Tuesday 7/15

Cultures
518 – make Lp2R ok ← streaked against λ-2
811 – make auxotrophs, ~~Lp2R~~
1655 – look for gal –
1485 – natural λ, assay.
750 – galdiction as function of cell nos.
518tK-12 – stability check ok (streaked on EMB gal from overnight cult.)
lysate ok (aerated cult. begun)
811tK-12, stability check ok
lysate ok

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

Purification 1

	expected
10 gal –	14
73 gal +	73

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

Restreaked –

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

1692 X 1402
Purification – restreaked 3rd time EMB gal – 2 representative of 3 originally received.
2062

Wednesday July 16 - 52

- 518t K-12 X 1436 — See previous page
  20 gal - } from primary streakings - restreaked -
  20 gal + } unstables with mosaics, picked first in order - than apparent stables.

- 811t K-12 x 1436
  12 gal - picked from 1st streakings and restreaked.
  Out of EMB gal - store gal + until 7/17 -

- 518t K-12 } lysate - cleared after 4 hours.
- 811t K-12 }

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

EMB gal	1924 cell not aerated, 11/ml	heated K-12 λ	K-12 λ	no lap 2 days
1	0.1	0.1	—	29
2	0.1	—	0.1	129

← check for lysogenicity -

Galg -

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

Thurs. -
518t K X 1436 - ABOVE - 2nd streaking.
20 gal - streaked - 19 grew (1 lost) all pure gal -
20 gal + - streaked - 20 grew — all mixed ± col. less mosaics (3) Than on 1st streaking
of 5 1st streaks showing mosaics - one shows mosaic

811t K-12 x 1436 ABOVE.
12 gal - picked + streaked purif. - 2
- of 12, 11 pure gal -, - 1 mixed -

518/λ-2 λ-2 resistant col apparent.

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

Thursday 7/17/52

- 518 t K-12 x 1436
  19 gal - from Purification 2 - restreaked (6/plate)
  20 gal + " " " " (no particular order)
- 811 t K-12 x 1436
  18 gal - from Purif. 2 - restreaked
- 518 t K-12
- 811 t K-12 from stock cultures
  1. streaking ~~[illegible]~~ from first bottle culture shows mixed
  2. single colony picked, restreaked.
- 1924 - gal dried previous page.
  all control plate papillae picked, streaked EMB gal = 29 pap.
  equivalent no of " in gal dried picked. = 29 pap.
- 811 t K-12
  gal + passed up yesterday
  2 discarded, remainder streaked (P-2) on EMB gal.

Friday 7/18/52

Report on above

518 t K-12 x 1436
  19 gal - all gal -
  20 gal + 19 appear mixed, one gal + pure -

P-2 { 811 t K-12 x 1436
  18 gal - all gal -
  64 gal + (1 plate worth lost from original 72) 62 unstable, mixed,

- 1924 t K-12
  29 untreated plate pap. streaked - 28 grew - all ranges of +, from dark to lightl
  15 appear mixed, no mosaic colonies noted.
  29 from K-12 λ plate streaked - 21 grew - all ranges of +
  10 appear mixed, maybe a mosaic colony.
- 518 t K-12 } streaks streaked on EMB gal
  811 t K-12 } still mixed

Friday - 7/18

- 1924 - treated and untreated papillae restreaked - P2
  ~~29~~ 21 treated papillae streaked against 1485
- 811 tK-12 x 1436 -
  papillae picked from P-2, stored overnight in refrigerator.
  streaked P3 Saturday 7/19

Saturday.
7/19 - 1924 above - of streaking P2
Spontaneous 28 gal +, all pure gal +, varying degrees of gal-+-ness
K-12 gal dual 12 +, 3/12 mixed, also varying degrees of gal +-ness ← suggestion of greater stability?
← plate with 8 dropped (contained few if any unstable)

Streaking against 1485
~~28~~ 21 gal + from K-12 duction - none showed evidence of phage action on 1485

1924 above restreaked.

Sunday-Monday 7/20-7/21
- ~~1924~~ 2062 Attempted proline duction -

isolate 1.	EMB gal	D(0)	heated K-12 λ	K-12 λ	no pap 2 day
—	+	—	0.1	—	9
	+	—	—	0.1	271
	—	+	0.1	—	} solid grow - protophic?
	—	+	—	0.1	
isolate 2					
	+	—	0.1	—	2
	+	—	—	0.1	180
	—	+	0.1	—	no growth
	—	+	—	0.1	solid growth —

Gal 4 - $\frac{2.7 \times 10^3}{1.9 \times 10^{14}} = 2/10^7 \lambda$

- 7/21
  1924 tK-12
  ~~29~~ 12 P-2 papillae on EMB gal - only one appears mixed - discard and repeat hoping for lower background.
  28 P-2 spontaneous papillae - all pure gal + - discard -

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

- lysate streaked out in NSA

- 750 Check on stable gal+ from transduction

EMB gal	nothing	K-12 λ	no pop. 2 days
1	0.1	—	2
2	—	0.1	c. 254

$\frac{2.54 \times 10^3}{1.4 \times 10^{10}} = 2 \times 10^{-7}$

$1/5.5 \times 10^6 \lambda$

- 2062-1, 2062-2

	EMS lac	heatal λ	K-12 λ
-1	1	0.1	—
	2	—	0.1
-2	1	0.1	—
	2	—	0.1

no growth again see pg 106

again shows prototrophy - discarded

7/22 Tuesday

- Repeat crosses -

811tK-12 X 1436

518tK-12 X 1436

- Repeat 1924

- Repeat 2050

See next page

Thursday July 24 -

1924

- Gal duction repeat -

EMB gal	Addition	no of papillae after 3 days
1	—	40
2	0.1 ml K-12 λ	161
3		

L9

$\frac{1.2 \times 10^3}{2.3 \times 10^{12}} = 1/10^7$ Gal 4 -

- 2062

attempted protein duction -

EMB factor	Addition	Observation after 7 days
1	—	no growth
2	0.1 ml K-12 λ	no growth

L9

- 2050 gal 3 -

EMB gal	Addition	No papillae 7 days	
1	—	11	11
2	0.05 K-12 λ	1/4 = 172	688
3	0.1 K-12 λ	1/4 = 223	892

$\frac{8.8 \times 10^4}{2.3 \times 10^{12}} = 1/2.6 \times 10^6 \lambda$ Gal 3 -

$\frac{1.86 \times 10^4}{2.3 \times 10^{12}} = 1/1.7 \times 10^6 \lambda$

- 518 tK-12 X 1436 repeat on cross

- unconcentrated cell suspensions used = 10^9/ml, 0.1 ml plated -

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

EMB gal	Additions	no. colonies	no gal +	no gal -
1	0.1 ml 518 tK-12 cells	0	0	0
2	0.1 ml 518 tK-12 + 0.1 ml 1436 cells	35	0	35
3	" " " "	30	0	30
4	0.1 ml 1436 cells	0	0	0

- 811 tK-12 x 1436 repeat cross

- unconcentrated cell suspension as in above cross -

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

EMB gal	Additions	no. colonies	no gal +	no gal -
1	0.1 ml 811 tK-12 cells	0	0	0
2	0.1 ml 811 tK-12 + 0.1 ml 1436	12	11	1
3	" " " "	17	16	1
4	0.1 ml 1436 cells	0	0	0

approximate results obtained previously -

- Phage lysates examined for sterility - streaked on NSA

Mad. λ tested

Source	
1821	- no growth
heated K-12	- no growth
892	- no growth
750 tK 1821	- 1 small colony =
1439	- no growth
811 (gal+)	- no growth
K-12	- no growth
811-2	- no growth
811	- no growth
750	- no growth
902	- no growth
1736 gal+	- no growth

Irrad. K-12 lysate

Dose	
60	- no growth
120	- no growth
180	- no growth
240	- no growth

Order of stability 750 > 2050 > 1655 x 902 > 811, 513

~~gal 1 > gal 2 > gal 3 > gal 4~~

Saturday 7/26

- 750 from pg 105 - Check on stability of galducees from treated plate 40 papillae picked.

1st streaking - 12 appeared unmixed - ~~streaked~~ restreaked sep from below non-mixed
28 appeared mixed -

7/27 2nd streak 28/29 mixed

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

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

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

Gal 1 -
$\frac{1.9 \times 10^3}{1.4 \times 10^{10}}$ $\frac{1}{7.6 \times 10^6}$ λ

- 750 - Stability and nature of irred. λ galduced - cells from overnight incubated culture.

EMB gal	Addition (0.1)	no. of pap	2 days
1	—	1	1
2	K-12 λ	186	186
3	m 60 K-12 λ	1/8 = 107	856
4	m 120 K-12 λ	1/8 = 208	1664 — Pap picked from here for stability check.
5	m 180 K-12 λ	1/16 = 161	2576
6	m 240 K-12 λ	1/16 = 183	2928
7	λ-2	0	- (resistant colonies 1000

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

discarded - attempt with 513 to see if possible to pick up galt λs

- 811 + K-12 X 1436 -

carried through 8 single colony isolation after purification - still segregating gal -

discarded -

Sunday 7/27

test not very satisfactory

Salt

811EK-12 X 1436 – check on purified proto trophs

	loc		D(0)	λ	λ-2
1.	–		G 1.		
2.			G 2.		
3.			G 3.		
4.			G 4.		
5.			G 5.	λ L	λ-2 S
6.			N. G	—	—
7.			G 6		
8.			7.		
9.			8	(9)	
10.			9.		
11.			G 10.	failed to grow	
12.			~~N. G~~	λ R	λ-2 S
13.			G. 11	failed to grow	
14.			16	λ R	λ-2 S
15.			17		
16.			18	failed to grow	
17.			14	λ R (12)	λ-2 S
18.	all here –	one result on each	~~N. G~~		
19.			G.		
20.			~~N. G~~		
21.			~~N. G~~		
22.				failed to grow	
23.				λ R	λ-2 S
24.					
25.					
26.					
27.					
28.					
29.				(19)	
30.				failed to grow	

	no. D(0)	λ	λ-2
31		failed to grow	
32	G with D(0)	λ R	λ R
33		λ R	λ-2 S
34			
35			
36			
37			
38			
39			
40	all here		
41			
42	salt	failed to grow	
43		λ R	λ-2 S
44			
45			
46			
47			
48			
49			
50			
51		λ R	λ-2 S
52			
53			
54			
55			
56		(A3)	
~~57~~			
~~58~~			
~~59~~			
60			

- Tuesday 7/29 UMP

~ 518tK-12 X 1436 = I

purified "prototrophs"

20 gal + examined, 19 prototrophic

all lac-

i all lysogenic when replicad on 1485

20 gal - examined, 18 prototrophic

all lac-

i all non lysogenic when replicad on 1485

~ 811tK-12 X 1436

purified "prototrophs"

12 gal - examined 9 prototrophic, all lac- all λ-2 sensitive

5 lysogenic when streaked against 1485 } ones lysogenic found λ^R from below

4 sensitive when " " λ } ones found sensitive, found non lysogenic, above

i

from page 108 { 56 gal + picked, all lac-, 6 failed to grow on D(0)

of those growing in D(0)

43 tested successfully against λ - all λ^{-R}

" " " " λ-2 - " λ_2^R.

i 56 gal replicad against 1485 - all lysogenic.

~ 750 X 1503 on EMS gal

~ 1177 X 1655 on EMS meal

~ 750tK-12

gal + 6 stables } from pg 107 - picked and restreaked - further ✓ instability and progress unstable → stable

6 unstables } EMB gal

i → 6 unstables - unstable after 3 transfers - mixed gal+, gal-

→ 6 appear stable, with possibility of being mixture.

~ 1924tK-12

of 24 galdrices - pg 107.

8 unstables on EMB gal - streaked against 1485 - no evidence of lysis - all presumed non lysogenic

8 apparently remain unstable

i → continued streaking of 8 = streaking #4

streaking not shows 8 unstables - (gal+, gal-)

Wednesday July 30 -

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

EMB gal	addition	no. pop. 2 days
1	0	9
2	0.1 ml K12 λ	158

↑ assume 1.4×10^{10}

- 8 streaked out for stabl. ✓
- 24 streaked out ..

$\frac{1.5}{1.4} = 1/9.3 \times 10^{6}$ λ

- Thursday 7/31
1655 X 902 Lp2^S above
8 spontaneous streaked (1) - all mixed
24 + K-12 " - all mixed

} see pg 111

Cross - mirror page
750 X 1503 to get TLB$^-$ gal$_1$ - EMB gal
control plates bacon
mixed plates 1. 2 gal +, 13 gal -
2. 2 gal +, 9 gal -

pick gal + streaks on EMB gal -

1177 X 1655 to get mal$^-$ - - - for gal direction run -
control plates barren
mixed plates 1. c. 1000 colonies, (8-9 gal + to 1 gal -)
2. c. 1000 " " "

- 518 with mad. lambda to get gal direction without lysogenicity

EMB gal plate	addition	no. pop. 2 days
1	0	19
2.	0.1 ml 1/100 mitos λ	61

← pick and examine to
1. see if sensitive, stable - if so spontaneous mutation
2. if lysogenic, stable, probably spontaneous
3. if lysogenic, unstable, probably is

pick 29 - examine for
1. stability
2. λ^S
see pg 111

NOT INDEXED
BEYOND HERE

Thursday 7/31
Friday 8/1

- 518 t in 240 K-12 λ - 29 papillae picked in attempt to find λ^s gal$^+$ unstable?
  all 29 appeared mixed
  1st streaking 7 streakings showed mixed colonies ⊛ - these streaked against ~~[illegible]~~, λ, (8/2) (all λ^R) discarded
  all restreaked.

- 902 X 1655 Lp_2^S
  ✓ on stability of gal ducees -
  of 30 papillae picked
  2nd streaking - 5 unstable still in gal duced - 4 unstable 3rd
  restreaked -
  1 unstable in 6 spontaneous - not unstable 3rd streaking } discard

- 750 x 1503 - isolation of diploid for purpose of securing TLB, Gal, -
  only suspicious colony from 1st cross found stable EMS gal - discard

- 1177 X 1655 -
  - 1st 32 ~~ga~~ mal - prototrophs picked, streaked over EMB mal - picked and streaked against λ-2 - all λ_c^R discard.
  - 14 additional picked and streaked - tested against λ-2 -
    - these 14 also λ-2 resistant - pick more mal -

8/10

- 518 t K-12 in 240 above
  8 unstables noted 2nd streaking - streak against λ
  1 proved to be sensitive, and probably unstable - picked restreaked and tested against λ - probably sensitive, doubtfully unstable - recheck.
  found λ^s but stable - repeat entire exp -

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

EMS gal	Addition	no pop 2 days
1	—	8
2	0.1 K-12 λ	245

assumed 1.4 x 10^5

1/5.8 x 10^6

2.4 x 10^5 / 1.4 x 10^5

Gal

~ from the crosses of gal ducees X 1436 - check gal - segregants from the prototrophs obtained by the cross for λ^s

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

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

- Monday 8/4

- In the crosses of the unstable, non lysogenic gal ducess, of 1924 (Lp^R) 8 unstable forms obtained.

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

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

16 gal+/c. 952

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

poor medium may actually be larger number of +

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

11/992

- Tuesday 8/5

From the cross 1177X1655 to get Mal- Lp_2^S

30 additional gal- prototrophs picked and streaked out (see pg 111) - tested against λ_{-2} all λ_2^R - total to date picked 90 - all Lp_2^R

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

26/30 gal - prototrophs successfully tested against 518 for lysog. - all showed no evidence of lysis 1 possible ?? plaque

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

(#2)

30 prototrophs picked at random - streaked/518 - all showed no evidence of lysis

Thursday - sick

- Friday 8/8

14 36 Gal ductions - Test of 1954 and 2046 λ for Leucine ductions -

EMB gal	Addition	no papillae 2-days	
1	-0-	5	(halo about 1 pap.)
2	-0.1 K-12 λ	1/4 = 324 = 1296	(some pap c̄ halos, some without - no apparent reason)
3	0.1 1/50 m 240 λ	77	(no halos)
4	0.1 1954 λ	1/4 = 279 = 1116	(some pap with and some without halos)
5.	0.1 2046 λ*	38	(no halos about pap.)

8 picked, streaked 2nd streaking all strong, 2 segregating; 8 picked 2nd streaking all - [illegible]

Reexamined

D(0)+B,T		no. col.
1	-0-	1
2	~~0.2~~ 2046 λ*	1
3	1954 λ	2

- New lysate - from 8/7

K-12	mod.	30 sec	$> 46 \times 10^8 = 4.6 \times 10^9$
	"	35 sec	$> 11 \times 10^9 = 1.1 \times 10^{10}$
	"	40 sec	$> 3 \times 10^9 = 3.0 \times 10^9$
1954			$> 140 \times 10^8 = 1.4 \times 10^{10}$
2046			205, 206 $\} \times 10^8 = 2.1 \times 10^{10}$

8/11 23 picked, streaked and single colonies tested for λ^s - all were resistant to λ and presumed lysogenic

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

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

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

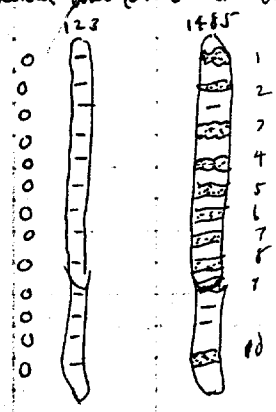
40 add. mal - picked, streaked, picked and streaked against λ^s

all Lp_2^s total to date 80
40
120

Wednesday 8/13

- 1924 t K-12
  - 6 unstable gal-ducees from pg 112 and before (these 6 are the 6 not yet crossed with 1436)
  - all cross streaked against 1485 on EMB lac - reaction slightly interfered with but all showed no evidence of lysogenicity

- 1436 t 2046 of pg. 113
  - the 13 gal ducees picked and streaked - against NTCC 123 and 1485



none lysogenic on 123

$10^7/13$ lysogenic on 1485

= ~~2070~~

- 1673 gal - made by exposing c. 10^8 cells ~~to~~ 8 sec exposure to U.V lamp (Hanovia) picked and purified 3 times. -

NO GALDUCTION

EMB gal	addition	no pop. 2 days
1	—	0
2	Δ K-12 λ = 0.1	0
3	811 λ = 0.1	0
4	K-12 λ = 0.1	1 (small)

sensitivity of this culture to λ verified

probably in error but gal slow not gal -

- From the cross 1177 x 1655

  24 additional Mal - picked, streaked, picked and tested against λ-2 all Lp_2^R

  total to date 144 - Recross and try again!

Friday 8/15

- 1267 galduction

EMB gal	Addition	no pop. 2 days
1	—	23
2	0.1K-12 λ	1/4 = 367 1468

- 1487 galduction λ^s S^r selected by S^r

EMB gal	addition	no pop 2 days
1	—	30
2	0.1K-12 λ	1/4 = 221 884

$\frac{8.8 \times 10^{-}}{2.3 \times 10^{-}} = 3.8 \times 10^{-7}$

- 518 galduction with mal. λ

EMB gal	Addition	no pop 2 days
1	—	41 (+ many minute)
2	0.1 ml in 1-50 240 K-12 λ	41 (+ no minutes)

what happened?

41 × 50 = 2005 pop/ml

doesn't work out this way

approx equivalent to [illegible] unimal λ

- W1673 gal -

1673 gal I × 750 gal₁ - 750 barren on EMS gal

EMS gal-1 3+, 7-
EMS gal-2 14-
} 3/24 gal+ = 1/8 = 12.5%

1673 gal - × 902 gal₂ - 902 gal₂ - barren on EMS gal

EMS gal-1 12+, 143-
EMS-2 13+, 235-
25/4038 gal+ = 6.2%

1673 gal - × 892 gal₃ - 892 gal plate shows 9+ col

EMS-1 2+, 57-
EMS-2 4+, 22
6/85 = 9.0%

1673 gal - × 811 gal₄ -

EMS-1 1+, 19-
EMS-2 —, 35-
= 1/55 = 1.8%

1673 gal slow

1673 gal slow

Wednesday - 9/3

- 1655 mal - - continue purification to obtain low ~~ga~~ mal + background and reexamine for mal direction -

- 1177X 1655, second crosses to obtain a mal - Lp_2^s for the purpose of attempting mal-direction -

Thursday 9/4 -

- Adsorption characteristics of 811
  - washed cells, resuspended in saline - conc. of 16 hr cult in Pu.
  - phage titer = dil 10^7 → 0.1 ml + 0.1 ml 1485 cells →

    cell titer - dil 10^6 → 0.1 ml plated on EMB lac - →

    1 ml cells mixed 1 ml phage -

    at time 1 min - 0.1 ml mix added to 10 ml broth - centrifuged 15 min - (all sample at once)

    2 min 0.1 ml as 1 min

    5 min - 0.1 ml as above

    10 min - 0.1 ml as above

Time sample	dilution
1	10^7
2	↓
5	
10	

factual

Monday 9/22

EML's crosses of 518 X 1956 - for mal-Lpz's on EMS mal

plate	mal+	mal-	totals
1	9	43	52
2	6	29	35
3	14	68	82
4	8	64	72
5	7	28	35
	44		276

0.159 = 15.9% Mal+

276) 44.0
276
1640
1380
2600
2208

EML's cross of 518 X 1817 (F+) - for Mal-Lpz's on EMS mal

1.	24	5	29
2.	35	0	35
3.	38	2	40
		7	104

92.3% Mal+

0.067 = 6.7% Mal-

104) 7.00
624
760
728

1655 mal- incidental obs. on EMB mal -
Obtained from J.L. - purified through 3 single colony isolations
2071 Entered in stock book — no. 2071

Wednesday 9/24/52

- Characterization of Ag 16 mutants saved up from p. 75-68

cultures revitalized by fresh growth, single auxotrophic colonies of each picked and streaked on EMB(·) - Replicated to D(·) + A1 - 1 grew } same
A2 - 1 grew }
A3 - none
A4 - 10 of 11 remaining grew for certain (thin plate) - rechecked.
~~A5~~ - Can't find must have forgotten or lost - recheck if A4 gives unidentified
Vit. - all failed to grow
HC - all grew

- recheck

811 gal duction by 811 galt λ -

811 galt lambda presumably contaminated - checked below

EMB gal	addition	no papillae 2 days
1	none - cells 811	39
2	0.1 ml 811 galt λ (no cells added)	64 (colonies)
3	0.1 ml 811 galt λ + 0.1 811 cells	78

apparently no activity of gal + 811 λ on 811 suggesting that this galt is not a true reversion but a suppressor mutation. Cross 811 galt × 1436 gal4 - if gal - occur further confirmation of suppressor -

- Summary of Mal - hp_2^S from 1177 to date

Since pg 114 about another 60 mal - picked from 1655 X 1177 cross

total	p. 114	144
since		c. 60
total		204

From the cross plates EML p 119 ← this streaked on EMB and replicated against λ-2

picked and - 56 in this lot one possible λ-2 sensitive.

260

Thursday 9/25/52

- 811 - Effect of heat on lambda - on galduction effect
  - K-12 λ lot labelled #7 titer approximately 2.3×10^{10}
  - Immersed in water bath at 56C for 30 minutes

no pasteurization likely

EMB gal	Addition	no pop. after 2 days	increase
1	→ none	37	
2	0.1 ml K-12 λ	56	19
3	0.1 ml K-12 λ heated as above	256	219

$\frac{2.1 \times 10^3 \text{ gal+}}{2 \times 10^{10} \lambda} \frac{1 \text{ ml}}{\text{ml}} = \frac{1}{9.5 \times 10^6}$

Apparently the activity in lambda lysates is destroyed by this heat treatment. It remains to be seen what has happened to plaque forming lambda.

λ titer = $2 \cdot 10^9$ dil = 1 = 2×10^9 λ/ml = 10^{-1} reduction in phage titer

- 811 Spontaneous gal+ above - picked, streaked on EMB gal to isolate a few gal+ of both suppressor and true back mutation type - make lysate of both types for galduction (by reversion only?)

- Wg 16 mutants collected - see 119
  1. Mutant growing on both A_1 + A_2 -
     On breakdown of these two groups - this mutant grew in both methionine, leucine } better in methionine judging by turbidity -
  2. Remaining 11 mutants judging by recheck on D(0) + A_4 replication are all A_4 -
     Examination in liquid med D(0)+pr, nos 1 + 7 grew indicating proline dependence - discard incidently these two appear different on A_4 plate - more opaque - (from remainder)
     remaining 9 are list - transferred to stock.
     (meth or leuc - one list -)

- 1655 X 1177
  Mal - Lp_2^5 search continues.
  32 addition mal - picked - now tested against λ-2

Friday 9/26

- 811 galt sp papillae from 121
  galt as picked into water - streaked on EMB lac -
  16 in all

discarded

- 2020 = 1673 gal -
  EML suspicions that in her 2020 λ+ is not gal - but gal's
  or something - ✓ of 1673 gal - on gal -
  picked from slant into Hot streaked on gal - not gal but slow

- Wg 16 pr - X_1 -
  Wg 16 pr - X_2 - } picked from slant and streaked out on EMB lac.

Sat 9/27
- Cross

811 galt X Y-10 - washed cells - 10 ml cult susp in 2 ml saline

811 galt #2

EMS gal plate	Addition	No gal +	No gal -
1	Y-10	0	
2	811 galt	0	
3	both	18	0
4	"	13	0
5	"	21	0
6	"	21	0
		73	0

sort of gal sl?

- Wg 16 pr X_1 - replicated to D(0) + pr } X_1 - failed to grow on replication to D(0) + pr
  Wg 16 pr X_2 - " " - } ← X_2 not diauxotrophic

Sunday 9/28

- 1177X 1655 - to obtain Mal- Lp_2^S
  24 gal- additional tested - all Lp_2^R -

  total to date 260
  32
  24
  316

- Wg16 pr- X_1 on EMB lac - a single lac^s or lac- noted. picked and restreaked on lac - odd appearance - some colonies with no dark centers most lac^s but with sectoring ⊙ O. ⊙ opaque sectors etc. discarded

- Wg 16 meth - penicillin run —
  control grew -
  plate out EMB lac und., 1-10, 1-100

- Comparison of K-12 lysates.

$\frac{1.44 \times 10^3}{2.8 \times 10^9} = \frac{1}{2 \times 10^6}$

λ titers (p. 124)	811 galg - EMB gal	Addition	No. of papillae	These cells retested 2 days later for ability to be gal duced
	1	none	42	33
2.8×10^9	2	0.1 ml X λ	125	72
1.1×10^{10}	3	0.1 ml 30 λ	186	
2.8×10^9	4	0.1 ml 35 λ	164	
2.9×10^9	5.	0.1 ml 40 λ	131 ← contaminated.	

- 2nd Cross

811 galt X Y-10 on EMS gal - control plate barren -
washed cells, resp'd in saline 3 ml, 0.1 ml / plate

EMS gal	galt	gal
1	215	0
2	161	0
3	142	0
4	112	0
	630	

	+	-
Cross 1	73	0
Cross 2	630	0
	703	0

Sat. 10/4

- Comparison of m λ on 811 and 518
  use m180 λ this time

EMB gel	811	518	m180 λ	m. pag. 2 days
1	0.1	–	–	52
2	0.1	–	0.1	2808
3	–	0.1	–	56
4	–	0.1	0.1	932

not much clarity added by this expt. see previous efforts

- Wednesday 10/8
  Wg16 methionine-thre- previously isolated -
  assigned #2097 - culture to D. Shaw.

- Phage titers - -

	lysate from K-12	del.	count (0.1) ml
u.v. dose	X	10^7	~~[illegible]~~ 33 33 = 2.8×10^9 λ/ml
	Y (2 pooled)		43, 67 = 5.5×10^9
	30-1		116, 98 = 1.1×10^{10} λ/ml
do	35-1		15, 40 = 2.8×10^9 λ/ml
do	40-1		(0.1)26, (0.3)94 = 2.9×10^9 λ/ml
0.5 ml tested in Pen-formed steele	15		0, 6 = 3×10^8 λ/ml
	25		176, 141, = 1.6×10^{10} λ/ml
	35		189, 78 = 1.3×10^{10} λ/ml — this stock given to ETIL
also 811tK-12 λ	811tK-12	10^7	5, 13, 9×10^8 λ/ml

Thursday 10/9

- Friday prep - Spontaneous

811 gal+ - (1) re-isolated from lysate used - stock, broth culture for X Y-10 (on EMB lac (-) reaction)
" (2) - stock labelled #2 - previously X's x Y-10
make broth cultures for lysate prep
" (3) new isolate - purified 3X + broth culture for X Y-10
" (4) " " " " " " " "
" (5) " " " " " " " "
" (6) " " " " " " " "
" (7) " " " " " " " "
" (8) " " " " " " " "
" (9) " " " " " " " "
" (10) " " " " " " " "

1st five started

- Wg 16
meth - A_2 -

1 is apparently valine - (forms pelicule - contam?)
1 is isoleucine -

stocks made

broth added to remaining 3 in the hope of resurrection since original plate lost.

- Wg 16 meth - Trive - x Wg 16 pr - X_1 -

EMS lac	addition
1	
2	
3	
4	no //
5	

- 811 galductions with 0.1 ml of various lambda prep.

	addition	no pupil as 2 days	Comment (pg 129)	ratio gald/λ
1	—	53		
2	15' K-12 λ	58	3×10^8 λ/ml lots	
3	25' K-12 λ	145	1.6×10^{10} } λ/ml	0.9×10^{-7}
4	35' K-12 λ	146	1.3×10^{10} } λ/ml	
5	750 E 1821 λ	247	c. 6.5×10^9 λ/ml according to prev. assay	

$\frac{9.2\times10^2}{1.6\times10^{10}} = X_{.7\times10^7}$

$\frac{9.3\times10^2}{1.3\times10^{10}} = X_{.4\times10^7}$

- 518 as 811 above

1	—	20
2	15' K-12 λ	78
3	25' K-12 λ	691+
4	35' K-12 λ	587+
5	750 E 1821 λ	963+

$\frac{6.7\times10^3}{1.6\times10^{10}} = \frac{4}{10^7} = \frac{1}{2.5\times10^6}$

- 750 E 1821 lysate - 0.1 ml plated EMB gal - no col.