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 broth and incubated. 1 loopful at dist 50 cm 20 second and spotting loop on indicator.

N16 - 10 colonies picked — 7/10 colonies give strong reaction ± 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, - in N tested - N12 suggestive. Re ✓

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

in 7' HFT gal_2^- λ used. - Dil 1-10 from original probably.

(7) cells from unaerated 10ml culture sedimented - resuspd. in 1ml saline 0.5ml cell + 1.0ml HFT λ - Mixed and diluted out cells plated - a dil series of cell grown 12 hrs + and then pen run 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. pen run.

[redacted]

219a

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

– 0.1 ml of lysate /5ml on EMB(0) – gave 354 plaques/¼ plate = 2.8×10⁴/ml

Against 2175
no add – 9
0.1 1924 – 17

Plaques appear to be of two sizes(?)

2281 vs 750 λ to see if HFT property is 902 peculiarity a property
1210 of 2_{gal^-} in general.

% Transductants

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

24 pop picked from each for purification and HFT examination — apparent more frequent HFT

Checking on 2251

2305 = (2319) × 2251 on EMB gal.

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

0(+)/1434(−)

1673 gal - produced by irrad. Previous reference page 206

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

1 Recheck - not transduced by N1, N16, 518, 592 (+), 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+ using 1st lot - new one made. 9/3/53 - lysate made - tested

	750	2175	811	2070 = gal -
0.1 ml lys ½ plate / control ½ plate	37/4	7/8	74/17	121/3

↖ some suggestion that lysate inhibitory to 0 part. 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.

Rckt.	λ Source	Result	Comment
	1. N1 (1-10)	no transd.	- area of lysis = spot size
	2. N16 (1-10)	" "	- " " " = c. 300-500 plaques.
	3. 518 (1-10)	" "	- area " " = spot size
	4. 2175 750 (+)	" "	- " " " = c. 30 plaques.

2062 - attempted positive detection - c̄ HFT λ's on D(0)

1. N1 und. no colonies
2. N16 " "
3. N7 " "
4. 518 " "
5. 2175 750 (+) " "

since pr - gal probably > 1% transduction likely

9/13/53

1673 gal - #11

2318 X 811 control 5h

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

9/16/53

2251 lysate - Is crossability of 2251 (SRP) nearly as in an S[r] deletion?

Check against 578 on B gal and B gal sm

1. B gal - ½ plate = 65 pfu = 520
2. B gal sm " = 0 " = 0
3. Replica B gal on B gal sm — no confluent growth - Couple minute colonies

~~~

2238 lysate #2 tested against 2175, 578

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

578 1. control half 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 not appear to ~~t~~ other gal - see pg. 220

lysate appears unrelated to 2175. Re checked in abbrev. type eff.

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

2312 made λ[t] - This is second time - Culture (λ[t]) used for lysate above discarded by mistake.

9/17/53

2317 X 811 - controls on EMB gel

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

1/84
0/114
1/198

See also page 206

2238 transduction see page 221
2175 2237
24 papillae picked

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

518 by 2238 1 Spread plates

1. no add	19
2. 2238 1	26

811 by 2238 1

1. no add	46
2. 2238 1	55

} doesn't go?

750 by 2238 1

1. no add	4
2. 2238 1	24

2nd 1/24 +a
3rd 13/24 +a

2317 X 902 - controls on EMB gel

4 plates (-) : 11 : 66 = 528 X 4 = 2112

(+) = 1 confirmed on stock on EMB gel.

1/2112 (-)

9/20/53

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

1st	(+) 39	total cells 64×4 = 1312	Cil. $10^6 \times 10$	1.3×10^{10}

= 1312 / 3900. = 3% recovered

2nd	(+) 7	64×4 = 256		

276 / 0.003 / 7.00 = 3.0%

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

Colony	/578	simult. Streaked	(+)	4 Colonies picked from each by streaking across streak of 578 (+)	(−)	(−)
1.	str. lytic	mixed	+	+	+	+
2.	" "	"	+	+	+	+
3.	" "	"	+	+	+	+
4.	non lys.	"	−	−	−	−
5.	str. lytic	"	+	+	+	+
6.	wk. lytic	"	+	+	+	+
7.	sh. lytic	"	+	+	+	+
8.	wk lytic	"	+	+	+	+

use of shill 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

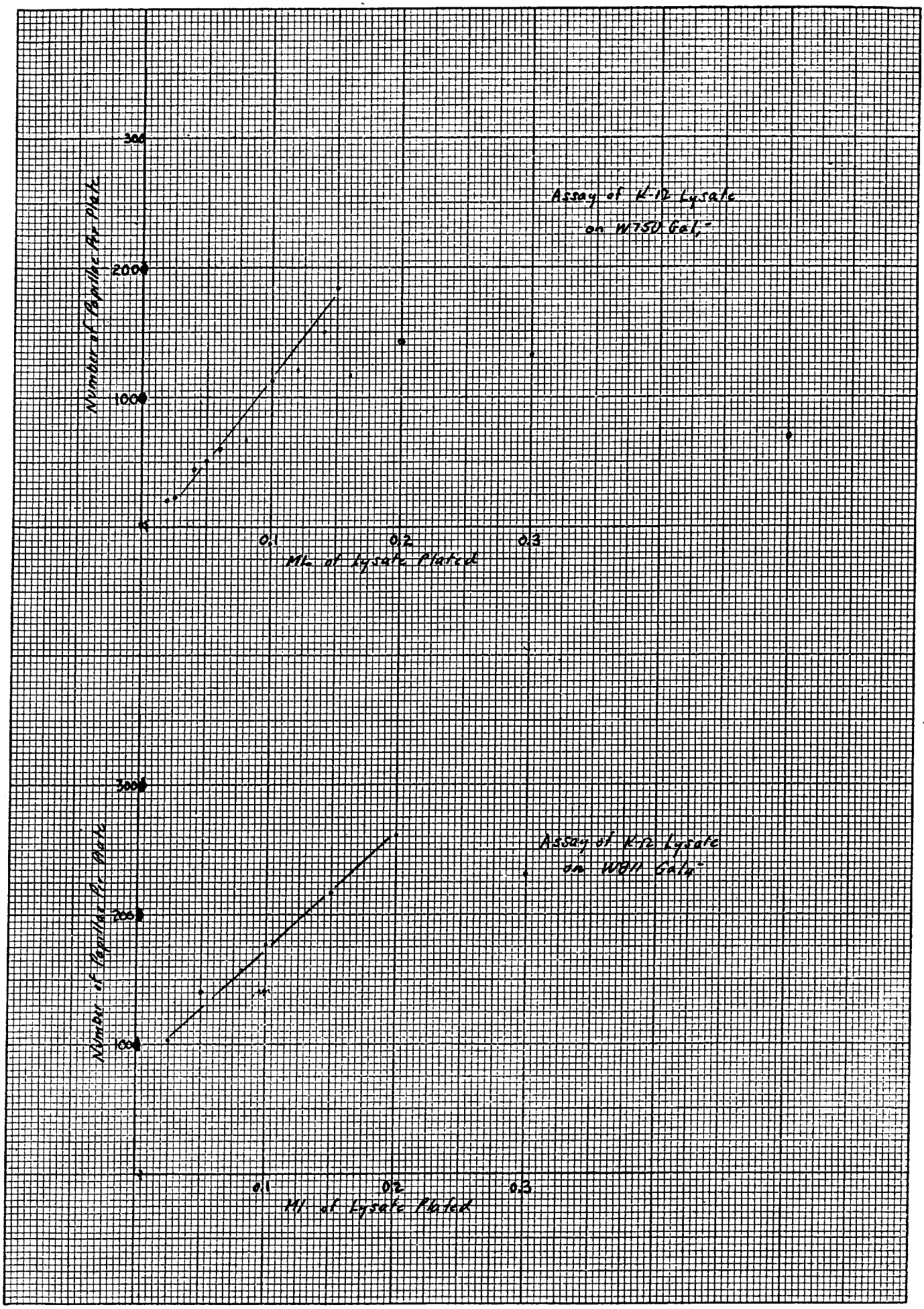
			Δ
1.	0	99	0
2.	0.025	104	5
3.	0.05	141	42
4.	0.1	177	78
5.	0.15	218	119
6.	0.2	261	162
7.	0.3	231	132

1027 - a second tp^r - Lwoff attempt

	streaked/518
1. Supernat broth before irrad. -	0
2. Centrifugate (after 3 hours inc.) of irrad. 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 to eight eight broth 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



9/24/53

2312 – a double minus – gal₂⁻ and galₓ⁻ outside the region of transduction? Cross and examine ∈ [illegible] to see if two classes of gal⁻ can be recovered. gal₂⁻, galₓ⁻ in which galₓ⁻ does transduce gal₂⁻

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-12 λ 5/30 – 3.6 × 10[10] [illegible] + [illegible] p 228

[illegible] 10⁻¹, 10⁻⁴, 10⁻⁶, 10⁻⁷ 0.1 272, 347 = 310 × 10 × 10⁷ = 3.1 × 10[10] cells/ml

1. 0.8 ml cells + 0.8 ml K-12 λ
   0.1 ml = 108 papillae
   41
   net 67
   birth control = 41 — 0 — 67 — (67 / 78)

[illegible] after [illegible] 0.8 ml [illegible]

2. 0.7 ml cells + 0.7 ml K-12 λ
   = 150 papillae
   108
   net 42 — 109

3. 0.6 ml cells + 0.6 ml K-12 λ
   = 132 papillae
   no adsorption — 91

3.6 / 66 / 2.7 — 3.6 / .86 / 216 / 288 / 3.096

1st ad. = 3.1 × 10[10] λ adsorbed
2nd ad. = .7 × 10[10] λ adsorbed
3.8 × 10[10] λ Total

3.8 × 10[10] adsorbed / 3.1 × 10[10] cells = ave 1.2 λ/cell

2297 ∈ K-12 λ

½ plate = control — slight diff. at
½ " = 0.1 ml K-12 λ — at all in [illegible]. – suggestion of inhibition by lysate. [illegible] around edge.

9/27/53

Adsorption K-12 / 5/80

750 cell assay $10^3, 10^4, 10^6, 10^7$ = 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-12 / 5/80 = 0.1 ml = 45
   0.1 ml both 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

275 cell assay $10^3, 10^4, 10^6, 10^7$ = 99, 180 = $115 \times 10 \times 10^7 = 1.15 \times 10^{10}$

as above

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

Cross

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

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

Streaks of 2312 / + and 2076 / + made after testing 8 colonies of each +

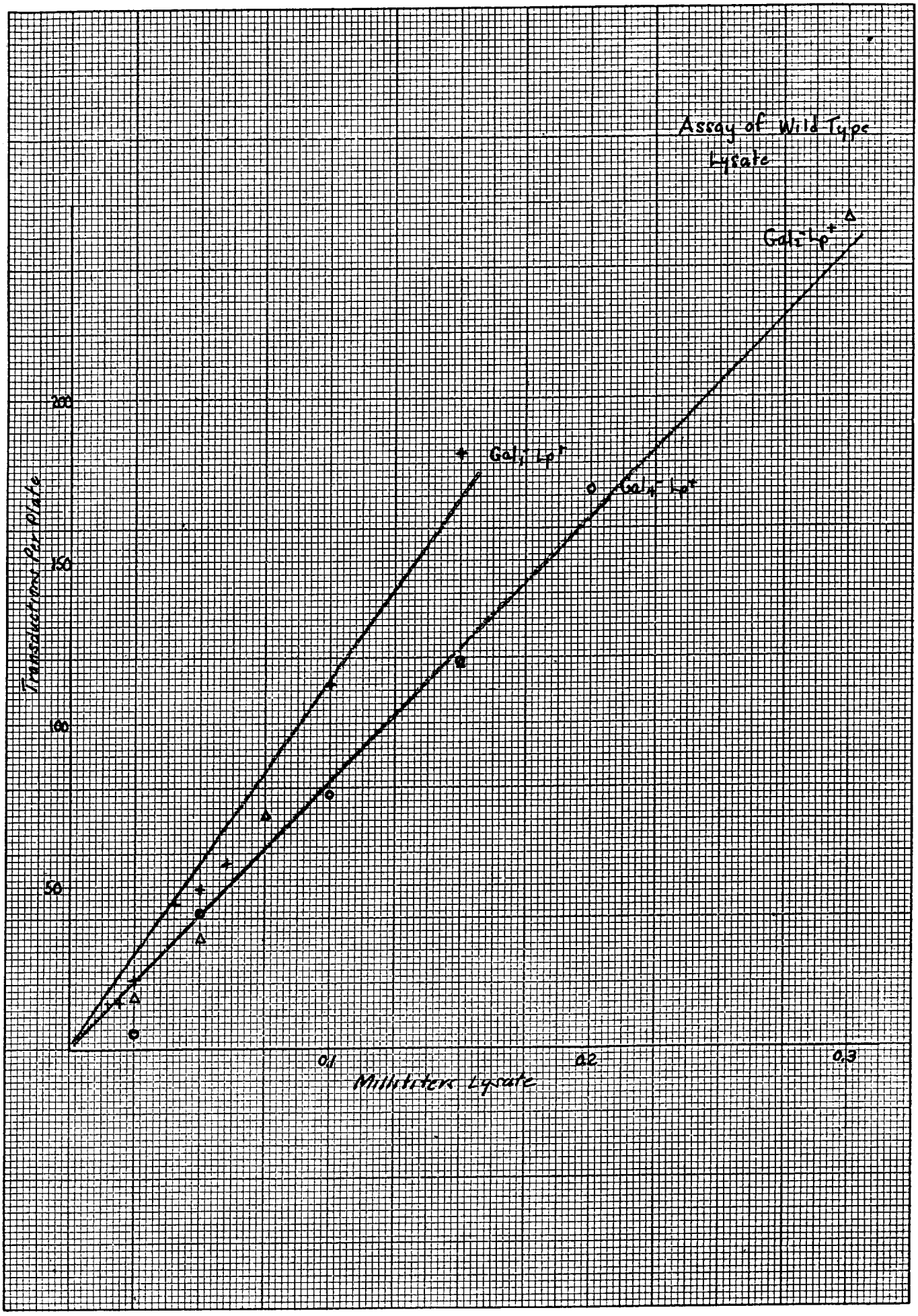
ve if are lysogens

1436 lost its S+

+ for gal allele

/N1	/N16	S1+
++	++	00

apparently stick gals



9/28/53

~~adsorption K-12 λ 5/30 λ 250 ... +~~

250 transduction with 902 λ to isolate Nl HFT — also crossable no

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 × 10 × 10^6 5.09 × 10^9/ml

	no pap	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

2175 - duction versus variable cell no.
cell assay 10^7 = 257, 217, = 237 × 10 × 10^7 = 2.37 × 10^10

Cell no.	Dilution	Sp. pap	Transd. plate	Δ
2.4 × 10^9	und.	23	83	60
1.2 × 10^9	1-2	22	106	84
6 × 10^8	1-4	13	104	91
3 × 10^8	1-8	18	129	111
2.4 × 10^8	1-10 (?)	17	102 ?	85 ?
1.2 × 10^8	1-20	14	135	121
6 × 10^7	1-40	14	115	101
3 × 10^7	1-80 (?)	14	92	—— ?
2.4 × 10^7	1-100	19	109	90
1.2 × 10^7	1-200	15	93	78
6 × 10^6	1-400	16	87	71
3 × 10^6	1-800	8	83	75
2.4 × 10^6	1-1000	11	84	73

518 Lytic λ ?

c. 6 × 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 adsorpt. - Resuspended in NSB (10 ml)
with aeration — 5 hours - non-turbid

Titrated /518 (B(o)) after ~~wash~~ centrifugation

/2175 on EMB gal — control ½ = 10, 0 ml lysed = 12

/2062 D(o) — no growth either half of plate

10^8 = 331 ? 3.3 × 10^10
10^7 = 150 × 16 = 2400 × 10^7 = 2.4 × 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

} pulled and streaked -

2281 t K12	2281 t 750	2281 t 811
12/18? + 4" after 2	24/24 + 4" dil. 2	20/24 + 4" dil. 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 811 λ-9 twice centrifuged & resuspended in NSB - Equivalent no cells both treated, used as control - Incubated with aeration - c. 4.5 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 activ.

	control ½ plate	phage ½ plate : 0.1 ml
750	3	2
518	9	5
2175	7	8

229

A

10/6/53

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

2281 transduced by K-12

Segregant	Lac	/S18	/NIL	Allele
1.	r	+	0	2-
2.	r	Gal +		
3.	s	+	+	contaminated with +?
4.	r	+	0	2-
5.	r	+	0	2-
6.	s	-	+ ?	?
7.	s	+	+	contaminated ō + ?
8.	s	+	0	2-
9.	s	+	0	"
10.	s	+	0	"
11.	r	+	0	"
12.	s	0	0 ?	?

75 +++ 5
25 r III 4

↑ Gal+ ↑ Gal-

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

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

10/23/53 what goes? Sensitive segregants

2281 t 750 (-) seq -

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

mix up 1st run (14.–17.)

contaminated? (8.)

This ligate probably not ? ??? (/N7)

Gal$_1^-$ Gal$_2^-$

disregard

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

229
C

22014011 Seq

Seq #	tp	/N16		/S18	Allele
1.	r	0	S	+	2
2.	r	0	S	+	2
3.	r	+		0	4
4.	r	+		0	4
5.	r	0	S	+	2
6.	r	0	S	+	2
7.	r	0	S	+	2
8.	r	0	S	+	2
9.	r	0	S	+	2
10.	r	0	S	+	2
11.	s	0	S	+	2
12.	s	0	S	+	2
13.	r	[illegible] +		→	–
14.	r	0	S	+	2
15.	r	+		0	4
16.	r	0	S	+	2
17.	r	0	S	+	2
18.	s	0	S	+	2
19.	r	0	S	+	2
20.	r	0	S	+	2
	3s 16r	↑ [illegible]		↑ [illegible]	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 this referred to as NA-4

(−) pap #	/NE 1	/N16	Allele		/2175	/750
1	o	+	gal₁ −	1.	o	o
2	o	+		2.		
3	o	+	"	3.		
4	o	+		4.	(+)?	
5	+	o	gal₂ −		o	
6	−	+	gal₁ −	5.		
7	o	+		6.		+
8	gal +	clone →			+	+ o
9	o	+		7.	o	o
10	o	+		8.		
11	o	+		9.		
12	o	+		10.		
13	o	+		11.		
14	o	+		12.		
15	gal +	clone →			+	+
16	o	+		13.	o	o
17	o	+		14.		
18	o	+		15.		
19	o	+		16.		
20	o	+		17.		
21	o	+		18.		
22	gal +	→			+	+

once plated in HFT test

{lysate made and behaves as HFT on 2175, 518 - (numerous on 2175)

11/20/53 Second lysate made also HFT gal₁ - Stock made - Entered in stock book as W2346

W2346

18 gal₁
1 gal₂

W2346 = 4, 7, 9, 11 } 6/20/54 failed to survive in storage

10/10/83

Linearity –

250 – K-12λ 5/30

	pfu	Δ		
1. no add	3	6		
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 pipette
6. 0.1	87	(84)		84
7. 0.12	123	120	10/12	100
8. 0.14	152	148	10/14	105
~~9. 0.16~~	~~117~~	~~114~~		507/5 = (101)

2175

		Δ		
1. no add	21			
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 pipette
5. 0.1	118	(97)		97
6. 0.12	101	[illegible]		348/4 = (87)
7. 0.14	101	[illegible]		
8. 0.16	159	138		

811

		Δ		Converted to 0.1 ml
1. no add	37	0		
2. 0.02	52	15	x5 =	75
3. 0.04	79	42	x2.5	104
4. 0.06	81	44	x 10/6 =	73
5. 0.08	135	98	10/8 =	122
6. 0.1	157	(120)		120
7. 0.12	137	[illegible]	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 if Lp2^R by attempt. detection

one plate

1. no add ½ = 12
2. K12λ 5/30 = 2

} looks like inhibiting effect

W1436 ✓ on 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	26

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 resusp. in 1.1 ml saline
c. 10^{10} cells/ml

N16

Dilutions
10^8
↓
10^6
↓
10^4 ⟶ in 15″ → dil → 10^2 NSB incubate 37c → duction assay; → λ assay
→ cell survival.
→ phage assay -
→ transduction assay

	Pre Irradiation - No's given for irrad. dose. N16	NA4	Post - Irradiation N16	NA4
Plaque Assay				
Cell Assay	1.34×10^3	2.9×10^3	3.37×10^2	3.87×10^2
T'duction Assay				

didn't work

see p 232 for orig.
24 pedia $16/24$ $+^4$

10/21/53

2281 t K12 Batch II Repeat exam. because of Tp_1^s seg.

Seg	Nb	SIf	Tp_1	Arab
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	"
		NS	9 s / 7 r	16 2⁻

56% Tp^s

For Crosses X 902

	① (+) 8 plates per (−) X		② cross	③
233-3	[illegible]	> 4665	> 3140 (−)	
-7	0	> 4992		
-13	0	106	20 (−) 6 plate	276 / 9 plates 902
-16	0	> 4552		

Control tubes ok except 902 has c 10 (+)
~~[illegible] 902 contam?~~
ok
due to lack of neutralization of 13 cult.?
ok

} 4 plates all few (ind.?) survivors in stock

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

233-B a Freehal?
cross c 902 F+

2

10/21/53

2281 t 750 Batch II Repeat for tp characterization

Seq	N6	N1	tp	active
1	0	+	r	2⁻
2	0	+	r	"
3	0	+	r	"
4	0	+	r	"
5	0	+	r	"
6	0	+	s	"
7	0	+	r	"
8	0	+	s	"
9	+	+	r	?
10	+	0	r	1⁻
11	0	+	r	2⁻
12	0	+	r	"
13	0	+	r	"
14	0	+	s	"
15	0	+	r	"
16	0	+	r	"
17	0	+	r	"
18	0	+	r	"
19	0	+	r	"
20	0	+	s	"
21	0	+	s	"
			16 r	20 2⁻
			5 s	1 1⁻
			23% s	

reexamine - 2⁻ on reexam. after purification

Official Expt

2281 t 750 p. 236 B

~~four crosses X-902~~

~~21~~
~~4.8~~
~~14~~
~~0~~

10/21/53

2281 + 811 Batch II - Repeat for Vp. ✓

Seg	NIb	SIf	Vp	Allele
1	0	+	s	2⁻
2	0	+	r	"
3	gal + →			
4	0	+	r	2⁻
5	0	+	r	"
6	0	+	r	"
7	0	+	r	"
8	0	+	r	"
9	0	+	r	"
10	0	+	r	"
11	0	+	r	"
12	0	+	r	"
13	gal + →			
14	0	+	s	2⁻
15	0	+	s	"
16	0	+	r	"
17	0	+	r	"
18	0	+	r	"
19	0	+	s	"
20	0	+	r	"
21	0	+	r	"
22	+ ?	+	r	?
23	0	+	r	2⁻
			4 s 17 r	21 ~~20~~ 2⁻ ~~1~~ ?
			19% s	

2⁻ on reexamination after purification

discard

(2~~3~~6A)

10/27/53 Batch III

2281 E K12 Repeat - as in (2 ~~3~~6B)

Papillae	2281	? streaked out	Segregant	NK	N1		
~~1~~	~~lys~~	~~mixed~~	~~(mixed +)~~	~~—~~	~~—~~	~~mixed +~~	~~—~~
~~2~~	~~"~~	~~"~~	~~mixed +~~	~~—~~	~~—~~	~~mixed +~~	~~—~~
~~3~~	~~"~~	~~"~~	~~(mixed +)~~	~~—~~	~~—~~	~~mixed +~~	~~—~~
~~4~~	~~"~~	~~"~~	~~(+)~~	~~—~~	~~—~~	~~[illegible]~~	~~—~~
~~5~~	~~√ non lys~~			~~—~~	~~—~~	~~(+)~~	~~—~~
(1) 6	lys	"		—	+	Lp+	(1)
(2) 7	"	"	[illegible]	—	+	"	(2)
(3) 8	√ non lys	— a	gal(-) mixed	—	+	"	(3)
(4) 9	lys	mixed		—	+	λ[illegible]	(4)
(5) 10	"	"		—	+	lys+	(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[illegible]	13
~~23~~	√	"	~~mixed~~		—	mixed	—

~~[illegible]~~ ~~[illegible]~~ 2 Lp^s/13

11 Lp+ 11 Lp+
2 Lp r+s 2 Lp^s

discarded by accident

officard

~~2281 E K12~~
(Expt. 233)

0 Official
1 gt

10/26/53

Batch III - pap, 2(+) picked, 3(+) tested/1485, streaked out on gel and a (-) picked and tested

2281 t 750

Papillae	1485	pap 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~~	—	—	~~[illegible]~~	~~Lp~~	+	0 →
24	"	"	lys	"	"	"

20 pap./tested: 19 Lp+ ; 1 Lp r+s

3/22 non lys.

All = gl2- = 20

Out
Recheck for seg. analysis

Crossing tests x 902 I (-) pick II

4	906	131 = 1037
8	>10.572	
12	contaminated E+	
16	" "	
21	110	
6	>100 x 8 = 7800	

out (12, 16)

6/20/54 on stock slants

out 5/13/55

10/26/53

2281 t 811 ← As 2281 t 700) on 236B

papillae	1485	pap streaked out	Segregants	new segregants	N16	S1f	Allele
1	lys	mixed	lys	lys	0	+	2-
2	"	..	..	..	..	..	..
3	"	..	..	..	..	..	..
4	"	..	..	..	..	..	..
5	"	..	..	..	..	..	..
6	..	..	..	..	..	..	..
7	"	..	..	..	..	..	..
8	..	..	..	..	..	..	..
9	..	..	..	..	..	..	..
10	..	..	..	..	..	..	..
11	..	..	..	..	..	..	..
12	..	..	..	..	..	..	..
13	..	slow, (-1) ?	..	..	..	..	..
14	..	.. .. ?	..	..	0	0	? --> or v u lp2?
15	..	.. .. ?	..	..	0	+	2-
16	..	mixed	..	..	..	..	..
17	..	..	..	..	..	..	..
18	..	..	..	..	..	..	..
19	..	..	..	..	..	..	..
20	..	..	..	..	..	..	..
21	..	..	..	..	..	..	..
22	..	..	..	..	..	..	..
23	..	..	..	..	+	0	4-
~~24~~							

0/23 non lys

W2350

21 gal₂-
1 gal₁-
1 gal ? -?

Maybe ole?
No
Disregard - any discarded before classification - lyt

For cross X 902, 1485.

	X 902
6	0/117
10	>137x8 = >1096
14	(150x8) x 10 = >12000 (really prototrophs? lot of bkgd growth)
23	~~300~~ / 3914?

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

out 7/13
" "

2/9/54

Lysates	/811 XV	/2175	trans
23.	1/1	9/1	4-
14.	0/1	8/1	-- ?

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

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

1. 750 transduced by N16 gal₂⁻
   (a) 18 papillae picked = after purification 15 segregating = ~~[illegible]~~ 15/18 5/6 = 83.3%
   (b) examination of segregants from test λ'

	WT	TN16	Tests
1.	—	+	1⁻
2.	—	+	1⁻
3.	—	+	1⁻
4.	mixed		
5.	—	+	1⁻
6.	—	+	1⁻
7.	+	—	2⁻
8.	—	+	1⁻
9.	mixed		
10.	—	—	? double? in re V-
11.	+	—	2⁻
12.	—	+	1⁻
13.	—	+	1⁻
14.	—	+	1⁻
15.	—	+	1⁻

10 1⁻
2 1⁻
1 ?

{ not c N1, N16, possibly by S18 - a double(-)?
lysate of this in
2175 = 1/3 (exp/control)
750 = 1/1
This culture also found to be tp_2^s
Cross X 902 - controls ok.
1 plate = 207 x 8 = 1656 (-) no (+)

This is W2586

2. 750 c S18 gal₄⁻
   (a) after purification 9/18 = 50% segregating
   (b) examination of segregants

	/N1	/S18
1.	—	0
2.	—	+
3.	mixed c (+)	
4.	—	+
5.	—	+
6.	—	—
7.	—	+
8.	—	+
9.	—	+
~~10~~		

10/27/53 HFT durham

(3.) 2175 transduced by N1 gal₁⁻
(a) after purification 10/18 stbl segregating = all not λˢ
(b)

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

~~(−)(−) on recheck - but ... S1F ... ~~
on subsequent test not + N16, ± by N1, S1F = gal₁

(−)(−) on recheck - transd. by S1F - a double

6 1⁻
3 2⁻
1 ?

(4.) 2175 transduced by S1F gal₂⁻
a. after purification 13/18 stbl segregating
(b) all not λˢ

	N16	S1F	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 transduction

(5.) 811 transduced by NIB gal_2 -

(a) after purification 15/18 still segregating

(b) Reisolation

	NIB	SIP
1.	+	-
2.	+	-
3.	+	-
4.	+	-
5.	+	-
6.	+	-
7.	+	-
8.	+	-
9.	+	-
10.	+ ?	-
11.	+	-
12.	+	-
13.	+	-
14.	+	-
15.	+	-

10/29/53

1765 Gal- mutants. Induced on B gue c̄ U.V. c 10 seconds exposure

Isolate #	518	1316	N1	51Ft892	Genus?	Allele?
1 not (+)					not t_1 t_2? p^s	
2	+ 0	+ 0	− 0	− 0	Lp^r? or slow?	
3					not t_2?	
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						

Isolate #	518	1316	N1	51Ft892	Genus?	Allele?	Stocks
2	—	—	—	—	Lp^r or slow?	?	2645
~~4 not Gal(+)~~							
5	+	+	+	+	Lp^s	new	2646
6	+	+	—	+	growth too thin	Gal_1^-	2647
contaminant ~~7~~	—	—	—	—	Lp^r or slow	~~[illegible]~~ ?	~~[illegible]~~
8	+	+	+	+	Lp^s	new	2648
— not slow ~~9~~	—	—	—	—	Lp^r or slow	~~[illegible]~~ ?	~~[illegible]~~
10	+	+	+	+	Lp^s	new	2649
11	—	+	—	+	Lp^s	Gal_4^- or 1^-	2650
12	+	—	+	+	Lp^s	Gal_2^-	2651
13	+	—	+	+	Lp^s	Gal_2^-	2652
	4^-	2^-	1^-	+			

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 Reexamination - 3 1485 lytic lambda reported previously. Isolated from 1485 - (became contaminated, re CsCl, ed - EAL 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. 2·10⁸ and exposed to 2 1.0 ml portions of 518 - in adsorption technique - sedimented and resuspended in 3 NSB - Incubated 3 hours - Centrifuged (not much clearing) and chloroformed.

2. Tested again:

	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	exptl (0.1 ml) ½	
W750	1	15	apparent action on 750, 518, not on 2175
W518	6	38	or on standards
W2175	10	13	

In each case on the 1st streaking numerous were found - considered as confirmatory evidence that action was genuine transduction. The low number of transductants is compatible with the assumption that the λ 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	20/24 (4th) picked after 3 ½ picked
3. W2175	18	10	

Effect of lysin on gal⁻ transduction

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

11/6/53

— Reversals —

750 gal+^{R} - 1

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

2175 gal+^{R} - 1, 2

Actin 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$_2^-$

		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 portion of the gal$_2^-$ (in exp N) in which cross X 2251 have been made (arrow) equaled (=) in 2251 distinct from 750?

— In control line —

	(-)	(+)
1.	~~173~~	± 0
2.	651	0
3.	989	0
4.	1463	0
5.	17.81	0
6.	2092	0
7.	2405	0
8.	2743	0
9.	3197	0
10.	3606	1
	3606	1 ✓ in streaking on EMB gal

Cumulative count

$3.6 \times 10^3 \overline{)\ 1.0.0}$ ~~0.27~~ $\times 10^{-3}$ = 0.027% (+)

72

250

previous estimate of gal$_2^-$ ↔ gal$_1^-$ = < 0.1%

10/8/53

518 correlation of transduction with lysogenicity
N16 used
c. 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. ductive 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[4)] on strk

C 24 mini 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 ρ 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, not lysogenic
4 (-) seg → λ sensitiv

---

750 prototroph transduced by 90 r λ

24 (-) picked after purification –
exp inoculated and plated against 2175, 750
2. segregants appear to be gal_2 – HFT = #14; #19
stocks made –

W1765 – NA4 – 2 ← Conflict ? W2373

– negative transduction to obtain gal – in a line crossable with W750 –
1. Three colonies obtained on second streaking from HFT gal_1 (N1 5) and 1765 on 13 (+)

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

3. Crossed with aerated W750 on EMB gal – (1765 gal^- 1)
controls ok. on 15 EMB gal, plates a total of 152 (-) colonies – has this culture gone F-
Neomys-using non aerated 750

11/11/53

518 E 1210 Analysis of the segregants.

Segregant	tp	1416	518	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.	0 (+)	—
17	"	+	—	4
18	"	+	—	4
19	"	+	—	4
20	"	—	+	2

17 4–
2 2–

Crosses

8 x 90r, 1436,
20 x " , 1436,

5 x 90r, 1436
9 x " , "

6/18/54
Stocks of
two
died discarded

Lysates:	4/10/54		
	811	2175	Crosses
-8	1/1	1/0	
-20	7/1	0/	2–

11/12/53

750 t 1210 (-) seq analysis -

Segment #	Hp	N1	N16	Allele	
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-	

Lysate	2/10/54 /84	/2175	/750	Hom	
3	19/1	1/0	—	2-	6/nov/53
6	17/1	0/0	—	2-	faible
7		2/2	90/1 (2%)	2-	+ survivors