D $\frac{20}{?}$ - 811EK-12

$\frac{1}{5}$ 518EK-12

Tuesday 7/15

Checks — streaked against λ^{-2}

518 — make Lp2R ok
811 — make auxotrophs, [illegible]
1655 — look for gal -
1485 — natural λ, assay
750 — gal deletion as film [illegible] } cell no's
518EK-12 — stability check ok } streaked on EMB gal from overnight cult.)
lysate ok } aerated cult. beyond)
811EK-12, stability check ok
lysate ok

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

Purification 1

		expected
10 gal -		14
73 gal +		73

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

Restreaked -

Of the cross 518EK-12 X 1436
shortage of EMB 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%) (per [illegible])

1692 X 1402
Purification — restreaked 3rd time EMB gal — 2 representative of 3 originally received.
2062 7/12

Wednesday July 16 - 52

See previous page

- 518tK-12 X 1436
  20 gal - } from primary streaking - restreaked -
  20 gal + } unstable, with mosaics picked first in order - then apparent stable. ✓

- 811tK-12 x 1436
  10 gal - picked from 1st streaking and restreaked.
  Out of EMB gal - store gal + until 7/17.

- 518tK-12 } lysate - cleared after 4 hours. ✓
- 811tK-12 }

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

EMB gal	1924 cell (not aerated, 10⁸/ml)	1924 cell + K-12	K-12 λ	no. of lap
1	0.1	0.1	—	29
2	0.1	—	0.1	129

← check for lysogenicity -

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

Gal + -

Thurs. -
518tKX1436 - ABOVE - 2nd streaking
20 gal - streaked - 19 grew (1 dirt) all pure gal - ✓
20 gal + - streaked - 20 grew all mixed ± col. less mosaics (3) Than on 1st streaking
1 of 5 1st streaks showing mosaics - one showed mosaic

811tK-12X1436 ABOVE.
12 gal - picked + streaked pure gal - 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
  15 gal - from purif. 2 - restreaked

- 518 t K-12
- 811 t K-12 from stock cultures
  1. streaking ~~[illegible]~~ from first broth culture direct mixed
  2. single colony picked, restreaked.

- 1924 - galduced previous page.
  all control plate papillae picked streaked EMB gal = 29 pap.
  equivalent no of " in galduced picked. = 27 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 X1436
  19 gal- all gal-
  20 gal+ 19 appear mixed, one gal+ pure-

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

- 1924 t K-12
  29 untreated plate pap. streaked - 28 grew - all range of +, from dark to light
  15 appear mixed, no mosaic colonies noted.

  27 from K-12 λ plate streaked - 21 grew - all range of +
  10 appear mixed, maybe a mosaic colony.

- 518 t K-12
  811 t K-12 } stocks streaked on EMB gal
  still mixed
~~~~~~~~~~

Friday - 7/18

- 1924 - treated and untreated papillae restreaked - P2
  ~~29~~ 24 treated papillae streaked against 1485

- 811 EK-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 +, are 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?
(12 +: plate contk. 8 dropped (contained few if any unstables))

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

1924 above restreaked.

Sunday-Monday 7/20 - 7/21

- ~~1924~~ 2062 Attempted proline duction -

isolate 1	EMB gal	D(a)	heated λ K12	K-12 λ	no pap 2 day
—	+	—	0.1	—	9
	+	—	—	0.1	271
	—	+	0.1	—	} solid grow - prototrophic?
	—	+	—	0.1	} solid grow - prototrophic?
isolate 2					
	+	—	0.1	—	2
	+	—	—	0.1	180
	—	+	0.1	—	no growth
	—	+	—	0.1	solid growth

gal 4 - $\frac{2.7 \times 10^{7}}{1.9 \times 10^{14}}$ = 2 / 10⁷

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

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

- lysate streaked out on NSA

- 750 Check on stable colt from transduction

	EMB gal	Nothing	K-12 λ	no prop. 2 days
	1/2	0.1 —	0.1	ca. 254 —

$\frac{2.54 \times 10^3}{1.9 \times 10^{10}} = \frac{2}{?}$ $\frac{1}{5.5 \times 10^6}$ λ

- 2062-1, 2062-2

	EMS loc	neutral λ	K-12 λ	
-1	1/2	0.1 —	0.1	no growth again (see pg 106)
-2	1/2	0.1 —	0.1	again shows prototrophy - discarded

7/22 Tuesday

- Repeat crosses -

  811 EK-12 X 1436

  918 EK-12 X 1436

- Repeat 1924

- Repeat 2050 (see next pg)

Thursday July 24 -

- 1924 Gal duction repeat -

EMB gal	Addition	no. of papillae after 6 days
1	—	40
2	0.1 ml K-12 λ (L9)	161
3		

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

- 2062 attempted proline duction -

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

- 2050 gal₃ -

EMB gal	Addition	No. papillae 7 days	
1	—	11	11
2	0.05 K-12 λ	¼ = 172	688
3	0.1 K-12 λ	¼ = 223	892

(L9) $\frac{8.8 \times 10^3}{2.3 \times 10^{10}} = \frac{1}{2.6 \times 10^6}$ λ - Gal 3 -

$\frac{1.86 \times 10^4}{2.3 \times 10^{10}} = \frac{1}{1.7 \times 10^6}$ λ

- 518 tK-12 X 1436 repeat on cross
  - unconcentrated cell suspensions used = 10^9/ml 0.1 ml plated -
  518tK-12 suspension from streaking on EMB gal appears mixed (ca. 10 gal+/1 gal- ?)

EMB gal	Addition	no. colonies	no gal +	no gal -
1	0.1 ml 518tK-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 -
  811tK-12 suspension from streaking on EMB gal appears mixed (ca. >20 gal+/1 gal- ?)

EMB gal	Addition	no. colonies	no gal +	no gal -
1	0.1 ml 811tK-12 cells	0	0	0
2	0.1 ml 811tK-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

(irrad. λ tested)

Source	
1821	no growth
heated K-12	no growth
892	no growth
75UC1821	1 small colony = .
1437	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 > 1655X1922~~ 811, 518

~~gal₁ > gal > gal > gal~~

Saturday 7/26

- 750 from pg 105 – Check on stability of galducees from treated plate 40 papillae picked.
  1st streaking – 12 appeared unmixed – restreaked sep from below
  28 appeared mixed –
  7/27 2nd streak non-mixed 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 galducced picks – all mixed 1st streaking
– 9/10 appear pure 2nd streaking
10/10 appear pure 3rd "

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

Gal₁⁻ $\frac{1.9\times10^{3}}{1.4\times10^{10}}$: ~~λ / 1~~ / 15 $\frac{1}{7.6\times10^{6}}$ λ

- 750 – Stability and nature of mixed λ galducced – cells from overnight unaerated culture.

EMB gal	Addition (0.1)	no. of pap	2 days
1	—	1	1
2	K-12 λ	186	186
3	uv 60 K-12 λ	½: 107	856
4	uv 120 K-12 λ	⅛: 208	1664 — Pap picked from here for stability check.
5	uv 180 K-12 λ	1/16: 161	2576
6	uv 240 K-12 λ	1/16: 183	2928.
7	λ-2	0	— (ct 1000 resistant colonies

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

discarded – attempt with 513 to see if possible to pick up galT λ's

- 811 Ē K-12 X 1436 –

Carried through 8 single colony isolation after purification – still segregating gal –

discarded –

Sunday 7/27

test not very satisfactory

gal+
811 EK-12 X 1436 – check on purified prototrophs
lac ~~gal~~

	D(0)		λ	λ-2
1.	G	1.		
2.	G	2.		
3.	G	3.		
4.	G	4.		
5.	G	5.	λ^R	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.		11	λ^R	λ-2^S
15.		12	↓	↓
16.		13	failed to grow	
17.	↓	14	λ^R (12)	λ-2^S
18.	~~N G~~			
19.	G			
20.	~~N G~~			
21.	~~N G~~		λ^R	λ-2^S
22.			failed to grow	
23.			λ^R	λ-2^S
24.				
25.				
26.				
27.				
28.				
29.			(19)	
30.	↓		failed to grow	

all lac –

also gal+ on reisolation

	N.G. D(0)	λ	λ-2
31		failed to grow	
32	G with D(0)	λ^R	λ^R
33	↓		
34			λ-2^S
35			
36			
37			
38			
39			
40	all lac–		
41			
42	gal+	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		↓ (43)	↓
~~57~~			
~~58~~			
~~59~~			
~~60~~			

-Tuesday 7/29

518 EIC-12 X 1436 = I

purified "prototrophs"

20 gal+ examined, 19 prototrophic
all loc-
all lysogenic when replicaed on 1485 ✓

20 gal- examined, 18 prototrophic
all loc-
all non lysogenic when replicad on 1485

811 EIC-12 X 1436

purified "prototrophs."

12 gal- examined 9 prototrophic, all loc- all λ-2 sensitive
5 lysogenic when streaked against 1485 } ones lysogenic found λ^R from before
4 sensitive when " " X } ones found sensitive, found non lysogenic, above

from page 108 { 56 gal+ picked, all loc-, 6 failed to grow on D(-)
of those growing on D(-)
43 tested successfully against λ - all λ^{-2}
" " λ-2- " λ^{2R}.

56 gal replicad against 1485 - all lysogenic.

750 X 1503 on EMB gal

1177 X 1655 on EMB gal

—

750 EIC-12

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

↓ 6 unstables - unstable after 3 transfers - mixed gal+, gal-

↓ 6 appear stable, with possibility of being mixture.

1924 EIC-12

of 24 guidances - Pg 107.
8 unstables on EMB gal - streaked against 1485 - no evidence of lysis - all presumed non lysogenic
8 apparently remain unstable
↳ continued streaking of 8 = streaking (#4)
streaking not to show 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. pap. 2 days
1	0	9
2	0.1 ml K12 λ	158

↑ assume 1.4 X 10[10]

- 8 streaked out for stabil. ✓
- 24 streaked out " "

1.5 / 1.4 ... = 1/9.3 X 10[6] λ

- Thursday 7/31
1655 X 902 Lp2[S] above
8 spontaneous streaked (1) - all mixed

24 EK-12 " - all mixed

} see pg. 111

Cross - (mirror page)
750 X 1503 to get TLB⁻ gal⁻ - EMB gal
control plates pdown
mixed plates 1. 2 gal +, 13 gal -
2. 2 gal +, 9 gal -

pick gal + streak on EMB gal -

1177 X 1655 to get mal⁻ - - - for gal duction run -
control plates barren
mixed plate 1. c. 1000 colonies, (8-9 gal+ w. 1 gal -)
2. c. 1000 " " "

- 518 with mad. lambda to get galduction without lysogenicity

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

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

~~discard~~

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

NOT INDEXED
BEYOND HERE

Thursday 7/31
Friday 8/1

- 518 t m240 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 λ, (all $λ^R$) 8/2 discarded
  all restreaked.

- 902 X 1655 Lp_2^S

  ✓ on stability of gal duces -
  of 30 papillae picked

  2nd streaking - 5 unstable still in galduced - 4 unstable 3rd
  restreaked -

  1 unstable in 6 spontaneous - not unstable 3rd streaking } discard

- 750 x 1507 - isolation of diploid for purpose of recurring TLB, Gal, -
  only suspicious colony from 1st cross found stable EMB gal - discard

- 1177 x 1655 -
  - 1st 32 mal- prototrophs picked streaked are EMB mal- picked and streaked against λ-2 - all $λ_2^R$ discard.
  - 14 additional picked and streaked - tested against λ-2 -
    - these 14 also λ-2 resistant - pick more those -

8/2

- 518 K-12 m 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 - Galduction done in EMS gal

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

1/1.5 x 10^{10} 6
4 x
1.4
Gal
(1.4 x 10^5)

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

811 t G12 X 1436 -
7 gal- segregant picked and tested all $λ^R$

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

- Monday 8/4

- On the cross of the unstable, non lysogenic galdu cross, of 1924 (tp.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) cells	barren
2	0.1 ml of "1436, "1924tK-12	11 gal+, c. 384 gal-
3	" "	5 gal+, (1/4 = 142 gal-) = 518
4	0.1 ml 10X 1436	barren

16 gal+/c. 952

poor medium may actually be larger number of +

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

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

11/992

~~~~

- Tuesday 8/5

From the cross 1177X1655 to get Mal- tp.S

30 additional gal- protrophs picked and streaked out (see pg 111) - tested against 1-2 all 1-2R - total to date picked 60 all tp.R

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

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

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

30 protrophs (#2) picked at random - streaked/518 - all showed no evidence of lysis
~~~~

1.3 x 10^? 0.6 x 10^-6

Thursday – sick

– Friday 8/8

14 36 Gal duction – Test of 1954 and 2046 λ for leucine duction –

EMB gal	Addition	no papillae 2-day		
1	-0-	5		(halo about 1 pap.)
2	-0.1 K12 λ	¼ : 324 :	1296	(some pap c̄ halos, some without – no apparent reason)
3	0.1 1/50 m240 λ	77		(no halos)
4	0.1 1954 λ	¼ : 279	1116	(some with and some without halos)
5.	0.1 2046 λ*	38		(no halos about pap.)

8 picked & streaked, 2nd streaking all stormy, 2 sectored; 8 picked & 2nd streaked – all – [illegible]

Reexamined

DC(0)HB,T		no. col.
1	-0-	1
2	~~0.2~~ 2046 λ*	1
3	1954 λ	2

– New lysate – from 8/7

K-12 mal	30 sec	> 46 x 10^8 = 4.6 x 10^9
..	35 sec	> 11 x 10^9 = 1.1 x 10^10
..	40 sec	> 3 x 10^9 = 3.0 x 10^9
1954		> 140 x 10^8 = 1.4 x 10^10
2046		205, 206 } x 10^8 = 2.? x 10^10

8/11 ~~AA~~ 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 galt 2nd streaking

– From the cross 1177 X 1655 to obtain Mal- Ly_2^S

40 add. mal- picked, streaked, picked and streaked against λ~

all ly_2^L total to date 80

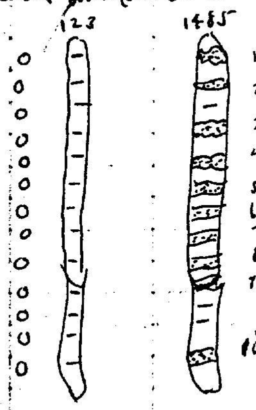
40

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)
  are 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

10/13 lysogenic on 1485

- 1673 gal- = ~~2070~~ made by exposing c. 10^8 cells to 5 sec exposure to (Hanovia) U.V lamp.
  picked and purified 3 times. -

NO GALDUCTION

probably in error - but gal slow not gal-

EMB gal	addition	no pap. 2 days
1		0
2	Δ K12 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

- From the cross 1177 x 1655
  24 additional Mal- picked, streaked, picked and tested against λ-2 all ly_2^R
  total to date 144 - Recross and try again!

Friday 8/15

- 1767 galduction

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

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

EMB gal	addition	no. pap 2 days
1	—	30
2	0.1 K12 λ	1/4 = 221 884

8.8×10^? / 2.3×10^? = 3.8×10^?

- 518 galduction with mal. λ

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

← (what happened?) ✓ — 41 × 50 = 2005 pap/ml

(doesn't work out this way) approx equivalent to ... unimal. λ

- W1673 gal-

1673 gal_1 × 750 gal- — 750 barren on EMS gal

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

1673 gal- × 902 gal_2- — 902 gal_2 - barren on EMS gal

EMS gal-1	12+, 143-	25/403 gal+	= 6.2%
EMS-2	13+, 235-		

1673 gal- × 892 gal_3- — ~~892 gal plate shows 9+ col~~

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

1673 gal- × 871 gal_4-

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

1673 gal ... (left margin)

} ... 1673 gal slow

Wednesday - 9/3

- 1655 mal- - continue purification to obtain low γa mal+ background and re-examine for mal- deletion -

- 1177x 1655, second cross to obtain a mal- tp_2^s for the purpose of attempting mal- deletion -

Thursday 9/4 -

- Adsorption characteristics of 811
  - washed cells, resuspended in saline - conc. of 16 hr culture in broth.
  - phage titer = dil $10^8 \rightarrow$ 0.1 ml + 0.1 ml 1485 cells $\rightarrow$
    cell titer - dil $10^6 \rightarrow$ 0.1 ml plated on EMB loc. $\rightarrow$
    1 ml cells mixed 1 ml phage -

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

Monday 9/22

EMC's cross of 518 X 1956 - for mal- typ in EMS mal

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

0.158 = 15.9% Malt+

276 / 44.0
276
1640
1380
2600
2208

EMC's cross of 518 X 1817 F+ - for Mal- typ in EMS mal

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

92.3% Malt

0.067 = 6.7% Mal-

104 / 7.00
624
760
728

1655 mal- incidental stor. in 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 Arg 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 O(-) +
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 - rechecked if A4 grew unidentified
Vit. - all failed to grow
MC - all grew

- recheck

811 galductase by 811 galtt λ -

811 galt lambda presumably contaminated - checked below

EM13 gal	addition	no. papillae 2 days
1	none - cells 811	39
2	0.1 ml 811 galtt λ (no cells added)	64 (colonies)
3	0.1 ml 811 galtt λ + 0.1 811 cells	78

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

— Summary of Mal- typ.D from 1177 to date.

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

total p. 114		144
since total		c. 60
		204

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

picked and - 56

260

on this lot are possible λ-2 sensitive.

Thursday 9/25/52

- 811 - Effect of heat on lambda - a goldschein effect.

- K-12 λ lot labelled #7 titer approximately 2.3×10^{10}
- Immersed in water bath at 56C for 30 minutes.

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

$\frac{2 \times 10^7 \text{ phage}}{2 \times 10^{10} \text{ λ}} \cdot \frac{1 \text{ λ}}{1 \text{ ml}} = \frac{1}{9.5 \times 10^6}$ 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 = $\frac{10^{+1}}{2}$ 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 goldschein (by necessity?)

- Wg 16 mutants collected - see 119

1. Mutant growing on both A_1 + A_2 -

grew in both — On breakdown of these two groups - the mutant methionine / leucine better in methionine judging by turbidity -

2. Remaining 11 mutants judging by recheck on O(i) + A_4 replication are all A_4 -

Examination in liquid med O(i) + pr, has 1 + 7 gives indicating proline dependence - discard - incidentally these did appear different on A_4 plate - more opaque -

remaining 9 are list - (from remainder) have been saved to stock.

(methionine-leuc- no list -)

- 1655 X (177)

Meal - 4p.s search continues.

3rd addition meal - picked - now tested against λ-2

Friday 9/26

- 811 galt sp papillae from 121
  galt al picked with water - streaked on EMB Lac -
  16 in all
  discarded

- 2070 = 1673 gal -
  EML suspicious that her 2070 λ+ is not gal - but gal^s or something - & of 1673 gal - on gal -
  picked from slant with Hot streaked on gal - not gal, but slow

- W916 pr- X_1 - } picked from slant and streaked out on EMB lac.
  W916 pr- X_2 - }

Sat 9/27

- Cross
  811 galt X Y10 - washed cells - 10ml cult susp in 2 ml saline

811 galt #2 EMB gal plate	Addition	gal +	gal -
1	Y-10	0	
2	811 galt	0	
3	both	18	0
4	"	13	0 — sort of gal sl?
5	"	21	0
6	"	21	0
		73	0

- W916 pr X_1 - replicated to D(0) + pr } X_1- failed to grow on replication to D(0)+pr
  W916 pr X_2 - " " " } & X_2 not diauxotrophic

Sunday 9/28

- 1177 X 1655 - to obtain Mal⁻ λp_2^S
 ·24 gal⁻ additional tested - all λp_2^R -
 total to date 260
 32
 24
 316

- Wq16 pr⁻ X_1 on EMB lac - a single lac⁻ or lac- noted. picked and restreaked on lac - odd appearance - some colonies with no dark centers most lac⁻ but with sectoring ⊘ O. opaque sectors etc. discarded

- Wq 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 gal⁻ EMB gal	Addition	No. of papillae	These cells retested 2 days later for ability to be gal dured
	1	none	42	33
2.8 X 10⁹	2	0.1 ml X λ	125	72
1.1 X 10¹⁰	3	0.1 ml 30 λ	186	
2.8 X 10⁹	4	0.1 ml 35 λ	164	
2.9 X 10⁹	5.	0.1 ml 40 λ	131 ← contaminated.	

- 2nd Cross

811 gal⁻ X Y-10 on EMB gal- control plate barren -
 washed cells resusp in saline 3 ml, 0.1 ml/plate

811 plate #	EMB gal	gal +	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 gel	518	m180 λ	m. pq. 2 days
1	0.1	–	–	5
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 expts

- Wednesday 10/8

Wylb methionine-three- previously isolated -
assigned #2097 - culture to D. Shaw.

- Phage titers

	lysate from 11-12	dil.	count (0.1) ml
u.v. dose	X	10^7	~~(too many)~~ 33 83 = 2.8×10^9 λ/ml
	Y (2 probed)		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
osmo tested in Pen-found stable	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
also 811 ER-12 λ	811 ER-12	10^7	5, 13, 9×10^8 λ/ml

this stock from D. ER-12 L

Thursday 10/9

- Friday prep -
Spontaneous 811 gal+ - (1) - re-isolated from lysate used - on EMB lac(-) weaker - broth culture for X Y-10 stock,
" (2) - stock labeled #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 time started

- Wg 16
meth - A_2 -

1 is apparently valine - (forms pellicle - certain?)
1 is isoleucine -

stocks made

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

- Wg 16 meth Thre - X Wg 16 pr-X_1 -

EMB lac addition
1
2
3 no //
4
5

- 811 galductions with 0.1 ml of various lambda prep. Comment (pg 129) ratio gald/λ

	addition	on papillae 2 days			
1	—	53			
2	15' K-12 λ	58	$\frac{9.2\times10^2}{1.6\times10^{10}} = X.7\times10^7$	3×10^8 λ/ml both	
3	25' K-12 λ	145		1.6×10^{10} λ/ml	0.9×10^{-7}
4	35' K-12 λ	146	$\frac{9.3\times10^2}{1.3\times10^{10}} = X.4\times10^7$	1.3×10^{10} λ/ml	
5	750 E 1821 λ	247		c. 6.5×10^9 λ/ml according to prev. assay	

- 518 on 811 alone

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.