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

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

1831 + 882 of previous page.
Both N and P picked to water - streaked out on EMB-0 for colony isolation - o.k.

Colony inhibition? forms
dark, sharp edge = B
light, fuzzy = A

Senci of each picked to PX - incubated - ~~5 hrs~~ ~~$5 \cdot 10^7$/ml~~ dilute each 2,4 → A + B
A - ca 300 colonies ē 10 type B
B - ca 300-900 colonies ē $5 \cdot 10^8$ type A.

K-12 Post irradiation effect

Culture aerated 2: hours - Centrifuged - resusp. in W-D - dilute to 10^8/ml - Irrad. dilute both in PX - Incubate ē 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 10^{-1} ca. Indicates no effect of post incubation on survival - colony survival

inc 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
inc : 1:30
out : 4:00 - partially cleared

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

2/21

10 colonies from N plates of 1831 + 882 picked and ~~cultured~~ streaked with 1831 to No. lys due to 882 – All appeared to be like 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 streak valve —

Cultures started K-12, 1831, 1405

2/23 Cultures of Wg 14 and Wg 16 started in PX from culture of 2/14 –
0.5 ml + 10 ml PX at P: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 Pro + 0.1 ml Pr
DO + 0.1 ml Pr
DO

Wg-16 – dil 1-100 (same cells) add 1.0 ml to DO + 0.3 ml Pro.
DO

Growth 2/24	
–	–
+	
+	←
–	
+	

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 colonies sensitive

Diploid 1432 · 1482 DM cultures streaked on EMB and EMB – 2/25 – Segregation on EMB,

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 LWP TSA on ½ plates

				No's	growth at 24 hrs	proline added 2/27 growth 2/28
Wq-14	0	2	colonies picked to D(0)	5	+	~
				6	+	~
	2	0				
	4	0				
Wq-16	0	3	" " "	1	0	0
				2	0	+
				3	0	0
				4	0	0
	2	1				
	4	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 (A / B) forms. dilute 10^6 – Plate 1. 343 c̄ 81A = 0.24A
2. 279 c̄ 54A = 0.19A
3. 290 c̄ 53A = 0.18A

Cultures started for 2/26/52
[1831 + 882] N
(1831 + 882) P

K-12

1483

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

Aerated culture of K-12 – A / B started 8:15

Irradiation of P, N – Cultures – Centrifuge, resuspend W-D ca. dilute to contain 2·10^8 cells/ml

	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 unusual and not representative of other isolates from 1655 — or since DA came from 58-161 and also has Dose ed. 1.0, the order of establishing lysogenicity is important. All

$\lambda^s 882^s$ → $\lambda^s 882^+$ → $\lambda^+ 882^+$ Dose Ext.

$\lambda^s 882^s$ → $\lambda^+ 882^s$ → $\lambda^+ 882^+$ have dose ext. 1.0

2/26/52

K-12 A + B forms - centrifuged and resuspended in N-D
Dilute to 10⁸ cells/ml - Irradiated 15 sec -

Mix 1.0 ml + 1.0 ml 1485 - spread 0.1 ml on TSA plate

A.	Dose	Plate	Cells/Plate	Phage/plate
	0	A-0	127 (8B/119A)	—
	15	A-15	53 (4B/49A) ✓	—136 X 2 = 272

.41
127 | 53.0
50.8
220

Neither A nor B agglutinated when re-suspended in ca 9% NaCl

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

0.52
200 | 104.0
100.0
4

Indicates 200% yield - 2 plaques/1 cell - may possibly be due to contamination of irradiation suspension with free phage - doesn't seem likely from standpoint that cells were sedimented and resuspended - would be equivalent to 90% dilution in phage titer

K-12 Lwoff for high titered phage stock.
Irradiate conc. suspension - ca 10⁹
35 seconds - Incubate c̄ air after adding 5.0 ml irrad + 5.0 ml PX

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

titer ≠ $10^6 \cdot \underline{0.1}$ = 10^7 X count. titer = (3.0X10¹⁰)

no plaques > 3,000

similarly, this would indicate that the original culture had a titer of about
272 } X 10^7 = ca 3 X 10^{9}
470 }

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 picked to PX to observe rate of A ⇄ B
[see 2/26]
B } " " "

Cultures started

W-1655 → to begin $\delta\gamma z^{+}$ → λ^{+}

K-12

Wg-14

Wg-16

[?] see page 33
see previous page

2/28 Wq-16 Of 4 colonies surviving penicillin treatment.
none grew in synthetic - After 2 days
proline added - #2 grew - transferred to agar slant.

Aerated Cultures started K-12, Wq14, Wq16 - 8:30 AM
↓ ↓ penicillin run

PX
K-12A
K-12B } cultures of 2/27 plated out for examination of purity.
↳ 0.1 ml added to 10 ml PX = K-12A-2 (75B/250)
K-12B-2 (3A/156) } serial transfer to observe rate of decay

K-12 Lwoff - Viscous material.
Wash W.D. etc Irrad. 35 sec - add 10 ml PX - incubate with aer - noticed. 11:55
Suspend in WD - 10^8 cell

1. PX broth not viscous immediately after irradiation 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 hyd (HCl) with Benedict's - negative
tested with Stumpf DNA reaction - negative

		Plaques	
Titration of Lwoff-2	- 2,4,6,7 $\xrightarrow{0.1}$	337	= 3.4×10^{10}
Lwoff-3 (this one)	- " $\longrightarrow$	38	= 3.8×10^{9}

Cultures Started
K-12
1485

3/29 K-12 lysate ↗ # - Aerated cult. 2 hours (ca 10^8/ml) 2 cultures
Centrifuge - resusp'd in MD (15ml) - Incubate 45 sec (more dense than usual) divide into 2 portions 1 in PX
1 in D(0)

Incubate 2-3 hours - Some clearing in both.

3/29 P655 + 442 - plaques not large or centered with growth - growth not good on EMB-0. Incubation continued discarded as of no value

---

3/2 Titer of lysate #:

Spn $10^6 \xrightarrow{0.1}$ 1 plaque : 1×10^7

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

~~Beta~~

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

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

K-12 plated out for EMB A + B from

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 : $\frac{2}{12} \times 3.4 \times 10^{10} = 6 \times 10^9$ 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 resuspended in sol tubes added to D(M) incubated in air 12:55 – out at 2:15 – Dilute 1-10 add 0.1 ml
P(0) + 0.1 ml A + 0.3 Phen sol – plated undil 1-10 3/4
D(0) sol – 3/4 colonies
abs. 1.0 ml for reversion of pro⁻ – plated on Complete agar

WB-1 + WB-4
Turbid cultures at 12:45 Centrifuged and cells resuspended in D(M) - Incubation with aeration to study lysis

Cleared at 2:00 PM
3/4

Cultures started -
K-12
1485
Wg16
W1655
WB-1
WB-4

3/5 Primary cultures on Wq 14 (originally mixed up with Wq 16)
all colonies from all. labeled Wq16 are pro⁻
in addition [illegible] + pro, 3 are in A_3 group –
require either β alanine, tryptophane or tyrosine – [illegible]

All cultures grew tryptophane – all are pro⁻ tryp⁻

Wq-14-1
Wq-14-3
Wq-14-4

Culture originally considered Wq 16 pro⁻ [illegible] Wq 14 stock [illegible]
is [illegible] pro⁻ 1.0 ml D(o) culture of 3/3 plated on D(o) for reversion of pro
large no. colonies > 8000 – large (pro⁺) and small (prob pro⁻) – Large col picked to broth.

Aerated cultures of K-12 1485 started 10:30

1655 + 882 –
2 plaque [illegible] picked and streaked on EMB lac

K-12 A = 22/142 } 45 colonies of each picked to PX – [illegible] 11:15 AM
B = 110/142 }

Aerated culture of 1485 – (1:30 PM) 1.0 ml [illegible] 2 (3 × 10^11) added [illegible]
culture placed at room temp [illegible]
never cleared –

K-12 A centrifuged 2:30 [illegible] in H2O – —— A·0 – 230
A dilute 10^6 → plate [illegible] for col EMB
plate viewed 0.1 + 0.1 1485 for phage —— A·0 – 1
" —— A·15 – 265
[illegible] plate for phage
" " col EMB A·15 – 209

B Same [illegible] cells before [illegible]. —— B-0 – 270
phage before [illegible]. —— B-0 – 3
" —— [illegible] – >1000
col [illegible] —— [illegible]
[illegible] – 228

Don't understand
See earlier exp

Suggestion that cells are clumped

3/6 Colony from Wg-14 streaked on D(0) agar for reversion picked from broth.
D(0)

2/8 - single colony picked from broth

1655 + 1652 - 1st picking → 2 streakings
↓
6 colonies picked from each and streaked on EMB -

Aerated cultures of K-12, 1485, Wg16, W3-1 (D(0)) at 9:00 ← clear on 3/7 3/8

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

K-12 turbid 35 sec.
live at 11:45
slight clearing 1:15 - not 2:00 becoming turbid

Wg-14 - 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 58-161 - 3 hours aerated culture - centrifuged and resuspd in WD
turbid 35 sec. partial clearing after 2 hours -

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

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

Cultures
W-1655
W-19 prt

3/10

Streaks of W7-14 Pen mm 2 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

Stock culture W7 14 Pr+ from broth growth tube -
PX ← D(0) agar ← D(0) broth ← heavy inoculated D(0)

W7-16 Pen 1st mm -
inoculated col count = 18
1-10 dil = ca 250
① inoculated replica'd to D(0)
② 30 colonies of 1-10 dil picked, spotted on TSA

Aerated cultures of K-161 and K-12 started 1:30 PM
(1.0 ml + 10 ml PX)

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

Cleaning 6:00 PM
out 6:45

Cultures started -
K-12
58-161
W1655
W1485

3/11

Wq-14 Pr⁻ — auxotrophs - picked from TSA plates to
2nd streaking on TSA for Replica -

Lwoff 58-161 L2 } centrifuged — dil 10^6 → plaques on 1485 → $600 \times 10^7 = 6 \times 10^9$
dil 10^4 → colonies on EMD lac → $15 \times 10^5 = 1.5 \times 10^6$
K-12 L5⁻ → dil 10^6 → plaques on 1485 → ca $400 \times 10^7 = 4 \times 10^9$
→ dil 10^4 → colonies ~~plaques~~ on EMD lac → 0×10^5

Wq-16 - Colonies picked from Pen survivor plate
5 picked to carry over to TSA spotting
- Plate replicated to D(o)

W1655 + 882 — Prep of 882 on 1661 lys λ.

~~W~~
W1655 } Aerated culture 4 hours - Centrifuged and resusp'd in W
58-161 } mod 50 sec. Add 1.6×10^{10} λ = 1.6×10^9 λ/ml
add PX - Incubate 1:15 PM -
no clearing 3:15.
Clearing 4:15

3/14

1655+882 – 12 plaques picked and streaked on EMB(0).
from streaking 12 colonies picked streaked on EMB(0)

Wq-16 Pm pm 2
15 colonies surveyed on EMB – Picked to AOA and
streaked on D(0) - 1 failed to grow – Picked to slant.

Wq-19 Pm summary – replicate to A_1, A_2, A_3, A_4, A_5
growth only on A_3 – ϕ clear, typ. or turpt

Wq16 Pm summary
30 additional col. picked to TSA – replicate to
D(0) – all grew.

3/15 Wg-14
Ten [illegible] + tryp1 r pro tubes [illegible] with
pen survivors determined ̄c A₃

1655 + 882
40 colonies picked from 1st purification
of plaque pickings cross streaked on 1655 for lys. exam.

(One showed plaques on 1655 3/17)

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

Wg16 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	YWA	Vit.	
	-	-	-	+	-	-	—	

3/16 Wg 16.
No streaks on NA. replica'd to EMS-low (no D(o) avail) All grow 3/17

Wg 19 Ten A3 mutants on D(O) + 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

Wg-16 A4 breakdown

cmt	~~pro~~ asp	thre	glut
−	+	−	−

Wg16 pro-

3/18 Alg 14 pm - tryptt - 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 2:30
K-12 .. .. 3:30 } 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-tc	1-10	1 colony

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

K-12 L6 Pen
58-161 L3 Pen
} centrifuged and plated in tubes (20 ml each)

Assay

58-161 $10^2 - 10^4 - 10^6 - 10^7$ 0.1 → plaques :
0.1 → cells :

K-12 $10^2 - 10^4 - 10^6 - 10^7$ 0.1 → plaques :
0.1 → colonies :

very low

K-12 Plated out for repeat of A - titroff
B - Effect

W-1655 - culture (1.0 + 10 ml) started in Pen + 0.3% agar
1.0 ml (3×10^{10}) phage added after turbidity about 5×10^8
Immediate loss of flow lines and partial clearing.
(discarded)

3/20

W4 14 pro- tryp+ -

Pen summary colonies streaked on EMB for purity to replicating

Observed that W4 14 is lac- -

K-12A (rough col) - 2 colonies picked in Pen and aerated P: 70 } 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) in Pen began 8:40

K-12A Centrifuged, resusp in sal- dilute 2, 4, 5S

Shake ½ min on shaker.

			Cell Survival	K-12 as phages
A-0	0.1 ml	- 61	1.0	0.33
A-0	0.5ml + 0.5ml 1/45 → 0.1 ml	- 10 × 2 = 20		
A-15	about	- 35	0.57	
A-15	0.5ml + 0.5ml 1/45	- 41 × 2 = 82 / 62		

K-12B Centrifuged and as above

B-0		65	65	
B-0	0.5ml + 0.5ml 1/45	- 6 × 2 = 12		
B-15		21	21	0.32
B-15	0.5ml + 0.5ml 1/45	- 42 × 2 = 84		

(1831 + 882) N aerated cult. ca 5×10^8 - diluted $10^2, 10^4, 10^5$/2

Dose	Survival
0 - 485	
10 - 398	8.2×10^{-1}
20 - 202	4.2×10^{-1}
30 - 87	1.8×10^{-1}
40 - 49	1.0×10^{-1}

3/27 Monday -

Wg 14 pro- trypt -

16 survivors on EMB

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

3/22 all grew xcept #6 + #8

on 3/24 inoculated #6, #8 onto fresh trypt + pro + D(0) for recheck

(Both grew 3/25)

1655 + 882 -

1.0 ml + 10 ml Pen - aerated 90 min.

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

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

~~Why? recovery due to a temp. difference? Plated and [illegible] incubator [illegible].~~

Dose extension lies between 2. - 3.0

Tuesday 3/26

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

K-12B - Effect of agar growth etc on runoff.

Colonies removed from K-12 plate (EMB) to broth
Spread on EMB - incubated ——— hrs - Washed off, centrifuged and resuspend in sol - dilute to 10^7 cells/ml - inoculate

Not done

K-12 L6 - in Pen - Tiber
Flattened 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
~~xx~~ colonies 2/27 - discarded

Centrifuge - add ~~0~~ ml Sol - Centrifuge

	plated	broth
Plate 0.1 ml → D(0)		
Dil 1-10 → D(2) (not broth)		—

W1655 + ~~A~~ + gal agar - partial clearing at 2 hours - still particles clear 3/2

3/26

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

(1655+882) + λ discarded because of above

2/27 Mix up concerning Hott tubes. 15 tubes suspicious of being contam. spotted on
- EMB lac – all neg
- EMB mal – all pos.
- D(0) – all failed to grow

streak out of another batch of the base showing turbidity – no growth on EMB

W9/16 pr– few 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 30 min to (0.1ml)

observe transduction effect.

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

[illegible] cultures

3/29 K-12 - Lac⁻ K-12 observed(?) - picked and streaked in EMB lac

slight pink reaction on EMB lac
↳ streaked again 2/30 on EMB-0

Wq 16 Pr⁻ - Pen selection survivors (1.9 streaks)
replica'd to D(G) + Pr
EMB lac.
all grew -

2/30 colonies of 1635 + λ82 on EMB lac picked
for purification - Streaked on EMB-0
one a small colony formed(?) 2/30

Cultures of Wq 19 pr⁻ tryp⁻
58-161 made for crossing

Small colony from 1635 + λ transduction picked
to broth - In case transduction ~~[illegible]~~ requires a 2 step
process - one for each requirement (that is BM really exists)

3/31 1655 + 8-12
5 colonies streaked on EMB

Picked 5 from each and restreaked

poor streaking
questionable results - repeat

58-161
Wq 14 pr⁻ trypt -
Centrifuged, resuspended in Saline, centrifuge, resuspended

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
	1		

plate out 0.1 ml on 3 EMB - 2 useful (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