pick and streak 3 ~~[illegible]~~ spottings marked (yellow circle)
this is on EMB lac and spottings are lac - how?
maybe lac oh left and faded on plates

~~2055~~ 2062 "

2/3 grew

Sunday 7/13

169tK140² restreaked on EMB gal -

~~2055~~ 2060 =

2/3 grew