11/16/50

W2252 = 1895 Hfr Lp^s

(-) transduction with HFT)s ~~to~~ obtain Hfr gal-

2341 { 1. Several (-) colonies with N16 HFT gal₂- isolated and purified - HFT λ tests indicate gal₂- cross with W902

Results indicate HFT cross - almost solid smear (with dilute cells also) no (+) in probably 100,000 colonies

(2.) Streaked against λ - not sens.
518 - not lysogenic = Lp₁^R Lp₂^r
λ2 - sensitive

(3) "Lysate" prepared - very viscous

0.1 ml / 2281 = no effect, neither plaques nor pap
" 518 = " " (?) 14/9 = ratio of pap/control area

11/25/50 Repeat with NA-4 lysates 1+2

W 2345 = From lysates 1 - one gal₁- isolated - tested against N16, NA-1 = gal₁-

From lysate 2 - 5 gal₁- isolated - " " " -gal₁-

also nonlysogenic

NA-1
NA-2 } lysates

sterility confirmed

11/30/53

Transductions for segregant analyses

A W1765 gal⁻ (made by ~ transduction) W2373

	λ	cured ½	plage ½
1.	811	1	30
2.	1210	0	56
3	902 (cur lysate)	1	11
4	K-12	1	33

W 750

1.	K12	1	46

W1210

1.	K-12	4	~~21~~ (23)
2.	750	2	65
3.	811	5	56

12/5/53 See also next page

T18} λs against 2070 - to see if both 1⁻, 2⁻ is in these apparent doubles.
T19}
(-2) lysates not very active

245B

T18-2 5/1
T19-2 9/2

1. Analysis of transductants of 2070 by T19-2 λ

	segregants	/N1	/N16	Locus
1.	+	0	2⁻	
2.	+	0	2⁻	
3.	0	0	-- ?	
4.	+	0	2⁻	
5.	+	0	2⁻	
6.	+	0	2⁻	
7.	+	0	2⁻	
8.	+	0	2⁻	
9.	+	0	2⁻	
10.	+	0	2⁻	
11.	+	0	2⁻	
12.	+	0	2⁻	
13.	+	0	2⁻	
14.	+	0	2⁻	
15.	+	0	2⁻	
16.	+	0	2⁻	

1. Analysis of segregants from 2 transd. of T18/2070

	/N16	/N1	λ Rx	Allele
1.	+	0	R	1⁻
2.	+	0	"	1⁻
3.	0	+	"	2⁻
4.	0	+	"	2⁻
5.	+	0	"	1⁻
6.	+	0	"	1⁻
7.	+	0	"	1⁻
8.	contaminated (+)		"	

12/2/53

2175E750 λ -

A. $TI\delta^{=}$ transduced by mixture N16, ~~N4~~ N1, but not by single λ's

Proposed Constitution: ① 18 pop (gal+) picked and streaked. Many mosaic colonies — all appear to be segregating

lac⁺ $2^- 1^-$ / $2^- 1^+$ / $2^+ 1^-$ } 3m on lac should be lac slow if gal⁻ present – which is the case. Can't test gal segregation

② One of $+^u$ on gal streaked out on lac, gal – segregating on gal – also on lac, (+) appears to be segregating lac slow

	On lac	Gal-Segregants ~~segregation~~		~~by transd~~
lac⁺ 2^-1^-	s	no	–	wt.
lac⁺ 2^+1^-	s	no		1^-
lac⁺ 2^-1^+	+	no		2^-
lac⁺ $2^-/2^-\ 1^-/1^+$	+	seg lac slow		2^-, wt.
lac⁺ $2^-/2^+\ 1^-/1^-$	s	no	–	1^-, wt.

see next page 246A for detail

B. 2345 / 2251 λ to make HFT, Hfr

0.1 ml 2251 λ = 56, control Y_2 = 0

On second streaking only 4/24 $+^u$ – greater stability of transd. than expected

The 4 restreaked and segregants examined.

12/9/53 T18 continued.

1. The lac slow -

#	β lac	β gal	Transd. Assay /N1	/N16	locus
1	slow	(−)	o	+	1⁻
2	slow	"	o	+	1⁻
3	slow	"	o	o	double
4	slow	"	o	o	"
5	"	"	o	+	1⁻
6	"	"	o	o	double
7	"	"	o	o	"
8	"	"	o	+	1⁻
					4 1⁻
					4 (−−)

On standing (12/14) these are papillated quite heavily.

Lac character not good criterion

Ignore

according to later ... should have ... no. probably #14 + #15 mixed up

On 2 examinations the following marked ⊕ were found stable — Why stable?

2. The Lac + ON

#	β lac	β gal	Transd. Assay /S92ε+	HFT /N1	HFT /N16	locus	
1	+ (?) slow	+ᵘ	+	o	+	1⁻	✓1 ⊕
2	neg	"	o	o	o (1 pap)	−−	✓2
3	neg	"	o	o	o stretch thin	−−	✓3
4	"	"	+	o	+	1⁻	✓4 ⊕
5	"	"	⊖	⊖	⊖	−−	✓5
6	"	"	o	+	o	2⁻	✓6 ⊕
7	"	"	+	o	+	1⁻	✓7 ⊕
8	"	"	o	+	o	2⁻	✓8 ⊕
9	"	"	o	o	o	−−	✓9 +ᴿ gave stable + only
10	"	"	o	+	o	2⁻	✓10 ⊕
11	"	"	o	o	o	−−	✓11
12	"	"	o	o	o	−−	✓12 +ᴿ gave stable + only
13	"	"	o	o	o	−−	✓13
14	"	"	o	+	o	2⁻	✓14 ⊕
15	"	"	+ 1 pap	o	+	1⁻	✓15 ● Rev. unstable
16	"	"	o	o	o	−−	✓16
17	"	"	o	o	o	−−	✓17
18	"	"	+ 2 pap	o	o	? −−	✓18
19	"	"	+	o	+	1⁻	✓19 ⊕
20	"	"	o	+	o	2⁻	✓20 ⊕
21	"	"	o	o	o	−−	✓21
22	"	"	o	o	o	−−	✓22
23	"	"	+	o	+	1⁻	✓23 ⊕
24	"	"	o	o	o	−−	✓24

24 lac al picked from here and ⊖ gal tested

Comparison between ... here and later ... Results still suggest two 2⁻

Lion reversion in double ?

1st 12 says (−−)

don't know what to think about this.

13 −−
6 1⁻
5 2⁻

3/5/54 Lysate made of 246A-15
Lopped
/700 solid spot
/1210 0
/811 solid spot
} indicates HFT gal₂⁻

6/18/54
246A-15 +ᴿ stocks discarded
246-A-15 on stock

Correlation of double above with 1⁻ only. This lysate is not + ... Lp₁ but that this is a ... Lp⁺ lysate

5 R
4- +
2+ -

S R
4- +
2- -

(5
4-
2-) R + +

(247A)

12/6/53

See pg 245 - Sample size = 22
6/22 + ...

W/1210 transduced by K12

Transduct	Transd 518	Segregant /N16	N1	Segregant by Rx	Allele
1.	lys	0	+	r	2-
2.	..	0	+	↓	2-
3.	..	0	+	↓	2-
✓ 4.	..	0	+	↓	2-
5.	..	0	+	↓	2-
6.	..	0	+	↓	2-
7.	.. pure galt →				
8.	.. pure galt →				
9.	..	0	+	r	2-
10.	..	0	+	↓	2-
11.	..		+	↓	2-
12.	..		+	↓	2-
13.	.. weak	0	+	↓	2-
14.	..	0	+	↓	2-
15.	.. pure galt →				
16.	.. pure galt →				
✓ 17.	..	0	+	r	2-
18.	.. weak pure galt →				
19.	.. pure galt →				
20.	.. gal + se →				
21.	.. pure galt →				
22.	..	0	+	r	2-
23.	..	0	+	↓	2-
	18/23 +"		15/15 gal2-		

6/18/54 Stock discarded
See ↙

Cross x 902 — 6/28/54 — out
247A - 4
247A - 13] Those in stocks — 5/13/55
247A - 22] — "
247A - 33

Lysates 4/22/54

	Spotted 2175	/841
4	1/<0.1	3/0.5
13	0/<0.1	0.70/0.5
22	0/<0.1	0.20/0.5
23	0/<0.1	0.40/0.5

} these two lysates give large numbers of small papillae } possibly not sterile? partially HFT?

12/6/53

W1210 t 811

See 245 for origin

(247B)

Transd.	Transd. /518	seg ↑ NK+	/SIR	seg to R	
1	lys.	+	0	r ← 9/10 (58?)	NFT segregant found gal4 −; t^R of NFT segregant found stable.
2	"	0	+		
3	"	0	+		
4	"	0	+		
5	"	0	+		
6	"	contaminated with galt →		r	
7	"	0	+		
8	"	0	+		
9	"	0	+		
10	"	0	+		
11	"	0	+		
12	"	0	+		
13	" } rx bad	contaminated with galt →			
14	"	0	+		
15	"	0	+		
16	"	0	+		
17	lys	0	+		
18	"	0	+		
19	"	0	+		
20	"	0	+		
21	"	0	+		
22	"	0	+		
23	"	0	+		
24	"	0	+		
	all +ᵘ	22 gal₂ −; 1 gal₄ −			

Crosses × 1176, 901

1
8
14
18

} 6/18/54 Stocks discarded

2/9/54

Lysates	/SM	/2175	
-7	0/0	solid smear	= HFT gal4 − (ALSO as the result F⁺ — × F^x)

See 245

12/6/53

W1710 t 750

Transd. #	Transd. $Lp^{R}x/98$	Seg / N16	Seg / N1	Seg Lp Rx	Locus
1	Lgv	o	+	r	2-
2	"	o	+	↓	2-
3	"	contaminated w. gal		r	[illegible]
4	"	o	+		2-
5	"	o	+		2-
6	"	o	+		2-
7	"	o	+		2-
8	"	o	+		2-
9	"	o	+		2-
10	"	o	+		2-
11	"	o	+		2-
12	"	o	+		2-
13	"	o	+		2-
14	"	omitted			(but segregating)
15	"	o	+	r	2-
16	"	contaminated with gal			[illegible]
17	"	o	+		2-
18	"	o	+		2-
19	"	o	+		2-
20	"	o	+		2-
21	"	o	+		2-
22	"	+	o		1-
23	"	contaminated with gal			—
24	"	+	o		1-
	all +	19 gal 2-			
		2 gal 1-			

6/10/54 These are on stock

	cut
1	5/13/55
3	"
[illegible]	
24	"

12/5/53

4750 transduced by K12 λ

Transd. #	Transd. Rx /578	/N1	/N16		Attitude
1	lys.	0	+		1⁻
2	"	pure gal+		→	
3	"	" "		→	
4	"	" "		→	
5	"	contaminated with gal+		→	
6	"	0	+		1⁻
7	"	0	+		1⁻
8	"	0	+		1⁻
9	"	0	+		1⁻
10	"	0	+		1⁻
11	"	0	+		1⁻
12	"	0	+		1⁻
13	"	0	+		1⁻
14	"	0	+		1⁻
15	"	pure gal+		→	
16	"	0	+		1⁻
17	"	0	+		1⁻
18	"	0	+		1⁻
19	"	0	+		1⁻
20	"	contaminated with gal+		→	
21	"	pure gal+		→	
22	"	0	+		1⁻
23	"	0	+		1⁻
24	"	0	+		1⁻
	19/24 +ᵘ				16 1⁻

Spotted

lysate	/2175	/750
1	1/0.5	0/0
7	3/0.5	0/0
9	2/0.5	0/0
11	5/0.5	0/0

6/20/54
← only cell line surviving in stock of the four

12/8/53 — W2373

249 A

W1765 gal₁⁻ (by c-s transd. with NA4 lysate) transduced by 841 λ - See pg 245

Transd.	Transd by R/S	Stab. types	Seg.	SIF
1	S	non lys	stable	N1
2	R	lys	"	
3	R	lys	"	
4	R	lys	"	
5	R	lys	"	
6	R	lys	unstable	+ (?) λᴿ = gal₁⁻
7	S	non lys	stable	
8	R	lys	"	
9	R	"	"	
10	S	non lys	"	
11	R	lys	"	
12	R	"	"	
13	R	"	"	
14	R	"	"	
15	R	"	"	
16	R	"	"	
17	R	"	"	
18	R	"	"	
~~19~~	~~skipped~~		"	
20	R	S non lys	"	
21	R	S "	"	
22	S	r non lys	"	
23	S	"	stable	

extra? → ~~stable~~

Lp⁺ = 17 1/23⁺ᵘ
Lp^str = 5 1 Lp⁺

5 s
17 +
0 r

6/8/54 } stocks discarded

0.50
59/41.0
35 4
.560

.41
.59

23, 24, 12, 23 → (82), 23

22, 16, 12, 13 → (63), 23, (40/82)

12/8/53 W2373

W1765 gal- (by (-) transd c NA4) transd. by 1210 λ

Transd #	Transd Lp R/S		Transd. stability		Seg.-amng N1	N16	allele		
1	lys	R	s						
2	H	R	u		−	+	λ^R	1−	
3	H	R	s						
4	H	R	s						
5	\ non lys	S	s						
6	lys	R	s						
7	\ non lys	S	s						
8	lys	R	u		−	+	λ^R	1−	
9	H	R	u		contaminated c		(+)		
10	\ non lys	R	S	x	+	−	λ^R	2−	✓
11	\ H	R	s						
12	lys	R	u						
13	lys	R	u		−	+	λ^R	1−	✓
14	\ non lys	S	s						
15	lys	R	v						
16	H	R	s						
17	H	R	s						
18	H	R	s						
19	\ non lys	R	s	x					
20	lys	R	u		−	+	λ^R	1−	✓
21	H	R	u		−	+	λ^R	1−	✓
22	H	R	s						
23	H	R	s						
24	H	R	u		−	+	λ^R	1−	
			8/24 +^u				6	1−	
							1	2−	

$Lp^+ = 18$

$Lp^{s/r} = 6 = 3 Lp^s$ 8 Lp^+

what gives ?

an Lp^R transduction segregating the allotypic allele and Lp^R simultaneously.

10/26/56 Probably an error in recording

S may refer to segregating and not stable

See next page as well.

Lyse 249B
10
13
20
21

6/18/54 stocks discard

12/10/53 W2373

1765 gal⁻ transduced by 902

Transd #	Transd. Lys.	Lp/518	Transd. stability	Segregant analysis		/518
1	- lys	R	S			
2	- "	R	"			
3	- nm lys	R	"			
4	- nm lys	S	"			
5	- lys	r	"			
6	- lys	r	"			
7	- nm lys	s	"			
8	- nm lys	s	"			
9	- lys	r	"			
10	- lys	r	"			
11	- nm lys	r	"			
12	- lys	r	u	−	+	Ly* ← this is the only segregant obtained λR 1⁻

7 Lp^r
2 Lp^r
3 Lp^s

6/18/54

(Stocks discarded)

12/10/53 W2373

say samples = 23

W1765 gal⁻ transduced by K12

Transd. #	/1485 Lp14	/λ	Transd. Stab	/W16		/W1	Allele		
1	lys	r	s						
2	lys	r	s						
3	lys	r	s						
4	non lys	s	u	−	+	−	λR	1⁻	✓ Lp
5	lys	r	s						
6	non lys	s	u	−	contam. c̄ (+)				
7	lys	r	s						
8	..	..	u	−	+	−	λR	1⁻	
9	..	..	s						
10	..	..	s						
11	..	..	s						
12	..	..	u	−	+	−	λR	1⁻	
13	..	..	s						
14	..	..	u	−	+	−	λR	1⁻	
15	..	..	u	−	+	−	λR	1⁻	
16	non lys	s	s						
17	lys	r	u	−	+	−	λR	1⁻	
18	"	"	s						
19	"	"	s						
20	"	"	u	−	+	−	λR	1⁻	
21	"	"	u	−	+	−	λR	1⁻	
22	"	"	u	−	+	−	λR	1⁻	
23	"	"	s						
24			10/23 + u						

20 Lp⁺ 3 Lp^s 8 Lp⁺ 2 Lp^s 9 Lp⁺

Lysates 4, 8, 12, 14 } none have any action on 1st prep

{ 6/18/54 Stocks discarded }

Exp. 642

F+ F⁻ Lp+ Lp^v

250 A

12/12/53 EMC^v cross 1210 × 2234 Gal2⁻ × Gal4⁻

1. The small colony "prototrophs" (probably require B_1)

#	M16	Sit	Lp Rx	Locus	
1.	+ (few)	0	s weak	4	
2.	+	0	s	4	
3.	+	0	s	4	
4.	+	0	s weak	4	
5	+	0	s weak?	4	
6.	0	+	R	2	—
7.	+	0	s	4	
8	+	0	s weak	4	
9.	+	0	s	4	
10	0	+ R	s	2	+
11.	+	0	s weak	4	
12	+	0	s	4	
13	+	0	s	4	
14.	+	0	s	4	
15.	+	0	s	4	
16	+ D	0	s weak	4	
17	0 2	+	~~s~~	2	—
18	+	0	s weak	4	
19	+	0	s	4	
20	+	0	s	4	
21	+	0	s	4	
22	+	0 (?)	s	4	
23	+	0	s	4	
24	+	0	s	4	
25	+	0	s	4	
26.	0 v	0 (4+4)	s	?	
27.	0	+	R	2	—
28.	0 v	0 v	s	?	
29.	+	0	s	4	

23 = 4⁻ all Lp^v

4 = 2⁻ 3 Lp+ 1 Lp^v

2 = 2⁻ 4 Lp^v

F+ Gal2⁻ Gal4⁻ Lp+ × F⁻ Gal2+ Gal4⁻ Lp^s

250 B

Cont. of 250A.

2. The Large colony proto-type.

	M16	Sit	Lp	Locus
1.	0 ✓	0 ✓	S	— — ?
2.	0	+	r	2 —
3.	+	0	S	4
4.	0	+	r	2 —
5.	+	0	S	4
6.	+	0	S	4
7.	+	0	S	4
8.	0	+	r	2 —
9.	0	+	r	2 —
10.	+	0	S	4
11.	+	0	S	4
12.	0	+	r	2 —
13.	+	0	S	4
14.	+	0	S	4
15.	+	0	S	4
16.	0	+	S	4
17.	0	+	r	2 —
18.	+	0	S	4
19.	+	0	S	4
20.	+	0	S	4 ✓
21.	0	+	r	2 —
22.	0	+	r	2 —
23.	+	0	S	4
24.	+	0	S	4
25.	0	+	r	2 —
26.	+	0	S	4
27.	+	0	S	4
28.	+	0	S	4
29.	0	+	r	2 —
30.	+	0	S	4
31.	+	0	S	4

20 = 4 = all Lp^S

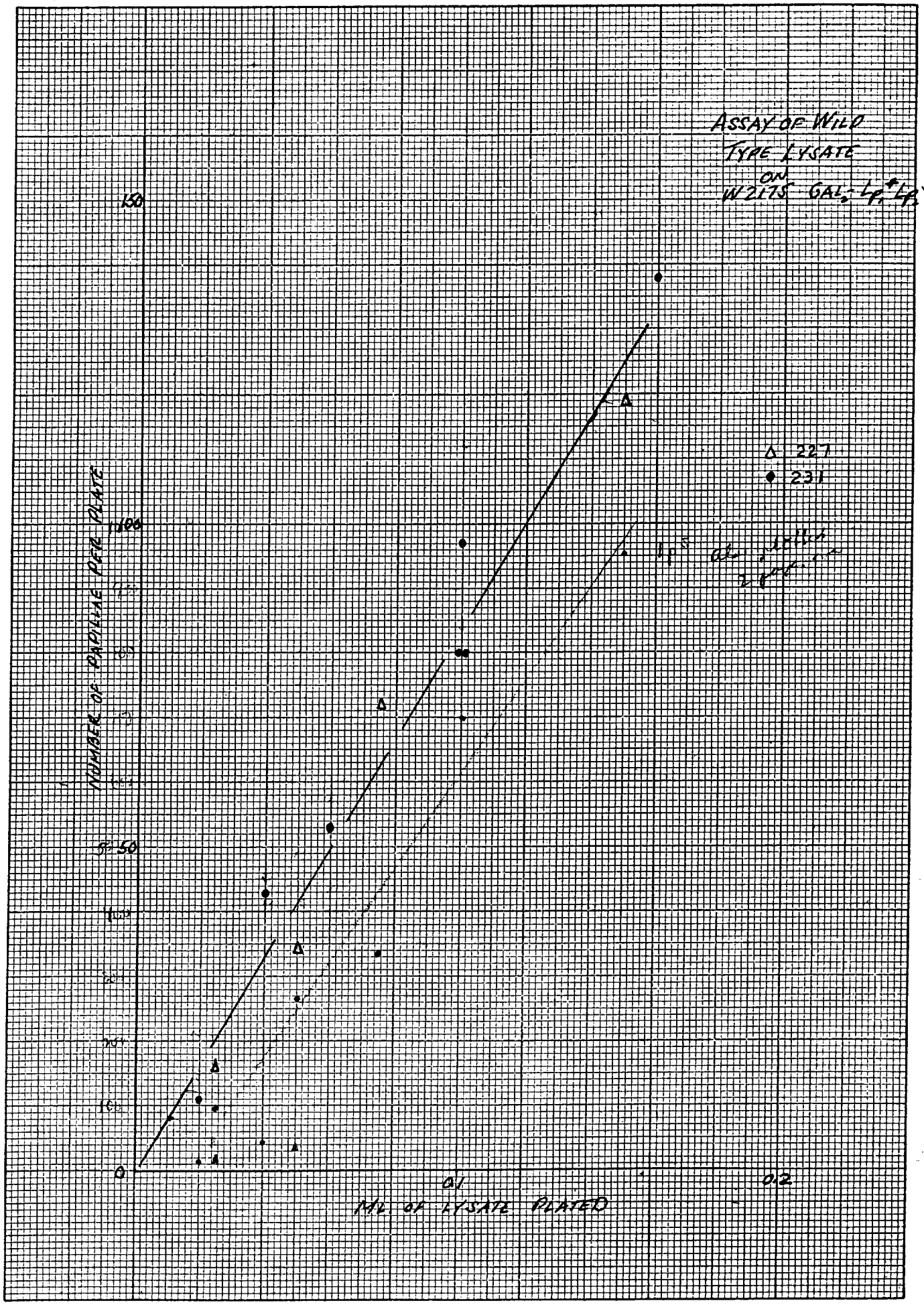
10 = 2 = all Lp^R

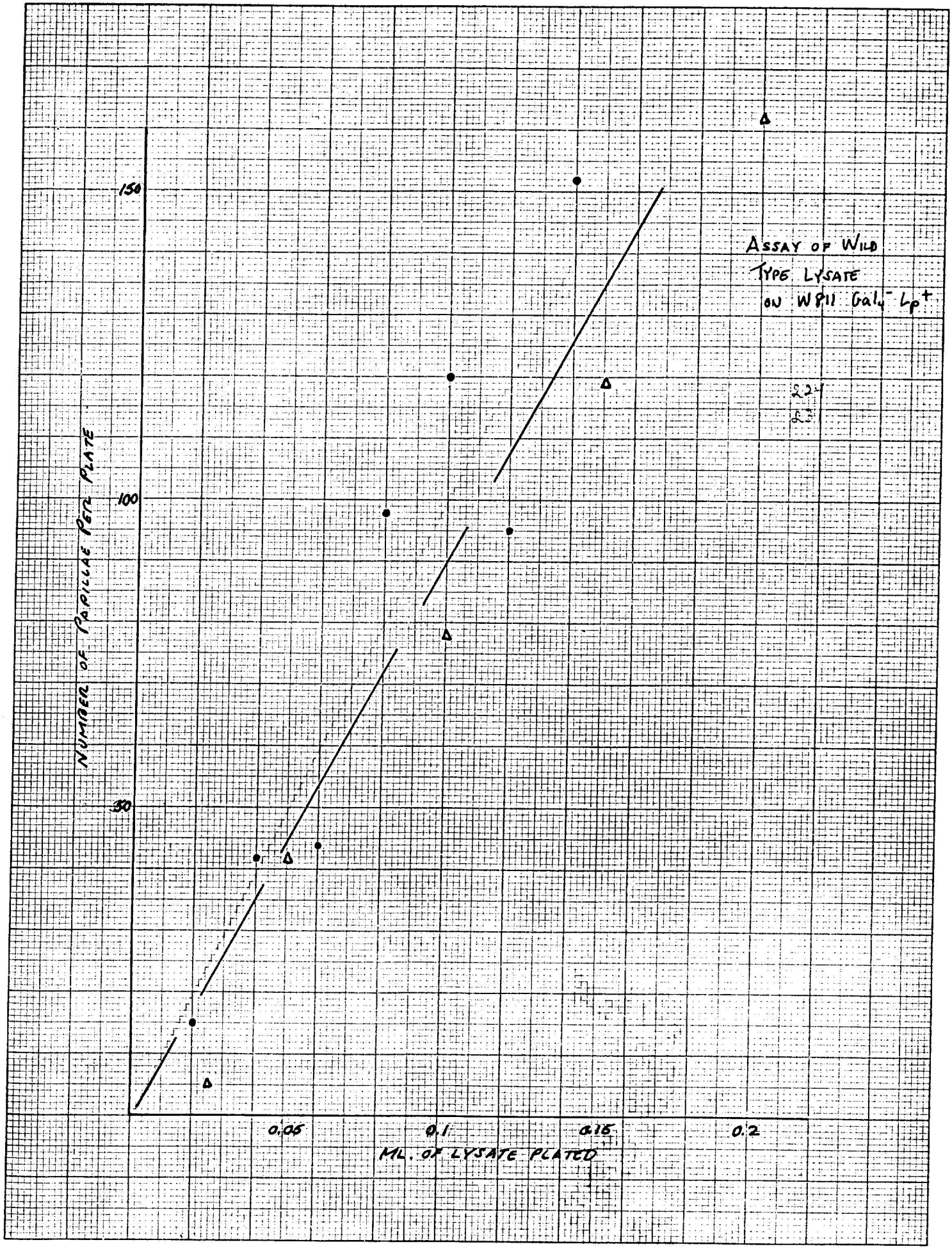
1 = 2 = Lp^S

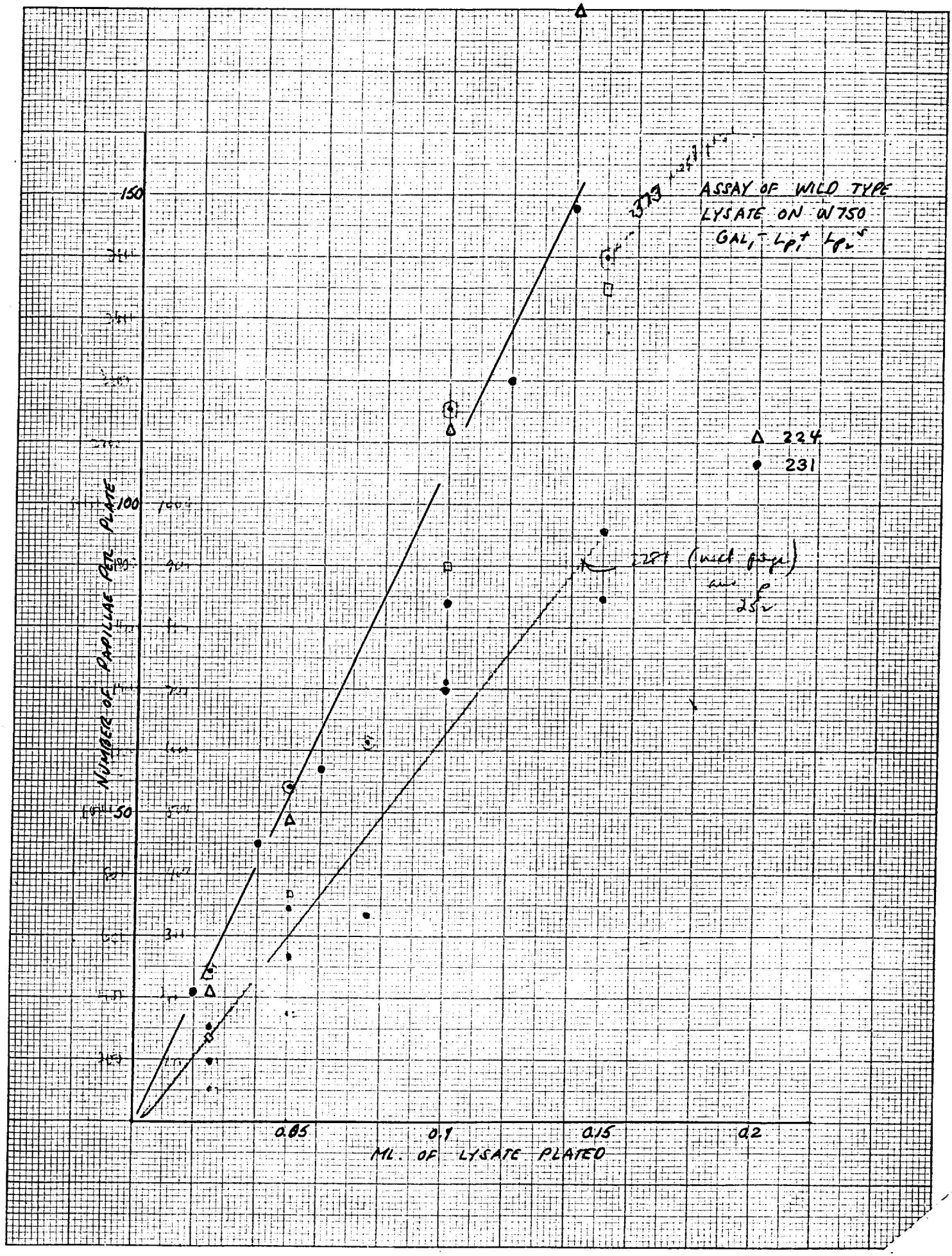
$4^- Lp^V \times 2^- Lp^+$

Total of 250A + 250B

43	4^-	Lp^V
13	2^-	Lp^+
1	2^-	Lp^V
3	$2\text{-}4^-$	Lp^S







Assay - KHz 5/30/53 3.6x10^10 λ/mil

2175

ML	227	231
0.025	16	0.02 11
0.05	34	0.04 43
0.075	72	0.06 53
0.100	—	0.1 97
0.15	119	0.16 138
0.20	—	
0.3	255	

Lpk

750

224	231
0.025 - 21	0.02 - 21
0.05 - 99	0.04 - 45
0.1 - 112	0.06 - 57
0.15 - 183	0.08 - —
	0.10 - 84
	0.12 - 120
	0.14 - 148

84

2500

P 253 KHz 12/28/53

224	231
0.025 5	0.02 - 15
0.05 42	0.04 - 92
0.1 72	0.06 - 94
0.15 119	0.08 - 91
0.2 162	0.1 - 120
	0.12 - 95
	0.14 - 152

0 —
0.025 = 5
0.05 = 9
0.1 = 2
0.15 = 2

Grp -

2281

Lp 5 KHz 12/28/53 1.7x10^10 λ/mil

0	0
0.025 - 96	151
0.05 - 266	345
0.075 - 334	—
0.1 - 703	706
0.15 - 955	842

2377

Lp 5 / 252 KHz 12/28/53 1.7x10^10 λ/mil

0.025 -	483
0.05 -	1079
0.075 -	1223
0.1 -	2314
0.15 -	2799

578 KHz 12/28/53 1.7x10^10 λ/mil P. 253

0	0
.025	276
.05	738
0.1	1778
0.15	2689

1924 P. 253 KHz 12/28/53 1.7x10^10 λ/mil

0	0
.025	53
.05	72
0.1	143
0.15	249

1.7
1.5
8.5
2.5

12/17/53

2345 - Hfr gal₁ - Use as a allele tester by
growth with prototrophic gal - for several hours and then plating out on B gal gal_x

1. Preliminary encouragement.

Tube	From fresh cultures	No. of (+) pap. Plated on B gal after 6 hours
1. 2175 (- gal₁ -)	0.1 ml	12
2. 2345 (- gal₁ -) Hfr	0.1 ml	1
3. 2175 + 2345	0.1 ml each	166

} suggest method may work

12/20/53 2. Cultures apparently need purification in some places
Expt as above.

purify	Parent 2		0	Gal_2 - HfR 2341		Parent gal_1 - 2345-1	
✓	2341		47	—		—	
	2345		3	—		—	
	2343		3	(+) >500	✓	(0) (3)	✓
✓	750		26	(+) >1000	✓	(0) 14	✓
	Lp^+ 1210	2	9	51 (107)	?	(+) >500	
	2175	2	14	(0) 17	✓	(+) >1120	✓
	Lp^s 2281	2	1	(0) 41		(17) 21	
	578	4	11	(+) >10^4	✓	(+) >250	✓

12/29/53

Are lysates "decaying" at room temperature? Inability to repeat massive dilution 578 and the inability to obtain expected ~~amount~~ no. of transduction of Lp^s as compared to Lp^+ says perhaps. This would also argue that transduction of Lp^+ is affected by particles different from those affecting Lp^s dilution.

(Margin note: No transd. of 578 — 2281, 1765 gal₁ - by K12 5/26 λ)

NEW lysates of N16, K12 made - designated 12/28
approximate titers

1. K12 - 167 × 10 × 10^7 = 1.67 × 10^{10} against 2281
2. N16 - >50 × 10 × 10^7 - >5 × 10^9 " "

N1765 gal₁ - made by (-) transd. cultured into stock broth a

W2373

12/31/53

Assay against λ^s - use of K12 λ 12/28/53

1. 2373 - gal$_2$ - Lp^s test Conc. - No. cells in suspension

λ added	pfp/plate	Δ
0.	3	0
0.025	486	483
0.05	1082	1079
0.075	1226	1223
0.1	2314	2311
0.15	2799	2796

$= \frac{1376 + 1104}{2} = 1243 \times 10^7 = 1.2 \times 10^{10}$.

added / plate = 1.2×10^9

2. 2281 - gal$_2$ - Lp^s (Colt) - No cells in suspension - $2145 \times 10^7 = 2.1 \times 10^{10}$

2.1×10^9 added

λ added	pfp/plate
0	0
0.025	96
0.05	266
~~0.075~~	~~344~~
0.1	700
0.15	955

Suspension appears mixed - large and small clones

← 18 ? Nos. picked and added - tabs against slit - all reg λ^+

1/9/53

Purified 2211 - small colony from cell assay $\frac{221}{200} 211 \times 10 \times 10^7 = = 2.1 \times 10^{10}$

2.1×10^9 added

0	0
0.025	151
0.05	345
0.1	542
0.1	706

1/4/54

Cells from aerated 10× conc. cultures.

Assays – K12 λ 12/24/53

1. 881			Δ
	no add.	40	0
	0.025′	94	54
	0.05′	138	98
	0.1	247	207
	0.15′	319	279
2. 1974			Δ
	no add	27	0
	0.025′	80	53
	0.05′	105′	72
	0.1	170	143
	0.15′	276	249
3. 2341			Δ
	no add.	34	0
	0.025	217	183
	0.05′	423	389
	0.1	945	911
	0.15′	1398	1364
4. 518			Δ
	no add.	55′	0
	0.025	331	276
	0.05′	793	738
	0.1	1833	1778
	0.15′	2744	2689

1/11/54

518 Transduction of —, with HFT gal_2- lysate of 1/9/54

① One ml lysate added to one ml of cell suspension – 1/12/54 Incubated at 37C for 10 min centrifuged and resuspended in 1.0 ml broth. diluted and plated.

Non λ exposed cell titer = 708, 889, 794, 889

3280/4 = 820 × 10 × 100 × 100 × 50 = 410 × 10^7 = 4.1 × 10^9/ml

λ exposed cell titer 761, 651, 670, 719

2801/4 = 700 × 10 × 100 × 100 × 50 = 3.5 × 10^9/ml

② Distribution of colony cell types –

	gal (−)	gal (+)	gal- λ culture	Total
1. Control (broth exposed)	3280	0	0	3280
2. λ exposed	2801	31	~~28~~ 54	2886

31 (−) picked and streaked /518 – all non lys.
/λ – all sensitive

30 (+) picked

	#	/518	/λ	Segregating?	#	/518	/λ	Segregating?	#	/518	/λ	Segregating?	Summary
Lp+	1.	lys	R	yes	11.	non lys	s	no	21.	lys Lp+	R	"	+
Lp+	2.	"	"	"	12	Lp+ lys	R	yes	22.	— —	— s	a (−) (no)	23 Lp+
Lp+	3.	"	"	"	13	Lp+ "	"	"	23.	~~lys~~ lys Lp+	R	~~[illegible]~~ yes	3 Lp R
Lp+	4.	"	"	"	14	Lp+ "	"	"	24	lys Lp+	"	"	
Lp+	5.	"	"	"	15	Lp+ "	"	"	25	" Lp+	"	"	
Lp R	6.	non lys.	"	"	16	Lp R non lys	R	"	26	" Lp+	"	"	
Lp+	7.	lys	"	"	17	Lp+ lys	"	"	27	non lys p+	some s plaques?	"	
—	8.	non lys.	s	" ?	18	Lp+ "	"	"	28	~~[illegible]~~ lys Lp+	R	"	
—	9.	" "	s	no (int. 8)	19	Lp+ "	"	"	29	lys Lp+	"	"	
—	10.	—	— s	a (−)	20	Lp+ "	"	"	30	lys Lp+	"	"	
									31	lys Lp+	"	"	

254B Lytic λ – Retest (#3) of lytic λ preps previously reported inactive –
no control spread done.

		non lys
1.	750	3
2.	2281	9 ← all discarded as slow and slow growing
3.	2373	4 1/4
4.	518	6 4/4 tested stable 2 appear slow (+)
5.	811	39 ← all stable (+)

Stocks made

2/4/54 Stocks of these discarded excepting #s 6, 16, 27. Stocks made of these

254-5 = W 2866

254-16 = W 2867

1/13/54

Crosses - check on Lp Sal aggregation

A 1. 1765 X 750 in S sal

39 particles - all (-)

B 2. 2281 X 2035

25 (-)

329 (+)

C 3. 902 X 1655

not counted - many small - appear about 50-50

1/22/53

246A-15 Reversions continued

Reversion	Segregating?	Segregant Character	Proposed Genotype
1	yes	(- -)	1 2 / - - ; + +
2	"	(- -)	
3	"	(- -)	
4	"	(- -)	
5	"	(- -)	
6	"	(- -)	
7	? no	stable +	-
8	? yes	mixed ±	
9	? no	stable +	
10	yes	(- -)	1 2 / + - ; - +
11	"	(- -)	
12	? no	stable +	
13	yes no	"	
14	"	(- -)	a 1-6, 10
15	"	(- -)	
16	"	(- -)	
17	"	(- -)	

2/4/54

811 t 902 (New lysate of 902 - 3 bottles)
for the purpose of isolating HFT gal₄ -
1. no add ½ = 19
2. 0.1 902 λ = 89

2/8/54

750 t 902 gal₂ - (segregants sitting around about a month) suspected of HFT gal₂ - Origin is now cloudy - Originally 3 but one lost
Both in need of purification

#1 Test for HFT		750	#2 Tests for HFT		750
257-1 →	Col. 1.	NFT		1.	HFT
	2.	NFT		2.	"
	3.	HFT		3.	"
	4.	"	257-3 →	4.	NFT
	5.	" (?)	257-4 →	5.	HFT
	6.	"		6.	"
	7.	"		7.	"
257-2 →	8.	"		8.	NFT
	9.	NFT		9.	HFT
	10.	HFT		10.	NFT

2/10/54
NFT cultures examined
257B-1 = gal₂⁻, +R stbl
257-3 - gal₂⁻, +R stbc

W2869

518 transduced with N16 λ 1-9-54 for the purpose of establishing that the transduced colonies contain both Lp⁻, Lp⁺ cells and gal⁻ cells - (segregation for both ?)
Fresh unaerated cul. 518, 0.2 ml cells + 0.4 ml λ incubated at rm temp. 10'
Then diluted 10⁶ and plated β gal

C

plate	(+)	(-)	(-) nibbled	total
1.	1*	28	2	31
2.	1**	32	0	33
3.	0	39	2	41
4.	0	43	1	44
	2	142	5	149 / 4 = 37 X 10⁷ X 10⁶ X 3/1

109 X 10⁷ = 1.0 X 10⁹ cells
3 X 10⁷

* a colony such ●
** " " " ◐
} both streaked - 10 (+) and 10 (-) colonies picked + crossfed /λ 518
see p. 262 for consideration

after 10

	1. λ (+) /518	λ (-) /518		2. λ (+) /518	λ (-) /518
1.	R Lyso.	R lysog.	← these	R nonlys.	sens. nonlys. ← these
2.	"	"		"	"
3.	"	"	(257C-5 +" Lp⁺	"	"
4.	"	"		"	"
5.	"	"		"	"
6.	"	"		"	"
7.	"	"		"	"
8.	"	"		"	"
9.	"	"		"	"
10.	"	"		"	"

257C-6 +" Lp⁺ ? segregant from this

W2868

2/13/54

W2350 - double deletion

parent culture streaked out and 10 colonies examined to eliminate possibility of including reversions at one locus in the inoculum → and therefore transducible by one of the 2 (-) lysates.

1. All 10 colonies not transducible by HFT 2⁻, 4⁻

2. Loopfuls of overnite broth placed on B gal. 2350 = 4⁻ 8⁻

Cells - (c.60 pap) (0 pap.) (c. 20 pap.)

HFT 2 λ — HFT 4 λ — HFT2 + HFT 4 λ

4⁻		−	+
8⁻		+	+
2⁺		+	−

Are the pap on HFT 2 due to reversions at the 8 locus being transduced? If so they should segregate 4⁻ segregants predominantly and no (−−). Pick up 12 and see -

258-A. Of the 12 - 6 found unstable segregants

	HFT 2⁻	HFT 4⁻				Pap	
1.	0	+		0	+	(2⁻)	hard to explain
2.	0	+					
3.	contaminated with (+)			0	+	(2⁻)	
	0	+		−	−	(−−)	
4.	0	+		0	+	(2⁻)	
5.	0	0		−	−	(−−)	
6.	+	0		+	0	(4⁻)	

3 (2⁻
2 (−−
1 (4⁻

3/18/54 stocks discarded 258-B ⇒ pap

3. Examine HFT 2 + HFT 4 for double deletion.

19 examined — Seg. characteristics

	HFT 2⁻	HFT 4⁻	Locus		Reversion stability + pap. charact.	
1.	0	0	(−−)			
2.	0	0	(−−)			
3.	0	+	2⁻			
4.	0	0	(−−)			
5.	0	+	2⁻			
6.	0	+	2⁻			
7.	0	+	2⁻			
8.	0	0	(−−)			
9.	0	+	2⁻	9	0 (+) pap	
10.	+	0	4⁻	10.	46 (+)	⁶⁄₆ stable
11.	+	0	4⁻	11	40	⁶⁄₆ stable
12.	0	0	(−−)			
13.	0	+	2⁻	13	22	⁶⁄₆ stable
14.	0	+	2⁻	14.	9 (+) pap	⁶⁄₆ stable
15.	0	0	(−−)			
16.	+	0	4⁻	16	11 (+) pap	5/5 mixed
17.	0	0	(−−)			
18.	0	+	2⁻	18	cultures went (+)	
19.	0	+	2⁻	19		

autolo: 6, 7, 8 — minute papillating

Contol c minute papillae 18, 19

9 2⁻
7 (− −)
3 4⁻

↑ Tubes labelled 1L

* recleaned (B)
9 (2⁻ or 8⁻ 2⁻8⁻)
7 (2⁻4⁻ - 8⁻4⁻)
3 (4⁻)

3/5/54 Lysate made of #16. Loop spotted

Ac... =	/750	/1210	/841
	0	0	0

2/13/54

W2350 lysate on 2070 - After about 4 days c. 30 small plqs on lysate half - 1-5 larger plq (reversions?) and on control ½ about 5 larger plqs - no smaller -

24 picked to gal for purification - all (-)

2/22/54 Rpt HFT duction with N16

N16 lysate 1/9/54 - 0.5 ml

N16 titer - 2x10^9 $\frac{200}{8.9}$ = c. 20x multiplicity 0.5 ml N16 + 0.5 ml cells

Cell titer = 892 x 10^5 = 8.9 x 10^7

EML analysis EML 867

control plate c. 900/plate - all (-)

exposed plate ¼ of (1) = 213 = 852 per plate - indicates little killing

no. of (+) 21, 23, 35, 24 = $\frac{103}{4}$ = 26 870 | 26.00 (0.03) 2610 = 3.0% (+)

S18 HFT duction with N16 1/9/54

Aerated culture 1/10 - 1 ml sedimented - resusp'd in 0.5 ml N16 added - adsorb. c 3 min centrifuged - Rpt 3 times except no centrifugation after the 3rd - dil 10^2, 10^2, 0.5/10 → plate

See above

Control plates:

37C 371, 341, 319 = 1031/3 = 344 x 10^6 x 2 = 688 x 10^6 = 6.8 x 10^8 $\frac{36}{344}$ = 10.4%

30C 350, 398, 361 = 1109/3 = 369 x 10^6 x 2 = 738 x 10^6 = 7.3 x 10^8 $\frac{39}{369}$ = 10.5%

N16 Phage Plates

37°C 63/763, 22/309, 23/207 = 108/1279 = 8.4% (+) /3 = 426 (/3 = 36)

30°C 31/175, 45/197, 41/194 = 117/566 (= 39) /3 = 189

Esther's Page 667

N16 Assay -

λ 10^7 dil = 106, 130; 236 = 120 x 10^7 = 1.2 x 10^9

Testing of (-) in plate - lp^k and (+) present in any (-) colony?

30 gal - no no

7/28 found mixed lp^+ + lp^s (- in S18) - self plaquing

Transd. — only 2-fold diff? 0.2 ml pipette?

10^7 dil = plaques = 240, papillae = 104

no control plate for spont. done on assumpt = 84 ratio $\frac{pap}{plaques}$ = $\frac{60}{240}$ = $\frac{1}{4}$

Looking for Recombination between hp^r by selecting for (+) recombinant when two diff. gal- ade hp^R

1. This is second attempt - previously no hp^+ found in (+) recombinants between 2341 $gal_1 - hp^R$ x 1924 $gal_2 - hp^R$

2. In this case 2341 $gal_2 - hp^R$ x W1 (= 1924 + 902 $hp^R \rightarrow hp^+$ $gal_2 - hp^R$) out of 33 gal(+) on a Bgal mixed culture of these (probably nicklaus sp. reversion) none found hp^+. Not expected? some in 1st test hp^R_x x hp^R_{1924} failed to give hp^+.

What goes here? 2341 = $\frac{Gal_2 - hp^R}{Gal_2 - hp^+}$

Where did Gal+ come from?

518 + N16 - again for higher fraction (+)

0.4 ml N16 11/9/54 + 0.1 ml of 518 culture c. 10^8 cells/ml

cell assay of above	$10^2, 10^4$	$\rightarrow$	3 plates	1 = 600 mm (+)
Luchsi plate	" "	$\frac{+1}{5}$	3 plates	1 = 163 x 5 815 mm (+)

failure? why?

2/19/53

246A-15 gal+^{R}-1 }
-11 } ~~[illegible]~~
(probably +^{R}-11) ww

246A-15 - labelled 11 reversions examined

~~reversion~~ colony	single seg (+)	HFT (2-)	Structure		Label
1	0	0	++ / --	—	246A-15+^{R}-1A
2	+	0	[illegible]	— a crossover	" " -1B
3	0	0	as 1		
4	0	0	as 1		
5	0	0	as 1		
6	0	0	as 1		

Because gal five? check segregants from culture labelled 246A-15+^{R}-1 to see if conforms to original description of 246A-15+^{R}-11

261 C 3/5/54
Lysate of 261C →

	reversion col.	Minus	characteristics	
	1	6/7	(- -)	1 (gal$_2$-)
	2	stable		
	3	7/7	(- -)	
	4	7/7	(- -)	

} according to this it is 246A

/750	solid spot	} indicates HFT
/120	" "	
/811	" "	

Lysates of 261A + 261B made

Ratio of (+) to minus colonies in	(+)	(-)	Examination of the (-)	
261A	82	44	11 (2-)	4 (- -)
261B	103	63	10 (2-)	6 (- -)

Acting lysate	261A drop	261B drop
750	HFT	HFT
2175	HFT	HFT
578	HFT	HFT

N16 [illegible] According to [illegible] 270 [illegible] NFF
[illegible] N16 [illegible] 1⁻ 2⁻. Since N16 [illegible]
[illegible] from $1^- 2^+ \rightarrow 1^+ 2^-$

2/21/54

257C-2 518 t N16 → + ᵃ lp ᴿ - Examination of the segregants from this

Segregant	518 non lys	N16 NFT 2⁻	518 NFT 4⁻	lp ᴿˣ		
1	non lys	0	0	s	1	+
2	"	0	+(?)	s	mt	—
3	"	0	+	R	✓	—
4	"	+	0	s	2	—
5	"	0	0	s	2	—
6	"	0	+ (poor result)	s	mt	
7	"	0	+	s	mt	
8	"	+	0	s	2	—
9	"	0	+	R	✓	—
10	"	0	+	R	✓	—
11	"	0	0	s	3	
12	"	0	0	s	4	
13	"	0	(0)	(s)	5	
14	"	(0)	(+)	R	✓	
15	"	+	0	s	2	
16	"	0	+	R	✓	
17	"	0	+	R		

2	4	r	
-	-	s	(5)
-	+	R	(6)
+	-	s	(5)

The NFF results suggest that the original [illegible]: $1^- 2^-$ / $1^+ 2^-$

On this hypothesis #2, 6, 7 in the table could be $1^- 2^-$

In which case the [illegible] must have been: $1^+ 2^+ 4^-$ / $1^- 2^- 4^+$

$1^- 2^- \; 1^+$

[illegible] been observed before

Not certain that these segregations came from separate (+) -

In recent reactions on p 242 and in any case [illegible] 1⁻ 4⁻ [illegible] are not (+).

2/2/54 — this is the original (−) that is diploid for some portion of this gal region

246A-15 — Streaked out and (−) colonies picked and tested against Both AFT gal₁ − and gal₂ −. (−) also gal₂ − found − no gal₁ − [disagrees with earlier classification]

The gal − by the present test were plated and (4) revertants obtained and streaked on B gal

gal_1 −	stability of revertants	
1	unst.	+
2	"	+
3	stab.	
4	unstab.	+
5	"	+
6	stab.	
7	unstab.	+
8	"	+
9	stab.	
10	"	
11	unstab.	+
12	stab	

6/18/53 These stocks discarded −

(5 stable
7 unstable)

prototroph obtained!

(246A-15) cross with 1503 gal₁ − EMS gal

10/26/56

246A-15 —— 2 1 / − − —— =+ → arrested = F −

1503 gal − —— + − F+

control plates ok

	(+)	(−)
plate 1.	27	0
2.	3	3
3.	25	3

where did these colonies come from if one is prototroph?

2/22/54

1503 - (-) ductions to obtain gal- in Het stocks?

1. 2 gal_2- obtained one Lp^s — To Stock W2428; one Lp^r — W2429
2. 1 gal_1 - obtained one Lp^u — W2430

Also this day
2431 = 2478-1
2432 = 1673 gal_2-
2433 = T18
2434 = T19

Crosses of gal - Control streaks on B gal = pure

1. Ⓧ 750 aerated - c. 30 (small) / plate × 11 = $0^+/330$
2. Ⓧ 1210 aerated - 9, 8, 13, 12, 12, 14, 12, 6, 6, 8 = $0^+/101$
3. Ⓧ 811 - 3/17, 3/141, 0/15, 1/28, 1/17, 4/15, 0/16, 5/23, 0/29, 2/16 = 19+/190 - (+) growth not all of 18 checked & to see if diploids. 7 found (+) 5 of which may be diploid. (4 were)

Analyses of these diploids by examination of segregants

#2 9 (-) segregants tested against HFT 1-, HFT 4- - all $Lp^{+ or R}$ and gal_4-
#3 10 (-) segregants " " " - all $Lp^{+ or R}$ and gal_4-
#4 8 (-) segregants " " " - all $Lp^{+ or R}$ and gal_4-
#5 12 (-) segregants " " " - all $Lp^{+ or R}$ " gal_4-

~~~~~~~~

W2360 - a Lp^u form of W2350 gal_2- gal_4-
6 colonies tested from old broth streaking out from culture all Lp^u and not transduced by HFT 2 or HFT 4 in two days.

Stock made
~~~~~~~~

3/4/54

Examination of Transduction Complexes (+[4]) for HFT nature of at all

Stocks have been maintained in slant for considerable time (6 mo?)

	Designation Stock Slant	Loop spotted /750	/1210	/811	Characteristic of pp. used to make lysate (−)	(+)	mosaic
	518tK12	solid spot	almost solid spot	solid spot	19	53	5
	750tK12-1	solid spot	solid spot	solid spot	13	79	8
not sterile?	750t902-1	solid spot	solid spot	solid spot	26	110	8
not sterile	750t902-2	0	0	0	14	c.50	4
not sterile?	2175t750-1				4	c.50	1-2 per plate
not sterile?	2175tFW-1				+	2 kinds +++	?
both labelled 2175t811-1	518t750-1	+++	+++	+++	18	83	?
	750tK12-2	+++	+	+++	14	32	5
	750t902-2	+++	+ ck	+++	27	63	6
	2						

HFT Stocks

Designation	Page	Genotype	Comment	Autotrophic Markers
2342 (N16)	192B	gal_2 -		none
2346 (N14)	230	gal_1 -		M -
(01)	153	gal_2 -	from 592A — × 57F	M -
(S1P)	202	gal_4 -	probably NFT now	M -
(#14)	242	gal_2 -		none
(#19)	242	gal_2 -		none
246A-15	246A	gal_2 -		none
20[illegible]-16H	202	gal_2	516 has an NFT E it	M -
257-2	257	gal_2 -	has an NFT E it	M -
257-4	257	gal_1 -	" " " "	M -
247B-1	247	gal_4 -		M -
261A	261	+ u		none
261B	261	+ u		none
261C	261	+ u		none
2175 2750 + u		+ u	750A — × 2175	none
57B E1412	263	+ u		M -
750E 1412-1	263	+ u		M -
750E R02-1	263	+ u		M -

3/21/53

Tests of some lysates for HFT and allele — See also page 265

Lysate	Spotted against 750	1210	811	Comment	Cell Characteristics Making up lys	Stability of Gal+ Revertant*	Yellow crayon #
in NSB → 2342 (3/16/54)	+++	0	+++	Known gal₂⁻ HFT	—		1
Pen- 5 blls. 2342 (3/17/54)	+++	0	+++	Known gal₂⁻ HFT	—	64 12/12 +ᴬ ②	2
2175t811-1	++	small	+++		mixed		3
750t612-2	+++	++	+++	behaves as + HFT	mixed		4
NSB → 750t902-2	+++	+	+++	" " "	mixed		5
247B-1 prop 2	1 pap	2 pap	0	gal₂⁻ HFT lost?			6
2175t F11-1	0	0	2	NFT ?	mixed		7
518t750-1	+++	+++	+++	behaves as + HFT	mixed		8
750t902-2	1 pap	0	2	NFT?	mixed		9
258-16	5 pap	1 pap small	0	gal₁⁻ unstable reversion			10
246A-15	+++	0	+++	gal₂⁻ giving +ᴬ reversion			11
261C	+++	++	+++	segregates (−−) principally			12
518tK12	+++	c. 100 pap	+++	behaves as + HFT	mixed (+)(−)		13
750tK12-1	+++ pap	++	+++	" " "	" "		14
750t902-1	18 pap	2 pap	c. 30	NFT ?	mixed		15
2346					—	5 4/5 +ᴬ ②	

+++ = solid drop
++ = some discrete pap

Margin notes: "two dif. cultures mislabelled here"; "the same?"; "One of these is 2175t811 other 2175t751"; "4/10/54 all tested streak on Pen. 0.5ml sample".

* Gal+ Reversion Stability — no obs., no found +ᴬ/no. examined

Lysate	750	1210	811	Yellow crayon #
4/8/54 750t902-1	0	0	0	16 also tested streak
4/8 750t912-2	+++	+	—	17

3/22/54

Transduction with 2342 (=W16) in NSB

(1.) W 518	No (+)	No. (-)	No(-) contam.	No(-) cul. pap	dilution
Lysate	8	140*	9	1	resusp. in 10 ml, 10^{2}, 1-50 → 2 dil
Broth	0	679*	0	2	"

$8/140 = 140\overline{)8.00}$ 0.057, 700, 1000 = 5.7% (+) transd. on Lp^{S}

* difference in numbers probably due in part to loss in centrifuge tube decantation

2. W 811

	No (+)	No. (-)	No(-) contam.	No(-) cul. pap	dilution
Lysate	1	426 *	0	5	above
Broth	0	448 *	0	1	"

$426\overline{)1.000}$ 0.0023, 852, 1480 = 0.23% (+) transd.

* two colony type - large and small.

3/25/54 Experiment with TCN - Transmission of "injectate" in crosses.

($Hfr\ Lp^{+}Lp_2^{S}$ S treated) exposed to 2342 λ ⊗ $F^{-}\ Lp_1^{+}\ Lp_2^{R}\ TLB^{=}\ Gal_2^{-}$

Experiment Couldn't succeed in first place — gal₂

Absorption of 2342 λ on (2341 streptomycin treated) 1 ml phage + 1 ml cell suspension

[Before 10^{7} = 273, 214 = 2.44×10^{9} /2 1.22×10^{9} ‖ No adsorption $\frac{1.2}{1.2}$ = 0.0
After 5×10^{7} = 30, 26 = 1.15×10^{9} (on agar & on fast plating on B(c)).

on 2252
Before - above
After 5×10^{7} = 15, 13 = 7.0×10^{8}] $\frac{7}{12}$ $12\overline{)7.0}$ 0.58, 60, 100 = (42% adsorpt.)

3/30/54 Repeat examination of adsorption of s^{t} cells.

W 2341 and W 2342 HFT λ (Assays by pour tube)

1. Pre adsorpt. titer = 247, 167 × 10^{7} = 2.07×10^{9} = 2.1×10^{9} (compare with above)

2. S^{t} cells (6.3 ml) + 0.3 ml 2342 λ (NSB prep 1/9/54) - incub. 37°C 10' 9.4 ml broth added centrifuge. Serum
dil 10^{2}, 10^{4} → 0.1 = 444, 482
titer = 463 × 10 × 33 × 10^{4} = 3.3×10^{6} = 1.53×10^{9} % Not adsorbed. $\frac{15}{21}$ = 71% 29% adsorpt.