Research Notes Vol. II

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8/26/53

Beginning again.

1. Purification of HFT stocks.

Testing by picking single colonies - suspd. in HSH and mad. 1 loopful at dist 50 cm 20 seconds and spotting loop on indicator.

N16 - 10 colonies picked 7/10 colonies give strong + reaction 811 stocks made of #1 (1, 2, 3, 5, 7, 8, 10)

N1 - (1) 10 colonies picked 10/10 gave no reaction

(2) 10 colonies picked 10/10 gave no reaction

9/16/53 all remaining gal$_1^-$ in N tested - N12 suggestive. Rc v

2. Use of mad. HFT λ to find auxotrophic mutation which will be transducible.

in 7' HFT gal$_2^-$ λ used. - Del 1-100 from original probably.

(7) cells from unaerated 10 ml culture sedimented - suspd. in 1 ml saline 0.5 ml cell + 1.0 ml HFT λ - Mixed and diluted out cells plated - a dilution of cells grown 12 hrs + and then per min on grown cell.

a. cells used gal$_1^-$ prototroph, gal$_4^-$ prototroph - no evidence of gal + transduction platings - λ probably too dilute? both cases.

b. per min.

[redacted]

1924 lysate made – apparent clearing after 45" exposure – Viscosity to the lysate –

– 0.1 ml of lysate / 578 on EMB(0) – gave 354 plaques / 1/8 plate = 2.8×10^4/ml

plaques appear to be of two sizes(?)

Against 2175
no add – 9
0.1 1924 – 17

2281 vs 750 λ to see if HFT property is 902 peculiarity or property
1210 of gal$_2$ – in general.

1. Transduction

2281	no add	5
	0.1 750λ →	364
1210	no add	2
	750λ – 2	119

24 pop picked from each for purification and HFT examination

apparently none found HFT

Checking on 2251

2305' = (2319) X 2251 on EMB gal.

	(–)
1.	244
2.	197
3.	319
4.	183
5.	208
6.	283
	1434

0(+) / 1434(–)

1673 gal - produced by mad. Previous reference page 206

1673 gal - #5 = W2312 - possibly a non transducible locus

1 Recheck - not transduced by N1, N16, S18, 892 (+), K-12, λ's
9/17/53 re check NFT λ - no transd by 1, 2, 4, K-12
in confirmation and extension of previous

2. Made λ+ - orig λ+ stock lost - new one made. 9/3/53
- clysate made - tested

	750	2175	811	2076 = gal -
0.1 ml lys ½ plate	37/4	7/8	74/17	121/3
control ½ plate				

some suggestion that lysate inhibitory to 0 pmt. pop in phaged ½ of plate.

By t test a new locus - By lysate test gal ?

9/11/53

1673 gal - #11 = W2318 - another possible non transducible locus.

Retest.	λ Source	Result	Comment
1.	N1 (1-100)	no transd.	- area of lysis = spot size
2.	N16 (1-100)	" "	- " " " = c. 300-500 plaques.
3.	S18 (1-100)	" "	- area " " = spot size
4.	2175t 750 (+)	" "	- " " " = c. 30 plaques.

2062 - attempted proline direction - c̄ HFT λ's on D(0)

1. N1 mid. no colonies
2. N16 " " "
3. N7 " " "
4. S18 " " "
5. 2175t 750 (+) " " "

since pr — gal probably > 1% transduction likely

9/13/53

1673 gal - #11

2318 X 811 controls ok

	(-)
1.	32
2.	45
3.	49
4.	52
5.	62
6.	46
	286 (+) 0/286

9/16/53

2251 lysate - Is crossability of 2251 (SRP) really so or an S^r duction?
Check against 578 on B gal and B gal SM

1. B gal - 1/8 plate = 65 pop = 520
2. B gal SM = 0 = 0
3. Replica B gal on B gal SM — no conspicuous growth - Couple minute colonies

2238 lysate #2 tested against 2175, 578

2175 1. control half of plate = 7
2. lysate " " " = 48 - papular picked and streaked.

578 1. control half of plate = 7
2. lysate " " " 8 (lysis)

2312 lysate. (2312 = 1673 gal - #5) Tested against 2175

This lysate also does not appear to transduce 2175 previously found also - does appear to t other gal - see pg. 220
lysate appears inhibitory to 2175. Rechecked in adsorpt. type expt.

Broth Control = 19
Adsorbed tube = 28. 12 per level - are stable(+) after 2.

2312 made λ^+ - this is second time - Culture (λ^+) used for lysate above discarded by mistake.

9/17/53

2312 X 811 - controls ok EMB gal

S gal	(+)	(-)
1.	0	30
2.	0	14
3.	0	24
4.	0	18
5.	0	28
	0	114

See also page 206

1/84
0/114
1/198

2238 transductions see page 221
2175E2238
24 pupillar picked

1st S	2nd	3rd
8/24 +"	8/24 +"	9/24 +"

518 by 2238λ Spread plates

1. no add 19
2. 2238λ 26

811 by 2238λ

1. no add 46
2. 2238λ 55

doesn't go?

750 by 2238λ

1. no add 4
2. 2238λ 24

2nd	3rd
11/24 +"	13/24 +"

2312 X 902 - controls ok
EMB gal

4 plates (-) = 1/8 = 66 = 528 X 4 = 2112
(+) = 1 confirmed on stk on EMB gal.

1(+)
2112(-)

9/20/53

578. Adsorption of HFT 2 λ (3rd batch)

1st (+) 39 total cells 164 x 8 = 1312 dil. $10^6 \times 10$ 1.3×10^{10}

= 1312 / 39.00 = 3% transduced

2nd (+) 7 64 x 4 = 256

276 / 7.00 = 3%

276 / 7.00

8 (+) colonies picked and streaked out 2 times
Rep. colonies then picked and cross breaked on 578, and streaked out

Colony	/578	simult. Streaked	(+)	4 Colonies picked from each hypogenicity examined /578 (+)	(−)	(−)
1.	str. lytic	mixed	+	+	+	+
2.	" "	"	+	+	+	+
3.	" "	"	+	+	+	+
4.	non lys.	"	−	−	−	−
5.	str. lytic	"	+	+	+	+
6.	wk. lytic	"	+	+	+	+
7.	str. lytic	"	+	+	+	+
8.	wk lytic	"	+	+	+	+

see if still segregating

9/24/53

Assay of Lysates - Linearity

K-12 lysate 5/30/53

750

	Lysate	No pap	Δ
1.	0	2	0
2.	0.025	23	21
3.	0.05	51	49
4.	0.1	114	112
5.	0.15	185	183
6.	0.2	144	142
7.	0.3	132	
0.	0.5	70	

Comparison

λ	750	811
0.025	21	5
0.05	49	42
0.10	112	78
0.15	183	119
0.20	142	162

811

			Δ
1.	0	99	0
2.	0.025	104	5
3.	0.05	141	42
4.	0.1	177	78
5.	0.15	218	119
6.	0.2	261	162
7.	0.3	231	132

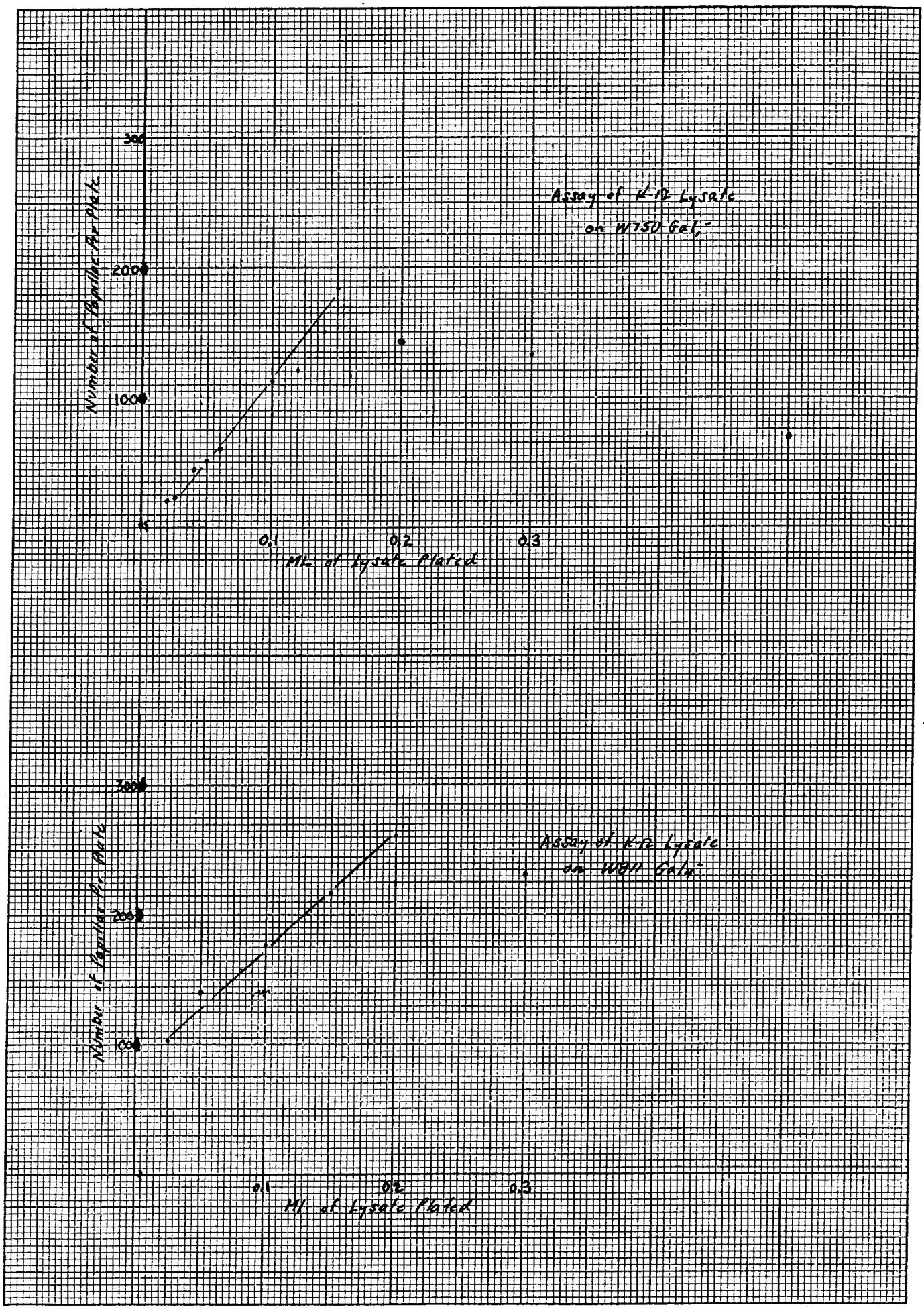
1027 - A second tpt - Lwoff attempt

		streaked 518
1.	Supernat. both before inad. -	0
2.	Centrifugate (after 3 hours inc.) of inad. culture -	7 plaques.

Does gal- transduce gal- and vice versa?
In case usual technique allows inhib. of transd. expres. adsorption type experiment done - "No add" plate run as exptl exept both used.

750

	no add	1
	811 λ-8	9
811	no add	162
	750 λ-2	201
518	no add	>40
	750 λ-2	73
1924	no add	33
	750 λ-2	43



Form No. 999 — 20 Squares to Inch
AMERICAN PAD & PAPER CO., HOLYOKE, MASS.

9/24/53

2312 – a double minus – gal_2^- and gal_x^- outside the region of transduction? Cross and examine ei prototrophs to see if two classes of gal – can be recovered.

gal_2^-, gal_x^- in which gal_x^- does transduce gal_2^-

1655 X 2312 controls ok.

	(+)	(-)
1.	2	0
2.	3	0
3.	2	0
4.	7	0
5.	3	0
	17	0

Repeat using pr addition to medium –

811 adsorption of K-12λ 5/30 – 36 x 10^10 (see UV NTG Sept) + array p 224

array 10^2, 10^4, 10^6, 10^7 0.1 272, 347 = 310 x 10 x 10^7 = 3.1 x 10^10 cells/ml

1. 0.8 ml cells + 0.8 ml K-12λ

0.1 ml = 108 papillae
41
net 67

broth control = 41

0

67 (67/78)

2. 0.7 ml cells + 0.7 ml K-12λ
= 150 papillae
108
net 42

109

3. 0.6 ml cells + 0.6 ml K-12λ
= 132 papillae
no adsorption

91

36
66
27

3.6
.86
216
288
3.096

1st ad. = 3.1 x 10^10 λ adsorbed
2nd ad. = 5.7 x 10^10 λ adsorbed
5.8 x 10^10

Total

5.8 x 10^10 adsorbed / 3.1 x 10^10 cells = ave 1.9 λ/cell

2297 E K-12λ

1/2 plate = control – slight diff. it
1/2 " = 0.1 ml K-12λ – at all in nos. – suggest ion of inhibition by lysate. Cap around edge.

9/27/53

Adsorption: K-12λ 5/30

750 cell assay $10^2, 10^4, 10^6, 10^7$ = 122, 127 $125 \times 10 \times 10^7 = 1.25 \times 10^{10}$

centrifuged resuspended in orig. volume

1. 1.0 ml cells + 1.0 ml K-12λ = 0.1 ml = 45
5/30 0.1 ml broth exposed = 3
net = 42

2. 0.9 ml + 0.9 ml 0.1 = 159 net 156

3. 0.8 + 0.8 0.1 = 159 156

2175 cell assay $10^2, 10^4, 10^6, 10^7$ = 99, 130 = $115 \times 10 \times 10^7 = 1.15 \times 10^{10}$

as above

1. 1.0 ml + 1.0 ml K-12λ = 52 broth control = 20 net = 32
2. 0.9 ml + 0.9 ml K-12λ = 103 net = 83
3. 0.8 ml + " = 97 net = 77

Cross

1655 X 2312 Repeat. Proline added Aerated 1655 on EMS gal controls ok.

	(+)	(-)
1.	2	0
2.	2	0
3.	0	0
4.	1	0
5.	2	2 (?)
6.	1	1 ?
	8	3

} check

Stocks of 2312 λ+, 2076 λ+ } made after testing 8 colonies of each to see if are lysogenic

1436 lost its Sr

✓ for gal allele

/N1	/N16	S18
++	++	oo

apparently still galy

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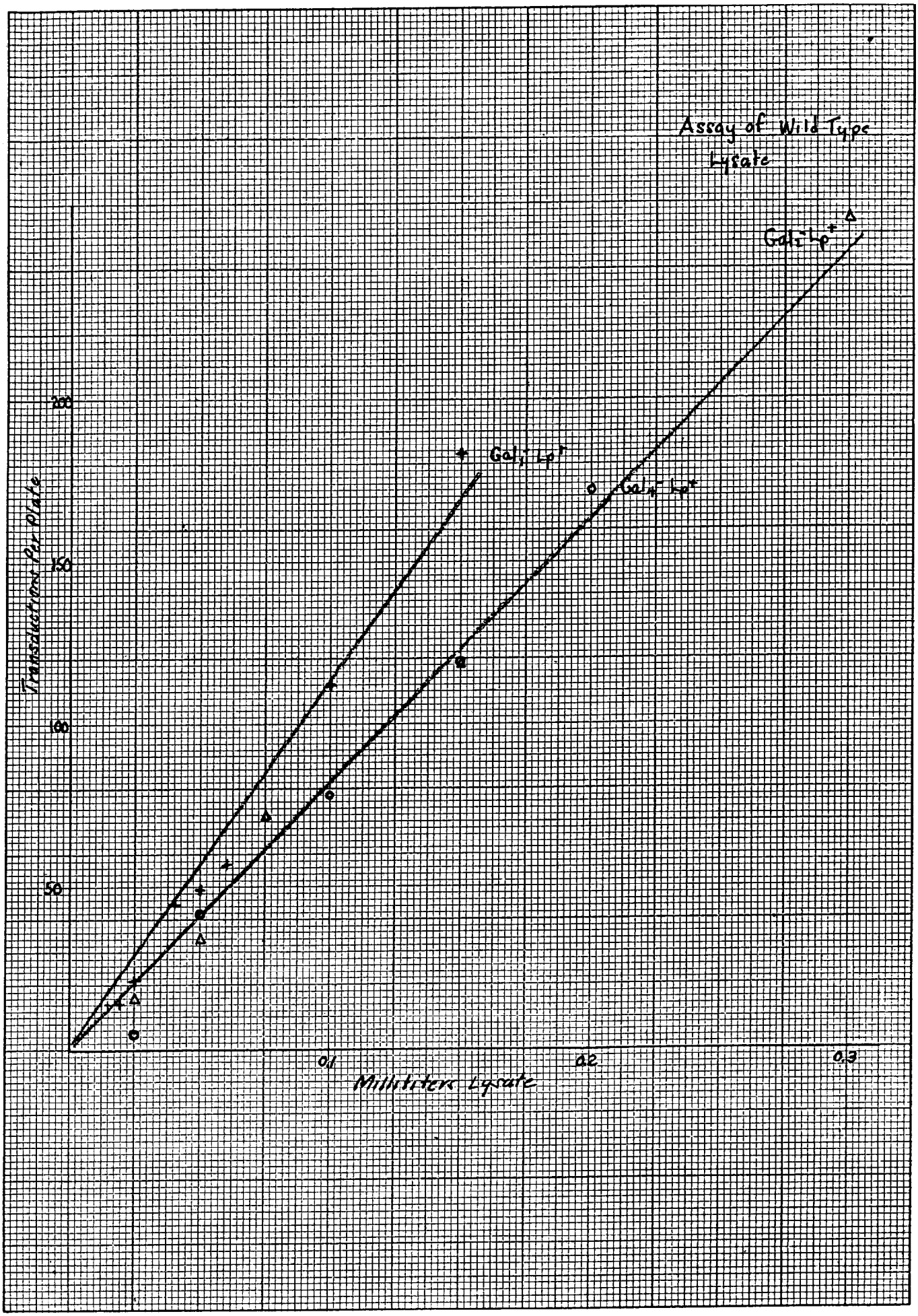

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9/28/53

~~adsorption K-12 5/30~~
~~750 [illegible]~~

750 transduction with 902 λ to isolate NI HFT – also crossable one

1. no add half of plate = 1
2. 0.1 ml 902 λ = 126 pick 24 and test

2175 ~~adsorption~~ Linearity of assay · K-12 λ 5/30

cell assay 10^6 568, 450 = 509 X 10 X 10^6 5.09 X 10^9/ml

	no pop	Δ
1. no add	18	0
2. 0.025 M	34	16
3. 0.05	52	34
3. 0.075	90	72
4. 0.100		
5. 0.15	137	119
6. 0.2		
7. 0.3	273	255

5/4 90

2175 - duction versus variable cell nos.
cell assay 10^7 = 257, 217, = 237 X 10 X 10^7 = 2.37 X 10^{10}

Cell nos	Dilution	Sp. pop	Transd. plate	Δ
2.4 X 10^9	und.	23	83	60
1.2 X 10^9	1-2	22	106	84
6 X 10^8	1-4	13	104	91
3 X 10^8	1-8	18	129	111
2.4 X 10^8	1-10 (?)	17	102 ?	85
1.2 X 10^8	1-20	14	135	121
6 X 10^7	1-40	14	115	101
3 X 10^7	1-80 (?)	14	92	
2.4 X 10^7	1-100	19	109	90
1.2 X 10^7	1-200	15	93	78
6 X 10^6	1-400	16	87	71
3 X 10^6	1-800	8	83	75
2.4 X 10^6	1-1000	11	84	73

518 Lytic λ ?

c. 6 X 10^9 - 10^{10} cells 518 exposed to 1.0 ml K-12 λ 5/30 twice
centrifuged after each exposure – supernat discarded
after second adsorption – Resuspended in NSB (10 ml)
with aeration – 5 hours – non-turbid

Titrated /518 (BCo) after ~~[illegible]~~ centrifugation

/2175 on EMB gal
control 1/2 = 10
0.1 ml lytic λ = 12

/2062 D(0)
no growth
either half of plate

10^8 = 331 ? 3.3 X 10^{10}
10^7 = 150 X 16 = 2400 X 10^7 = 2.4 X 10^{10}

10/1/53

2281 transductions

1. 902 λ = 0
2. K-12 λ = 21
3. 750 λ = 256
4. 2238 λ = 19
5. 811 λ = 97

} picked and streaked -

2281 t K12	2281 t 750	2281 t 811
12/18? + 4" attr 2	24/24 + 4" att. 2	20/24 + 4" att. 2
19?/1	22/24 + 4" 3	23?/24

1485 lytic λ

1. c. 5×10^9 - 10^{10} cells exposed (via adsorption technique) to 811λ-9 twice centrifuged, resuspended in NSB - Equivalent no cells both treated, used as control - Incubated with aeration - c. 45 hours. After 5 hours phage tube transparent but dark, control turbid.

Assay.

10^7 dil - 1/16 = 150+ = $2400 \times 10^7 = 2.4 \times 10^{10}$
10^8 dil - 294 = $294 \times 10^8 = 2.9 \times 10^{10}$

Transducing action.

	control ½ plate	phage ½ plate = 0.1 m
750	3	2
518	9	8
2175	7	8

229

A

10/6/53

Segregants from 2281 - Picked after 2 (+) picking and streaked.

2281 transduced by K-12

Segregant	Lp	/S18	/N16	Alkle
1.	r	+	o	2-
~~2.~~	~~r~~	~~Gal +~~		
~~3.~~	~~s~~	~~+~~	~~+~~	contaminated with +?
4.	r	+	o	2-
5.	r	+	o	2-
6.	s	-	+ ?	?
~~7.~~	~~s~~	~~+~~	~~+~~	contaminated ō + ?
8.	s	+	o	2-
9.	s	+	o	"
10.	s	+	o	"
11.	r	+	o	"
12.	s	o	o ?	?
	7s ~~IIII~~ 5 5r IIII 4	↑ Gal+	Gal-↑	

$$\frac{5+9}{12+16} = \frac{14}{28} = 50$$

Added to 233 for correlation of lysogenicity and transduction in table summary

10/20/56 What goes? Sensitive segregants

229
(B)

2281 t 750 (-) seq -

Seq #	Lp	/N7	/N16	Attack
1.	r	0	0	?
2.	r	0	0	?
3.	r	0	+	1-
4.	r	0	0	?
5.	s	0	+	1-
6.	← gal + →			
7.	r	0	0	?
8.	r	+3	+3	
9.	r	+ 15	0 1	2-
10.	r	0	+	1-
11.	r	0	0	?
12.	r	0	0	?
13.	r	+ 5	+ 7	?
14.	r	0	0	?
15.	r	0	0	?
16.	r	0	0	?
17.	—	—	—	?
18.	r	0	0	?
19.	r	0	0	?
20.	r	0	0	?
21.	r	0	+	1-
22.	r	0	0	?
23.	r	0	0	?
24.	r	0	0	?
	1 s	↑		4 1-
	22 r			1 2-
				17 ?

mix up 1st run

This legate probably not cure [illegible] ?

Gal_1^- Gal_2^-

contaminated ?

disregard

$$\frac{22+16}{23+21} = \frac{38}{44}$$

229
C

2281t 811 Seq

Seq #	tp	/N16	/S18	Athlete
1.	r	o s	+	2
2.	r	o s	+	2
3.	r	+	o	4
4.	r	+	o	4
5.	r	o s	+	2
6.	r	o s	+	2
7.	r	o s	+	2
8.	r	o s	+	2
9.	r	o s	+	2
10.	r	o s	+	2
11.	s	o s	+	2
12.	s	o s	+	2
13.	r	Gone +	→	–
14.	r	o s	+	2
15.	r	+	o	4
16.	r	o s	+	2
17.	r	o s	+	2
18.	s	o s	+	2
19.	r	o s	+	2
20.	r	o s	+	2
	3 s 16 r	↑ Gal 2⁻	↑ Gal 4⁻	16 2⁻ 3 4⁻

discard

$$\frac{16+17}{19+21} = \frac{33}{40}$$

750t902 In search of HFT

Note 6/18/54

Culture and lysates of this referred to as NA-4

(−) seq #	NA-1	N16	Allele		Grace related in HFT test /2175	/750
1	0	+	gal$_1^-$	1.	0	0
2	0	+		2.		
3	0	+	"	3.		
4	0	+		4.	+ ?	
5	+	0	gal$_2^-$		0	
6	−	+	gal$_1^-$	5.		
7	0	+		6.		+
8	gal +	clone			+	+ 0
9	0	+		7.	0	0
10	0	+		8.		
11	0	+		9.		
12	0	+		10.		
13	0	+		11.		
14	0	+		12.		
15	gal +	clone			+	+
16	0	+		13.	0	0
17	0	+		14.		
18	0	+		15.		
19	0	+		16.		
20	0	+		17.		
21	0	+		18.		
22	gal +				+	+

18 gal$_1^-$
1 gal$_2^-$

{ lysate made and behaves as HFT in 2175, 518 - (more so on 2175)

11/20/53 Second lysate made also HFT gal$_1$ - Stock made - Entered in stock book as W2346

W2346

W2346 = 4, 7, 9, 11 } 6/20/54 failed to survive on stock

10/10/53

Linearity –

750 – K-12λ 5/30

		prop	Δ		
1.	no add	3	0		
2.	0.02	24	21		105
3.	0.04	48	45		113
4.	0.06	60	57		
5.	0.08	68	65	low – remain portion (0.2 – 0.12) of pyrette	
6.	0.1	87	84		84
7.	0.12	123	120	10/12	100
8.	0.14 (2)	152	148	10/14	105
~~9.~~	~~0.16~~	~~117~~	~~114~~		507/5 = 101

2175

			Δ		
1.	no add	21	0		
1.	0.02	32	11		55
2.	0.04	64	43		108
3.	0.06	74	53	10/6	88
4.	0.08	62	–	low – remain portion of 0.2 ml pyrette	
5.	0.1	118	97		97
6.	0.12	101	80		348/4 = 87
7.	0.14	101	80		
8.	0.16	159	138		

811

			Δ		Converted to 0.1 ml
1.	no add	37	0		
2.	0.02	52	15	×5 =	75
3.	0.04	79	42	×2.5	104
4.	0.06	81	44	× 10/6 =	73
5.	0.08	135	98	10/8 =	122
6.	0.1	157	120		120
7.	0.12	137	98	10/12	80
8.	0.14	189	152	10/14	108
9.	~~0.16~~	~~161~~	~~124~~		682/7 = 97.4

W945 (relative of W902) Test to see of Lp_2^R by attempt. direction
one plate
1. no add ½ = 12
2. K72λ 5/30 = 2 } looks like inhibiting effect

W1436 ✓ in nutrition

D(0)	no growth	
D(0)+TB₁	no growth	
D(0)+LB₁	slight growth	+
D(0)+LB₁	growth	++
D(0)+LB₁	growth	+++

10/13/53

Comparison of 2238 and 2297

	Control half	0.1m KCl half
1. 2238	62	53
2. 2297	7	36

2281 ductions - Repeated to examine segregants
control halves are all barren

1. 750λ =	214	–	~~21/24~~
2. K-12λ	46	–	~~18/24~~
3. 811 λ =	98	–	~~24/24~~

N16, NA4 Yield of HFTλ from

Procedure - Aerated cultures - centrifuged and resuspd. in 1.1 ml saline
c. 10^{10} cells/ml

Dilutions

N16

10^8
↓
10^6
↓
10^4 → in 15" $\xrightarrow{\text{dil}}$ 10^2 NS8 incubate 37c → ductions assay
→ λ assay
→ cell survival.
→ phage assay -
→ transduction assay

Pre Irradiation - No's given for irrad. tube. Post - Irradiation

	N16	NA4	N16	NA4
Plaque Assay				
Cell Assay	1.34×10^3	2.9×10^3	3.37×10^2	3.87×10^2
Induction Assay				

deduct work

see p 232 for [illegible]
24 picked 16/24 +4

10/21/53

2281 t K12 Batch II Repeat exam. because of Lp.s seq.

Seq	Nib	Sif	Lp.	Ankle	
1.	0	+	s	2⁻	
2.	0	+	s	"	
3.	0	+	r	"	
4.	0	+	s	"	
5.	0	+	s	"	
6.	0	+	r	"	
7.	0	+	s	"	
8.	+	+	r	?	re examine 2⁻ on reexamination after purification
9.	0	+	r	2⁻	
10.	0	+	s	"	
11.	0	+	s	"	
12.	0	+	r	"	
13.	0	+	r	"	
14.	0	+	r	"	
15.	0	+	s	"	
16.	0	+	s	"	
			9 s	16 2⁻	
			7 r	~~1 ?~~	
			56% Lp.s		

For crosses X 902	(1) (+) 8 plates per (−) X		Control stks. oh except 902 has c. 10 (+)	(2) cross	(3)	
233-3	~~[illegible]~~	> 4665	~~[illegible] 902 contam?~~	> 3140 (−)		[illegible]
-7	0	> 4992	oh			
-13	0	106	due to lack of revitalization of 13 cult.?	20 (−) 6 plates	276 | 9 plates / 402	
-16	0	> 4552	oh			

(−) Counts made on smallest plate, therefore (−) #'s are minima. Variation among plates ± 10%

233-B a F retrial? cross c̄ 902 F+

survive in stock

2

10/21/53

2281 t 750 Batch II Repeat for tp. characterization

Seq	N16	N1	tp.	acute	
1	o	+	r	2⁻	
2	o	+	r	.	
3	o	+	r	..	
4	o	+	r	..	
5	o	+	r	..	
6	o	+	s	..	
7	o	+	r	..	
8	o	+	s	..	
9	+	+	r	?	reexamine - 2⁻ on reexam. after purification
10	+	o	r	1⁻	
11	o	+	r	2⁻	
12	o	+	r	.	
13	o	+	r	.	
14	o	+	s	.	
15	o	+	r	.	
16	o	+	r	.	
17	o	+	r	.	
18	o	+	r	.	
19	o	+	r	..	
20	o	+	s	..	
21	o	+	s	..	
			16 r	10 2⁻	
			5 s	1 1⁻	
				?	
			23% s		

Official Expt

2281 t 750 p. 236B

[illegible] x 902

61

48

14

10

10/21/53

2281 + 811 Batch II - Repeat for Lp. ✓

Seq	Nib	Slt	Lp	Allele
1	o	+	s	2⁻
2	o	+	r	"
3	gal +	→		
4	o	+	r	2⁻
5	o	+	r	"
6	o	+	r	"
7	o	+	r	"
8	o	+	r	"
9	o	+	r	"
10	o	+	r	"
11	o	+	r	"
12	o	+	r	"
13	gal +	→		
14	o	+	s	2⁻
15	o	+	s	"
16	o	+	r	"
17	o	+	r	"
18	o	+	r	"
19	o	+	s	"
20	o	+	r	"
21	o	+	r	"
22	+ ?	+	r	?
23	o	+	r	2⁻
			4 s	21
			17 r	~~20~~ 2⁻
				~~1 ?~~
			19% s	

discard

2⁻ on reexamination after purification

2~~X~~36A

10/27/53 Batch III

2281tK12 Repeat - as in 2~~X~~36B

Papillae	2281	pep sheathed out	Sequigant	NH	NI		
~~1~~	lys	mixed	(mixed +)	—	—	~~mixed +~~	—
~~2~~	"	"	mixed +	—	—	mixed +	—
~~3~~	"	"	(mixed +)	—	—	mixed +	—
4	"	"	(+)	—	—	lost	—
~~5~~	✓ non lys	"		—	—	(+)	—
(1) 6	lys	"		—	+	Lp+	(1)
(2) 7	"	"	non lys	—	+	"	(2)
(3) 8	✓ non lys	a gal(-) mixed		—	+	"	(3)
(4) 9	lys	mixed		—	+	λ↑?	(4)
(5) 10	"	"		—	+	Lp+	(5)
~~11~~	"	"		—		gal [illegible]	
(6) 12	"	"		—	+	Lp+	(6)
~~13~~	"	"	mixed (+)	—		gal +	—
~~14~~	"	"		—	—	mixed (+)	
~~15~~	✓ non lys	a pure gal +		—	—	lost	—
(7) 16	lys	mixed		—	+	Lp+	7
(8) 17	"	"		—	+	"	8
(9) 18	"	"		—	+	"	9
(10) 19	"	"		—	+	"	10
(11) 20	"	"		—	+	"	11
(12) 21	"	"		—	+	"	12
(13) 22	✓ non lys	"	non lys	—	+	λ	13
~~23~~	"	"	~~[illegible]~~	—	—	mixed	—
	~~[illegible]~~		~~[illegible]~~			2 Lp+/13	
	11 Lp+					11 Lp+	
	2 Lp r+s					2 Lp+	

discarded by accident

Official

~~2281tK12~~

Expt. 233

Original
1 gal

10/26/53

Batch III - pop, 2(+) picked, 2(+) tested / 1485, streaked out on gal and a (-) picked and tested

2281 + 750

Papillae	1485	pop streaked out	Segregant	New Segregants	N1	N16
1	lys	mixed	lys	Lp⁺	+	0
2	"	"	"	"	"	"
3	"	"	"	"	"	"
4	"	"	"	"	"	"
5	"	"	"	"	"	"
6	"	"	"	"	"	"
7	"	"	"	"	"	"
8	non lys.	"	non lys	sensitivity	"	"
9	lys.	"	lys	Lp⁺	"	"
10	"	"	"	"	"	"
11	"	"	"	"	"	"
12	"	"	"	"	"	"
13	"	"	"	"	"	"
~~14~~	~~non lys~~	"	non lys	—	lost	→
15	lys	"	lys	"	+	0
16	"	"	"	"	"	"
17	"	"	"	"	"	"
~~18~~	~~non lys~~	~~pure (+)~~	~~non lys~~	—	lost	→
~~19~~	~~non lys~~	~~(+) (-) intermed~~	~~non lys~~	—	~~lost~~	→
20	lys	mixed	lys	"	+	0
21	"	"	"	"	"	"
22	"	"	"	"	"	"
~~23~~	~~"~~	~~"~~	~~Dotman Lp⁺~~	~~Lp⁺~~	~~+~~	~~0~~
24	"	"	lys	"	"	"
20 pop/s	19 Lp⁺		3/22 non lys.			All = gal₂⁻ = 20
1/2	1 Lp⁺ +5					

Not
Recent seg.
analysis

Chromosomes	X902	I (-) pure	II
4		906	131 = 1037
8		>10 572	
12		contaminated Ct	
16		" "	
21		110	
6		>100 x 8 = >800	

6/20/54 on stock stands

out 5/13/53

236C

10/26/53

2281 t 811 - (As 2281 t 700) on 236B

Papulae	1485	pap streaked out	Segregants	new Segregants	N16	S18	Atlete
1	lys	mixed	lys	lys	0	+	2-
2	"	"	"	"	"	"	"
3	"	"	"	"	"	"	"
4	"	"	"	"	"	"	"
5	"	"	"	"	"	"	"
6	"	"	"	"	"	"	"
7	"	"	"	"	"	"	"
8	"	"	"	"	"	"	"
9	"	"	"	"	"	"	"
10	"	"	"	"	"	"	"
11	"	"	"	"	"	"	"
12	"	"	"	"	"	"	"
13	"	slow, (-) ?	"	"	"	"	"
14	"	" " ?	"	"	0	0	? --? on V in Lp_2^{5}
15	"	" " ?	"	"	0	+	2-
16	"	mixed	"	"	"	"	"
17	"	"	"	"	"	"	"
18	"	"	"	"	"	"	"
19	"	"	"	"	"	"	"
20	"	"	"	"	"	"	"
21	"	"	"	"	"	"	"
22	"	"	"	"	"	"	"
23	"	"	"	"	+	0	4-
~~24~~							

0/23 non lys

21 gal_2-
1 gal_4-
1 gal_x-?

W2350

may by ok?
No.
Segregant-seg
discarded before
classification - hyst

For cross x 902, 1483.
x 902
6 0/117
10 >137x8=>1096
14 (150x8) x 10 = >12000 (really prototrophs? lot of blyd growth)
23 3(?)/39144.

6/18/54
Stocks of
these made
and saved
Remainder discarded
ok. 6/30/54

out 7/13
" "

2/9/54	811	2175	locus
hysates	~~XV~~		
23.	1/1	9/1	4-
14.	0/1	8/1	-- ?

10/27/53 Examination of the Segregants from HFT ductions

In each case pap., and 3 (+) colonies picked in purification

1. 750 transduced by N16 gal_2^-

(a) 18 papillae picked – after purification 15 segregating = ~~[illegible]~~ 15/18 5/6 = 83.3%

(b) examination of segregants from [illegible] not λ^s

	N1	N16	Fresh
1.	–	+	1⁻
2.	–	+	1⁻
3.	–	+	1⁻
4.	mixed		
5.	–	+	1⁻
6.	–	+	1⁻
7.	+	–	2⁻
8.	–	+	1⁻
9.	mixed		
10.	–	–	? double? on rev ✓
11.	+	–	2⁻
12.	–	+	1⁻
13.	–	+	1⁻
14.	–	+	1⁻
15.	–	+	1⁻
			10 1⁻
			2 1⁻
			1 ?

not t N1, N16, possibly by S18 – a double(–) ?
lysate of this in
2175 = 7/3 (exptl/control)
700 = 1/1
This culture also found to be Lp_2^s
Cross × 902 – controls ok.
1 plate = 207 × 8 = 1656 (+) no (+)

This is W2586

2. 750t S18 gal_4^-

(a) after purification 9/18 = 50% segregating

(b) examination of segregants

	N1	S18
1.	–	0
2.	–	+
3.	mixed c̄ (+)	
4.	–	+
5.	–	+
6.	–	–
7.	–	+
8.	–	+
9.	–	+

3
2~~1~~7B

10/27/53 HFT duchain

(3.) 2175 transduced by N1 gal$_1$ -

(a) after purification 10/18 still segregating = ~~[illegible]~~ all not λ^s.

(b)

	N1	N16	locus
1.	— ?	—	?
2.	—	+	1⁻
3.	—	+	1⁻
4.	—	+	1⁻
5.	—	+	1⁻
6.	—	+	1⁻
7.	+	—	2⁻
8.	—	—	?
9.	—	+	1⁻
10.	+	—	2⁻
			6 1⁻
			3 ~~2~~ 2⁻
			1 ~~4~~ ?

~~(-)(-) on recheck - but transduced by S18 - λ double~~

on subsequent test not + N16, ± by N1, S18 = gal$_1$

(-)(-) on recheck - transd. by S18 - a double

(4.) 2175 transduced by S18 gal$_4$ -

a. after purification 13/18 still segregating

(b) 7. all not λ^s

	N16	S18	locus
1.	— mixed		
2.	+	—	4⁻
3.	+	—	4⁻
4.	—	+	2⁻
5.	—	+	2⁻
6.	—	+	2⁻
7.	+	—	4⁻
8.	+	—	4⁻
9.	—	+	2⁻
10.	—	+	2⁻
11.	—	+	2⁻
12.	—	+	2⁻
13.	—	+	2⁻
			8(~~2~~)
			4 (4⁻)

10/27/53 HFT transductions

(5.) 811 transduced by N16 gal_2 –
(a) after purification 15/18 still segregating
(b) Examination

	N16	S18
1.	+	–
2.	+	–
3.	+	–
4.	+	–
5.	+	–
6.	+	–
7.	+	–
8.	+	–
9.	+	–
10.	+ ?	–
11.	+	–
12.	+	–
13.	+	–
14.	+	–
15.	+	–

10/29/53

1765 Gal- mutants - Induced in B gal c̄ U.V. c 10 seconds exposure

Isolate #	518	516	1N1	518+892	Growth	Article?
1 not (+)						
2	0	0	− 0	− 0	Lp^r ? or slow?	
3						
4 not (+)	+	+	+	+		
5	+	+	+	+	Lp^s	
6					growth too thin	
7	+?	+?	+?	+?	Lp^s	
8	+	+	+	+	Lp^s	
9	−	−	−	−	Lp^s	
10	+	+	+	+	Lp^s	
11	+	−	+	−	Lp^s	
12	+	+	−	+		
13						

2	−	−	−	−	Lp^r or slow?	?	
4	not gal(+)						
5	+	+	+	+	Lp^s	new	
6	+	+	−	+	growth too thin	Gal₁⁻	
contaminant 7	−	−	−	−	Lp^r or slow	~~[illegible]~~?	
8	+	+	+	+	Lp^s	new	
— not slow — 9	−	−	−	−	Lp^r or slow	~~[illegible]~~?	
10	+	+	+	+	Lp^s	new	
11	−	+	−	+	Lp^s	Gal₄⁻ or 1⁻	
12	+	−	+	+	Lp^s	Gal₂⁻	
13	+	−	+	+	Lp^s	Gal₂⁻	
	4⁻	2⁻	1⁻	+			

Stocks

2645

2646

2647

~~[illegible]~~

2648

~~[illegible]~~

2649

2650

2651

2652

Crosses

		Cumulative counts					
#11	X 518 −	1255	2405	3639	4810	6050 (−)	no (+)
	X 750 −	581	722	1176	1771	2683 (−)	no (+)

7/14/54

{ Cultures of above entered in stock }

11/2/53

Lytic λ Reclamination of 1485 lytic lambda reported previously. galt-λ grown on 1485 - (became contaminated, re CHCl3 ed - EML says transduces diploid and λ 47

	control half	0.1 ml λ half
W750	2	0
W2175	6	2
W518	13	8

Again says no transducing activity of lytic λ.

HFT λ - Does it grow lytically?

1. Fresh 518 cells from aerated culture sedimented - c.2·10^10 and exposed to 2 1.0 ml portions of 518 - in adsorption technique - c 5x10^9 λ Sedimented and resuspended in NSB - Aerated 3 hours - Centrifuged (not much clearing) and chloroformed.

2. Tested against

	control half of plate	0.1 ml half of plate	
1. W750	1	90	no plaques noted
2. 2175	10	130	- " "
3. W518	12	7	263 small plaques

Results suggest no reproduction of HFT λ on W518. Apparently transduction of 750, 2175 residual non-adsorbed phage.

Lytic λ - Wild type grown on W518

assayed previously on 2175 with no action

Reassay

	control ½	0.1 ml/½
W750	1	15
W518	6	38
W2175	10	13

apparent action on 750, 518, not on 2175 ✓ on stabilities

In each case on the 1st streaking mosaics were found - considered as confirmatory evidence that action was genuine transduction. The low number of transductions is compatible with the assumption that the t were due to desorbed or unadsorbed phage in the prep.

W1210 λ -

	control ½	0.1 ml / ½
1. W750	1	92
2. W518	4	128
3. W2175	18	10

20/24 (+)" ~~picked~~ after 3 (+) picked

Effect of Lp2^R on galt - transduction

	control ½	0.1 ml ½
1. 2238	9	16
2. 2297	3	45

11/6/53.

— Reversions —

750 gal+R – 1

Action of lysate · control = 0 , 0.1 ml = 648

2175 gal+R – 1, 2

action of lysates 1. control = 10, 0.1 ml = 96
2. control = 6 , 0.1 ml = 552

84 gal+R on 750 – A means of distinguishing gal_1^- from gal_4^-

		control	0.1 ml lysate
1.	gal+R – 1	1	5
2.	" – 2	1	3
3.	" – 5	0	146
4.	" – 8	1	153

11/8/53

2251 X 750 – Since the gal_1^- prototrophs (in expt N) in which crosses X 2251 have been made haven't yielded (+) is 2251 distinct from 750?

— no controls done —

	(–)	(+)
1.	173	± 0
2.	651	0
3.	989	0
4.	1463	0
5.	1781	0
6.	2092	0
7.	2405	0
8.	2743	0
9.	3197	0
10.	3606	1
	3606	1 ✓ on streaking on EMB gal

Cumulative count

$3.6 \times 10^3 \overline{)1.00}$ = 0.27×10^{-3} = 0.027% (+)

72
280

previous estimates of $gal_2^- \leftrightarrow gal_1^-$ = < 0.13

10/8/53

518 correlation of transduction with lysogenicity
N16 used
e. 10^{10} cells used — exposed to about 10^9 N16

1. Cell assay — 10^8 dil = 98, 121, 141, (all (-)) = 360/3 = 120×10^8 = 1.2×10^{10}

2. N16 assay (on 518). 10^7 dil = 7, 4 = 11/2 = 6 $\times 10^7$ Assay probably low —

3. duction plates
A picked { 2 def (+) colonies; 1 possibly (+) .. neither were; 1 partially lysed col neither were } total no of colonies suggests little if any killing of cells by phage

B 12 picked on 2nd day some papillating colonies — appear intermed (+) on stock

C 24 minus colonies picked
against/518 / λ
2 def (+) both non lys r
12 picked all non lys not done —
24 tested 1 Lp^+ 1 r
2? $Lp^{r\ or\ s}$ 23 s — 3 of intermediate sensitivity — stock made of one

11/17/53 In a repeat expt. 1 (+)^u obtained. Analysis of 4 (+)^u → λ resist, non lysogenic
4 (-) seg → λ sensitive

750 prototrophs transduced by 902 λ

24 (-) picked after purification —
loop inadiated and plated against 2175, 750
2 segregants appear to be gal_2 — HFT = #14; #19
stocks made —

Conflict ?

W2373

W1765 —
— negative transduction to obtain gal_1 — in a line crossable with W750 —
1. Three colonies obtained on second streaking from HFT gal_1 (N1) 5 and 1765 on B(s)

2. Checked against HFT 1, HFT 2, HFT 4. Not transduced by HFT 1 — therefore suspected gal_1 —

3. Crossed with aerated W750 on B gal — (1765 gal_1 — !)
controls ok. on 15 EMS gal plates a total of 152 (-) colonies — has this creature gone F.
Recross — using non aerated 750

11/11/53

518 E 1210 Analysis of the segregants.

Segregant	Lp	N16	S18	Allele
1	r	+	0	4
2	r	+	0	4
3	r	+	0	4
4	r	+	0	4
5	s	+	0	4
6	r	+	0	4
7	"	+	0	4
8	"	0	+	2
9	"	+	0	4
10	"	+	0	4
11	"	+	0	4
12	"	+	0	4
13	"	+	0	4
14	"	+	0	4
15	"	+	0	4
16	"	contam.	E (+) →	—
17	"	+	—	4
18	"	+	—	4
19	"	+	—	4
20	"	—	+	2
				17 4⁻
				2 2⁻

Crosses
8 x 902, 1436,
20 x " , 1436,

5 x 902, 1436
9 x " "

6/18/54
Stocks of
Fun
Expt discarded

Lysates: 2/10/54

	/811	/2175	Locus
-8	1/1	1/0	
-20	7/1	0/0	2⁻

11/12/53

750t 1210 (-) seg analysis -

Segregant #	Lp	/N1	/N16	Attack	
1	+	0	+	1-	
2	"	0	+	1-	
3	"	+	0	2-	✓
4	"	0	+	1-	
5	"	0	+	1-	
6	"	+	0	2-	✓
7	"	+	0	2-	✓
8	"	0	+	1-	
9	"	0	+	1-	
10	"	0	+	1-	
11	"	0	+	1-	
12	"	0	+	1-	
13	"	0	+	1-	
14	"	0	+	1-	
15	"	0	+	1-	
16	"	0	+	1-	
17	"	0	+	1-	
18	"	0	+	1-	
19	"	0	+	1-	
20	"	0	+	1-	
21	"	0	+	1-	
				18 1-	
				3 2-	

hysate	2/10/54 /811	/2175	/750	Lowm	
3	19/1	1/0	—	2-	6/20/54 failed to survive
6	17/1	0/0	—	2-	
7		2/2	90/1 (28/1)	2-	