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 1/10 HFT med. 1 loopful at dist 50 cm 20 second 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 gal₁⁻ 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 1-100 from original probably.

① cells from unaerated 10 ml culture sedimented - despd. in 1 ml saline 0.5 ml cell + 1.0 ml HFT λ - Mixed and diluted out cells plated - a dilution of cell grown 12 hrs + and then pen run on grown cells.

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]

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 = 2.8x10^4/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 or property
1210 of gal2 - in general.

1. Transductions

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

24 pap 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 [illegible]. Previous reference page 206

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

1 Recheck - not transduced by N1, N16, S18, 592 (+), K-12, λ's
9/17/53 re check NFT λ - no transd. by [illegible] 2,4, K-12
in confirmation and extension of previous

2. Made λ+ using λt [illegible] but new are 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 spont. pap 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.

Rekt. λ Source	Result	Comment
1. N1 (1-10)	no transd.	- area of lysis = spot size
2. N16 (1-10)	" "	- " " " = c. 300-500 plaques.
3. S18 (1-10)	" "	- area " " = spot size
4. 2175t 750(+)	" "	- " " " = c. 30 plaques.

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

1. N1 und. 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 control 6h

	(-)
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 deletion?
Check against 518 on B gal and B gal Sm

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

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2238 lysate #2 tested against 2175, 518

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

518 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 ~~to~~ appear to + other gal - see pg. 220
lysate appears inhibitory to 2175. Recheck of in adsorpt. type expt.

Broth Control = 19
Adsorbed tube = 28 . 12 picked - all 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

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

See also page 206

1/84
0/114
1/198

2238 transduction see page 221
2175E2238
24 papillae picked

1st 5	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

| all [struck through]

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 strk on EMB gal.

1(+)/2112(−)

9/20/53

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

1st . (+) 39 . total cells 164 × 8 = 1312 . dil. 10⁶ × 10 . 1.3 × 10¹⁰

= 0.03 / 1312 / 39.00. = 3% transduced

2nd (+) 7 64 × 4 = 256

276 / 7.00 = 0.003 = 3.%

276 / 7.00 = 0.03

8 (+) colonies picked and streaked out 2 times
Rep. colonies then picked and cross brushed on 518, and streaked out

Colony	/518	simult. Streaked	(+)	4 Colonies picked from each (+)	(−)	(−)
1.	str. lytic	mixed	+	+	+	+
2.	" "	"	+	+	+	+
3.	" "	"	+	+	+	+
4.	non lys.	"	(−)	−	−	−
5.	str. lytic	"	+	+	+	+
6.	wk. lytic	"	+	+	+	+
7.	str. lytic	"	+	+	+	+
8.	wk lytic	"	+	+	+	+

no sign of segregation

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

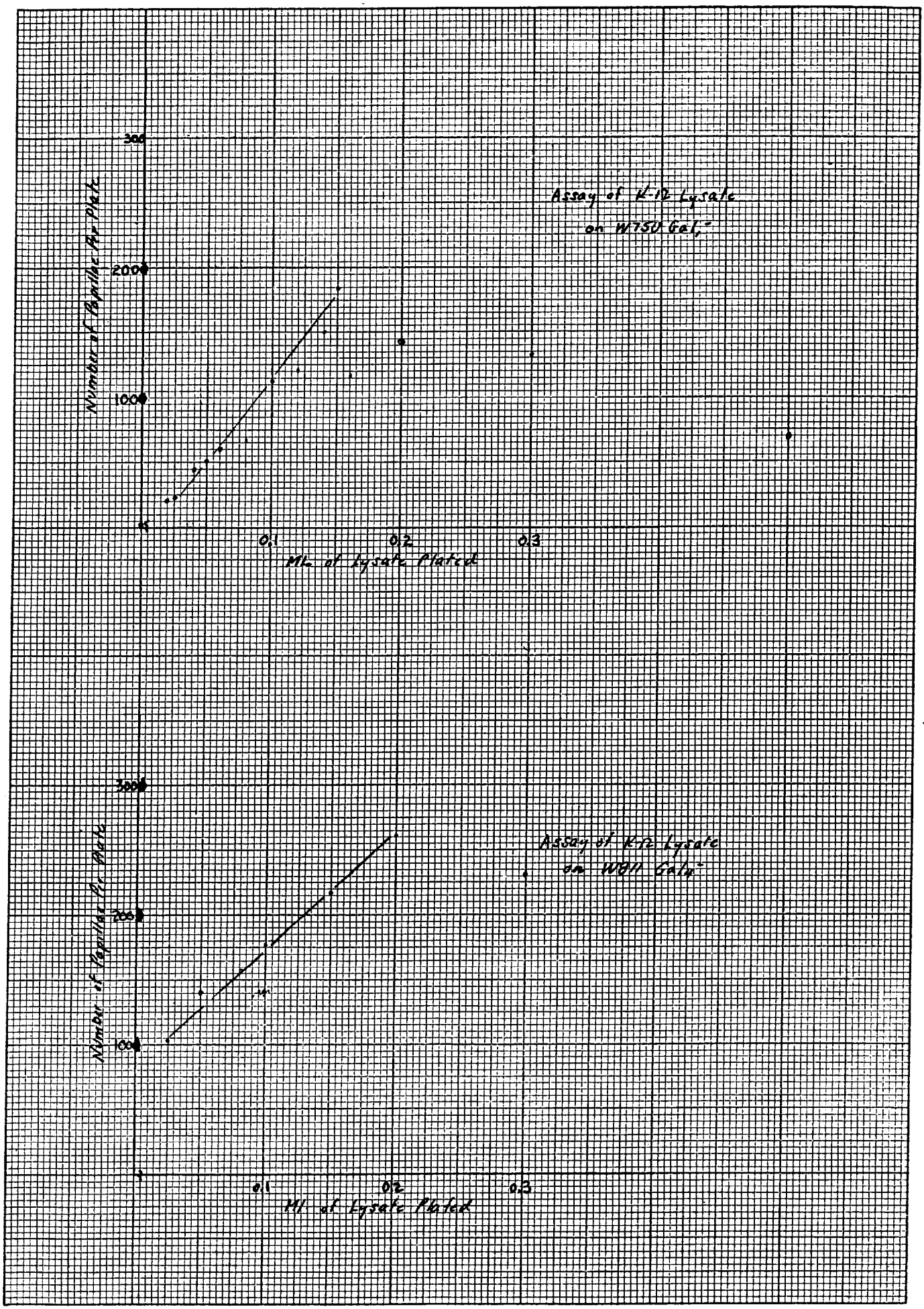
			Δ
1.	0	99	0
2.	0.025	104	5
3.	0.05	141	42
4.	0.1	177	78
5.	0.15	218	119
6.	0.2	261	162
7.	0.3	231	132

1027 - a second tp⁺ - Lwoff attempt

	shocked/518
1. Supernat broth before irrad. -	0
2. Centrifugate (after 3 hours inc.) of irrad. culture -	7 plaques.

Does gal⁻ transduce galy⁻ and vice versa?
In case usual technique allows inhib. of transd. expression adsorption type experiment done - "no add" plate run to expt except 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 mutant – gal₂⁻ and galₓ⁻ outside the region of transduction? Cross and examine if possible to see if two classes of gal – can be recovered. gal₂⁻, galₓ⁻ in which galₓ⁻ does transduce gal₂⁻

16 SSX 2312 controls etc.

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

Repeat using pr addition to medium –

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811 adsorption of K-12 λ 5/30 – 36 × 10¹⁰ (... UV NTG (kt)) + assay p 224

assay 10², 10⁴, 10⁶, 10⁷ 0.1 272, 347 = 310 × 10 × 10⁷ = 3.1 × 10¹⁰ cells/ml

1. 0.8 ml cells + 0.8 ml K-12 λ
   0.1 ml = 108 papillae
   41
   net 67
   birth control = 41
2. 0.7 ml cells + 0.7 ml K-12 λ
   = 150 papillae
   108
   net 42
3. 0.6 ml cells + 0.6 ml K-12 λ
   = 132 papillae
   no adsorption

(assayed after cent in 0.8 ml ... ...)

0, 67, (67/78), 109, 6%, 91

3.6 × 66 = 216 ...

1st ad = 3.1 × 10¹⁰ λ adsorbed
2nd ad. = 0.7 × 10¹⁰ λ adsorbed
3.8 × 10¹⁰ Total

3.8 × 10¹⁰ λ adsorbed / 3.1 × 10¹⁰ cells = ave 1.2 λ/cell

2297 C K-12 λ

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

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. 1.0ml cells + 1.0ml K-12λ 5/30 = 0.1 ml = 45
0.1ml broth exposed = 3
net = (42)
2. 0.9ml + 0.9ml 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. 1.0ml + 1.0ml K-12λ = 52 broth control = 20 net = 32
2. 0.9ml + 0.9ml K-12λ = 103 net = 83
3. 0.8ml + = 97 net = 77

Cross
1655 X 2312 Repeat ~~Aerated~~ Proline added 1655 on cross gal controls ok.

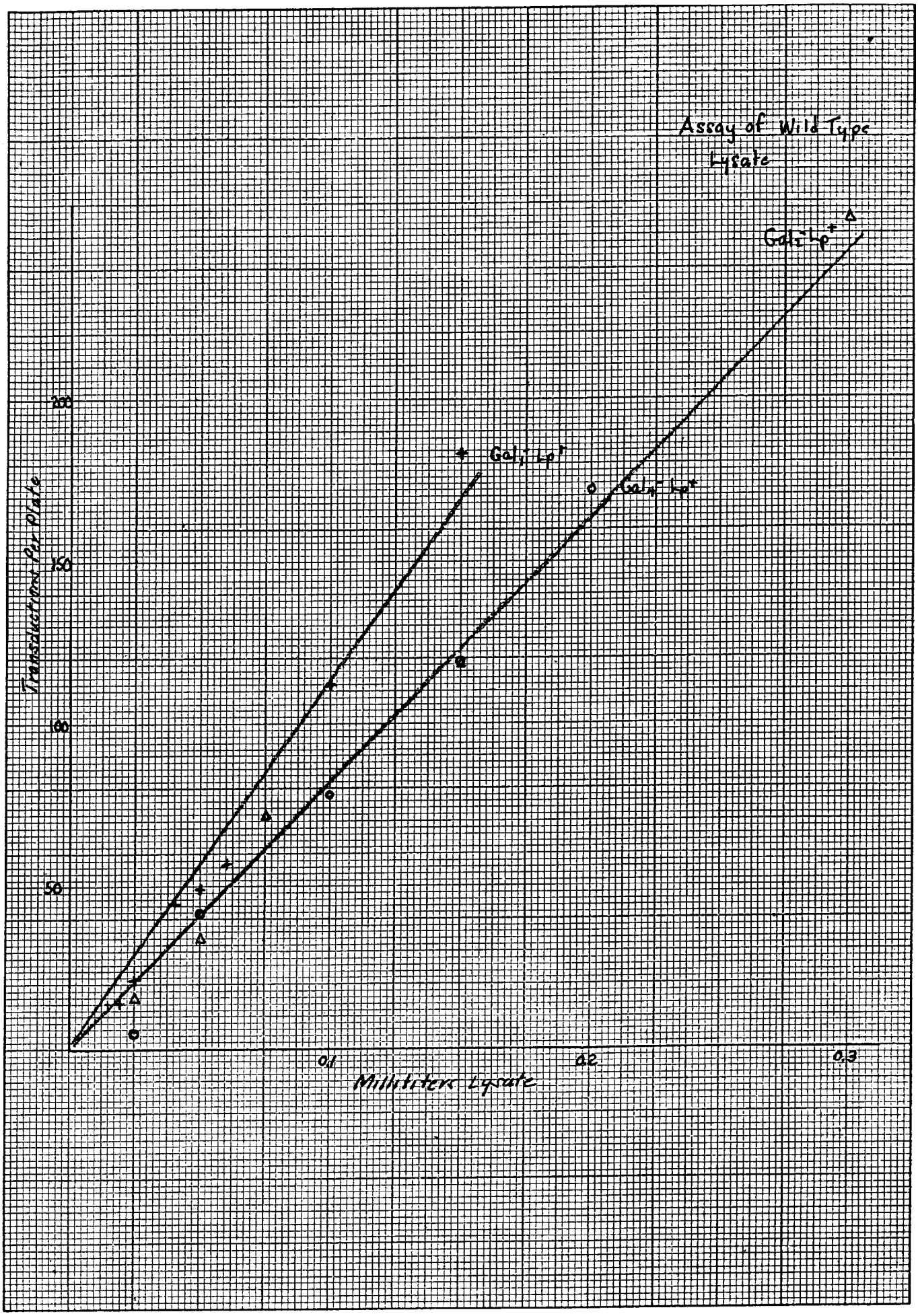
	(+)	(-)
1.	2	0
2.	2	0
3.	0	0
4.	1	0
5.	2	2(?) } check
6.	1	1 ?
	8	3

8 tubes of 2312/+ 2076/+ } made after testing 8 colonies of each to see if are lysogenic

1436 lost its S^r
✓ for gal allele

/N1	/N16	S/F
++	++	00

apparently still gal+



9/28/53

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

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 ml	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 { /2062 D(o) } 10^8 = 331 ? 3.3 × 10^10
control ½ = 10 } no growth } 10^7 = 150 × 16 = 2400 × 10^7 = 2.4 × 10^10
0 ml lytic λ = 12 } either half of plate

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 M1
12/18? +" al-hr2	24/24 +" al-h.2	20/24 +" al-h.2
19?/λ	22/24 +" (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. 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 action

	control ½ plate	phage ½ plate: 0.1 ml
750	3	2
578	9	8
2175	7	8

229

(A)

10/6/53

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

2281 transduced by K-12

Segregant	Lp	/518	/W12	Allele
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 𝍸 ||| 8 5r |||| 4		Gal₁⁺?	Gal₂⁻?	

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

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

10/23/56 what goes? Derivative segregants

229 (B)

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 [illegible] ?

Gel₁⁻ Gel₂⁻

disregard

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

229
c

2201t011 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
	3 s 16 r	↑ [illegible] 2		↑ [illegible] 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
this referred to as NA-4

(–) pap #	1/NB	/N16	Allele		once plated in HFT test /2175	/750
1	0	+	gal₁⁻	1.	0	0
2	0	+		2.	↓	↓
3	0	+	11	3.		
4	0	+	↓	4.	(+)?	
5	+	0	gal₂⁻		0	
6	–	+	gal₁⁻	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 + →				+	+

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

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

W2346

18 gal₁⁻
1 gal₂⁻

} [illegible]

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

10/10/83
Linearity –

700 – K-12λ 5/30 pfu Δ

			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
~~1.~~	~~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	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	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	~~96~~	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 Lp2R by attempt. detection
one plate
1. no add ½ = 12
2. K12λ 5/30 = 2 } looks like inhibiting effect

W1435 ✓ 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 KCN half
1.	2238	62	53
2.	2297	7	36

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

1. 780 λ	214	-	3/24
2. K-12 λ	46	-	1/24
3. 871 λ	98	-	24/24

N16, NA4 Yield of HFT λ from

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

Dilution — N16

10^8 → 10^6 → 10^4 → uv 15" → dil 10^2 N58 incubate 37C → duction assay; → λ assay
uv 15" → cell survival.
10^4 → phage assay -
10^4 → transduction assay

	Pre Irradiation - 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				

No's given for irrad. dose

delete work

see p 232 for agri
24 predict 16/24 +[u]

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	?
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	16 2⁻
			7 r	~~1 ?~~

re examine 2⁻ on reexamination after purification

56% Tp^s

For Crosses X 902

	① 8 plates per X (+)	(−)		② cross	③	
233-3	~~[illegible]~~	> 4665	Control ~~tks~~ ok except 902 low c 10 (+) ~~[illegible] 902 contam.?~~	> 3140 (−)		} chief F+ all females or
-7	0	> 4992	ok			
-13	0	106	due to lack of neutralization of 13 culture?	20 (−) 6 plate	276 9 plates / 402	
-16	0	> 4552	ok			

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

233-B a F rechecked? cross c 902 F+

discard in stock

2

10/21/53

2281 t 750 Batch II Repeat for tp, characterization

Seq	N4	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	? reexamine - 2⁻ on reexam. after purification
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	

Official Expt
2281 t 750 p. 236B

~~Pur cultures x 90r~~

~~21~~
~~4.8~~
~~1.4~~
~~0~~

10/21/53

2281 + 811 Batch II - Repeat for tp. ✓

Seq	NI6	SIF	tp	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	21
			17 r	~~20~~ 2⁻
				~~1~~ ?
			19% s	

22 on reexamination after purification

discard

236A

10/27/53 Batch III

2281 E K12 Repeat - as in (236B)

Papillae	2281	pay streaked out	Segregant	NK	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 small (-) inside		—	+	λ° ??	(3)
(4) 9	lys	mixed		—	+	λ° ?	(4)
(5) 10	"	"		—	+	lys+	(5)
~~11~~	"			—		~~pale clear~~	
(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~~	√	"	~~mixed~~		—	mixed	—
	~~19 Lp+~~					2 Lp°/13	
	11 Lp+ 2 Lp r+s					11 Lp+ 2 Lp°	

discarded by accident

officead

~~2281 E K12~~ Expt. 233

0 Official
1 gt

10/26/53

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

2281 t 750

Papillae	/1485	pap streaked out	segregant	new segregants	/N1	/N16
1	lys	mixed	lys	Lp^r	+	0
2	"	"	"	"	"	"
3	"	"	"	"	"	"
4	"	"	"	"	"	"
5	"	"	"	"	"	"
6	"	"	"	"	"	"
7	"	"	"	"	"	"
8	↓ non lys.	"	non lys	sensitivity^r	"	"
9	lys.	"	lys	Lp^r	"	"
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]~~		+	0 →
24	"	"	lys	"	"	"

20 pap/lys: 19 Lp^r; 1 Lp^{r+s}

3/22 non lys.

All = gal₂⁻ = 20

out — Record of seg. analysis

Crossing tests x 902

	I (-) pap	II
4	906	131 = 1037
8	>10,572	
12	contaminated E+	
16	"	"
21	110	
6	>100 x 8 = >800	

6/20/54 on stock stands

out 5/13/55

236C

10/26/53

2281 t 811 — As 2281 t 700) on 236B

papillae	/1485	pap streaked out	segregants	new segregants	/W16	/S14	Allele
1	lys	mixed	lys	lys	0	+	2⁻
2	"	..	..	..	..	..	..
3	"	..	..	..	..	..	..
4	"	..	..	..	..	..	..
5	..	..	..	..	..	..	..
6	..	..	..	..	..	..	..
7	..	..	..	..	..	..	..
8	..	..	..	..	..	..	..
9	..	..	..	..	..	..	..
10	..	..	..	..	..	..	..
11	..	..	..	..	..	..	..
12	..	..	..	..	..	..	..
13	..	slow, (-) ?	..	..	..	..	..
14	..	.. .. ?	..	..	0	0	?
15	..	.. .. ?	..	..	0	+	2⁻
16	..	mixed	..	..	..	..	..
17	..	..	..	..	..	..	..
18	..	..	..	..	..	..	..
19	..	..	..	..	..	..	..
20	..	..	..	..	..	..	..
21	..	..	..	..	..	..	..
22	..	..	..	..	..	..	..
23	..	..	..	..	+	0	4⁻
~~24~~			0/23 non lys				

→ ? or V h l_{p2}^{s} ← W2350

~~21~~ gal_2^-
1 gal_4^-
1 $gal_?$ - ?

Maybe ok? No
Disregard - my discarded before classification - lys?

For crosses X 902, 1485.

	X 902
6	0/117
10	>13768 = >1096
14	(150x8) x 10 = 12000 (really prototrophs? lots of bkgd growth)
23	~~3M~~/391 ~~4~~.

6/10/54
Stocks of these made and saved
Remainder discarded on 6/22/54

out 1/13
" "

2/9/54	/811	/2175	locus
lysates 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 pop., and 3 (+) colonies picked in purification

1. 750 transduced by N16 gal₂⁻

(a) 18 papillae picked - after purification 15 segregating = 15/18 % = 83.3%
(b) examination of segregants from

	HT	TNDI	Locus
1.	—	+	1⁻
2.	—	+	1⁻
3.	—	+	1⁻
4.	mixed		
5.	—	+	1⁻
6.	—	+	1⁻
7.	+	—	2⁻
8.	—	+	1⁻
9.	mixed		
10.	—	—	? double?
11.	+	—	2⁻
12.	—	+	1⁻
13.	—	+	1⁻
14.	—	+	1⁻
15.	—	+	1⁻

10 1⁻
2 1⁻
1 ?

no rev. - 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 Lp₂ˢ
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.	—	o
2.	—	+
3.	mixed c (+)	
4.	—	+
5.	—	+
6.	—	—
7.	—	+
8.	—	+
9.	—	+

10/27/53 HFT durham

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

	N1	N16	locus	
1.	−	−	?	~~(+)(−) on recheck - but ... SIK - λ ...~~ on subsequent test not +N16, t by N1, SIF = gal₁
2.	−	+	1⁻	
3.	—	+	1⁻	
4.	—	+	1⁻	
5.	—	+	1⁻	
6.	—	+	1⁻	
7.	+	—	2⁻	
8.	—	—	?	(+)(−) on recheck - transd. by SIF - a double
9.	—	+	1⁻	
10.	+	—	2⁻	

6 1⁻
3 2⁻
1 ?

(4.) 2175 transduced by SIF gal₄⁻
a. after purification 13/18, still segregating
(b) all not λˢ

	N16	SIF	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 $CaCl_2$ –
(a) after purification 15/18 still segregating
(b) Streptomycin

	N16	51F
1.	+	–
2.	+	–
3.	+	–
4.	+	–
5.	+	–
6.	+	–
7.	+	–
8.	+	–
9.	+	–
10.	+ ?	–
11.	+	–
12.	+	–
13.	+	–
14.	+	–
15.	+	–

10/29/53

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

Isolate #	518	1316	N1	51Ft892	Genes	Allele?
1 wt (+)					wt + ? γ^s	
2	+ 0	+ 0	− 0	− 0	Lp^s? or slow?	
3					wt + ?	
4 wt (+)	+	+	+	+	Lp^s	
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	Genes	Allele?	Stocks
2	−	−	−	−	Lp^s or slow?	?	2645
~~4 wt~~	~~grid (+)~~						
5	+	+	+	+	Lp^s	new	2646
6	+	+	−	+	growth too thin	Gal_1 −	2647
contaminant - ~~7~~	−	−	−	−	Lp^s or slow	~~[illegible]~~ ?	~~2648~~
8	+	+	+	+	Lp^s	new	2648
− not slow - ~~9~~	−	−	−	−	Lp^s or slow	~~[illegible]~~ ?	~~2649~~
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 }

13/2/53

Lytic λ — Recontamination of 1485 lytic lambda reported previously. gal+ 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. 3·10[11] 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	expt (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 no vaucer were found — considered as confirmatory evidence that action was genuine transduction. The low number of transductions is compatible with the assumption that the + were due to denatured or unadsorbed phage in the prep.

W1210 λ —

	control ½	0.1 ml / ½	
1. W750	1	92	
2. W518	4	128	20/24 (+)[s] ~~picked~~ after 3 (-) picked
3. W2175	18	10	

Effect of Lp2R on gal+ 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 , 0.1ml = 648

2175 gal+R - 1, 2

action of lysates 1. control = 10 , 0.1ml = 96
2. control = 6 , 0.1ml = 557

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

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

11/8/53

2251 X 750 - Since the gal- prototrophs (in expt N) in which crosses X 2257 have been made haven't equalled (+) is 2257 distinct from 750?

— no controls done —

		(-)	(+)
	1.	~~[illegible]~~ 173	± 0
	2.	651	0
	3.	989	0
Cumulative count	4.	1463	•
	5.	1781	0
	6.	2092	0
	7.	2405	0
	8.	2743	•
	9.	3197	•
	10.	3606	1
		3606	1 ✓ on streaking on EMB gal

$3.6 \times 10^3 \overline{)1.0.0}$ = 0.27×10^{-3} ; 72 ; 280

= 0.027% (+)

previous estimate of

$gal_2^- \leftrightarrow gal_1^-$ = < 0.13

10/8/53

518 correlation of transduction with lysogenicity
N16 used
c. 10^{10} cells used – exposed to about 10^7 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(4) on strk
   C 24 minute colonies picked

	against/518	/λ
2 def (+)	both non lys	r
12 picked	all non lys	not done –
24 tested	1 lp+ r or s / 2: lp	1 r / 23 s – 3 of intermediate sensitivity – ↖ stock made of one

11/17/53 In a repeat expt. 1 (+)" obtained. analysis of 4 (+)" → λ resist, non lysogenic
4 (–) seg → λ sensity

~~~~~~

750 prototroph transduced by 902 λ

24 (–) picked after purification –
exp irradiated and plated against 2175, 750
2 segregants appear to be gal₂ – HFT = #14, #19
stocks made –

~~~~~~

Conflict ?

W2373

W1765 – NA4 – 2
– negative transduction to obtain gal – in a line crossable with W750 –
1. Three colonies obtained on second streaking from HFT gal, (N1/5) and 1765 on B(?)
2. Checked against HFT 1, HFT 2, HFT 4. – Not transduced by HFT 1 – therefore suspected gal, –
3. Crossed with aerated W750 on B gal – (1765 gal – !)
controls ok. on 15 EMS gal, plates a total of 152 (–) colonies – has this culture gone F. Nervous – using non aerated 750

11/11/53

518 E 1210 Analysis of the segregants.

Segregant	tp	N16	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 907, 1436,
20 x " , 1436,

5 x 907, 1436
9 x " , "

6/18/54
Stocks of
four
died discarded

Lysates: 4/10/54

	811	2175	Crosses
-8	1/1	1/0	
-20	7/1	0/0	2–

11/12/53

750 t 1210 (-) seg analysis -

Segregant #	Lp	/N1	/N16	Allele	
1	r	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	trans	
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⁻	

11/16/50

W2252 = 1895 Hfr lp^s

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

2341 { 1. Several (-) colonies with N16 HFT gal_2^-
isolated and purified - HFT λ tests indicate gal_2^-
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 = Lp_1^R Lp_2^S
λ2 - sensitivity

(3) "Lysate" prepared - very viscous

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

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

W 2345 = From lysates 1 - one gal_1^- isolated - { tested against N16, NA-1 = gal_1^-

From lysate 2 - 5 gal_1^- isolated - { " " " = gal_1^-

also nonlysogenic

NA-1
NA-2 } lysates
sterility confirmed

11/30/53

Transductions for segregant analysis

A W1765 gal₁⁻ (made by ~ transduction) W2373

	λ	control ½	phage ½
1.	811	1	30
2.	1210	0	56
3	902 (new 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

T18 } λ's against 2070 - to see if both 1⁻, 2⁻ is in these apparent doubles. See also next page
T19 }

245B

(-2) lysates not very active

T18-2 5/1
T19-2 7/2

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

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

2. Analysis of segregants from ~ transd. c 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 c (+).		"	

12/2/53

2175E750 λ-

A. TLδ transduced by mixture N16, ~~M4~~ N1, but not by single λ's

Proposed Constitution: ① 18 pp (gal) picked and streaked. Many mosaic colonies — all appear to be segregating lac+
2- 1-, 2- 1+, 2+ 1- } 3n on lac should be lac slow if gal- present - which is the case. Can't test gal segregates

~~Segregants~~ 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	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 Y2 - 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	Lones
1	slow	(–)	0	+	1⁻
2	slow	"	0	+	1⁻
3	slow	"	0	0	double –
4	slow	"	0	0	"
5	"	"	0	+	1⁻
6	"	"	0	0	double –
7	"	"	0	0	"
8	"	"	0	+	1⁻
					4 1⁻
					4 (– –)

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

Lac character not good one...

2. The Lac +

#	β lac ON slow	β gal	I guess Transd. Assay /S92+	HFT₁ /N1	HFT₂ /N16	Lones	
1	+ (?)	+"	+	0	+	1⁻	1 ⊕
2	say	"	0	0	0 (1 pap)	– –	2
3	say	"	0	0	0 stretch thin	– –	3
4	"	"	+	0	+	1⁻	4 ⊕
5	"	"	0	0	0	– –	5
6	"	"	0	+	0	2⁻	6 ⊕
7	"	"	+	0	+	1⁻	7 ⊕
8	"	"	0	+	0	2⁻	8 ⊕
9	"	"	0	0	0	– –	9
10	"	"	0	+	0	2⁻	10 ⊕
11	"	"	0	0	0	– –	11
12	"	"	0	0	0	– –	12
13	"	"	0	0	0	– –	13
14	"	"	0	+	0	2⁻	14 ⊕
15	"	"	+ 1 pap	0	+	1⁻	15 ● Rev. unstable
16	"	"	0	0	0	– –	16
17	"	"	0	0	0	– –	17
18	"	"	+ 2 pap	0	0	? – –	18
19	"	"	+	0	+	1⁻	19 ⊕
20	"	"	0	+	0	2⁻	20 ⊕
21	"	"	0	0	0	– –	21
22	"	"	0	0	0	– –	22
23	"	"	+	0	+	1⁻	23 ⊕
24	"	"	0	0	0	– –	24

24 lac ol picked from here and (β) gal tested

Comparison between not thirty here and latter ... results still suggest this is 2

according to table would 261 have been ... no. probably #14 + #15 switched

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

Lones reversion in double ?

+ᵈ gave stable + only

+ᵈ gave stable + only

1st 12 says (– –)

13	– –
6	1⁻
5	2⁻

don't know what to think about this.

3/5/54 Lysate made of 246A-15

lyophil		
/750	solid spot	indicator HFT gal₂⁻
/1210	0	
/811	solid spot	

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

Correlation of duction above with 1ᵈ ... This lysate is not + ... Lp₁ but that this is a ... Lp⁺ lysate

5 R
4− +
2+ −

5 R
4+ +
2− −
→ 5 R
4− +
2− +

(247A)

12/6/53

See pg 245 − Sample size = 22
6/22 r+t

W/1210 transduced by K12

Transduct	Transd. /518	Segregant /N16	N1	Segregant of Rx	Mlelt
1.	lyz	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.	..	0	+	↓	2−
12.	..	0	+	↓	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 r+u		15/15 gal2−		

6/18/54 Stock discarded
See ↙

Cross x 902 6/22/54 out
247A − 4
247A − 13] These in stocks 5/13/55
247A − 22] "
247A − 33

lysates 4/22/54

	spotted /2.75	/811
4	1/<0.1	2/0.5
13	0/<0.1	0.70/0.5
22	0/<0.1	0.20/0.5
23	0/<0.1	0.40/0.5

4, 13 — } these two lysates give large numbers of small papillae } possibly not sterile? partially HFT?

12/6/53

W1210 × 811

See 245 for origin

(247B)

Transd.	Transd. /518	seg ↑ /Ml +	/S1r 0	seg to R r ← 9/10	
1	lys.				NFT segregant found gal4⁻; t^R of NFT segregant found stbl. (5? circled)
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	" }	contaminated with galt		→	
14	" } rx	0	+		
15	" } bad	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, 902

1
8
14
18

} 6/18/54 Stocks discarded

2/9/54
Lysates /811 /2175
-1 0/0 solid smear = HFT gal4⁻ (ALSO as the result $F^+ \times F^x$)

See 245

(247C)

12/6/53
W1710 t 750

Transd. #	Transd. Lp Rx/98	Seg N16 / [illegible]	Seg N1 / [illegible]	Seg Lp Rx	locus
1	Lp v	o	+	v	2⁻
2	"	o	+	↓	2⁻
3	"	contaminated w. [illegible] gal →		v	[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	+	v	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⁻

6/20/54
These on stock agar {

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

12/5/53

4750 transduced by K12 λ

Transd. #	Transd. RX /578	/N1	/N16	Arbitrary
1	lys.	0	+	1⁻
2	"	pure galt →		
3	"	" " →		
4	"	" " →		
5	"	contaminated with galt →		
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 galt →		
16	"	0	+	1⁻
17	"	0	+	1⁻
18	"	0	+	1⁻
19	"	0	+	1⁻
20	"	contaminated with galt →		
21	"	pure galt →		
22	"	0	+	1⁻
23	"	0	+	1⁻
24	"	0	+	1⁻
	19/24 +ᵃ			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 culture surviving in stock of the four.

12/8/53 — W2373 (249) A

W1765 gal$_1^-$ (by c-s transd. with NA4 lysate) transduced by 841 - See pg 245

Transd.	Transd. by R	Stab of G-S	Seg.	N1	S18
1	S	non lys	stable		
2	R	lys	"		
3	R	lys	"		
4	R	lys	"		
5	R	lys	"		
6	R	lys	unstable	+	+ (?) λ^R = gal$_1^-$
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 1/23rd
Lp^{str} = 5 1 Lp$^+$

5 s
17 +
0 r

} 6/4/54 stocks discarded

23	22
24	16
12	12
23	13
82	63
23	23
	40/82

0.50
59 | 41.0
35 4
.560

.41
57

12/8/53 W2373

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

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

8/24 +ᵘ

$Lp^+ = 18$
$Lp^{s/r} = 6 = 3 Lp^s$ 8 Lp^+

6 1⁻
1 2⁻

What give?

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

(249C)

12/10/53 W2373

1765 gal- transduced by 902

Transd #	Transd. ~~Lys~~ Lp/518	Transd. stability	Segregant ~~Lys~~	~~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	−	+	✓±	λR 1−

← this is the only segregant obtained

7 Lp[r]
2 Lp[r]
3 Lp[s]

6/18/54

(Stocks discarded)

12/10/53 W2373 — 249D

sug. Samples = 23

W1~~675~~ 1765 gal- transduced by K12

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

20 Lp+ 3 Lps — 8 Lp+ 2 Lps — 9 Lp+

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

{ 6/18/54 Stocks discarded }

Expt. 642

(250)
A

F+ F- Lp+ Lpv

12/12/53 EMLs cross 1210 X 2234 Gal2- x Gal4-

1. The small colony "prototrophs" (probably require B1)

#	/M16	/S17	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	+ R	0	s weak	4
17	0 S	+	r ~~weak~~	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 ✓	0 (4 pq)	s	?
27.	0	+	r	2 —
28.	0 ✓	0 ✓	s	?
29.	+	0	s	4

23 = 4- all Lps
4 = 2- 3 Lp+, 1 Lpv
2 = 2-4- Lpv

F+ Gal2- Gal4+ Lp+ X F- Gal2+ Gal4- Lps

Cont. of 250A.

2. The Large colony prototrophs.

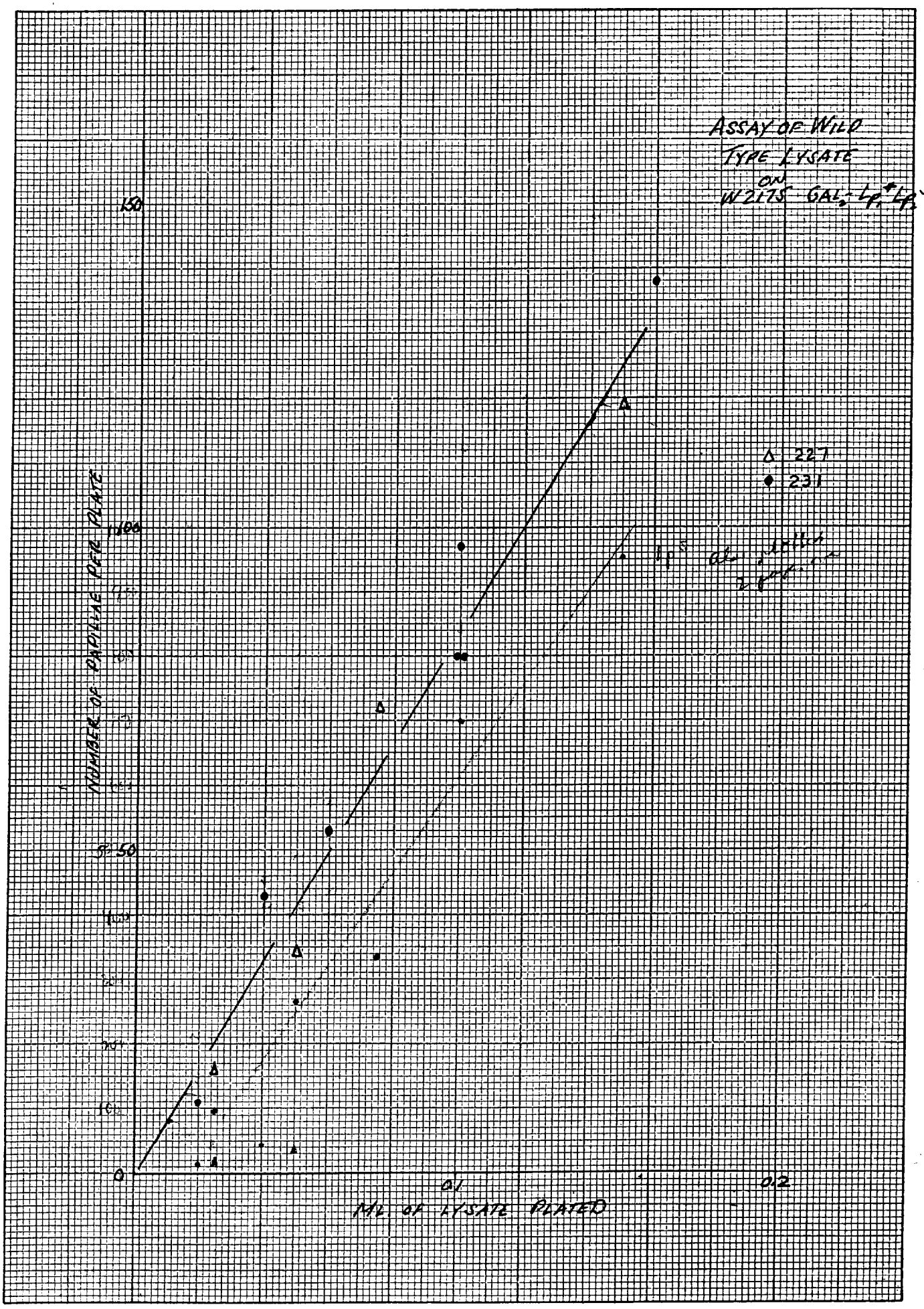
	-N16	-S18	Lp	Locus
1.	0 ✓	0 ✓	s	- - ?
2.	0	+	r	2 -
3.	+	0	s	4
4.	0	+	r	2 -
5.	+	0	s	4
6.	+	0	s	4
7.	+	0	s	4
8.	0	+	r	2 -
9.	0	+	r	2 -
10.	+	0	s	4
11.	+	0	s	4
12.	0	+	r	2 -
13.	+	0	s	4
14.	+	0	s	4
15.	+	0	s	4
16.	0	+	s	4 -
17.	0	+	r	2 -
18.	+	0	s	4
19.	+	0	s	4
20.	+	0	s	4 ✓
21.	0	+	r	2 -
22.	0	+	r	2 -
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

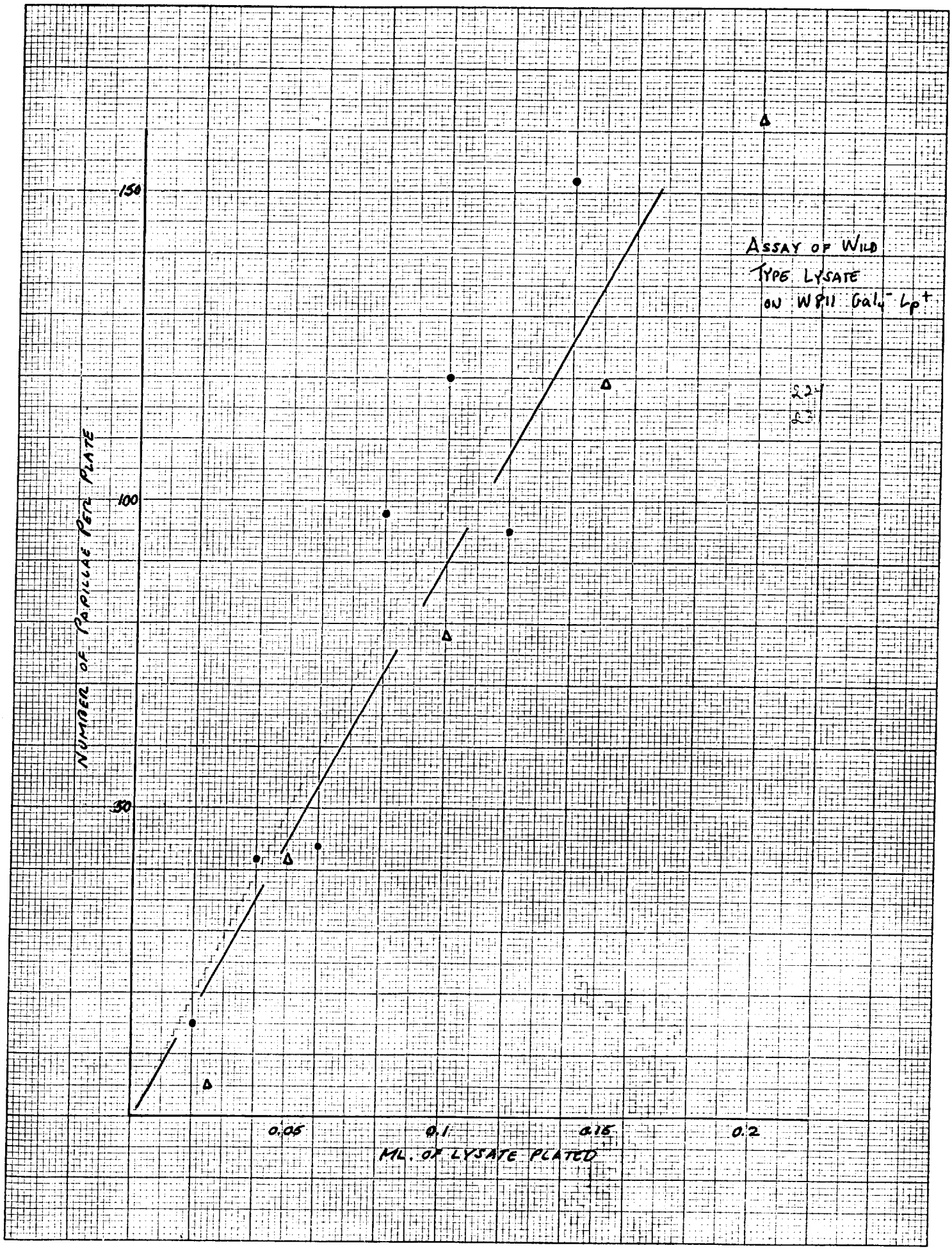
20 = 4⁻ all Lpˢ
10 = 2⁻ all Lp⁺
1 = 2⁻4⁻ Lpˢ

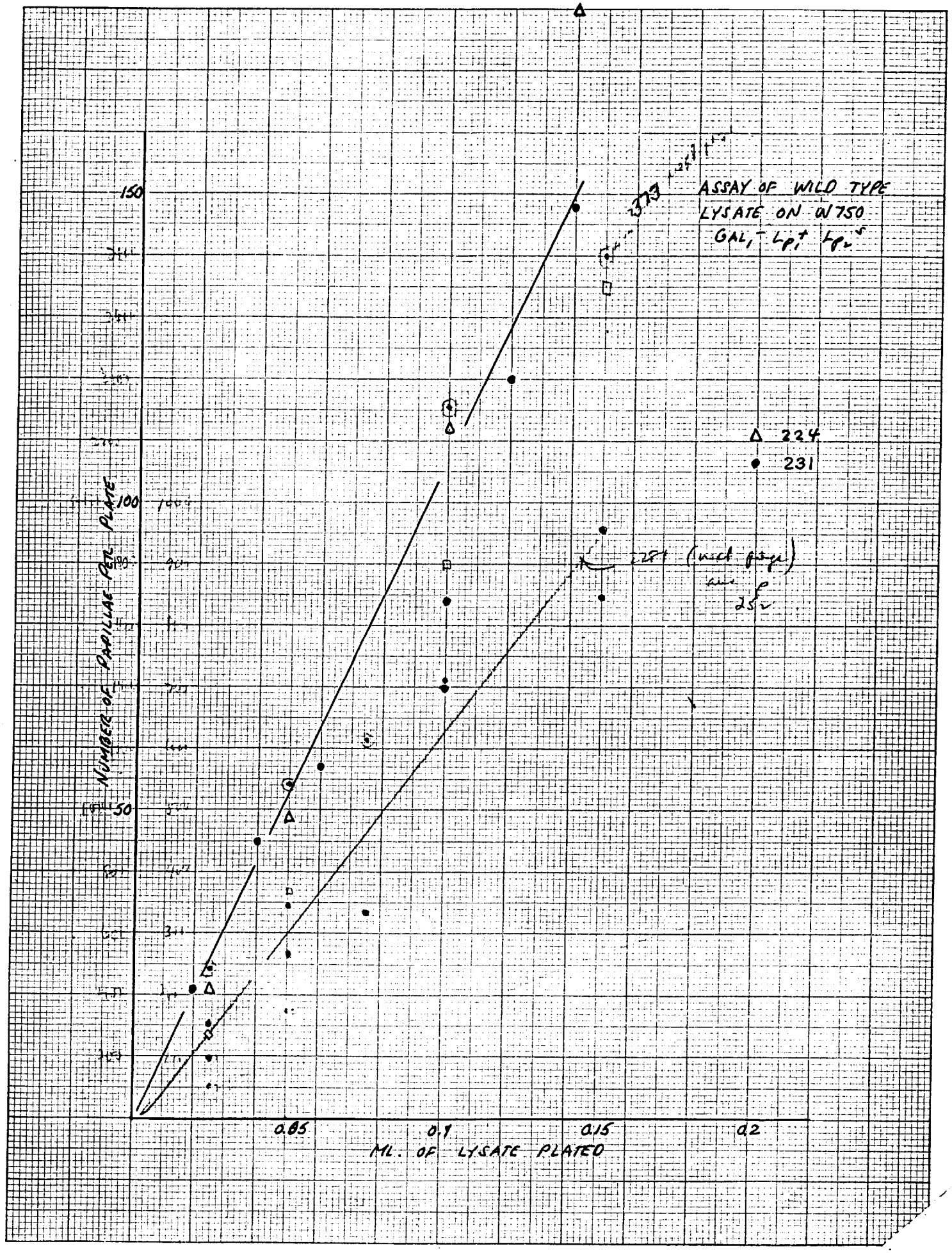
4⁻ Lpˢ X 2⁻ Lp⁺

Total of 250A + 250B

43	4⁻	Lpˢ
13	2⁻	Lp⁺
1	2⁻	Lpˢ
3	2⁻4⁻	Lpˢ







Assay - K12λ 5/30/53 3.6×10^10 λ/ml

Lp^K

250C

2175 → 227 / 231

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		

750 → 224 / 231

	224		231
0.025	21	0.02	21
0.05	49	0.04	45
0.1	112	0.06	57
0.15	183	0.08	—
		0.10	84
		0.12	120
		0.14	148

84 → 224 / 231

	224		231
0.025	5	0.02	15
0.05	42	0.04	42
0.1	72	0.06	44
0.15	119	0.08	51
0.2	[illegible]	0.1	120
		0.12	95
		0.14	152

P 253 K12λ 12/28

0 —
.025 = 5
.05 = 9
0.1 = 2
0.15 = 2

Gaeg -

2281 Lp^S K12λ 12/28/53 1.7×10^10 λ/ml

	0	0
0.025	96	151
0.05	266	345
0.075	334	—
0.1	700	706
0.15	955	842

2373 ~~2224~~ Lp^S 252 K12λ 12/28/53 1.7×10^10 λ/ml

0.025	483
0.05	1079
0.075	1223
0.1	2314
0.15	2799

578 K12λ 12/28/53 P.253 1.7×10^10 λ/ml

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

1924 P.253 K12λ 12/28/53 1.7×10^10 λ/ml

0	0
.025	~~[illegible]~~ 53
.05	~~[illegible]~~ 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_x - for several hours and then plating out on B gal

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	Gal₂ - Hfr Parent 2341	Gal₁ - 2345
✓	2341	47	—	—
	2345	3	—	—
	2343	3	(+) >500 ✓	(0) 3 ✓
✓	750	26	(+) >1000 ✓	(0) 14 ✓
	tp⁺ 1210	9	51 107 ?	(+) >500
	2175	14	(0) 17 ✓	(+) >1120 ✓
	4 2281	1	(0) 41	(17) 21
	518	11	(+) >10⁴ ✓	(+) >250 ✓

12/29/53

Are lysates "decaying" at room temperature? Inability to repeat massive dilution 518 and the inability to obtain expected ~~amount~~ 710.5 of transduction of tp^s as compared to tp⁺ says perhaps.
This would also argue that transduction of tp⁺ is affected by particles different from those affecting tp^s dilution.

No trans'd. of 518 - 2281, 1765 gal₁ - by K12 - 5/30 λ

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

1. K12 - 167 X 10 X 10⁷ = 1.67 X 10¹⁰ against 2281
2. N16 - >50 X 10 X 10⁷ - >5 X 10⁹ " "

N1765 gal₁ - made by (-) transd. entered into stock book as

W2373

12/31/53

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

1. 2373 - gal, - Lp^s host cure - - no cells in suspension

= 1378 + 1108 / 2 = 1243 X 10^7 = 1.2 X 10^10

added / plate = 1.2 X 10^9

λ added	pap/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

2. 2281 - gal₂ (Gal₂⁻) - Lp^s - no cells in suspension - 2145 X 10^7 = 2.1 X 10^10

2.1 X 10^9 added

suspension appears mixed - large and small colonies

λ added	pap./plate	
.0	0	
0.025	96	← 18 of these picked and added - tests against 511 - all neg. λ^+
0.05	266	
? ~~0.075~~	~~377~~	
0.1	700	
0.15	955	

1/9/53
Purified 2281 - small colony from cell assay 221/201 } 211 X 10 X 10^7 = = 2.1 X 10^10

2.1 X 10^9 added

0	0
0.025	151
0.05	345
0.1	642
0.1	706

1/4/54

Cells from aerated 10× conc. cultures.

Assays – K12 λ 12/24/53

		Δ	
1. 581			
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₋ lysate of 1/9/54

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

Non λ exposed cell titer = 708
889
794
889
$3280/4 = 820 \times 10 \times 100 \times 100 \times 50 = 410 \times 10^7 = 4.1 \times 10^9$/ml

λ exposed cell titer
761
651
670
719
$2801/4 = 700 \times 10 \times 100 \times 100 \times 50 = 3.5 \times 10^9$/ml

(2) Distribution of colony types –

	gal (−)	gal (+)	gal-[illegible]	Total
1. Control (broth exposed)	3280	0	0	3280
2. λ exposed	2801	31	~~58~~ 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 Lp+	R	lys	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^R	some s^t present?	"	
—	8.	non lys	S	" ?	18	Lp+ "	"	"	28	lys Lp+	R	"	
—	9.	" "	S	no (int. θ)	19	Lp+ "	"	"	29	lys Lp+	"	"	
—	10.	—	—S	a (−)	20	Lp+ "	"	"	30	lys Lp+	"	"	
									31	lys Lp+	"	"	

254B Lytic λ – Retest (#3) of lytic λ lysogens 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 = W2866
254-16 = W2867

1/13/54

Crosses - check on Lp gal segregation

A 1. 1765 X 750 on S gal

39 prototrophs - all (-)

B 2. 2281 X 2035

25 (-)
379 (+)

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	"	2–	
12	? no	stable +	
13	yes no	"	
14	"	(– –)	a 1-6, 10
15	"	(– –)	
16	"	(– –)	
17	"	(– –)	

2/4/54

811t902 (New lysate of 902 - 3 bottles)
for the purpose of isolating HFT gal$_4$-
1. no add ½ = 19
2. 0.1 902λ = 89

2/8/54

750t902 gal$_2$- (segregants sitting around about a month) suspected of HFT gal$_2$ - Origins now cloudy - Originally 3 but one lost
Both in need of purification

#1 Test for HFT				#2 Tests for HFT	
257-1 →	Col. 1.	750 NFT		1.	750 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 = gal$_2$-, +^{R} stble
257-3 = gal$_2$-, +^{R} stbe

W2869

518 transduced with M6λ 1-9-54 for the purpose of establishing that the transduced colonies contain both lp^u, 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 10^6 and plated β gal

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 10^1 X 10^6 X 3/1   100 X 10^7 = 1.0 X 10^9 cells   3 X 10^7

C

1 * a colony such ●
2. ** " " " ◐ } both streaked - 10 (+) and 10 (-) colonies picked + ~~streaked~~ brushed 1λ / 518
see p. 262 for consideration.

	1. λ (+)	518	λ (-)	518		2. λ (+)/518	λ (-) /518		
1.	R	lysis	R	lysis?	← then	R	nonlys.	sens. nonlys	← then
2.	"		"			"	"	"	
3.	"	"	"	"		"	"	"	
4.	"	"	"	"		"	"	"	
5.	"	"	"	"		"	"	"	
6.	"	"	"	"		"	"	"	
7.	"	"	"	"		"	"	"	
8.	"	"	"	"		"	"	"	
9.	"	"	"	"		"	"	"	
10.	"	"	"	"		"	"	"	

257C-5 +^{u} lp$^+$
257C-6 +^{u} lp^R
? segregant from this
W2868

after 10

W2350 - double ducks:
parent culture streaked out and 10 colonies examined to eliminate possibility of including reversions at one locus in the inoculum — and — and therefore transducible by one of the 2 (-) lysates.
1. All 10 colonies not transducible by HFT 2⁻, 4⁻
2. Loopfuls of overnite broth plated on B gal.

2350 = 4⁻8⁻

Cells — (c.60 pap) HFT 2 λ; (0 pap.) HFT 4 λ; (c. 20 pap.) HFT 2 + 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 be (− −). Pick up 12 and see —

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

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

3 (2⁻
2 (− −
1 (4⁻

2/18/54 stock discarded

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

258-B → pap

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. (control + minute papillating)	0	+	2⁻			
7.	0	+	2⁻			
8.	0	0	(− −)			
9.	0	+	2⁻	9	0 (+) pap	
10.	+	0	4⁻	10.	46 (+)	% stable
11.	+	0	4⁻	11	40	% stable
12.	0	0	(− −)			
13.	0	+	2⁻	13.	22	% stable
14.	0	+	2⁻	14.	9 (+) pap	% stable
15.	0	0	(− −)			
16.	+	0	4⁻	16	11 (+) pap	5/6 mixed
17. (control + minute papillae)	0	0	(− −)			
18.	0	+	2⁻	18	cultures went (+)	
19.	0	+	2⁻	19		

9 2⁻
7 (− −)
3 4⁻
↑ false labelled 1L

*
reclass. B3
9 (2⁻ w. 8⁻) 2⁻8⁻)
7 (2⁻4⁻ + 8⁻4⁻)
3 (4⁻)

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

	/750	/1210	/811
action =	0	0	0

2/13/54

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

24 picked to gal for purification - all (−)

2/22/54 Rpt HFT induction with N16

EML Analysis EML 667

N16 lysate 1/9/54 - 0.5 ml
N16 titer - 2X10⁹   200/8.9 = c. 20x multiplicity   0.5 ml N16 + 0.5 ml cells
Cell titer = 892 x 10⁵ = 8.9X10⁷

control plate c. 900/plate - all (−)

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

No. of (+) 21, 23, 35, 24 = 103/4 = 26   870 | 26.00 (0.03) 2610   = 3.0% (+)

↑ See above

51st HFT induction with N16 1/9/54

Aerated culture 1/10 - 1 ml sedimented - resuspended in 0.5 ml N16 added - adsorb. c 3 min centrifuged - Rpt 3 times except no centrifugation after the 3rd - dil 10², 10², 0.5/10 → plate

Control plates:

37C 371, 341, 319 = 1031/3 = 344 x 10⁶ x 2 = 688 x 10⁶ = 6.8X10⁸   36/344 = 10.4%

30C 360, 398, 361 = 1109/3 = 369 x 10⁶ x 2 = 738 x 10⁶ = 7.3X10⁸   39/369 = 10.5%

Esther's Page 667

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

N16 Assay -

λ 10⁷ dil = 106
130/236 = 120X10⁷ = 1.2X10⁹

↑ why 2-fold diff? 0.2 ml pipette?

Transd.
10⁷ dil = plaques = 240
papillae = 104

no control plate for spont. done on assumpt = ¼ ratio pap/plaques = 60/240 = 1/4

Testing of (−) in plate - λp^x and (+) present in any (−) colony?
30 gal - no no
7/28 found mixed λp^- + λp^s - self plaquing

2/17/54

Looking for Recombination between tp^+ by selecting for (+) recombinant when two diff. gal- are tp^R

1. This is second attempt - previously no tp^+ found in (+) recombinant between 2341 gal_1^- tp^R x 1924 gal_4^- tp^R

2. In this case 2341 gal_2^- tp^R x W1 (= 1924 + 902 tp^R → seg gal_2^- tp^R) out of 33 gal(+) in a Bgal mixed culture of these (probably includes sp. reversions) none found tp^+. Not expected? since in 1st test tp^R_x x tp^R_{1924} failed to give tp^+.

What goes here? 2341 = $\frac{Gal_2 - tp^R}{Gal_2 - tp^J}$

Where did Gal+ come from?

518 + N16 - again for higher fraction (+)

0.4 ml N16 1/9/54 + 0.1 ml of 518 culture c. 10^8 cells/ml

cell assay of above	10^2, 10^4 a'	3 plate	1 = 600	no (+)
dilution plate	" " $\frac{x1}{5}$	3 plates	1 = 163 x 5 815	no (+)

Failure? why

2/19/53

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

246A 15 - labelled 11 reversions examined

~~Reversion~~ colony	single seg (+)	~~HFT~~ (-)	Structure		Labelled
1	0	0	[illegible]	—	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+ve / Check segregants from culture labelled 246A-15+^R-1 to see if conforms to original description of 246A-15+^R 11

		reversion col.	Minus	characteristics		
261 C	3/5/54	1	6/7	(- -)	1 (gal₂ -)	} according to this it is not 246A
	Lysate of 261C →	2	stable			
		3	7/7	(- -)		
		4	7/7	(- -)		

~~750~~ solid spot } indicate
~~120~~ " " } HFT
~~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 p. 270 [illegible] NFF
[illegible] N16 [illegible] $1^- 2^-$. Since N16 [illegible]
[illegible] $1^- 2^+ \rightarrow 1^+ 2^-$

2/21/54

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

Segregant	518	N16 HFT 2⁻	518 HFT 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	7	—
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) (8)
- + R (6)
+ - s

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

The ideal readings on p 242 and in any case from $1^- 4^-$ indicated are not (+).

The HFF result suggest [illegible]

$1^- 2^- \rightarrow \overline{1^+ 2^-}$

On these [illegible] #1, 2, 6, 7 in the table would be

$\rightarrow \overline{1^- 2^-}$

In which case the [illegible] have been

$1^+ 2^+ 4^- \rightarrow \overline{1^- 2^- 4^+}$

$\rightarrow \overline{1^- 2^- 1^+}$

[illegible] been observed before.

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 stained and streaked on B gal

gal_2−	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)

246A-15 — prototroph stopped! cross with 1503 gal1− EMS gal

246A-15 $\frac{2\ 1}{-\ -}$ ——• $\frac{}{=+}$ → aerated = F−

1503 gal− ——• + − F+

control plate sh

	(+)	(−)
plate 1.	27	0
2.	13	3
3.	25	3

10/26/56 ~~this~~ ~~[illegible]~~ where did these counts come from if we is prototroph?

2/22/54

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

			To Stock
1. 2 gal₂ - obtained	one	Lp⁻	W2428
	one	Lp⁺	W2429
2. 1 gal₁ - obtained	one	Lp^v	W2430

Also This day
2431 = 2478-1
2432 = 1673 gal₂⁻
2433 = T18
2434 = T19

Crosses of gal - Control streaks on B gal = pure

1. (X) 750 - c. 30 (small) / plate × 11 = 0+/330
  aerated

2. (X) 1210 - 9, 8, 13, 12, 12, 14, 12, 6, 6, 8 = 0+/101
  aerated ↑

3. (X) 801 - 3/17, 3/14, 0/15, 1/28, 1/17, 4/15, 0/16, 5/23, 0/29, 2/16 = 19+/190 - (+) questionable of 18 checked ✓ to see if diploids. 7 found (+) 5 of which may be diploid. (4 were) ←

Analysis of these diploids by examination of segregants

#2 9 (-) segregants tested against HFT 1⁻, HFT 4⁻ - all Lp⁺ ʷᵏ and gal₄⁻
#3 10 (-) segregants " " " " - all Lp⁺ ʷᵏ and gal₄⁻
#4 8 (-) segregants " " " " - all Lp⁺ ʷᵏ and gal₄⁻
#5 12 (-) segregants " " " " - all Lp⁺ ʷᵏ " gal₄⁻

W2360 - a Lp^v form of W2350 gal₂⁻ gal₄⁻
6 colonies tested from old broth streaking out from cell all Lp^v and not transduced by HFT 2 or HFT 4 in two days
Stock made

3/4/54

Examination of Transduction Complex ($+^{4}$) for HFT nature if 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	per plate 1-2
not sterile?	2175tFW-1				+	2 kinds +++	?
both	518t750-1	+++	+++	+++	18	83	?
labelled	750tK12-2	+++	+	+++	14	32	5
2175t801-1	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 -
(D1)	153	gal_2 -	from 592A — × 57F	M -
(S18)	202	gal_4 -	probably NFT now	M -
(#14)	242	gal_2 -		Arg
(#19)	242	gal_2 -		none
246A-15	246A	gal_2 -		none
202-16H	202	gal_2	S16 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
217S2750 + u		+ u	750A — × 217S	none
57BE1K12	263	+ u		M -
750E1K12-1	263	+ u		M -
750CR02-1	263	+ u		M -

3/21/53

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

	Lysate	750	1210	811	Comment	Cell Characteristics / Making up lys	Stability of gal+ Revertant*	Yellow crayon #
in NSB →	2342 (3/16/54)	+++	0	+++	Known gal_2^- HFT	—		1
Pen- 5bth.	2342 (3/17/54)	+++	0	+++	Known gal_2^- HFT	—	64 12/12 $+^a$ ②	2
	2175t811-1	++	[illegible] small	+++		mixed		3
	750t612-2	+++	++	+++	behaves as + HFT	mixed		4
two / NSB →	750t902-2	+++	+	+++	" " "	mixed		5
dif. cultures	247B-1 pap 2	1 pap	2 pap	0	gal_1^- HFT lost?			6
mislabelled	2175t F11-1	0	0	2	NFT?	mixed		7
here	51Ft750-1	+++	+++	+++	behaves as + HFT	mixed		8
	750t902-2	1 pap	0	2	NFT?	mixed		9
	258-16	5 pap	1 pap small	0	gal_1^- unstable reversion			10
	246A-15	+++	0	+++	gal_2^- giving $+^u$ reversion			11
the same?	261C	+++	++	+++	segregates (– –) principally			12
	51Ft K12	+++	c. 100 pap	+++	behaves as + HFT	mixed (+) (–)		13
	750tK12-1	+++ [illegible] pap	++	+++	" " "	" "		14
	750t902-1	18 pap	2 pap	c. 30	NFT ?	mixed		15
	2346					—	5 4/5 $+^u$ ②	

+++ = solid drop

++ = some discrete pap

One of these is 2175t811, other 2175t751

Beside the yellow crayon numbers: 4/10/54 all tested streaks in Pen. [illegible] 0.5 ml sample

* Gal+ Reversion Stability – no. obs., no. found $+^u$/no. element

	Lysate	750	1210	811		Yellow crayon #
4/19/54	750t902-1	0	0	0		16
4/19	750t912-2	+++	+	—		17

16, 17 also tested streaks

3/22/54

Transduction with 2342 (=W16) in NSB

① W518	No (+)	No. (–)	No(–) contam.	No(–) col. pap	dilution	
Lysate	8	140*	9	1	sampled in 10 ml, 10^2, 1-50 → plate	8/140 = 140/8.00 = 0.057 ... = 5.7% (+) transd. on Lp^s
Broth	0	679*	0	2	"	

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

2. W811						
Lysate	1	426 *	0	5	above	426 / 1.000 = 0.0023 ... = 0.23% (+) transd.
Broth	0	448 *	0	1	"	

* 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
[After 5×10^7 = 20, 26 = 1.15×10^9 (average of our final plating on B(c)).   1.2/1.2 = 0.0

on 2252
Before - above
After 5×10^7 = 15, 13 = 7.0×10^8] 7/12. 12 / 7.0 = 0.58 ... : (42% adsorpt.)

3/30/54 Repeat examination of adsorption of s^c cells.

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

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

2. S^c cells (6.3 ml) + 0.3 ml 2342 λ (NSB prep 1/9/54) - incub. 37° 10', 9.4 ml broth added, centrifug. 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. 15/21 = 71%   29% adsorpt.

3/28/54

p 184

518C750-1 (Look up origin) Nature of segregant.

Seg.	/HFT-1	/HFT-4	Locus
1	o	+	1⁻
2	o	+	"
3	o	+	"
4	o	+	"
5	o	+	"
6	o	+	"
7	o	+	"
8	o	+	"
9	o	+	"
10	o	+	"
11	o	+	"
12	o	+	"
13	o	+	"
14	o	+	"

what happened to 4⁻?

all λ^R

lysate made of these to see if HFT-Hfr & has any additional properties

2341 transductions (for TCN) using his cel here which was found $\frac{L}{E}$ 5⁻) Use in crosses of added segment as a test of elim. and passage of segment in 5⁻ crosses.

269-1 1. K12 lambda 1. mock = 4
2. 0.1 K12 (6/30?) = 32×58 = 116 = 1856
174/1856

just done beyond induction

2345 (as above) 1. 902 lambda - (update HFT Hfr)

1. no add 2
2. 0.1 902λ 42

4/7/54

269-3 In Relation to 518C750-1 Above Transduction of 811 with HFT 1⁻ 2346
Examination of stability - Is the (1⁻ / 1+ 1⁻) combination +? Does 1⁻ and 4⁻ segregate from such a complex? Are the observed (+) in 4⁻, 1⁻ transductions (+) after elim. of a (-) allele?

1. 8 papillae examined 6/8 stable.

	/HFT 1⁻	/HFT 4⁻		HFT/1⁻	HFT/4⁻
A 1.	−	+	B 1.	+	−
2.	−	+	2.	+	−
3.	−	+	3.	+	−

3/28/54

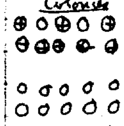
Examination of HFT stocks.

1. Previously noted (in last week) that cultures of N16 (= 2342) and NA-4 (= 2346) were segregating NFT lines (spot plate test) (need. on plate possible. (dropful added to indicator, plate irradiated). In each case of 10 colonies 8 were HFT - From each a NFT and HFT selected and placed on slants and stored in refrigerator to lessen segregation. A portion of each plated on Bgal and reversion examined - (See pg 267)

2. 241-14 (a gal₂ - HFT) tested against 1210, 750 - 10 colonies

Colonies

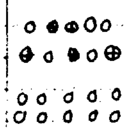
against 750 · against 1210



= 8/10 HFT. Stocks made of an HFT, NFT

3. 241-19 (a gal₂ - HFT) as 2 above

against 750 · against 1210



Examination of NFT, HFT stocks above - also 2342, 2346 - See page 267 also

Culture.	No Reversions	Stability	/4 HFT	/1⁻ HFT	/2 HFT	4⁻ HFT	Allele
			Transduction anal. ?				
241-14 HFT	~~19~~ (Some)	12/12 +ᴴ	(+)	+	0	(+)	2⁻
" NFT	~~#~~ 12	12/12 stable	+	+	0	+	2⁻
241-19 HFT	12	12/12 +ᴴ	(+)	+	0	(+)	2⁻
NFT	0	—	+	0	0	~~(?)~~ +	1⁻2⁻
2342 HFT	62	12/12 +ᴴ					
2342 NFT	~~#~~ (0)	—	+	0	0	+	1⁻2⁻
2346 HFT	~~#~~ 5	4/5 +ᴴ					
2346 NFT	8	8/8 stable	+	0	+	+	~~[illegible]~~ 1⁻

4/5/54

Examination of HFT lysate:

1. 750tKR-2 - Assay on different indicators - 0.1ml of overnight unaerated cult. used.

dil	0.1ml No pap.	Assay cult	dil	0.1ml No pap.	Assay Cult.	dil.	0.1ml No pap	Assay cult
no	0	750	no	0	2433(1⁻2⁻)	—	3	1210
10^2	+++	"	10^2	++	"	10^1	+	"
10^4	365	"	10^4	214	"	10^4	23	"
10^6	12	"	10^6	4		10^6	1	"
Titer	1.2×10^8			4×10^7 2.1			2×10^6	

2. 750t902-2 4/8/54 (Lysate not worked)
Titered on U2 (sp. λ^s) $10^7 = 717$ - 7.2×10^9 - Lysate tested stock · p267

Constitution presumed $\frac{1^- 2^+}{2^- 1^+}$

Assay	750 (1⁻)	1210 (2⁻)	2433 (1⁻2⁻)
no add	0	3	0
0.1ml of dil 10^2	+++	$\frac{1}{8}$ 200 = 1600	183
" 10^4	++	13	1
" 10^6	12	?	0
titer	1.2×10^8	1.0×10^6	1.83×10^5

suggests

4/11/54.

U2. - spont. lp^s? from 2175t811 → (-) seg lp^s gal$_4$ - Only useful marker for elim. possibility of contamination is prototrophy. This culture grows on EMS gal - Presumably this is a valid recurrence of lp^s - No other gal$_4$ - lp^s prototrophs about at time of isolation.

4/5/54

In search of triple (-) (1⁻2⁻4⁻)

1. W2350 ~~backcrossed to~~ exposed to HFT 1⁻ (2346) - Segregants examined

seg #.	HFT 1	HFT 2	HFT 4
1	+	0	+
2	+	0	+
3	0	+	+(weak)
4	0	+	+ (weak)
5	+	0	+
6	+	0	+
7	+	0	+
8	+	0	+
9	+	0	+
10	+	0	+

8 2⁻
2 1⁻

2⁻
1+
4⁻

X − + / − / +

In these cases a few small papillae noted similar to the rx of 2350 against HFT 2⁻ Significance?

2. T18 = W2437 (1⁻) gal₁⁻ gal₂⁻ ~~backcrossed~~ exposed to HFT 4⁻ (2478-1) - Segregants exam.

	1⁻		4⁻
1	0	0	+
2	0	0	+
→ 3	0	0	+
4	0	0	+
5	0	0	+
6	0	0	+
7	0	0	+
8 →	0	0	+
9	0	0	+
10	0	0	+

minute papillae in these - also on control spots.

272-2

all 1⁻2⁻

2⁻ +
1⁻ +
4+ −

4/7/54 - and several days earlier

Recovery of Lp_2^s from Lp_2^R culture

W902 -

1. transduced c 2346 AFT 1° λ - Reaction suggest fairly large fraction of Lp_2^s present.
2. Streaked onto B gal - seg +° observed.
3. Tested for seg and (c.b.) all gal_1^-
4. One tested for nutrition - D(0) no growth; + TZ growth 2 days; + LB no growth; + TB, no growth; + TLB, growth 24 hrs. } nutrition the same stock — tested on B Mal = (mal +)
5. Examination of more segregants

W2584

W945

1. transduced c 2346 AFT 1° λ - React. also suggest fairly large no. Lp_2^s present.
2. streaked B gal - +° observed.
3. 6 seg tested /2346 λ /2342 λ - all gal_2^-
4. Examination of the culture for sugar reactions.

Mal + Xyl - Mtl -

3 additional colonies also found Mal +

W2580

4/9/54 In search of phage mutants.

Platings on Endo - all platings gave no plaques with the following cultures. 1485, 578, 2281

Assays on NSA (pours)

	$/10^2$ dil	$/10^1$ dil
2281	274	10
1485	237	10
578	281	6

4/10/54

Repeated at lower dilution

	10^3	10^5
Spont. Lp^s → U2		
578	0	0

4/14/54

Is the complex $\frac{1^+ 4^+}{1^- 4^+}$ phen. (+) ? All (+) appear to be secondary - segregating out (-)

51F × 2746 λ - Single exposure to phage - unaerated broth, near sat. per cent.

(A)

Treat	51F cell dil	No. (-) papillating	No (+)	No. pure (-)	Total
1. broth	10^6	0	0	67	67
2. 2746 λ HFT 1⁻	"	9	2 (papillate)	41	52

(B) Examine (-) on treated plate to see what they are segregating

1. Single (-) appearing colonies (up to 2 days - then giving a papillating appearance thus ⊛, and turning darker in the following days.
2. Streaked out and giving
   a. (+) some of which appear to be segregating (-)
   b. (-) non papillating (?)
   c. parental type (-) which papillate
3. 10 (-) colonies picked and streaked against HFT 1⁻ + HFT 4⁻

#	HFT 1⁻	4⁻	Lp Rx	Comment	Allele
1.	+	0	S	Lp to segregating also	4⁻
2.	+	0	S		4⁻
3.	mixed (+) and (-)		R?		— (?)
4.	+	0	S		4⁻
5.	mixed (+) and (-)		R(?)		— (?)
6.	"	"	R(?)		—
7.	"		R(?)		—
8.	0	+	?	Some R?	Rc ✓ —
9.	intermed (+) turning dark after 3 days				—
10.	"	"	R?		—

Only segregants are 4⁻ Lp^S

Lp^R preventing segregation of 1⁻ ?

parental / reexamine from streaks / parental / parental / parental

4/28/54

902 Mal- → Mal+ = Lp_2^R → Lp_2^S ?

10 well isolated colonies (-) on Mal, tested and found Lp_2^R streaked on Mal for reversion exam. (1/streak) and on gal coated with HFT λ.
1. Exam papillae on gal for instability indicating Lp_2^S + transduction.
2. Exam Mal+ for Lp_2 reaction.

①

Original Colony	No. reversions B mal		Lp_2 Reaction of Mal+	No. pap. B gal + HFT λ	Stability of Gal pap. (6 picked)	Mal + Lp_2 character of unstable pap. (Control colonies)		Transd. of Segregant from λ HFT	λ HFT
1	+		Lp_2^S	31	2/6 stbl	mal-	Lp_2^R	0	0
2	0		—	16	4/6 stbl	"	"	0	0
3	+		Lp_2^S	18	0/6 stbl	"	"	0	0
4	+	intermed (+)	faint reaction	12	5/6 stbl	"	"	0	0
5	+		Lp_2^S	14	2/6 stbl	"	"	0	0
6	0	intermed	—	10	3/6 stbl	"	"	0	0
7	+	(+)	faint reaction	16	3/6 stbl	"	"	0	0
8	+		Lp_2^S	26	1/6 stbl	"	"	0	0
9	+		Lp_2^S	12	1/6 stbl	"	"	0	0
10	+		Lp_2^S	21	4/6 stbl.	"	"	0	0
			6 Lp_2^S 2 internal sens.					All non transducible	

Σ. mal+ reversions are Lp_2^S or intermediate sensitive

Selection here may not be for the occurrence of Lp_2^S under conditions of extreme purity of cell population but may be for host range mutants in the HFT phage population.

② Lysate made of #1 (unstable, and seg(-))

	0/ml	results
1.	750	solid smear
2.	578	" "
3.		

5/6/54 W2331 mal- tested for transd. with N16 4/9/54 λ (HFT
not found transd.

5/4/54

J.L. Single cell expt.

1. 2342 (1/9/54) + 578 cells
2.4×10⁹
0.5 ml + 0.5 ml → Adsorption 10 min. 37C → Centrifuge 10 min. → resuspend 0.5 ml pen. →

Total cells: 213 185 / 2 99 = 2.0×10⁸ — No. (+): 16 (18)

dil 10² 10⁴ 10⁶ 425 TK · 3.89/10 (?) dil ÷ 10⁶

2. Control
0.5 ml Pen + 0.5 ml no → → → → 401 851 / 2 = 4.25×10⁸ — 0

24 hour count

JL reports no. [illegible] present in his sample of cells studied c̄ the microscope.

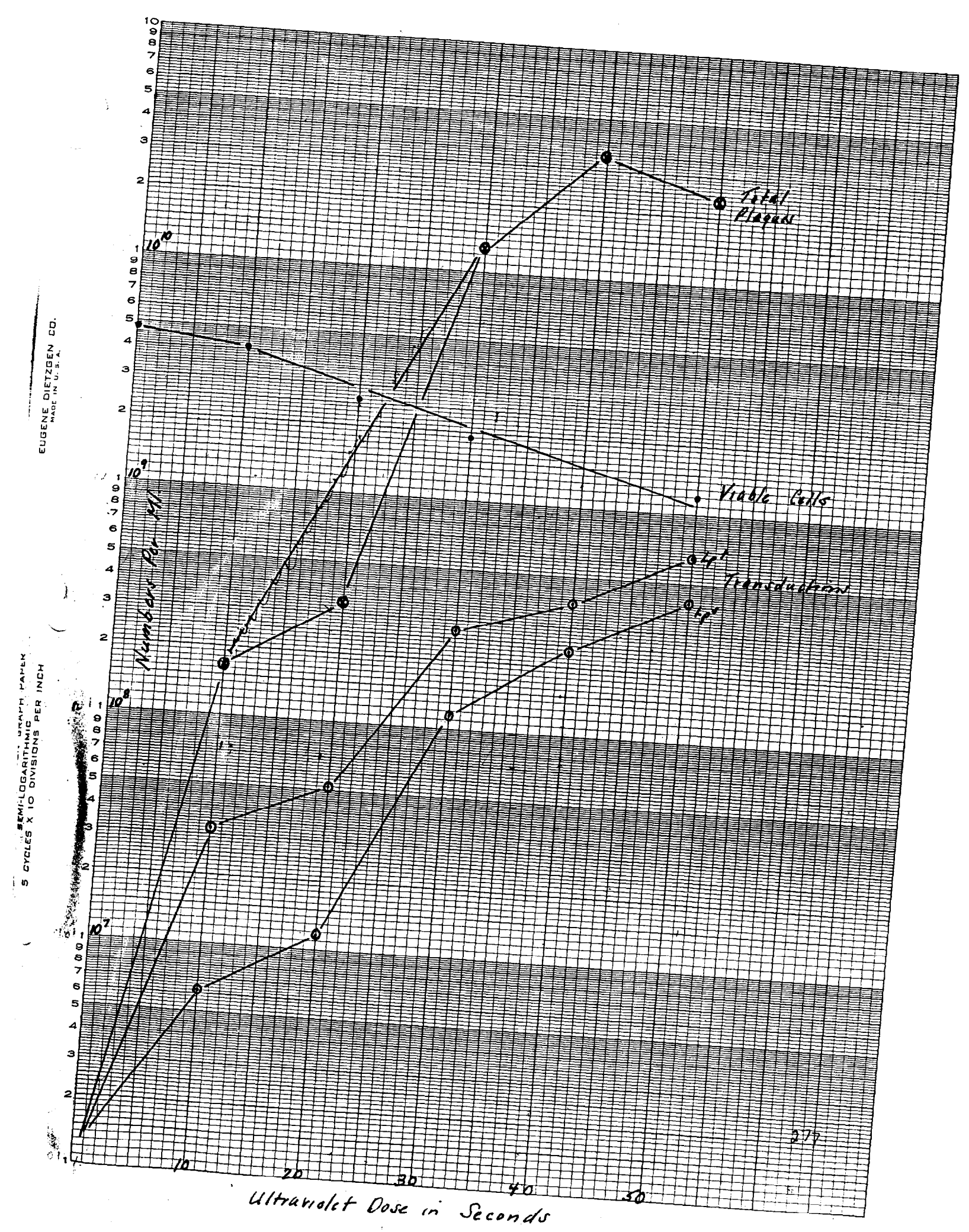
5/6/54

This day 2252× — Hft gal₄⁻ (247 B4) entered in stock book as W2487 presumably made and tested c. Mar. 24, 1954 – Culture to TCN at this time also.

5/6/54 For the purpose of studying the transmission of the [illegible] in crosses. As sugg. by JL. Transf. 2252 (=Hfr gal+ [illegible]) to ++/−+ and then cross to ++/−+ ⊗ +−

1. 2252 treated Hft gal₄⁻ (W16) colonies examined for "phage contam" appearance – some found streaked out.

2. On second streaking, all + [illegible]


Total Plaques
Viable Cells
4h Transduction
1½h
Number per ml.
Ultraviolet Dose in Seconds
10
20
30
40
50
10^{10}
10^{9}
10^{8}
10^{7}
EUGENE DIETZGEN CO.
MADE IN U.S.A.
SEMI-LOGARITHMIC
5 CYCLES X 10 DIVISIONS PER INCH
277

5/26/54

234z. Test in survival to see if inability to get lysates with high titers is caused by application of wrong u.v. dose.

1. Aerated culture - nearly saturated - centrifuged and resusp. in saline
2. Irrad.

0.1 ml added to 10 ml Pen incubated → dil 10^4 add. in NSB for cell Assay

Irrad.	Single plate Count	N/No	Plaques (total) 1-100 dil	Transd./plate x 10^3	Δ		Plaque/1-100
0	477	1.0	0	50	0		0
10	417	0.88	103X16 = 1648x10^5	112	62	dil x10^3x10^4	~~1.65x10^8~~
20	263	0.55	c. 2X10^?	164	114		
30	194	0.41	1284x10^7 = 1.3X10^10 [illegible]	1200	1150		
40	(256) -	—	3456x10^5x10^2 ~~[illegible]~~	2368	2318		
50	117	0.25	—	4160	4110		

1-100 10^6

Time	Viable Cells Present	"Induced" cells	Transd. produced	Plaques produced
0	4.77X10^9	0		
10	4.17X10^9	0.6x10^9	6.2x10^6	1.65X10^8
20	2.63X10^9	2.14X10^9	1.14x10^7	c3.3X10^8
30	1.94X10^9	2.83x10^9	1.15x10^8	1.28X10^10
40	est. 1.51x10^9	3.18X10^9	2.32X10^8	3.46X10^10
50	1.17X10^9	3.60X10^9	4.1X10^8	2.4X10^10

Transd. Assay on 8U

			Plaque / Transd.	Ratio
no add	17			
Irrad. Dose				
10'	16x200x10^4 =	3.2x10^7	16.5/3.2	5.2
20'	32x163x10^4 =	5.2X10^7	33/5.2	6.4
30'	169x16x10^5 =	2.7X10^8	103/2.7	38
40'	379X10^6 =	3.8X10^8	340/3.8	90
50'	654X10^6 =	6.5X10^8	240/6.5	37.

6/1/54 Transduction of lp_2^R with HFT lysates - An indication of host range change in λ?

1. 1412 (lp_1^S lp_2^R Gal$_4^-$) transd c̄ 2342 λ
   - no add 40
   - 2342 λ 456

#1, #2 2. 30 papillae picked - after 2 streakings all stable on Gal
3. 24 Gal papillae " - after 4 " 2 unstable - both non-lys on 518, both mal-
#3 4. 24 " " " 3 streakings 1 " - Mal(-), non lys. on 518
#4, #5 5. 24 " " " " 2 - , non lys on 518
also 23 stable gal+, non lys. on 518

6. Examine Mal + Reversion of above and see if sens.

Mal Revers		Mal Reversion	lp_2	Gal		Mal Rev	lp_2^R of Mal+ rev	lp_2 Recomb	518 Mal Rev.	Gal+ of
internal	1.	+	R	seg	}	full	full sens.	R	non lys	+w
full +	2.	+	R	seg		"	" "	S	non lys	—
full +	3.	+	R	seg		"	" "	R	" "	+w
internal	4.	+	R		?	internal	internal sens.	R	" "	+w
~~full~~	~~5.~~	~~+~~	~~R~~			?	?		?	?

Suggests that transd. accomplished by lp^R forming phage

278 - 1

- 2 (7) Examine to see if segregation of lp occurring - Tested against λ, 518

seg.	Transd	518	2	λ	518	λ	518 4	5
	1.	S	non lys	r	non lys	r	non lys	
	2.	S	"	.	"	r	"	
3	3.	S	"	.	"	S	"	
	4.	r	"	.	"	v	"	
	5.	V	"	.	"	—	—	

Indicate some cultures look for active phage

Stocks made as 278-1, 2, 3, 4

(7) Lysate made #2 gal+ Mal+ lp^R - no plaques found before irradiation
7/7/54 " " #1 pre irrad. streak indicates mixed for gal - lysate showed no evidence of plaques or transd. in 518 B gal

10/26/54 - In testing for whether selection for (Mal- lp^R) intermediate from absorbing allele λ selected by HFT expt above. Stocks found to be revertants of Mal and lp_2^S. Two gal$_4^-$ lp^R seg found from #3 #4. Revertants of both found stable such that it is likely these lp^R are not diploid (3x repeat)

801 λ for lytic lambda prep. Labelled 6/1/54

1. Titer - 209 x 10^8 = 2.1x10^{10}

W2877
2878
2879
2880

518 c̄ 2342 - Attempt to get higher fraction of transduction

		(-)	(+)	total	Cell count	
1.0 ml cells add to 1.0 ml lysate. Inc. 5 min centrifuge decant resusp in 1.0 ml saline	(1) No add -	216	0	216	$\frac{216}{3} \times 10^5$ = 7.2x10^6	
	(2) 2342 lysate (65")	206	10	215	$\frac{215}{3} \times 10^5$ = 7.1x10^6	c.5% (+)

6/17/54

279-1 1412t 2342 plate - the spontaneous (+) - 18 picked Revert for Mal and examine lp, & react.

279-2 1412t2342 plate - 12 picked from lysate portion of plate - to see nature of lp of the stable gal (+) in lysate portion of plate: See previous data 278

1. All 12 mal - gal + were found to be ly_2^R

2. De Mal+ R (of varying degrees of + ness)	518	λ2		on Bone	Re v		ABOVE 1321	λ sens.	λ2 sens.	Mal + rev.
1. nonlys	R ?	s	wk	+		-	1. nonly.	sens.	sens.	
2. "	R	s		seg		-	2. "	"	"	
3. "	R	s		seg		-	3. "	"	"	
4. "	s not confirmed →	s	wk	seg !	rev rev	seg. + R -	4. "	"	"	
5. "	s	s	"	+		-	5. "	"	"	
6. "	s	s	"	+		-	6. "	"	"	
7. "	R	s		seg		-	7. "	"	"	
8. "	R	s	wk	+	rev	stabl, + R	8. "	"	"	
9. "	s	s		(-)			9. "	"	"	
10. "	s R not confirmed →	s	wk	+	rev	stabl, s -	10. "	"	"	
11. "	s	s		+		-	11. "	"	"	
12. "	R	s	wk	+	rev	stabl, R -	12. "	"	"	
							13. "	"	"	
							14. "	"	"	

Σ
4 spont + λ^s — 1, 5, 6
3 transd. + λ^R
4 " +" λ^R } Refer to 278

free Mal+ revertants ... above not obtained

Σ 14 spont (+) λ^s

General summary
transd. appears to be gal + λ^R
gal +" λ^R → seg gal- λ^R
gal +" λ^R → seg gal- λ^s

279-3 Stock (2580tK12 F+) X 1321 3 plates
Control plate in 2580tK12F+ for distribution of (+), (-) gave 109(+) 5(-)

(279-3) 1. Three plates gave "prototrophs" early (1st day) - all (-)
After 2 days - c. 200/plate - 1 apparent (+) isolated (+) on streaking on B gal - Segregating gal - noted after (2)
6 segregants of this (+) tested against HFT 2-, 4-
all transduced by 4-, ∴ Gal- from 2580tK12 F+

W 2896

279-4 Stock (2580tK12 F-) X 518 3 plates (discarded one, omitted to other because of thinness.)
Control on (+), (-) in 2580tK12 indicates 115(+) 13(-), seg. col. in apparent

(279-4)	(+)	(-)	total
Plate 1.	300	53	353
2.	241	46	287
3.	—	—	—
	541	99	

} 30 (+) picked from this cross to find (-) allele - of the 30, 4 were apparently stable (+), of the remaining 26, 25 tested successfully against HFT 2-, HFT4- - all transd. by HFT 4-, ∴ Gal- from 2580tK12 F-

W2897

6/21/54 2342 purified. Of 10 E colonies examined 7 were HFT in 84 - new stock prepared

For repeating above transmission experiments
1321 transd. by K12 → (1321tK12 F-)

Stock - 279-5

W2898

6/24/54

280-1 Are the transd. of Lp^+ really of Lp^R/Lp^+ nature? Examine an Lp^+ transd. W2570 TK12

① The Gal (-) seg (20)
280-2 ② The Gal $+^{u}$ (20)

— (280-1) the Gal - (In General these were poor tests) all found to be Lp^+ and λ^R

(280-2) the Gal $+^{u}$
18 found to be Lp^+ and λ^R - 2 tests were too poor to judge

280-3 Lytic λ - 1485 - 0.5 ml of this lysate into Pen - Sterile after 2 days.

① 1485 grown in NSB overnight - then aerated culture in NSB started until nearly full density, - centrifuged resusp in 2.0 ml. SH λ lambda (52.78) titer = 2.1 X 10^{10} - Centrifuge resusp in c.20 ml NSB aerate. After c 4 hours partially cleared. Centrifuged and CHCl₃'d

② Assay.
10^7 = 757 - titer = 7.6 X 10^9

③ Transduction exp.
on D(0): 0.1ml this lys + 0.1 ml 811 overnight broth culture.
- no colonies after 48 hours - No M duction

④ Galduction

	Expt/Control	Stability ✓	
1. W750	2/0		After 2 streakings - 2 were seg stbl (+)
2. W1210	2/1	2 very faint (+)	discarded
3. W.811	10/14	1, a very faint (+)	9 stbl (+) after 2 streakings

Further evidence that lytic λ does not transduce

Aerated 2342 diluted to [illegible] [illegible] in [illegible]
[illegible] add. 0.1 ml + 10 [illegible]

281-1 Production of HFT λ – Assay plates Bucks 518

Time	Cell Assay	Infective Centers *		Transd. Centers (no. =49)		Transd. 1-100	SP = 25		Plaques 1/100	corrd	A x100	N/Inf Centers	HFT/NFT Assay of Colony of Ecoli
0	622	3	0	45	0	97	72		6	0	0	0	3/25 = 0.12 ← will do here 25 others
10	381	193	190	51	2	47	22		~~42~~ 11	5	500	2.6	10/25 = 0.4
20	170	372	369	87	38	38	13		~~48~~	41	4100	11	9/25 = 0.36
30	63	517	516	136	87	28	3		68	62	6200	12	9/25 = 0.36
40	20	449	446	128	79	25	0	60	59	53	5300	12	4/20 = 0.20
60	1	259	256	98	49	26	1	30	35	29	2900	11	
70	1	230	227	80	31	16	0	33	24	18	1800	9.	

* Some of these infective centers appear to have papillae in them. There also appears to be papillae [illegible] infective centers about them.

↑ These values cannot be trusted since they represent some growth effects after incubation [illegible]

D	Total N/N₀	P/N₀	T/N₀	x3.5
0	1.0	—	–	
10	0.61	0.31	.0032	0.11
20	0.27	0.59	0.061	0.21
30	0.10	0.83	0.14	0.48
40	0.032	0.72	0.13	0.45
60	0.0016	0.41	~~0.066~~ 0.079	0.27
70	—	0.36	0.050	0.17

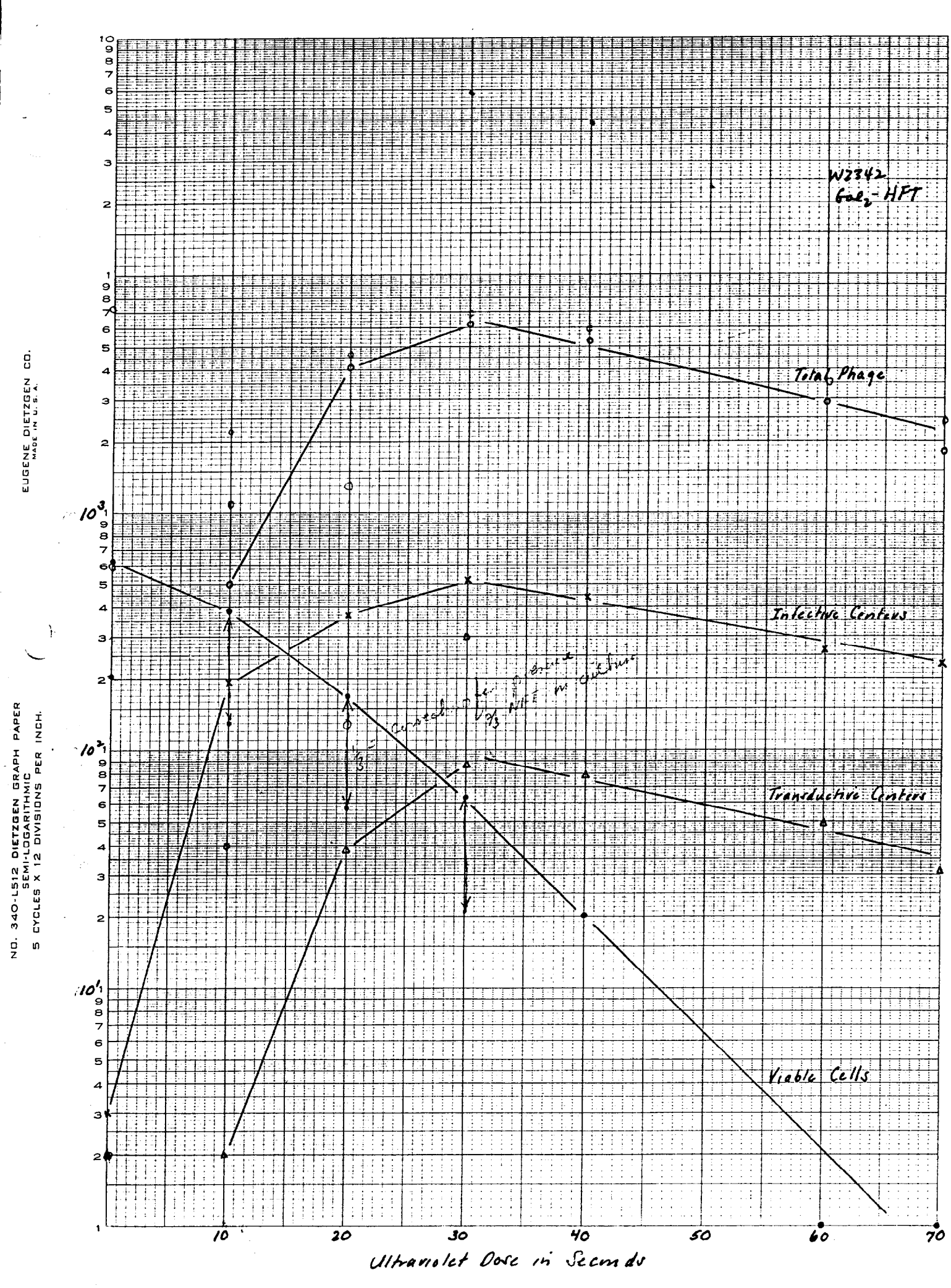
Estimate of fraction HFT

$$\frac{3+10+9+9+\cancel{5}}{\frac{\cancel{25}}{25}} = \frac{36}{5} = \frac{7.2}{25} = 0.29\ \text{HFT}$$

Corrected for LFT

Inf $N_0 = 622 \times 0.29 = 180$

D	N/N₀	Inf x0.29	P/N₀
0	1.0	0	
10	0.61	55	
20	0.27	103	
30	0.10	150	
40	0.032	129	
60	0.0016	74	
70	—	66	


W2342
Gal2- HFT
Total Phage
Infective Centers
Transductive Centers
Viable Cells
Ultraviolet Dose in Seconds
10
20
30
40
50
60
70
EUGENE DIETZGEN CO.
MADE IN U.S.A.
NO. 340-L512 DIETZGEN GRAPH PAPER
SEMI-LOGARITHMIC
5 CYCLES X 12 DIVISIONS PER INCH.

282-1 In the $\frac{1}{4}$ data so far suggests lysates of $\frac{1}{4}$ carry more often than not the (+) of the segment. Really so?

1. 2175E 750-1 Streaked out and 5 colonies picked - Cultures made and lysates of these prepared.

Culture	Assay/750 (no add. = 0)	Assay/1210 (no add. = # small)	Plaques/578	Comment	Ratio Pl/Tr	Comment 2
1 (lysate cultures)	11264: 448	49 (small)	533x10^6 x 4: 2.1x10^8	(about 2x as many minute plaques)	2132/497 = 4.3	Higher titer in Gal₄⁻ than 2 suggest that fragment is 2⁻ or Gal₂⁺
2	460	26 (small)	307x4x10^5: 1.2x10^8		1228/486 = 2.5	
3	336	36 (")	136x4x10^5: 5.4x10^7	-	544/372 = 1.5	
4	281	57 (")	217x4x10^5: 8.7x10^7	-	868/338 = 2.6	
5 (lysate cultures)	382	34 (")	191x4x10^5: 7.6x10^7	-	764/416 = 1.8	

due to lower... ? ... with 1210? ... W2175

2. Single colony isolated from each and tested for allele - All were gal₂⁻

also 60% duvate...

W2852 | W2851

Lysate = 282-2

282-2 Transd. of W518 with HFT 1⁻ λ 408 (-), 2 (±) Control 440 (-) ... , no (+) (B gal)

① W518 cells exposed to HFT 1⁻ λ (NA-4 page 2) plated out. Many partially lysed colonies after 3 days. 6 colonies showing some evidence of (+) pockets. Of the 6 4 appear to be reversions. Other 2 possible transd., both predominately (+) in streaks, also appear to be segregating. Original colonies were not (+), but pink in color, also of large size as if had selection advantage on Gal.

② Search for $\frac{1^- 4^+}{1^+ 4^-}$
ⓐ 1 discussed labelled #2. Lysate prepared - Streak of culture before drum c. 50-50 (+)/(-) Started from (-) colony - Assay of lysate (spotted) on 578, 1210, 750 - all HFT reactions

③ Single (-) pap. colony (the original) → (restreak start) streaked out → single (+) pure colonies streaked out: 24 in number
(6) 6/24 stable (+)
Remember these (-) came from pure (+) (5) (-) colonies from remainder tested for allele

	HFT 1⁻	HFT 4⁻	λ Resist.	Comment	
1.	+	0	Resist.	Gal 4⁻ $\frac{1^+4^-}{1^-4^+}$	④
2.	not gal (-), but reverting			parental	2d
3.	..	..		"	
4.	0	+	resist.	Gal₁⁻	①
W2851 5.	+	0	"	Gal₄⁻	④
283-1 → 6.	0	0	"	Gal₁⁻ Gal₄⁻	①④
7.	+	+	"	Gal₁⁻	①
8.	?	?	resist	?	?
Discarded 9.	+	0	"	Gal₄⁻	④
283-2 → 10. (5/13/55)	0	0	resist.	Gal₁⁻ Gal₄⁻	①④
11.	0	+	"	Gal₁⁻	①
12.	+	0	"	Gal₄⁻	④
13.	0	+	"	Gal₁⁻	①
14.	not gal (-), but reverting			$\frac{1^+4^-}{1^-4^+}$ parental	
15.	0	+	resist.	Gal₁⁻	①
16.	+	0	"	Gal₄⁻	④
17.	+	0	"	Gal₄⁻	④
18.	not gal (-) but reverting		—	parental $\frac{1^+4^-}{1^-4^+}$	

Σ	
parental	4
Gal₄⁻	6
Gal₁⁻	5
Gal₁⁻ Gal₄⁻	2
Questionable	1
	18

all tested again

These are not cleaved ... separate ... crosses 1210 and found NFT

7/11/54

283-1 }
283-2 } from previous page - are presumably (Gal₁⁻ Gal₂⁻)

283-1 Transd. c̄ HFT c̄ to get triple (-).
ⓐ Reaction weak - (+) not strong in cross br.h. - suggestion $\frac{1^-4^-2^+}{1^+4^+2^-}$ is not full + ?
ⓑ Streaking out - suggestion that (+) may be of two classes. - one full (+), other intermed.

A 283-1 cross A = ⊗ 2234
B cross B = ⊗ 902 gal₁⁻

Ⓐ 1st cross not good - heavy bkgrd. - no (+) observed Ⓑ 1/8 = 83, = 664 x 10 plates = 6640 (-), no (+) observed.

c 283-1 Further test - transd. 283-1 c̄ HFT gal + λ and obtain (+) - this showed then segregate phenotypic (-) which revert rapidly - whose genetic constitution is $\frac{1^+4^-}{1^-4^+}$
① transd. c̄ SIT+K12 [(#13) p. 267] - dil 10^0 = solid smear, dil 10^4 = c 100, dil 10^6 = 20 (large full)
ⓐ 24 (+) seg streaked out. all appear to be stable (-) - allele = all found 1^-4^-
ⓑ restreaked 12 (-) seg all stable (-)

283-3 Continuation of 282-1 - #2 lysate. The small plaque? a λ mutant. Attempt to obtain lysogenic culture of - Plaques picked and streaked out, colonies tested

1. 1st 24 colonies found non lys. /518
2. 2nd " " " " /518

7/16/54

-A Repeat examination of $+^4$ — after picking single colonies —
Check on lysate and population:

			Sterility	Assay 0.1 ml 750 c. 0		10' dil 2175 c. 5		811 c. 8
1.	518 E 750-1	6(+) col.	sterile	14	14	20	15	32
2.	518 E 902-1	neg, mostly +	"	0	0	17	17	23
3.	518 E K12	" "	not sterile / dense	—		4	—	[illegible]
4.	750 E 902-1	" "	sterile	2	2	9	4	26
5.	750 E K12-2	"	"	5	5	11	6	26
6.	811 E 902-1	" "	"	3	3	11	6	16
7.	2175 E 750-1	" "		9	9	15	10	27
8.	2175 E 811-1	" "	not sterile / not dense	—				

? lky — deletion too great

Lysates Repeat assay 10' dilution 0.1 ml — not for plaque — 518 for phage

		0.1 ml			no cell 13	wet	world: 37		
→ 1.	518 E 750	(5)		148 : 1.5×10^8	88	75	111	74	— active on 1⁻, 2⁻, 4⁻
2.	518 E 902-1	0		62 : 6.2×10^7	18	5	45	8	—
3	518 E K12	0		113 : 1.1×10^8	10	0	39	2	
4	750 E 902-1	1	~~344 : 3.4×10^8~~	99 : 9.9×10^7	~~11~~	0	25	0	
→ 5	750 E K12-2	plaque → 0 (4V?)	~~99 : 9.9×10^7~~	577 : 5.8×10^8	32	18	50	13	— active on 2⁻, 4⁻
6	811 E 902-1	0	~~577 : 5.8×10^8~~	344 : 3.4×10^8	10	0	35	0	
→ 7	2175 E 750-1	3 plaque 24 (?)	~~615 : 6.2×10^8~~	615 : 6.2×10^8	160	147	170	133	— active on 1⁻, 2⁻, 4⁻
8	2175 E 811-1	6	~~116 : 1.1×10^8~~	116 : 1.1×10^8	12	0	30	0	

~~Also repeat / chromosomes / ultimate cond~~

Reassay 750 0.1 ml (1) 10^4 = 501, 5×10^7
Control = 0 (5) 10^4 = 24 = 2.4×10^6
(7) 10^4: 433 = 4.3×10^7

presumably the 750 culture I obtained such that assay aspect — other experiments about this time suggest this is true also

284-B
1. Cross 2487 × 2593 — Gal$_4$-Hfr × Gal$_1$-Hfr — to secure diploid? not plen. (+)?
2. Cultures grown together c 3 hours. plated not EMS lac
10² dil — c 10³ prototrophs.
10⁴ dil — c. 50 " "
10⁶ dil — c. 20-70 " "
3. Streakings out of grp. colonies — slightly positive in appearance, larger. 14 in number

	EMB	S. Gal
1.	(−)	(−)
2.	(−)	no growth
3.	(−) one pap. col.	no growth
4.	+ also?	+ (few)
5.	+ " "	+ (intermed)
6.	+ " "	+ (intermed.)
7.	+	no growth
8.	no growth	" "
9.	+ also (−)	+, also (−)
10.	+ " "	one (−) col.
11.	(−)	minute col.
12.	(−)	(−)
13.	(−)	(−)
14.	(−)	(−)

7/18/54

2277 / 2299 = Gal,- tp^s - Repeat of 518t AFT, using Gal,- cells instead of Gal4

1. Actively growing culture of 2277 in Pen. c. 10^8 cells/ml. 0.5 ml cells + 0.5 ml NA-4 phage 2. Adsorb 10', dil 10^2, 10^3, 10^4. Control cells similarly treated

(a). Control cells - one of three plates - 155 - 1.55x10^2 x10x10x10^4 1.6x10^8 no (+), lysed, or phage exposed colonies observed, nor any weak (+) colonies

(b) expt. cells - av. of three plates - 118 on all three plates {38 partially lysed colonies observed; 2 colonies papillating in appearance, slightly pink brown - similar to appearance of gal4- transd. c AFT 1- = the 2 colonies in streaking out gave many (+), and parental (-), and possibly non-parental (-). Some of the (+) appear to be segregating

285-1 / 285-2 Streaks

Picking (+) to examine (-) seg.

2nd batch (-) seg picked from gal+ segregation ↓ 9 in all

6 were parental / 3 were gal,- tp^s

285-1 (+) colony	stability	seg(-) test 1-	14-	Allele	285-2 stbl	(-) Segreg tested 4-	
1.	s = stable	—	+	—	u	parental	
2.	u = unstable	0	+	1-	s	—	
3.	s	—		—	s	—	
4.	u	+	0	4-	u	parental	
5.	u	0	+	1-	u	parental	
6.	u	+	0	4-	u	parental	
7.	u	0	+	1-	u	0 tp^s	Many of parental show evidence of lysis - Segregation is that this is an tp^R transduction, segregating (-) seg that are tp^s
8.	u	0	+	1-	s		
9.	s	—		—	u	parental	
10.	s	—		—	u	"	
11.	u	reverting to + also transd. by 4-?		parental?	u	"	
12.	u	0	+	1-	u	"	
13.	s	—			s	—	
14.	s	—			u	parental	
15.	s	—			s	—	
16.	u	0	+	1-	s	—	
17.	s	—			u	parental	
18.	s	—			u	"	
19.	s	—			s	—	
20.	u	0	+	1-	u	parental	
21.	s	—			u	"	
22.	u	0	+	1-	s	—	
23.	u	0	+	1-	u	parental	
24.	u	0	+	1-	u	"	
	11 stbl / 13 unstbl			10 1- / 2 4- / 1 parental?	8 stbl / 16 unstbl	1 1-4+? / 15 parental	

As in the case of the 1st β

gal,- λ —x gal4- where the transduction was also tp^R and segregating tp^s haploid types

no heterotype recovered. Crossing over apparently takes place with

From the above information it appears that these (-) seg. are not derived from separate crossover

7/22/54

Linearity of Gal₂-? in Gal₁- and Gal₄-?

3/17/54 902A Q	a purified 750 2	Δ	84 26	Δ
0.025	62	60	357	325
0.05	81	79	415	387
0.075	206	204	725	699
0.100	243	241	1392	1366
0.125	157	155	1734	1708
0.150	130	128	1566	1540

From the above 12 papillae picked - to observe if transd. of ly⁺ are by ly^R particles examine transd. for lysogenicity and also Seg to confirm the phenomena of reduced lysogenicity with in Gal⁺ transtab. Examine seg to see if these seg are ly^R or ly^s. Also lyse one of each to see behavior of + unstab. Make stock.

286-1 = 750 t 902 | of the 12 picked - after 2 stab. 11/12 unstab (+)
286-2 = 84 t 902 | " " " " " " " 6/12 " (+)

1. In both cases - no clear distinction as regards lysogenicity response between transduction and segregants. No cases of ly^R segregants observed. On the assumption that ly^R particles are transducing ly⁺ cells, perhaps the heterotypic segregants should be ly^R. However previous evidence indicates that such segregants need not be ly^R, since lysates were made of them - see 84 t 902

286-1 titer against 518 10⁷ AS = 1 plaque = c. 1 X 10⁷

Assays

		10²	0.1 ml of 10⁴	10⁶	titer
1	750 (control:0)	41	18	0	1.8X10⁶
	2175 (cont.:11)	63	11	13	6.3x10⁴

750 t 902

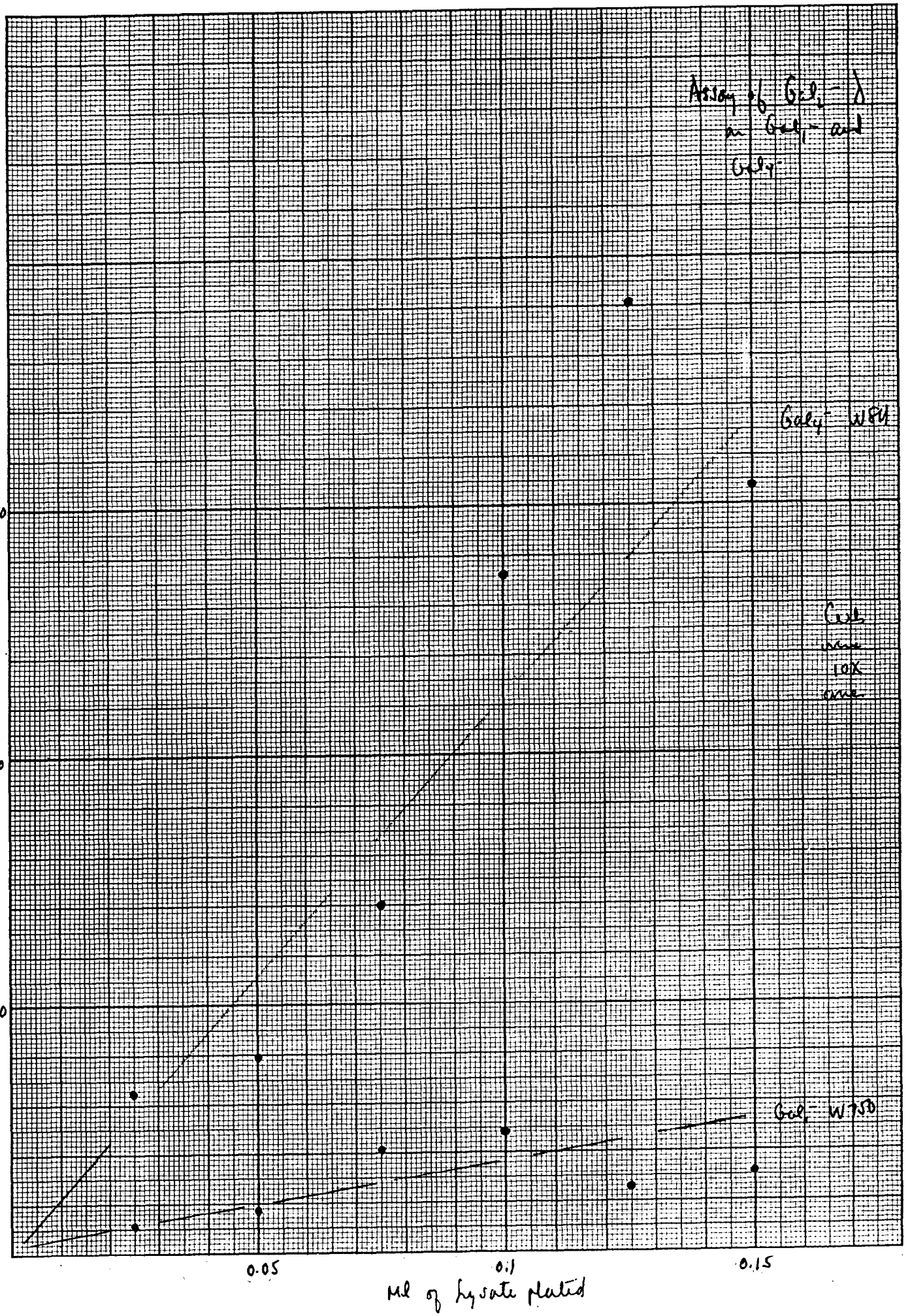
286-2 titer against 518 0.1 ml of 10⁷ = 73 = 7.3X10⁸

Assays

	10²	10⁴	10⁶	
750 (control:1)	41	251	2	2.5X10⁷
84 (control:11)	276	13	14	2.8 X 10⁵

84 t 902

||| Assay culture



9/9/54

2341 Its constitution. Isolated as ~~Hfr~~ Hfr gal - lp^R - TN isolated lp^s segregant from. Is this an indication of lp^R/lp^s?

(1) Streaked out and single col tested.
94 lp^R
1 lp^s

(2) One lp^R and one lp^s grown up and plated B gal to obtain revertants.

(3) Revertants (obtained from ~~single~~ separate colonies)
(a) lp^R - 8/8 appear to be seg ± on streaking
(b) lp^s - 6/6 " " stable on streaking

∴ 2341 is $\frac{2^- \; lp^s}{2^- \; lp^R}$ coming from the following Rx

$\frac{2^- \; lp^R}{} \times \frac{+ \; lp^s}{} \rightarrow \frac{+ \; S}{- \; R} \Rightarrow \frac{- \; S}{- \; R}$

9/10/54 7 col of lp^R of 2341 placed in stock
Labelled as 2341R

9/12/54 Lysate of 2341 lp^R (made as with an lp^+)
assay against 518
no plaques
no transduction

9/13/54

F+ 1924EKR - Recheck and cross c̄ F+ 1436 to see if fragment is ±R by recovery of $\frac{- \; S}{+ \; R}$

8 possible trans. strains - all non lysogenic 1518
(1) crossed c̄ 1436 - both cultures not aerated. B gal
3 plates -

1/8 of plate = 106(+)/39(-)

The (-) 23 picked, streaked out once and tested (2 may be gal slow) - 21 lp^s 2 lp^R

The (+) 24 picked streaked out 2x, (-) seg (gal) = 24 (3 appear either mixed or reverting others (mixed))
21 were λ^R

stock plate of these
saved for further

9/20/54

2341 - 2344

2344 is a V_1^R derivative of 2341. Since 2341 is $\frac{2^- \; Lp^s}{2^- \; Lp^r}$ what is 2344 with regard to this?

① testing of colonies of 2344 streaked out

17 colonies tested / λ - all Lp^s. Suggestion that only Lp^s seg. of 2341 can be made V^R since according to Cavalli segregation crossing over between fragment and chromosomal gene results in dependence for V_1 and Lac?

② Plate 2341 / T1 see if any Lp^r V_1^R exist.

~~str~~ ③ ~~31~~ V_1^R colonies picked, purified once and tested against λ
10 were mucoid and phage reaction suspect
1 lambda sens
20 Lp^R

Better repeat with repeated Gal so as to judge segregation

{811 - Making Hft by adding fragment?
{518 (9/15/54) -
The run with 518 exposed with Hft 4 - 48 tested found NFT against 2175 -

811 (9/20/54) - 24 colonies tested against 2175 - all NFT
single { 18 .. .. .. - .. NFT
run { 2 .. .. .. - possibly HFT - No
9/21/54 20 .. .. 1210 - all NFT

c. 9/18/54

518

To make HFT stocks of other gal — 289-1 ← W2870

289-1 → ① 518 against E71C's 2238 λ — of 20 pap picked (very more pap in exposed portion of plate) (1 found segregating)

10 segregants tested are transduced by HFT 4⁻, must be gal₃⁻?

Lysate made — Tested for transd. / 2297, 811

② 2070 λ — no + found of 24 tested

	no add	0.1ml of lat 10^4	10^6	titer
811	25	73	33	4.8×10^6
2297	223	313	251	9.0×10^6

9/23/54

2350 — stock to see contaminated c revertants

10 colonies tested against 2 and 4 HFT

} to make gal₂ – HFT by segregation from double duction

① after 36 hours in transduction
② " 72 " transductions by HFT 2⁻ — of 4⁺ revertants?

This behavior is consistent with previous.

9/23/54.

Recheck on probability that reversion lysates give more stable transduction than wild type as typified by K-12

1. The trans ductions:

		Lysate	Control	0.1 lysate	Stability	
290-2	750	750 g+-1	0	384	21/24	+u
290-1		2175 g+-1	0	80	22/23	+u
290-3	2175	750 g+-1	3	540	21/24	+u
290-4		2175 g+-1	3	93	23/24	+u

(cf. 291-2)

no's ↑ out
stocks out 5/12/57

Stocks 290-1→8

1210 - transd. c̄ 2070.1 to Blair $\frac{2-6+}{2+6-}$ →

		Lysate	Cont	0.1 ml		
290-5	1. 1210	2070.1+	1	17	9/12	+u

Single sep clone picked - lysate prepared

7 sep from sep. transd. tested against HFT 2 - all not transd. (see 2.)

1010 t 811 for Cavalli Expt

291-A control 3
811 λ 62

saved 5/13/55

[1, 2, 3 cultures made 3/4 x 4"] 291A1, ~~291A2, 291A3~~ out

1. 291A-1 ① 8 seg tested by uv. irrad. spots on 811 – all NFT — ① c. 8 found stable
8 spotted on 8 gel to see if reversions stable ② c. 5 reversions found stable
② 12 addnl. tested a la HFT – none HFT
2. 291A-2 ① 10 seg as 291A-1 NFT, spotted for reversions. ① c. 8 found stable
② 12 additional tested via HFT test – none HFT ② 4 reversions found stable
3. 291A-3 ① 8 seg as 291A-1 NFT, spotted for reversions. ① c. 8 found stable
② ② 4 reversions found stable

HFT strains 2 } 202-16, 241-14, 241-19 } lost found stable

Effect of λ-2 Resistance on adsorption

811 vs. 1439

1. Expt. 0.5 ml K12 λ (7/27/54 Prep 1) + 0.5 ml 10X conc. cell from non-aerated cult.
2. λ prep titer /578
   Reassay 10^7 = 113, 66 = 8.9×10^8
   10^7 dil. = 66, 16 = 41×10^7 = 4.1×10^8 (better assay. plaques/ml
3. Adsorb. cell titers
   811 10^7 dil = 927, 793 = 860×10^7 = 8.6×10^9 cell/ml
   1439 10^7 dil = 961, 967 = 964×10^7 = 9.6×10^9 cells/ml
4. Adsorpt. tubes contains
   811 4.3×10^9 cells + 2.1×10^8 λ
   1439 4.8×10^9 cells + 2.1×10^8 λ
5. Assay after 15 min adsorpt. 37C, 10' centrifugation
   811 10^7 dil = 0, 1 = ~~.5~~ 0.05 $\times 10^8$ λ est. $\frac{5}{210}$ λ remain.
   1439 10^7 dil = 85, 56 = 7.0×10^8

W2888 (292.3 really?)

292

10/10/54

(1) Reexamination of 257C-6 $\frac{4-2+s}{+-R}$, its segregants to study crossing over - 24 colonies picked and streaked out from original streaking of stock. Single (+) seg selected.

	HFT 2	HFT 4	Ly	Genotype	Seg. Re-str.		2⁻	4⁻	Ly	Genotype
292-1 1.	0	+	R	✓ 2⁻ R		16	0	+	R	✓ 2-R
2.	+	0	S	– 4⁻ S		17	0	+	R	✓ 2-R
3.	0	+	S	✓ 2-S	S(+)	18	0	+	S	✓ 2-S
4.	0	+	R	✓ 2-R		19	0	+	R	✓ 2-R
292-3 5.	0	+	R	✓ 2-R		20	0	+	R	✓ 2-R
6.	+	0	S	– 4-S	S(1)	21	0	+	R	✓ 2-R
7.	0	+	S	✓ 2-S	S(1)	22.	0	+	partial S	2-seg ly? (292.22)
8.	0	+	R	✓ 2-R						
9.	0	+	S	✓ 2-S	S(1)					
10.	0	+	R	✓ 2-R						
11.	0	+	R	✓ 2-R						
12.	0	+	R	✓ 2-R						
13.	0	+	R	✓ 2-R						
14.	0	+	R	✓ 2-R						
15.	0	+	R	✓ 2-R						

292- Ly (?)

Idiotype	4⁻ S	= 2	1/1 $+^R$ S(b)
Allotype	2 - R	= 15	11/12 $+^R$ ...
Allotype ly^S	2 - S	= 4	3/2 $+^R$ S(b)
Allotype seg?	2 - R/S	= 1 ?	not done.

See also 298

CONTINUED 292A

(2) Transduction of ly^R - 1924 x gal₂⁻ → gal₂⁻ ly^R. Culture is W1 from Expt W. Is this culture heterogenic ~~among~~ for gal or ly? Plated out on B gal and revertants examined. 12 picked.
1. 6 obviously slow (+)
2. 6 fine (+). Of these 1 appears to be seg.

292-3 3. The seg clone of 2 above - 3 cl examined for lambda reaction
1. seg +
2. pure +
3. pure (-)
/ 2279 All non lysogenic

Attempt to isolate $\frac{2^- ly^R}{2^- ly^R}$? and see character of phage

(3) 1503 Lu... Insertion of gal 4 - After c. 100 colonies examined from single colony experiment (cells + HFT 4 plated out) - failure to find (-) spotting on B(o) of cells and HFT - After about 30 streakings out. 2 (-) observed also a segregating (+), perhaps $\frac{+}{-}$. Stock of (+) made ~~(-...)~~ discarded

~~Test of the gal ... 292-1~~ N16 1/1/53 292-7

transduced by HFT 1, 2, 4 → says (-) is spontaneous gal_x^-

292-1, 22

292-1 - The original spot of this in association with the transd. test was pure (-), no suggestion of papillation, (+)

- on streaking out for colony test to see if λp segregating - streak found mixed (+), (-). Pure (-) colonies tested against λ and 1 (+)

1. The (+) - weakly sensitive (segregating probably λp^R/λp^s)
2. 1 of 10 (-) colonies sensitive λp^R/λp^s #6 (-), #7 from beginning
3. The above (-) spotted ~~against~~ on Gal to check reversion stable

{6 reversion obtained. 2 obviously unstable others may be. Streaks incubated too long}

292-22 - The case obviously segregating λp^R/λp^s
1. 10 (-) colonies tested 7 out of 10 λp^s #s 1, 2, 5, 6, 7, 9, 10 -
2. The above (-) spotted to check reversion stability.

{Reversion found seg = #8, #3, #4; non-seg = #1, 5, 6, 9, 10; no reversion obtained in others (2, 7)}

2734 ← EML selected a better growing isolate for this and labelled 2734

292A - 1503 → original = W2733

{Apparently two cultures of 292A-1503 retained and one of the transd. gal of 292}

1503 In search of gal+ Het 1503

From the spotting of HFT 4 on 1503 a (-) obtained not transd. c

9-30 /54 HFT 1-, 2-, 4+ apparently sensitive to λ (less so than usually found)

Cross with 2279 - gal, - λp^s to see 1. non-gal, - 2. diploid obtained

(A.) controls OK on B gal. after 3 days both show occurrence of reversions (papillae) ←

(B) the cross B gal.

	(+)	(-)
1.	12	171
2.	6	57
3.	7	81
4.	17	127
5.	4	56
6.	23	160
	69	592

Actual no (+) may be higher since small colonies appear to be intermed in appearance and may be immature (+), counted here as (-).

$592 \overline{)69.0}$ = 0.114 ; 592 ; 880 ; 592 ; 2880

= 11.4% (+)

12 (+) examined and found stable for gal

10/17/54

Search for HFT 4 - (After many unsuccessful attempts)
2175 ?? - transd. plate = 40/10 (old lysate)

Of 15 segregants tested (transd. saved also to see if only certain transd. give rise to HFT, as distinct from a rare segregational event ~~going~~ from any transduction - two found to be HFT in 2175. Stocks made and also streak out for purification (result could also be certain E (+)).

Stocks. 293-11 = transd., 293-11S = HFT 4 }
293-13 = " , 293-13S = " " }

also found 293-12 - lysates prepared of 12, 13 (poor lysates since cultures were aerated 5-6 hours before incubation.)

		lysate 12	13
- unsterilized lysate streaked against	2175	++	0
	811	0	??

SEE 365

11/5/54 293-1, 2, 3 are transductions from which NFT seg were obtained. 9/13/55 ?? 293-12

Behavior of transductions in giving HFT or NFT - Separate segs. from diff. (+)

transd. seg. #	293-1 /1210	293-1 /811	293-2 /1210	293-2 /811	293-3 /1210	293-3 /811	293-11 /1210	293-11 /811	293-12 /1210	293-12 /811	293-13 /1210	293-13 /811	/280	/811	
1	0	(55)	0	0	0	(15)	0	9	(55)	1 A	0	0	0	0	total
2	0	c(30)	1	6	0	(18)	0	2	(55)	0 B	0	(c.18)	0	0	
3	2	5	0	9	0	(13)	0	7	(55)	2 C	0	0	0	0	9 HFT 4
4	0	(18)	1	(15)	0	(23)	0	3	(55)	2 D	0	0	0	0	25
5	0	(14)	0	(55) A	0	11	0	3	0	(12)	0	0	0	0	
6	0	c(14)	3	3	0	4	0	3	(18)	2	0	(c.20)	+	0	
7	0	(10)	0	(55) B	0	(30)	0	6	(55)	0 E	0	0	0	0	
8	0	~~?~~	1	(12)	0	6	0	3	(8)	6	0	(c.20)	0	0	
9	0	~~?~~ 1	1	1	0	2	0	A (55)	(5)	2	0	(c.20)	0	0	
10	0	(10)	0	(10)	0	(c.40)	0	0	(55)	1 F	0	~~0~~	+	0	
11	0	(10)	0	7	0	(c.30)	0	0	1	1	~~?~~		0		12/16/54
12	—		—		—		—		(55)	0 G	—		0	0	
													0		
	1 HFT 2		2 HFT 2				1 HFT 2 (poor plate)		7 HFT 4, 3? HFT						
no. of papillae out	0	2	0	3	0	6	0	5	0	3	0	3			

Seg. picked, spotted on 1210 ?? and read 15 sec.

(811 has high bkgnd)

SS = solid smear

293-12A substituted for W2431 as HFT 4 stock

8 col picked and streaked out all found HFT on 1210 and all 8 lysogenic

Repeat assay	293-1 A /750	293-2 A B /750	/750	293-11 A /750	293-12 A-G (all)	/750	/750
1	(55)	1	0	0	SS	25	0
2	2	5	3	1	SS	11	0
3	0	4	2	2	SS	6	0
4	0	6	3	2	SS	c 40	0
5	0	(55)	0	1	—	1	1
6	1	4	1	1	—	0	1
7	1	(55)	0	2	SS	c 30	0
8	0	4	0	0	—	2	3
9	2	9	2	(55)	—	0	2
10	0	7	4	0	SS	c. 50	0
11	0	3	1	0	0	1	1
12	—	—	—	—	SS	12	—
	✓	✓	✓			ch	✓

11/7/54

Recombination between tp^u to give λ

1. Attempted by growing tp^u cultures together - tp^u cultures known to have differences, 2281 more difficult to lysogenize, 2373 gives stable transductions more frequently, etc. All tp^u believed to be separate mutational events or cases where the tp^u had been in contact with transducing phage and had emerged as tp^u again

2. Proced. - cultures grown in all combinations in penassay. Starting from 0.1 ml of overnight culture. Cultures centrifuged after 24 hours and loopful of supernatant spotted on 2281

3. Set up

	A W2281 F^+ Gal₂⁻	B W2373 F^+ Gal₁⁻	C W578 F^+ Gal₄⁻	D W2344 HFR Gal₂⁻	E W1673 F^+ Gal+	F W2280 F^+ Gal₁⁻
A +		+	+	+	+	+
B +			+	+	+	+
C +			+	+	+	+
D +					+	+
E +						+
F +						

+ = culture or combination . .

4. Results
   a. Spottings on 2281 showed no obvious evidence of λ plaques
   b. mixed Cultures transferred and grown to saturation - loopfuls spotted on 578 and irradiated no plaques found on irradiated plates

11/24/54
At Luca Cavalli's suggestion transduction of Hfr or F+ [c̄ HFT]

1. W1321 (578 F⁻)
   a. untreated
   b. 0.3 ml ~~[illegible]~~ of overnight culture ~~[illegible]~~ } incubated 10' at 37, dil c̄ 10 ml broth added
      1.0 ml of HFT 2 (2342)
   c. as b. only using 269-1 lysate - 2342 t K12 Hfr HFT with the same conditions.

2. a, b, c incubated at 37C overnight, centrifuged resp. in saline 10X

3. a, b, c, crossed with W945 S gal.
   a, b, c also spotted on S gal separately, 945 as well, as controls, - no growth

4. After 3 days no suggestion of prototrophs - indication of no F. duction or HFT duction

1412 attempts to transduce c̄ λ-2

1. Do internal malt^R of (Mal- λ^R) have interaction with λ-2 such that transductions might be obtained?
2. 8 Mal +R obtained with varying degrees of +-ness
   1 = extreme (+) 8 = (−)
3. λ-2 prepared by growth on 1405. 0.1ml plated B gal

# Rev-t	Mal phenotype	Control area	Lysate area
1	(++)	8	19
2	(++)	6	18
3	+	5 (many small blue)	19
4	±	10 (many small blue)	29
5	±	10	29
6	±	8	16
7	(−)	14	25
8	(−)	20 *	26

* with the exception of this plate all plates show evidence of lysis by the phage.

W2872

1210 transduction of, with 1-4 lysate. ADDITIONAL RUN -297

1. lysate 283-1 (appears to be a good lysate, cleared promptly at 2 hours, viscous)
2. control lysate
   6 81 ← after 24 hours no signif. diff.
   " 36 " lysate >> control
   " 48 " " ????

3. Analysis of segregants

The transd. these (−) came from are: 295-1 ← 1.; 295-2; 295-3; 295-4; 295-5

	HFT 1	HF 2	HFT 4	
295-1 1.	+	−	+	found NFT
295-2 2.	−	+	−	NFT
295-3 3.	+	−	+	
295-4 4.	+	−	+	
295-5 5.	−	+	−	found NFT
6.	+	−	+	
7.	+	−	+	
8.	+	−	+	
9.	+	−	+	
10.	+	−	+	
W2853 11.	+	−	+	
295-12 12.	−	+	−	found NFT
13.	+	−	+	
14.	+	−	+	
15.	+	−	+	
16.	+	−	+	
17.	+	−	+	
18.	+	−	+	
1 19.	+	−	+	
2 20	+	−	+	
3 21	+	−	+	
4 22	−	+	−	found NFT
5 23	+	−	+	

Σ: 15 idis, 3 allo

		HFT 1		HF 2		HFT 4	
6	24	+		−		+	
7	25	+		−		+	
8	26	+		−		+	
9	27	−		+		−	found NFT
10	28	+		−		+	
11	29	+	W	−	2-3	+	W
12	30	+	F	−		+	F
13	31	+	W	−		+	W
14	32	+	F	−		+	
15	33	+	F	−		+	
16 (control population)	34 (control)	−		+		−	possibly HFT or contam. No ✓ indicates contamination
17	35	+	F	−		+	
18	36	+	F	−	2	+	
19	37	−		+		−	found NFT
20	38	+	W	−		+	W
21	39	+	W	−	2	+	W
22	40	+	W	−		+	W
23	41	+	F	−		+	F
24	42	+	W	−		+	W
25	43	+	F	−		+	W
26	44	+	F	−		+	F
27	45	+	W	−		+	W
28	46	+	F	−		+	F
29	47	+	F	−		+	F
30	48	+	W	−		+	W

W2853

* These reactions for HFT required a day extra for full development suggesting that the combined is not full (+)

295A

$\frac{1^+4^-2^+}{1^+4^+2^-}$

135 idio
14 allo
3 amphi (all +)

F = full
W = weak

1210 + 283-1 Combined.

Reg	HFT1-		AFL-		AFT4-	
31 49	+	F	−	4	+	F
32 50	−		+		+ found HFT	W
33 51	+	F	−	2	+	F
34 52	+	F	−	1	+	F
35 53 cont	+	F	−	3	+	F
Re ✓ 36 54	−		+		+ found HFT	W
Re ✓ 37 55 cont.	+	W	−		+	
38 56	−		+		− found HFT	
39 58	+	F	−		+	F
41 58	+	W	−		+	W
41 59 blue	+	W	−		+	W
42 60	+	F	−	2	+	F
43 61 blue	+	W	−		blue +	W
44 62 blue	+	W	−	1	blue +	W

52 idio + 6 = 58 idio
8 allo ? + 1 = 9 allo
2 amphi + 0 = 2 amphi

Reg	HFT1-	AFL-	AFT4-	
(45) 63	+	−	+	
(46) 64	+	−	+	
(47) 65	+	−	+	
(48) 66	+	−	+	
(49) 67	−	+	−	found HFT
(50) 68	+	−	+	
(51) 69	+	−	+	

Batch II

1.	+	−	+	
2.	+	−	+	
3.	+	−	+	
4.	+	−	+	
5.	+	−	+	
6.	+	−	+	
✓ 7.	+	−	+	
✓ 8.	+	−	+	
9.	+	−	+	
10.	+	−	+	
11.	+	−	+	
12.	−	+	−	found HFT
13.	+	−	+	
✓ 14.	+	−	+	
15.	+	−	+	
16.	+	−	+	
17.	+	−	+	
18.	+	−	+	
19.	−	+	−	found HFT
20.	+	−	+	
21.	+	−	+	
22.	+	−	+	
23.	+	−	+	
24.	+	−	+	
25.	+	−	+	
26.	+	−	+	
27.	+	−	+	
28.	+	−	+	

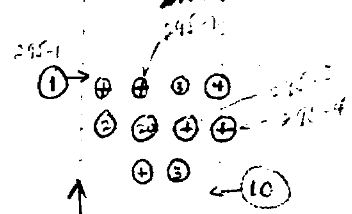
29	+	−	+	
30	+	−	+	
31	+	−	+	
32	+	−	+	
33	+	−	+	
34	−	+	+	[illegible] (circled)
35	+	−	+	
✓36	+	−	+	
37	+	−	+	
38	+	−	+	
39	+	−	+	
40	+	−	+	
41	+	−	+	
42	+	−	+	
43	−	+	−	found HFT
44	+	−	+	
45	+	−	+	
46	+	−	+	
47	+	−	+	
48	+	−	+	
49	+	−	+	
50	+	−	+	
51	−	+	−	found HFT
52	+	−	+	
53	+	−	+	
54	+	−	+	
55	+	−	+	
56	+	−	+	
57	+	−	+	
58	+	−	+	
59	+	−	+	
60	+	−	+	
61	+	−	+	
62	+	−	+	
63	+	−	+	
64	+	−	+	
65	+	−	+	
66	+	−	+	
67	+	−	+	
68	+	−	+	
69	+	−	+	
70	+	−	+	
71	+	−	+	
72	+	−	+	
73	+	−	+	
74	+	−	+	
75	+	−	+	
76	+	−	+	
77	+	−	+	
78	−	+	−	

49 idio
4 allo
1 amphi

78 idio ?
5 allo
1 amphi

none of these found HFT in 2175

4/23/86
Retest of these stocks shows no HFT rx after extra days digest



Repeat examination of 10 colonies, 6 found HFT in 2175 Stocks made of 4 of them for safety's sake
295A-1, 2, 3, 4

All tested

	1	2	4
1.	−	+	−
2.	−	+	−
3.	−	+	−
4.	−	+	−
5.	−	+	−
6.	−	+	−
7.	−	+	−
8.	−	+	−
9.	−	+	−
10.	−	+	−

1412 Repeat transduction with HFT 2 to observe the nature of transduction

1. β gal
   no odd ½ = 41
   HFT 2 (2342 pap)½ = 317 pap gal

2. Batch I 24 papillae picked and streaked β gal ② - all stable, all non lysogenic /518, all Mal+ ② revert and test λ^s -

3. Batch II 22 papillae picked and streaked β gal ② - all stable, all non lysogenic /518
   ~~23 Batch III papillae picked ② all~~ W2881
4. 24 papillae .. .. ② 1 +^s found, all non lysogenic /518 (296-1)

5. 24 papillae picked .. .. ② 3 +^s found, all non lysogenic /518 (296-2,3,4) W2882 W2883 W2883

6. tests of 296-1,3,4 ~~above~~ gal- seg flamed from each and brushed against HFT 2 4 - after 2 days

	HFT 2	HFT 4
1	4 pap	0
3	1 pap	0
4	3 pap	2 pap
2		

Since this HFT 2 is the lysate to make the original selection these results do not indicate that selection has been for a mutant of the cell adsorbing λ

1st experiment HFT 2	HFT 4
3	0
8	0
contamination	
0	0
0	0

7. 296-1,2,3,4 gal+ seg used as indicators in plating with conc. (>10⁹) particles of λ in the hope of selecting host range plaque mutant. None found on either 1,2,3,4

Batch I for reversions

Renumber in terminology	#	Appearance of gal on Mal	Reversion obtained	Reversion phenotype	Gal transd. λ-2	Mal+R λ /518	
1	1	pink	+	full +	resistant	S	
2	2	blue	+	full +	"	S	
296-5 3	3	pink	+	full +	"	S	
296-6 4	4	blue	+	full +	"	R	
296-7 5	5	pink	+	full +	"	S	
6	6	pink	+	intermed (+)	"	S (weak)	
7	7	pink	+	full +	"	S	
	8	..	−	—	"	—	
8	9	..	+	full +	"	S	
9	10	..	+	full +	"	S	
10	11	..	+	intermed (+)	"	S (weak)	
11	12	..	+	full +	"	S	
12	13	..	+	full +	"	S	
13	14	..	+	int. (+)	"	S (weak)	
	15	..	−	—			
14	16	..	+	full +	"	S	
15	17	..	+	full +	"	S	
	18	blue	−	—	"		
	19	blue	−	—	"		
16	20	pink	+	full +	"	S	
	21	pink	−	—	"		
17	22	pink	+	full +	"	S	
18	23	pink	+	full +	"	S	
19	24	pink	+	full +	"	S	

W2884
W2885
W2886

K-12 Lysate 11/12/54 - harvest titer about 150/ml 0.1
against 1210. Papillae slow in development. 3 days required and small. Other 'derivatives' with 1210 were also slow at this time. 1210, bad medium?
SEE BELOW

1210 transduced with 283-1 - same culture as ↑ (1210)

1.	no add	3
2.	283-1 0.1ml	71

SEE 297A

K12 lysate above: Test of layer plating method.
Assays. 0.1 ml lysate + 2.5 ml B gal (0.6% agar) poured on B gal plate

817	① no add	38	
	② 0.1 ml	294	256
1210	① no add	4	
	② 0.1 ml	44 very small	40
750.	① no add	4	
	2 0.1 ml	346 (quite a few small	342
2289	① no add	7 c. 400 small plaques)	
	② 0.1 ml	1/4 = 162, 1296	

Contaminated culture?

11/30/54

For J.L. tests of some segregants from TCN diploids 3

(1) 3-144 Gal- against HFT 1⁻, 2⁻, 4⁻ - cultures not gal-, no suggestion of transduction

(2) Lysates of cultures TCN II-2 and TCN VII-94, also lysate of 583.
Lysate additions = 0.1 ml, control = 0.1 ml broth spotted on film

Lysate	/150	/2175	/PM	/2307	/3-144
control	0	2	0	0	not
583	0	49	9	0	
II-2	1	34 *	1 *	0 *	gal-
VII-94	c.120	c.150	c.120	c.100	

* a little chloroform transferred to plate causing inhib. of growth

Attempted transduction of Eliminated arabinose and also direct attempt at (Mal- Lp₂ᴿ).

1. 2347 = 583 mal- ara- Lp₂ᴿ Lp+ Sᴿ <- obtain tractable gal+ for Sᴿ (This removes a doubt of HFT)

	4 days	
control	many small	} no transduction
on Broth 0.1 ml HFT 2⁻	" "	

2. 2307 = 583 mal + Lp₂ˢ ara- Sˢ

	HFT 2⁻	4 days
on B ara	control	0
	0.1 ml	6

2307 against HFT 1, 2, 4 - Transd. well by 2, 4, less by 1⁻ after 3 days

257C-6 - Continuation. see also 292
seg from dipl. (+)

	HFT 2⁻	HFT 4⁻	A. Resist.	seg from rev 1	seg from rev 2				Page 292	total
1.	−	+	R	1 2⁻S	2⁻S	Jdis type	4⁻ S	1	2	3
2.	−	+	S	—	—	allotypic	2⁻ R	6	16	c 22 Lpᴿ
3.	−	+	S	—	—	allotypic	2⁻ S	2	4	6
4.	−	+	R	2 2⁻S	2⁻R	amplin	4⁻2⁻S	1	?	1
5.	−	+	R	3 —	—					32
6	−	+	R	4 2⁻S	2⁻S					
7.	−	+	R	5 2⁻S	—					
8	−	−	S	(Rev) —	—					
9.	−	+	R	1 or 2R	2⁻S					
10	+	−	S	don't include						

298 (beside items 1–7); 298-8 → (beside item 8)

W2840 also got done.

On the basis of the reversion study the 2⁻R were derived thus

1 2 × 3 4
S S × R R
4⁻ 4⁻ 4⁺ 4⁺
2⁺ 2⁺ 2⁻ 2

(3 4) → S R / 4⁺ 4⁺ / 2⁻ 2⁻ → Rev → S R / 4⁺ 4⁺ / 2⁻ 2⁺

S R / 4⁺ 4⁺ / 2⁺ 2⁻ not seen

S / 4⁺ / 2⁻

"
2⁻ R

S S × R R / + + + + / − − + + → 1,3 S R / + + / − −

12/2/54

Spontaneous lambda from HFT stocks.

241-14. Supernatant from overnight unaerated culture plated against 2343 on S gal / 578 on B gal (borrowed from EML)

Addition	2175	578
none		0
0.1 1-100 sup.		700
0.1 1-10 "		[illegible]
0.1 und. "		

} poor assays. Redone (e. 2000 = 2 × 10^5 / also = 10^5-10^7 transductions)

Necessary to use selective method since plate liberation of λ HFT appears to be confusing things.

Test of HFT stocks.

12/12/54

202-16 streaked out, 10 col tested /500

HFT = 2, 3, 4, 6, 7, 8, 9, 10
NFT = 1, 5

241-14 maltose, Ly_2^R by mal-selection. 6 colonies tested

HFT = 2, 3, 4, 5, 6
NFT = 1

2431 - HFT gal₄ - stock. Streaked out from 2931-2A and colonies tested. The broths given to H. Bernstein for immediate lysates. Ceil ~~tubes~~ colonies tested HFT. after about 2 weeks 2 lysate tubes opened and tested - all still HFT. Titers from lysate suspensions not high

12/7/54

One Step c̄ HFT 241-14

1. 241-14 Streaked out single colonies picked to broth and tested for HFT by mad. a 750. Broths saved. After determ. which were HFT 2 broths were added to 10 ml saline incubated 15 min at 37C. Faintly turbid = <10^7/ml. Irradiated 45 sec to obtain approx. 10^{-2} survival. Dilute 0.1 + 10 sal. . <10^5 } dil.
0.1 + 10 sal. . <10^3
1.0 + 10: = ~10^2

Assay against 750, 2279

Time	/750	/2279	colonies
10:24 0			10/0.1 ml
5	Skip		
10:34 10			
10:39 15			
10:44 20			
10:49 25			
10:54 30			
10:59 35			
~~11:04 40~~			
~~11:09 45~~			
~~11:14 50~~			
~~11:19 55~~			
~~11:24 60~~			
~~11:29 65~~			
~~12:29 125~~			

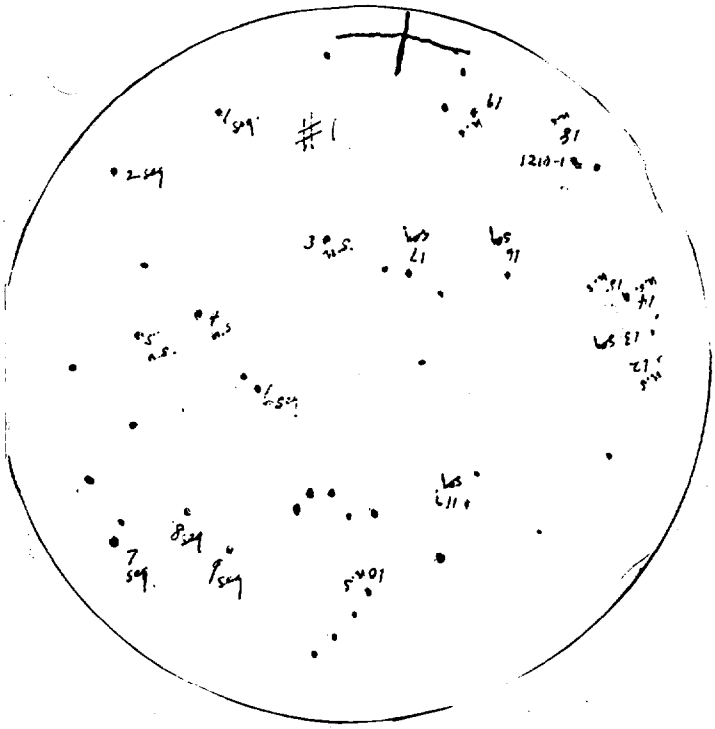
picked over diluted [illegible] 10 colonies [illegible] plaques [illegible] suggesting [illegible] state

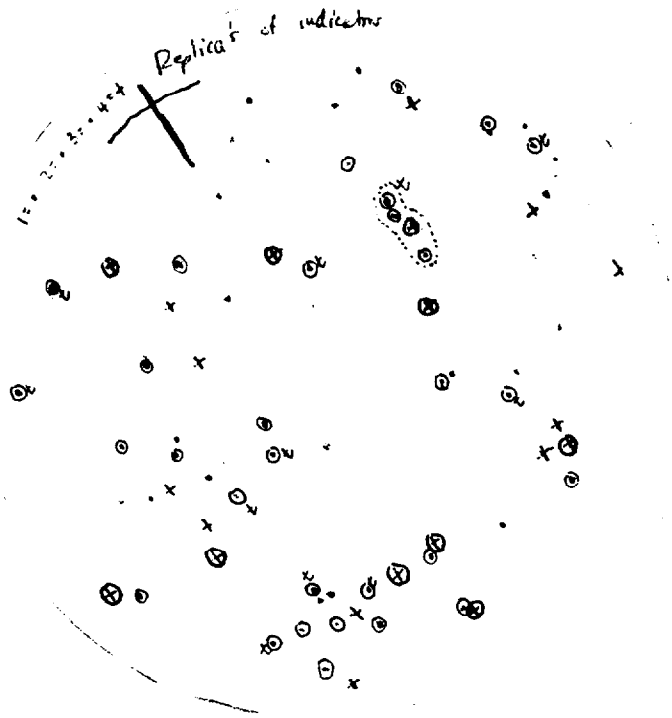
2. 241-14 - attempt to make Gp*.
Streaked out on λ-2 m. B mal.
1. Mostly Mal + seen -
2. 2 mal - col picked and streaked out
3. pure pure mal - 6 col streaked on B gal - all gal (-)

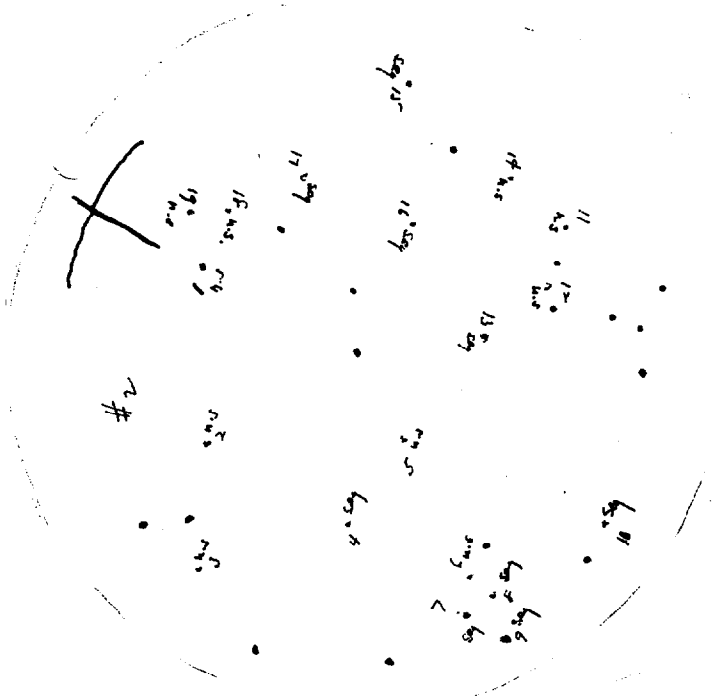
4. Repeat — Supernat. broth of culture (checked) used to make this: 0x10^5 plaques (2x10^5 trans?)
(b) Repeat assay

5. On next run pure mal - obtained - tested for HFT all HFT except 1
6. Stock made called (241-14 Mal-)
1. Lysate 12/18/54 53 x 10^7 plaques/2279 = 5.3x10^8
trans. assay 13 x 10^7 = 1.3x10^8

3. 202-1H - (as 241-14 above. plated against λ-2, mal- obtained. Tested 7/7 col as HFT against 750 - For use in one step growth culture


#1


Replicas of indicators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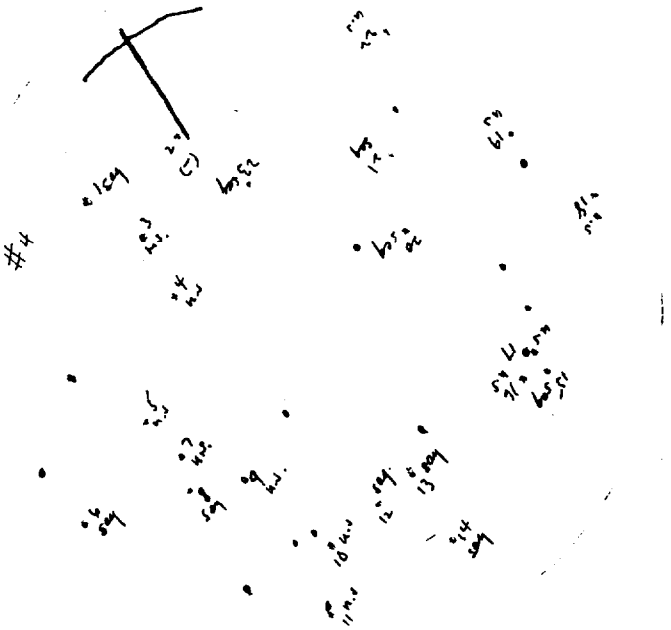
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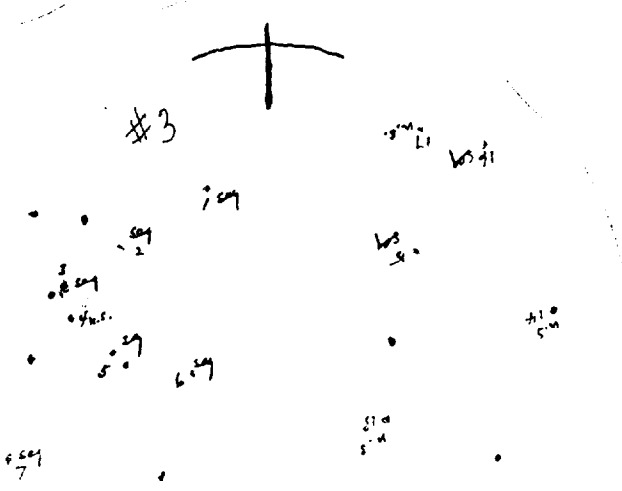

#3

12/11/54

Are the NFT cells giving transducing phage clonally related?

1. W1210 - spread 0.1 a B gal from overnight culture.
2. Incubated about 12 hours
3. replicated to 4 pre warmed B gal
4. after 30 min. 4 replicas irradiated 30" - 50 cm sterilamp, incubated 10'
5. Irradiated plates replicated to B gal plates spread with W758 cells as indicator transducing. W758 cells = ½ dilution, 0.1 of overnight culture, spread ½ hour before replication
6. Result of replica comparisons in attached figure.
7. All colonies on indicator plates picked, streaked out twice to see which are segregating

12/12/84

Observations on 292-3 = 4+ 2- 5 (from 510 S_y 2 -X)
against 295A-4 = presumed HFT 1-4- to see of initial complex to phen. (+).

1. c. 10^3-10^4 cell exposed to lysate of 295A-4 (titer unknown) 1.0 ml, assoc. 15 minutes, 0.1 ml plated B. gal. Broth exposure used as control

		After 3 days
1. control	= 3x10^3 (-) col.	c. 12 pap on colonies repre c. 12 reversions
2. control	.. ..	c. 14 .. .. 14 ..
3. 295A-4	= 200-300 col	c. 12 .. .. .. representing ?
4. ..	.. ..	c. 8 .. .. .. ?

2. Presumably lysate not active either through low titer or NFT nature - Repeat c̄ good lysate.

W2814

Observations on 2307. = 583 ual+ L_{p2}^{s} gal$_7$- L_p+

302-(1),2,3,4,5. 1. 2307 diluted to count 10^3 cells / ml
2. Exposure.
a. broth = control
b. 1.0 ml 2342
c. 1.0 ml NA-4 prop 2

3. Exposure

		2 days	3 days
1. broth	c. 400 (-) col.	c. 5 reversions →	
2. "	" (-) col.	c. 3 " →	
3. HFT 1-	c. 500 col (-)	0	c. 30(+) - also most colonies papillating
4.			
5. HFT 2-	"	0	c. 1 pap. - also at the end of 2 days all colonies seemingly papillating and reverting to (+) colonies almost stellate in appearance because of (+) growth. At the end of 3 days c. 9.2 of colonies (+)

305-1-4 = 5a
302-1
Are transductions

5 colonies picked and streaked - Seg. test against HFT2 all neg. or 7-. Spotted and read on 811, apparently all HFT - Do lysates and see
1. 1st lysate = 302A-1, not HFT (1,2,4,(1,9))

For further information see p 309.

4. Examine $\frac{1^- 7^+}{1^+ 7^-}$ phenotype also
2308 = L_p^s 2391

302A →
1. Expt. interrupted for about a week - 2308/HFT (2) plate had after 3 days about 3050 papillating pale pink colonies / about 1000 colonies - Controls about 56 per the same number.
2. pick the presumed (+) and see if $\frac{1^- 7^+}{1^+ 7^-}$ phenotype can be discovered
1. the phen-of total clone streaked out, 24 clonal cell picked and streaked out to obtain discrete changes to (+), 22 mixed streaks obtained, 2 pure (-) streaks
2. Single (+) picked from each of the 22 streakings
1st (+) streaking 7/22 show "slight" segregation, i.e. few (-) col.
3rd (+) .. 3 apparently stable, 3 "slightly" seg., one lost.
" 1/15 seg., stable (+)

some of the (+) appear to be the parental (± ∓)

SEE PAGE 307

12/12/54

Observations on lp^{+} transductions and stable transduction

292-3 = 518 gal_2 - Assay of K-12 λ on 0.1ml =
298-8 = 518 gal_2 - gal_4 -
518 also

285-2 Streaked out on B(0) [B gal] after 3 days, 20 colonies picked

① Pure (-) were 8 in number - Presumably derived in part directly from $\frac{+}{-}\frac{-}{+}$

combination	+	1-	λ RR	Adsole	~~Recession tolerance~~
1.	+	-	s	1-	cultures mixed up
2.	+	-	s	1-	and partially
3.	+	-	s	1-	lost, in attempting
4.	+	-	s	1-	to recover,
5.	+	-	s	1-	tested against
6.	+	-	s	1-	λ, 4/4 (-) not
7.	+	-	s	1-	grow, $λ^s$
8.	-	+	R	4-	3/3 (-) λ4(?)

apparently 4- lp^c lost somewhere during this procedure. Correct!

Save one of the 1 lp^s 7 518

A

②

Gal (+) λ	Gal Segregating?	Segregant	Seg. lp
1. R	yes	1-	S
2. S	no	—	—
3. R	yes	1-	S
4. R	yes	1-	S
5. R	yes	parental?	[illegible]
6. R	yes	1-	S
7. R	yes	—	—
8. R	yes	1-	S
9. R	yes	parental	R
10. R	yes	1-	—
11. S	no	—	—
12. S	no.	—	—

B

298-8 - Gal₄⁻Gal₂⁻ Ly^s from 257C-6

For the purpose of settling two problems, possibly 3
1. Lyˢ transductions?
2. Stable transductions, how many?
3. Size of the fragment.

1. 298-8 — 4 days (lysis here also)
Donald. 0
K12(11/13/54) 0? (perhaps 50 very small)

A a. This is odd since in 297, this lysate approximated at 150/0.1 ml
found 256/811
40/1210
842/750
c1300/2299

this lysate titered against 2300 ?
1/8 = c. 2000 = titer = 2000
control = 0

304A - b. the small paps, inhibited by something?
(1) 6 picked, 5 found to contain (+) in first streak
(2) 3 found segregating (one of these appears to be

	phenotype	2	4	λRR
1.	-	-	-	R
2.	-	-	-	R
3.	-	-	-	S
4.	+	-	-	R
5.	+	-	-	R

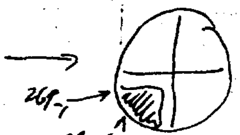
W2892

	2+	4+
LyR		
Lyˢ	-	-

Stock: 304A-1

B.
269-1 - because of EML's finding of unusual phage in lysate of this.
1 pap of 269-1 streaked and transduced with 10^{-5} dil. of 2478-1
no add = 10
0.1 2478-1 lys = 53

	Plated against	269-1 lys.	trop of 269-1 lys.	probably not sterile supernat. of 269-1	area control
on Bgal	1. 2297	cleared, c.60 col.	lysis, odd film of contact, pink in color	contam. c (+), on the back ground are also about 100-200 plaques.	ok
on Bgal	2. 750	c. 20 col remaining	no lysis, contam. c +	no lysis contam c (+)	ok
on B(1)	3. 269-1	c. 4 short col.	no change	no change	ok
	4. 1412	c. 10 unst. col.	no lysis contam. c +	no change	



Clear for 269-1 lysate against all

1/9/54 Additional lysate made as in NSP, another in Pen, also using actinic glass tubes —

	Pen	NSP
2279	λˢ	λˢ ← usual λ lysis
811	λᴵᴵ	λR
1177	λR	λR

Presumably the new phage is not in the supernat or the new lysate. In addition, sup. appears not sterile

305

12/29/54

2341 × ——— wild type HFT to Salani o S z⁻ / R z⁻ / + z+ } and see segregants

1. 2341 fresh culture Pen overnight cul/11ml incubated in water bath, no air, for 3 hours, diluted to c. 10^6 cells/ml
2. 750 EK12 lysate = 5.8×10^8 plaques/ml (see pg 284)
3. 0.1ml cell susp + 0.8 ml lysate incubated at 37C for 10' (1.8×10^9 phages/ml)
4. control (broth) exposed cells = (?) 275 × 4 × 10^3 = 1100×10^3 = $\frac{1.1 \times 10^6 \text{ cells}}{5.8 \times 10^8 \lambda}$ =
   plate
   3 plates about the same no of colonies, no (+) on any of them, no evidence of lytic activity.

5. expt. plates:
   1. ¼ = 216, probably not significantly different in no. from control, evidence of lytic activity
      no. (+) = 8
   2. no. approx. same as control
      no. (+) = 4
   3. no approx. that of control
      no. (+) = 6

6. purified twice

	Stbl	/λ	/2279	single seg /λ	single seg /2279		Stbl	/λ	/2279	single seg /λ	single seg /2279
1.	u	R	n lys.	R	nl	13.	u	R	lys	R	lys
2.	s	R	..	—		14.	u	R	n lys	s	nl.
3.	u	R	..	R	nl	15.	u	R	lys	R	lys
– 4.	u	R	..	R	lys	16.	u	R	lys	R	lys
– 5.	u	R	lys	s	nl	17.	u	R	n lys	s	nl
6.	u	R	n lys	s	nl	18.	u.	R	n lys	s	nl
7.	u	R	..	R	nl						
8.	u	R	..	s	nl						
9.	u	R	..	R	nl						
10.	s (?)	R	..	—							
– 11.	u	(S)	n lys	R	lys						
– 12.	u	R	lys	s	nl						

(S) → found not segregating – It should be noted that segregants are derived from the same clone as the (+), not from the (+) shown

283-1 Cont. 304.
lysate K12 12/18/54 on,

B

no cell = 0
0.1ml K12 = 175

	Lp⁵ 292-3	2175	Lp⁵ 2281
no add	27	9	6
1-10 dil 0.1	520	65	263
net	493	56	257
/0.1ml	4930	560	2570

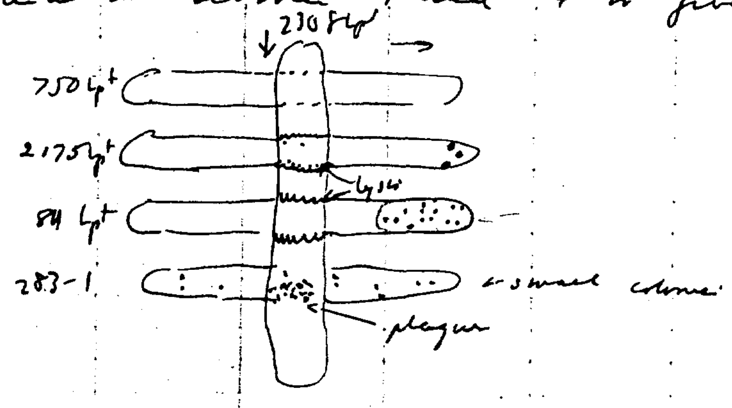
On to → 307

see pg. 304

/2279	c 1300
/750	342
/120	40
· 84	256

2308

previous test of some T- segregants against 811 to see if they were HFT suggested they were, but a lysate made of one of the segregants was not. Is there a phenotypic interaction between 7 and 4 to give (+)?



2202-16A mal - One step - aerated culture from overnight Pen.

$10^9 \to 10^7 \to 10^5 \to 10^3$ moderate → 0.1 + 10 ml Pen Incubator

0.1+10 ml Penn → Pen

Time	0.1 ml /2279 (Post)	0.1 ml /750	0.1 ml plated (O assay)
0	3	5 (1)	0
15	4	5 (1)	—
38	4	3 0	—
45	4	5 1	—
60	1	5 1(9?)	—
75	5 (ave 3.5) (5 pg)	7 1.75	—
	Prod. = 35 sec.		
210	58 (6 trnsd)	14 10	
Burst size	c. 16 plaques	c. 3 trnsd. part	

3 of these were also marked as plaques.

0.1/2279	0.1/750	0.1/plated
0 (3 pg)	4	20

750 no. cod = 3

C /2279	/750	/plated
0 (2)	30	59

10X above Expt

		P	pg	T	C
	0	15 (6)	8	6	9
- 2:15'	60	21 (14)	16	6	—
- 2:35'	80	33 (6)	8	10	—
2:55'	100	165	0 2	13	—
3:15'	120	145 (9)	6	13	—
Burst size		c. 10		4	

Prod. = 25"

4 in the places marked for plaques from top to bottom 3, 1, 2, 1, 0

$\frac{1^+ 7^-}{1^- 7^+}$ ← position effect strains

307-1A, 1C ute

($1^- \times 7^-$)

(307)

307A Continued.

Tried	segregation	Segregant 1	Segregant 2	ϕ		Re ✓ /1	/7	Genotype
1	"slightly"	−	+	1^-, w 1^-7^-	all of these spotted on	−	+	1^-
2	"	parental di-heterozygote			2178	[struck out]		—
3	"	−	+	1^-, w 1^-7^-	on	+ −	+	1^-
					5 gal	—		—
4	"as usual"	parental di-het.		−	and	—		—
5	"	+	+	7^-	used.	+	−	7^-
6.	"	+	+	7^-	none	+	−	7^-
7.	"	−	+	1^-, 1^-7^-	found	−	−	double
8.	"	−	+	1^-, 1^-7^-	HFT	−	+	1^-
9.	"	−	+	1^-, 1^-7^-		−	−	double
10.	"	−	+	1^-, 1^-7^-		−	+	1^-
11.	"	+	+	7^-		+	−	7^-
12.	"	+	+	7^-		+	−	7^-
13	"	−	+	1^-, 1^-7^-	307-1 →	−	−	double
14.	"	+	+	7^-		+	−	7^-
15.	"	parental di-heterozygote		−		—		—
16.		+	+	7^-		+	−	7^-
17.		+	+	7^-		+	−	7^-
18.	"as usual" → stable (+)		−		~~7 7 / 7 1⁻, 1⁻7⁻~~	all these 7^- are (minor R) pap. after 4 days.		
19.	"slightly"	} → stable (+)						
20.	"							
21.	"							

7 7^-
4 1^-
3 1^-7^-
3 parental
4 stable +

← W2854 = 307-1

Also λ⁺ derivative W2855

Continued from 305 - the 2341 × (+) [illegible]

① # 4, 5, 11, 12 restreaked from original tailing from "brush"
② single colonies (+) picked and tested /λ, 2279, streaked out ∅ gal

	2279	/λ	segregating
slower growing → 4	lysog	R	+
5	nonlys	Sens	~~no~~
11	lysog	R	+
12	nonlys	R	+

307-4, -11 streak out 4, 11, see if λ present

Stocks 1/4/55 (307A)

K12
58-161
W67
W518
W518 galt #1
W583
W588
W677

84 t HFT 7⁻ (309-1) to obtain 4⁻7⁻
seg. removed from a cross brush.

1. 7 sep. seg. transd. /12 picked
single seg. from each 3 7⁻ ← stock of this create H⁻ gal⁻
4 4⁻ 307A-1 ← W2792

2. Restreaked to obtain 7 more seg.
6 obtained, 3 7⁻
3 4⁻

7 4⁻ (6 7⁻)

5° ?

1/9/54

Position effect between Gal_1^- and Gal_6 (2070)

1. NA4 pap-1 mixed with cells of 2070 with λ in excess (c. 10^7 cell / 10^8 λ), incubated for 15 min at room temp., diluted and plated. Broth used as control

	(−) col	orange col. after 2 days
broth	95	0
	82	0
	100	0
	277	0
HF71 lysate	73	1
	104	2
	90	0
	267	3

Stocks of the diheterogenotes 308-1, 308-2, 308-3

Stock gal+ discarded Sept 1, 1964

2. Segregants from pap. (+) organisms. tested again

1.	0 pap	0 pap	papillating parental
2.	+	0	6−
3.	−	+	1−
4.	−	+	1− ← 308-4
5.	0 pap	0 pap	papillating parental
6	+	0	6−
7.	+	0	6−
8	0 pap.	0 pap.	papillating parental
9.	+	0	6−
10.	+	0	6−
11.	+	0	6−
12	0	0	pap. parental
13	0	0	1-6− : some pap and revertants
14.	0	0	1-6− ← 308-5
15.	+	0	6−
16	0	0	pap. parental
17.	0	0	1-6−
18.	0	+	1−
19	0	0	pap. parental

24

5 stable gal+

Σ 6 parental pure heterogenotes
8 6−
2 1−
3 1-6−

← W2856

+ −
− +
↓
+ + or − −
− − or + +

← W2857 also lp derivat

+ −
+ +

1/9/54

Continuation of 302 -

302-1, 2, 3, 4, 5 - In search of HFT gal₂ -

1. Since gal₂- cultures when spotted on either 2, 5 and irradiated give "apparent" false positive reactions for HFT, experiment was done on S gal using prototroph 2175 gal₂ -

2. Above cultures streaked, 6 individual (+) picked from each and streaked out to obtain 6 separate segregational events. The (-) obtained spotted on W2175 on S gal and irradiated. After 3 days result were

302-1	302-2	302-3	302-4	302-5	derived from 2307
no HFT	no HFT	no HFT	no HFT	1/6 appear HFT ←	309-1

1/26/54 To test L.L. Cavalli's hypoth. about crossing over between fragment and chromosome.

7. 309-1 plated on B lac, xyl, gal, arabinose to obtain reversion and to test their stability

A. no reversions obtained B lac, xyl, several on B gal. B arab. react. appears to indicate mixed culture (how - this came from single colony above). Streaking out of the growth on the plate suggests segregation for gal going on. Poss. of reversion gal?

B. 2307 streaked against HFT 1, 2, 4, 7 on B arab.

C. 309-1 gal +ʳ should be checked (after purification) on B arab. - Apparently 309-1 is ara + as 2307 has been on ✓. Streaks of 309-1 after showing different colony type, do not show any (-)

D. Gal +ʳ of 309-1 are unstable 6/6

Prototrophs 2⁻ 1⁻ 4⁺ / 2⁺ 1⁺ 4⁻ 309 B-1

4⁻ 8⁻ ← 1⁻

2350 × ← 1⁻ See 272 for order of loci. Gather additional information on segregants.

2⁻4⁻ × ← 1⁻

W2859 also 4ʳ den

10⁵ mould dil. of NA-pap

B

Seg.	1⁻	2⁻	4⁻	Alleles		1⁻	2⁻	4⁻	Alleles
1.	mixed with + ? ND - position effect -				20	+	− *	+	2⁻
2.	+	− *	+	2⁻	21	+	− *	+	2⁻
3.	mixed cult + ? Discarded →				22	−	− *	−	1⁻2⁻4⁻
4.	+	− *	+	2⁻	23	−	− *	−	1⁻2⁻4⁻
5.	+	− *	+	2⁻	24	+	− *	+	2⁻
6.	−	− *	−	1⁻2⁻4⁻	25	−	−	−	1⁻2⁻4⁻ (then spray)
7.	−	− *	+	1⁻2⁻	26	−	+	+	1⁻
8.	+	+	−	4⁻	27	+	− *	+	2⁻
9.	+	− *	+	2⁻	28	−	− *	−	1⁻2⁻4⁻
10.	+	− *	+	2⁻	29	−	+	+	1⁻
11.	+	−	+	2⁻	30	+	− *	+	2⁻
12.	−	+	+	1⁻	31.	−	+	+	1⁻
13.	−	+	+	1⁻	32	−	+	+	1⁻
14.	+	− *	+	2⁻	33.	−	+	+	1⁻
15.	+	− *	+	2⁻	34	−	+	+	1⁻
16.	+	− *	+	2⁻	35.	+	− *	+	2⁻
17.	+	− *	+	1⁻2⁻4⁻	36.	+	− *	+	2⁻
18.	+	− *	+	2⁻	37.	+	− *	+	2⁻
19.	+	− *	+	2⁻					

* plus (2,4) ...

309B-11 triple (-) plus 31 ... 2

Classes

(idio)	2⁻	19	27
(allo)	1⁻	8	10
(allo)	1⁻4	0	0
amphi	1 2 4	6	6
	1⁻2⁻	1	1
	4⁻	1	1
		35	45

Previously
idio 2⁻ 8
allo 1⁻ 2

1/9/54

2433 × 4⁻ For order of loci - see 272 and also other comb. on 309, test sy

Seg	no add dil of 2470-1 /1	0 17 /2	~~Segregant~~ – 2/17 stable, 1 lost, 14 tested /4	Comment
1	—	—	+ ?	heavily papillating after 3 days
2	—	—	+ ?	" " " "
3	contam ē	(+)		
4	—	—	+ ∀	as (1)
5	—	—	+	as (1)
6	—	—	+	no pap.
7	—	—	+	as (1)
8	—	—	+	as (1)
9	—	—	+	no pap.
10	—	—	+ ∀	as (1)
11	—	—	+	as (1)
12	—	—	+	no pap
13	—	—	+ X	as (1)
14	—	—	+	no papillation

A

Also suggesting on 2nd day that some of the papillating forms are Lp^s or slow to something. Pap. forms usually have thinned growth. Restreak or look at pap. again - are there Lp^s in these clones?

Rpt tests: confirm these as to allele, but be prep. this script #3.

By making purification before restreak, more secondary pap. forms were selected.

See if pap occurs in 3 on B(0). Also if this cult. auxo, in Nopin?

2308 × 2070λ - to see if Gal₆ is distinct from Gal₇ -

B

	no add	7
old lysate	2070 λ+ (0.1)	30 (lysis? also)

27 picked - all appear stable (~~??~~)

	/λ	/1321		/λ	/1321
1	R	lysogenic	15	R	lysogenic
2	↓	↓	16	↓	↓
3			17	S	nonlys
4			18	S	"
5			19	R	lysogenic
6			20	↓	↓
7			21		
8			22		
9			23	↓	↓
10			24	S	nonlys
11			25	R	lysogenic
12	↓	↓	26	↓	↓
13	↓	↓	27	↓	↓
14	↓	↓			

2360 t K12 - to see incidence of stable (+) and lysogenicity c2000/0
reactions. transd. reg. 304

transd.	λ	/1321	? Segregating
1	R	lysogenic	+
2	↓	↓	+
3			+
4			−
5			+
6			−
7			−
8			+
9			−
10			+
11	↓	↓	−
12	S	non lys	−
13	R	lys	+
14	↓	↓	+
15			+
16			−
17			+
18			+
19			+
20			+
21			+
22	↓	↓	−
~~23~~			
~~24~~			

+ = yes total 8 nonseg
14 seg

2070 λ+ - for making HFT 6− ~~onto~~ 2175

① no add 11
311-10 (old lysate) 0.1 2070λ+ 97 ← This titer higher than in 2305 lp+, but again this may be indication of decay of lysates in standing (only measured
311-13 on lp+ cells) or a positional effect between 6− and 7−.

②. pick and streak to obtain HFT 6−

③ 13 reg tested /2175 on 5701, 2 possible HFT, #10, #12

311-2 ④ 17 " tested /2175 Bgal, 1 #2 appears HFT

1/20/84

283-1 against 7⁻ - If the order is 1⁻ 7⁺ 4⁻, then only double crossover were gave (+)
#3 must be ...

A

mixed = 0
0.1 (1-10 dil)
309-1

Pap.	stable (2)	Streaks 10 (−)	/1	/4	/7		Genotype
1	+	—					
2	single (+)	(−)	— (small pap)	—	—	1 pap	1⁻7⁻ or 1⁻4⁻?
3	c.10(−)	If portion crosses pap.					—
4	+	—					—
5	single (−)		—	—	—	2 pap	1⁻7⁻ or 1⁻4⁻?
6	c. 10 (−)		—	+	—		1⁻7⁻
7	single (−)		—	+	—		1⁻7⁻
8	" "		—	+	—		1⁻7⁻
9	2(−)		—	— c.3 very small pap	—		1⁻4⁻7⁻ or (1⁻4⁻)?
10	+	—					

3 1⁻7⁻
3 (1⁻4⁻)?
3 gal(+)

~~In search of ...~~

2020 cross brushed with HFT dipoles - Gal₆ distinct from Gal₇

/1	/2	/4	/7
++	solid once	+++	++++

1/25/5?

307-1 × 4⁻ to obtain order

1⁻ ⁻x+1, 7+ ⁻+1!, 4+ +x⁻⁻ ⁻+, ⁻+ X ⁻+ ⁻+

① the 4⁻ (247R-1) lysate diluted 1-100 gave c. 5 × 10³ pap < Control Rei ε 1·4⁻ × 7⁻ where double crossovers are required on order !

② 24 papillae picked, streaked ③ ; on agar ; (-) picked streaked ①

③ (-) tested

	/1	/4	/7
1	−	+	−
2	−	+	−
3	−	+	−
4	−	+	−
5	−	+	−
6	−	+	−
7	−	+	−
8	−	+	−
9	−	+	−
10	−	+	−
11	−	+	−
12	−	+	−
13	−	+	−
14	−	+	−
15	−	+	−
16	−	+	−
17	−	+	−
18	−	+	−
19	−	+	−
20	−	+	−
21	−	+	−
22	−	+	−
23	−	+	−
24	−	+	−

24 1⁻ 7⁻

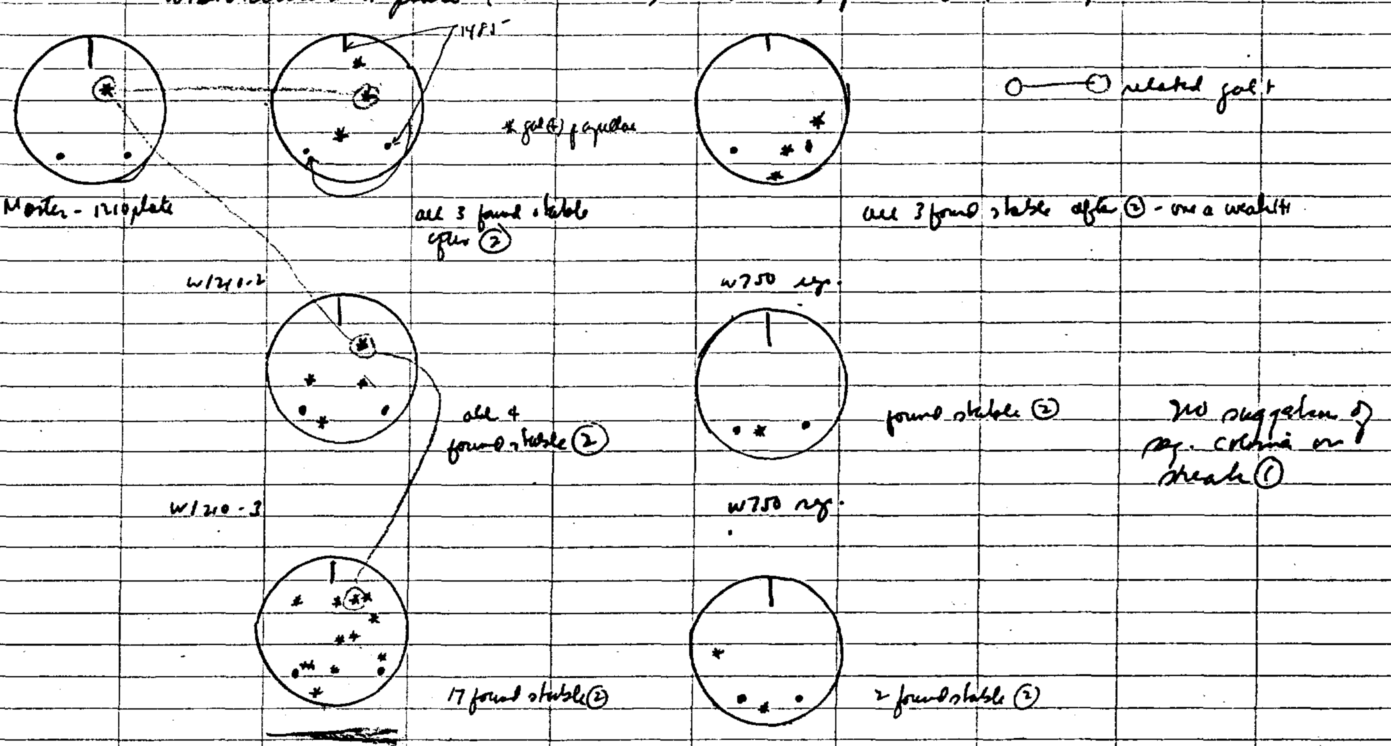
~~The results suggest the order is 1 4 7, but the absence of the 1/3 should be seen in other crossings~~

DATE: 1/26/54 REF:

Repeat examination of Expt 301 – to see if transd. particles in NFT lysates are distributed "as mutants" in clones.

1. Cultures of 1210 750
2. Overnight culture of W1210 (in B gal) replica'd to 3 prewarmed B gal plates and incubated c. 45 minutes. At this time the three plates were irrad. 20' with U.V. (50 cm, Sterilamp). Reincubated about 30 minutes and then replicated to plates (previously spread c W750, 30 min) and the plates incubated 3 days.
3. Positioning of the replica's aided by marking original 1210 plate with 3 streaks of W1485 (gal+) thus. 

4. Results:



Where are the (+) streaks from?

DATE: 1/31/55 REF:

1210 against lysate of 307-1. Completion of Position Effect c̄ 1⁻, 7⁻
307-1 = 1⁻7⁻. Is the complex 2⁻ 2⁺ / 1⁺ 1⁻ / 7⁺ 7⁻ phenotypically (+)?

What are segregants?

The results say yes

A

1. no add = 0
2. 0.1ml 307-1 = 33
3. Segregants

	/1	/2	/7	Phenotype
1	+	−	+	2⁻
2	+	−	+	2⁻
3	+	−	+	2⁻
4	+	−	+	2⁻
5	+	−	+	2⁻
6	+	−	+	2⁻
7	+	−	+	2⁻
8	+	−	+	2⁻
9	+	−	+	2⁻
10	+	−	+	2⁻
11	+	−	+	2⁻
save M-1⁻7⁻ 12	−	+	−	1⁻7⁻
13	+	−	+	2⁻
14	+	−	+	2⁻
15	+	−	+	2⁻
16	+	−	+	2⁻
17	+	−	+	2⁻
18	+	−	+	2⁻
19	+	−	+	2⁻
20	+	−	+	2⁻
21.	+	−	+	2⁻
22.	+	−	+	2⁻
23.	+	−	+	2⁻
24.	+	−	+	2⁻
25.	+	−	+	2⁻
26.	+	−	+	2⁻
27.	+	−	+	2⁻
28.	+	−	+	2⁻
29.	+	−	+	2⁻
30.	+	−	+	2⁻

29 2⁻
1 1⁻7⁻

3 (dashed)

1210 = (+)g8⁻ / not (+)g2⁻

W1210 = gal8⁻

315-12

W2861

750 EMC 12-1 λ Prey 1/28/55
Pooled / 750 = 2.6 × 10⁷
0.1 ml in Pen + Sterile

143 x100 (100 x100 ~ 1.4x10^5)

DATE: 2/1/54 Induct of 241-14 mal- lysate REF:

Dose	spont. = 3 pap/2279 (dil 10)	spont = 2 pap/750 (dil 10)	spont = 0 Plaques 2279 ↓ 10^2 dil	spont = 0 pap 2279 10^2 dil	Exam of papillae stable/total	sex	Lambda	Reactions
0	28	67	155	1	1/1	— mostly (-) 14 p^R		
15	54	140	151	15	12/15	s, rr	2 p⁺, 7 p^s, 3 p^R	
30	78	184	172	22	17/18	R	— , 14 p^s, 2 p^R	
45	108	293	66	25	18/18	—	— , 17 p^s, 1 p^R	
60	176	434	28	22	17/18	R	, 16 p^s, 1 p^R	
75	230	638	39	19	17/18	R	1 p⁺, 16 p^s, —	
90	221	711	14	24	17/18	s: stable (+)	— , 17 p^s, —	
105	237	536	17	37	18/18	discarded ↓		
120	252	958	12	10^2 31	17/18			
135	184	798	10^1 = 45	10^1 201	18/18			
150	163	699	und: 305	und 247 (1/4)	18/18			
165	163	717	und: 231	ca. 4	18/18			
180	148	589	und = 141	ca. "	17/18			
~~195~~								

0 dose

/ml = 155×10^3 plaques
= 900×10^2 hand.

1 - 10 dil /ml = 15500 plaques
9000 hand

$1.55 \times 10^5 \times 10^3 = 1.55 \times 10^8$ /ml

1.6×10^8 plaques/ml

6.7×10^6 hand/ml

$10^4 \times 10^3 = 1.$

probably 2279

Multiplicity Aspect.

(1) 2297 + 0.1 ml 10^2 0 dose plated. To 1/2 of plate 0.1 ml 750-2 λ added (Per Plate) see also
plaques on control (no 750-2) = 37
papillae on 750-2 half = 175
papillae on control = 2
2.4 > than the highest value for plaques or hand. given above in 0, 15, 30 (155, 151, 172)

(2) 0 above 10^2 dil
(1) 0.5 ml + 0.5 ml cells (c. 10^8) adsorbed 10'
(2) add 0.5 ml 750-2 (= 2/3 dil of original), chill 5'
(3) plate 0.1 ml. no. papillae. 188 × 3/2 = 282

DATE: 2/5/55 REF:

298-8 see that page and 304

1. 298-8 made lysogenic with λ from 84
2. To complete the study of gene order.
   a. 298-8 × HFT 1
   b. 298-8 × " 6
   c. " " " 7
   } undiluted 0.1 ml
3. Results. no papillae on any of the plates. What goes?

2279 against HFT 6, 7 to obtain recip. test of pret. effect.
0.1 ml c. 10^8 cells + 0.5 ml lysate diluted 1-100 ... ml plated
1. 2279 control 3 plates c. 600 colonies/plate are (-)
2. 2279 × 6⁻

Failed no haloed. Inoculation observed. Killing was very light here

W2862 also got derivative

list 7

	/2	/7	∅
1	0	+	2⁻
2	0	+	2⁻
3	0	+	2⁻
4	0	+	2⁻
5	0	+	2⁻
6	0	0	2-7⁻
7	0	+	2⁻
8	0	+	2⁻

317C-6

317C-4

8 additional segregants from the same transductions

2175 against HFT 6, HFT 7⁻ to make double (- -) for positive gene under test

1. Strong reaction between 6⁻, 7⁻ and 2
2. 12 colonies picked from each

6⁻

	/2	/6		
1.	+	0	6⁻	8 cln
2.	0	+	2⁻	1 allo
3.	0	+	2⁻	1 amph
4.	0	0	2-6⁻ Separate	
5	0	+	2⁻	
6.	0	+	2⁻	
7	0	+	2⁻	
8	0	+	2⁻	
9.	0	+	2⁻	
10.	0	+	2⁻	

7⁻

/2	/7	∅	
0	+	2⁻	7 cln
0	+	2⁻	2 allo
0	+	2⁻	
0	+	2⁻	
0	+	2⁻	
0	+	2⁻	
+	0	7⁻	
+	0	7⁻	
0	+	2⁻	
—	—		

DATE: 2/8/55 REF:

Crosses to see distribution of (-)

~~1. 578 fag-F+ x 902 gal-F-~~
~~a. [illegible]~~
~~b. [illegible]~~

750E902-1 x 2274

1. controls ok. (B gal), 2274 (-), 750E902-1 mixed (mostly +)

A 2. Poor cross plate - heavy bkgd of small (-) colony, irreg. shaped c. 50 gal+ per plate

12 colonies picked. after ③ 3 reg. - All good (+)

	stable	λ	/2279
1	u	R	lys
2	u	R	lys
3	u	R	lys
4	s	s	non lys
5	s	s	" "
6	s	R	lys
7	s	s	non lys
8	s	s	..
9	s	s	..
10	s	s	..
11	s	s	non lys
12	s	s	..

~~2. 902 F+ gal- x~~

279-3 x 1321

1. controls ok (B gal) (1321 (-), 279-3 mixed (mostly +))

B 2. Poor cross plate - heavy bkgd of (-) colony, irreg. shaped about 20-30 weak (+) per plate

11 (+) colonies picked - after ③ 10 stable, 1 segregating. Only 2 strong (+), one of these segregating.

		stable	λ	/2279
	1	u	R	lys
	2	s	s	non lys
	3	..	..	
	4	↓	↓	↓
	5			
	6			
	7			
	8			
	9			
	10			
weak gal (+)	11	s	R	non lys

DATE: 2/16/58 REF:

1	2	3	4	5	6	7	8	9	10

1. On the order of the loci
308-5 = 1⁻6⁻

(A) a Cropful (c. 0.01 ml) of HFT 7⁻ streaked on ½ plate β gal
control ½ : 0 (a faint background of very minute pap)
HFT 7⁻ lysate ½ : 31 (on single gal(−) HFT 7⁻ plus have given c. 10^4)
The low titer here suggests the order 1.7.6 and that the pap are the result of double crossovers.

B. The segregants. Of the 30 pap examined, 18 were stable but at (2) RETESTS AFTER PURIFICATION

A

	/1	/6	/7	∅	/1	/6	/7	∅
1.	−	−	0 (1 pap)	1-6-7-	0	0	(2 pap) 0 *	16⁻ ?
2.	−	+	− (2 pap)	1-7- (?)	0	+	5 pap *	1⁻
3.	parental pap. (−)			1-6-7-	0	0	0 *	1-6-7-
4.	−	−	−	1-6-7-	+	+	0	7⁻
5.	−	−	−	1-6-7-	0	+	0 *	1-7-
6.	− vol +			—	0	0	2 pap *	1-6⁻ ?
7.	−	−	−	1-6-7-	+	+	0	7⁻
8.	+ (6 pap)	+	−	7⁻	2 pap	+	0	7⁻ ?
9.	−	−	−	1-6-7-	0	0	0 (1 pap) *	1-6⁻ ?
10.	−	−	−	1-6-7-	0	0	0 (4 pap) *	1-6⁻ ?
11.	−	−	+	1-6⁻	0	0	0 (2 pap) *	1-6⁻ ?

0 = (−)
(−) ≠ 0

12. 7 (1-6-7-) amplitype — 1-6⁻ ? = 5
RETEST (?) 4 (1-7-) amplitype — 1⁻ = 1 ampli
1 (1-6-) idem — 1-7⁻ = 1 ampli
1 (7⁻) amplitype — 7 = 3 ampli
* many minute papillae throughout — 1-6-7⁻ = 1 ampli

(B) a cropful (0.01 ml) of HFT 4⁻ streaked on ½ plate β gal
control ½ = 0
HFT 4⁻ lysate ½ = 500-1000 — this high titer suggests the order 1-6-4 and these pap. are the result of single crossovers } the overall order appears now to be 2-1-7-6-4

1. The segregants. – After (2) 6 of 24 stable, the segregating strains in each case have only 1 or 2 (−). Is this segregation from allotype? Similarity of order is correct

	/1	/4	/6	∅
1	0	+	0	1-6⁻
2	0	+	0	
3	0	+	0	
4	0	+	0	
5	0	+	0	
6	0	+	0	
7	0	+	0	
8	0	+	0	
9	0	+	0	
10	0	+	0	
11	0	+	0	
12	0	+	0	
13	0	+	0	
14	0	+	0	
15	0	+	0	
16	0	+	0	
17	0	+	0	
18.	0	+	0	18 (1-6-)

Most of these have many small pap., in control and elsewhere. reminiscent of 2433 - 4 - 1

DATE: 2/12/55 REF:

On the order of the loci — To complete the analysis of the position effect.
With regard to the assays of this lysate on other (−) see 305, 304

1. 307-1 x — (+)
no add = 0
0.1ml K12 = 294
(11/12/54)

This finding indicates the structure $\frac{- -}{+ +}$ is ∅ gal +

2. The segregants

A

	/1	/7	/+
1	0	0	+
2	0	0	+
3–18	↓	↓	↓

	/1	/7	/+
19	0	0	+
20–24	↓	↓	↓

24 1-7

1. 308-5 x — (+)
no add = 0
0.1ml K12 = 238
(11/12/54)

The structure $\frac{1\ 6}{\frac{- -}{+ +}}$ is ∅ gal (+)

← 3708-1 presumed heterogenote — W2876

2. The segregants

B

	/1	/6	/+
1	0	0	+
2	0	[illegible]	↓
3	0	[illegible]	↓
4	0	[illegible]	↓
5–11	↓	↓	↓
12–20	↓	↓	↓
21	mixed c̄ (+)		

3 stable (+) 20 1-6

DATE: 2/13/55 REF: 321

Gal1- Gal6- interaction. Gal2- x —— Gal1- Gal6-, is the product Gal+ ?

W1210
no odd - 0
0.1ml sp. 308-5 = 50

W1210 = gal8-

Seg	/1	/2	/6	Genotype				
1	+	0	+	2-				
2	+	0	+	"				
3	0	+	0	1-6-	← 321-3	M-1-6-	←—	W2864
4	+	0	+	2-				
5	0	+	0	1-6-				
6	+	0	+	2-				
7	+	0	+	"				
8	+	0	+	"				
9	+	0	+	"				
10	+	0	+	"				
11	+	0	+	"				
12	+	0	+	"				
13	0	+	0	1-6-				
14	+	0	+	2-				
15	0	+	0	1-6-				
16	+	0	+	2-				
17	lost							
18–21	stable(+)							

12 2- idio
4 1-6- allo
7 stable(+) ?

DATE: 2/21/55 REF:

Multiplicity Effects

Reagents:
1. 750-2 λ (old prep) 10^7 = 34 41 = 3.8×10^8
2. cell Assay = 10^7 = 172, 188 = 1.8×10^9
3. Transd. lysate = 10' dil of 0 dose 241-14 p 316, = 15500 plaques/ml

Procedure

	Tube 1	2	3	4
R_x 3	0.5	0.5	—	—
Penassay	—	—	0.5	0.5
R_x 2 cells	0.5	0.5	0.5	0.5

Incubate 10' at rm. temp.

dilute as follows, 0.1ml of above to

	1	2	3	4
0.4ml 750-2λ	*	0.1	—	0.1
0.4ml Pen	0.1	—	0.1	—

Incubate 5' and then plate 0.1ml + 0.1ml of R_x2 cells

	1	2	3	4
Plates: 24hr	all (-), slight evidence of λ ⊕	all (-) partially lysed		
Count:	187 plaques / 6 papillae	— / 12 papillae	— / 6 papillae	— / no papillae
	X10X5X2	X10X5X2		
	18700 plaques	1000	0	

DATE: 2/21/55 REF:

1	2	3	4	5	6	7	8	9	10

Multiplicity Effect and also Reciprocal case of position effect between 1-6, 1-7

2279 diluted = 10^6 cell

		tube	1	2	3	4	5
	cells		+	+	+	+	+
	HFT 6⁻	0.5 ml	+	+	o	o	o
	HFT 7⁻	0.5 ml	o	o	(+)	+	o
adsorb. 10 min							
dil 100 (HFT p. 322)							
add 0.1 ml + 0.9 ml 253-2			o	+	o	+	o
add 0.1 ml + 0.9 ml penassay			+	o	+	o	+
adsorb 5 min							
plate 0.1 ml							

	Plates:		1⁻ × 7⁻	1⁻ × 7⁻	
24 hr	col(-), slight evidence of ⊙	(-) col. all partially gal	as 1	as 2	(-) col
			323-1		
26 hr	256 (-) col	287 (-) col	1 col pap. slightly; 246 (-) col. some partially gal.	1 col pap. 306 (-) col	231 (-) col.
48 hr	1 col. c̄ 2 central pap ⊙	1 col. c̄ 5 central pap ⊙	no further change	no further change	
	323-3	323-4 ← stock gal+ discarded Sept 1, 1964			

323-2 ← stock gal+ discarded Sept 1, 1964 (isolated this ⊕ colony)

Streaked out 24 parental (-) picked in each case to obtain (+) ✓ ✓

No (+) obtained 22 23

323-4 = 1⁻ × 6⁻ for position effect analysis
1 segregant 6/22 +ᵘ

323-2 = 1⁻ × 7 as above
1 segregant 4/23 +ᵘ → 4 seg.

W2791

6 segregants	/1	/6	type	Genotype
1.	o	+	sens	1⁻
2.	+	o	sens	6 - 323-5
3.	+	o	resist	6 - 323-6
4.	o	+	resist.	1⁻ 323-7
5.	+	o	sens	6⁻
6.	+	o	sens	6⁻

4 seg.	/1	/7	genotype
1.	+	o	7⁻
2.	+	o	7⁻
3.	+	o	7⁻
4.	o	+	1⁻

all λˢ

For further seg see 383B

W2894

W2895

of 2 6⁻ revertants both stable gal+

of 6 gal+ revertants, 1 unstable

DATE: 2/27/55 REF: (324)

1	2	3	4	5	6	7	8	9	10

Is 1210 really gal_1- or a closely linked Γ-1. In the past, particularly with W2350 and W2760, Gal_1- Gal_2- derived from W1210 it has been noted that tests against HFT 2- (all HFT 2- lysates are derived from the (-) of W902) gave (delayed) positive papillae few in number. Purification of the cultures did not always remove this effect. It may be (despite the crossing data (11,000 prototrophs) between 1210 x 902 that 1210 gal- is not a recurrence of gal_2. To add further information 3 separate gal_2- HFT lysates derived from (W902 gal-), known to have originated from separate single gal- HFT colonies were put onto 1210

	Lysate	1210
2342	3/17/54 lys	c 50 pap after 3 days
2342	3/20/54 lys	.. ..
	241-14 lysate	.. ..

Mal- L_{p}^{r} breakthrough — also selection for λ mutant — Serial selection for these.
296-1 gal(-) seg.) 296-1 = HR t 2342
~~HFT~~ (not purified) exposed to 0.1 ml 2342 (3/17/54)

no add = 23
0.1 ml HFT 2- = 78 X 8 = 624 slightly higher than previously but this is probably a different lysate

1	2	3	4	5	6	7	8	9	10

Observations on Erotic Induction

1895 Hfr Lp+ X 2234 F- Lp s

1. Freshly grown cultures, by dilution of overnight culture
2. 5.0 + 5.0 ml

Post - Mix
0.5 ml samples

	Pre mix Plaques	Pre mix Col.	Plaques 0	Col	Plaques 15	Col	Plaques 30	Col	Plaques 45	Col.
1895	2	72	4	23		17		17		23
	0.1 ml		0		0		1		5	
2234	0	99		19		23		23		17

dil = 1/4

Suggestive —

2nd run. Dilutions the same overnight

(1)	Pre mix 1895	2234	0	15 Post mix	30	45	60	75	90
plaques	3	6	0	1	2	1	2	3	4
2234	~~(9)~~	—	111	—	—	—	—	97	—
1895	~~(28)~~	—	18	19	24	21	31	16	c. 24

(2). After 90' culture diluted and plated on gal to see if and how are Lp R. 3 plates

Not suggestive

replication of this plate shows c. 10 prototrophs.

	(+)	(−)
1, 2, 3 about same	2	1/4 = 142

On streaking out some of these prototrophs, 28(−), 2(+), most of the (−) were greatly contaminated with (+) — the origin of these (+) is puzzling since the culture was diluted before plating and there should have been few (+) auxotrophs — the cross plate and the pickings were discarded — Re do —

1210 c 84 to make HFT of 1210 (−)

1. no add 2
UV λ (old) 22

2373 - to [illegible] on stability of [illegible].

1. no add : 8
0.1 ml K12 (7/29/54) 280

2. as control the same / 2279
2279
no add 0
0.1 ml K12 (7/29/54) 281
} many small [illegible]

DATE: 3/5/55 REF:

307-1 Lp^5 (made c UV) = gal₁- gal₂-. To determine order and type of selection operating against certain ²strand class
1. Transduced with both 4-, 6- (HFT) = 2478-1 for 4, 311-2 for 6-)

overnight culture ↓ 1-100 ↓ [0.2ml + 0.2ml HFT] TC inc. 20' rm temp ↓ dil H₂O 1-50 ↓ plate 1. gal follow ed. app.

		(−)	(+)	papillating(−)
A. Control not exposed to λ. exposed to broth plate	1	79	0	0
	2	77	0	0
	3	87	0	0
B. Exposed to HFT 4-	1	47	4	0
	2	48	5	1
	3	69	0	0
		164		
C. Exposed to HFT 6-	1.	32	0	0
	2.	23	0	0
	3.	37	1	0

326C - analysis on p 332

DATE: 3/5/55 REF:

As on 326 only using 308-5 ϕ^s (abs. by UV) = 1:6

		(−)	(+)	papillation	
(A.) Control - cells exposed to broth	1	101	0	0	
	2	101	0	0	
	3	92			
(B) Exposed to HFT 4′	1.	77	0	0	} 5 susp. col. after 5 days 327-B
	2.	82	0	0	
	3.	75	0	0	
(C) Exposed to HFT 7′	1.	95	0	2	
	2.	90	0	1	
	3.	84	0	1	327C-1, 2, 3 for analysis of 336
					327C-1 see [illegible]

DATE: 3/8/55 REF:

2344 MI - at J.L. Request to test multiplicity yield and to get information for run.

1. Cleaved 2344 → dil. from overnight
2346λ (2/17/54) →

	1	2	3	4	5	6	7	8	9
					1-10	1-100	1-1000	1-10000	→ 1-100
					+ 0.2 ml	↓ 0.2 ml	↓ 0.2 ml	↓ 0.2 ml	↓ 0.05 ml
					0.2 ml	0.2 ml	0.2 ml	0.2 ml	(2 X 10⁷)
					← Incubate 15' at 37C →				
					↓	↓	↓	↓	
					dil 10^5	10^4	10^3	10^2	
				Id. 1X10⁷	← plate 0.1 ml →				
					not done, no plate available				
						① ②	① ③	④	134
				no. colonies	—	46 | 47	37 | 38	35 | 30	120
		Result		no. gal(+)	—	3 | 7	2 | 2	2 | 2	1.27 X 2 X 10⁷
				no cells / tube / 2 HFT / tube	—	~~2.54 X 10⁷~~	~~2.54 X 10⁶~~	~~2.54 X 10⁵~~	2.54 X 10⁹
				No. survivors	—	4.7×10^6	3.8×10^5	3.3×10^4	Plate Rem'd.
				Fract. surviving		0.36	0.29	0.25	Rem'd
				Fract. (+) survivors		0.11	0.053	0.061	Rem'd
				Fract. transd.		0.04	0.015	0.015	Diff. 1/2

DATE: 3/9/55 REF:

Reexamination of 282-2 (The ± was reisolated from stock and a new purifying stock made.)

1. Early tests do not say separate segregational events were examined.
2. 24 ± colonies streaked out and single (+) from each restreaked. After 2 (+) pickings a (-) stamina to classify for locus.

2nd (+) clone	Stability	Stability Rev	/1 (say)	/4	Allele	
1	s	u	~~repopulating penetrated (-)~~			penetrated
2	u	—	0	0	1⁻ 4⁻	
3	u	—	+	0	4⁻	
4	u	—	+	0	4⁻	
in white ± 5	u (s?) —	u				✓
in Rev 6	s	~~m~~ s	~~+~~	~~+~~		
7	u	—	+	0	4⁻	
8	u	—	0	+	1⁻	
9	u	—	+	0	4⁻	
10	u	—	repopulating —	± ?	—	penetrated
11	u	—	+	0	4⁻	
12	s	s	———	———		
13	u	—	+	0	4⁻	
14	u	—	+	0	4⁻	
15	s	s	———	———		
16	s	s	———	———		
17	u	—	+	0	4⁻	
18	s	s	———	———		
19	s	u	~~ooooooo~~			✓
20	s	s	———	———		
21	u	—	0	0	1⁻ 4⁻	
22	u	—	+	0	4⁻	
23	u	—	+	0	4⁻	
24	s	s	———	———		
	9 s	7 s			10 4⁻	✓
	15 u	17 u			2 1⁻ 4⁻	✓
					1 1⁻	✓
					2 penetrated	
					2 not done	
					17	

DATE: 3/14/55 REF:

1 ?

1	2	3	4	5	6	7	8	9	10
2344 M1 ← 2346 λ			3/17/54	Repeat to see frequency of transd.					
	Ancestral 2344 M								
	↓		Culture		Extr				
	1-100	→	0.3		0.3				
	Birth		0.3		—				
	2346 λ		—		0.3				
		Adsorb	15 min	37 C					
		Ab	10^4		10^3				
			↓						
			0.05 ml plated →		0.05 ml				
	Clones:	Plates	1 2		1 2				
		(+)	0 0		9 2				
		(−)	43 26		220 190				
		total	43 26		229 199				
			35		184 / 428	= 4.2% (+)			

DATE: 3/14/55 REF:

Repeat analysis of 285-2 – 24 pg. (–) picked and streaked to obtain a single gal (+) from each one.

Gal +	Stability ①		Stability ②			1	14	Lp^{RX}	
1	u			✓		parental (–)		—	
2	u			✓		o	+	res	1–
3	s	1			s				
4	u			✓		o	+	res	1–
5	s	2			s				
6	s	3			s				
7	u	—	~~stable~~	sy ✓		o	+	res	1–
8	s	4			s				
9	s	5			s				
10	u			✓	s	o	+	res	1–
11	s	6			s				
12	s	7			s				
13	s	8			s				
14	u			✓		o	+	res	1–
15	u			✓		o	+	res	1–
16	s	9			s				
17	s	10			s				
18	s	11			s				
19	s	12			s				
20	s	13			s				
21	s	14			u	+	o	res.	4–
22	u			✓		– parental (–)			
tot	14 s		13 s					6	1–
	8 u		9 u					1	4–

This is wrong cell – as Lp^R instead of Lp^+ should have been used. 285-1

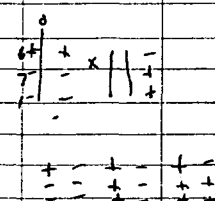
DATE: 3/16/55 REF:

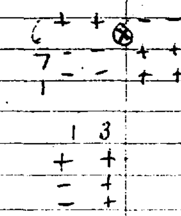
1-7-x-6-

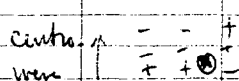
see [redacted]. Analysis of segregants from [redacted] 326c from p 326c

1. 24 (-) pap. ce picked and streaked
2. from these streakings 24 (+) obtained
3. 24 (+) streaked out.

+col streak out	stability	Rev stability	1-	6-	7-	
1.	s	s	o	+	o	
2.	s	u	mixed c (+)			
3.	u		o	+	o	✓
4.	u		o	+	o	✓
5	s	s	—	—	—	
6	s	s	—	—	—	
7	s	s	—	—	—	
8	s	s	—	—	—	
9	u		o	+	o	✓
10	u		o	+	o	✓
11	u		o	+	o	✓
12	s	s	—	—	—	
13	u		o	+	o	✓
14	u		o	+	o	✓
15	u		o	+	o	✓
16	u		o	+	o	✓
17	u		o	+	o	✓
18	u		o	+	o	✓
19	u		o	+	o	✓
20	u		o	+	o	✓
21	u		+	o	+	
22	u		o	+	o	✓
23	s	s	—	—	—	
24	u		not done			
	8 s	7 s			14	1 7-
	16 u	8 u			1	6-







DATE: 3/18/55 REF:

1321 X 902 F+ – to obtain reciprocal cross data. Previously 518 X 902

1. Control streaks ok on B gal
2. Cross plates bad – many small (–), ca. 20 large col., probably (+)

Don't know what to make of this? Easier indeed..?

518 X 2035 to look for "unlikely" recombinant class" – namely gal+ Lp^{s} S gal.

1. Cross plate ok. ca. 90% gal+ – no record of control plate
2. 50 (+) col. picked streaked out B gal, same suspension also streaked against λ!

sensitive Streak #	No. (–) in B gal streak	Tests of the (+) on the streak (1)	(2)
# 3	1/ca. 50	sens.	sens
# 5	0/ca 100	sens.	sens
# 20	0/ca 200	sens.	sens
# 41	5/ca 100	sens	resist.

replicated to S gal, all prototrophic

4/50+ are recombinants between Lp and Val

Gal$_4^-$ Lp^{s} X Gal$_4^+$ Lp^{+}
↓
Gal$_4^+$ Lp^{s}

296-1 = 1412 t AFT2

1. Hoping to find λ host range mutant. Repeated transd. of gal- seg from this heterogenote

1. transd. with gal(+) HFT 1 (#1) → gal- seg obtained →(transd. #2) Gal(+) HFT → rpt. #3

→ from #3 8 transd. picked and single gal- seg obtained. Each plated with 0.1 ml NFT gal(+) λ to see if plaques observed on USA.

1. On one (#3) single faint plaque (?) noted.
2. on one (#6) several " plaques (?) observed near edge

abandoned after the 4th passage.

DATE: 3/18/55 REF: W2790

1	2	3	4	5	6	7	8	9	10

W2580 = gal_2 - 988 $t/2^s$ - mutated and lys^- obtained

↑ W2580 lys^-

DATE: 5/20/55 REF:

Lysates

811 t 902	Plaques (2×10^5)	Plaque Titer	Transductions ($td = 2\times10^5$)			5/21/55 $td = 2\times10^4$		Lysate streaked	
			/811	/2580		td /811	/2580	2580	750
335-1	1. 110, 116	2.3×10^7	34	30	19 = 3.8×10^6	22	20	+++	+
2	2. 87	1.5×10^6	26	20	9 = 1.8×10^6	24	17	0	0
3	3. 129, 134	2.6×10^7	20	23	12 = 2.4×10^6	21	25	±	++
4	4. 148, 158	3.1×10^7	19	26	15 = 30×10^6	41 [illegible]	23	+++	+

2580 t 811	Plaques (2×10^5)		2×10^5			$td = 2\times10^5$			
5	1.	?	115	16	[illegible]	13	18	0	0
not streaked 6	2. 229 = 2.3×10^7			33		33	48	±± 2×10^6	+++ 4.4×10^6
7	3. 211 = 2.1×10^7	?	69	78		28	96	1.2×10^6	1.4×10^7
8	4.		35	32		12	16	0	0
Spontaneous			29	11		22	26		

Better start from scratch

2236 for HFT 3

1. no add = 2 when these were tested, initially fair percentage unstable (12/12? or 12/24)
2. 902 λ* = 342 plate stored in fridge c. month, then 24/24, 17/17 found stable.

↑ why? see next page also/811

2062 1. no add 4
2. 902 λ* = 85 81

2580 against 811 λ 6/1/54 Rpt 4/25/55

1. no add 11 9
2. 0.1 ml λ 140 170

2580 Ly^3 against 811 λ 6/1/54

1. no add 19
2. 0.1 ml λ 4 x 986 = 3944

DATE: REF:

327C-1 = 1⁻6⁻ × 7⁻ - ~~From~~ 24 pap. presumed parental (-) streaked out - 20 actually were. From each parental (-) a single + obtained.

Co. Gal(?)	stability (1)	(2)	/1	/6	/7
1	ø	ø	——	——	——
2	ø	ø	——	——	——
3	ø	ø	——	——	——
4	ø	ø	——	——	——
5	ø	ø	——	——	——
6	ø	ø	——	——	——
7	ø	ø	——	——	——
8	"	"	0	0	2 pap
9	"	"	0	0	1 pap
10	"	"	0	0	0
11	"	"	0	0	0
12	"	"	0	0	0
13	"	"	0	0	1 pap
14	"	"	0	0	3 pap
15	"	"	0	0	0
16	"	"	0	0	2 pap
17	"	"	parental (+)		——
18	"	"	0	+	6 pap
19	"	"	0	0	1 pap
20	"	"	0	0	1 pap

13 4
7 ø.

(9–19: all Lp^s except parental (−))

This culture presumably Lp^r/Lp^s

6⁻ - +⁻
7⁺ + - -
1⁻ - + +

1⁻
4 1⁻6⁻
3 1⁻6⁻7⁻

Sd by 902 λ *

1. no add	29	
2. 0.1 ml λ	88	60

518 by 902 λ

518?

1. no add	49	
2. 0.1 ml λ	234	185

DATE: REF:

Linearity of HFT at high dilution

750 cc 1-10 dil of 241-14 (0 dose - rad. expt.) see 316

6/22/55 1-10² dil
Plaques against W2792

ml lysate	pfu	Δ				
0	2	0	10.1		0	0
0.02	16	14	70		0.02	90
0.04	38	36	88		0.04	183
0.06	68	66	110		0.06	257
0.08	74	72	90		0.08	354
0.10	95	93	93		0.1	459

see plates, broth @ 37
total plate addition made up to 0.2 with broth

2279 undil of 241-14 (0 dose rad. expt) see 316 Rpt. 4/8/55 Rpt. II Rpt III

total particles 38 × 35 15030/0.1 ml

ml lysate	pfu	Δ						
0	1	0		3	0	0	2	0
0.02	28	27				30	37	35
0.04	61	60		66	63	37 ?	73	71
0.06	93	92		140	137	68 ?	145	143
0.08	201	200		154	151	91	135	138
0.1	316	315		159	156	238	158	157
			0.12	175	172	312		
			0.15	287	284	(0.14) >350		

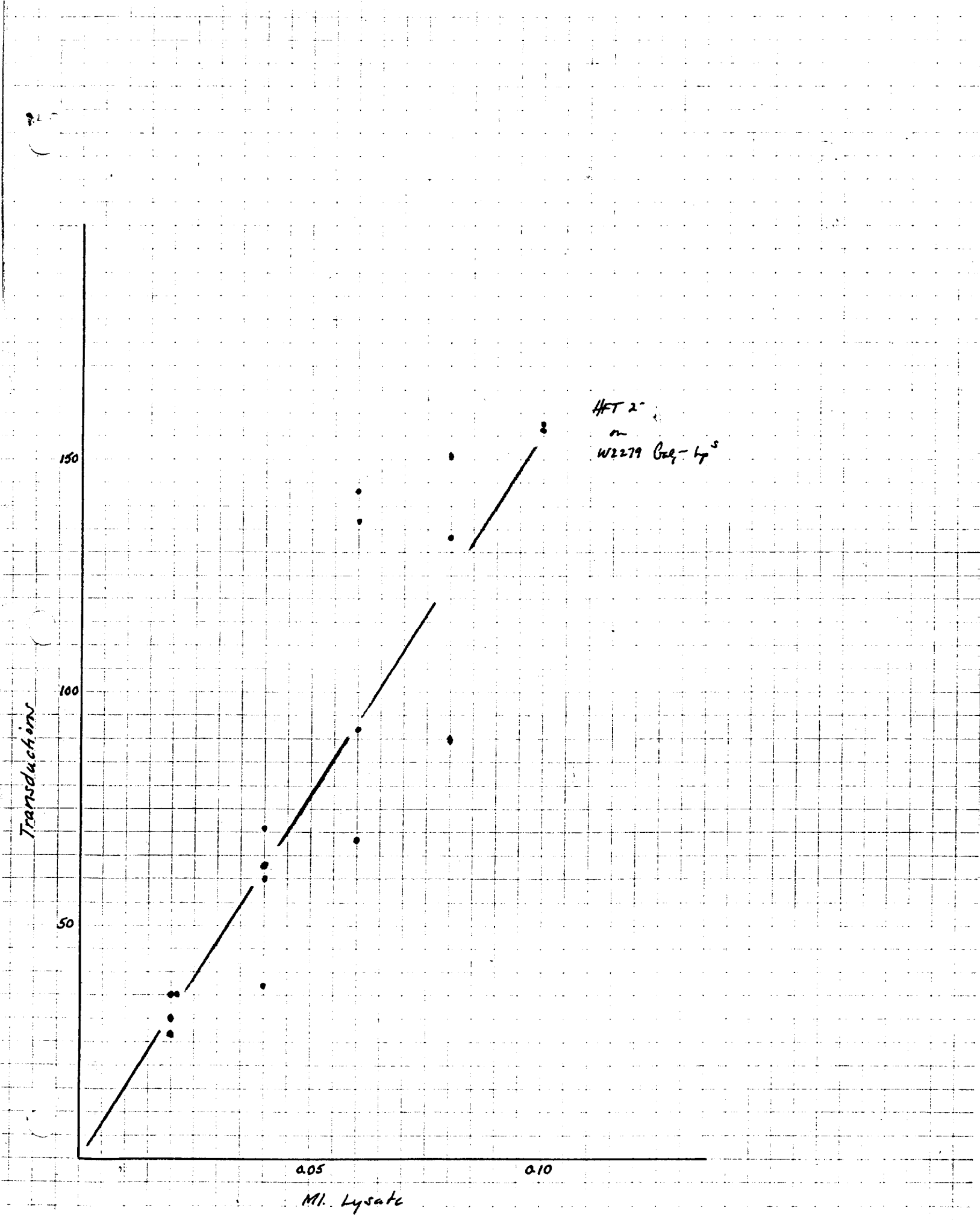
1402 X 902F+

7 controls on B gal ok.

2.	(-)	(+)
Photographs ①	521	1
②	512	1
③	603	1
④	466	2
⑤	538	0
⑥	575	0
⑦	556	2
	3771	7

$$3771 \overline{)7.000} = 0.0018$$
3771
32290

= 0.18%


HFT 2°
on
W2279 Gal-Lys
150
100
50
Transduction
0.05
0.10
Ml. Lysate

DATE: 3/23/55 REF:

Linearity with NPT.

1. 814 overnight cult. wt curve. lysate = K12 11/12/54

	lysate	plaques	Δ		
	0	23	0	-	
	0.02	63	40	20	
	0.04	112	89	22	
	0.06	151	128	21	
	0.08	plate contaminated			
	0.10	234	211	21	

2. 2580	0	22	0	K12	11/12/54
	0.02	163	141	70	
	0.04	270	248	62	
	0.06	345	323	55	
	0.08	460	438	55	
	0.10	527	525	53	

3. 2580 λ^3	0	10	0	K12	11/12/54
	0.02	1467			
	0.04				
	0.06				
	0.08				
	0.10				

518

0	49	0		
0.02	499	450		
0.04	714	665	186	plate contaminated
0.06	888	839	139	
0.08				
0.10				

DATE: 4/8/55 REF:

1	2	3	4	5	6	7	8	9	10

1439 to see if Mal+ S_2^R (λ-2 Resist) are also found. By HFT since the mal- S_2^R may all be temperature dependent (ETC).

1. streaked out on B mal - mixed.

1. 4 (+) } streaked / λ-2, mal+ = λ-2 sens.
2. 4 (-) } mal- = λ-2 resist.

1439 spread with λ-2 to obtain Mal+ λ-2 resist.

Tests of some HFT cultures (2-) obtained on 293. Stabs streaked out B gal - all pure (-). Colonies picked and tested / tested for HFT using u.v.

⊕ = HFT , O = NFT

293-1A.
O O ⊕ ⊕
⊕ ⊕ ⊕ O — O may be the presence of too few cells
O O ⊕ ⊕ — HFT 4/4 gal+R segregating at ②

293-2A
⊕ O ⊕ ⊕ — O = too small inoculum (no growth in spot plate)
⊕ ⊙ ⊕ ⊕
⊕ O O ⊕ — HFT 2/3 gal+R segregating at ②

293-2B
⊕ ⊕ ⊕ ⊕ — HF /2 /4 /+
⊕ ⊕ ⊕ ⊕ — O = good inoculum 0 + + 2-
⊕ ⊕ O ⊕ — HFT 3/3 gal+R segregating at ②

293-11A
⊕ ⊕ ⊕ ⊕
⊕ O ⊕ ⊕ — O = good inoculum 0 + + 2-
⊕ ⊕ ⊕ ⊕ — HFT 3/4 gal+R segregating at ②

	1		2			/2	/4	/+	2-
1A	⊙	⊕ ⊕	⊙		1	0	+	+	2-
	⊕	⊕ ⊕	⊕	⊙ good inoculum	2	0	+	+	2-
	⊕	⊕ ⊙(3)	⊕		3	0	+	+	2-

		1							
2A	⊕	O	⊕ ⊕		1	0	+	+	2-
	⊕	⊕	O(2) ⊕	O good inoculum	2	0	+	+	2-
	⊕	⊕	⊕ ⊕						

DATE: 4/13/55 REF:

1436 - originally (see pg 137, 113) 1436 E/K12 gave -/ or weak (+) sound.
- suspicion that something unusual here. Reexamine

(1) no add c. 40
K12 λ c. 1000

(2) Streaks. 12/12 strong pptz? past, aggregating

2799: mal+ λ-2^R 2279 no breakthrough:

Designate bp_4^R

1. no add = 3
2. 0.1 ml
2342 3/17/54 = 4

241-14 mal - Spontaneously produced λ - HFT?

/750
1. no add (?) 6
2. 10^2 (0.1 ml) 41 35
3. 10^3 (0.1 ml) 3

rpt.

$35 \times 10 \times 10^2 = 3.5 \times 10^4$

/2279 10^2 (0.1 ml) 0 plaques
5 pap.

DATE: 4/28/55 REF:

2580 for checking on diploidy of other markers after crossing over to give aelotypic HFT gal(-)

1. Origin of duchess in 335
2. a second plate made see p 335
3. to aid in understanding phenotype of this culture on arabinose comparison made between K12 +

341C - control
341/4
341/2

W945 (94° stock) mt. −
W945 (stock culture) mt −
W2580 untreated −
} turning dark after 2 days

4. 17 easy clones obtained. Spotted on F11 and 2580 − tried.

Previously diploids for peculiar phenotype [illegible] 2580

Summary

Ⓐ F11 o o ⊚ o / o o o o
341-9 → ⊕ o o o 1 HFT 2−
© o © © / o
Ⓑ 2580 o o ⊚ o o / o o o o / © o ©©
ⓞ = HFT
© = Contaminated
O = NFT

Ⓑ F11 o o o o / o o o o / o o o ⊕ / o o o o
some of o have several pap. 1 HFT 2− 341-12 (thrown out before tested)
B 2580 o / o o ⊚ o / o o o o / o o o o / o

Ⓒ o o o o / o o o / o o o / o o o o } most of these have c. 20 papillae WHY?

TESTS OF REVERSIONS

341-9 reverted on
B gal 4/6 reversion +
B lac 6/6 reversion std
B xyl 6/6 " "
B ara 6/6 " "

2/17/56
LFT seg of 341-9
8/5 [illegible]
5/5 gal+ of LFT std

2062 - see 335 for duchess.

1. check of HFT derivatives for diploidy for prototrophs
2. 5 separate hand. picked - segregants tested

Segregants from hand # → 1, 2, 3, 4, 5 (each /F11 /2580)

All NFT, spotting on 2580 show 10-20 pap. Is this an indication they are 4−?

Expt. 1

as above

Expt. 2.

DATE: 4/30/55 REF: (342)

1. Are there position effects between 4, 6, 7?
2. The directions.

A. Overnight broth culture diluted 1-100 ĉ pen. 0.1 ml cells + 0.1 ml lysate → adsorb 10' at ~~[illegible]~~
Dilute 1-100, 0.5 + 10 ml, and plate 0.7 ml on 2 B gal plates.

(B) lysates of 2478-1 = 4
311-2 = 6
309-1 = 7

(3). → x Gal₇⁻ 2308?

		(–) col	36 hrs (+) "pap col."		Phenotype
	A. broth	302, 260	0		
HFT 4⁻	B. 2478-1 λ	192, 198	6, 5	none of these (+) were + in 24 hrs.	The phenotype here may be weak (+) 342-A1 → X
HFT 6⁻	C. 311-2 λ	254, 169	5, 4		papillated (–) ?* 342 B1 → * 4

(4) → x Gal₆⁻ 2070

A.	Broth	483, 431	0	
B.	HFT 4⁻	256, 251	4, 2	pap (–) ? * 342C1 → * C4
C.	HFT 7⁻	283, 332	3, 3	pap (–) ? * 342D1 → * D3

(5) → x Gal₄⁻ 518

A.	Broth	108, 124	0	
B.	HFT 6⁻	199, 99	2, 1	pap (–) ? * 342E1 – * E2
C.	HFT 7⁻	85, 105	0, 1	untransformed (+)? 342F1 –

* on 2nd streak gave papillating colonies.

DATE: 5/11/55 REF:

Hfr 6⁻ × 7⁻ (342B #1) Of 24 presumed P.E. (−) colonies picked to obtain separate crossover events 3 were true (−), 21 were P.E. (−)

P.E. (−) #	+ obt. seg?	(−)	Segregant 6⁻	7⁻	1+				
1	0 = no								
(Streaked λ_p plate code) 2	0	−							This clone
3	0	−							
4	0	−							$\lambda p^{N}/s$
5	0	−							
6	+ = yes	3	0	+	+	λp^s	Gal 6⁻	← 343-1	Use λp^t.
7	0	−							
8	+	2	0	+	+	λp^R	Gal₆⁻	← 343-8	
9	+	2	+	0	+	λp^s	Gal₇⁻		
10	0								
11	0								1/5/56
12	+	1	+	0	+	λp^s	Gal₇⁻		
13	0	−							W3058
14	0	−							
15	+	1	0	0	+	λp^s	Gal₆-Gal₇⁻	← 343-15	
16	0	−							
17	0								
18	0								
19	0								
20	0								
21.	+	3	0	+	+	λp^s	Gal₆⁻		

6 seg (slight) — 3 Gal₆⁻, 2 Gal₇⁻, 1 Gal₆-Gal₇⁻

15 non seg.

Rpt 342B4

#	+ obt seg	#/Stk				Stk II (5/27/55 to rest, Seg? streaked)	Stk III			5/29/55
1.	0	−				−	−			After Stk III, spotted for
2.	0	−				−	−			both on 275 on 5Gal
3	0	−				−	−			and incub. 14/16
4	0	−				−	−			appear Ab⁻ on 275 (some papillae)
5	+	1	0	+	+	+	nd	Gal₆⁻		
6	0	−				+	nd	16 − 17 − 18 +	← Gal 6-7⁻	
7.	+	5	0	0	+	+	nd	Gal₆-Gal₇⁻		
8	0	−				−	−			
9	0	−				−	−			
10	+	1	0	+	+	+	nd	Gal₆⁻		
11.	+	750	0	+	+	+	nd	Gal₆⁻		
12	0	−				−	−			
13	0	−				−	−			
14	0	−				−	−			
15	0	−				−	−			
16	0	−				−	−			
17	0	−				−	−			
18	0	−				+	nd	16 0, 17 +, 18 +	Gal₆⁻	
19	0	−				−	−			
20	0	−				−	−			
21	0	−				−	−			
	4 seg					6 seg	6	4 6⁻		
	17 non seg					15 nonseg	15	2 6-7⁻		

DATE: 5/11/55 REF:

HFT 7⁻ —x 6⁻ 348 D – Of 44 P.E. (–) picked for (+), 4 were true (–), 20 P.E. (–)

Procedure as in 343

	(+) At. seg?	no.	/6	Segregant /7	/4		
P.E(–) #1	0	–	+	+	+	–	–
2	+	7	0 ✓	+ ✓	+ ✓		6⁻
3	+	10	~~0~~	~~+~~	~~+~~		lost
4	+	3	0 ✓	0 ✓	+ ✓		6-7⁻
5	0	–				– ~~[illegible]~~	–
6	+	12	0 ✓	0 ✓	+ ✓	~~[illegible]~~	6-7⁻ ← 344-6
7	+	7	0	+	+	~~[illegible]~~?	6⁻
8	+	4				P.E. (–)?	
9	0	–					–
10	+	4				P.E. (–)?	
11	0	–					–
12	+	9	+	+	+	P.E (–)?	
13	0	–					–
14	0	–					–
15	0	–					–
16	+	4	0	+	+		6⁻
17	+	5	0	+	+		6⁻
18	+	730	0	+	+		6⁻
19	+	750					
20	+	10				P.E (–)?	

13 seg – one lost
7 nonseg.

5 6⁻
2 6-7⁻
4 PE (–)?

344B = [illegible] ±–– = (+) / 2⁻ ++

(A) 344-6 above, the 6-7⁻, × K12λ 7/27/54
no. add = 0
o.line λ = 44 see p. 325 for previous use of this lysate
many small pap.

Seg.	/6⁻	/7⁻	/4	Genotype
1	0	0	+	6-7⁻
2	0	0	+	"
3	0	0	+	"
4	0	0	+	"
5	0	0	+	"
6	0	0	+	"
7	0	0	+	"
8	0	0	+	–
9	0	0	+	"
10	0	0	+	"
11	0	0	+	"
12	0	0	+	"
13	0	0	+	"
14	0	0	+	–
15	0	0	+	"

(B) 344-6 —× 2580
no. add = 10
o.line = 215

Segregants	/2	/6	/7	Gen
1	0	+	+	2⁻
2	0	+	+	2⁻
3	0	+	+	2⁻
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⁻
344-13 → 13	+	0	0	6-7⁻
14	0	+	+	2⁻
15	0	+	+	2⁻
16	NA+	0	0	6-7-2⁻?
17	0	+	+	2⁻
18	0 0	0 +A	0 0	2-7⁻ [illegible]
19	0	+	+	2⁻
20	0	+	+	2⁻
21	0	+	+	2⁻

nonseg. 18
344 [illegible]

18 2⁻
2 6-7⁻
1 2-7⁻ A

DATE: 5/21/55 REF:

HFT 4⁻ → ×6⁻ 24 P.E. (-) picked segregants, 7 were true (-) 17 P.E. (-). to m 344,343

342C1

	(fert. [illegible]?)	no. cy.	/4	/6	/+
P.E.(-) #1.	+ eyes	13	+	o	+
2.	+	5	+	o	+
3.	+	6	+	o	+
4.	+	12	+	o	+
~~5.~~	~~+~~	~~10~~			
5 6.	o	–			
6 7.	+	3	+	o	+
7 8.	+	24	+	o	+
8 9.	+	13	+	o	+
9 10.	+	11	+	o	+
10 11.	o	–			
11 12.	+	14	+	o	+
12 13.	+	28	+	o	+
13 14.	+	11	+	o	+
14 15.	+	6	+	o	+
15 16.	+	9	+	o	+
16 17.	+	6	+	o	+
17.	o	–			
	14 sex				14 6⁻
	3 nonsex.				

DATE: 5/11/55 REF:

342E1

HFT 6 — x 4 — 24 P.E.(−) picked, 1 real(−), 27 C.E.(−). as in 345, 44, 43.

	+obt. seg?	no.	Seg. /4	/6	/+			
P.E.(−) #1	0 = no	−						
2	0	−						
3	0	−						
4	0	−						
5	+ = yes	9	−	−	−	P.E.(−) ?		
6	0	−						
7	0	−						
8	0	−						
9	0	−						
10	+	3	0	+	+	Ly^s	Gal4	
11	0	−						
12	0	−						
13	+	1	+	0	+	Ly^s	Gal6 −	[illegible]
14	+	1	+	0	+	Ly^s	Gal6 −	
15	0	−						
16	0	−						
17	0	−						
18	+	1	0	+	+	Ly^s	Gal4 −	
19	+	2	0	+	+	Ly^R	Gal4 −	← 346-19
20	0	−						
21	0	−						
22	0	−						
23	0	−						
	6 seg / 17 non seg.		strk II	strk III				

Lyt. Syt. using culture met in Lyt

This is Ly^R/S. Use Lyt

3 Idn — 2 Ly^s, 1 Ly^R

2 Gal4

SEE 347

342E2 II — Picked to ... [illegible] tested 5/26/55

	+obt. seg	no./strk	Seg.			/4	/6	/+			
1	0	−				+	0	+		~Gal6 − self agglut.?	
#2	+	1	+		nd					Gal6 −	
3	+	7	−	0	nd		+		+	Gal4 −	I
4	+	1	−	0	nd		0		+	Gal4-Gal6 −	II
#5	0	−	+		nd	−	−	+		Gal4-Gal6 −	
6	0	−	+		nd	−	+	+		Gal4 −	
√7	0	−	−		−						
8	+	8	−	0	nd		+		+	Gal4 −	I
9	0	−	+		nd	−	+	+		Gal4 −	
10	+	1	−	+	nd		0		+	Gal6 −	I
11	+	1	+	0	nd		+		+	Gal4 −	I
12	0	−	+		nd	+	−	+		Gal6 −	
13	0	−	+		nd	+	−	+		Gal6 −	
√14	0	−	−		−						
√15	0	−	−		−						
√16	0	−	−		−						
√17	0	−	−		−						
18	0	−	+		nd	−	−	+		Gal4-Gal6 −	
√19	0	−	−		+	+	−	+		Gal6 −	
20	0	−	+		nd	+	−	+		Gal6 −	
21	0	−	−		−						
22	0	−	−		−						
23	0	−	−		+	+	−	+		Gal6 −	
	17 non seg / 6 seg		↑ 13 Total dis seg.		16					8 Gal6 − / 5 Gal4 − / ? Gal4-Gal6 −	

all apparently stable + were picked and spotted on W2589 (gal2 − Mal+ S^R) on B gal staphlymyci, mod. 20 sec. incub. 2 days. No. papillae at spots observed — indicating NFT

Won't mature so?

W2589 found Gal − Mal −, λ-? Resist.

5/19/55. After streaking III, remaining 7 cul (from broths) were spotted on W2175 on 5 Gal + mod. 20 sec. After 2 days all show papillae - suggesting HFT

W3163
346-4 re ✓
5/16/56 found 4-6-7
← 346-4

More results 346-4 × 346-7

5/27/55
Lysate of 346-4 × 2580
no add. : 14
0.1 ml : 71

Seg.	/4 −	/6 −	/+	Gen
1	0	0	+	4-6-
2	0	0	+	"
3	0	0	+	"
4	0	0	+	"
5	0	0	+	"
6	0	0	+	"
7-16	0	0	+	"
16	all 4-6-			

DATE: 5/25/55 REF:

1. Continuation of 346.

A. 346-4 × — K12 7/27/54 $\frac{++}{--}$ = + ?

[illegible] = 0

0.1 ml = 46 of 344-6

B. results on P. 346 under 346-4 —× 2580

C. 346-4 —× 2580

1. 1st 24 segregants were 2⁻

2. Rpt. streaking of above yielded 16(−) = 15 gal_2^-, 1 gal_6^- } is this a culture? [lysate of a gal_6^-]

3. Rpt. " " " " 15(−) = 13 gal_2^-, 1 gal_6^-

See 418 4. Rpt experiment using same lysate and applying to 2580 20− 19 gal_2^-, 1 gal_6^-

Retest 346-4

See 346 for retest

Note, this 6⁻ requires 2+ days to be [illegible], have the tests of 346-4 been allowed to go long enough?

DATE: 6/6/55 REF:

285-1 Rpt clone of 4⁻ x 1⁻
Cells removed from stab, streaked to obtain P.E. (−), restreaked to give 24 P.E. (−), from these 24 Gal+ obtained

Gal+	Seg.? (+)/abh		/1	/4		
1	0					
2	0					
3	0					
4	+	2	0	+	.1⁻	
5	+	5	0	+	1⁻	
6	0					
7	+	8	0	+	1⁻	
8	0					
9	0					
10	0					
11	+	6	0	+	1⁻	
12	+	10	0	+	1⁻	
13	+	5	0	+	1⁻	
14	+	3	0	+	1⁻	
15	0					
16	+	1	0	+	1⁻	
17	+	5	dark	dark	?	P.E. (−) and Gal +
18	+	5	0	+	1⁻	
19	+	+	0	+	1⁻	
20	0		0	+	1⁻	
21	+	2				
22	0					
23	0					
24	0					
	+ = yes 0 = no				11 1⁻	
	12 stable 12 seg.					

DATE: 6/13/55 REF:

1. Do mixtures of the Gal- ferment galactose?
NSB + 1.0% Gal + fermentation vials

			Reaction after 2 days	
1.	W 750	Gal₁ -	small bubble	Apparently no fermentation in mixtures by two's
2.	W 811	Gal₄ -	no bubble	
3.	W 2580	Gal₂ -	small bubble	
4.	W750 + W2580	1 + 2 -	small bubble	
5.	W750 + W811	1 + 4 -	small bubble	
6.	W811 + W2580	4 + 2 -	no bubble	

2. Tested by Iro
293-12-5 - Gal₄ - HFT. Found equivalent or better than 247B-1

DATE: 6/20/55 REF:

1. Testing for segregation of λp in isolated heterogenotes.
Also effect of temperature shift on frequency of transduction.

A. Overnight cultures 2279, 2790, 518 at 37C in incubator.
1. Cultures divided and portion placed at rm temp for ca. half hour.
RM temp cells; 37C cells.

B. 1. Lysates
Gal_2^- λ for 2279 518 = 2342 3/20/54
Gal_1^- λ for 2790 = 2346λ
2. Lysates 0.4ml ~~plated~~ in small tubes, caps off incubated at 37C for half hour.

C. Technique.

0.4ml lysate + 0.1ml cells ← overnight cult { 0.1+10 for 2790 2279 ; 0.5+10 for 518 }

↓ incubated 37C 10 min → diluted 0.1+10, 1.0+10 → 0.1ml plated

Control counts: 0.1+10, 0.1+10, → 0.1ml plated

Exptl counts

37C Culture	Plate 1	Plate 2	3	
W518	1/443	3/460	0/428	← these col. also plaqued.
W2790	1/460	1/372	1/422	← also contain with plaques
W2279	8/173	1/166	5/142	← Just about every colony has plaques - multiplicity too high

Control Counts.

Cultures	RM temp		37C	
W518 =	112, 103	(1.1×10^7)	119, 130	(1.2×10^7)
W2790	35, 42	(3.9×10^6)	173, 184	(1.8×10^7)
W2279	180, 144.	(1.6×10^7)	338, 290	(3.1×10^7)

No gal+ on these plates

RM temp. cult.	Plate 1	Plate 2	3	
W518	2/ca. 50	3	4	← these plates have contaminant - no count of total made
W2790	3/263	1/292	0/262	
W2279	1/55	3/56	0/50	← these colonies all are plaqued. mult. too high

The multiplicities in this experiment were too high to be used for the purpose of testing λp seg.

DATE: 6/23/55 REF:

1. Observations on segregation rate ..

A. Culture : 348 idio type
B. Streaked out and single pure col. inoculated into Pen - assay overnight
C. Diluted $10^2 - 10^4 - 10^6 - 10^8$

10 . 0.3 ml samples incubated 37C 8:30 AM → 1:00 PM

Plated 0.05 ml of

1.	361
2.	61
3.	219
4.	122
5.	855
6.	1279
7.	441
8.	676
9.	319
10.	336

Below 60 min lag. div every 30 min, from 9:30 AM - 11:30 / 12:00
2^5 - leave until 1:00 PM
$2^8 = (256X)$

5 0.1 ml samples plated B gal

1.	100
2.	108
3.	134
4.	136
5.	108
	586 / 5 = 117

Therefore ave. of 1.2 cells/0.1 ml ↑ = 3.6 cells/tube

1.05 ml

0.6 cell

6/24/55

2. Repeat experiment using 780tKR-1
A. Overnight culture from stock.
B. diluted $10^2 - 10^4 - 10^6 - 10^8$

5 0.1 ml samples incubated in horizontal 0.2 ml pipette at 37C. 9:15 AM
20 in number
Plated (and mixed in broth) 1:45 PM

Assay 0.05 ml

1	98, (2-)
2.	112, (5-)
3.	106, (4-)
ave	106 (4-)

212 (B-) / 100 = 2 cell / 0.1 inoculum, 0.08 Gen-

(1)

Samples incubated in pipettes	(−)	total
1	*	*
2	6	1169
3	3	595
4	4	251
5	0	237
6	*	*
7	23	1252
8	* 103	* 2730
9	319	1622
10	↓	↓
11		
12		
13		
14	15	¼ = 141
15	9	1113
16	all (−)	
17	22	1966
18	15 (19)	¼ = 479
19	est. 100	est. 2000-3000
20	19	897

*. These platings have ca. 3000 cells

The no. (−) appears to vary from ca 10-200

(2) At the same time 5 0.1 ml ~~pipettes~~ samples plated in drop form on B gal, respread after platings ca. 2:15 PM by adding a little broth & spreading

	(−)	(+)	
1.	0	89	2 seg. colonies
2.	0	52	2 seg. colonies
3.	7	1295	
4.	22	1222	
5.	52	730	

Calculations on next page - also rates -

351A

$a = 0.602\,(\text{no. mutants}) / N \log N$

DATE: REF:

A. Incubation in pipettes

No. (–)	Total cells	N log N	0.602 R	5/3	Recalculated
6	1169	1169 (3.06781) = 3580	3.612	1.0×10^{-3}	1.0×10^{-3}
3	595	595 (2.77452) = 1650	1.806	1.1×10^{-3}	1.1×10^{-3}
4	251	251 (2.39967) = 600	2.408	4.0×10^{-3}	4.0×10^{-3}
23	1252	1252 (3.09760) = 3880	13.846	3.6×10^{-3}	3.6×10^{-3}
9	1113	1113 (3.05308) = ~~3400~~ 3391	~~5.418~~ 5.418	~~1.1×10^{-3}~~	✓ 1.6×10^{-3}
19	897	897 (2.95279) = 2650	11.438	4.5×10^{-3}	4.3×10^{-3}
0	237	— —	—	—	

B. Respreading

No. (–)	Total cells	N log N	0.602 R	5/3	Recalculated
7	1295	1295 (3.11227) = 4030	4.214	1.0×10^{-3}	
22	1222	1222 (3.08707) = 3780	13.244	3.5×10^{-3}	
52	730	730 (2.86332) = 2085	31.304	1.5×10^{-2}	
0	89				
0	52				
103	2750	2750 (3.439) = 9430	62.00	6.6×10^{-3}	6.6×10^{-3}
319	1622	1622 (3.21005) = 5200	~~192~~ 192.	3.7×10^{-3}	✓ 3.6×10^{-2}
22	1966	1966 (3.29358) = 6460	13.2	2.0×10^{-3}	2.3×10^{-3}

$\frac{32.0}{9} = 3.5 \times 10^{-3}$

Estimated P_0.

using 200: $a = \frac{2.3}{200} \log \frac{1}{\frac{1}{12}} = (0.012)(\log 12) = (0.012)(1.08) = 1.3 \times 10^{-2}$

using 1024: $a = \frac{2.3}{1024} \log \frac{1}{\frac{1}{10}} = \frac{2.3}{1024} \log 10 = \frac{2.3}{1024}(1) = 2.2 \times 10^{-3}$

DATE: 6/22/55 REF:

1	2	3	4	5	6	7	8	9	10

Yield of HFT λ from heterogenote

1. Culture 241-14 (λ/λ) - streaked from stock and 10 colonies picked, tested for HFT against 2279 - Colonies stored Pen assay frig

1 ○ ○ ○ ○ ○
○ ○ ○ ○ ○

2. Reaction not apparent 24 hours - select most likely appearing col and proceed. Broths incubated 24 hours - Estimated titer 10^9

3. Exptl.
   a. dilute 1.0 ml pen to Blanc ca. 1000 col/ml in Saline. Irrad 35 seconds and plate 60B gal with 750 col. - Make pre irrad. count
   b. Inc. 2 hours - respread 3 plates

4. Results:

Pre irrad count/0.1 ml = 67, 50, 46 } ave. 53

Post irrad plaques/2279

			Cap.	PAP wi plaque	352A 6 ½ hrs
0.1 ml:	22	ave 16	5	3	(1) 3/5 +ʰ
	12		8	4	(2) 5/8 +ʰ
	14		5	2	(3) 2/5 +ʰ

Post irrad. pap /737

				352B stk 1
NOT SPREAD	(1)	2	ave 3	1 0/2 +ʰ
	(2)	5		2 1/5 +ʰ
	(3)	2		3 1/2 +ʰ

				352C (stk 1)
	(1)	3	ave 2	(1) 3/3 stkd
SPREAD	(2)	2		(2) 1/2 stkd ←
after 2 hours	(3)	3		(3) 2/3 stkd, 1(-)

DATE: 6/22/55 REF:

Low multiplicity infection, - does λ segregate?

1. Culture - 2279 overnight

λ = 241-14 (10/12/54) titer 5.3 X 10^8 plaques / 1.3 X 10^8 trans'd } see p 300

ⓐ 0.4 ml 2279 + 0.1 ml HFT 2^-
" " 0.1 ml broth }

ⓑ incubate 37C 10 min.

ⓒ dilute 10^2, 10^4, 10^6 → plate 0.05 ml

λ - 6 plates

① control 3 plates — 53, 47, 49 on Gal - 50 X 20 X 10^6 = 1.0 X 10^9

② Expt'l plates

(+)	total
1	66
0	54
0	40
0	41
0	54
0	56
①	311

} no (-) found contam. with λ or part. lysed.

tested against 2279 and found not lysogenic

353-1

Rpt.

1. Culture = 2344 JL's δ stock Gal₂⁻ Ly⁵ Hfr
taken from his stock culture labelled δ

2. Lysate = 750HFR-1 1/28/55 titer = trnsd. titer = 2.6 X 10^7 see 315

3. Expt 0.1 ml lysate + 0.1 ml cells inc. 37C 15' - 10 ml Pen added, diluted 10^2, 10^4 → 0.1 ml

4. Results -

A. control plating - exposure to broth

Plate 1. 237
2. 326
3. 283 } 282 X 10 X 10^4 X $\frac{10^2}{2}$ = 1.41 X 10^9 cell/ml overnight cult.

B. Lysate exposed

	+ *	(-)	(-) partially lysed	total
1.	3	92	0	95
2.	2	84	4	90
3.	0	76	1	77
4.	3	71	4	78
5.	1	61	0	62
6.	0	94	1	95
7.	0	96	2	98
	9		12	595

750 HFR-1 5.6 X 10^7 from Pomona titer estimated

* the + appear to be in two categories: (1) Appearing ca. 18 hours and represented by intact (not lysed) colonies that are almost all (+) (2) appearing in (-) colonies, λ contaminated and partially lysed, after ca. 24 hours. In the above there were 5 were early, 3 late.
This correlation was noted.

Mutation	Lysis of 1st	Fraction Gal+
early	2/5	predom. (+)
late	3/3	" (-)

} are trnsd. not mutant - total = 8

C. Plates = primary stocks of (+) given to EML.
\# 1-5 early - 2/5 ly⁺ as from
6-8 late - 3/3 ↑

EML 900
give letters A-H

DATE: 6/30/55 REF:

Crossover rate in P.C. heterozygote
34rE2 6 → 4
Exptl as in 357 with 750CKR-1

1. Overnight culture from single colony
2. Dilute $10^2 - 10^4 - 10^6 - 10^8$ → 20 0.1ml samples

(incubated 9:30 → 2:30)

3 plates for estimate of ratio

	(+)	total cells/0.1ml	
1.	4	67	(8 bal-)
2.	4	78	(7 bal-)
3.	6	78	(8 bal-)
		ave 71	= 0.71/0.1ml

Note all 20-30% low because log values used

	(+)	total	Max Prob
1.	0	0	
2.	0	1406	1.3×10^{-4}
3	0	ca. 3000	6.0×10^{-5}
4	0	1/8 = 193 = 1544	—
5	0	1/16 = 193 3088	6.0×10^{-5}
6	0 contaminated		—
7	ca 1000	ca 3000	—
8	0	ca. 150	—
9	4	1/8 = 643 5144	ca. #3 1.3×10^{-4}
10	0	ca 4000	ca. #3 4.1×10^{-5}
11	2	1/8 = 841 6728	5.3×10^{-5}
12	5	2266	4.0×10^{-4}
13	0	0	—
14	1	1/16 = 370 5920	3.0×10^{-5}
15	0	ca. 7000	2.5×10^{-5}
16	ca 1000	ca 2000	—
17	0	0	—
18	0	2245	-
19	0	748	-
20	0	34	—
	total	29128/10 = (2913)	

Using null method and N = 5900 (from culture #4)

~~$a = \frac{2.3}{5900} \log \frac{1}{12/18}$~~

~~$a = (0.00039)(0.176) = 7.0\times10^{-5}$~~

$a = \frac{2.3}{2.9\times10^3}\ 0.176 = \frac{0.405}{2.9\times10^3} = \frac{4.05\times10^{-4}}{2.9} = 1.4\times10^{-4}$

B Reciprocal 34rC1 As above - except stab picked to dilute per and this grown overnight

10:30 AM
1:45 PM

		(+)	total
Control	1.	0	577
	2	2	595
	3	0	556
			583 = 5.8/0.1ml

	(+)	total	cells	
1.	2	728	276 (100%)	4.0×10^{-4}
2	0	818	62 (810)	
3	0	1/16 = 110	660	
4	0	1/8 = 86	688	
5	0	1/8 = 80	640	
6	0	1/8 = 49	392	
7	0	1/8 = 80	640	
8	0	1/8 = 91	728	
9	0	1/8 = 83	664	
10	0	1/8 = 149	1192	
11	0	1/8 = 62	496	
12	0	1/8 = 83	664	
13	0	1/8 = 137	1096	
14	0	1/8 = 74	592	
15	0	1/8 = 90	720	
16	0	1/8 = 152	1216	
17	1	185	185	
18	0	1/8 = 87	696	
19	1	1249	1.4×10^{-4}	
20	0	1/8 = 62	496	
		20 | 15588	1559/2	(779)

Using null method, and ~~N = 1200~~ (AA)

~~$a = \frac{2.3}{1200} \log \frac{1}{17/20} = (1.9\times10^{-3})(0.13) = 2.6\times10^{-4}$~~

$a = \frac{2.3}{779} \log \frac{1}{17/20} = \frac{2.3}{7.79\times10^2} \log 1.2$

$= 2.4\times10^{-4}$

(353)

DATE: 6/30/53

REF:

1	2	3	4	5	6	7	8	9	10

1924 ← 2346 λ to look at single heterozygotes

1 0.1 cc overnight culture + 10 ml

0.1 cc + 0.1 cc 2346 λ 11/10/54

↓

incubated 37C - for 15'

↓

add 10 cc Pen → dil 10^2, 10^4 → 0.1 ml samples

2. Back - control

2346 λ 11/10/54 - Expll.

DATE: 7/6/55 REF:

Segregation from w2730

1. Expt as before - see page. 357

2. Overnight cult

$10^{2}-10^{4}-10^{3}-10^{8}$ → 20. 0.1ml sample.

0.1ml	(+)	total
1.	17	122
2.	19	168
3.	21	151
	19	$\frac{441}{3}$ = 147

= 1.5 cells/0.1ml

28/19

Using null fraction

$$a = \frac{2.3}{100}\left(\log \frac{1}{p_0}\right) = \frac{2.3}{100}\left(\log \frac{1}{0.5}\right) = 0.012$$

$= 1.2 \times 10^{-2}$

Estimated clone size → 40, 80, 200, 150, 40, 30, 150, 100, 30

ave at. 100

	(−)	total	
1.	all	323	8.1×10^{-3}
2.	2	176	3.0×10^{-3}
3.	0	0	—
4.	8	1669	8.9×10^{-4}
5.	3	317	2.3×10^{-3}
6.	52	1236	8.1×10^{-3}
7.	0	10	—
8.	0	0	—
9.	ca. 300	ca. 700	? —
10.	36	1055	6.6×10^{-3}
11.	0	0	—
12.	0	0	—
13.	3	299	2.4×10^{-3}
14.	0	0	—
15.	0	0	—
16.	6	386	3.6×10^{-3}
17.	0	0	—
18.	0	0	—
19.	55	1965	5.1×10^{-3}
20.	0	0	

$\frac{46.2}{9} = 5.1$

8 failed to grow
1 contained only Gal+
10 contained both Gal+ & Gal−

DATE: 7/13/55 REF:

1. Rpt 2344M1 (SC stock) fm X HFT +
lysate = 293-125 –

2. 0.3 ml lysate + 0.3 ml overnight cult.
↓
add 10 ml broth
↓
$1\text{-}10^2\ 10^4$
↓
0.1 ml

broth as control.

Plates bad fro wet.

3. Result. A. Control. $4/10 \cdot 10^? \cdot 10^2 \cdot 10 = 6 \times 10^7$

1. 457
2. 540 5.0×10^9 ?) av(+)
3. wet plate

B. Exptl.

	(+)	total (−)	contam. (−)	Decay
1.	2	322	2	⊕2 ●1
	1	ca 300	0	⊕3

Rest of plates have been in cold count—(−5) about the same as above – after 2 days some (+) appeared – may be reversion – This culture seems to revert more than other Gal₂ –

DATE: 8/10/55 REF:

Penassay broth Test tubes – Blank = water – PC-4 filter

λ	OD	Aristol Gel(?) ca. 10^9/ml)
650	0.051	0.74
600	0.071	0.81
550	0.11	0.90
500	0.19	1.04
450	0.365	1.23
400	0.630	1.45

Lysates	1210	K12	2236
10:50AM 0	0.58	0.73	0.80
1:10 PM 2:20	0.75	0.52	0.80
1:25PM 2:35	0.69	0.44	0.64
1:45PM 2:55	0.67	0.40	0.53
	0.67	0.40	0.48

$\frac{0.2}{20.0} \times 5$ 0.05% RNA

9/25/55.

750EK12-1 grown on B gal + 0.05% RNA

1. no RNA 5 gal – + ca 10 sg + ca 60 gal +
2. RNA 6 gal – + ca " + ca 70 gal +

} No effect of RNA at this conc

1210 λ – ×750 in search of HFT λ

no odd 2
0.1ml 18 per lysate

HFT test	No. tests	against?	HFT ?	
I	10	750, 2341	possibly one/2341 - lysate made 358-1	] none HFT - reversion of [illegible]
2.	10	750, 1210	possibly one/750 358-2	both made - [illegible]

2279 + 750 λ ← K12 8/10/55

1. 750 0.1ml: 142×4: 568
2. 2279 0.1ml: 426

→ Rather low titer in lys. – This lysate has been at room temp since it was made

no Control

DATE: 8/17/55 REF:

1	UV	3	4	309-1 = (HFT?)	6	7	8	9	10

Assay of irradiated HFT lysate - Irradiated suspension from agar - Preliminary assay.

(359A) ← ← confirmed at later

UV Dose	1-100 dil (0.1?) /750		0.05 /2307	0.05 ml 10^4 2580	0.05 ml 10^4 2790	0.1 ml /2790 purified 2790
0 min	21	0	13	257	37 ca. 500+	196
1 ..	273	252	14	729	190 ca 500	297
2 ..	517	496	6		227 584	994 ca 1000
3 ..	593	572	6		341 436	
4 ..	885	864	10		413 (1st plate) 234	
5 ..	950	929	11		175 16	
6 ..	809	888	11		148 7	

750 spontaneous + = $ frequency ← 1.6×10^7

spont = 8 sp = 22 sp 14 sp. 17

← RPT = 359/2

← at max. 413×20×10^5 = 8.2×10^8 (?)

these with premarked plates

See 359A →

1-100 dil too concentrated on 2790 - dil at least 10^2 more

359A test λ test rev against 2279 →

λ /2790 ; + = lysogen; 0 = non-lys.

ss seus / rs rand (?)

ns = not segregating

	Spont (+) λ	/2790	0 λ	/2790	1 λ	/2790	2 λ	/2790	3 λ	/2279	4	/2279	5 λ	/2279	6 λ	/2279
1.	s ns	0	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
2.	s	0	r s	0	r? ns	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
3	s	0	r s	0	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
4	s	0	s ns	0	r ns	r+	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
5	s	0	s ns	0	r s	r+	s ns	0	s ns	0	r ns	6	s ns	0	s ns	0
6	s	0	r s	0	r s	0	s ns	0	s ns	6	r ns	+	s ns	0	s ns	0
7	s	0	r s	0	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
8	s	0	r s	r+	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
9	s	0	s ns	0	r s	0	s ns	0	r ns	0	s ns	0	s ns	0	s ns	0
10	s	0	r s	0	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
11	s	0	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
12	s	0	s ns	0	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
13			s ns	0	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
14			s ns	0	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
15			r s	0	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
16			s ns	0	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
17			r s	r+	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
18			r s	r+	r s	0	s ns	0	s ns	0	r s	0	s ns	0	s ns	0
19			s ns	0	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
20			r s	0	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
21			r s	0	r s	0	s ns	0	s ns	0	r s	0	s ns	0	s ns	0
22			r s?	0	r s	0	s ns	0	r s	0	s ns	0	s ns	0	s ns	0
23			s ns	0	s ns	0	r s	0	s ns	0	s ns	0	s ns	0	s ns	0
24			r s	0	r s	0	—		s ns	0	s ns	0	s ns	0	s ns	0

Spont	0	1	2
12/12 lp^s	9 lp^s	3 lp^s	22 lp^s
	3 lp^+	2 lp^+	1 lp^r
	12 lp^r	19 lp^r	

↑ The surviving growth from 16 of these streaks, streaked on B sol - found pure Gal+ - adding λ does not restore stability

Spont	0	1	2	3	4	5	6
	-5.6 lp^s	-1.7 lp^s	-1.44 lp^s				
12 ns	9 lp^s ns	3 lp^s ns	22 lp^s ns	23 ns - 22 lp^s 1 lp^r	22 ns - 20 lp^s 1 lp^+ 1 lp^r	24 ns	24 ns
	3 lp^+ s	1 lp^+ ns	1 lp^r [illegible]	1 s lp^r	2 s lp^r		
	12 lp^r s	1 lp^r ns					
		1 lp^+ s					
		18 lp^r s					

2915 used for ψ rx

359A

DATE: 9/28/55 REF:

Rpt. of 359 - 2' dose / 2790 48 papillae picked streaked out single col tested Δ 1229 streaked out on B gal for stability. This dose taken because less likelihood of contam. from nearby plaques - Early doses require more dilution to avoid

r = lys, o = non lys

Rev. seg. second streak		1 Dose 2' (359A2') /2279	2 Gal-neg Δ	3 Pltes 359A0 /3748	4 359A1' /3915
ws	1	s w o		r s o	r s o
s	2	r s o	Lp^s	r s +	r s +
ws	3	s w o		r s o	r s o
ws	4	s ws o		r w +	r s o
ws	5	s ws o		r s o	r s o
s	16	r s o	Lp^s	r s o	r s o
w	7	s ws o		r s o	r s o
s	8	r s o	Lp^r	r s o	r s o
ws	9	s w o plq		r s +	r ws o
ws	10	s ws o		r s o	r s +
ws	11	s ws o		r s o	r s o
ws	12	s ws o		r s o	r s o
ws	13	s ws o		r s o	r s o
ws	14	s ws o		r s +	r s o
ws	15	s ws o		r s o	r s o
—	16	no growth		r s o	r s o
ws	17	s ws o		r s o	r s o
ws	18	s ws o		r s o	r s o
ws	19	s ws o		r s o	r s o
ws	20	s ws o		r s o	r s o
s	21	r s o	Lp^s	r s o	r s o
ws	22	s ws o		r s +	r s o
ws	23	s ws o		r s o	r s o
ws	24	s ws o		r s o	r s o
s	25	r s o	Lp^s	r s o	r s o
ws	26	s ws o		r s o	r s o
ws	27	s ws o		r s o	r s o
ws	28	s ws o		r s o	r s o
s	29	r s o	Lp^s	r s +	r s o
ws	30	s ws o		r s o	r s o
ws	31	s ws o		r s o	r s o
ws	32	s ws o		r s o	r s o
ws	33	s ws o		r ws +	r s o
ws	34	s ws o		r s +	r s o
s	35	r s o	Lp^s	r s o	— —
ws	36	s ws o		r s o	— —
ws	37	s ws o			
ws	38	s ws o		8 Lp^+	2 Lp^+
ws	39	s ws o		78 Lp^r	32 Lp^r
ws	40	s ws o			
ws	41	s ws o		2 ws Lp^+	33 s Lp^{+r}
ws	42	s ws o		34 s Lp^{+r}	1 ws Lp^r
ws	43	r s o	Lp^s		
s	44	r s o	Lp^s		
ws	45	s ws o			
ws	46	s ws o			
ws	47	s ws o			
ws	48	s ws o			

total: 10 Lp^r s, 60 Lp^s ws

Lp^+ = 0
Lp^r s = 9
Lp^s w = 38

Recross 10/3/55
0.1 ml 10^4 dil / 2145

	c	= 25	ca. 5pl : 1t
359A0	0	= 355	
359A1	1	= 662	ca 2Pl : 1t
	2	= 678	ca 1Pl : 1t

Assay of these for pract. seg., ψ rx or ←

3.12
2010.253

359B

DATE: 10/6/55 REF:

Continuation of 359 359A

Platings of UV treated 309-1 lysate on 2580 (lp+) - 0.1 ml 10^5 dilution of irradiated lysate.

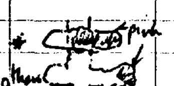
UV dose	Papillae	net	Assay/2341 (same dose)
control	31		?
0	75	44	35
1	183	142	
2	388	347	
3	576	545	
4	710	689	253
5	563	532	
6	412	381	

#10 streaked out - 10 col. v Agar 2279 all similar. 359B(4) 7th seq #10

55 54 ns = not seen to lysogenic 0 = ...

on new not work

	0 dose /2279		4' dose		/2279	Seq	1st seq	2nd seq	3rd seq	4th	5th	6th	7th	
1	ns	+	r	s	+		2-lp+	7-lp+	2-lp+	7-lp+	7-lp+ (wk)	2-lp+	7-lp+	359B1 Cl... 2 S/b
2	s	+	r	s	+	x	2-lp+	2-lp+	2-lp+	2-lp+	2-7-lp+	2-lp+	2-lp+	
3	s	+	r	s	+	x	2-lp+	2-lp+	not obt.	2-lp+	2-lp+	not obt.	2-lp+ wk	
4	s	+	r	s	+	x	2-lp+	2-lp+	2-lp+	2-lp+	2-lp+	2-lp+	2-lp+	
5	s	+	r	s	+	x	2-lp+ *	2-lp+	2-lp+ *	2-lp+	2-lp+	2-lp+	2-lp+	
6	ns	+	r	s	+	x	2-lp+	2-lp+	none	2-lp+	2-lp+	2-lp+	2-lp+	
7	s	+	r	s	+	x	2-lp+	contam.	2-lp+	2-lp+	2-lp+	2-lp+	2-lp+	
8	s	+	r	s	+	x	2-lp+	2-lp+	2-lp+	2-lp+	2-lp+	2-lp+	2-lp+	359B10
9	s	+	r	s	+			2-lp+	2-lp+	2-lp+	2-lp+	2-lp+	2-lp+	Cl...
10	ns	+	r	s	+		2-lp+ *	2-lp+	2-lp+	2-lp+	2-lp+	2-lp+	2-lp+ R	S/b
11	s	+	r	ns	+			none	none	none	none	none	none	
12	s	+	r	s	+	x	7-lp+	2-lp+	2-lp+	not test	2-lp+	2-lp+	2-lp+ wk	1
13	ns	+	r	ns	+			none	none	none	none	none	none	
14	s	+	r	ns	+			none	none	none	none	none	none	
15	ns	+	r	s	+	x	2-lp+ *	2-lp+	2-lp+	2-lp+	2-lp+	not obt	2-lp+ wk	
16	s	+	r	ns	+			none	none	none	none	none	none	
17	s	+	r	s	+	x	7-lp+	2-lp+	2-lp+	2-lp+	2-lp+	2-lp+	2-lp+	1
18	ns	+	r	s	+	x	2-lp+ *	2-lp+	2-lp+	2-lp+	2-lp+	2-lp+	2-lp+	
19	s	+	r	s	+			not obtained	2-lp+	2-lp+	2-lp+	2-lp+	2-lp+	
20	s	+	r	ns	+			none	none	none	none	none	none	
21	s	+	r	s	+	x	2-lp+	2-lp+	2-lp+	2-lp+	2-lp+	2-lp+	2-lp+	
22	s	+	r	s	+	x	2-lp+ *	2-7-lp+	2-lp+	2-lp+	2-lp+	2-lp+	2-lp+	←359B-22
23	s	+	r	s	+			contam	none	none	none	2-lp+	none	
24	s	+	r	s	+	x	2-lp+ *	2-lp+	2-lp+	2-lp+	2-lp+	2-lp+	2-lp+	
	6 ns 24+ 18 s		5 ns 24+ 19 s											



1st seq	2nd seq	3rd seq	4th	5th	6th	7th	
14 endo	14 endo	16 endo	16 endo	16 endo	17 endo	17 endo	110
2 exo	1 exo		1 exo	1 exo		1 exo	6
	1 amphi			1 amphi			2

55 17
2 1
1 2

DATE: 8/19/55 REF:

1924 × — HFT 1⁻ to study lysogenization + crossing over
a-4⁺ × — 1-4⁺

1. Transd. plates not recorded - ca. 300 colonies/plate
   no gal+ in control, lysate agreed.
2. After 2 days in the lysate plate several papillating colonies
3. Result:
   Papillating colonies streaked out and from the primary streaks 8(−) and 8(+) tested for lysogenicity against 2279

	8 gal+lys?	Gal+seg?	Gal-lys?	day ∓ plan?
Pap. Col. 1	no lys	no seg	no lys	—
2	no lys	no seg.	no lys	yes.
3	no lys	no seg.	no lys	—
4	no lys	no seg	no lys	—
5	no lys	no seg	no lys	—
6	no lys	yes	no lys	yes
7	no lys	yes	no lys	yes

6–7: } discarded

(circled note, with an arrow to Pap. Col. 2: "Gal+ 1984 Aug 26 discarded"; "360-2")

4. 360-2 – 24 Gal+ colonies from 24 diff. ∓ colonies tested /2279 for lysogenicity
   all found not lysogenic.

	stbl?	/2279	−seg/2279	HFT /1	HFT /4	Genotype	Lysogenic spotted /2279	Gal^L stbl?	
1	stbl	non lys							
2–12	↓	↓	not done ↓						
13	unstbl		non lys	0	0	1-4⁻	ca. 1000 plaques	—	← 360-3
14				0	0	1-4⁻	"	—	360-4
15				0	+	1⁻	"	(1)	1 found stbl
16				0	+	1⁻	"	(1)	1 found stbl
17				0	+	1⁻	"	(2)	2 found stbl
18				+	0	4⁻	"	(9)	8 found stbl
19				+	0	4⁻	"	(8)	7 found stbl
20				0	0	1-4⁻	"	—	
21				0	+	1⁻	"	(1)	—
22				— (E(−)	—	—	"	[illegible]	
23				+	0	4⁻	"	(5)	4 found stbl
24	↓	↓	↓	+	0	4⁻	"	(9)	8 found stbl.

Unaerated cultures (ov. nite) inoc. and incubated without air density ca. 10⁸ → lysogenic spotted /2279

4 [illegible]
4 ets
3 [illegible]

DATE: 8/21/55   REF:

Preliminary Expt. on seg freq. from $\frac{-+}{+-}$ both Lp^+ and Lp^R/s

1. Culture of W2868 $\frac{4^- 2^+ Lp^s}{4^+ 2^- Lp^R}$

A. Overnight culture diluted $10^2 - 10^4 - 10^6 - 10^8$ → 10 0.1 ml sample

assay 0.1	(+)	(−)	total	
1.	40	3	43	mean/sample
2.	76	2	78	
3.	52	3	55	$= \frac{59}{100} = 0.6$
	168/3 = 56	8/3 = 3	176/3 = 59	

B. Samples 10 (0.1 ml) plated after 5.25 hours

	(−)	(+)	
1.–6	no growth		
361-7 — 7.	5	1208	
361-8 — 8.	1	462	← this seg tested + 0 + Lp^s – Gal – Lp^s ↑ 4 $+^R$ obtained, 3 pure +, 1 intermed. 4/4 not segregating
361-9 — 9.	63	648	
streaked on a plate from 10.	ca 19	1116 ca 1185	

stored test + 361-9 ↑ these are ok of 205 [illegible]

This a colony – almost pure (−) noted = ①

Analysis of this colony is on 362 B

Reversions – the (−) seg from 361-8, 361-9, 36188 streaked on B gal and 4 $+^R$ obtained from each – examined for Gal stability – 10 tested

$1+$	1_{2-}	1_{4-}	Lp	4 $+^R$ from each of 5 ...
+	−	+	S	1 3/4 intermit, not seg
+	−	+	R	2 3/4 int. (+), not seg
+	−	+	S	3 2/4 int. (+), all not seg
+	−	+	S	4 3/4 " " " "
+	−	+	S	5 3/4 " " " "
+	−	+	R	
+	−	+	S	
+	−	+	S	2–S = 8
+	−	+	S	2–R = 2
+	−	+	S	1. 3/4 int (+), 1(+) seg. 2. 3/4 int. (+) 2(+) seg

2 Culture of W2869 $\frac{4^- 2^+ Lp^+ ?}{4^+ 2^- Lp^+ ?}$

A. Overnight cult. diluted $10^2 - 10^4 - 10^6 - 10^8$ → 10 0.1 ml sample

0.1 ml assay	+	−	total	
1	18	7	21	
2	22	8	30	0.33/0.1 ml
3	22	20	42	
	62/3 = 21	35/3 = 12	97/3 = 33	

B. sample 10 (0.1) plated 5.25 hours

1.–5	no growth		
6–7	all (−)	(+) 1 c ca 2000 col	1 c ca 300
361-138 — 8.	(−) 4	361	← these 4 seg — $1+$ 1_{2-} 1_{4-}: + + 0 result = 4–...
9.	14 = 66	ca 101	
10.	ca 1500	ca 700	

1. 4/4 int. (+) none seg
2. 4/4 int (+) none seg
3. 2/4 int. (+) none seg
4. 4/4 int. (+) none seg

SEE 362 ALSO

Using inocula from above – 0.1 ml of 10^6 dil in each case added to 7 ml Pen and incubated 20 hours at 42C

2868 inoculum ratio $56^+/3^-$ 20 hour ratio: $167^+/10^-$, $155^+/10^-$, $175^+/12^-$

2869 " " $21^+/12^-$ " " " $214/176^-$ +?−/ +?−/

DATE: 8/24/55 REF:

1	2	3	4	5	6	7	8	9	10

Repeat study of segregation from $\frac{2^x 4^-}{+ +}$ Lp+ - as in 361 - W2869

Culture streaked out and single (+) colony selected. Overnight culture from this colony diluted:

$10^2 - 10^4 - 10^6 - 10^8 \rightarrow$ 20 0.1 ml samples

incubated 4.5 hours, plated ppt washed with 0.1 ml broth

Cell assay (OD) made next day

	Sample	(−)	(total)	allele ✓			(−)	(total)
	1–5	no growth						
	6	0	354					
	7	1/4 = 157	ca. 300 X 4	discarded	2^-4^-	1	14	140
11(−) picked →	8	~~10~~ 19	1209	8 tested HFT + 8 +	+ 0 = 8(4⁻)	2.	2	136
	− 9	2	464	both Gal$_4^-$		3	16	118
	− 10	2	540	Gal$_4^-$	HFT + 2^-4^-		$\frac{32}{3}$ = 11	$\frac{394}{3}$ = 131
~~8 (−) picked~~	11	19	1089	4 tested	+ 0 + 0 = 12⁻; + + 0 = 34⁻			
	12	17	1357	discarded			HFT + 5 4⁻	
6(+)	13	297	933	10⁻ picked	2 contain E(+)		8 + + 0	= 8(4⁻)
these are ?	− 14	4	428	4 Gal$_4^-$				
	15	12	840	discarded				
	16	11	1525	"				
	17	33	796	discarded	A 12⁻ 14⁻			
12(−) picked 6(+)	18	441	690	"	12(−) + +	0 = 12 4⁻		
	19	494	1722	"				
	20	18	604	"				

362B the (+) colony of 361-9

\# 1. the (+) colony streaked out gave almost all (−)

2. from #1 10 (−) colonies tested A 12⁻ 14⁻ all 2⁻ Lp^s

from #1 4 Gal+ colonies obtained and streaked out - Extreme (−) seg from each to test for secondary non disjunction - 6 Gal+ picked from each of the 4 and streaked to obtain 6 separate segregation as evident

Gal+ #	observation	single seg		
1	4/6 no (−) seg	2/2 Gal$_2^-$ Lp^R	2	0
2	1/6 no (−) seg	5/5 Gal$_2^-$, 2/5 Lp^s, 3/5 Lp^R	2	2
3	1/6 "	5/5 Gal$_2^-$, 2/5 Lp^s, 3/5 Lp^R	2	2
4	0/6 "	6/6 Gal$_2^-$, 2/6 Lp^s, 4/6 Lp^R	2	2
		6 Lp^s + 12 Lp^R		

DATE: 8/25/55 REF:

1	2	3	4	5	6	7	8	9	10

Observation of erotic induction

1895 ⊗ 2790 Aerated, anti-foam added, cultures 3 hours from overnight

1.0ml + 5.0ml + 4.0ml Pen - aerated no anti-foam (4.x10⁸ Hfr) (2.x10⁹ F-)

$10^2, 10^4, 10^6$ → 0.5 ml; 1. 206; 2. 206; 206

Samples taken – ml – diluted $10^2, 10^4$ → 0.05 ml plated; 1. /2279 m B Gal; 2. / D(0)

$10^2 - 10^4, 10^6$ → 0.05 ml; 1. 209; 2. 157; 396 198

Optical Density Read / 10ml Pe at 650

Time	O.D.	Plaques/2279	Proto. D(0)	Proto/phages λ	λp
0	0.95	—	—		
15	—	7 *	3	35	Contam.
30	1.10	19	1	15	with
45	1.7	36	0	—	phage
60	2.2	69	3	35	
75	2.0	147	11	115	
90	2.2	ca 110	6	65	

Ratio of Hfr/F- : 20.6/99 = 1/5

4.12 x 10⁷ 4.1 x 10⁹ 4.00 x 10⁹

* since these plated on B gal, estimation of 1895 Gal+ component can be made: appear to be ca 100 at 0 and no reduction in number up to 90 min noted.

7 x 20 x 10⁴ = 140 x 10⁴ = 1.40 x 10⁶ λ / ml at Zero

150/7 x 1.4 = 29 x 10⁶ = 2.9 x 10⁷

2.9/41 = 7%, Hfr

2341 ⊗ 2308 Mal- Ind. 2341 to look for translocations and seg behavior of Heterozygote

1. 2341 aerated wet. incd. 60 sec. - Mixed equivalent volumes of Hfr & F- incubated 30 min 37C diluted 1/100 and plated B gal, S Mal - S Mal plates discarded - difficult to Malt from Mal-.
2. 20 Gal+ prototrophs on S Gal picked and tested /2279 for lysogenicity all non lysogenic

The 20 Gal+ are divided into several groups on degree of Gal-ness

363 B → 12 full Gal+ — these do not segregate
3 intermed Gal+
5 weak Gal+ — these contain a few full Gal+

} all appear to be seg.

DATE: REF:

Segregant diploidy. Test by rescreening segregant, ✓ stability

Culture			Rev. stable	0 = no	+ = yes
1	518E1612		0		
2	518E750		0 (wk +)		
3	518E902-1		0		
4	518E902-2		0		
5	2175E811-1		0 (wk +)		
6	2175E811-2		—		
7	750E1612-1		0		
8	750E1612-2		0 (wk +)		
9	750E902-1		—		
10	750E902-2		0		
11	811E902-1	all +	no		
12	811E902-2	all +	0 (wk +)		
13	286-1		—		
14	286-2	failed to grow	—		
15	2175E750-1		0 (wk +)		
16	2175E750-2		—		
17	290-1		—		
18	295-2		—		
19	302-2	7×2⁻	—		
20	334B	2⁻×6-7⁻	0		

10/10 successful tests no seg = 10 seg not diploid

311-2 = HFT 6⁻ =

streaked out 2 Gal+ reversions obtained

both segregating Gal+/Gal⁻

HFT 6⁻ +R

	/4	/2	/6	
2 HFT seg obtained both	+	+	0	= 6⁻

309-1 = HFT 7⁻

streaked out 8 Gal+ reversions obtained

2/8 segregating Gal+/Gal⁻

HFT 7⁻

2874 looking for Gal_2^- in 583 line

1. first 8 seg = Gal_2^-
2nd 6 " = "

DATE: 8/31/55 REF:

Segregation – non disjunction? Obtain clone with early seg and test the Gal+ component to see if it segregates 50% homozygotes.

7. Culture W2869 – from single (+) colony → overnight Pen.

dilute: 10^7 — 10^6 — 10^4 — 10^2

↓ Spot 0.01 ml samples B Gal; incubate 3 hours and respread. – look for clones with many (–)

→ 0.1 ml for Gal+ : Gal- ratio

	Gal-	total
1.	19	117
2.	17	142
3.	19	139
	55/3 = 18	398/3 = 133/0.1 ml

Respreading

1. failed to grow or regrowth – 19 — $\bar{X} = 1.3$, $e^{-x} = 19.4$, obs 19
2. are gal+ – 32 — Gal+ $\bar{X} = 1.2$, $e^{-x} = 21.6$, (19+6) = 25
3. are gal- – 6 — Gal- $\bar{X} = 0.18$, $e^{-x} = 59.76$, 13 Gal-
4. mixed Gal+ + Gal- – 15 (72)

No's used for seg. studies

	total clone size		ratio		Discarded	4 Gal+ picked to obtain (–) clone	TEST OF ORIGINAL SEGREGANT COUNT 1	2	4	
1	14	1.	(1-) : 13+	no other (–) on plate		(1) +	+	+	o	Gal-
	46	2.	8- : 38+	(–)	+					
2	11	3.	(2-) : 9+	no other (–) on plate		2 +	(2) +	+	o	2 Gal-
	45	4.	8- : 37+	same plate	+					
3	8	5.	(1-) : 7+	same plate		3 +				
	39	6.	15- : 24+		+		+	+	o	Gal-
	24	7.	10- : 14+	(–) same plate	+					
	41	8.	10- : 31+	(–) same plate	+					
4	33	9.	(1-) : 32+	(–)		4 +	+	+	o	Gal-
5	11	10.	(1-) : 10+	(–) same plate		5 +	+	+	o	Gal-
	9	11.	7- : 2+	(–)	+					
	35	12.	13- : 22	(–) same plate	+					
6	12	13.	(1-) : 11+	(–) same plate		(6) +	+	o	+	Gal_2-
	5	14.	4- : 1+	no other (–) on plate	+					
	12	15.	1- : 11+	no other (–) on plate	+					

2 Gal+ recessives selected from each – all found Gal stable.

Localization of clone on plate and relationship to other Gal- in case of spattering from one streak to another.

Segregant from these Gal+

#6 col A = 1 (2-), 5 (4-)
B = 3 (2-), 3 (4-)
C = 2 (2-), 1 (4-) 1 no test
D = 1 (2-), 4 (4-)

tested against +, 2-, 4-

Segregant from #1 Gal+ i
A. 8 (2-) 5 (4-)
B 2 (2-) 3 (4-)
C 4 (4-)
D 5 (4-)

OR PLET

A1 (2-), D1(4) } A repeat, 6 seg analyzed: 1-4 (4-) 3 4-; 5-6 (2-) 3 2-

B1 (4-), B2-4 (2-), B5-6 (4-)

Gal+ Revertants (2) obtained from these and tested for stability

2 des of A1 unstable ← 16(+) col. tested 1/750 → 4 contain (+), 5 NFT, 7 HFT

A2-A6 stable; B1 (–) stable; B2-B4 unstable – NFT segregants obtained 1/6 Gal_2-; also B56

10(+) col. tested 1 HFT/811

C1 (4-), C2-3 (2-) } C repeat, 5 seg anal. 1 = 4- 4(4-); 23 = 2-, 45 = 4- 4 (2-) } 1/6 stable

D1-2 (4-), D3- (2-), D45 (4-) } 10/10 stable

DATE: 9/25/55 REF:

A

293-12 (which see) gives increased frequency of HFT Gal4⁻
Use to test for diploid of distal markers as a result of C.O. between Gal's.

1. A mal obtained by λ² resistance in B mal.
2. 12 seg obtained, tested for HFT against 2580, 811

control	2580	811
o o ⊙ o	o o o ⊕	o o ⊙ o
o o o o	o ⊕ o o	o o o o
o o o o	o ⊕ o ⊕	o o o o

⊙ = contam
⊕ = HFT

4/12 HFT 4⁻ — malt populace

Total: 13 HFT 4⁻, 37 +, 13/49 or 16/49

after mal + R V gal alleles

3. Streak HFT 4⁻ on B mal v mal +R sttd — from leg. colonies
#4 - 6/6 malt + R sttd, 3/3 Gal + R sttd; #6 6/6 malt + R sttd, 3/3 Gal + R sttd; #10 6/6 mal + R sttd, 3/3 Gal + R sttd; #12 6/6 malt + R sttd, 3/3 Gal + R sttd

A1)
4. Test these seg /2⁻ /4⁻ /+ (these are mal⁻)
A. Despite mal reaction — after 3 days at 37C, reactions obtained — all appear to be transduced by +, 2⁻, but not by 4⁻ — means all Gal4⁻ - Gal4⁻ is in exogenote
B. Reversions of #4, 6, 10, 12 obtained to check HFT.

The HFT above (A1) and 3 from A2 grown with 1177 24 hours and plated on SMal Sm: no growth — indicating these cultures not Hfr.

Reversion above say culture not homogenote why?

A2

5. Rept. on 12 additional seg.

control	2580	811
o o o o	o o ⊕ o	o o o o
o ⊙ ⊙ o	o ⊕ ⊕ o	o o o ⊕
o o o o	o o o o	o o o o

A2 Malt R: 6/6 + R sttd #3; 6/6 + R sttd #6; 6/6 + R sttd #7; 6/6 malt R #8 sttd
Gal + R: 5/6 " " ; 5/6 sttd; 6/6 " " ; 3/2 sttd 4
1 unstable; 1 unstable

the Gal⁺ are from the malt R

CONTINUED PAGE 366

6. 293-12 irradiated on B4gl, Base, Bott. — 14gl obtained, (malt?)
not gal+/gal⁻

2852/2 = 365B = Gal1⁻ Gal4⁻ × 2⁻ to obtain (- - -) for expt. to show crossing over between genotes, as: $\frac{+}{2}$ = | 1± 1∓ = not Gal+, c.o. between genotes to give +/+ yields Gal+.

B

1. 2852 streaked / HFT 2⁻
2. 10 streaks made from cross lines.
7 yielded 4p⁺ trans.
3 " 4pR/4ps some seg 1⁻ 4⁻ 4ps
3. Obtain 1⁻ 4⁻ 52ps (1 1+ 4+ 2⁻ 4p+ line)
a. seg. 11 1⁻ 4⁻ 4ps → Reverse 365B-1
1 2⁻ 4ps
1 2⁻ 4p+ → reverse B gal → 7 seg obt. 6 = 1⁻ 2⁻ 4ps, 1 1⁻ 4⁻ 4ps — more of 365B-2

DATE: 10/1/55 REF:

1. 1578 – to obtain F⁻ 1⁻4⁻ and F⁻1⁻ for EML

0.1 ml O.N. 1578
1578 X — 2346 X (Gal,⁻) – Exposed to 0.3 ml 2346 – incub. 10'
dilute 10⁻², 10⁻³, 10 0.1 ml – No contro done.

	/Plate	(–)	2 days pos.	
Segregation from #1	1.	14	0	366-1
11 Dstbl (+)	2.	15	2	(1 mosaic in appearance)
12 seg.	3.	17	1	366-2
Segregants tested	4.	12	1 but	
1 1⁻4⁻	5	8	0	
6 4⁻		66	4	(%) 4/70
1 1⁻				
2 P.E.				

control = 3, no lost

2 sub / to tested — stock gal. ... dried ... Sept

F⁻ Gal,⁻ Gal₄⁻ Lp⁺
= 366-3
W2994

K12 lysate 9/28/55

Assay 10/1/55 = 108 X 10⁸ – 1.1 X 10¹⁰
10/4/55 = 108 X 10⁸ – 1.1 X 10¹⁰

365A3 continued. 293-12 mal – → seg tested for mal diploids, etc.

A3 9 additional seg.

HFT-tested	/2550	/811	Not invaded	/2550	/811
phenotype of these exceptions not different from	o o ⊕ o	o o o o		o o ⊕ o	o o o o
	o ⊕ o o	o o o ⊕ 20		o ⊕ o o	o o o ⊙ 2
non exceptions or Bgal →	⊕	o		⊕	o

This growth, streaked out for gal +, also mosaic colonies

THESE APPARENT HFT SEGREGANTS ARE OBSERVED WITHOUT UV TESTING.

These were not numbered. 4 mosaics obtained from 3, 2 from one. Which?

A3 Gal+ᴿ ← of above (1) 2/4 +ᴿ seg, (2) 1/1 +ᴿ seg, (3) 3/4 +ᴿ seg.
↓
Malt⁺ᴿ

	from Gal+/–	Malt+Gal+/–?	→ Gal–
#1	6/6 stbl	6/6 Gal–	6/6 stbl
#2	5/5 stbl	2/5 Gal+/–	6/6 stbl
#3	5/5 stbl	1/5 Gal+/–	6/6 stbl

Does these seg. heterogeneity have a greater tendency to fall apart? Does mal have effect on seg.? Of 16 malt⁺ᴿ, only 2 were still Gal+/–

Return to 293-12 – are the cosegregants really Gal₂⁻? 365 suggests no.

/2550	/81
⊕ o o ⊕	o o o o
o o ⊕ o	o o o o
o o ⊕ o	o o o o
o o ⊕ o	o o o o
o o	o o

tested against HFT 2⁻, 4⁻ all Gal₄⁻

DATE: 10/8/55 REF:

1	2	3	4	5	6	7	8	9	10

2341 Lp+/Lp^s 2/2 x — (+) 750 CK12-1

1. 2 day cult. 2341 → 1-10
↓
0.1 ml + 0.1 ml 750 CK12-1 → 10², 10⁴ → 0.1 ml
inc. 10' 37C

2. same with broth as control →

		(-)	(+)		One streaking		
					last sp. cult. / λ 2341		
					Galt	Gal-	1st streak
Control	1.	150	0		1 Lp^s	Lp^s	→ Lp+, -s
	2.	164	0		2 Lp^s	Lp^s	→ Lp+, -s
Exptl.	1.	173	1	} (2-4) several partially lysed colonies / plate	3 Lp^s	Lp^s	→ Lp+, -s
	2.	158	2		4 Lp^s	Lp^s	→ Lp+, -s
	3.	162	2		5 Lp^s	Lp^s	→ Lp+, -s
	4.	123	2		6 Lp+	Lp+	→ Lp+, -+
	5.	189	2		7 Lp+	Lp+	→ Lp+, -+
					8 Lp+	Lp+	→ Lp+, -+
					9 Lp+	Lp+	→ Lp+, -+

(-), (+) picked / tested λ 2341

[These discarded]

A

365B2 = M⁻ Gal₁⁻ Gal₄⁻ Gal₂⁻ Lp^s x — HFT 7 to see quadruply (-)

1. Procedure as with 2341 above. 309-1 lysate used.

		(-)	pap. col.
1. Control	1.	428	0
	2.	454	0
	3.	503	0
2. Exptl	1.	171	15
	2.	189	27
	3.	196	20
	4.	206	33
	5.	200	46

} These at low, perhaps 30% most (90%) colonies partially lysed

B

$N_0 = 43 \times 30 \times 10^3 = 1.26 \times 10^6$

$N_s = 2 \times 20 \times 10 = 4 \times 10^2$

DATE: 10/10/55

REF: $\frac{N_s}{N_0} = \frac{4}{1.26} \times \frac{10^2}{10^6} = 0.3 \times 10^{-2}$ $= 0.3\%$

1	2	3	4	5	6	7	8	9

241-14 - (a colony picked for HFT) HFT λ yield

1. dilute to obtain 10^4-10^5/ml solution (10, 10^3 dilu) → dil 10^1, 10^2 → assay
2. Irradiate 60 seconds.
3. 1.0 ml + 10 ml Pen. ca 10^4/ml

N_0 = 1. 36 (1 gal+) 2. 40 3. 53 — 129/3 = (43)

0.05 ml sample → plated 1. 1 col. 2. 2 col ← forgot to add 3. 3 col. 7/3 = 2 all lost!

4.

Time	/750	PAP /2279	PLAQUES /2279		
0	52	79	>62	Plaques small	4. 33
15	30	81	>168	" "	5. 35
45	32	93	>107	" "	6. 34
55	43	88	>280	"	102/3 (34)
65	50	86	>334	wet plates	
90	56	93	>374	" "	

} no plaques

HOPELESS

SEE RESPEAD → 253/6 = 42

For EML - a gal⁻ in F-750 lines.

2902 × HFT 4
2 heterogenotes (P.E. -) obtained

368-1
368-2 ← Lp^R/Lp^S

Gal + August 26 1964 McCord

plaque + S Gal⁻ 2902/4 18/22 + str

13 Gal⁺ tests to λ 2279 3 Lp^S, 10 Lp^R

4 ag dil. Babys' tel. P.E. (-)

2915 × HFT 7 (309-1) - Do Lp^R/Lp^S heterogenotes come from clones were there is also $(Lp^+)\lambda^+$? Recovery of 4 strands.

1. Freshened culture of 2915 ca. 10^8 ~~diluted~~ 0.1 ml + 0.4 ml 309-1 λ (prep.) incubated 10' 37°C, dil 10^2, 10^3, 10^4 → 0.1 ml plated B Gal

		(+)	(-)	(-)/lysed	total
1. Broth control	1.	0	92	0	92
	2.	0	94	0	94
	3	0	96	0	96

2. Lysate spotted.

		lysed colony			
1.	4	(3)	ca 80 wet plate	12	ca 16
2.	3	(2)	ca 60 wet plate	14	ca 77
3.	6	(6)	65	10	71
4	2	(1)	41	9	[illegible]
5.	5	(3)	ca 62 wet plate	ca 16	ca 93

CONTINUED →

3. Testing the heterogenotes - streaked / 2902, streaked out BGal.

	2902 lysogenicity				
1	+	Gal seg	11	+	Gal-Gal seg
2.	+	"	12	+	Gal seg
3	+	"	13	+	"
4	+	"	14	+	"
5	+	"	15	+	"
6	+	Gal-Gal seg	16	+	"
7	+	Gal seg			
8	+	"			
9	+	"			
10	+	"			

} these last 4 came from intact col., the others from partially lysed colonies.

DATE: 10/18/55 REF: 368 A

1	2	3	4	5	6	7	8	9	10

(barlodge?)

Continuation of 368 - Do lp^s/lp^r transductions come from λ^+ particles?

1. Examination of the heterogenotes - 6 gal+, 6 gal- taken from the 1st streaking out of the isolated (single colony) heterogenotes; and streaked against λ, 2278

		het # 1	2	3	4	5	7	8	9
Gal+	1	+	+	+	R	+	+	+	+
	2	+	nt	+	R	+	+	+	+
	3	+	+	+	R	+	+	+	+
	4	r ?	+	+	R	+	+	+	+
	5	+	nt	+	R	+	+	+	+
	6	+	+	+	R	+	+	+	+
Gal-	1	+	+	s	s	s	+	+	+
	2	s	s	+	s	+	+	+	+
	3	s centr.	s	+	s	s	+	+	s
	4	+	+	nt	s centr.	nt	+	+	nt
	5	nt	+	+	s	nt	+	+	+
	6	s centr.	s	+	s	nt	+	+	s centr.

+ = lp^+, r = lp^r, s = lp^s, nt = no test

Reason of plate on which these were tested ... showed good lysis [illegible]
Re. #16, the plate showed [illegible] and [illegible]

		#10	#12	#13	#14	#15	#16	#18
Gal+	1	+	+	+	+	+	R	+
	2	+	+	+	+	+	R	+
	3	+	+	+	+	+	R	+
	4	+	+	+	+	+	R	+
	5	+	+	+	+	+	R	+
	6	+	+	+	+	+	R	+
Gal-	1	s	s	+	nt	+	s	+
	2	s	s	+	s	s	s	+
	3	+	s	+	s centr.	s	s	+
	4	+	+	+	s	s	s	+
	5	+	s	+	s centr.	+	s	+
	6	+	s	+	s	+	s	nt

(SEE 371)

For #4, #16 { The original colony (from the first plate) grown up in broth and spotted (post centrifugation) on 2278. One set of plates [illegible], one not. Both showed λ, the [illegible] = 0 [illegible].

For A. Rechler

To AR. new Hfr (W) 3 X — Gal₂⁻ (241-14) W

also Gal₄⁻ (247R-1) — X

From the Gal₂⁻ — X 4 Gal₂⁻ [illegible] 3 lp^s 1 lp^{R+?} none found lp^+

368A-3

DATE: 10/17/55 REF:

Irradiation of 309-1 lysate (Same lysate as previously)

1. Dil 0.1 ml + 10 ml saline, irradiate, 0.1 ml + 10 ml Pen.

Dose Pop 2915

0
15"
30"
45"
60"
75"
90"
105"

P. =

1	2	3	4	5	6	7	8	9	10

1. One step with 241-14 - assay M Gal using 2343 Gal.- prob a cul. 2915 as λ plaque assay

1. 241-14 streaked out - 10 colonies picked to broth, tested for HFT. 6 found HFT, broth of one used for Gal

2. Dilute fresh cult. from broth to [ca 5·10⁸/ml.] saline

Pre-ind. assay.

		Plate	Count	3 days	36 hr MGal
1. gal rev. 2343 0.1ml on MGal =		✓ 1	0	3	+
2. HFT lambda assay 0.1ml + 0.9ml 2343 cult. - 0.1ml =		✓ 2	0	8	+
3. λ plaque assay. 0.1ml + 0.9ml 2915 0.1ml =		✓ 3	7		+

3. Post-Ind. = 40 seconds. ADD 5ML + 5ML 2X PEN

Time			½ dilution			
0'	1. Trnsd. assay 0.1ml + 0.9 2343 =		4. ✓	0		+
	2. λ " " " 2915 =		5. ✓	348	(9)	+
15'	3. Trnsd.		6 ✓	0	(8)	+
	4. λ		7 ✓	341		+
30'	5 T		8 ✓	0	(10 cut pst)	+
	6 λ		9 ✓	ca 300-400		+
45'	7 T		10 ✓	1	(6)	+
	8 λ		11 ✓	321		+
60	9 T		12 ✓	1	(2)	+
	10 λ		13 ✓	360		+
75'	11 T		14 ✓	0	(8) cut pst	+
	12 λ		15 ✓	>400		+
90	13 T		16	1	(5) cut pst	+
	14 λ		17	>417		+
105'	15 T } omit		18			+
	16 λ }		19			+

3a. 6 M Gal plate 0.1ml each 20-25. 6
✓ To 3 add 0.1ml 2343 8-20° ca 600 all Gal — 4, 2, 3
After 90' add 0.1ml 2343 Respread 3 more. 24-26 3, 5, 8 122, 91, 105

Gal+ in ca. 600(?) or clear

B

2915 × K12 lysogenization - Does lysog. result in lp+/lp-?

or low multiplicity - ca. 3-4 contam. cells per 1000-1500.

1. 16 colonies picked for exam. - 10 colonies from each streaking;

	#	+	lp S	R	#	+	S	R		#	+	lp S	R
	1	9	1	0	8	0	10(1)	0	*	15	3	7(5)	0
*	2	3	7(1)	0	9	2	8(1)	0		16	0	10(7)	0
*	3	3	7(5)	0	10	0	10(2)	0					
*	4	10	0	0	11	7	3(1)	0					
*	5	10	0	0	12	——— mixed							
*	6	5	5(4)	0	* 13	0	9(13 contp)						
	7	0	10	0	14	0	10(6)	0					

wk (+) selected after streaking out 2 per each

* = wk lp+ reaction. Streaked out and 2 colonies picked from each.

all lp+ reactions stronger

contaminants in parent ( )

0.4   1.20 × 10^8

$6.54 \times 10 \times 10^4$

6.5×10^7/ml

DATE: 10/22/55   REF: 371

lp^r/lp^s heterogenotes - always accompanied by λ^+?

1A 2279 Gal_1^- lp^s λ ← HFT Gal_2^- from 241-14 Mal- lysate titer at 1.3×10^8

A. Overnight 2279 dil 0.4 ml + 10^{-1} ... – 0.5 ml + 0.1 ml 241-14 Mal-

A

2. Result.   dil $10^2 \to 10^4$ → 0.1 ml samples ... gal

		Gal+	Gal- not ...	... + Gal+
A. Broth control	1.	0	624	0
	2.	0	684 (65?)	0
B. 241-14 lysate	1.	6	633	26
	2.	5	608	18
	3.	7	592	25
	4.	2	498	16

+ = lysis, 0: no lysis

Heterogenote /2279

1	+	(11)	+
2	0	(12)	+
3	+	13	0
4	+	14	+
5	0	15	0
6	+	16	+
7	0	17	+
8	+	18	0
(9)	+	19	0
10	+	20	+

7 0   13 +

analyses → Continued 372

A

O omitted from ... because ... lp^s ... in each clone ... or ... Gal ...

The lp^r clones from 371A, 371B grown up in broth, centrifuged and spotted on 2279. One set of spots ... , one ... (30' UV). None of these ... showed λ lysis. At the same time 368A-4, 368A-16 showed λ lysis.

These cultures retested /2279 (not ...) on 12/2/55. No evidence of plaque lambda found.

B. Same as above but W518 Gal_4^- lp^s

A. Broth control	1.	0	90	0
	2	0	82 (86)	0
B. 241-14 Mal- lysate	1.	0	59	1
	2	2	47	5
	3.	3	131	9
	4.	1	42	2
			279 / 4 = 69	

Heterogenotes /2279   From 1st streak

		6 Gal+	6 Gal-	
1.	+	6 (r)	1(r) 5(s)	
2.	0	6 (r)	6 (s)	
3	+	5/5 (r)	3 (r), 2(s)	
4	0	5/5 (r)	4 (s)	
5	0	1 (r)	1(s)	but lost
6	0	6 (r)	6 (s)	

2 +   4 0

4 lp^r/lp^s

2 lp^r

← Given to JL 11/24/55

B

DATE: 10/28/55 REF:

371A Continued - Analysis of (+) and (-) from [illegible]

+ = lysis 0 = unlysis

	#1 (1/279)	#2	#3	#4	#5	#6	#7	#8	#10
6(+) 1.	R +wk	R 0	R +wk	R +wk	S 0	R +wk	S 0	R +wk	R +wk
2.	R +wk	R 0	R +wk	R +wk	S 0	R +wk	S 0	R +wk	R +wk
3.	R +wk	R 0	R +wk	R +wk	S 0	R +wk	no test	R +wk	R +wk
4.	R +wk	R 0	R +wk	R +wk	S 0	R +wk	no test	R +wk	R +wk
5.	R +wk	R 0	R +wk	R +wk	S 0	R +wk	S 0	no test	R +wk
6.	R +wk	R 0	R +	R +wk	S 0	R +wk	no test	no test	R +wk
6(-) 1.	R +	R 0	S 0	R +	R 0	R +	R 0	R +wk	R +
2.	R +	R 0	no test	—	R 0	R +	R 0	—	S 0
3.	R +	R 0	R — 0	—	R 0	R +	R 0	—	S 0
4.	R +	R 0	—	—	R 0	R +	R 0	—	S 0
5.	R +	R 0	—	—	R 0	R +	no test	—	R +
6.	R +	R 0	—	—	R 0	R +	no test	—	R +
	↑ (-) seg. (+) result stronger than Opel +				↑ Gre(+) and Gre(+) [illegible] change	(-) (+) change from Gre+ [illegible]	↑ Gre + and [illegible] order reversed		
1st [illegible]	Lp (+)	Lp R	Lp +	Lp +	Lp R	Lp +	Lp R	Lp +	Lp +

	#13 (1/279)	#14	#15
1	R 0	R +wk	R 0
2	R 0	R +wk	R 0
3	R 0	R +wk	R 0
4	R 0	R +wk	—
5	R 0	R +wk	—
6	R 0	R +wk	—
1	S 0	R +	S 0
2	S 0	R +	S 0
3	S 0	R +	S 0
4	S 0	R +	S 0
5	S 0	R +	S 0
6	S 0	R +	S 0
	Lp R	Lp +	Lp R

DATE: 11/8/55 REF:

1	2	3	4	5	6	7	8	9	10

Testing of some heterogenotes by crossing - Cmp. c VL expt.
Theoretically het. for lp^- could be two type or lp of lysogenic

$\frac{A\ A}{b\ b}$ $\frac{A\ A}{B\ b}$ $\frac{A\ A}{B\ B}$ × $\frac{A}{b'}$ → $\frac{A\ A}{b'\ b}$ + $\frac{A\ A}{B\ b'}$ + $\frac{A\ A}{B\ B}$

lp^R (not considered) lp^+ lp^+ lp^R lp^+ lp^+

W3011 × HFT (+?) from 7[illegible]1217-1 4 heterogenotes obtained · crossed ⊗ 2274

1. Grown together ca. 2 hours diluted 1-10 plated S Gal - only 2 Gal+ on all plates (0)
2. Mating mixture replated immed. after resusp in H₂O on M Gal (3 plates each cross)

A. Results.

		Cross 1	Cross 2	Cross 3	Cross 4	Rpt. #2 (11/27/55)
No Gal+	1.	14	3	22	26	3
	2.	ca. 85	4	19	69	7
	3.	ca 100	2	19	51	—
total		199	9 (11)	60	146	

#2 is lp^+, Gal (segregation)

MORE Seg[illegible] ... not lys[illegible]

B. Pick 24 from each. Strain 1 and test for 3 lysogenicity.

	Cross 1 /2279		Cross 1 /λ	Cross 2 /2279		Cross 2 /λ	Cross 3 /2279		Cross 3 /λ	Cross 4 /2279		Cross 4 /λ	Rpt. /λ	seg	/2279	/λ	seg	/2279
1	0	ns	S	+	s	R	+ (+)	s	R	0	s	R	s	ns	0	R	s	+
2	0	s	R	+	s	R	0	ns	(S)	+	s	R	R	s	+	R	s	+
3	0	ns	R	+	s	R	+	s	R	+	s	R	s	ns	0	R	s	+
4	0	ns	R	+	s	R	+	s	R	+	s	R	R	s	+	R	s	0
5	0	ns	R	+	s	R	0	s	R	0	s	R	(s/c)	ns	0	R	s	+
6	0	s	R	+	s	R	+	s	R	+	s	R	R	ns	+	R	s	+
7	0	ns	R	+	s	R	+	s	R	+	s	R	R	s	+	s	ns	0
8	0	ns	S	+	s	R	+	s	R	0	s	R	R	s	+	R	s	+
9	0	ns	S	+	s	R	0	ns	R	+	s	R				R	s	0
10	0	s	R	+	s	R	+	s	R	+	s	R				wt	ns	wt
11	0	ns	R	+	s	R	wt	s	R	+	s	R				R	s	+
12	0	ns	S				+	s	R	+	s	R				R	s	+
13	0	ns	R				0	wt	R	+	s	R				R	ns	0
14	0	ns	R				+	s	R	+	s	R				s	ns	0
15	0	ns	R				+	s	R	+	s	R				R	s	+
16	0	s	R				0	ns	R (S)	+	s	R				R	s	0
17	0	s	R				wt	s?	R	+	s	R				R	s	+
18	0	s	R				+	s	R	+	s	R				s	ns	0
19	0	?	R				+	s	S	+	s	R						
20	0	?	R				+	s	R	+	s	R						
21	0	?	R				0	s	R	+	s	R						
22	0	s	R				+	s?	R	+	s	R						
23	0	s	R				+	s	R	+	s	R						
24	0	s?	R							+	s	R						

12 colonies tested all gave weak sensitivity reaction.

seg transf. by 2-, 4-, & lp^s

Retested /λ /1- /6- /7-: (5) + 0 + +

seg transf. 2-, 4-, to lp^R + + +

Retested /λ /1- /6- /7-: (10) + 0 + + 4 [illegible]

seg not trans by 2-, 4-, to lp^S } transfer by 4, 1-, 6, 7- lots

+ = lys, 0 = not lys

Mistake 2 sets of 373-1 to 12 [illegible]

Pencil marked ones are deduced from [illegible], 2 plates 13-24 and 1-12 between #1 and #3

S = segregates Gal+/−
ns = non seg.
#17 mixed

373-1 tested /λ, 2279 lp^R 11 lp^+ Gal+/−

373-3 /λ /2279: R + lp^+; /λ /2279: R + lp^+

12/5/55

Use /λ reaction. These results not meaningful re test. One culture not tested /λ (lp^s, seg)

Retest completely

DATE: REF: 374.

1	2	3	4	5	6	7	8	9	10

360-3

1. Enlarging the P.E. loci data — Combinations of 1⁻ & 4⁻

1. 360-3 - 24 PE (-) picked streaked out. 19 Gal+ Stained.

9 Galt stbl 10 Gal- seg.

	1⁻	4⁻		
1	P.E(-)	——		
2.	o	o	?	
3.	o	+	contam.	
4.	PE	——		
5.	o	o	?	
6.	PE	——	3	PE.
7.	o	+	3	1⁻4⁻ ?
8.	o	o	3	1⁻
9.	o	+	1	4⁻
10.	+	o		

1924HHFI⁻

2. 368-1 As above. 9/15 Galt stbl.

6 Gal- seg stbl.

	1⁻	4⁻		
1.	—— PE.	——		
2.	o	+		
3.	+	o	3	1⁻
4.	o	+	2	4⁻
5.	+	o	1	PE.
6.	o	+		

3. 366-2 As above 6/28 Galt stbl. ← This clone $\lambda p^R/\lambda p^S$

12 Gal- seg B.

	1⁻	4⁻		
1	o	+		
2	o	+		
3	—— PE	——		
4	contam -			
5	—— PE.	——	4	1⁻
6	+	o	3	4⁻
7	o	+	2	1⁻4⁻
8	+	o	2	PE.
9	+	o		
10	o	+		
11	o	o		
12	o	o		

These all λp^S except P.E.

6 Galt stables were tested against λ — are λp^S, also recheck slms Galt stbl

2234 checked [illegible] orig. [illegible]

DATE: 11/19/55 REF: 375

More 11g Gal2 +/- x F- Lp s. (7) (375-1 → 375-7)
x 2274 (Grown together, [illegible], plated M Gal

	1	2	3	4	5	6	7	8
	375-1	375-2	375-3	375-4	375-5	375-6	375-7	
Re ✓ λ/2279	Lp+	Lp+	Lp+	Lp+	Lp [illegible] s	Lp+	Lp+	λ/2279, λ
Segregating?	YES	YES	YES	NO	(gal) NO	YES	YES	← Segregating?

Cross Matz. 0.1 ml 375-x + 0.1 ml 2274 in Pen. Inc. 6½ hours. Centrifuge, resusp. [illegible]. 0.1 ml EMGal. (2 Matz)

	1	2	3	4	5	6	7	8
Gal+ in EM COR → 1	8 (mostly wk?)	81	70	152	ca. 1000	113	128	RPT on #
2	15 (+)	71	40	103	ca. 1000	88	117	add. prob. from same cross.

10 Test Prototrophs.

	λ seg? /2279	λ seg? /2279	λ seg? /2279	λ seg? /2279	λ seg? /2279	λ seg? /2279	λ seg? /2279	seg? /2279
•1	R s +	R ns +	•R s +	R ns +	s ns 0	•R 2-4-lp5? s 0	R s +	s +
•2	R s +	R? s 0	•R s +	R ns +	s ns 0	•R 2- s +	•R 1- s +	s +
3	R s +	R s +	R s? +	R ns +	s ns 0	R s +	•R 4- s +	s +
•4	R s +	R [illegible] 0	•R s 0	R ns +	s ns 0	•R 1- s 0	•R 2- s +	s +
(2) 5	R s +	ut (ut?) ut	•R s 0	R ns +	s ns 0	•R 2- s +	•R 2- s +	s +
6	ut ng ut	R (ut) 0	•R ns 0	R ns +	s ns 0	R ns +	R s +	s +
7	R ns +	R (ns) +	•R s +	R ns +	s ns 0	R ns +	•R 2-4- s +	s +
8	R s +	R (s) +	R s 0	R ns +	s ns 0	•R 2- s +	R s +	s +
209	R s +	ut (s) ut	R ns +	s ns 0	s ns 0	•R 2- s +	•R 2- s +	s +
(2) •10	R s +	R seg (ut) 0	R ns +	R ns +	s ns 0	•R 2- s? +	•R 2- s +	s +
(2?) 11	R s +	R ns +	•R s +	R ns +	s ns 0	•R 2- s +	•R 2- s 0	s +
•12	R s *0	R s 0	•R [illegible] 0	R ns +	s ns 0	R ns 0	R s 0	s +
•13	R s +	R ut +	•R s +	R ns +	s ns 0	R s 0	•R 2- s +	s +
(2?) 14	R s +	R ut 0	s ut 0	R ns +	s ns 0	R s +	•R 2- s +	s +
•15	R s +	R s +	•R [illegible] +	R ns +	s ns 0	•R 2- s +	•R 2- s +	s +
16	ut s +	R s +	•R s +	R ns +	s ns 0	•R 2- s +	R ns +	s +
(2) 17	R s +	R s +	•R s +	R ns +	s ns 0	R ns 0	•R 2- s +	s +
(3) 18	R s +	R ut 0	R ut +	R ns +	s ns 0	•R 4- s 0	•R 2- s +	s s
(3) 19	R s +	R s +	s ns 0	R ns +	s ns 0	•R 4- s +	R ns +	
20		R s 0	s ns 0	R ns +	s ns 0	•R 2- s +	R s +	
21		R s +	•R s +	R ns +	s ns 0	R s 0	R s +	
22		R s +	R s 0	R ns +	s ns 0	•R 2- s +	•R 2- s +	
23			R ns 0	R ns +	s ns 0	R s +	•R 2- s +	
24			R s? 0	R ns +	s ns 0	•R 2- s +	•R 2- s +	
	17 lp+ 1 lpR	12 lp+ 8 lpR	13 lp+ 8 lpR 3 lps	23 lp+ 1 lps	24 lps	17 lp+ 7 lpR	22 lp+ 2 lpR	
40	{6 2- / 1 4-} Lp+		15 seg (more [illegible]) 9 2- < 8 lp+ 1 lp+; 2 2-4- 3 lp+	0 seg	0 seg	17 seg; 12 2- < 11 lp+ 1 lp+; 2 4- < 1 1; 1 2-4 lp?	22 seg (more [illegible]); 2 4-; 11 2-; 1 2-4-?	

Σ 35 lp+ 1 lpR

Thrown out before testing →

ut = no test
ng = no growth
+ = lp+, wk
0 = lp-
R = res
S = sens

Test for s or ns is based on elimination of [illegible] phenotype
no (-) = ns
(-) = s

50

DATE: 11/19/55 REF: 376

	1	2	3	4	5	6	7	8	9	10

Crossing over in 1-/4- PE. also ary if possible

A 368-1 overnight bu from single PE (-1 col. Inc. 9:50 AM → 2:20 PM

B 368-2 10² - 10⁴ - 10⁶ - 10⁸ → 10 0.05 ml sample
↓
0.05 ml
samples for lac-assay

		(+)	(−)	total			+	(−)	total
368-1	1.	0	2	75	368-2	1.	1	3	82
	2.	0	2	94		2.	5	0	~~II~~ 50
	3.	1	3	96		3.	4	0	69
				265/3 = 89					201/3 = 0.67/2 = 0.34

Plating (+) (−) total / Wash.

1. 7 5 483 0 340 375 ← NOTE change in ratio
2. ng
3. ng ng
4. ng ng
5. ng ng
6. 0 ca300 ca600
7. 0 " "
8. ng
9. ng.
10. 0 549 549

multiplicity: 0.15/sample

(+) (−) total
1. ng
2. 0 ca400 ca800 -
3. 0 8 794 -
4. 0 1 379 -
5. 346 0 ca. 500
6. 0 0 297 -
7. ng
8. ng
9. 0 0 208 -
10. ng

Rpt. on above culture following day. Expt. done by spotting 0.05 ml B. gal and inc. 5.5 hrs.

(+) (−) total
1. ng
2. 0 6 42
2. 0 5 314
3. 0 2 71
4. 0 226 229
5. 0 0 2
6. 0 0 9
7. 0 0 51

		(−)	(+)	Total
Ratio	1.	10	0	65
Parent	2.	17	0	108
Cult.				173

DATE: 11/30/55 REF:

Two strand co.?

2279 × 241-14 wal- overnight 2279 1-10 → 0.5ml + 0.1ml HFT 2', inc. 15min at Rm temp.
dil 0.2 + 10, 0.2 + 10 0.2 + 10 → 0.1ml / B gal plate

Plate	Gal+	Gal- center	Gal-	total
1	1	6	127	134
2	0	3	124	130
3	1	1	134	136
4	1	1	135	137
5	0	4	123	127
6	0	3	116	119
7	0	1	132	133
8	0	1	141	142
9	0	0	153	153
	3	20		1211

0.019 = ca. 2%
1281) 23.00
1211
10890

1st strk shows lysis? +1 → 4 ← one of the (-) col. showed + in streaking

10 colonies from the primary streak tested HFT /2', 1'

+ = yes
0 = no

1st strk shows lysis?		2'	1'	lp summary: 10 lp+ all centers		
0	Gal-cl 1	all	Gal-	10	0	0
+	2	"	"	10	0	1
+	3	"	"	2	8	0
+ (little)	4	"	"	10	0	0
0	5	"	"	10	0	0
+	6	"	"	10	0	0
+	7	"	"	10	0	0
+ (little)	8	"	"	10	0	0
+	9	"	"	10	0	0
+	10	"	"	10	0	9
+	11	"	"	10	0	1
+	12	all	Gal-	10	0	0
+	13	"	"	10	0	0
0	14	"	"	10	0	0
+	15	"	"	10	0	0
+ little	16	"	"	10	0	0
+	17	"	"	10	0	0
+	18	"	"	10	0	0
+	19	"	"	10	0	0
				10	0	0

2 cultures labelled #4
no #7
use in order: 2 = 2, 3 = 3, 4 = 4, 5 = 5, 6 = 6, 7 = 6, 8 = 8

1st strk shows lysis		10 colonies 5 Gal+	5+ 5- 5 Gal-
0	Gal+cl 1	5R 5 lys+	5 lp+
0	2	5R 5 unlys+	3 lp+ (all)
+	3	5 lp+ (wk?)	2 lp+ strng 3 lp+
+	4	5 lp+ (wk?)	5 lp+ (2 strng)

DATE: 11/30/55 REF:

$7^-/7^- \xrightarrow{Gal+} 7^-$; also lysogenization of 2308 by HFT 7 (309-1) does it give more Lp^4/Lp than 142λ?

1. Same procedure as on 376 c̄ 2279 × HFT (2⁻ 241 #4 wal-).

2.

Mate	Gal-λ curl	Gal-	Total
1.	1	35	36
2.	1	29	30
3.	2	37	39
4.	1	37	38
5.	2	26	28
	7		171

171) 7.00 = 0.04 = 4%

3. From each primary streak 10 colonies picked and tested, 2279, 142λ.

	Isolate 4 /2279	/λ	2 /2279	/λ	3 /2279	/λ	#4 /2279	/λ	5 /2279	/λ	6 /2279	/λ	7 /2279	/λ
1	ne	s	contam.	s	ne	s	ne	s	ne	s	ne	s	ne	s
2.	ne	s	ne	s	• ne	s	• ne	s	ne	s	ne (contam)	s	ne	s
3	contam	s	• ne	↓	• ne	s	ne	s	ne (Gal+)	s	ne	s	↓	s
4	ne	s	↓	↓	↓	↓	ly s	R	ne	s	ne	s	↓	s
5	ne	s	↓	↓	• ↓	↓	ne	s	ne	s	ne (contam)	s	↓	s
6	ne	s	↓	↓	↓	↓	↓	s	ne	s	ne	s	↓	s
7	ne	s	↓	↓	• ↓	↓	↓	s	ne	s	ne	s	↓	s
8	ne	s	↓	↓	↓	↓	↓	s	ne	s	ne	s	↓	s
9	ne	s	↓	↓	↓	↓	↓	s	ne	s	ne	s	↓	s
10	contam	s	↓	↓	↓	↓	↓	s	ne	s	ne	s	↓	s

ne = non lys
s = sens
contam. contaminated with λ

• papillating, mucoid

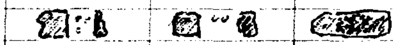
DATE: 12/7/55 REF:

1	2	3	4	5	6	7	8	9	10
W2892	tested this day (298-8 ← K12:λ → 4ª/4ˢ) 304A-1								
	9 colonies tested / λ all resistant.								
	10 colonies tested, all found not segregating.								
W2890	tested (298-8 = 2+ 4- 4ˢ from (257c-4?))								
	against + 2- 4- 4ˢ								
					7ª				
					7ᵃ				
					7ᵃ				
					7ᵃ				
					↓				

10 20 30 40 50

Rpt. titer of 269-1 lysate and attempt to recreate a 269-1 strain.

1. Retest of lysate die with — 10^2 (dilution) 10^4

	3	4	5
/2341			
	mucoid rest.	mucoid rest.	ca. 1000 minute plaques
/1412			
			few scattered plaques with lysis
/2279			

2. 2341 X — LFT K12 Gal+

1. Control ca. 30
2. Eff(0.1ml) 300

B 6 Colonies picked purified one strain – lysate made, UV (45") of overnight unaerated cult in broth. Inc. overnight in rotator.

Colony →	1	2	3	4	5	6
Seg.?	yes +	yes + lost in centrifuge	yes ++	yes +	yes +++	yes (+)
Supernat. of overnight cult. lyses 1412?	NO	—	NO	NO	NO	NO
Lysate strkd						
/2341	no lysis	—	no lysis	no lysis	no lysis	no lysis
/1412	no lysis		no lysis	no lysis	no lysis	no lysis
/2279	no lysis		no lysis	no lysis	no lysis	no lysis

DATE: 12/14/55 REF:

2341 X — HFT + from 750CK12-1

1. overnight 2341 ~~[illegible]~~ 0.4 ml + 0.1 ml HFT (+) → 2341 streaked out 3 gal, 10 colonies picked, tested against λ: 8 Lp^{s}/s, 2 Ly^{v}

↓ 0.1 ml plated undiluted for control ca 20

↓ dil 10^{2}, 10^{4} 10⁵ 0.1 ml

	Gal(+)	Gal-lysog	Gal-
1.	0	0	1/2 = 117 1st plate
2.	1	2	1/2 = 130 1st plate
3.	1	4	368
4.	0	2	373

2. The Gal+ streaked out — 6 + and 6 - colonies streaked / λ, / 2279

		λ ①	2279	λ ②	2279
Gal+	1.	r	ne	r	ne
	2.	r	ne	r	ne
	3.	r	ne	r	ne
	4.	r	ne	r	ne
	5.	r	ne	r	ne
	6.	r	ne	no test	
Gal-	1.	r	ne	r	ne
	2.	r	ne	s	ne
	3.	r	ne	r	ne
	4.	r	ne	r	ne
	5.	s	ne	s	ne
	6.	r	ne	r	ne

These clones do not carry a λ particle? Test entire mass.

from stock cult. collection: 2234 for reciprocal cross 373, 375 × Hfr heterogenotes

0.1 ml 10^{-4} dil HFT Gal(+) 750CK12-1

ca. 3000 plaques.

3 small papillae — apparently not to be segregating

Testing λ gal prior method to study segregation in P.C. clone - scoring both Gal+, Gal-. Also complete recovery

1. Gal+ readily scored 1st day.

1. After 2-3 days P.C. colonies Gal+, Gal- supposed colonies dark, but with light edges. Pick some to check.

18 picked — 16 pure —, 2 mixed +, ∓, mostly —

DATE: 12/14/55 REF: 381

Irradiation of HFT (+) from lysate 780 LKi2-1 — 5080 × 10² × 10² = 5.1 × 10⁷

Plated / 2308

1. Lysate diluted 0.1 ml + 10 ml D(0) – Irradiated UV 50 cm → 0.1 ml added 10 ml Pen

	Dose	Dil*	plaques 0.05 ml	λP/ml .05	free phage/plate (.05 ml)		phage/ml .05		RPT phage/2308 undiluted 0.1	
prelim. assay > 10³ plaques	0	1-10	254 (1 hr in ice)	2540	17	4	170	(80)	45	(30)
	10	1-10	274	2740	22	8	220	(160)	86	(71)
	20	1-10	174	1740	16	3	160	(60)	135	(120)
	30	1-10	135	1350	21	8	210	(160)	229	(214)
	40	1-2	777	1554	77	64	154	128	213	(198)
	50	1-2	511	1022	94	81	188	224	290	(275)
	60	1-2	197	394	56	43	112	172	257	(242)
	70	1-2	147	294	74	61	148	244	382	(367)
	80	1-2	123	246	78	65	156	260	422	(407)
prelim. assay 10² plaques	90	1-2	156	312	117	104	234	416	421	(406)
	Cell control = 0 plaques.	* 0.1 ml lys + 0.9 ml 2308 cells (10⁹/ml) or 0.5 ml + 0.5 ml.			control: 13				15	

312/300

DATE: 12/14/55 REF: 382

Analysis of Some P.E. clones. Gal₁⁻ // Gal₇⁻

1. Culture 307-1A. 21 Gal+ obtained from 21 separate P.E. Gal⁻
5 apparent stable Gal+ - Lysates made of apparent stbl (+) - Dilute lysates made by mixg. overnight cult in saline adding 0.1 ml to 75 ml Pen - incub 3 hrs at 37
16 seg Gal⁻.

2A The Gal⁻ / HFT I⁻ / HFT II

	/HFT I⁻	/HFT II
1	0	0
2	0	0
3	+	0
4	+	0
5	0	0
6	+	0
7	0	+
8	0	0
9	+	0
10	— P.E (-)	——
11	0	+
12	0	0
13	0	0
14	0	0
15	0	0

No. Stbl +	stbl B Gal *	Lysate cleared
1	seg	no
2	n seg	yes
3	n seg	yes
4	n seg	no
5	n seg	yes

* stbl on cult used for lysate

2 Gal₁⁻
4 Gal₇⁻
8 Gal₁⁻ Gal₇⁻

HFT I⁻ — × Gal₇⁻ 2708

2. Culture 307-1C 20 Gal+ obt. from 20 sep. P.E. Gal⁻
4 apparent stbl +
16 seg Gal⁻

The heterogenote is Lp^{2}/Ly_s

As above ↑

Stbl +	stbl Dgal	lysate clear	Gal Stabled Tested /λ
1	n seg	no	s
2	n seg	no	s
3	n seg	no	s
4	seg	no	r

2B The Gal⁻ / HFT I⁻ / HFT II

	/HFT I⁻		/HFT II
1	0	Ly s	0
2	+	s	0
3	0	s	+
4	+	s	0
5	0	s	0
6	0	s	0
⊙ 7	+	s	0
8	0	s	0
9	0	s	0
10	0	s	0
11	0	s	+
12	0	Ly^r	+
13	— PE (-)		——
⊙ 14	+	s	0
15	+	s	0
16	+	s	0

cont'd next page

3 Gal₁⁻
6 Gal₇⁻
6 Gal₁⁻ Gal₇⁻

A

B

DATE: 12/19/55 REF: 383

$Ly^+ \otimes Ly^+ \rightarrow Ly^R$ or Ly^S ?

811 X 2035 - Using replica plating to test.

1. The parents.

	no cells	no colonies not growing post UV	
W2035	51	9	20 random streaked/2279
	54	12	
	30	8	
	39	7	
	47	8	
	221	44	
W811	75	0	20 random streaked/2279
	85	2	
	73	8	
	76	0	
	62	6	
	88	5	
		21	

+ = Lyss
0 = no Lyss

	2035		811
1	+	R	+
2	0	R	all
3	0	R	+
4	0	R	↓
5	0	R	
6	0	R	
7	0	R	but
8	0	R	only
9	0	R	weakly
10	+	R	so
11	0	R	
12	0	R	
13	0	R	
14	0	R	
15	0	R	
16	0	R	
17	0	R	
18	+	R	
19	0	R	
20	0	R	20.
21	2 ly+0		—
	18 ly sorR		

one of these became 2035pt = W3171

Rpt. Test of 323-2. posture effect heterozygotes 7- × 1-

24 presumed P.E. (-) picked → 21 → 21 Gal+ → 8 seg → 1 of the 8 segregants obtained is P.E.(-)
↓ 13 Gal+ stbl.

Obtained after two single colony isolations. Indicates

(-+ → +- / -+ → -+)

Seg.	/1	/7
1.	0	0
2.	+	0
3.	0	+
4.	0	0
5.	0	+
6.	0	+
7.	0	0

1 7-
3 1-
3 1 7-

383B

DATE: 12/26/55 REF:

Yield from LFT culture.

1. Irradiated 2580 (F- Gal₂⁻) —× W3005 (F- Gal₁⁻ Gal₄⁻)

2. Expt. (Prelim. expt showed feasibility of expt. Irrad. survivors used as starting culture this expt.)

1. 2580 ca 5×10⁸/ml → centrifuged, resusp. saline - 40 seconds UV.
↓
spread for control = 109

2. 3005
↓ spread for control
= 0

3. Post irrad. → spread.
↓
5.0ml + 9ml Pa
Incubate 37C

(margin: prepared plates)

Time	pap/plate 0.1ml
10	247
20	227
30	230
40	198
50	354
60	390
70	369
80	579
90	546

0.1ml

		/0.1ml
1.	Zero	697
2.	"	585
3.	"	704
4.	"	ca 700
5.	Respread after 2 hrs	574
6.	"	719
7.	"	746
8.	"	ca 600

2686 / 4 = 672

2639 / 4 = 658

Expt. 2/10/56 — 4/5 :

DATE: 12/29/55 REF: 385

Allelism – Selfing.

Crosses of Hfr × F⁻ to establish that selfing does not give Gal⁺ recombinants.

1. Technique. Overnight Pen cultures → 1.0 ml [parent] + 1.0 ml [parent]
   ↓
   7 ml Pen → incubate 3 hours.
   0. Pl. B (Gal) for reversion
   1. dilute 10^2, 10^3 → plate 0.05 D(o)
   2. plate 0.05 ml undiluted M (Gal).

2. Cultures.

	Hfr	F⁻
Gal_1^-	2345	2958
Gal_2^-	2694	945
Gal_4^-	2487	1405

3. Expt. 1 –

		D(o) 10^3 0.05 ml	D(o) 10^2 0.05 ml	M Gal
(A) Gal_1^- × Gal_1^-	1.	22	192	80 Gal⁻
control spreads	2.	22	ca 200	80 Gal⁻
OK	3.	18	ca 200	ca 100 Gal⁻
		Ave. 21		
		= 420×10³ = 4.2×10⁵		
		prototrophs.		

(B) Gal_2^- × Gal_2^- – both parents contam. c̄ Gal⁺

(C) Gal_4^- × Gal_4^- apparently 2487 not pure Hfr population (Other lab people noticed this recently)

control spreads	1.	0	0
OK	2.	0	1
	3.	0	2
			Ave 1
			= 20×10²

Expt. 2

(A) Gal_2^- × Gal_2^-	1	—	60	1	0
both parents picked	2.	—	74	2	0
from single (–) on B gal	3	—	65	3	0
			199/3 = 67		
			134×10³ = 1.3×10⁵		

Pla[illegible] (–) [illegible]

Expt. 3

new stocks prepared 387 ↗

(A) Gal_6^- × Gal_6^-	1,	73	0
	2,	72	0
Controls B gal	3,	91	0
OK		236/3 = 79	
		158×10³ = 1.6×10⁵	

↘

(B) Gal_7^- × Gal_7^-	1,	72	0
	2	59	0
controls	3,	39	0
B gal		170/3 = 57	
OK		1.14 × 10⁵	

DATE: 12/30/55 REF:

1. Phage content of some lys^R cultures - Cultures grown up and centrifuged. The supernatant centrifuged, and shaken with chloroform.

Lysate spotted on 2279 + B gal

1.	1924	no plaques
2.	2035 lys^R	contam c (+) Gal
3.	1898	no plaques
4.	1027	contaminated c Gal(+)
5.	2587	no plaques

} Redo - make sure

Segregation of lys^0 from -x lys^s transductions.

1. Culture 373-3 grown in Pen - diluted and plated on B gal
2. Second day replicated to B(0) spread with 2279. Irradiated UV 25 seconds.

Plate	Gal(+)	Gal -	no. not lys^+ on 2279
1.	179	13	0
2.	153	13	0
3.	221	11	0
4.	194	8	0
5	186	13	0
6.	231	10	0
7.	225	6	0
8.	189	8	0
	1578	82	0

2nd Expt

ca 300 per plate ca 20-30 0

X20 plates = 6000 colonies total

DATE: 1/2/56 REF:

To obtain Hfr Gal₆⁻, Gal₇⁻

W2252 exposed to Hft 7⁻, 6⁻ on B(o). The spots streaked out.

				Hft / +	/6⁻	/7⁻	
1.	HfR 6⁻	2 gal⁻	obtained	+	0	+	λ^s weak
				+	0	+	λ^s weak
	Hfr 7⁻	2 gal⁻	obtained	+	+	0	λ^R
				+	+	0	λ^R

2. Test cross to see if Hfr. See page 385

$$\text{Hfr Gal}_6^- \text{ Lp}^s = W3056$$

$$\text{Hfr Gal}_7^- \text{ Lp}^{R \, or \, +} = W3057$$

2236 In search for HFT 3⁻

2236 ← LFT 750 Gal, prep 11/30/55

0.1 ml gave ca 60 small papillae on Bgal after 2 days

① 12 Gal+ papillae picked - all found stable after 2 streaks -
② 14 Gal+ papillae - - - - - - -

3, 14, 19, 18, 12, 7

0/70 seg { no HFT 1⁻ / no HFT 8⁻ }

B

I 14 additional obtained, no record of order - 2 contam E(+), 12/12 LFT

1. LFT 2. LFT 3. contam. 4. LFT 5. no seg. 6. HFT 750 (NO) [mixed?] 7. LFT 8. LFT 9. LFT
10. LFT 11. LFT 12. LFT 13. LFT 14. LFT 15. LFT 16. LFT 17. LFT 18. LFT
III 19. LFT 20. LFT

1210 ← 750λ prep 11/30/55

C

0.1 ml = ca 30 pap. small after 2 days

1. 20/22 colonies segregating - from the 2nd streaking 20 (-) picked, tested for HFT / 750, 1210

I Unless otherwise stated LFT results apply to both indicators

1. LFT 2. LFT 3. LFT (contam) 4. ::750 LFT 1210 5. contam. 6. LFT 7. LFT 8. LFT 9. LFT
10. LFT 11. LFT 12. LFT 13. LFT 14. LFT 15. LFT 16. LFT 17. contam. 18. LFT
19. LFT 20. LFT

2. 20 + pap. restreaked

II [illegible]

1. Contam. 2. LFT 3. LFT 4. wk Rx /750 5. no seg. 6. no seg. 7. contam. 8. wk Rx /750 9. LFT
10. LFT 11. wk Rx /750 12. no seg. 13. wk Rx /750 14. contam. 15. wk Rx /750 16. no seg. 17. wk Rx /750 18. /750 wk Rx
19. wk Rx /750 20. contam.

III N.S.: [illegible]

1. — 2. — 3. LFT 4. — 5. — 6. ~~LFT~~ 7. — 8. — 9. —
10. — 11. LFT 12. LFT 13. LFT 14. LFT 15. — 16. — 17. — 18. —
19. LFT 20. —

DATE: 1/10/56 REF: 388

2341 X 230c lg 3½ - lys/s xF⁻ 4 - lys

1. 1.0 ml of each parent to 10 Pen - grown ON. 4 times,
2. .05 ml plated MGAL
3. Colony counts 3 days:
1. 38 Gal+
2. 46 Gal+ } all small
3. 35 Gal+

Lys lysogenic
ml = non-lysogenic
R = resistant
S = sensitive

4 40 Gal+ picked - streaked once - isolated (1) taken, tested / λ/λ; for seg.

	/300S		↗ REF	seg ?1%	Sup	½	/4	
1.	nl	nl	n	n	+ = yes	o	—	
2.	nl	nl	n	n	+	+	o + R	lyr
3.	nl	nl	n	n	o = no	o	—	
4.	lys	lys	n	n	o	o	—	
5.	lys	ly	n	n	o	o	—	
6	nl	nl	n	n	o -	o	—	
7.	nl lys (pl.)		n	n	o	o	—	
8.	lys lys (pl.)		n	n	o	o	—	
9	lys	lys	n	n	+	+	o + R	lyr
10.	nl	nl	n	n	+	+	o + s	lyr
11.	lys lys (pl.)		n	n	o	o	—	
12	nl	nl	n	n	+	o	—	
13.	nl	nl	n	n	+	+	o + s	lyr
14.	lys	lys	n	n	o	o	—	
15.	nl	lys	n	n	o -	o	—	
16.	nl	nl	n	n	o -	o	—	
17.	nl	nl	n	n	+	o	—	
18.	nl	nl	n	n	o -	o	—	
19.	nl	nl	n	n	o -	o	—	
20.	ly	nl	n	n	+	+	o o s	lyr
21.	nl	lys	same	n	o -	o	—	
22	lys	nl	n	same	o	o	—	
23.	nl	nl	n	n	o -	o	—	
24.	nl	nl	n	n	o -	o	—	
25.	nl	nl	n	n	o -	o	—	
26.	nl	nl	n	n	+	+	o o? s	lyr
27.	nl	nl	n	n	+	+	o o s	contam. lyr
28.	nl	nl	n	n	+	+	o + sl/s	contam. lyr
29.	nl	[illegible]	n	n	+	+		
30.	lys	lys	n	n	+	o	—	
31.	nl	nl	n	n	+	o	—	
32.	nl	nl	n	n	+	o	—	
33.	nl	nl	n	n	+	+	o + s	lyr
34.	[illegible]		n	n	+	+	o o R	lyr
35.	lysogenic		n	n	o	o	—	
36	nl	nl	n	n	o -	o	—	
37.	non-lys	nl	n	n	+	o	—	
38.	lys	lys	n	n	+	+	o +? R	lyr
39.	lys	lys	n	n	+	o	—	
40	[illegible]				+	+	o + R	lyr

despite the order of appearance of lys clones are generated

order lysogenized / order lysogenized / retested

conj. lys / lys / 13 lys

26 lys < 18 / 13 lys < 43 / 1 lys < 5 | 21+ / 19 0

388A

DATE: 1/15/56

REF: MLM 360-2, 368-2

	0	1λ	2λ	No. clones	4 ke
A	–	⊕	–	4-	1.4-
	∓	⊕	∓	4-	
	–	⊕	–	4-	
	–	⊕	–	4-	
all λR	–	–	–	1.4- ①	
	–	–	–	1.4- ②	
	–	⊕	–	4-	
	∓	⊕	–	4-	
	∓	⊕	–	4-	
	–	⊕	–	4-	
	–	⊕	–	4-	
	–	–	–	1.4- ③	
	+/+	+	–	+	
	–	⊕	–	4-	
	–	(–)	–	1.4- ④	
	–	⊕	–	4-	
	–	⊕	–	4-	
	–	⊕	–	4-	
	–	⊕	–	4-	

Retests:

①	+	0	+
②	+	0	+
③	+	0	+
④	+	0	+

4 Gal1-

18 14 [illegible] 4 [illegible]

$Gal_1^- \times Gal_4^-$

I

Separate Gal- segregants obtained from a S.E. clone without going through Gal+

{14 4-; 4 1-}

II

368-2, all λ^3

	0	1λ	2λ	
1	–	–	±	④
2	–	–	–	①
3	–	–	–	①
4	–	–	–	wk 1-
5	–	–	–	②
6	–	–	–	①
7	–	–	–	0
8	–	–	–	②
9	–	–	–	①
10	–	–	–	②
11	–	–	⊕	⑧
12	–	–	–	①
13	–	–	±	②
14	–	w⊕	+	[illegible]
15	+/-			
16	–	–	⊕	
17	–	–	±	④
18	–	⊕	–	Gal4-
19	–	–	±	④

$Gal_4^- \times Gal_1^-$

Significance here is that the only 4- obtained is ly_3^-

Can indicate that the exogenote is ly_3?

$ly^3/ly_3 = \lambda^{-3}$,

HFT4

lysate wash

New prep being made

III

As above

368-1	/1		/4 Gal
1.	0	3	+
2.	0	0	+
3.	+	0	0
4.	0	3	+
5.	0		
6.		4	+
7.	0	2	+
8.	0	1	+
9.	+	0	0
10.	0	2	+
11.	0	2	+
12.	0	7	+
13.	0	3	+
14.	0	1	+
15.	0	1	+
16.	0	2	+
17.	0	3	+
18.	0	2	+
19.	0	1	+
20.	0	4	+
21.	0	1	+
22.	+	0	⊕ now +
23.	0	4	+
24.	0	6	+

$Gal_4^- \times Gal_1^+$

here - indicates Σ homogenote

{21 1-; 3 4-}

DATE: 1/12/55 REF: 389

For the purpose of obtaining F⁻ TLB₁ ≡ Gal₇⁻/Gal₇⁻ for cross with Gal⁺ Lp⁵

1. 307-1A, a P.E. heterogenote of 1+7⁺/1−7⁺ composition taken and Gal − segregant from this clone tested for HFT production

These are separate pos. seg. events

P.E. cl → 1 Gal − seg.

Seg	/1	/7	/+	HFT 7/2550
1	+	0	+	0 = no
2	+	0	+	0
3	+	0	+	0
4	0	+	+	0
5	0	+	+	0
6	+	0	+	0
7	0	+	+	0
papillating 8	+	0	+	0
9	+	0	+	0
10	+	0	+	0
11	+	0	+	0
12	+	0	+	0
13	0	+	+	0
14	+	0	+	0
15	0	+	+	0
16	+	0	+	0
17	0	+	+	0
18	0	+	+	0
19	+	0	+	0
20	0	+	+	0
21	0	+	+	0
22	0	+	+	0
23	0	+	+	0
24	0	+	+	0
25	+	0	+	0
26	+	0	+	0
		14 7⁻		0/26
		12 1⁻		

1/20/55

Additional 27 seg tested against 2550 - unclassified

389-1

1/22 HFT against 2550 - control spot Gal − = 389-1

Test further to see if 1⁻, 7⁻ (Gal₁⁻)

See 415

DATE: 1/25/56 REF: 390

1	2	3	4	5	6	7	8	9	10

To fill up ms table (again!) Observations on HFT 7⁻, 6⁻

1. 309-1 = HFT 7⁻ stock.

A. 12 colonies tested for HFT /2580 ⊕ ⊕ ⊕ ⊕ / ⊕ 0 0 0 / ⊕ ⊕ ⊕ ⊕ — ⊕ = HFT, 0 = LFT

LFT seg = 9 { /1 0, /+ + } = 1/9 LFT 7⁻

← may be closely related

LFT seg revertants 0/2 LFT Gal+[R] segregating at 3rd streak

Make indicators for lysylink

ALSO ENTER STOCK BOOK

MAKE LYSATE HFG⁻

2. 311-2 = HFT 6⁻ stock

A. 8 colonies /2580 0 0 0 0 / ⊕ ⊕ 0 ⊕

LFT seg revertants = 0/3 segregating at 3rd streaking

Preparation of Stocks for Kalckar, NIH, for study of the biochemistry of galactose fermentation

1. 2637 × HFT 1⁻, 2⁻, 4⁻, 6⁻, 7⁻

Overnight culture diluted 1-100, 0.05 ml + 0.5 ml of respective HFT stocks – subsequently (after 15 min. absorp. 37C) diluted 1-50 and plated 8 gal – 4 plates each.

2. Results. – On all plates, colonies are plaqued – probably > 90% indicating phage / cells > 1.

		total colonies	(−)	mosaic	Heterogenotes	HFT	(−)	mosaic
(A) HFT 1⁻ (2346)	1.	192	0	2	of these kept E.1	294	0	0
	2.	188	0	2		2. ca 300	0	0
	3.	211	0	0		3. ca 300	0	0 ①
	4	178	① to become 7(−) stock – initial streak showing phage	0 390-1		4. ca 300	0	② N 7 stock – initial white streak ca 20 plaque 390-7 (lys⁻/4)
(B) HFT 2⁻ (2342)	1.	330	0	0				
	2.	402	0	0				
	3	ca 300	0	0				
	4	ca 300	0 no ??	0				
(C) HFT 4⁻ (293-10A)	1	101	(1?)	2				
	2.	86	0	0				
	3.	94	0	0				
	4.	60	0	1	390-4 1st			
(D) HFT 6⁻ (311-2)	1	157	0	0				
	2.	205	0	0	6⁻ stock – 1st streak shows no λ			
	3.	201	0	2				
	4	234	0	① 390-6 this one is probably ly⁺/ly⁻				

2 repeated – no mosaics, suspicious (−), picked tested /4 /2⁻ + 0 slightly revertible? → λ[R]

SEE 396

DATE: 1/30/56 REF: 391-

1 2 3 4 5 6 7 8 9 10

The cross 2252 x 341-9
M Gal+ Hfr Gal$_2$/Gal$_2^-$ F$^-$ TLB$_1$ ≡ Lp$^+$

to see if Gal+/- Lp^R/Lp^S are generated

1. Parents grow together 3 hours (-plated not mistakenly on [illegible] SM)
2. Following day tubes (kept at room temperature) replated M Gal

Ⓐ MGal

	(−)	(+)
1.	ca. 200	ca 22
2.	..	ca 31
3.	..	ca 27
4.	..	ca 40

Ⓑ Pick Gal+ and see if seg. + Lp^R/Lp^S

		seg	non seg	Lp$^+$	Lp^S	Lp^R
1# 24	—	0 (1)	24 + 34.5	4 (16)	20 (19)	(2)

	self lysis 35° 0 = no + = yes	/λ R = none	/2677 small plaques	Genotype
1.	0 = no	R = none	+ [illegible]	Lp$^+$ wk
2.	+ = yes	self lysing	+ self lysing	Lp^s + Lp$^+$? Rec (1)
3.	0	R	n lys	Ⓛp^R Rec (2)
4.	0	S, [illegible]	n lys	Lp^s
5.	0	S	n lys	S
6.	0	S	n lys	S
7.	0	R	+	+
8.	0	R	+	+
9.	0	S	n lys	S
10.	0	R	lys	+
11.	0	R	n lys	Ⓡ Rec (3)
12.	0	R	lys	+
13.	0	S	n lys	S
14.	0	R	+ [illegible]	+
15.	0	S	n lys	S
16.	0	R	+	+
17.	0	S	n lys	S
18.	0	S	n lys	S
19.	0	S	n lys	S
20.	0	R	+	+
21.	0	R	+ [illegible]	+
22.	0	S	n lys	S
23.	0	R	+	+
24.	0	R	+	+
25.	0	S	n lys	S
26.	0	S	n lys	S
27.	0	S	n lys	S
28.	0	S	n lys	S
29.	0	S	n lys	S
30.	0	R	+ [illegible]	+
31.	0	S	n lys	S
32.	0	S	n lys	S
33.	0	S	n lys	S
34.	0	S	n lys	S
35.	0	S self lysing?	n lys	S Rec (4)

19 S
12 +
2 R 1?

DATE: 1/31/56 REF: 392

① HET from 2580

① Overnight culture diluted 1-50 incubated at 37C in rotator ca 3.4 hours
② Centrifuged, resusp. in saline, recentrifuged, resusp. saline
③ dilute 10^2, 10^4, 10^6 → 0.05 ml B gal A 1. 88 ; B 2. 83
~~Add 0.05 ml and BGalSM with 3005S^R lpt~~ ① 1. 0 ; ② 2. 0

④ irradiate 40 seconds →
⑤ dilute 10^2, 10^4 → 0.05 ml B gal C 1. ¼ ca 150 1200 ; D 2. ¼ ca 200 800
Add 0.05 ml und. B Gal SM with 3005S^R lpt trans. ③ 1. 0 ; ④ 2. 0

dilute 1.0 ml + 10 ml Pen - Inc. 37C

		Time	Plate 0.5 ml 3005S^R lpt on BGalSM	
	12:25	0	1. omit	2. omit
	12:40	15	⑤ 0	⑥ 0
plaque again	12:55	30	⑦ 0	⑧ 0
too soft - surface	1:18	45	⑨ 0	⑩ 0
plating worked	1:25	60	⑪ 0	⑫ 0
here after 0.1 ml inoculated	1:40	75	⑬ 0	⑭ 1
0.05 sample	1:55	90	⑮ 4	⑯ 1
	2:10	105	⑰ 9	⑱ 9

RPT.

1. Overnight culture diluted ca 1-50 incubated 37C - as above. After irradiation dilute 1-2 instead of 1-10

.05 ml plated — but remove samp + dilute 2x Pen

centers = .05 ml /3079 on B gal

	Pre irrad	Time	Irrad.	centers
freed	①② /3079 on SM	1:30 0 ✓	⑤⑥	⑦⑧
centers	③④ /3079 on B gal	30 ✓	⑨⑩	⑪⑫
		60 ✓	⑬⑭	⑮⑯
		90 ✓	⑰⑱	⑲⑳
		100 ✓	㉑㉒	㉓㉔
		110 ✓	㉕㉖	㉗㉘
		180 ~~120~~	㉙㉚	㉛㉜
		~~130~~		
		~~140~~		

Phage were surface plating not indicated in order of 1/30/56 on plates and B gal SM

to follow plaque production: a loopful taken out at the following intervals and spotted on 3079 on B gal SM

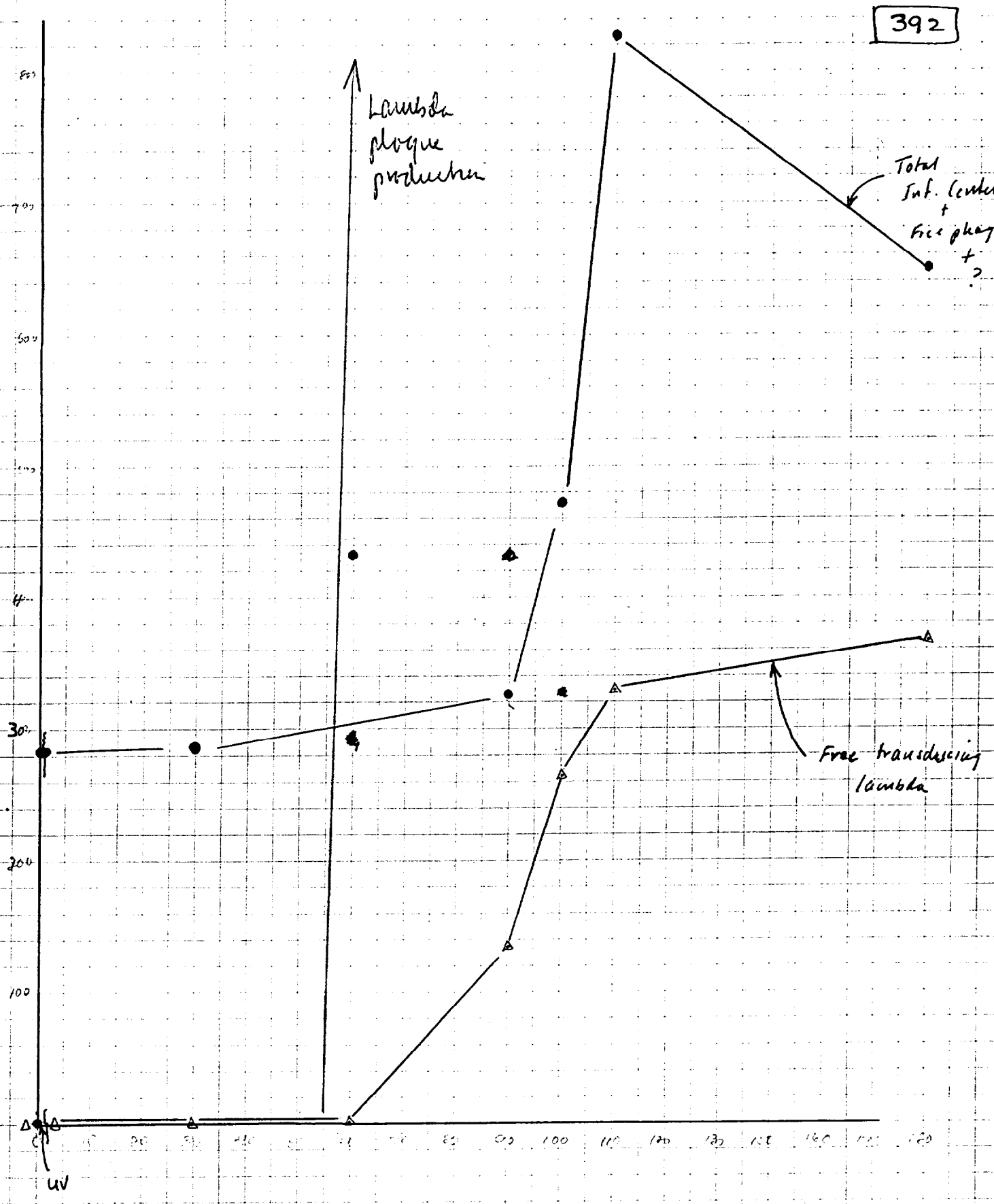
T	Spot
15	17 plaques
30	23 plaques
60	solid spot
90	" "
100	" "
110	" "

Count plaques

			count	ave			ave	
SM 1	ca 1000 pl, 6 pap (small)			3 ?	SM 7	112	134	
SM 2	ca 1000 pl, 1 pap (small)				SM 15	153		90
B 3	? conf.	9		8	B 19	271	327	
B 4	? "	7			B 20	382		
SM 5	ca 1000	0		0	SM 21	218	263	
SM 6	ca 1000	1(?)			SM 22	307		100
B 7	confluent?	252		285	B 23	472	471	
B 8	confluent?	321			B 24	470		
SM 9	ca 500 plaques	0		0	SM 25	298	328	
SM 10	ca 500 plaques	0			SM 26	358		110
B 11	con?	212		288	B 27	744	829	
B 12	–	363			B 28	913		
SM 13	conf.	3		2	SM 29	373	365	
SM 14	conf.	0			SM 30	356		
B 15	"	334		433	B 31	612	649	180
B 16	"	522			B 32	686		

reversed 3 + 4 (accidentally) ; 30 ; 60

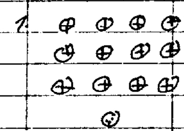
all plaques ~~confluent~~


Lambda plaque production
Total Inf. Centers + Free phage + ?
Free transducing lambda
UV

DATE: 2/11/56 REF: 393

Most of the HFT stocks obtained come from Lp^+ recipients. ~~[illegible]~~ Has there been selection for a lambda assaying on Lp^+ (high) and low on Lp^s? This is the usual experimental observation – in contrast LFT lysates assay highest on Lp^s cultures. Obtain a lysate from HFT culture obtained in Lp^s recipient. Such a culture is 364A 1 – Strain culture.

1. 13 colonies picked and tested for HFT /750

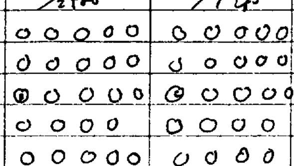


2/11/56

From EML to obtain an HFT (-) stock a culture labelled 7H -x 2961 which is lysogenic made by 7-Lp x 2-Lp^s. From the plate received 24 gal+ picked and streaked.

1. all segregated (-)
   a. the (-) were tested for HFT /2580, against 7-Lp^s stock (prepared for Kalckar study)

/2580 /7-Lp^s



lysis noted in all spots.

   b. 20 more tested with same result – all non HFT on 2580, 7-Lp- above.

From here to 412
B gal SM platings were
from method

DATE: 2/15/56 REF: 394

1	2	3	4	5	6	7	8	9	10

2580 – overnight, incubated 2.5 hrs with air. Centrifuged resusp'd in saline –

Pre-irrad. cell assay — Bgal — dil 10^6 – 0.05: (1) 107; (2) 110; $217 \times 10^7 = 2.2 \times 10^9$ — Bgal SM

" " trand assay undiluted → (3) 0; (4) 0 — 1/10 ca 300 plaques — on B gal (3a) 12; (4a) 21

+3079 — 22/13.0 = 0.59 — 11.0 — 59% survival

→ 5 ml + 9 ml 2x pen →

Irradiate 40 sec

Post irrad. cell assay dil 10^6: (5) 62; (6) 70; $132 \times 10^7 = 1.3 \times 10^9$ — λ assay Bgal (7) 26 plaques; (8) 45; 71 — $71 \times 10^7 = 7.1 \times 10^8$ plaque centers — 5/14 71 = 35.5 = 2.5×10^8

These undiluted sol. pen. w/ 2x Pen.

Post irrad. trand. assay und (9) ca 300 large 400 small; (10) " " — (11) 0; (12) 0 — almost continuous — This column B gal SM

time in 37° water bath

0 — 1:20 pm

20 — 1:40 pm — (13) 279; (14) 252; 531/2 = 266 — (15) 0; (16) 0 — 1/16 ca 300 plaques

dil 10^6 .05 + 3079: (15a) 137; (16a) 162; $299 \times 10^7 = 2.9 \times 10^9$

40 — 2:00 pm — (17) 350; (18) 397; 747/2 = 374 — (19) 0; (20) 0 — 1/10 ca 500 plaques

60 — 2:20 — (21) 419; (22) 488; 830/2 = 415 — (23) 1; (24) 3 — (21a) 163; (22a) 189; $352 \times 10^7 = 3.5 \times 10^9$

80 — (25) 312; (26) 248; 560/2 = 280 — (27) 108 (many small); (28) 81; 189/2 = 95

centrifuge 3 ml Bgal + 3079

100 — 0.05 ml + 3079 on D(0) (A) in (B) saline at 20 days — (29) 195; (30) 214; 409/2 = 205 — (31) 95 (many small); (32) 109 (many small); 204 — (29a) 57; (30a) 67 — without

120 — (33) 125; (34) 149 (150); 274/2 = 137 — (35) 68 (many small); (36) 127 (about 70 large); 195/2 = 98

dil 10^7 .05 + 3079 — (37a) 123; (38a) 139; $262 \times 10^8 = 2.6 \times 10^{10}$ plaque

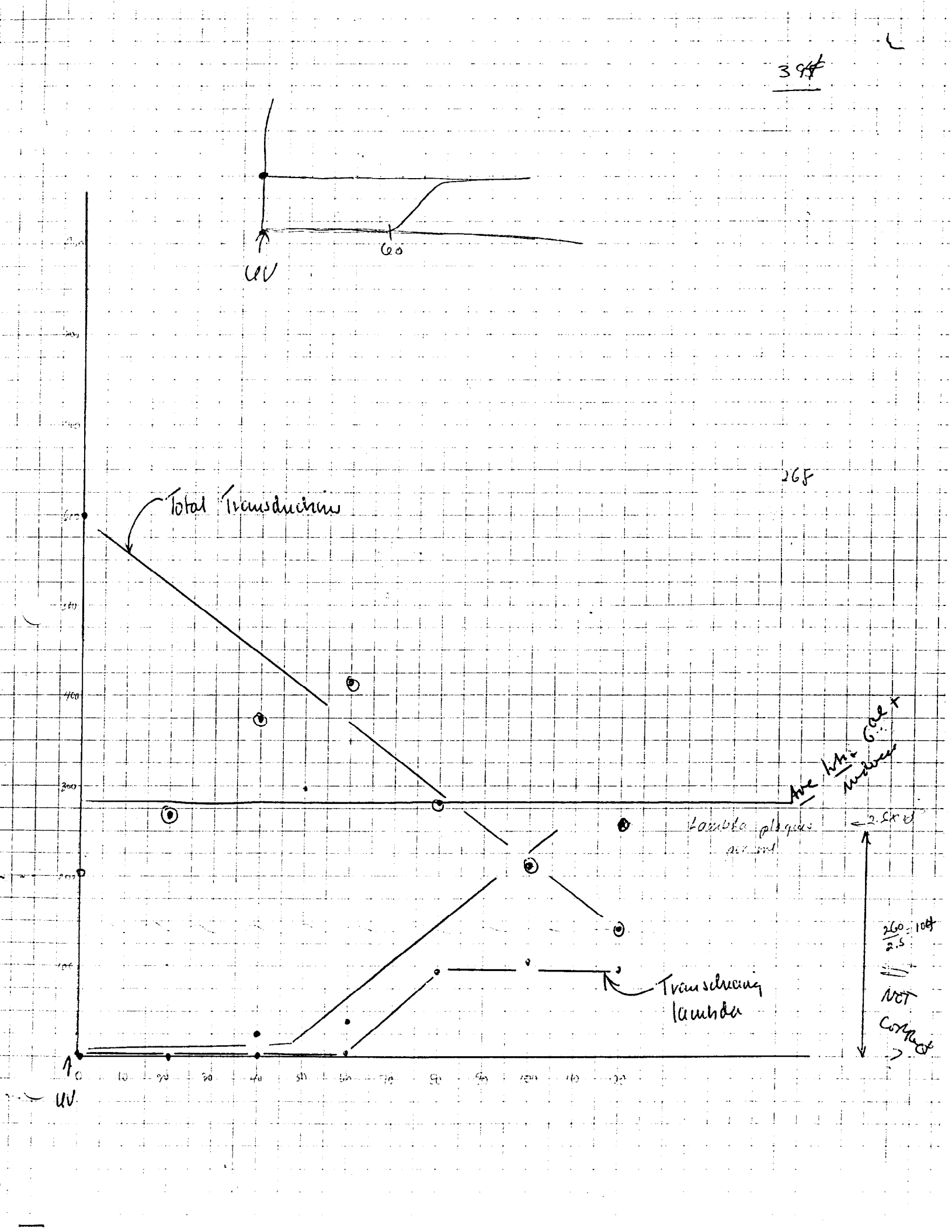
Total = 1677

Ave Total/6 = 1677/6 = 279

600 × 80 × 1/3 = 24000 = $\frac{2.4 \times 10^4}{2.2 \times 10^9}$ cells = $1/10^5$ cells

104, 95, 68; 267/3 = 99

99/279 = burst size — 279 / 99.0 = 0.36; 837; 1530


Total Transduction
Transducing lambda
UV

DATE: 2/16/56 REF: 395

1	2	3	4	5	6	7	8	9	10

1. Cross done 2/13/56 of 300 9 x 341-9
Lys Gal, Ly^v (R) TLB^s 2^-/2^- Ly^+

1. 1.0ml of each parent added 9.0ml pen, incubated 3 hours, diluted 1-100 with distilled H2O, 0.1ml

	(+)	(-)
2 (a)	x 7	ca 250
(b)	5	ca 300
(c)	5	ca 250

10^- colonies picked streaked B gal - grew up as Gal on B gal - 10 ca B one picked to D(c) - all grew therefore prototrophic
The 10^- tested against HFT 1^- HFT 2^-
10 + 0 = Gal_2^-

3 Gal + . After 2 streaks tested

	seg. ?	λ	3079		Genotype
1.	0	R	+		Ly^r
2.	0	R	+		Ly^+
3.	0	R	+		Ly^r
4.	0	R	+		Ly^+
5.	+	R	+	Ly^s	Ly^+
6.	0	R	+		Ly^+
7.	+	R	0	Ly^r	Ly^{R/S} ?
8.	0	R	0		Ly^{R/S} ?
9.	0	R	+		
10.	+	R	+	Ly^s	
11.	0	R	+		
12.	0	R	+		
13.	0	R	+		
14.	0	R	+		
15.	0	R	+		
16.	0	R	+		

(3+) 16 R 14 +
13 0 2 0

$2^+ 1^- s \times \frac{2^- 1^+ 1?}{2^- 1^+ +?} \rightarrow \frac{2^+ \; S}{2^- \; R}$

3 seg obtained	λ1	λ2	λ
5	+	0	R
7	+	0	S
10	+	0	R

DATE: 2/17/56 REF: 396

2637 derivatives from 390
labelled - after some re-arrangement, and re-arrangement

	Presumed	×3079	Growth λ(?)	λ	Genotype
W3091	Gal1- Lp+	ne	+	partially S	Lp^R
92	Gal2 " +	ne	+	R	Lp^R
93	—				
94	Gal4- Lp+	ne	+	R	Lp^R
95	—				
96	Gal6- Lp+	lysog.	+	R	Lp^r
97	Gal7- Lp+	lysog.	+	R	Lp+
98	—				
99	—				
W3100	Gal+ Lp+	lysog.	+	R	Lp+
3101	Gal1- Lp^s	~~[illegible]~~ ?			
3102	Gal2- Lp^s	~~[illegible]~~ ?			
3103	~~Gal~~				
3104	Gal4- Lp^s	— ?			
3105	—				
3106	Gal6- Lp^s	~~[illegible]~~ lys.	+	S	Lp^s
3107	Gal7- Lp^s	[illegible] lys.	+	S	Lp^s
3108	—				
3109	—				
3110	Gal+ Lp^s	[illegible] lys.	+	S	Lp^s

Frequency of homogenotes among stable (+)
LFT lysate : K12 8/10/55

1. 2580 spont. = 8
   0.1ml = 573

2. 3079 spont. = 0
   0.1ml 517

3. 750 spont. = 3
   0.1ml 356

DATE: 2/28/56 REF: 397

2580 one steps using 3077 as indicator. Survival 318 / 49.0 → 15.4%
overnight cult diluted 1-10, aerated 3 hours.

PRE-UV: dil 10⁶ - 0.05 ml: (1) 148, (2) 170, total 318 (B gal). Cell free assay: (3) 0, (4) 0 (B gal SM) — Pre transd. assay, plaque assay. Same plate. W. indicator.

$\frac{O.D.}{water} = \frac{4}{10}$ dil = 0.

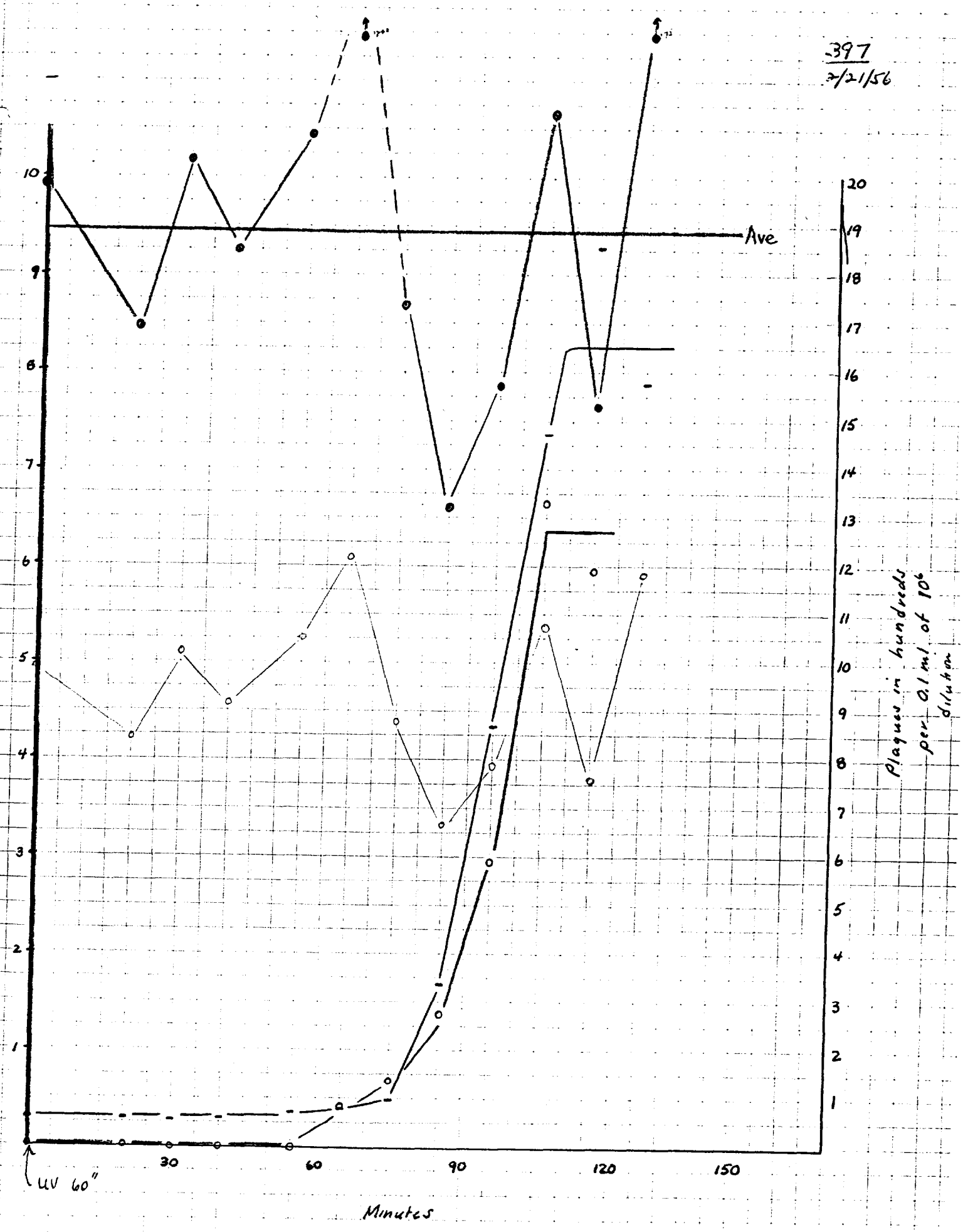
U.V. 60 seconds — add 3 ml + 9.0 ml Pen 2X

Post U.V. — not added to Pen.

dil 10⁶ — 0.05 ml	(3) 22, (4) 27 — 49	survival	(9) 58, (9) 64 — [61] B gal, for 0 plaque
0	(5) 887, (6) 1097 — 1984	transd. centers	(7) 0, (8) 0 (ca 1000 plaques free; ca 1000 transd. plaques)

dil 10⁶ — tube samples (B gal)		Time		
(9) 27, (10) 29 — 28 [56]	1:30 PM	20	(9) 476, (10) 368 — 844	(11) 0 (½:173 plaques), (12) 1 (ca 700 plaques)
(11) 32, (12) 21 — 27 [53]	1:40	30	(13) 467, (14) 552 — 1019	(15) 0 (½:195 plaques), (16) 0 (ca. ")
(13) 38, (14) 32 — 35 [60]	1:50	40	(17) 462, (18) 461 — 923	(19) 0 (ca 1000 plaques), (20) 0 (" ") (? #15, 16, etc)
(15) 33, (16) 36 — 35 [69]	2:05	50 (55)	(21) 528, (22) 517 — 1045	(23) 0, (24) 0
(17) 39, (18) 38 — 39 [77]	2:15	60 (65)	(25) 595, (26) 621 — 1216	(27) 23, (28) 18 — 41
(19) 35, (20) 63 — 49 [98]	2:25	70 (75)	(29) 427, (30) 441 — 868	(31) 29, (32) 39 — 68
(21) 109, (22) 226 — 168 [335]	2:35	80 (85)	(33) 296, (34) 364 — 660	(35) 46, (36) 91 — 137
(23) 452, (24) 413 — 433 [865]	2:45	90 (95)	(37) 388, (38) 395 — 783	(39) 136, (40) 159 — 295
(25) 772, (26) 701 — 737 [1473]	2:55	100 (105)	(41) 505, (42) 562 — 1067	(43) 313, (44) 352 — 665
(27) 960, (28) 873 [1833]	3:05	110 (115)	(45) 408, (46) 350 — 758	(47) 306, (48) 291 — 597
(29) 873, (30) 700 [1573]	3:15	120 (125)	(49) 612, (50) 563 — 1175	(51) 212, (52) 125 — 337

-397
2/21/56


Ave
UV 60"
30
60
90
120
150
Minutes
Plaques in hundreds per 0.1 ml of 10^6 dilution

DATE: 2/22/56 REF: 398

Better transduction indicators?

Lysate = 364A1 diluted 10^4 .05 ml taken

1. Effect of starving. Overnight cultures of 3079, 3080 sedimented, resusp'd in saline, sedimented, resusp'd in D(M). Aerated 6 hours. 0.1 ml used as an indicator. Broth control consisted of partly grown cultures (6 hours for both overnight unaerated) probably lower cell density than starved cells.

Plating on B gal SM

1. Plating	Pour	Surface	Broth	Starved	Counts	
3079	+	0	0	+	139	ca 1000 plaques each
	+	0	0	+	158	
	+	0	+	0	31	ca 1000 plaques
	+	0	+	0	24	
	0	+	+	0	52	
	0	+	0	+	164	
3080	+	0	0	+	200	
	+	0	0	+	222	
	+	0	+	0	65	
	+	0	+	0	149	
	0	+	?	?	138	plates not labelled
	0	+	?	?	127	

These results suggest that starved cells may be better as an indicator. Cell densities were not the same and it will be better to repeat using a density check.

DATE: 2/24/56 REF: 399

One step with 945 (culture from AR) technic and numbering as in 397 with the exceptions. Survival = 5/93 = ~5%

A. Exp.

(.05 ml samples)

1. Culture of 945 (mal- $λ_2^s$) instead of 2580 (mal+ $λ_2^s$)
2. Density of susp. (irradiated) adjusted to 0.58 "UV.-60"
3. Diluted 1-10 instead 1/12
4. Times (absolute) the same - samples taken at 50, 60, 70, 80 etc. instead of 5, 15 etc.

pre-cell (1) 44, (2) 49 — (93)
post cell → (3) 3, (4) 2 — (5)
(3) 0 (~4460 plaques) (5) 179; (4) 0 (" ") (6) 147 — 326
(7) 0 (4445 plaques) post UV free; (8) 0 (4153 plaques) free
traced centers removed — dilute 1-10

B. Results.

Time				3 Gal			B gal SM		
0	(7)	0		(9)	12		(11)	0	57 plaques
	(8)	0		(10)	15	27	(12)	0	31 plaques
20	(9)	6		(13)	27		(15)	0	71 plaques
	(10)	11	17 (17)	(14)	20	47	(16)	0	77 plaques
30	(11)	7		(17)	66		(19)	0	149 plaques
	(12)	11	18 (18)	(18)	69	135	(20)	0	146 plaques
40	(13)	8		(21)	47		(23)	0	½ = 66 plaques
	(14)	4	(12)	(22)	53	100	(24)	0	"
50	(15)	9		(25)	80		(27)	0	11? (small) almost confluent
	(16)	8	(17)	(26)	35	115	(28)		74? (small) " "
60	(17)	8		(29)	84		(31)	0	40? (small) confluent
	(18)	4	(12)	(30)	92	176	(32)	0	73? (small) "
70	(19)	8		(33)	93		(35)		1 large
	(20)	7	(15)	(34)	98	191	(36)		1 large — 2
80	(21)	10		(37)	60		(39)	22	
	(22)	9	(19)	(38)	63	123	(40)	9	31
90	(23)	12		(41)	58		(43)	28	
	(24)	8	(20)	(42)	38	96	(44)	28	56
100	(25)	62		(45)	88		(47)	78	
	(26)	23	(85)	(46)	47	135	(48)	84	162
110	(27)	74		(49)	80		(51)	84	
	(28)	84	(158)	(50)	60	140	(52)	90	174
120	(29)	184							
	(30)	145	329/16 = 20						

Transfers

Surface plating → plaques

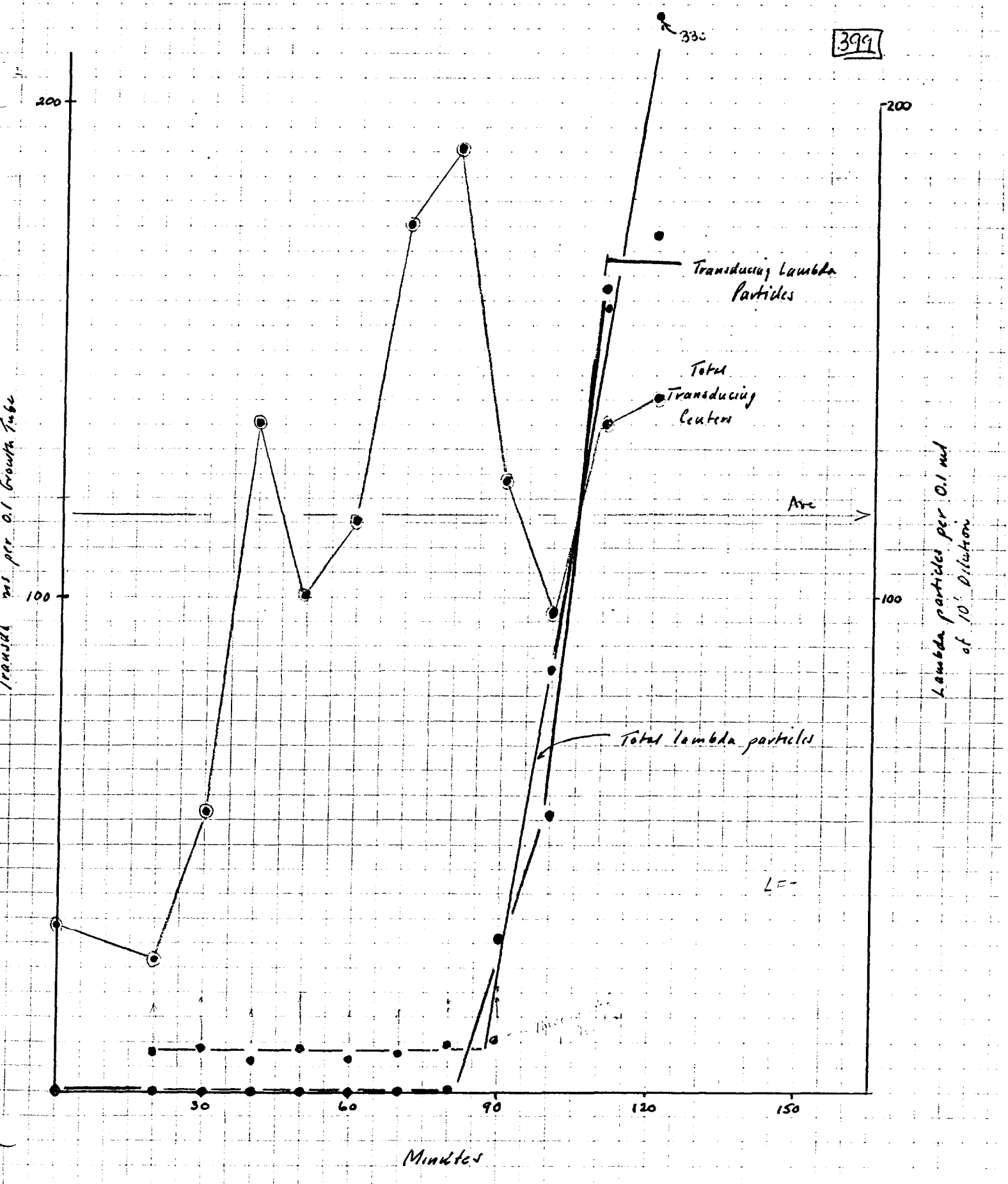
Total transd.

11 / 17.8 / 1/18

Free transd. λ

W3079

(in) circle plaques rotting


330
200
100
Transduc. per 0.1 Growth Tube
Transducing Lambda Particles
Total Transducing Centers
Ave
Total lambda particles
LF-
30
60
90
120
150
Minutes
200
100
Lambda particles per 0.1 ml of 10⁻⁷ Dilution

DATE: 2/27/56 Survival = $\frac{16}{40}$ = 0.40 REF: 400

One step with 341-9

Plaque count in red =

$\frac{45}{65}$, $\frac{42}{69}$ after med

1. Overnight culture diluted ca 1-10, incubated in rotator at 37C for 2 hours. Centrifuged, resuspd colonies to O.D. - 0.58

15/17 $\frac{18}{21}$ at 0

2. Pre assay Ⓐ dic 10^2 - 10^4 - 10^6 → 0.05 on pour for colonies ① 23 ② 17 — 40. B gal / B gal 34 — each of these 2.0 LFT. 4/40 = 10%

ASSAY THESE COL. FOR HFT →

Ⓑ transd. assay } / 3079 plaque (106 … 0.05) — 0 plaques ③ 1 ⑤ 0 0 plaques; 0 plaques ④ 0 ⑥ 0 0 plaques (transd. occurring in plaques)

3. Irradiate 60 sec

4. Dilute 10^2 - 10^4 → 10^5 — 0.05 surface for HFT. Incubate - plate 0.05 at various time intervals

10 LFT; 5 LFT

15/157 = ca 10%

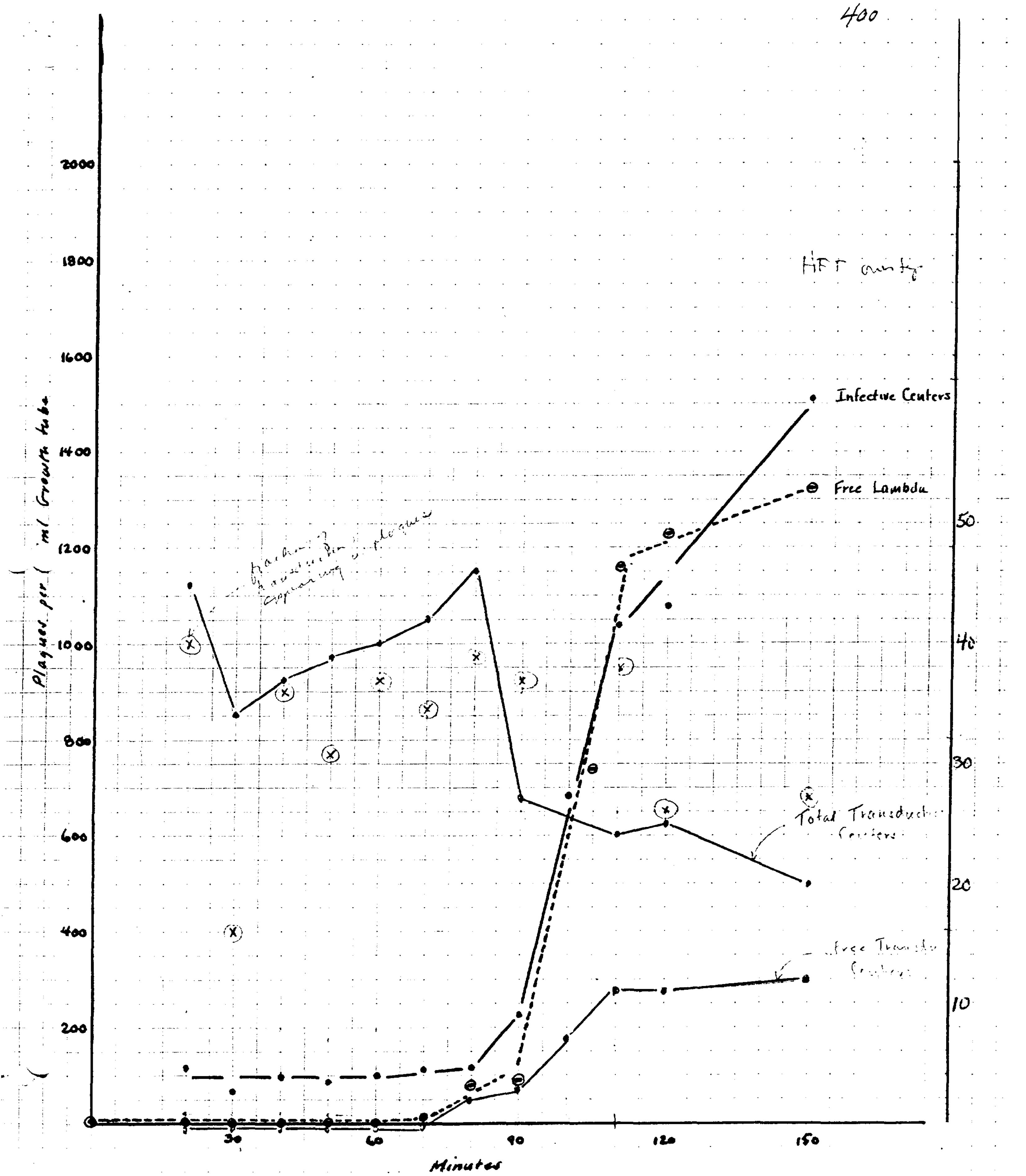
Cells disappearing, 40-16 = 24. Infect. cells producing 20' = 12; 30 = 6; 40 = 10; 50 = 9; 60 = 10; 70 = 11; 80 = 11; Ave = 57/6 = 10

(bad plate) partially sensitive on pouring both went off

Time	Min	Plates	Count	Plates	Count	Total
12:15	10	⑦ ⑧	76, 51 = 157	⑨ ⑩	0, 0	0 plaques, 0 plaques
12:35	20	⑪ ⑫	22 $\frac{13}{22}$ 57; 23 $\frac{18}{23}$ 59 (45) = 116	⑬ ⑭	0, 0	0, 0 = 0
12:45	30	⑮ ⑯	20 $\frac{4}{20}$ 27; 14 $\frac{9}{14}$ 37 (34) = 64	⑰ ⑱	0, 0	0, 0 = 0
12:55	40	⑲ ⑳	11 $\frac{10}{11}$ 42; 26 $\frac{15}{26}$ 56 (37) = 98	㉑ ㉒	0, 0	0, 0 = 0
1:05	50	㉓ ㉔	12 $\frac{4}{12}$ 32; 27 $\frac{18}{27}$ 53 (39) = 83	㉕ ㉖	0, 0	0, 0 = 0
1:15	60	㉗ ㉘	18 $\frac{9}{18}$ 50; 22 $\frac{8}{22}$ 49 (40) = 99	㉙ ㉚	0, 0	0, 1 = 1
1:25	70	㉛ ㉜	23 $\frac{16}{23}$ 64; 19 $\frac{13}{19}$ 49 (42) = 113	㉝ ㉞	0, 0	10, 6 = 16
1:35	80	㉟ ㊱	25 $\frac{17}{25}$ 55; 21 $\frac{13}{21}$ 55 (46) = 110	㊲ ㊳	1 $\frac{1}{1}$, 1 $\frac{1}{1}$	16, 66 = 82
1:45	90	㊴ ㊵	13 $\frac{11}{13}$ 103; 14 $\frac{10}{14}$ 122 (27) = 225	㊶ ㊷	1 $\frac{1}{1}$, 2 $\frac{2}{2}$	55, 38 = 93 (bad plating procedure)
1:55	100	㊸ ㊹ (plated 8 gal)	/ 264; / 412 = 676	㊺ ㊻	3 $\frac{3}{3}$, 4 $\frac{3}{4}$	435, 306 = 741
2:05	110	㊼ ㊽	7 $\frac{7}{7}$ 352; 17 $\frac{7}{17}$ 688 = 1040	㊾ ㊿	6 $\frac{3}{6}$, 5 $\frac{3}{5}$	536, 626 = 1162
2:15	120	51 52	13 $\frac{6}{13}$ 631; 12 $\frac{3}{12}$ 445 = 1076	53 54	7 $\frac{4}{7}$, 4 $\frac{3}{4}$	612, 621 = 1233
2:45	150	55 56	9 $\frac{5}{9}$ 771; 11 $\frac{6}{11}$ 740 = 1511	57 58	4 $\frac{3}{4}$, 8 $\frac{2}{8}$	656, 668 = 1324

(Crossed-out column 1 times: 0, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 150)

↑ transducing ↑ plaques


Plaques per ml Growth tube
2000
1800
1600
1400
1200
1000
800
600
400
200
30
60
90
120
150
Minutes
50
40
30
20
10
Infective Centers
Free Lambda
Total Transduction Centers
Free Transduction Centers

DATE: 3/8/56 REF: 401

1	2	3	4	5	6	7	8	9	10

Preparation of prototrophic F stocks with the gal markers – "Kalckar" stocks

Lp^+ , Lp^S , Lp^r (assumed)

1. The numbers proposed for them are 3091 → 98, 3101 → 3107, 31 → ?.
 3100 = Lp^+ Gal+
 3110 = Lp^S Gal+

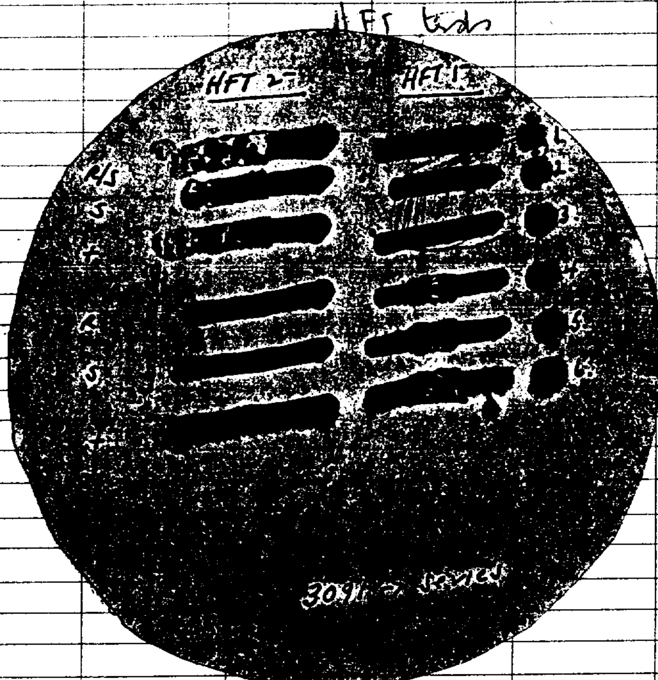
2. Beginning with 3110 = (F⁻ 1485) – the cells from an overnight culture exposed to HFT's and diluted and plated. See page 390 (also 396)

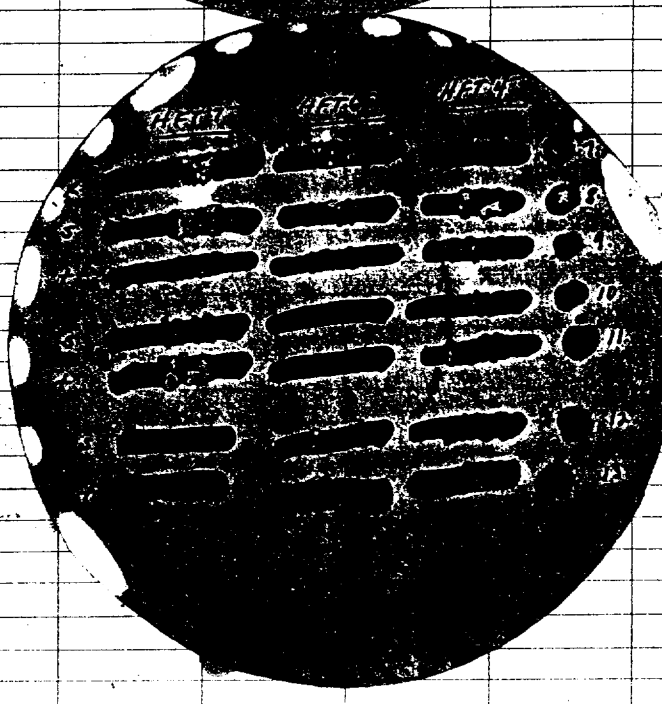
3. After all cultures obtained, they were tested:

3079		λ	Origin DTO	Original gen. designation
1.	ml	R	+	1⁻ r/s
2.	ml	S	+	1⁻ s
3.	Lys	R	+	1⁻ +
4.	ml ?	R	+	2⁻ r
5.	ml	S	+	2⁻ s
6.	Lys	R	+	2⁻ +
7.	ml	R	+	4⁻ r
8.	ml	S	+	4⁻ s
9.	Lys	R	+	4⁻ +
10.	Lys	R	+	6⁻ s
11.	ml	S	+	6⁻ +
12.	ml	S	+	7⁻ s
13.	Lys	R	+	7⁻ +
14.	ml	S	+	+ s
15.	Lys	R	+	+ +

(reverse order → 10., 11.)

HFT tests





Notes

① The Gal_2^- derivatives appear to be revertable

② The culture obtained by mad. 6⁻ Lp^r (to obtain Lp^s) appears no longer to be Gal_4^-; and the Lp^+ derived is also not Gal_4^-

Redo Gal_2^-
Gal_4^- Lp^S, Lp^+

THE CULTURES FIRST ISOLATED FOR Gal_2^- and Gal_4^- STOCKS #'s 4, 5, 6, 7, 8, 9 above given numbers 401-4, 5, 6, 7, 8, 9 etc

DATE: 3/11/56 REF: 402

Comparison of lysates from homogenote 364K1 - a homogenote obtained in a heterogenote line originating in an lys^0 recipient. If the higher titers of HFT lysates on ly^+ recipients (as compared with ly^0 recipients) is due to selection of phage particles entering ly^+ recipients (all HFT homogenotes have been obtained in ly^+ recip. lines), then there may not be higher assays on ly^+ recip. when the line originates in ly^0 recip.

1. 6 lysates made starting from single HFT colonies. (surface plated)
incub. 60 sec., incubated 4 hours
diluted 10^2, 10^4, 0.05 ml plated with 3079 ($1^-4^-ly^0 S^R$), 3080 ($1^-4^-ly^+ S^R$), on B gal S.T.

Lysate	3079 Plaques*	3079 Transduct.		3080 Transd.
1	ca. 15	2	(11)	29
2	20	7	(27)	28
3	29	4	(11)	7
4	26	3	(21)	25
5	17	5	(24)	14
6	7	5	(12)	19

It appears that HFT lysates obtained in ly^0 recip. this also assays higher on ly^+ recipients

2×10^5
17
3.4×10^5 = 3.4×10^6
[3.4×10^4]
20
1.7×10^3
20
$.8 \times 10^?$
20

$7 \times 20 \times 10^4$
140×10^4
1.4×10^6

* plaque assays low because plates were dry

$87 \times 20 \times 10^4$
3.4×10^6 / 3
1.1×10^6 trans.

Observation that Gal- grow in M Gal - test made to see what the source may be.

Comparison - using 1 proto homogenote, 1 ly^0 seg. derived from it, 2 ly^+ hom. derived in ly^0/ly^0 cross ly^- ⊕ ly^+ homogenote 341-9, and a ly^0 seg. from seg. of these 2-hom.

Medium	Obs
M (no gal)	no growth. crystals deposited along streak
M plus 0.01% glu	growth by all - colonies up to 1 mm in diam
M gal (gal sterilized by filtration)	growth by all - 2's better than ones - colonies up to 2 mm in diameter.
M gal stock	growth by all 2's better than 1's - colonies up to 2 mm

DATE: 3/13/56 REF: 403

The Kalckar stocks — Rpt.

3094, 3104 — 3092, 3102 needed.

1. Culture 390-4 = 4⁻ —x 3100 (Gal+ Ly^s) → heterogenote. This heterogenote not tested directly but previous segregants found Ly^R (see 401) indicative of this; seg. gave a non-only-Ly^s, also evidence of some phage.

2. Segregants re-obtained from 390-4. 4 picked from first streak and therefore clonally related.

	HFT(+)	HFT 4	Ly^{RX}	
1.	+	0	Sens	⇐ 403-1 = Ly^s (3104) — W3104
2.	+	0	"	
3.	+	0	"	
4	+	0	"	

Sens. genotype not expected — indicates that this heterogenote is Ly^s/Ly^s — If so where did phage and sens. come from in first expt.? Certain! what is genotype of 401-8?

DATE: 3/15/56 REF: 404

~~[illegible]~~

Irradiation of HFT lysate - lysate approx. titer 3.4 x 10^6 total λ/ml - see page 402. lysate no 5 from 364A1. Dilute the lysate 0.1ml + 10ml D(?) in adsorb and add 0.1ml samples to 1.9ml Pen. Samples (0.05ml) removed from these for the dilution and plated, by pouring on B gel with indicator.

	Dose	Plaques/3079	Transd/3079	Transd/3080	B gel SM: plaques/3079	/3080	transd/3079
1	0	314	13	101 (poor plate)	104	~~60~~	155
2	5 sec	344	34	188 ..	265	~~150~~	58
3	10 sec.	283	74	208 ..	170	~~126~~	38
4	15 sec.	205	83	256 ..	142		68
5	20 sec.	164	101	371 ..	99	~~221~~	89
6	25 sec.	151	136	378 ..	98		103
7	30 sec.	83	128	ca 390 ..	136		133
~~8~~	~~35 sec~~						
		4°	4°	4+			

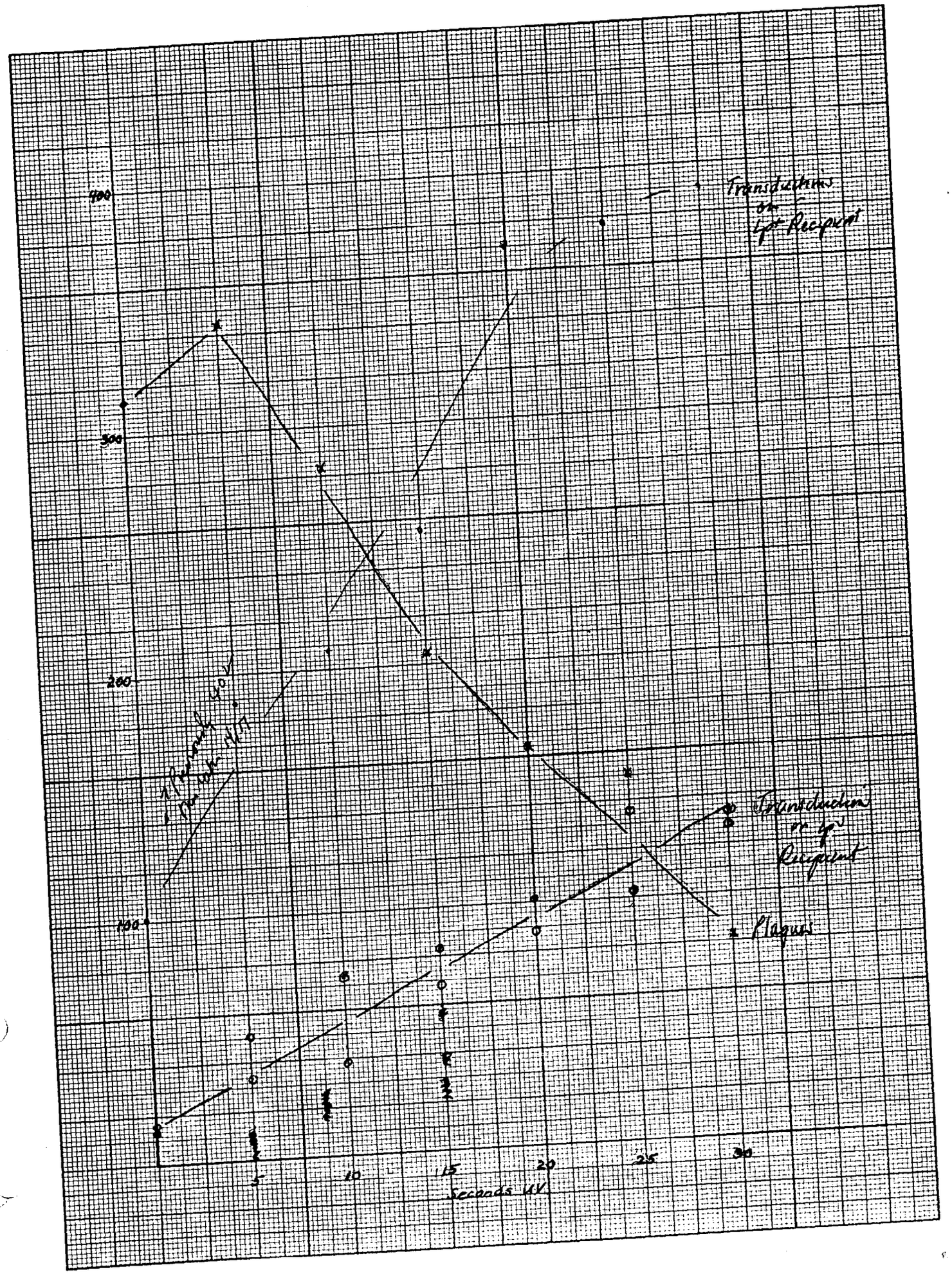
3080 apparently contam.!

Rpt. 3/26/56

	Plaques/3079	Transd/3079	Transd/3080	plaques/3079
1	36		16	4
2	68		56	0
3	14	11	25	0
4	26			0
5	14	16	50	0
6	11	8		0
7	31	32	99	7

Reason for the variability unknown.

These lysates have been exposed to a variety of conditions - including moving, etc. - storage at room temp, refrig, etc.



$\frac{5\times10^2}{3\times10^7} = 1\times10^5$

DATE: 3/16/56 REF: 405

Production of Transducing λ - LFT - Dosage study.

Y 945 grown overnight in aerator - dil 1-4, reincubate 2 hours - Centrifuge, resuspend in saline. Adjust density to 0.58, dilute 1-20 in adsol with UV. 50 sec.

All platings 0.05 on Bgal

Controls: 1. 945 nay. 2. 3079 3. 3080

Dose		Undiluted platings Bgal 3079		3080		Dilute 10^2, 10^4 plate 3079		cells	
0	①	3	②	5	③	0	④	128	2.5 × 10^7
15	⑤	26	⑥	23	⑦	14	⑧	78	
30	⑨	27	⑩	12	⑪	57	⑫	42	
45	⑬	14	⑭	15	⑮	60	⑯	13	
60	⑰	5	⑱	3	⑲	49	⑳	2	
75	㉑	2	㉒	2	㉓	31	㉔	1	
90	㉕	2	㉖	4	㉗	21	㉘	1	
105	㉙	1	㉚	6	㉛	7	㉜	0	

plaques appear ...

Dose 105 × 2×10^5

Fract. cells yielding transducing activity

$\frac{\text{no transd.}}{\text{no cells}} = \frac{5.4\times10^2}{2.6\times10^7} = 2.1\times10^{-5}$

Rpt. modification - overnight, aerated 3+ hours after 1-10, centrifuged, resusp. saline O.D. = 0.6

3/28/56 dilute 2/9 - irradiate as before - remove 0.05 ml + 4.5 ml, 10^2 → 0.05 for diluted; 0.05 as before for undiluted - Numbers as above.

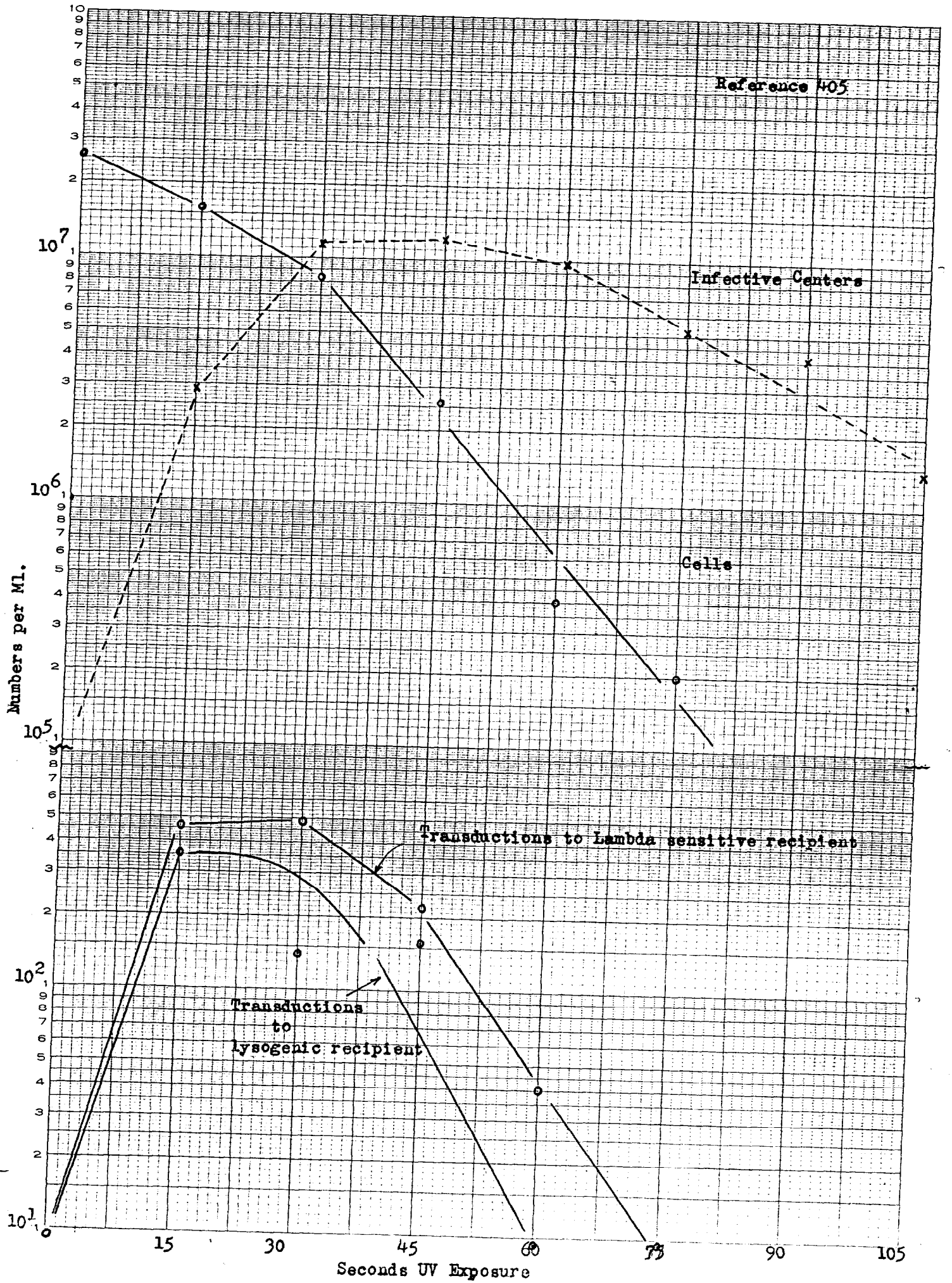
		Dose		Und. transd. 3079		3080		diluted 3079 = plaques		0 = cells	3.1×10^8	Fraction as plaques
0/3.1×10^8	0/3.1×10^8	0	①	13	②	19	③	1	④	790	1.0	0.0013
0.61×10^{-5}	0.34×10^{-5}	15	⑤	108	⑥	97	⑦	18	⑧	402	0.52	2.2×10^{-2}
1.4×10^{-5}	1.0×10^{-5}	30	⑨	226	⑩	179	⑪	77	⑫	291	0.37	9.8×10^{-2}
1.8×10^{-5}	0.61×10^{-5}	45	⑬	249	⑭	118	⑮	170	⑯	173	0.22	2.1×10^{-1}
1.0×10^{-5}	[illegible]	60	⑰	175	⑱	74	⑲	171	⑳	56	0.07	2.2×10^{-1}
[illegible]	[illegible]	75	㉑	130	㉒	26	㉓	71	㉔	23	0.029	4×10^{-2}
[illegible]	-	90	㉕	40	㉖	9	㉗	44	㉘	5	0.0063	5.6×10^{-2}
[illegible]		105	㉙	67	㉚	7	㉛	8	㉜	0	0 -	1.0×10^{-2}

this plate from diff batch Bgal

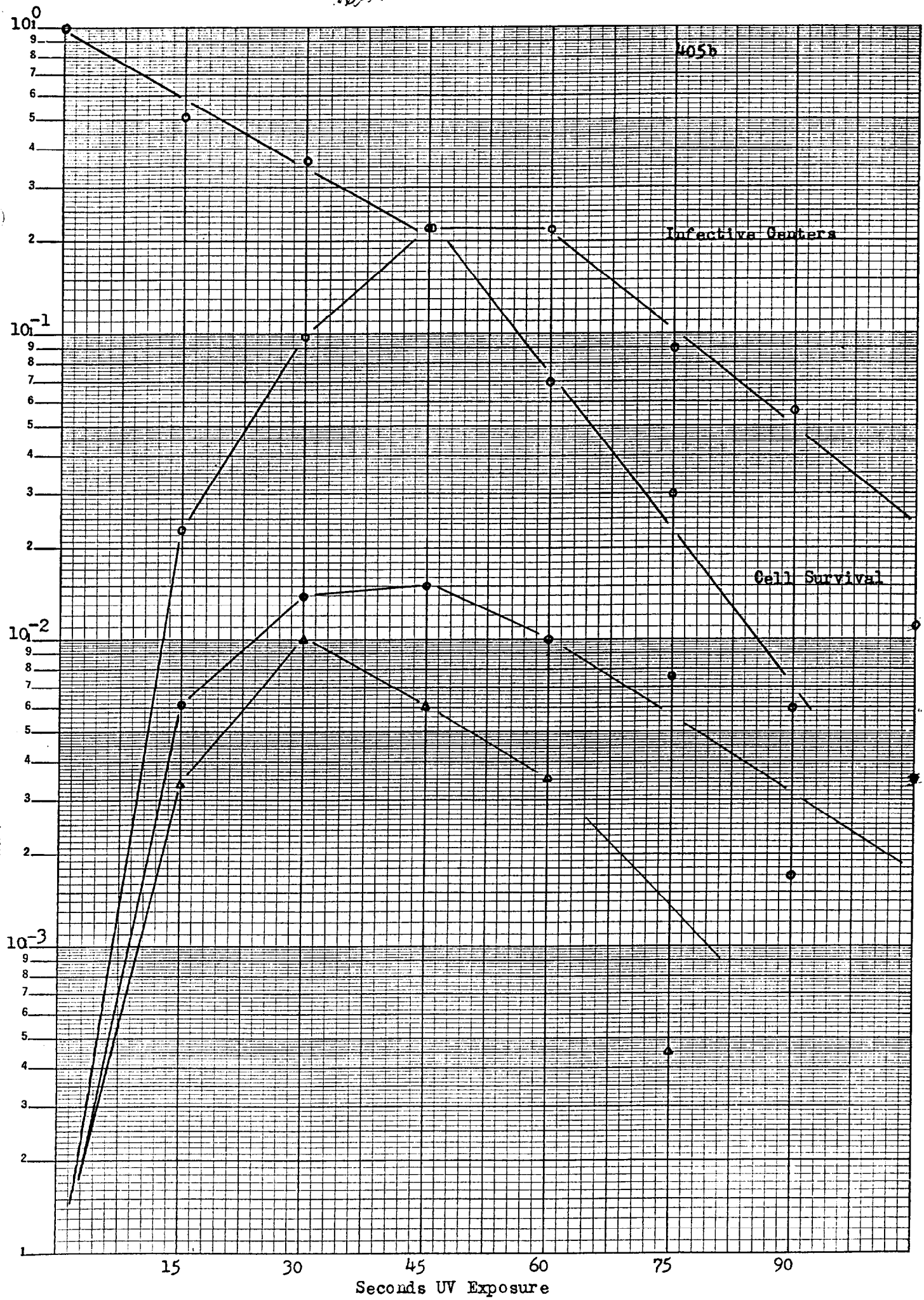
20×10^5 × 2×10^2

Fraction of cells yielding transduction

4×10^5 $\frac{\text{no. transd}}{\text{no. cells}} = \frac{472\times10^3}{3.1\times10^8}$



1602



DATE: 3/24/56 REF: 406

Comparison of U.V. Resistance of haploid and heterozygotic clones.

1. Set up. 341-9, a 2/2 homogenote streaked out, and a LFT segregant obtained. A galt revertant of this segregant by selection on B gal. The comparison for U.V. survival is between this segregant and 341-9.

2. Experimental.

(A) 341-9 started from HFT + clone (detected on B gal-HFT test) 341(+) from single colony.

(B) Overnight cultures diluted ca 1-20 incubated 37 in rotator for ca. 3 hours. Diluted 0.2 + 10 ml saline - each onto the first tube, and then 10^3, 10^4 additional. The first tube irradiated, and samples, 0.05 ml taken out and plated on B gal.

Time	Gal+ colonies: (1)	(2)	Total	Fraction	Gal- colonies: (1)	(2)	Total	Fraction	Fraction of Gal- Colonies HFT + /3080	
0	226	234	460	1.0	147	129	276	1.0	14/20	0.7
5	193	214	407	0.89	116	119	235	0.85	~~14/20~~	~~0.7~~
10	173	203	376	0.82	116	117	233	0.84	14/20	0.7
15	138	110	248	0.54	66	95	161	0.58	—	
20	110	111	221	0.48	76	64	140	0.51	16/20	0.8
25	55	73	128	0.28	53	43	96	0.35	—	—
30	38	22	60	0.13	27	11	38	0.14	16/20	0.8
35	16	14	30	0.065	17	8	25	0.091	—	
40	4	16	20	0.044	5	10	15	0.056	15/20	0.75
60	0	0	0	0	1	1	2	0.007	1/2	0.5

(Annotation: "same scale" — arrow from Gal+ colonies to Gal- colonies.)

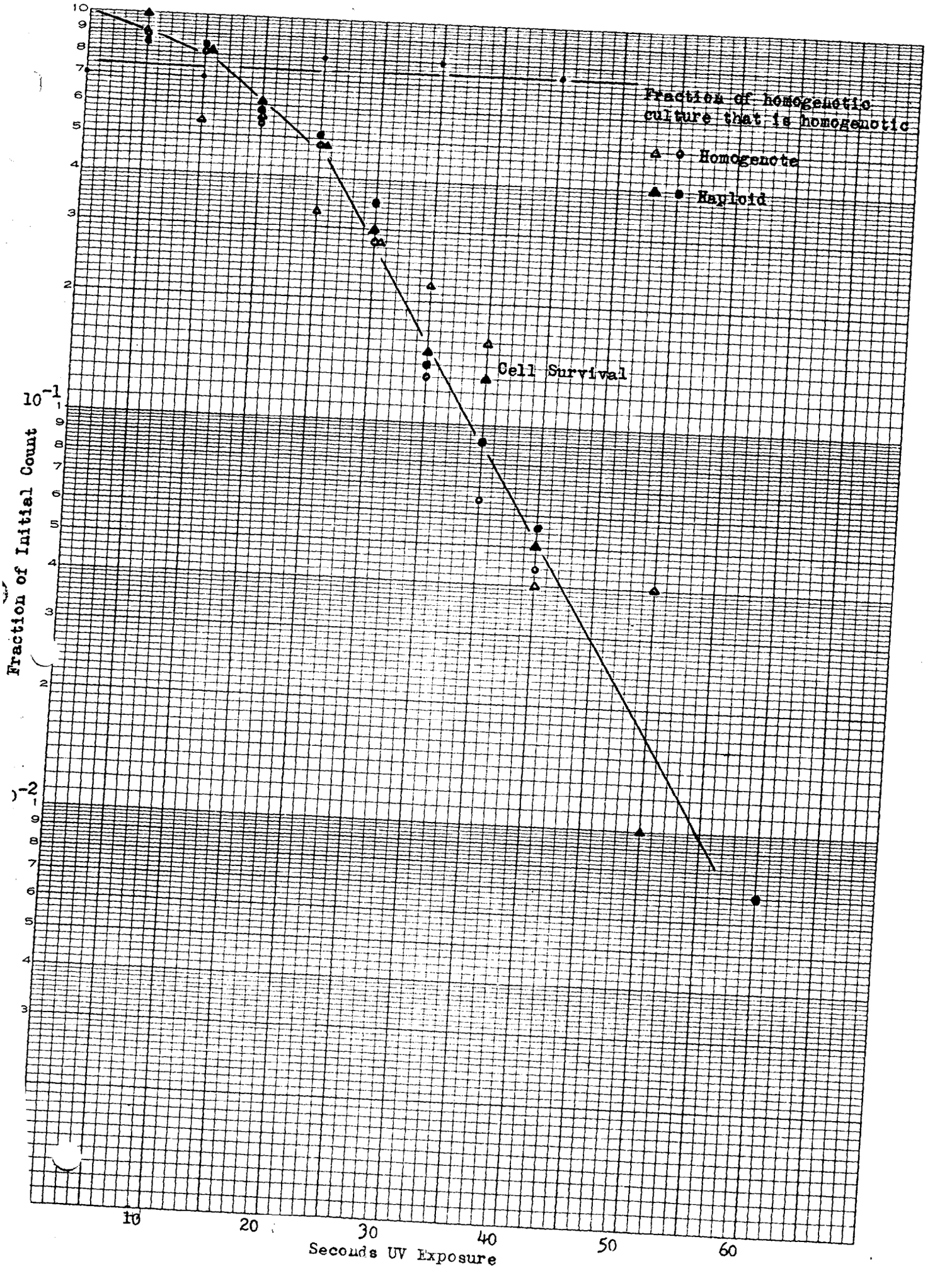
A second analysis (3/4/56) Using a $+^R$ of 341-9 and comparing with a (-) segregant of this

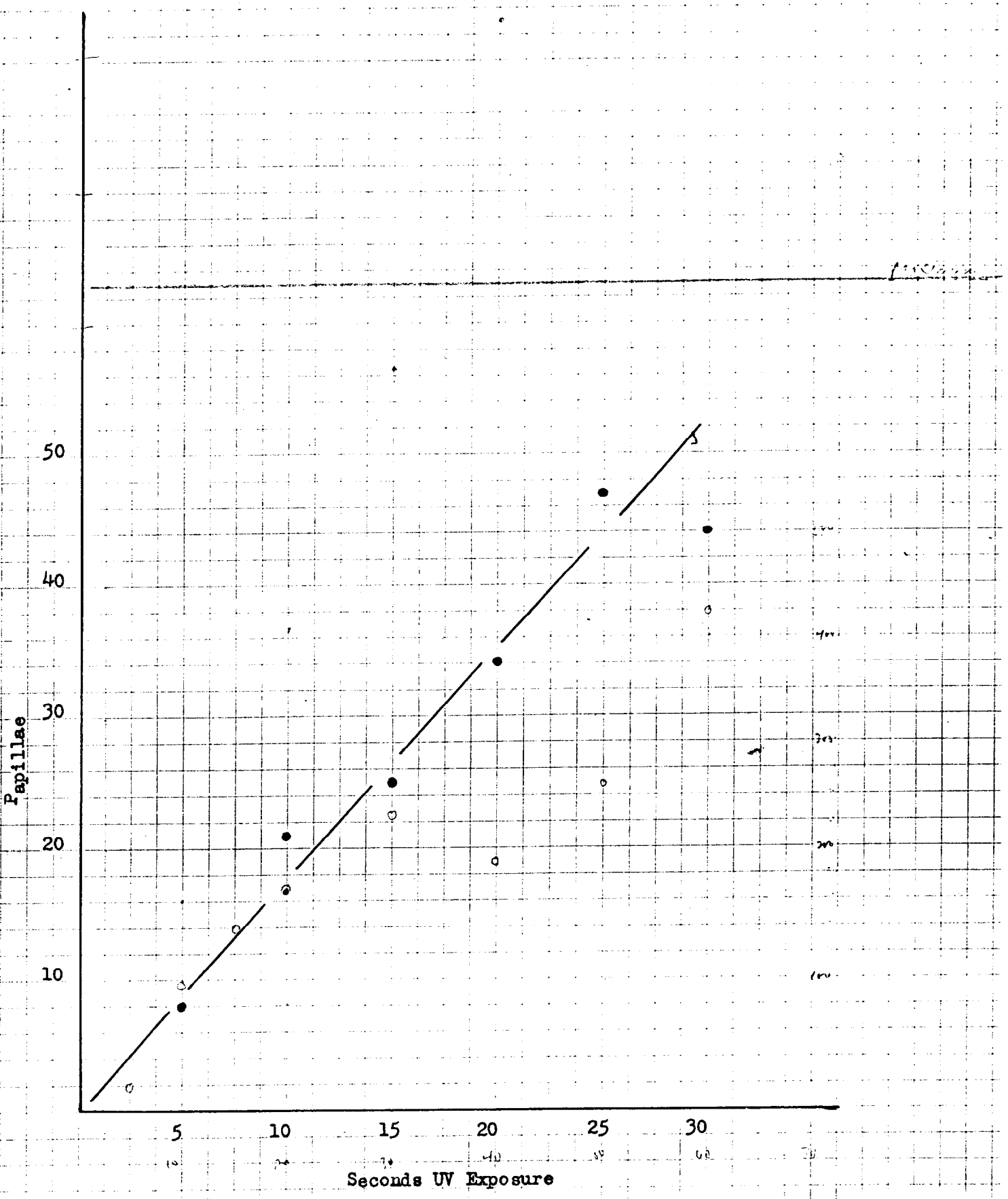
Time	Gal+ colonies:	Fraction	Gal- colonies:		Ratio +/-	Fraction
0	325	1.0	151	1.0	2.15	0.47
5	296	0.9	152	1.0	1.95	0.52
10	176	0.54	124	0.82	1.42	0.7
15	179	0.55	92	0.61	1.95	0.52
20	108	0.33	72	0.48	1.5	0.67
25	90	0.28	45	0.30	2.0	0.50
30	72	0.22	22	0.15	3.1	0.32
35	53	0.16	20	0.13	2.7	0.37
40	14	0.04	8	0.05	1.75	0.57
50	13	0.04	2	0.01	—6.5	0.16

In the ~~HFT~~ + culture, the ratio of +/- was:

1. 23/1
2. 19/3

42/4


Fraction of homogenetic culture that is homogenotic
Homogenote
Haploid
Cell Survival
Fraction of Initial Count
10^{-1}
10^{-2}
10
20
30
40
50
60
Seconds UV Exposure


50
40
30
20
10
Papillae
5
10
15
20
25
30
Seconds UV Exposure

DATE: 3/29/56 REF: 407

$0.58 = 2.6 \times 10^8$ cells/ml
$\times 20$
15×10^8
0.64

Test of conditions required to obtain "maximum" induction in B gal.

A

(1) Overnight aerated 945 – diluted ca 1-20 incubated in rotator ca 3 hours, centrifuge, resuspend saline. Adjust density to 0.6 diluted 1-20. (A)

(2) Susp A irrad. 45 seconds should yield [illegible] ca 500 [illegible]/ml [illegible]

(A) Test – irrad 45 seconds, plate 0.05 + 3050 (79)

(1)	77	
(2)	49	
mean	63	

(3). Plate 0.05 ml of suspension on surface of B gal – Irradiate varying doses, add 0.1 ml of 3050 (79) respread.

Dose	Plate	Count	Mean	
30"	(3)	99	112	
	(4)	124		
45"	(5)	115	116	Are some of these contaminated?
	(6)	117		
60"	(7)	86	62	
	(8)	38		
75"	(9)	57	56	
	(10)	54		

(4). Plate 0.05 ml of suspension of surface B gal – Incubate ~~3~~ hours – irradiate 60", add 3050 (79), respread
~~2 1/2 [illegible]~~
Wash two plates dilute and assay for total growth – ca wash 1.0 ml pooling ~~[illegible], [illegible]~~ once, 0.5 ml obtained, added to 10 [illegible]
dil 10^2, 10^4 → 0.05 ml

2:35
5:05

	Plate	Count	
respread	(11)	lost	
	(12)	ca 300	
	(13)	ca 300	
	(14)	ca 300	
Assay	(15)	—	
	(16)	—	
	(17)	27	$\frac{61}{2}$ = (30)
	(18)	34	
	(19)	34	(40)
	(20)	46	

Respread plus with spreader may have been contam.?

$20 \times 10^4 \times (\frac{6}{2}) \times 20$ $4 \times 10^6 = 120 \times 10^6 = 1.2 \times 10^8$ cells/plate

1.6×10^8 cells/plate

B.

(1) Also broad dosage response vs X [illegible]

Dose	0	5	10	15	20	25	30	35
papillae	5	13	26	30	39	52	49	28
circles	0	8	21	25	34	47	44	23

C.

(2) [illegible] incubation
(1) 6h dil 1-10 } irrad 45", + 0.1 ml 3079 = 7
" 1-100 x 10 [illegible] } = 0 ([illegible] plaque)

$\frac{.12}{4.8\times10^{7}}\times10^{-3} = 4.8\times10^{4}\times\frac{1}{7.5}$ (4×10⁸)

$\frac{0.1\times10^{2}}{2} = 5$

DATE: 4/1/56 REF: 408

1	2	3	4	5	6	7	8	9	10

945 for response to UV - linearity vs. exponential

[illegible] – Fresh culture – 3-4 hours aeration after being on the bench several days. Adjust density to 0.5 in saline ① dilute 1.2×10¹ and plate for transd. response. (0.6 = ca 5.0×10⁸ cells/ml) ② dilute 10¹ - 10³ for plaque-producing response – This last, read. (1-20×10²) and then add 0.1 to 7.5 ml.

read → plate and 0.1 ml 3079 – Incubate samples about 30 min before plating. Indicator = 3079

Est. cell 0.6 = 5×10⁸; 0.5 = 4×10⁸

Ⓐ Transduction: — B. Induction of λ — Too many cells ca. 2580

Time	Plate 1	Counts	Corrected	Time	Plate	Plaque	
0	1	14	0	as given ←	10	0 15	3516 ICh
5	2	28	14		11	143 158	3620 also IIa
10	3	26	12		12	349 364	3155 IIc
15	4	28	14		13	549 564	2437 IIc
20	5	28	14		14	816 831	1530
25	6	25	11		15	0.1: 2551; 0.05: 1267	1293
30	7	43	29		16	1661	816
35	8	39	25		17	1654	738
50	9	120	106		18	too many ca 1600	170

(2 types plaque and sep. plaque all reg.)

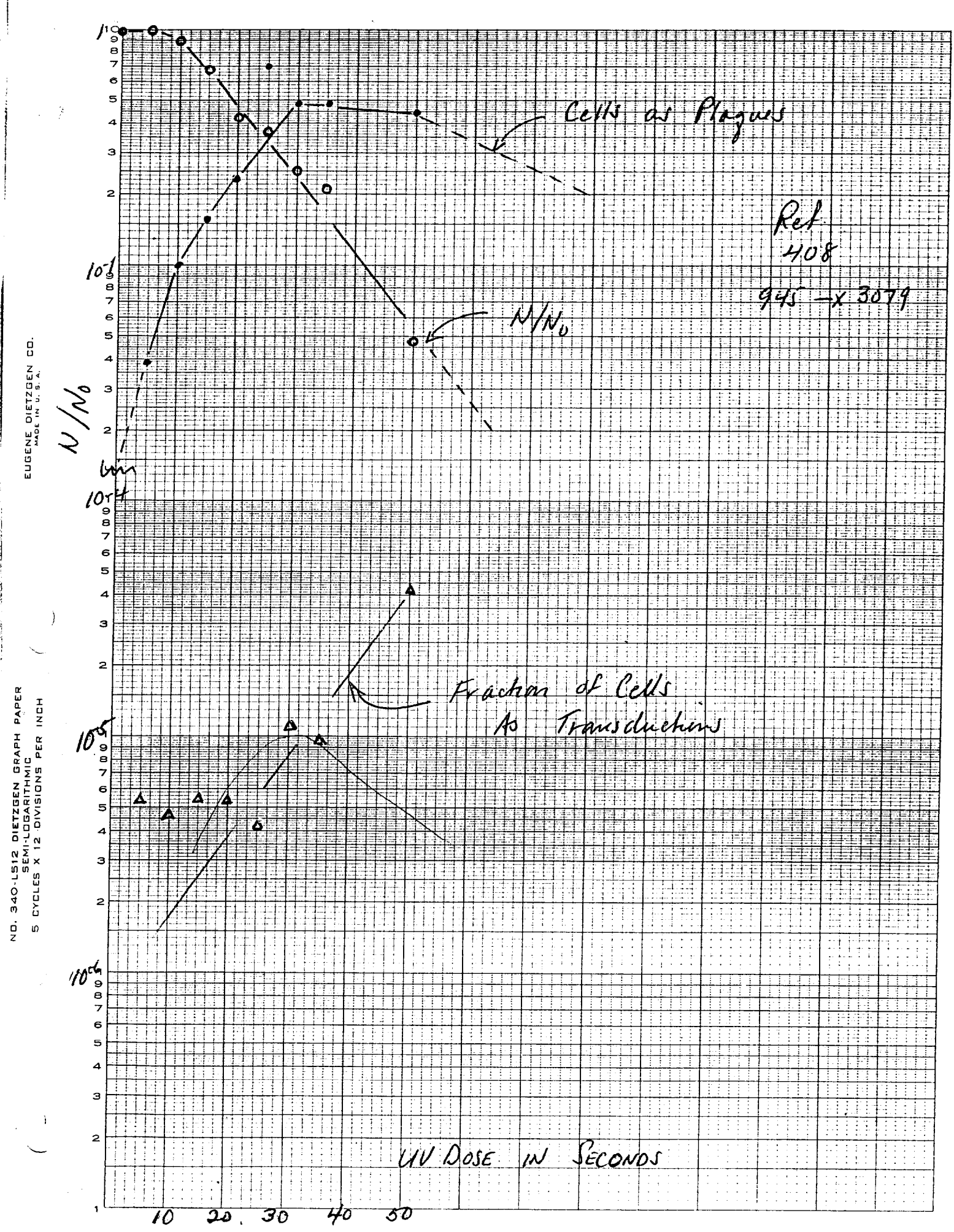
Total No cells/ml

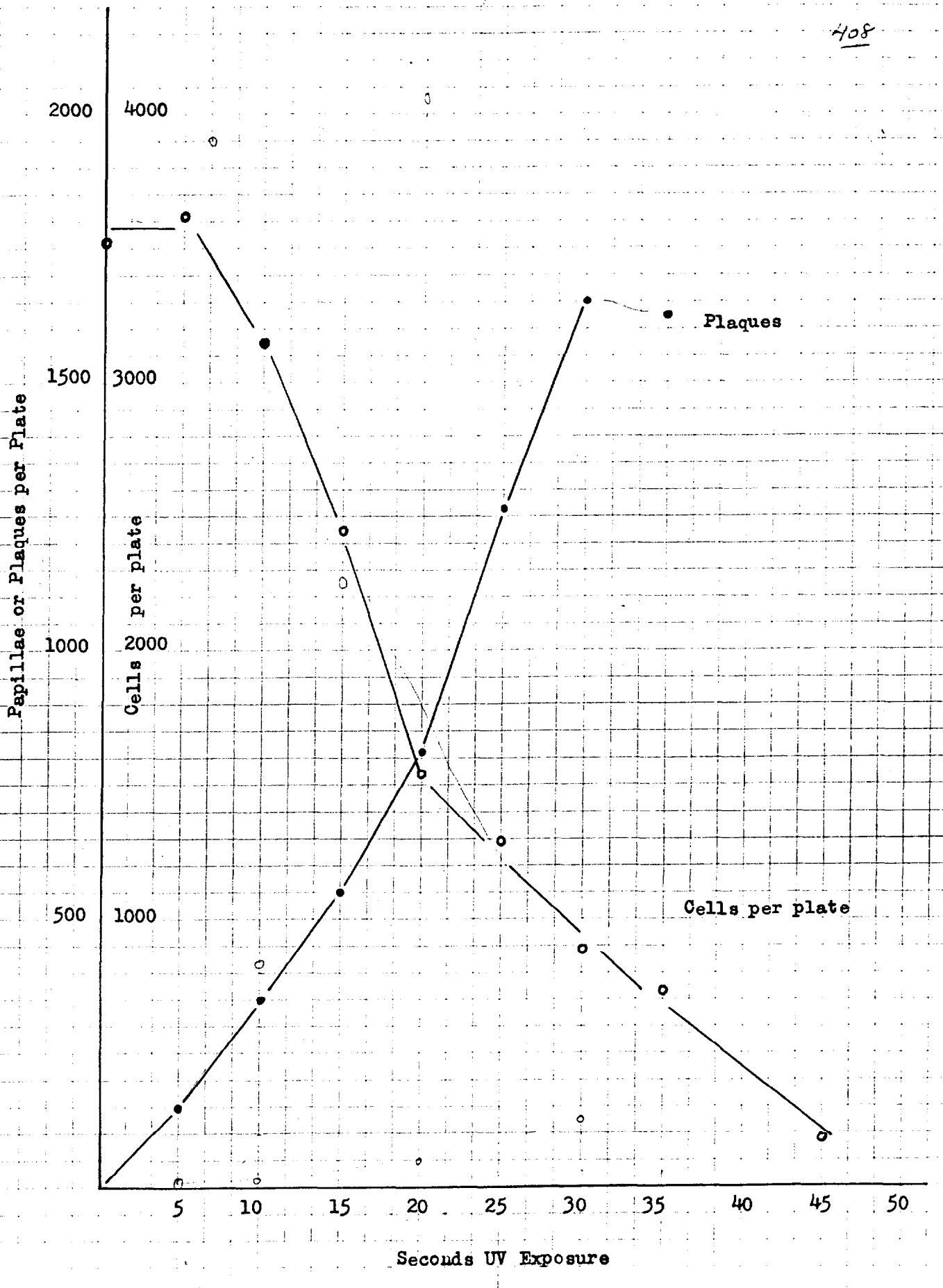
Transduction = 0.1 ml × $\frac{11.2}{1.2}$ = 100 $= 3.5\times10^{4}\times\frac{7.5}{0.1}\times10^{2} = 3.5\times7.5\times10^{8} = 2.6\times10^{8}$ cells/ml

2.6×10⁸

Fraction of Cells as Transd.	Transd	Fraction of Cells as Plaques	Time	Plaques/ml	N/N₀	N/ml
0	0	0	0	0	1.0	3.5×10⁴
5.4×10⁻⁶	1.4×10³	0.039	5	1.4×10³	1.0	3.6×10⁴
4.6×10⁻⁶	1.2×10³	0.099	10	3.5×10³	0.9	3.2×10⁴
5.4×10⁻⁶	1.4×10³	0.155	15	5.5×10³	0.68	2.4×10⁴
5.4×10⁻⁶	1.4×10³	0.230	20	8.2×10³	0.42	1.5×10⁴
4.2×10⁻⁶	1.1×10³	0.700	25	2.5×10⁴	0.37	1.3×10⁴
1.1×10⁻⁵	2.9×10³	0.48	30	1.3×10⁴	0.25	9.0×10³
9.6×10⁻⁶	2.5×10³	0.48	35	1.7×10⁴	0.21	7.4×10³
4.2×10⁻⁵	1.1×10⁴	0.45	50	1.6×10⁴	0.048	1.7×10³

Fraction of cells yielding transduction at 0 = $\frac{2.9\times10^{3}\text{ transd}}{2.6\times10^{8}\text{ cells}}$


Cells as Plagues
Ref
408
945 -x 3079
N/N0
Fraction of Cells
As Transductions
UV DOSE IN SECONDS
10
20
30
40
50
N/N0
EUGENE DIETZGEN CO.
MADE IN U.S.A.
NO. 340-L512 DIETZGEN GRAPH PAPER
SEMI-LOGARITHMIC
5 CYCLES X 12 DIVISIONS PER INCH


2000
4000
1500
3000
1000
2000
500
1000
Papillae or Plaques per Plate
Cells per plate
Plaques
Cells per plate
5
10
15
20
25
30
35
40
45
50
Seconds UV Exposure

DATE: 4/3/56 REF: 409

Examination of 4⁻ ×× 7⁻ heterogenotes

1. 4⁻ —× 7⁻ - Overnight culture diluted 10^2, 0.1 ml + 0.2 ml lysate -
293-12 lysate, W2308. Incubated room temp for 10 minutes, diluted 10^2, 10^4
0.1 ml plated 3 plates B gal.

A

	(−)	partial lysis	wk (+)
1.	31	(7)	(1)
2.	37	(5)	0
3.	29	(4)	(1)

3 additional possibilities appeared second day — 3 A

3 A

5/7/56 18 (−) seg obtained from the internal "+" typed - 18/18 Gal₇⁻

12 (−) seg obtained from the ~~bottom~~ free "+" and typed 12/12 Gal₇⁻

2. 7⁻ —× 4⁻ As above - 1st run no papillating or semi (+) colonies observed.
2nd run - 2 presumptives observed - slight papillation in the centers of two colonies noted. Picked and streaked total colonies ca 40, all phage contaminated.

C

DATE: 4/3/56 REF: 410

1	2	3	4	5	6	7	8	9	10

One step with W945, against 3077

1. From overnight culture, diluted 1-10 incubated 3 hours
2. Adjust [illegible] to 0.D. = 0.6, ~~dilute 1-10~~ [illegible] dilute 1-5 (3+9); irradiate 60 seconds, (Dil 1-2 with broth (Per))
3. Pre assay. 0.D.=.60 — dil 10^2, 10^4, 10^6 assay

Cells
Bgal 1. 38; 2. 38 } 7.4×10^8 cells in OD = 0.53 suspension
Plaque & Transd.
med SM 3. 0; 4. 0 } no indicator?

In a different expt at this time approx. 300 colonies 945 tested, all found inducible

4. Post-U.V. ← Incubate

(A) dil 10^3, 10^5-10^5 broth ←→ (B) Undiluted

cells ← 0.05 ml; Bgal Transd ← 0.05ml; → Bgal SM (trace transd.?)

Fraction cells yielding transd out. transd $\frac{3 \times 10^2}{7.4 \times 10^8}$

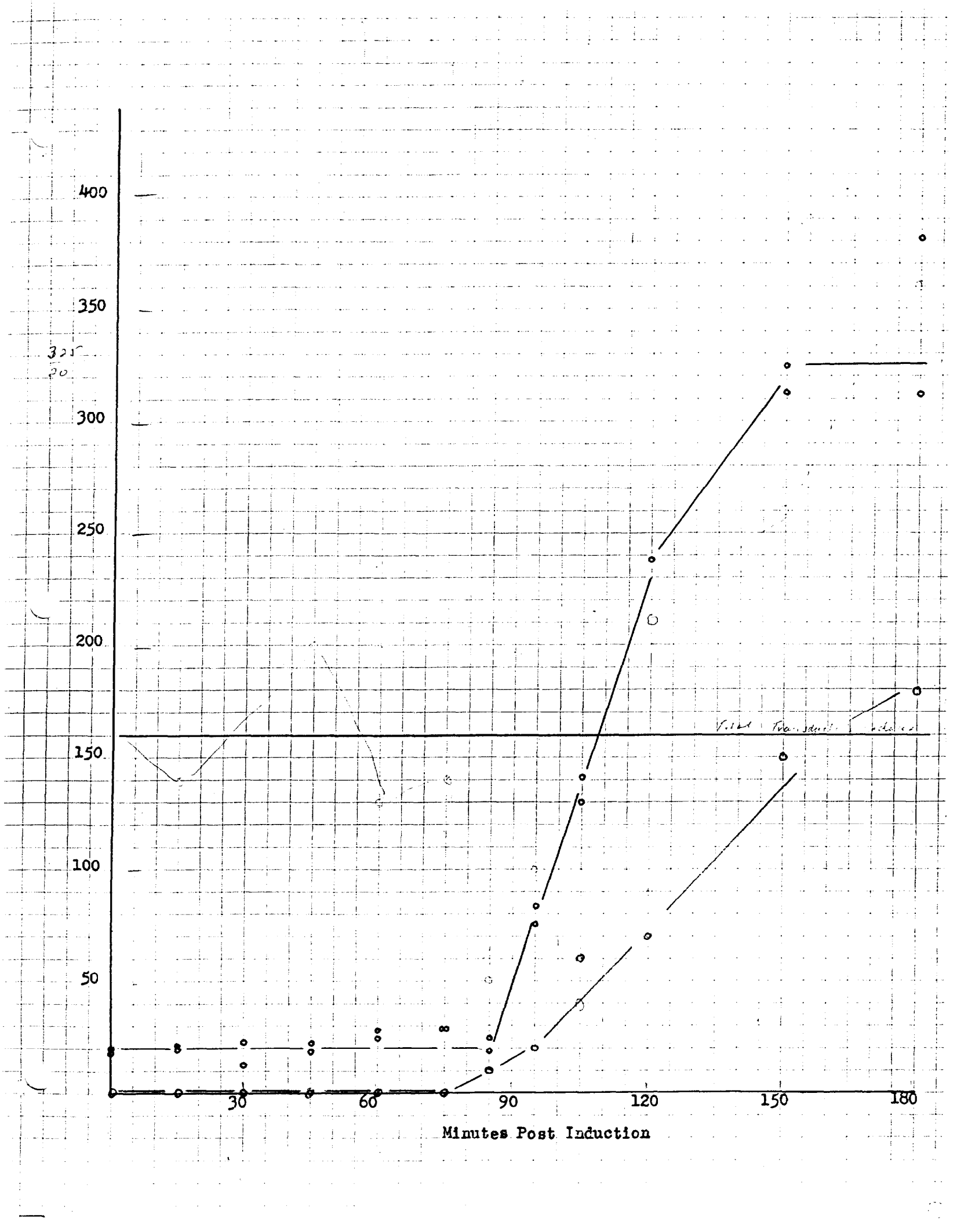
Time		Cells		Bgal Transd			Bgal SM		
2:15	0 time	5. 0			11. 12	16	11. 0	ca 200 plaques	
		6. 0			10. 19		12. 0 (ca 1000 plaques)	"	
	λ centers of (3077)	7. 19							
		8. 18							

3.04×10^8 projector centers induced / OD = 0.53 ; $\frac{3.04}{7.4}$ = fraction induced 0.41

TIME	min	Cells	Bgal Transd			Bgal SM			Trans.	Bgal
2:25	15	13. 20	15. 7	14		17. 0 (many small) ca 300 plaques				
		14. 20	~~16.~~			~~18.~~				
2:40	30	19. 23	—			21. 0	ca 200 plaques			
		20. 12	—			~~22.~~				
2:50	45	23. 19	25. 20	20		27. 0	ca 300 plaques.		Med. Total transd	
		24. 33	~~26.~~			~~28.~~				
3:10	60	29. 25	31. 13	13		33. 0 many small			16	16
		30. 28	~~32.~~			~~34.~~ ca 10^4 plaques			4	7
	75								20	20
3:25		35. 29	37. 7	14		39. 0			12	13
		36. 29	~~38.~~			~~40.~~			14	7
									5	4
									0	5
3:35	85	41. 25	43. 1	5		45. 1	1		4	2
		42. 19	44. 4			46. 0			21	21
									36	36
3:45	95	47. 75	49. 4	10		51. 2	2		36	38
		48. 83 (158)	50. 6			52. 2			159 / 16	157 / 14
3:55	105	53. 130	55. 2	4		57. 8	6			
		54. 141 (271)	56. 2			58. 4				
4:10	120	59. 238	61. 9 (21) pinned			63. 5	7			
		60. cut plate (76)	62. 33 surface			64. 9				
4:40	150	65. 313	67. 14 (36) pinned			69. 10	15			
		66. 326 surface plate	68. 38 surface			70. 20				

These were all surface platings

	Selected	Bgal
180.	71. 38 ✓	72. 37 (36)
	72. 3 ✓	74. 36
		Bgal SM
		75. 19 (18)
		76. 17


400
350
300
250
200
150
100
50
30
60
90
120
150
180
Minutes Post Induction

Gal+ r/s
vs
Gal- r/s

DATE:　　　　　　REF: 411

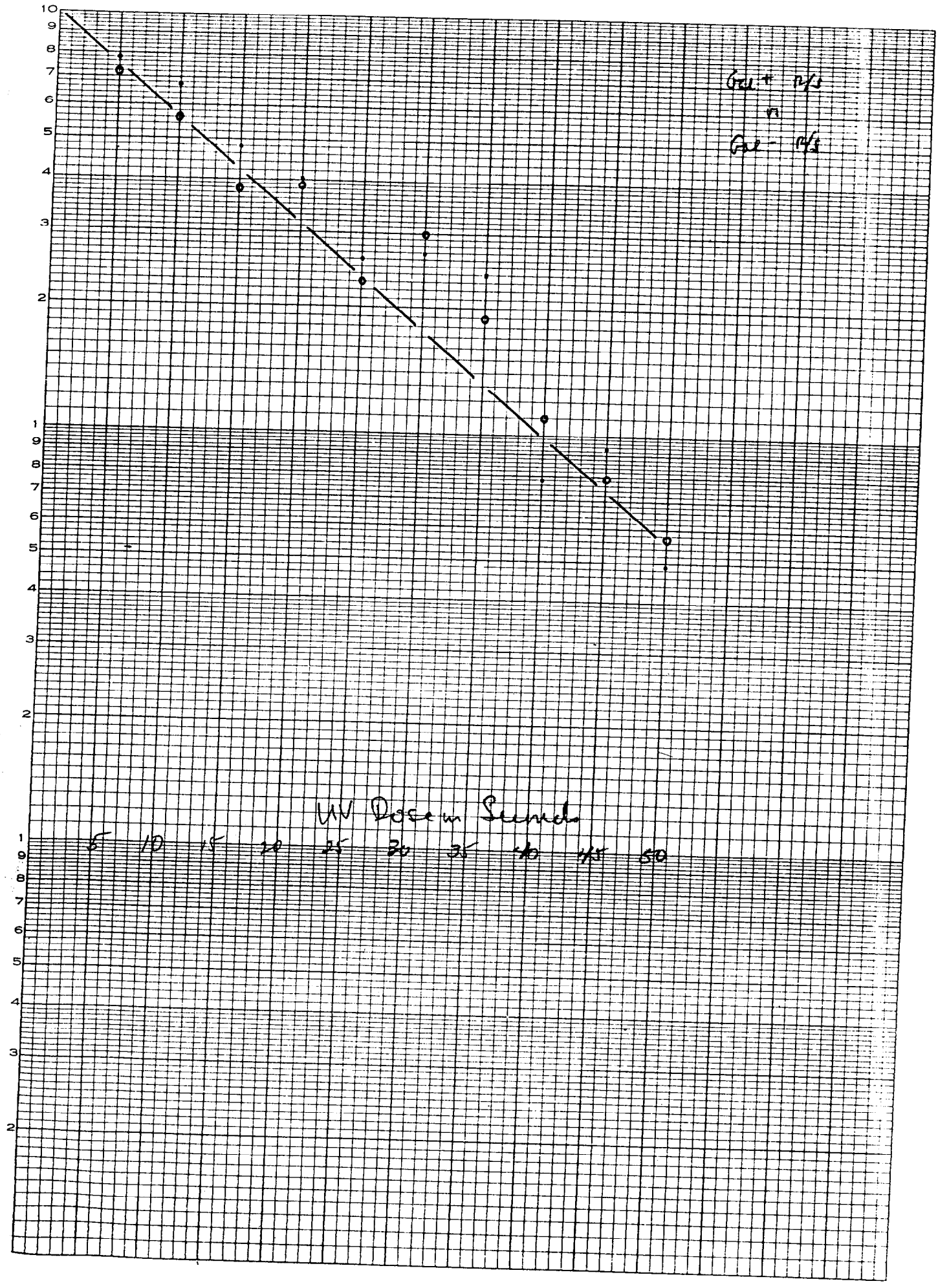
U.V. Resistance r/s heterogenote is s seg.
W2868 taken and a r/s seg of it. On testing against λ, 40°
the segregant formed r/s as well as the heterogenote

Both cultures diluted to 1-10 incubated 2 hours, diluted 50X 50X 50 in saline. The heterogenote first, 0.05 ml to 2 B gal plates to obtain Gal+/Gal- ratio. Then seg diluted in same tubes. The last tubes irradiated and samples taken (0.5) at the following times.

Time	Gal+	Gal-	Fraction Gal+	Fraction Gal-
0	600	204	1.0	1.0
5	431	158	0.72	0.78
10	336	139	0.56	0.68
15	226	99	0.38	0.49
20	236	81	0.39	0.40
25	138	54	0.23	0.26
30	178	55	0.30	0.27
35	115	48	0.19	0.24
40	66	16	0.11	0.078
45	48	19	0.08	0.093
50	34	10	0.057	0.049

Ratio Gal+/Gal- in 2868

Plate	Gal+	Gal-
1	229	10
2	259	1
	488	11



DATE: 3/10/56 REF: 412

One step with 945
Overnight culture diluted 1-5 incubated ca. 1 hour.
Centrifuge resuspend saline OD. .60
([illegible]), irradiate 60 seconds, dilute 1-2 with 2X [illegible]

Assay 1. Pre UV. dilute $10^3, 10^4, 10^5$ → 0.05 ml

Cells (1) 163 / (2) 186 Bgal

175 × 2 × 10^6 350 × 10^6
3.5 × 10^8 cells/ml OD 0.6

+ 3079
and Plaques (3) 0 (ca 1000 plaques) Bgal SM
Transd (4) 0 (ca 1000 ")

4.2×10^3 / 3.5×10^8 cells/ml

Post UV - dilute $10^2, 10^4, [10^5]$ Pre incubate Bgal; Unincub. Incubated Bgal SM

Time		Col. 2	Pre incubate Bgal		Incubated Bgal SM	
9:25 0 Time Cells	(5)	0				
	(6)	0	(9)	104 103	(11)	1 ca 1000 plaques
			(10)	101	(12)	0 " "
Ascites (?)	(7)	42				
	(8)	32				
9:40 15	(13)	43	(15)	85 117	(17)	0 ca 1000 plaques
	(14)	40	(16)	150	(18)	0 ca 1000 plaques
9:55 30	(19)	21	(21)	72	(23)	0 ca 1000 plaques
	(20)	56	(22)	98	(24)	0 " "
10:10 45	(25)	37	(27)	113	(29)	0 ca 1000 plaques
	(26)	47	(28)	112	(30)	0 ca 1000 plaques
10:25 60	(31)	37	(33)	93	(35)	0
	(32)	39	(34)	124 AVE = 105.2	(36)	0 (10^5 plaques)
10:40 75	(37)	53	(39)	88	(41)	2 [illegible]
	(38)	57	(40)	127	(42)	5
10:55 90	(43)	54	(45)	80	(47)	31
	(44)	64	(46)	67	(48)	24
11:05 105	(49)	99	(51)	71	(53)	29
	(50)	143	(52)	118	(54)	46
11:15 120	(55)	357	(57)	59	(59)	67
	(56)	448	(58)	81	(60)	78
11:25 120	(61)	1024	(63)	87	(65)	91
	(62)	930	(64)	65	(66)	124
11:55 150	(67)	1442	(69)	181	(71)	150
	(68)	937 / 2379. 1089	(70)	161	(72)	149
12:25 180	(73)	1422	(75)	203	(77)	193
	(74)	1196	(76)	174	(78)	208

no cells induced to yield transd
ave = 210 × 20 × 2 =

0 = no growth
+ = growth

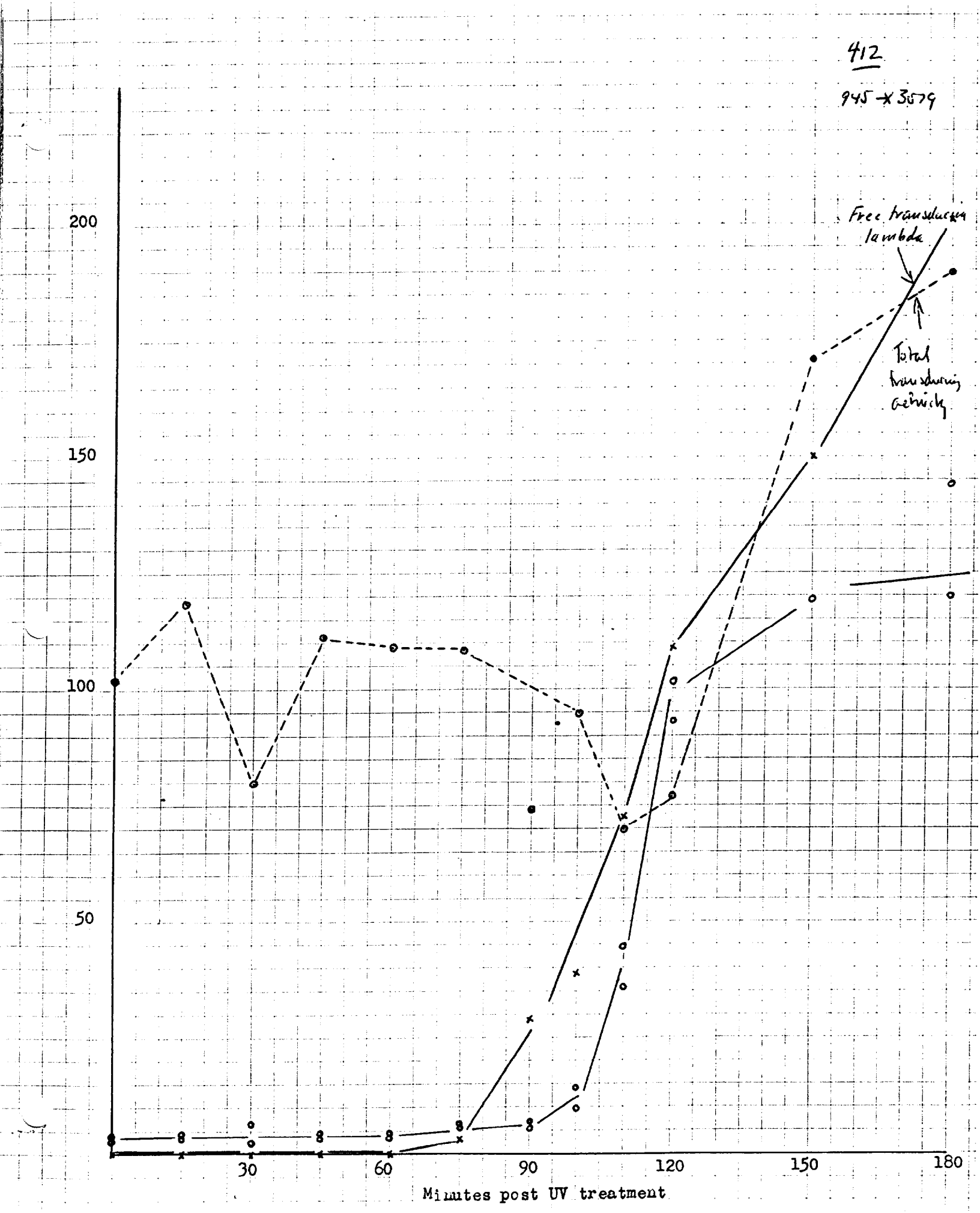
Some population checked

	Plate 46 Bgal	Bgal SM	48	75	
1	+	+	+	+	+
2	+	+	0	+	+
3	0	0	0	+	+
4	0	0	0	+	+
5	+	+	0	+	+
6	0	+	0	+	+
7	0	0	0	+	+
8	0	0	0	+	+
9	0	0	0	+	+
10	0	0	0	+	+
11	+	+	0	+	+
12	0	0	0	+	+

	77
1	+
2	+
3	+
4	+
5	+
6	+
7	+
8	+
9	+
10	+
11	+
12	+

412

945 × 3579


200
150
100
50
30
60
90
120
150
180
Minutes post UV treatment
Free transducing lambda
Total transducing activity

DATE: 3/14/56 REF: 413

1	2	3	4	5	6	7	8	9	10

Recheck on induction – as plaques – is it exponential?
– as transductions – " " linear?

1. Plaque induction – 945 rotated 2.5 hours post overnight, diluted 1-10. Centrifuged made up to O.D. .60 in saline – diluted 1-2, (2×10⁸) irradiated UV standard dist, etc., assay for cells: B gel (pour method
for plaques: B gel + 3079, also pour method.

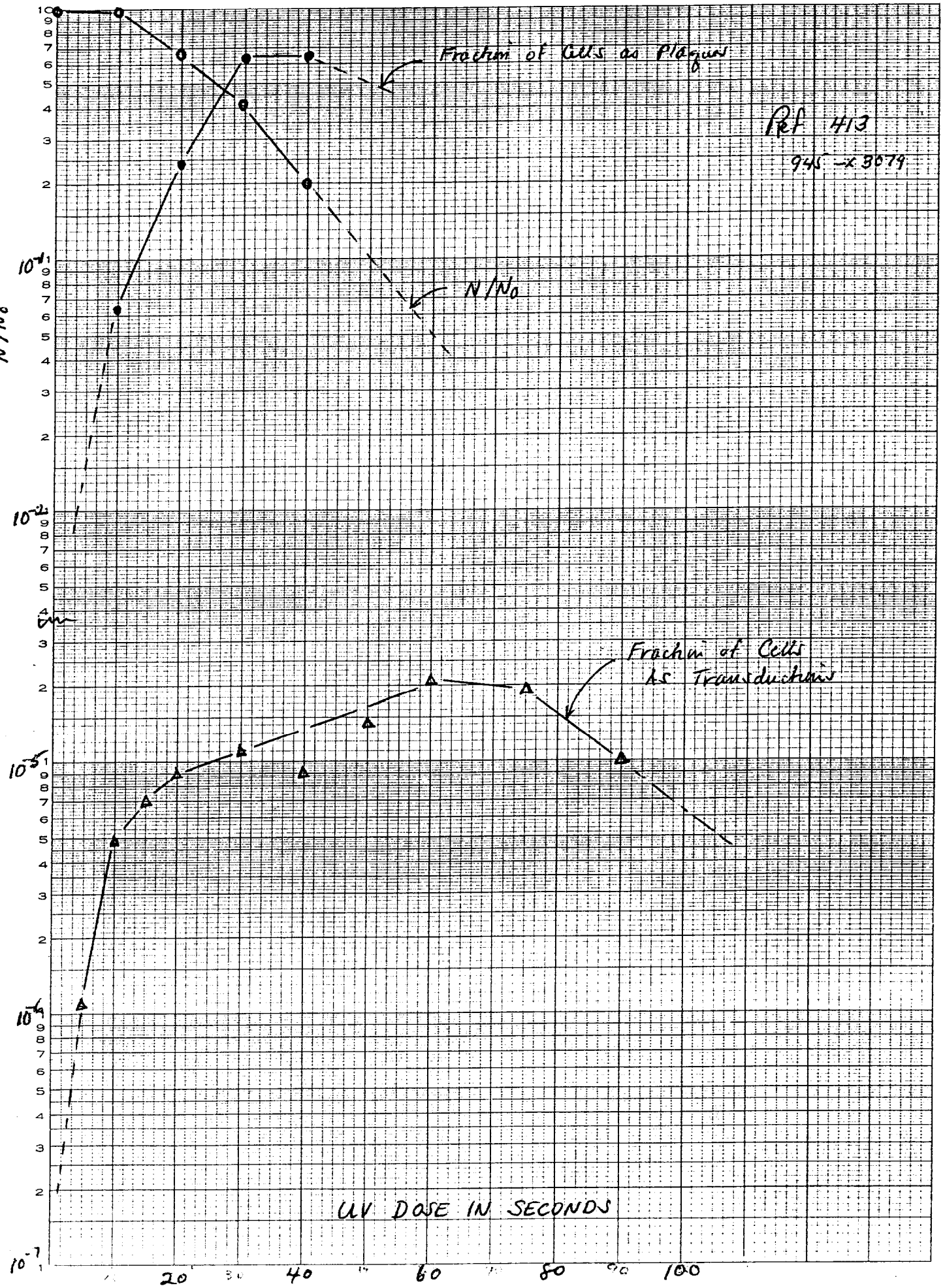
2.0×10⁸

Dose	plaques	Cells	Total cells + plaque	N/N_0	Plaques/ml	Fraction Cells to Plaques
0	0	198) 200	198	1.0	0	0
10	13	207)	220	1.0	1.3×10^7	0.064
20	49	136	185	0.66	4.9×10^7	0.24
30	127	86	213	0.42	1.3×10^8	0.64
40	130 many agar lumps	42	172	0.20	1.3×10^8	0.64

2. Transduction induction. dil 1-2 of OD=.60

2×10^3

Dose	Papillae	Δ	Transd/ml	Fraction Cells yielding trans.
0	23	0	0	0
5	45	22	2.2×10^2	1.1×10^{-6}
10	110	97	9.7×10^2	4.9×10^{-6}
15	161	138	1.4×10^3	7.0×10^{-6}
20	190	167	1.8×10^3	9.0×10^{-6}
30	247	224	2.2×10^3	1.1×10^{-5}
40	208	185	1.9×10^3	9.0×10^{-6}
50	292	269	2.7×10^3	1.4×10^{-5}
60	444	421	4.2×10^3	2.1×10^{-5}
75	411	388	3.9×10^3	1.9×10^{-5}
90	235	212	2.1×10^3	1.0×10^{-5}



Mikhopuri pages

DATE: REF: 413-1

Isolation of lyd from 2307

1. Overnight culture - diluted and plated to give ca 100 colonies per plate
2. 9 plates - two counted
   1. 97 colonies } all colonies gal-
   2. 109 colonies
3. replicated to B gal plate spread with 3079, UV'd 20 seconds 50 cm, etc.
4. On the nine plates 3 colonies found not to give a lysogenic reaction (2 on one plate, one on another)
5. The colonies were streaked on λ, 3079 after purification found λ^r and non lysogenic
6. Lysates made (not as conc. as usual) - after 4 hours spotted on 3079 on B gal

1.	ca. 20 small plaques
2.	ca. 17 " "
3.	ca. 9 " " 45/3 = 15 x at 0.01 = 1500 per ml = 1.5 = 1.5 x 10^4

W2307 lyd = W3172

About this time also 2345 treated in the manner above.

1. 1 colony of ca. 300 (on 3 plates) found non lysogenic
2. on subsequent test (1) found lyd

W2345 spont. - lyd = W3173

(2) 1 lfu with 945 after 3 hr growth in Pen and plated 1:100 on M gal, and on S gal

(3) tested against HFT 2, 1, + by 2, 0 by 1

(4) gave no phage when treated as above in lysate test

945 - Are the cells giving HFT lambda an LFT culture mutant? Ca 2x10^3 945 colonies per plate replicated to 3079 spread plate B gal UV'd 20 sec. Incubated 3 days gal + looked for. Many observed suggesting that some are UV induced reversions of 945 (As was the case previously with 1210) 15 plates in all representing ca 40,000 colonies. Areas on the 945 plate corresponding to the Gal+ on 3079 located and picked and retested by spotting on 3080 and irradiating - This to find a clone of 945 cells giving HFT lambda. Gal+ area picked from 3079 plate to confirm transduction. 12 945 areas picked per plate.

Results

1. first 5 945 areas - 60 total found LFT. None of the Gal+ areas on 3079 corresponding to these showed seg. clones.
2. to distinguish between gal+ due to reversion of 945 plates replicated to B gal SM, since 3079 is S^r. From the Gal+ on Gal+ SM reference made to (1) the original plates to obtain reference to original clone (2) the UV'd plates to see

to 413A

DATE: 3/27/56 REF: 413A

1 2 3 4 5 6 7 8 9 10

of HFT⁺ clone induced by UV.

Z series = original 945 plate - un UV'd
A " = 3079 + 945 UV'd
S " A series replicated to B gal SM

3. The SM Gal plates show Gal⁺, indicating transduction or reversion of the doubly - - (or something more complicated).
Examples ~~[illegible]~~

	Gal⁺			Gal⁺	
Z3	19		Z12	7	Theoretically
A3	24		A12	33	
S3	11 4/6		S12	18 3/6 streaked gave mosaics 1/5 striking	Z+S = A, however many many Gal⁺ are papillae and may not replicate well
Z5	9		Z4	11	
A5	ca 100		A4	40	
S5	58 3/6 gave mosaic 1/5 streak		S4	18 3/6 ch.	

4. From Z3, Z12, Z5, Z4, Z6

(A). 7 areas corresponding to the areas of transduction on 3079 (as determined by replication to SM) picked and spotted on B gal. Given to spot size (24 hrs), then from these spots growth picked, tested for HFT via UV on 3080.

(B) Results. of the 35 areas picked and tested, none found HFT⁺ after 48 hours.

Repeat this experiment adding HFT⁺ λ to obtain lower limits of resolution of the method. Suggestion is that cells producing HFT λ are not clonally selected.

5/2/56
Preparing for Kalckar experiments

Obs that 1, 6, 7 are all defective for enzyme II (Gal transferase?)
Prepare gal₂⁻, gal₃⁻ strains also 2734 (= non transferable gal⁻)

1. Because 3092 easily revertible, a new strain made by HFT₂⁻ × 3110
A. a sep. clone obtained.

2. ~~27~~ 2734 crossed with 1895 - growth in both, placed M gal⁻
A gal⁻ colony picked purified once, streaked on D(0) and tested against HFT(+)

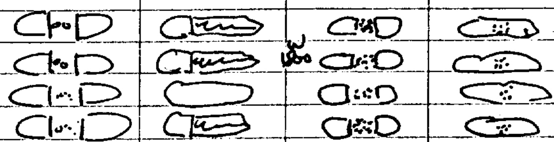
A. 6/6 colonies grew O(c) (labeled (413A-1) = W3142
B 6/6 colonies not transf. to + by HFT(+) - all resist. to λ.

DATE: 4/20/56 REF: 414

1	2	3	4	5	6	7	8	9	10

1. Recheck on 269-1 lysate · Ppt 269-1

	269-1	λ²	10⁻²	10⁻⁴
3079				
3080				
945				
(f/s) 343-8				



↑ Resistant colonies picked — very tiny plaques

2. Examination of the result of 1.
After two purification streaks — 2 from each culture

Specks → These tests in B gal — all cultures Gal⁻

		269-1 (1-100)	Mucoid	λ	λ-2	
3079	(1)	R	mucoid	S	S	414-1
	(2)	R	"	S	S	~~414~~
3080	(1)	R ?	"	R	S	-2
	(2)	R	"	R	S	~~—~~
945	(1)	R	slight mucoid	R	R	mucoid — a contaminant?
	(2)	S	mucoid	—	—	
343-8	(1)	R	"	S	S	-3
	(2)	R	"	S (pinkish)	S	-4

↖ Stocks discarded 3/9/59

341-9 Lysate 4/23/56 — To show that fragment size is constant — titers of 2⁻ × 1⁻4⁻, 4⁻, 1⁻ are the same

Assay

Dil 10⁴ → 0.05 ml surface plating)

① Preliminary — /3080 (1-4⁻) 101 trans.
/3101 (1⁻) 4 trans, ca 500 plaques.
/3104 (4⁻) 4 trans, ca 500 plaques

② Ppt. same dilution
/3094 5 trans.
/3091 8 trans
/3080 96 trans.

DATE: 5/1/56 REF: ~~[illegible]~~ 415

[307-1A made of 307-1C ... NS]

307-1C Segregation. (1⁻ → X7⁻) see 308A, 307

1. Overnight culture from a single colony B gal.

2. dil $10^2 - 10^4 - 10^6 - 10^8$ → 0.05 ml samples spotted on B gal, 6 per plate. (8 plates) Incubated 4.0 hours, 0.05 ml - 0.1 ml 1/50 added and then ~~plates~~ areas respread.

$\frac{10}{100 \times 2} = 0.05$ $e^{-.05} = 0.951$ $e^{-1.1} = 0.338$

3. Parent susp. ~~[illegible]~~ 0.1 ml

Calc titer

		Gal +	Gal-pop	Gal-	Total			
3 samples obs. 5? got a "+" cell	1.	10	209	10	229		230/100 × 2 =	$\frac{2.3}{2} = 1.1$ / sample (cells)
	2.	10	212	9	231			

4. Samples

(left margin: 3 samples obs. 4? got a G(?) cell; 16 sample got no cells.)

Plate		Gal+	Gal-pop	Gal-	Total cells		C.C.	
Plate 1	1.	220	0	0	220			
	2.	0	222	0	222			
	3.	0	6	0	6	?		
	4.	0	211	1	212			
	5.	58	ca 100	ca 100	–			
	6.	0	9	0	9	?		
Plate 2	1.	0	265	1	266			
	2.	0	0	0	0	NG		
	3.	0	185	0	185			
	4.	0	209	0	209	0.602	N log N	a
	5.	3	309	3	312	← 1.8	7.78×10^2	2.3×10^{-3}
	6.	0	2	0	2	?		
Plate 3	1.	2	192	0	192	1.2	4.38×10^2	3.6×10^{-3}
	2.	0	309	1	310			
	3.	2	164	0	164			
	4.	0	148	0	148			
	5.	0	17	119	136			
	6.	0	381	0	381			
Plate 4	1.	65	116	2	183			
	2.	0	0	0	0	NG		
	3.	0	272	3	275			
	4.	47	1	0	48			
	5.	0	183	0	183			
	6.	0	0	0	0	NG		
Plate 5	1.	3	252	4	259			
	2.	0	233	0	233			
	3.	0	77	0	77			
	4.	0	0	0	0	NG		
	5.	0	260	105	365			
	6.	0	21	0	21			
Plate 6	1.	0	158	3	162			
	2.	0	38	0	38			
	3.	0	245	1	246			
	4.	0	129	0	129			
	5.	0	468	1	469			
	6.	0	223	10	233			

DATE: 5/1/56 REF: 415A

	1	2	3	4	5	6	7	8	9	10
		Continuation —								
		Gal+	Gal-pos	Gal-	Total					
	Plate 7	0	0	0	0	NG				
	2.	0	153	0	153		This Het. is P/S (307-1C)			
	3.	0	68	0	68					
	4.	1.	ca 50?		ca 500		For comparison with Lp+			
	5.	0	450	1	451		transductants see 389 for 307-1A			
	6.	125 (MT)	25	0	150					
10	Plate 8 1.	0	223	1	224					
	2.	0	0	0.	0	NG				
	3.	0	72	0	~~25~~ 72					
	4.	0	--	ca 100	467 -					
	5.	0	272	5	277					
	6.	0	223	2	225					

II. Single Gal- pos obtained from as many clones as possible - 17 obtained from 17 clones.
Tested / HFT 6- / HFT 7-

20	6-	Lp^s	= 8	
	7-	Lp^s	= 8	
	7-	Lp$^{R/S}$	= 1	= 415A-1

30

40

3057 - m source of Lp^R

50

1. ca 2000 colonies tested by replication / 2730 pos (Gal$_2$-) - None found non Lp^s

DATE: 5/3/56 REF: 416

(4/13)

Redo a 945 experiment to see if "mutants" cells are responsible for +[illegible] activity in LFT cultures.

1. Technique – nearly confluent colony formation of 945, to which was added a few HFT+1- cells as a control – replicated to 3079 Bgal Gal UV'd. Incubated 2 days and replicated to Bgal, Bgal SM (Gal2+ prototroph) on

This plate then replicated to M Gal

2. Aerated 945 diluted $10^2 - 10^4$ → [2 ml + 9 ml] [1.0 ml] → 0.1 ml / 10 plates

10^6 → 0.05 ml 1. 113, 2. 118, 231

10 Exempt of Gal+ after 2 days

UV'd plate	300
Bgal replica	300
Bgal SM	10
Mgal replica	300

Overnight 389-1 $10^2 - 10^4 - 10^6$

0.05 ml = 108, 65, 173 — 25 col LFT, 11 col LFT

Replicated / 3079 UV'd

2. This contains 231 × 2/9 × 100/10 = 4620/9 = 512 945 cells

173 × 1/12

Original plate (non UV'd) ca 10

This experiment abandoned – Replication to Bgal SM showed ca. 10 Gal+ per irradiated plate which presumably are transductants of 945 → 3079. The irradiated plates (and B gal replica of them) showed at least about 200-300 Gal+ clones – of these replication to M Gal showed at least 200 were prototrophic and derived presumably

W 2872 (1210× dil)

in search of HFT 8-

1. 17 seg tested against [illegible] 3094 – are LFT but 8- appears to have a selective advantage of 4- as it grows through Gal- background.

2. 21 seg tested / 3094 – all LFT
15 seg tested / 3094 – all LFT

W 7550 × W 2854

no add	14	
0.1 ml lys	54	(pure lysate)

9. 13 seg tested – 12 Gal2-, 1 Gal7- Gal-

DATE: 5/11/56 REF: 417

Segregation in 323-2
as in previous expts

overnight cult 10^2 10^4 10^6 10^8 → 0.05 ml samples B gel - respread 4 hours.
↓
assay O.lux -

	+	-	pep -	Total
1.	18	30	99	
2.	22	24	116	

Respread Clones	+	-	pep -
#'s 7—11	no growth		
12.	21	0	0
13.	0	0	22
14.	0	33	0
15.	0	0	92
16.	0	0	30
17.	0	0	7
18.	0	1	0
19.	0	13	2
20.	0	0	42
21.	0	0	24
22.	0	0	81
23.	0	0	3
24.	0	0	16
25.	0	6	0
26.	0	0	4
27.	3	4	37
28.	20	0	22
29.	0	0	28
30.	0	0	43
31.	0	8	12
32.	0	0	18

DATE: 5/13/56 REF: 418

Segregation from W2874 ($2^{-}7^{-}$) heterogenote — It is full bolt, does it still contain both Gal — alleles?

	2^{-}	7^{-}	Number	
1.	+	o	14	} both allels present but segregation pattern reversed
	o	+	4	

2. 14/14 2⁻ tested for HFT / 3091 all LFT

4/4 7⁻ tested / 3092 all LFT

At this time W2308 × — HFT 2⁻ } to compare reciprocal case
W2915 × — HFT 7⁻

FOR SEG. ANALYSIS SEE 420

made in the "standard" way 0.1 ml of 1:10 dil overnight culture + 0.4 ml HFT lysate — diluted to obtain ca 100–300 colonies / B gal plate (1/50 & 1/60)
Results not recorded but ca 300 colonies / plate, >50% show contact with phage (partial lysis)

W2308 — 2 ~~apparent~~ transduction clone apparent [418-1, 418-2]

W2915 — 1 trand. clone obtained — not as "+" as the above [418-3]

A. The initial plates of this experiment replicated to M Gal + 3091 and irradiated with UV 60 seconds — This, to see if any homogenetic clone formed by transduction (all transduction clones for have been selected on the basis of plus phenotype, in the case of (−) recipient (−) or mosaic phenotype in "+" recipient. No evidence of HET (by gal+ clone on M Gal) indicating no Gal — homogenote formed.
The extent of the test: ca. 500 gal_2^- colonies (W2915) } Planned to replicate
ca 300 gal_7^- colonies
to Gal_2^- indicator
generated by reversion of recipient

5/14/56

1210 × — 308-5 } for stock for ...
8⁻ × — 1-6⁻

no add — 0
0.1 ml 308-5 lysate (original, perhaps 2 years old) 84 (small) ① 15/15 found 8⁻ 15 tested / 3091 and found LFT
② 7/7 found 8⁻ 7 tested / 3091 M Gal found LFT

1210 × — 344-6
8⁻ × — 6-7⁻
no add — 0
(old lysate) 0.1 ml 344-6 153 (small) ① 19/19 found 8⁻ 18 tested / 3091 and found LFT
② 11/11 found 8⁻ 10 tested / 3091 M Gal — and found LFT
③ 16/16 " "

2580 × — 346-4

no add — ~
0.1 ml 346-4 64 ① 20 ... tested 19 Gal_2^- } See 347
1 Gal_6^-
② 10 ... tested

DATE: 5/16/56 REF: 419

1	2	3	4	5	6	7	8	9	10

1. In the experiments to see if HFT+ cells are distributed as "mutants" in LFT clones (such as on 416) many gal+ clones possibly transductions, reversions or something else — observed in the UV irradiated mixtures of donor and recipient. The number appears too high. Both cultures used in these later experiments were F- and no crossing is possible. Are better crossings being found.

2. Experimental —

a. ~~to~~ avoid lambda transduction Lp^s cultures used.

W2915 - F- TLB: Gal₂ - Lac, Ara- Xyl- Mtl- Pr- Lp^s

W3079 F- M- Gal₁ - Gal₂ - Lac- V_1^R S^R Lp^s

		papillae	
W2915	UV	9	which of these UV'd not known, plates not labelled
W2915	~~[illegible]~~	11	
W3079	UV	0	which of these UV'd not known — both labelled as such
W3079		0	

W2915 / W3079 — UV — Two classes of papillae: large = ca 30 — Gal+ on restreak; small = ca 500 — most of these just faintly + on Bgal on restreak.

b. test some of the papillae for markers.

① 6 small papillae tested on Bara - 1 gal+, ara-; 4 gal+ʷ, ara-; 1 gal+ʷ, ara+ʷ

6 large papillae tested on Bara 6 Gal+, ara-

3551 X K12 (poor lysate) made 5/14/56
no add = 0
0.1 ml = 14

1. 15 segregant gal+ 4-

346-4 X K12
no add = 0 (some small) this culture very rough.
0.1 ml = 19

1. 21 seg found 4-6-

DATE: 5/23/56 REF: 420

Seg analysis of 418-1 $2^- \times 7^-$ This clone [illegible]R/s

Number	/2⁻	/7⁻		
16	+	0	= 7⁻	all [illegible]
2	0	+	= 2⁻	both [illegible] λ^R, probably [illegible]

Seg analysis 418-3 $7^- \times 2^-$

Number	/2⁻	/7⁻		
4	+	0	= 7⁻	all λ^r, probably [illegible]
8	0	+	= 2⁻	all [illegible]
1	0	0	= 2⁻7⁻ ?	appears [illegible]

2880 × 2851 $2^- \times 1^-4^-$ — [illegible]

	/1⁻	/2⁻	/4⁻	
19	+	0	+	= Gal₂⁻
2	0	+	+	= Gal₁⁻

no good

0.1ml 2817 50

Heterogenote for Photographic Illustration

Lysate of K-12

Culture	no agar	0.1ml
3091	0	45
3092	4	64
3094	0	63
3096	2	80
3097	0	44
2857	0	88
2854	0	55

DATE: 5/30/56 REF: 421

1	2	3	4	5	6	7	8	9	10

Segregation from some heterozygotes to check them before use in photography

2580 X — 308-5

Number	/1-	/2-	/6-	Genotype
16	+	0	+	Gal_2^-
2	0	+	0	$Gal_6^- Gal_7^-$

2580 X — 344-6

Number	/6-	/2-	/7-	Genotype
19	+	0	+	Gal_2^-
1	0	+	0	$Gal_6^- Gal_7^-$