From here to 412
B gal SM platings were
from method

DATE: 2/15/56 REF: 394

2580 – overnight, incubated 2.5 hrs with air. Centrifuged
resusp'd in soln –

Pre-mod. cell assay — Bgal — dil 10^6 – 0.05: (1) 107; (2) 110; 217 × 10^7 = 2.2×10^9

" " transd assay undiluted → Bgal SM: (3) 0; (4) 0 — 1/10 ca 300 plaques — on B gal: (3a) 12; (4a) 21

2 2/13.0 = 0.59; 110/200; +3079 — 59% survival

→ 3 ml + 9 ml 2x pen →

Irradiate 40 sec

Post. mod. cell assay dil 10^6 — (5) 62; (6) 70; 132 × 10^7 = 1.3×10^9 — λ assay Bgal: (7) 26 plaques; (8) 45 — 71 — 71 × 10^7 = 7.1×10^8 plaque centers

5/14: 71 = 35.5/14 = 2.5 × 10^8

Post. mod. transd. assay und. (9) ca 300 large 400 small; (10) " " — (11) 0; (12) 0 — almost confluent — This column B gal SM

These from undiluted sol. every 20 min.

time in 2X pen

time	Bgal		Bgal SM	
0 1:20 PM				
20 1:40 PM	(13) 279; (14) 252; 531/2 = 266		(15) 0; (16) 0	1/10 ca 300 plaques
40 2:00 PM	(17) 350; (18) 397; 747/2 = 374		(19) 0; (20) 0	dil 10^6 .05 + 3079: (18a) 137; (19a) 162; 299 × 10^7 = 2.9×10^9
60 2:20	(21) 419; (22) 488; 830/2 = 415		(23) 1; (24) 3	(21a) 163; (22a) 189; 352 × 10^7 = 3.5×10^9
80	(25) 312; (26) 248; 560/2 = 280		(27) 108 (many small); (28) 81; 189/2 = 95	
100	(29) 195; (30) 214; 409/2 = 205		(31) 95 (many small); (32) 109 (many small); 204	centrifuge 3 ml Bgal + 3079: (29a) 57; (30a) 67 — continue
120	(33) 125; (34) 149; 274/2 = 137		(35) 68 (many small); (36) 127 (small 70 lg); 195/2 = 98	dil 10^7 .05 + 3079: (37a) 123; (34a) 139 plaque; 262 × 10^8 = 2.6×10^{10}

0.05 ml + 3079 on D(0) (4) (8) colonies at 20 days

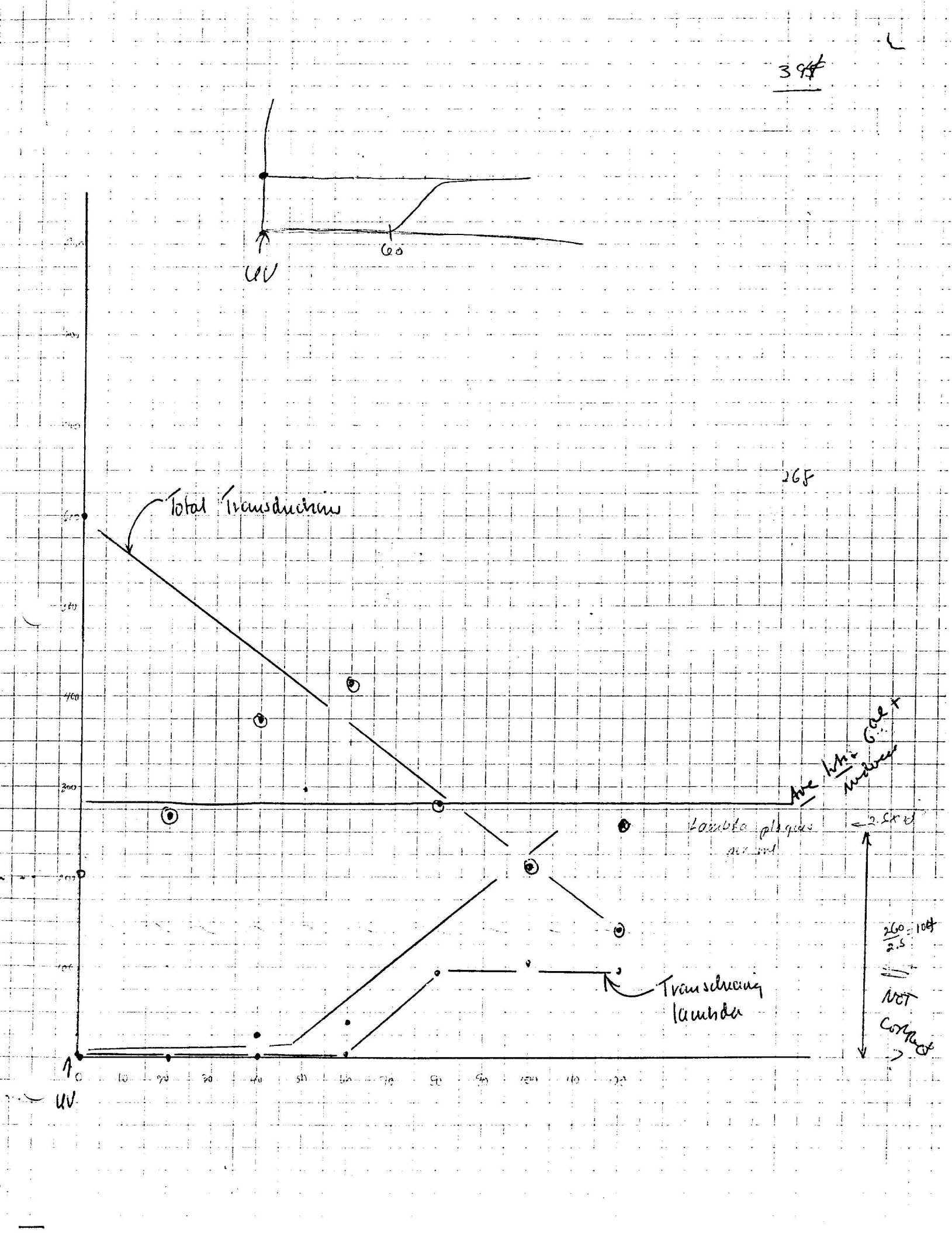
Total = 1677

Ave Total/6 = 1677/6 = 279

600 × 80 × 1/3 = 24000 = 2.4×10^4 / 2.2×10^9 cells = 1/10^5 cells

104 + 95 + 98 = 297/3 = 99

99/279 = burst size 279 / 99.0 = 0.36


Total Transduction
Lambda plaques
Transducing lambda
UV
60

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 Cd, ly^s (R) TLB^s 2^-/2^- ly^+

1. 1.0 ml of each parent added 9.0 ml pen, incubated 3 hours, diluted 1-100 with distilled H₂O, 0.1 ml

	(+)	(-)
2 (a)	* 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 picked to D(o) - all grew therefore prototrophic
The 10^- tested against HFT 1^- HFT 2^-
10 + 0 = Gal₂^-

3 Gal + after 2 streaks tested

seg. ?	λ	3079		Genotype
1. 0	R	+		ly^+
2. 0	R	+		ly^+
3. 0	R	+		ly^+
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	1	2	λ
5	+	0	R
7	+	0	S
10	+	0	R

DATE: 2/17/56 REF: 396

2637 derivatives from 290
labelled - after some arrangement and re-arrangement

	Presumed	3079	Growth ϑ(o)	^	Crossed gal
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+
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	~~nonly~~ lys.	+	S	Lp^s
3107	Gal₇ - Lp^s	~~nonly~~ lys.	+	S	Lp^s
3108	—				
3109	—				
3110	Gal+ Lp^s	nonlys.	+	S	Lp^s.

Frequency of transgenics 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 indicated — overnight cult diluted 1-10, aerated 3 hours.

Survival 318 / 49.0 = 15.4%

PRE-UV: 1 dil 10^6 0.05 ml — ① 148, ② 170 = 318 (B Gal) } cell used Assay .005 — ③ 0, ④ 0 (B gal SM) } Pre-trans'd, plaque assay } Same plate. (no indicator pre-coated)

O.D. / water = 4/10 dil = 0.58

1-10 dil

U.V. 60 seconds — add 3 ml + 90 ml Pen 2X

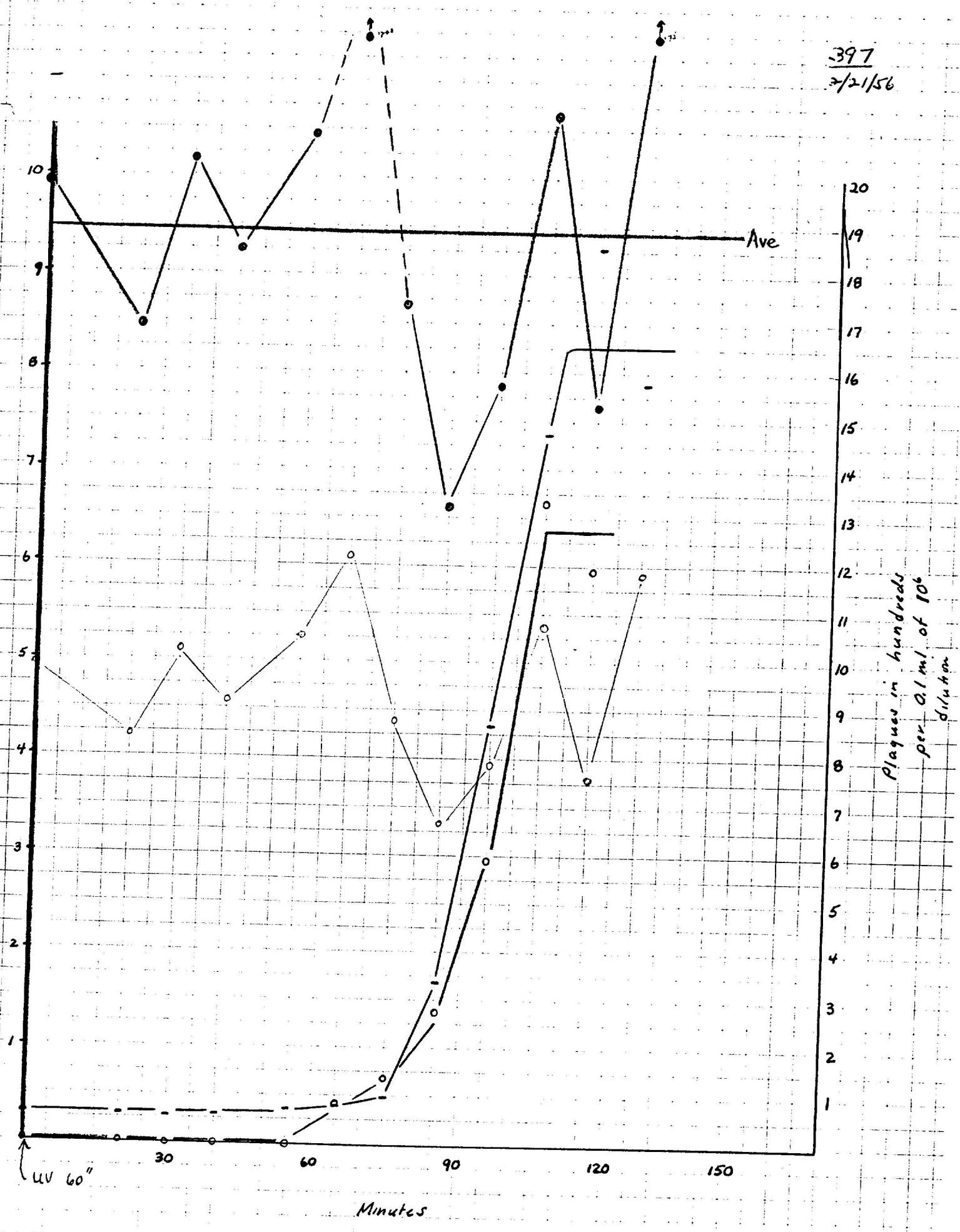
Post U.V. — not added to Pen.

dil 10^6 — 0.05 ml — ③ 22, ④ 27 = 49 } survival — ⑦ 58, ⑧ 64 [61] (Bgal, for 0 plaque)

0 — ⑤ 887, ⑥ 1097 = 1984 } trans'd centers — ⑦ 0, ⑧ 0 (ca 1000 plaques, ca 1000 plaques)

dil 10^6 — tube samples (Bgal)		Time				
⑨ 27, ⑩ 29 = 28	[56]	20	⑨ 476, ⑩ 368 = 844	⑪ 0 (ca 773 plaques), ⑫ 1 (ca 700 plaques)
⑪ 32, ⑫ 21 = 27	[53]	30	⑬ 467, ⑭ 552 = 1019	⑮ 0 (ca 196 plaques), ⑯ 0 (ca ")
⑬ 38, ⑭ 32 = 35	[60]	40	⑰ 462, ⑱ 461 = 923	⑲ 0 (ca 1000 plaques), ⑳ 0
⑮ 33, ⑯ 36 = 35	[69]	50 (55)	㉑ 528, ㉒ 517 = 1045	㉓ 0, ㉔ 0
⑰ 39, ⑱ 38 = 39	[77]	60 (65)	㉕ 595, ㉖ 621 = 1216	㉗ 23, ㉘ 18 = 41
⑲ 35, ⑳ 63 = 49	[98]	70 75	㉙ 427, ㉚ 441 = 868	㉛ 29, ㉜ 39 = 68
㉑ 109, ㉒ 226 = 168	[335]	80 85	㉝ 296, ㉞ 364 = 660	㉟ 46, ㊱ 91 = 137
㉓ 452, ㉔ 413 = 433	[865]	90 95	㊲ 358, ㊳ 395 = 783	㊴ 136, ㊵ 159 = 295
㉕ 772, ㉖ 701 = 737	[1473]	100 105	㊶ 505, ㊷ 562 = 1067	㊸ 313, ㊹ 352 = 665
㉗ 960, ㉘ 873	[1833]	110 115	㊺ 408, ㊻ 350 = 758	㊼ 306, ㊽ 291 = 597
㉙ 873, ㉚ 700	[1573]	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 10⁶ 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 freshly grown cultures (6 hours from overnight inoculum both) probably lower cell density than starved cells.

Plating on B gal SM

1. Plating	Pour	Surface	Broth	Starved	Counts
3079	+	0	0	+	139
	+	0	0	+	158
	+	0	+	0	31
	+	0	+	0	24
	0	+	+	0	52
	0	+	0	+	164
3080	+	0	0	+	200
	+	0	0	+	222
	+	0	+	0	65
	+	0	+	0	149
	0	+	?	?	138
	0	+	?	?	127

(ca 1000 plaques each colony plaques)

} plates not labeled

These results suggest that starved cells may be better as an indicator but densities were not the same and it will be better to repeat using a density check.

DATE: 2/24/56 REF:

1	2	3	4	5	6	7	8	9	10

One step with 945 (culture from AR) technic and numbering as in 397 with the exceptions. Survival = 5/93 = ca 5%

A Expt

1. Culture of 945 (mal- ly_2^R) instead of 2580 (mal+ ly_2^S)
2. Density of susp. (irradiated) adjusted to 0.28/58 - UV.-60"
3. Diluted 1-10 instead 1/12 (.05 ml samples)
4. Times (absolute) the same - samples taken at 50, 60, 70, 80 ck. instead 15, 15 ck

pre-cell (1) 44; (2) 49 — post cell (3) 3; (4) 2 — (32) 0 (4460 plaques) (7) 179; (42) 0 (11") (6) 147 (322) — (7) 0 (4545 plaques) post UV; (8) 0 (4153 plaques) free transd

B. Results

Time	Surface plates		Total Transd. (3gal) (93)	Free transd (3gal SM) (5)
0	(7) 0 / (8) 0		(9) 12 / (10) 15 / 27	(11) 0 57 plaques / (12) 0 31 plaques
20	(9) 6 / (10) 11 / 17	(17)	(13) 27 / (14) 20 / 47	(15) 0 71 plaques / (16) 0 77 plaques
30	(11) 7 / (12) 11 / 18	(18)	(17) 66 / (18) 69 / 135	(19) 0 149 plaques / (20) 0 146 plaques
40	(13) 8 / (14) 4	(12)	(21) 47 / (22) 53 / 100	(23) 0 1/2 = 66 plaques / (24) 0 "
50	(15) 9 / (16) 8	(17)	(25) 80 / (26) 35 / 115	(27) 0 (41? (small)) almost confluent / (28) 0 (74? (small)) " "
60	(17) 8 / (18) 4	(12)	(29) 84 / (30) 92 / 176	(31) 0 (40? (small)) confluent / (32) 0 (73? (small)) "
70	(19) 8 / (20) 7	(15)	(33) 93 / (34) 98 / 191	(35) 1 large / (36) 1 large / 2
80	(21) 10 / (22) 9	(19)	(37) 60 / (38) 63 / 123	(39) 22 / (40) 9 / 31
90	(23) 12 / (24) 8	(20)	(41) 58 / (42) 38 / 96	(43) 28 / (44) 28 / 56
100	(25) 62 / (26) 23	(85)	(45) 88 / (46) 47 / 135	(47) 78 / (48) 84 / 162
110	(27) 74 / (28) 84	(158)	(49) 80 / (50) 60 / 140	(51) 84 / (52) 90 / 174
120	(29) 184 / (30) 145 / 329/16 = 20			

transd centers measured — dilute 1-10

Surface plates → (30) plaques

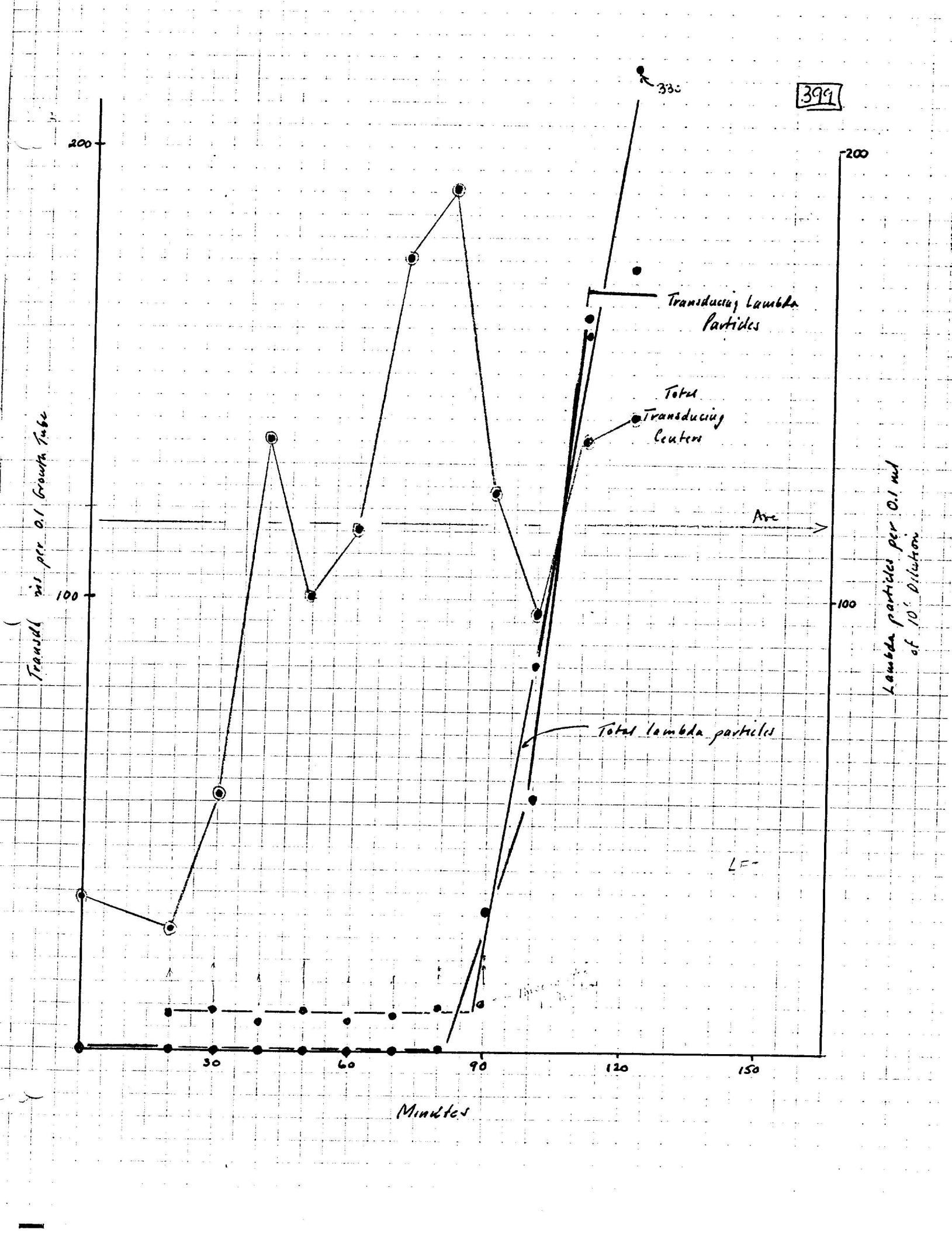
Total Transd.

11 17/28 18 1/18

Free transd CM

W3079

area plaques rating


330
200
100
Transduc. Lambda Particles
Total Transducing Centers
Ave
Total lambda particles
LF-
30
60
90
120
150
Minutes
Transduc. per 0.1 Growth Tube
Lambda particles per 0.1 ml of 10⁶ dilution

DATE: 2/27/56 Survival = 16/40 = 0.40 REF: 400

1	2	3	4	5	6	7	8	9	10

45/65, 42/69 after med

One step with 341-9

Plaque count in red =

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, 18/21 at 0

2. Pre assay (A) dil $10^2 - 10^4 - 10^6$ → 0.05 on surface for colonies (1) ✓ 23, (2) ✓ 17 (total 40) — B gal / B gal SM: — ; 4/40 = 10%
ASSAY THESE COL. FOR HFT →
each of these 2 of these LFT

(B) transd. assay} 10^6 dil 0.05 / 3079 + plaques
0 plaques ✓ (3) 1 ✓ (5) 0 — 0 plaques
0 plaques ✓ (4) 0 ✓ (6) 0 — 0 plaques
(transd occurring in plaques; transd occurring in plaques)

3. Irradiate 60 sec (10 LFT)

4. Dilute $10^2 - 10^4$ → 10^5 — 0.05 — surface ✓ for HFT
↑ Incubate - plate 0.05 at various time intervals (5 LFT)

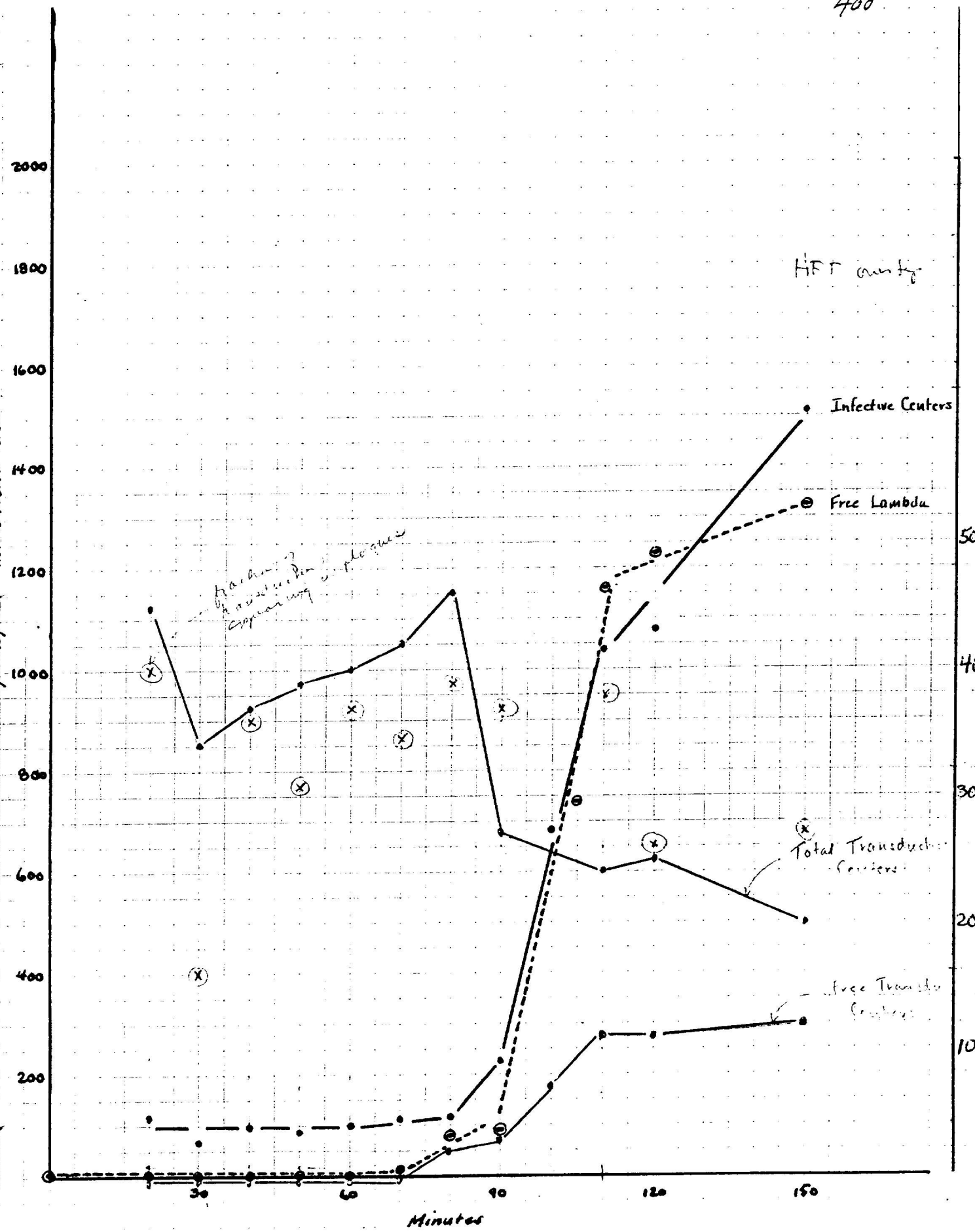
15/157 = ca 10%

(bad plate) partially solidified in pouring, both went off

Cells disappearing 40-16 = 24
Infect. centers produced 20' = 12
30 = 6
40 = 10
50 = 9
60 = 10
70 = 11
80 = 11
Ave = 53/4 = 10

	Time	Min	Plate / count	Plate / count	Total
~~0~~	12:15	10	✓ (7) 76	✓ (9) ~~0 plaques~~	0 plaques
			✓ (8) 81 — 157	✓ (10) 0	0 plaques
~~20~~	12:35	20	✓ (11) 22 19/22 57 ✓	(13) 0	0
~~30~~			✓ (12) 23 18/23 59 ✓ — 45 116	(14) 0	0 — 0
~~40~~	12:45	30	✓ (15) 20 4/20 27 ✓	(17) 0	0
~~50~~			✓ (16) 14 9/14 37 ✓ — 34 64	(18) 0	0 — 0
	12:55	40	✓ (19) 11 10/11 42 ✓	(21) 0	0
~~60~~			✓ (20) 26 19/26 56 ✓ — 37 98	(22) 0	0 — 0
~~70~~	1:05	50	✓ (23) 12 6/12 32 ✓	(25) 0	0
~~80~~			✓ (24) 27 18/27 53 ✓ — 39 83	(26) 0	0 — 0
	1:15	60	✓ (27) 18 13/18 50 ✓	(29) 0	0
~~90~~			✓ (28) 22 5/22 49 ✓ — 40 99	(30) 0	1 — 1
~~100~~	1:25	70	✓ (31) 23 16/23 64 ✓	(33) 0	10
~~110~~			✓ (32) 19 13/19 49 ✓ — 42 113	(34) 0	6 — 16
	1:35	80	✓ (35) 25 17/25 55 ✓	(37) 1 1/1	16
~~130~~			✓ (36) 21 11/21 55 ✓ — 46 110	(38) 1 1/1	66 — 82
~~150~~	1:45	90	✓ (39) 13 11/14 103	(41) 1 1/1	55
			✓ (40) 14 10/14 122 — 27 225	(42) 2 2/2	38 — 93 (bad plating technique)
	1:55	100	plated B gel ✓ (43) / 264 ✓	(45) 3 3/3	435
			✓ (44) / 422 ✓ — 686	(46) 4 3/4	306 — 741
	2:05	110	✓ (47) 7 7/7 352 ✓	(49) 6 3/6	536
			✓ (48) 17 7/17 688 — 1040	(50) 5 3/5	626 — 1162
	2:15	120	✓ (51) 13 6/13 631	(53) 7 4/7	612
			✓ (52) 12 7/12 445 — 1076	(54) 4 3/4	621 — 1233
	2:45	150	(55) 9 5/9 771	(57) 4 2/4	656
			(56) 11 6/11 740 — 1511	(58) 8 2/8	668 — 1324

↑ transductants ↑ plaques


HFT auto
Infective Centers
Free Lambda
Total Transducing Centers
Free Transducing Centers
Plaques per 1 ml Growth tube
Minutes
2000
1800
1600
1400
1200
1000
800
600
400
200
50
40
30
20
10
30
60
90
120
150

DATE: 3/8/56 REF:

Preparation of prototrophic F stocks with the gal markers – "Kalckar" stocks

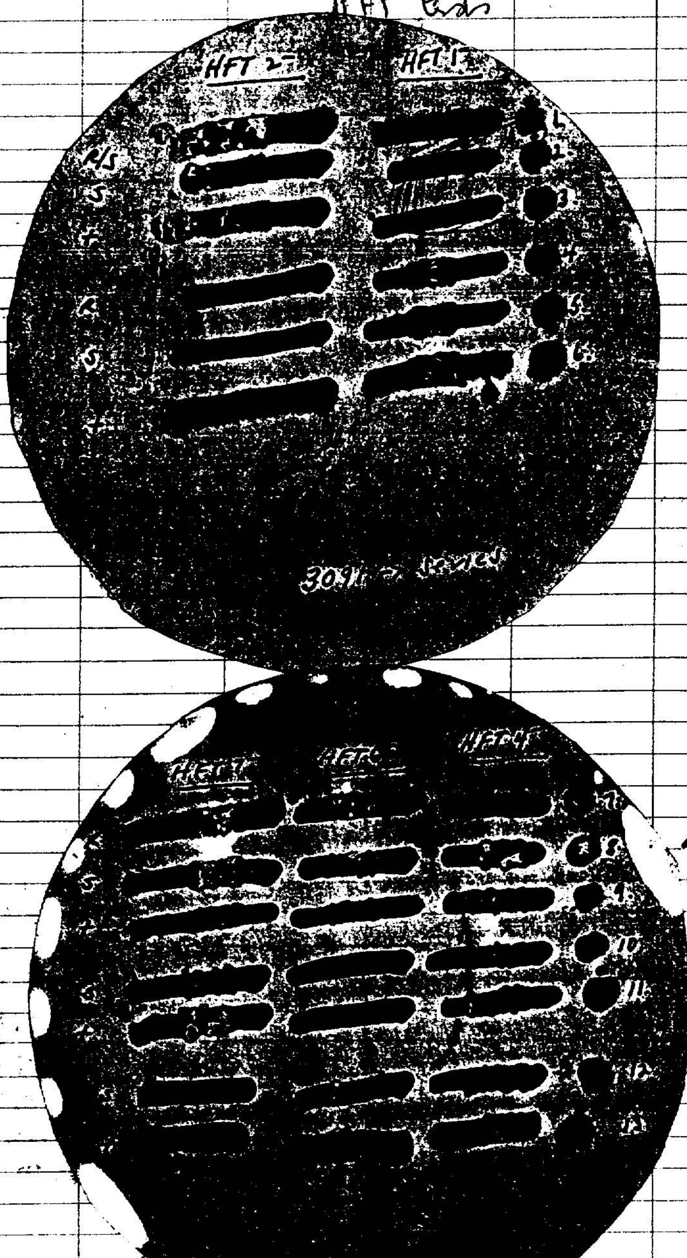
1. The numbers proposed for these are 3091 → 98 (λp^+), 3101 → 3107 (λp^S), 310→ ? (λp^2 (Assumed))
3100 = λp^+ Gal+
3110 = λp^S Gal+

2. Beginning with 3110 = (F⁻ 1485) – the cells from an overnight culture exposed to HFT's and diluted and plated. See page 390 a^{dn} 396

3. After all cultures obtained, they were tested:

3079		λ	D(o)	Original gen. designation
1.	wl	R	+	1⁻ r/s
2.	wl	S	+	1⁻ s
3.	lys	R	+	1⁻ +
4.	wl ?	R	+	2⁻ r
5.	wl	S	+	2⁻ s
6.	lys	R	+	2⁻ +
7	wl	R	+	4⁻ r
8.	wl	S	+	4⁻ s
9.	lys	R	+	4⁻ +
10.	lys	R	+	6⁻ s
11.	wl	S	+	6⁻ +
12.	wl	S	+	7⁻ s
13.	lys	R	+	7⁻ +
14.	wl	S	+	+ s
15.	lys	R	+	+ +

HFT tests



Notes

① The Gal₂⁻ derivatives appear to be revertable

② The culture obtained by mad. 6⁻ lys (to obtain lys) appears no longer to be Gal₄⁻; and the λp^+ derived is also not Gal₄⁻

Redo Gal₂⁻

Gal₄⁻ λp^S, λp^+

THE CULTURES FIRST ISOLATED FOR Gal₂⁻ and Gal₄⁻ 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 a gy^0 recipient. If the higher titers of HFT lysates on gy^+ recipients (as compared with gy^0 recipients) is due to selection of phage particles entering gy^+ recipients (all HFT homogenotes have been obtained in gy^+ recip. lines), then there may not be higher assays on gy^+ recip. when the line originates in gy^0 recip.

1. 6 lysates made starting from single HFT colonies
irrad. 60 sec, incubated 4 hours
diluted 10^2, 10^4, 0.05 ml plated with 3079 (1-4-gy^0 S^R), 3080 (1-4-gy^+ S^R), on B gal str. (surface plated)

Lysate	3079 Plaques*	3079 Transduct.		3080 Transd.
1	ca. 15	2	(11)	29
2	20	7	(27)	28
3	29	4	(17)	7
4	26	3	(21)	25
5	17	5	(24)	14
6	7	5	(12)	19

It appears that HFT lysates obtained in gy^0 recip. this also assays higher on gy^+ recipients

2×10^5 / 17 ; $3.4 \times 10^5 = 3.4 \times 10^6$; 3.4×10^4 ; 20 ; 1.7×10^3 ; 20 ; $.8 \times 10^3$; 20

$7 \times 20 \times 10^4$; 140×10^4 ; 1.4×10^6

$17 \times 20 \times 10^4$; 3.4×10^6 / 3 ; 1.1×10^6 trans.

* plaque assays low because plates were dry

Observation that Gal- grow on M Gal - test made to see what the source may be.

Comparison - using 1- proto homogenote, 1- seg. derived from it, 2- gy^+ hom. derived in cross lgy^+ (-) E- homogenote 341-9, and a heterogenote seg of this 2-hom.

Medium	Obs.
M (no gal)	no growth. crystal 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 + λ^d) → heterogenote. This heterogenote not tested directly but previous segregants found λ^R (see 401) indicating of this seg. gave a non-only-λ^d, also evidence of some phage.

2. Segregants re-obtained from 390-4. 4 picked from first streak and therefore clonally related.

	/HFT(+)	/HFT 4	λ^{RX}	
1.	+	0	Sens	← 403-1 = λ^d (3104) W3104
2.	+	0	"	
3.	+	0	"	
4	+	0	"	

Sens. genotype not expected — indicates that this heterogenote is λ^s/λ^d — 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

Irradiation of HFF lambda - lysate approx. titer 3.4×10^6 total pfu/ml - see page 402
lysate no 5 from 364A1. Dilute the lysate 0.1 ml + 10 ml broth (2), incubate and
add 0.1 ml samples to 1.9 ml Pen. - Samples (0.05 ml) removed from these periodically
and plated, by pouring on B agar with indicator

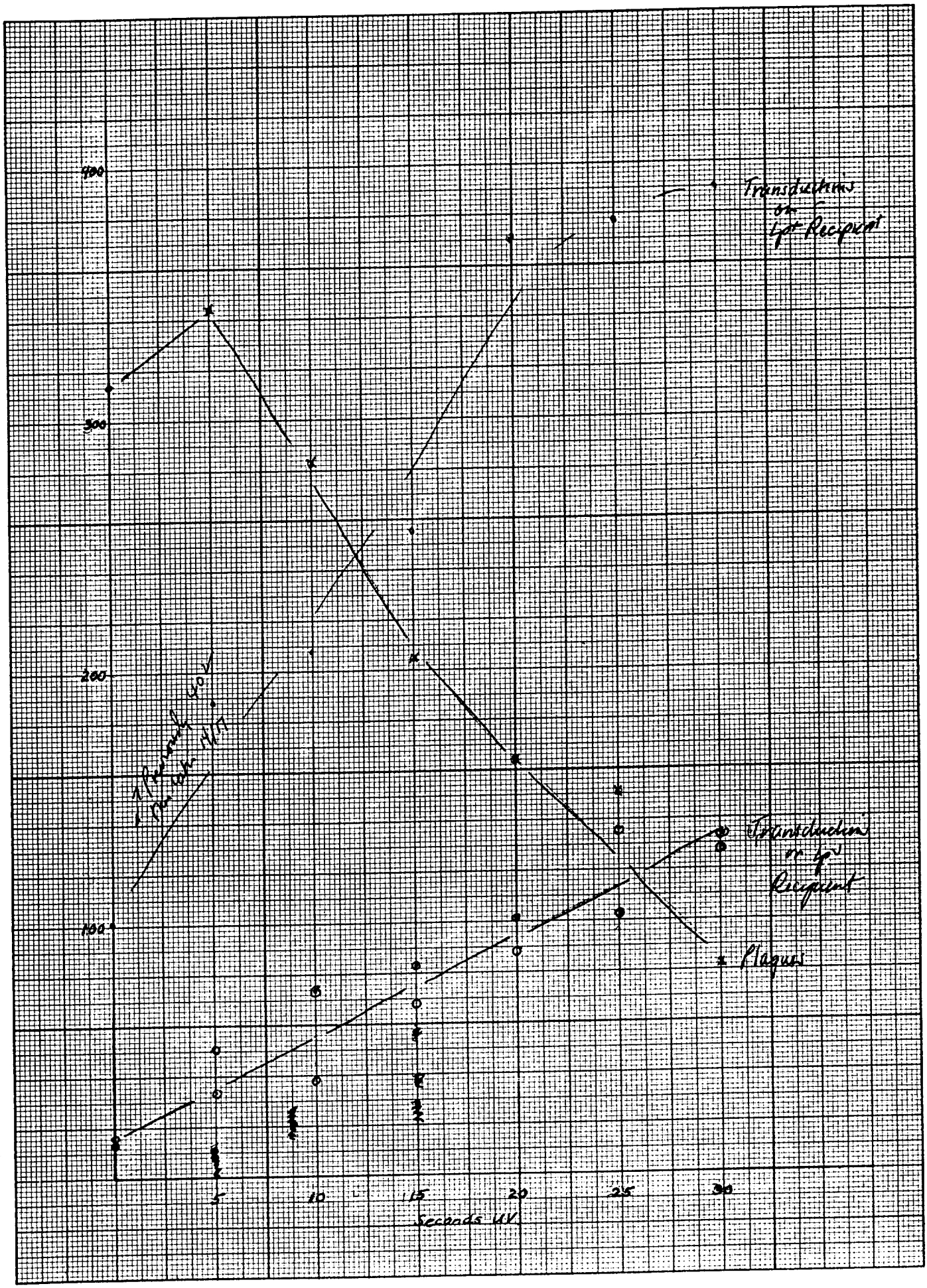
	Dose	Plaques/3079	Transd/3079	Transd/3080	plaques B agar SM /3079	/3080	Found/3079
1	0	314	13	101 (complete)	164	~~60~~	155
2	5 sec.	344	34	188 "	265	150	58
3	10 sec.	283	74	208 "	170	~~126~~	38
4	15 sec.	205	83	256 "	142		68
5	20 sec.	164	101	371 "	99	221	89
6	25 sec.	151	136	378 "	98		103
7	30 sec.	83	128	ca 390 "	136		133
~~8~~	~~35 sec~~				4		
		4+	4+	4+		3080 apparently contam.	

Rpt 3/26/56

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.



DATE: 3/16/56 REF: 405

5×10^2 : 1×10^5 / 3×10^7

Production of Transducing λ - LFT - Dosage study.

λ 945 grown overnight on aerator - dil 1-4, reincubate 2 hours - Centrifuge, resuspend in saline. Adjust density to 0.58 dilute 1-20 in adial with UV 5"

All platings 0.05 on Bgal

Controls		Dose	Undiluted plating Bgal 3079		3080		Dilute 10^2, 10^4 plate 3079		cells	
		0	①	3	②	6	③	0	④	128
1. 945		15	⑤	26	⑥	23	⑦	14	⑧	78
2. 3079		30	⑨	27	⑩	12	⑪	57	⑫	42
3. 3080		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
	plaques	105	㉙	1	㉚	6	㉛	7	㉜	0

$= 2.56 \times 10^7$: 2.6×10^7

Fract. cells yielding transducing activity

$\frac{\text{no trans}}{\text{no cells}} = \frac{5.4 \times 10^2}{2.6 \times 10^7} = 2.1 \times 10^{-5}$

Dose 105 x 2×10^5

Rpt with diff. plates - overnight, aerated 3-4 hours after 1-10, centrifuged, resusp. saline O.D. = 0.6 dilute 2/9 - irradiate as before - remove 0.05 ml + 4 sal, $10^2 \to 0.05$ for diluted; 0.05 as before for undiluted - Numbers as above.

3/28/56

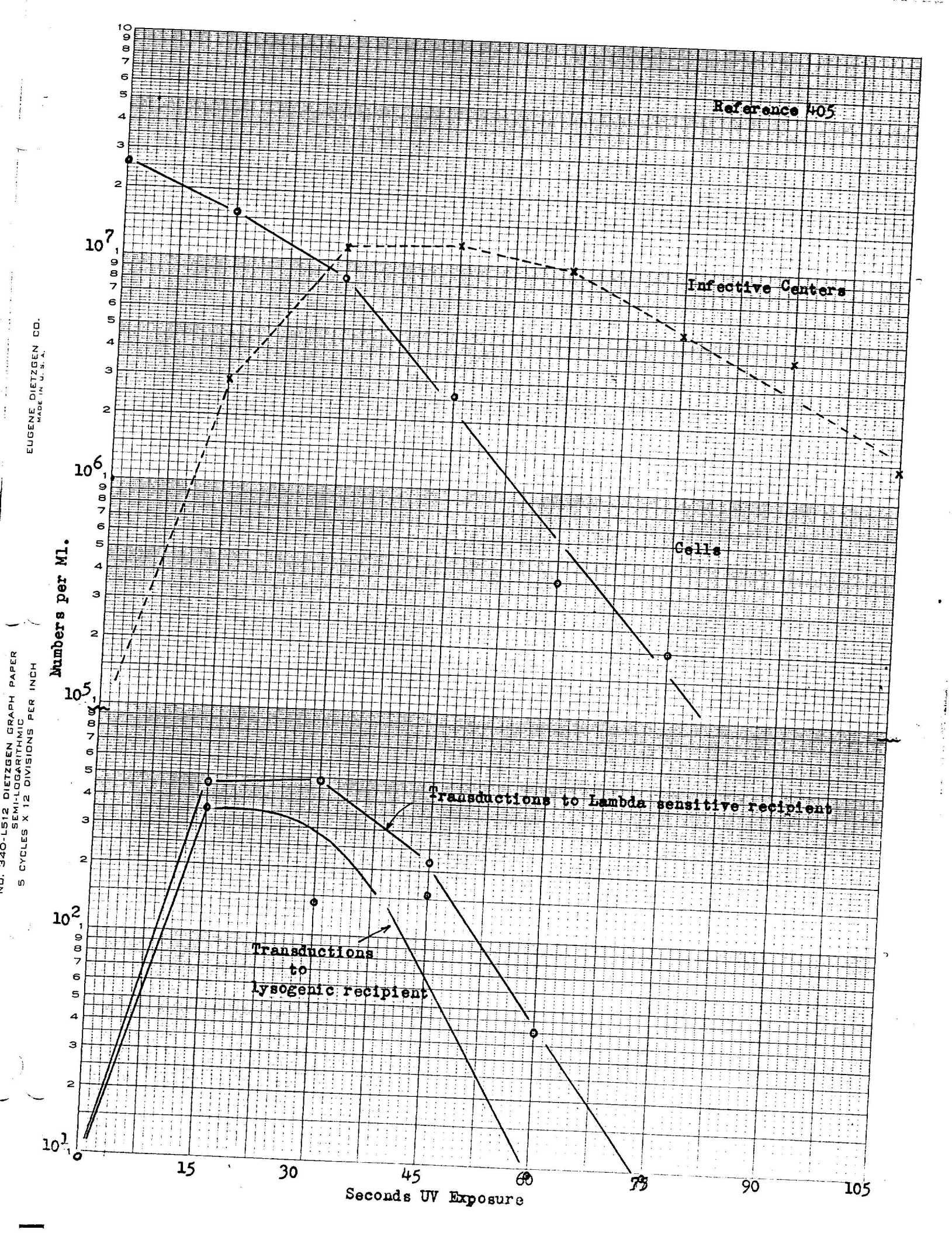
/3079 o/3.1 x 10^8	o/3.1 x 10^8	Dose	Und. trans 3079		3080		dilute 3079 = plaques		0 = cells	3.1×10^8	Fraction as plaques
		0	①	13	②	19	③	1	④	790	1.0	$\frac{1}{790}$ 0.0013
0.61×10^{-5}	0.34×10^{-5}	15	⑤	108	⑥	97	⑦	18	⑧	402	0.52	2.2×10^{-2}
1.4×10^{-5}	1.0×10^{-5}	30	⑨	226	⑩	179	⑪	77	⑫	291	0.37	9.8×10^{-2}
1.5×10^{-5}	0.61×10^{-5}	45	⑬	249	⑭	118	⑮	170	⑯	173	0.22	2.1×10^{-1}
1.0×10^{-5}	0.35×10^{-5}	60	⑰	175	⑱	74	⑲	171	⑳	56	0.07	2.2×10^{-1}
[illegible] $\times 10^{-5}$	[illegible]	75	㉑	130	㉒	26	㉓	71 low?	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

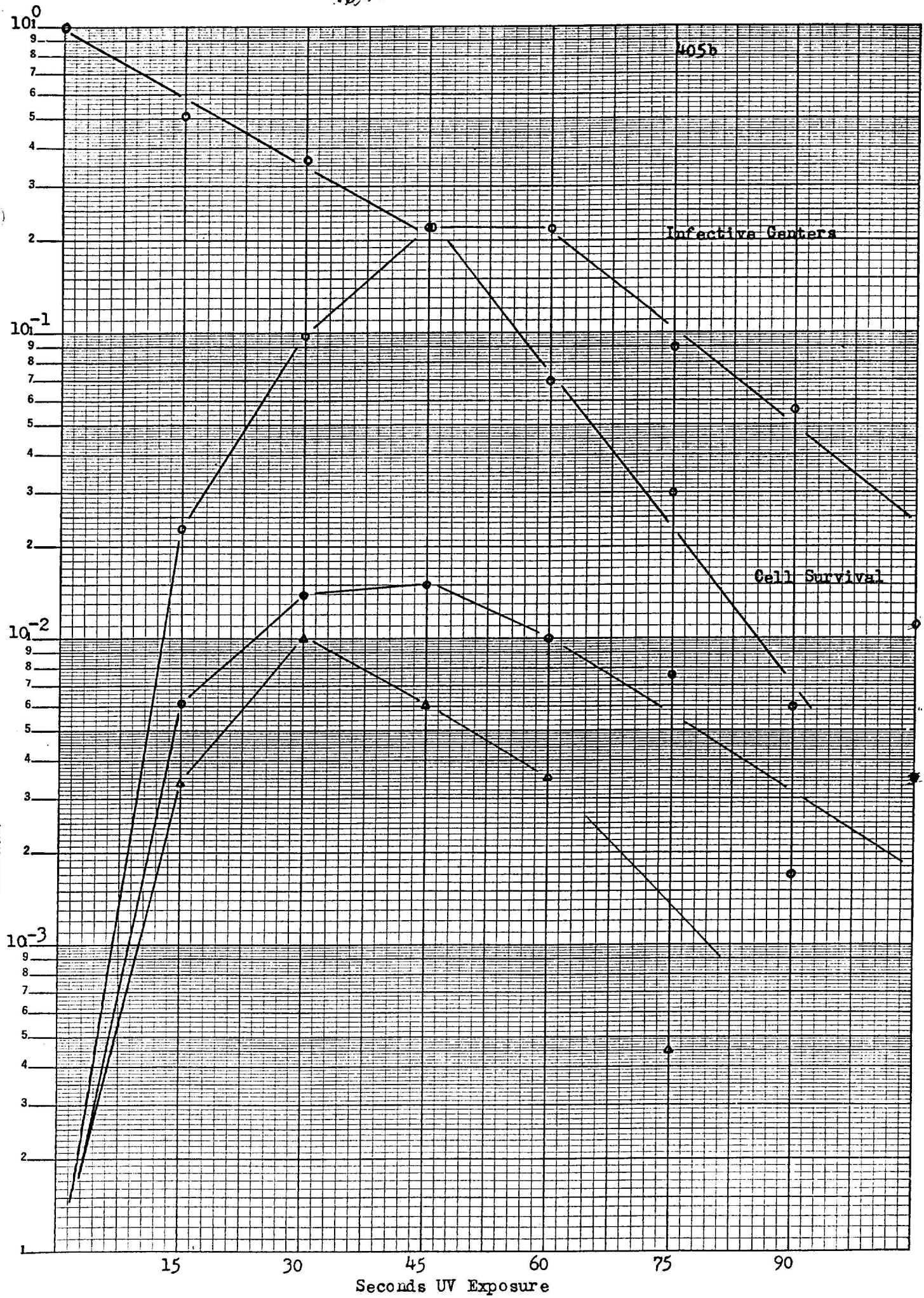
$20 \times 10^2 \times 2 \times 10^2$

Fraction of cells yielding transduction

4×10^5 $\frac{\text{no. trans}}{\text{no. cells}} = \frac{472 \times 10^3}{3.1 \times 10^8}$


Reference 405
Infective Centers
Cells
Numbers per Ml.
Transductions to Lambda sensitive recipient
Transductions
to
lysogenic recipient
Seconds UV Exposure
15
30
45
60
75
90
105
10^7
10^6
10^5
10^2
10^1
EUGENE DIETZGEN CO.
MADE IN U.S.A.
NO. 340-L512 DIETZGEN GRAPH PAPER
SEMI-LOGARITHMIC
5 CYCLES X 12 DIVISIONS PER INCH

1602



DATE: 3/24/56 REF: 406

Comparison of U.V. Resistance of haploid and heterogastic clone.

1. Set up. 3419, a 2/2- homogenote obtained out, and a LFT segregant obtained. A gal+ reversion of this segregant by selection on B gal. The comparison for U.V. survival is between these segregant and 341-9

2. Experimental.

(A) 341-9 started from HFT+ clone (detected on B gal - HFT test) 3418(+) from single colony.

(B) Overnight cultures diluted ca 1-20 incubated 37 on rotator for ca 3 hours. Diluted 0.2 + 10 ml saline - each into two test tubes, and then 10^3, 10^4 additional. Two final tubes unaerated, 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	~~HFT+ 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 0.9
35	16	14	30	0.065	17	8	25	0.091	—
40	4	16	20	0.044	5	10	15	0.016	15/20 0.75
60	0	0	0	0	1	1	2	0.007	1/2 0.5

A second analysis (3/14/56) using a t^R of 341-9 and comparing with a (-) segregant of this

Time	Gal+ Colonies	Fraction	Gal- colonies	Fraction
0	325	1.0	151	1.0
5	296	0.9	152	1.0
10	176	0.54	124	0.82
15	179	0.55	92	0.61
20	108	0.33	72	0.48
25	90	0.28	45	0.30
30	72	0.22	22	0.15
35	53	0.16	20	0.13
40	14	0.04	8	0.05
50	13	0.04	2	0.01

In the HFT+ cultures, the calculation of +/- was:

+/- 2.15 Prob. 0.47 1. 23/~~3~~1

1.95 0.52 2. 19/3

1.42 0.7 42/4

1.95 0.52

1.5 0.67

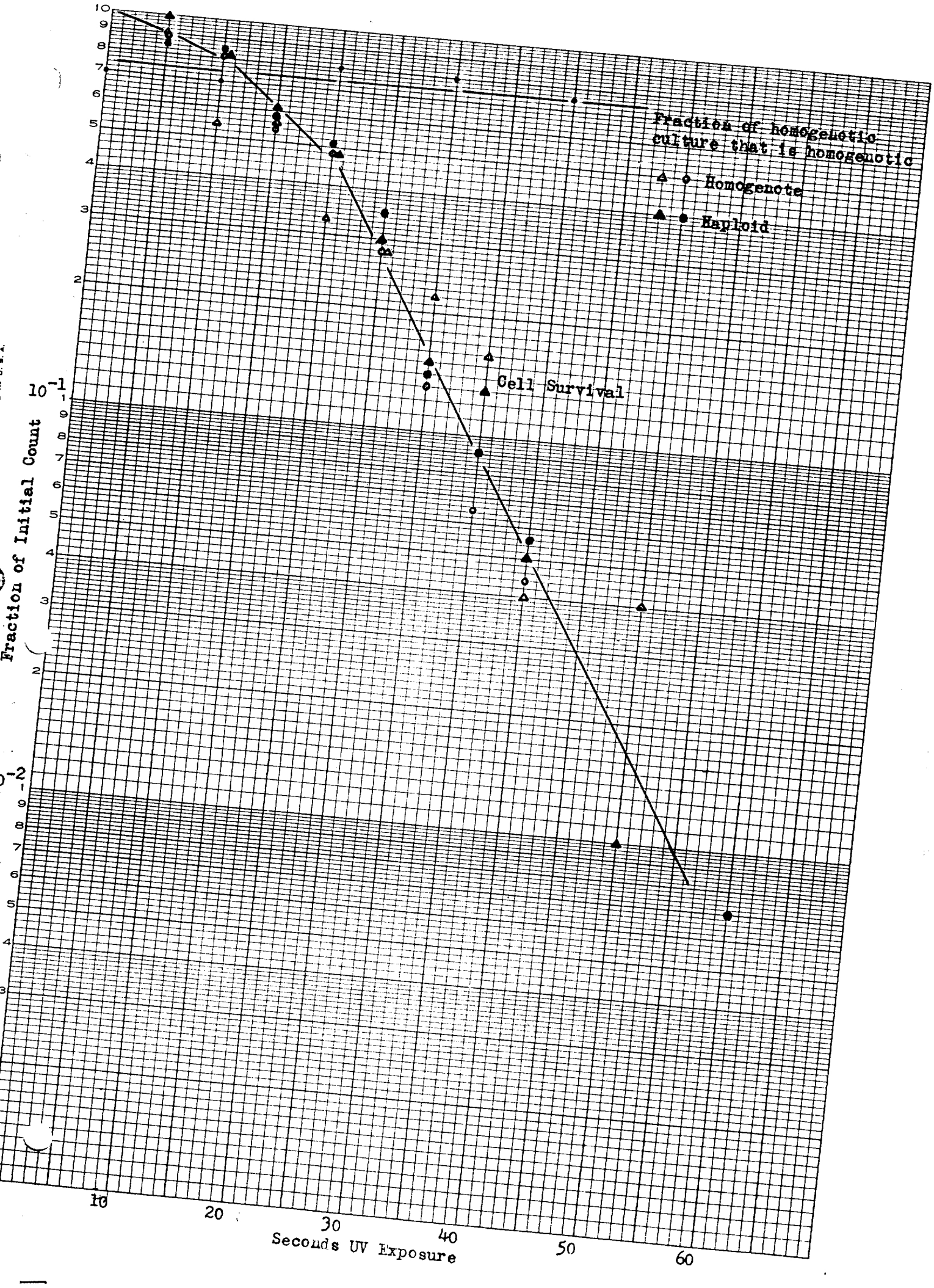
2.0 0.50

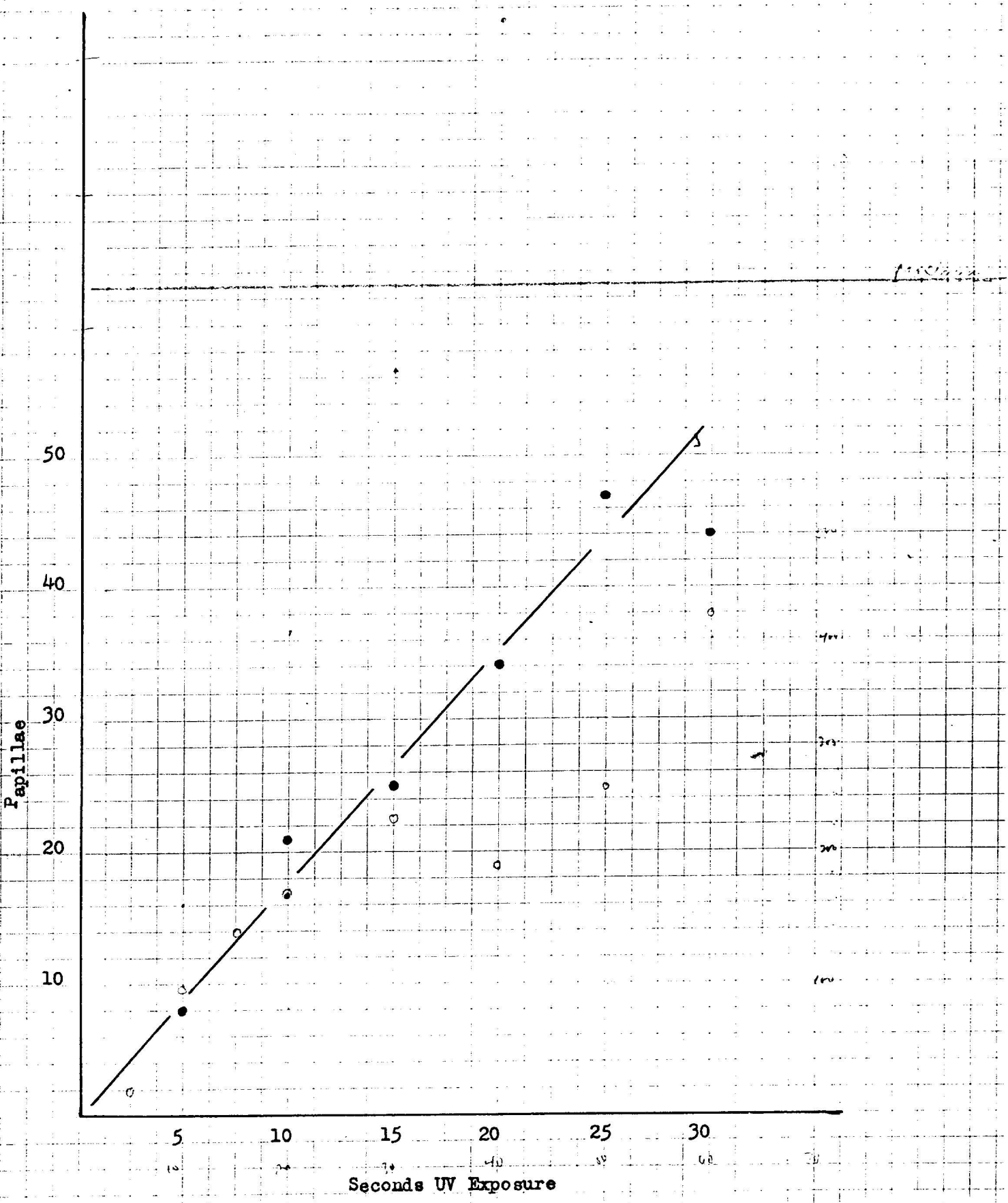
3.1 0.32

2.7 0.37

1.75 0.57

~6.5 0.16


Fraction of homogenotic 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

1 2 3 4 5 6 7 8 9 10

$0.58 = 2.6 \times 10^7$ cells/ml
×20
(5×10^8)
0.64

Test of conditions required to obtain "maximum" induction on β gal.

A ① Overnight aerated 943 – diluted ca 1-20 incubated in rotator ca 3 hours, centrifuge, resuspend saline. Adjust density to 0.6 diluted 1-20 – Ⓐ

② Susp A irrad. 45 seconds should could contain ca 300 [illegible] / [illegible]

Ⓐ Test – irrad 45 seconds, plate 0.05 + 3079 — ① 77
② 49
63

③ Plate 0.05 ml of suspension on surface of β gal – Irradiate varying doses, add 0.1 ml of 3079 respread.

Dose			
30"	③	99	→ 112
	④	124	
45"	⑤	115	→ 116
	⑥	117	
60"	⑦	86	→ 62
	⑧	38	
75"	⑨	57	→ 56
	⑩	54	

Are some of these contaminated?

④ Plate 0.05 ml of suspension on surface β gal – Incubate ~~3~~ hours – irradiate 60", add 3079, respread
Wash two plates dilute and assay for total growth – assay 1.0 ml portions ~~[illegible]~~, ~~[illegible]~~ once, 0.5 ml obtained, added to 10 ml
dil 10^2, 10^4 → 0.05 ml

2:35
5:05

respread
Assay

⑪	lost
⑫	ca 300
⑬	ca 300
⑭	ca 300
⑮	—
⑯	—
⑰	27
⑱	34
⑲	34
⑳	46

Respread plus with dispenser spreader may have been contam.?

$61/2$ = ㉚

$20 \times 10^4 \times$ (④) $\times 20$ 4×10^6 = $170 \times 10^6 = 1.7 \times 10^8$ cells/plate

1.6×10^8 cells/plate ㊵ =

B.

① Air bound dosage response vs. X-rays

	Dose	0	5	10	15	20	25	30	35
	papillae	5	13	26	30	39	52	49	28
	[illegible]	0	8	21	25	34	47	44	23

C.

② Co incubation
Co 64 dil 1-10 } dose 45", + 0.1 ml 3079 = 7
" 1-100 × 10^3 } = 0 (no [illegible] plaques)

$\frac{.12}{4.8\times10^7}\times10^{-3} = 4.8\times10^4$ (4×10^8) … 0.1×10^2 / 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 1.2×10^1 and plate for transd. response. (0.6 = ca 5.0×10^8 cell/ml) ② dilute 10^1 & 10^3 for λ plaque-producing response – This last, irrad. (1–20 × 10^2) and then add 0.1 to 7.5 ml. Incubate samples about 30 min before plating.

Induction = 3079

(margin: irrad. → plate cont 0.1 ml 3179)

Est. cell 0.6 = 5×10^8; 0.5 = 4×10^8

A. Transduction

Time	Plate 1	Counts	Corrected
0	1	14	0
5	2	28	14
10	3	26	12
15	4	28	14
20	5	28	14
25	6	25	11
30	7	43	29
35	8	39	25
50	9	170	106

B. Induction of λ

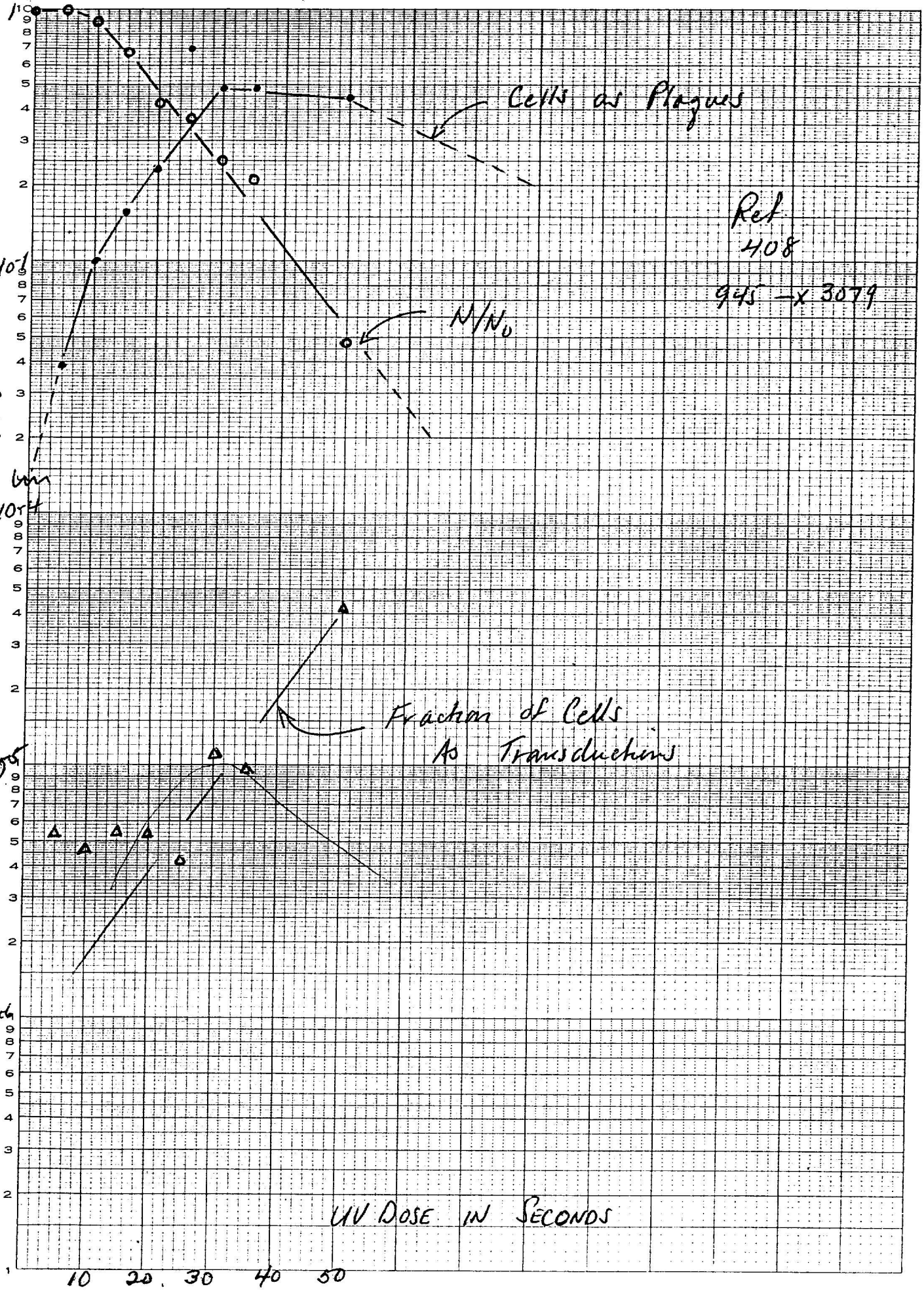
Time	Plate	Plaque	Cells (Too many ca. 2500)
as given ←	10	0 15	2516 ICh
	11	143 158	3620 also
	12	349 364	3155 2 type
	13	549 564	2437 plaque
	14	816 831	1530 large & small plaque
	15	0.1 = 2551; 0.05 = 1267	1293 all
	16	1661	896 neg.
	17	1654	738
	18	too many ca 1600	170

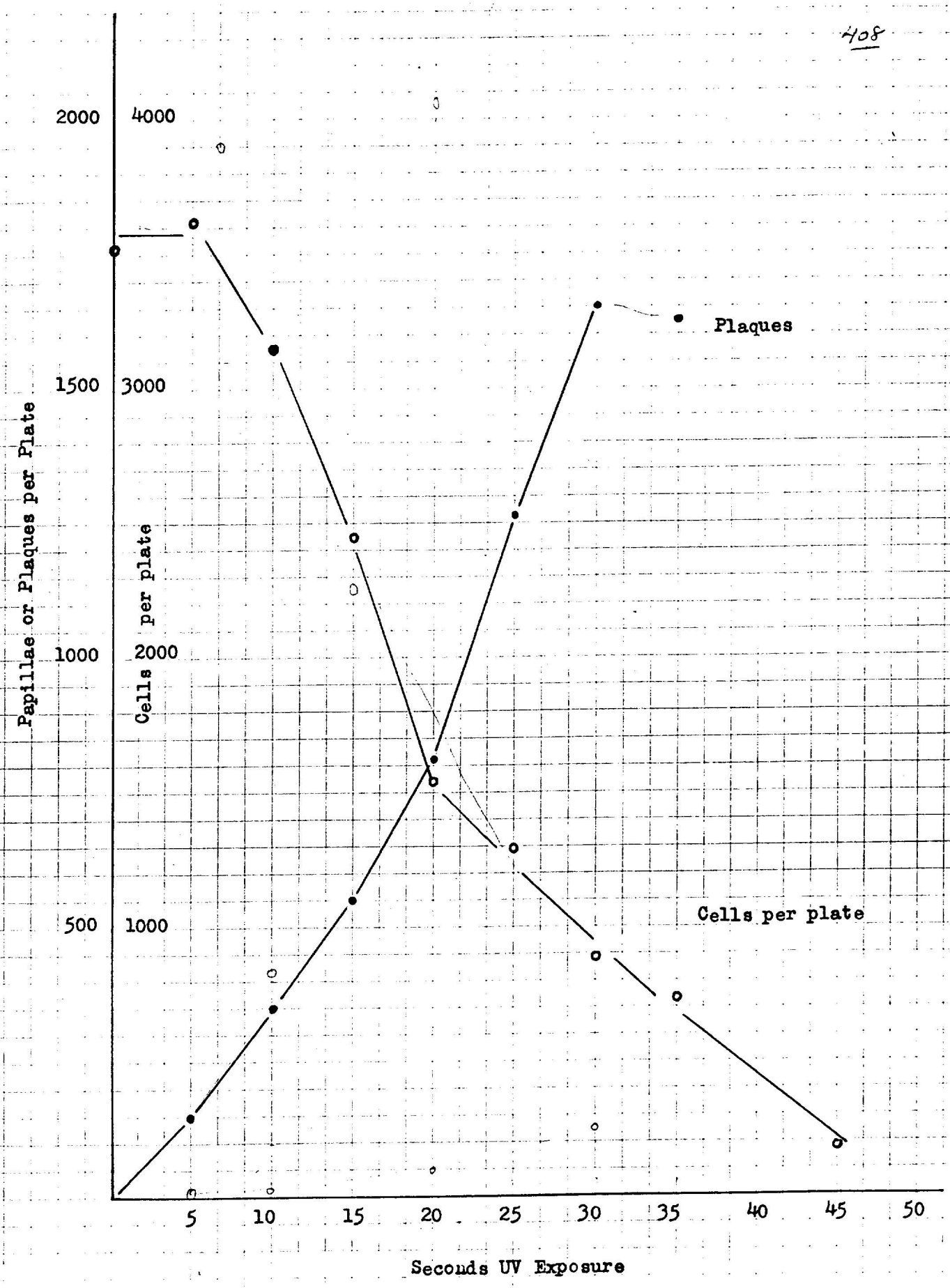
Total No cells/ml

Transduction = 0.1 ml × $\frac{11.2}{1.2}$ = 100   = 3.5×10^4 × $\frac{7.5}{0.1}$ × 10^2 = 3.5×75×10^6 = 2.6×10^8 cells/ml

2.6×10^8	Fraction of Cells as Transd.	Transd	Fraction of cells as plaques	Time	T. Plaques/ml	N/N₀	N/ml
	0	0	0	0	0	1.0	3.5×10^4
	5.4 × 10^-6	1.4×10^3	0.039	5	1.4×10^3	1.0	3.6×10^4
	4.6 × 10^-6	1.2×10^3	0.099	10	3.5×10^3	0.9	3.2×10^4
	5.4 × 10^-6	1.4×10^3	0.155	15	5.5×10^3	0.68	2.4×10^4
	5.4 × 10^-6	1.4×10^3	0.230	20	8.2×10^3	0.42	1.5×10^4
	4.2 × 10^-6	1.1×10^3	0.700	25	2.5×10^4	0.37	1.3×10^4
	1.1 × 10^-5	2.9×10^3	0.48	30	1.7×10^4	0.25	9.0×10^3
	9.6 × 10^-6	2.5×10^3	0.48	35	1.7×10^4	0.21	7.4×10^3
	4.2 × 10^-5	1.1×10^4	0.45	50	1.6×10^4	0.048	1.7×10^3

Fraction of cells yielding transducing act = $\frac{2.9\times10^3 \text{ transd}}{2.6\times10^8 \text{ cell}}$




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

A

Examination of 4⁻ xx 7⁻ heterogenotes

1. 4⁻ —x 7⁻ - Overnight culture diluted 10^2, 0.1 ml + 0.2 ml lysate -
293-12 W2308 Incubated room temp for 10 minutes, diluted 10^2, 10^4
lysate 0.1 ml plated 3 plates B gal.

	(−)	(partial lysis)	wk (+)
1.	31	(7)	(1)
2.	37	(5)	0
3.	29	(4)	(1)

} 3 additional possibilities appeared second day) - 3 x 5

3A

5/7/56 18 (−) seg obtained from the internal "+" typed - 18/18 Gal₇⁻

12 (−) seg obtained from the ~~outer~~ free "+" and typed 12/12 Gal₇⁻

2. 7⁻ —x 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. .5-0.6, ~~dilute 1-10 [illegible]~~ dilute 1-5 (3+9); irradiate 60 seconds, (Dil 1-2 with broth (Pen))
3. Pre assay. .53 0.056a — dil 10^3, 10^4, 10^6 assay

Cells: Bgal 1. 38, 2. 38 } 7.4×10^8 cells in OD = 0.53 suspension

Plaque + Transd.

wed 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 → (B) Undiluted

(A) dil 10^3, 10^3–10^5 broth

cells ← 0.05ml; Bgal Transd ← 0.05ml; → Bgal SM (true transd.)

2:10 Otime: 5. 0; 6. 0 | 9. 12 16; 10. 19 | 11. 0 ca 200 plaques; 12. 0 (ca 1000 ml)

λ centers + (3077): 7. 19, 8. 18

= 3.04×10^8 infective centers induced / OD = 0.53

$\frac{3.04}{7.4}$ = Fraction induced = 0.41

Fraction cells yielding transd out. Transd $\frac{3 \times 10^2}{7.4 \times 10^8}$ =

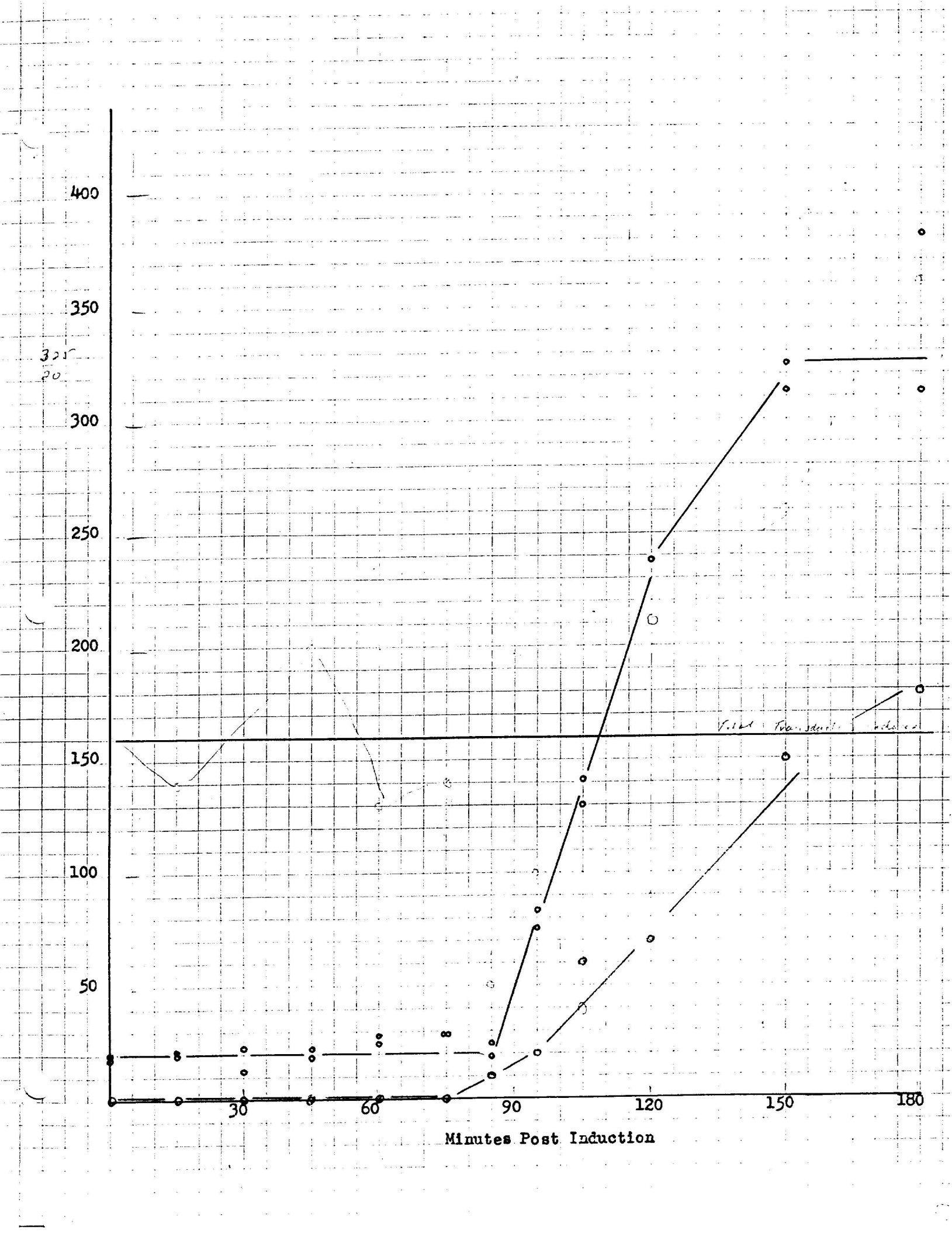
TIME		cells		Bgal Transd			Bgal SM		
2:25	15	13. 20		15. 7	14		17. 0	(many small) ca 200 plaques	
		14. 20		~~16~~			~~18~~		
2:40	30	19. 23		—			21. 0	ca 200 plaques	
		20. 12		—			~~22~~		
2:50	45	23. 19		25. 20	20		27. 0	ca 200 plaques	
		24. 33		~~26~~			~~28~~		
3:10	60	29. 25		31. 13	13		33. 0	many small	
		30. 28		~~32~~			~~34~~	ca 10^4 plaques	
3:25	75	35. 29		37. 7	14		39. 0		
		36. 29		~~38~~			~~40~~		
3:35	85	41. 25		43. 1	5		45. 1	1	
		42. 19		44. 4			46. 0		
3:45	95	47. 75		49. 4	10		51. 2	2	
		48. 83		50. 6			52. 2		
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. cont plate (76)		62. 33 surface			64. 9		
4:40	150	65. 313		67. 14 (26 pinned)			69. 10	15	
		66. 326 surface plate		68. 38 surface			70. 20		

Mea Total transd: 16, 7, 20, 13, 7, 4, 5, 2, 21, 26, 38; 159/11 = 14

These were all surface platings

Elution: 180. 71. 38; 72. 31 | Bgal: 72. 37, 74. 36 (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+ F/s
vs
Gal− F/s

DATE: REF: 411

1	2	3	4	5	6	7	8	9	10

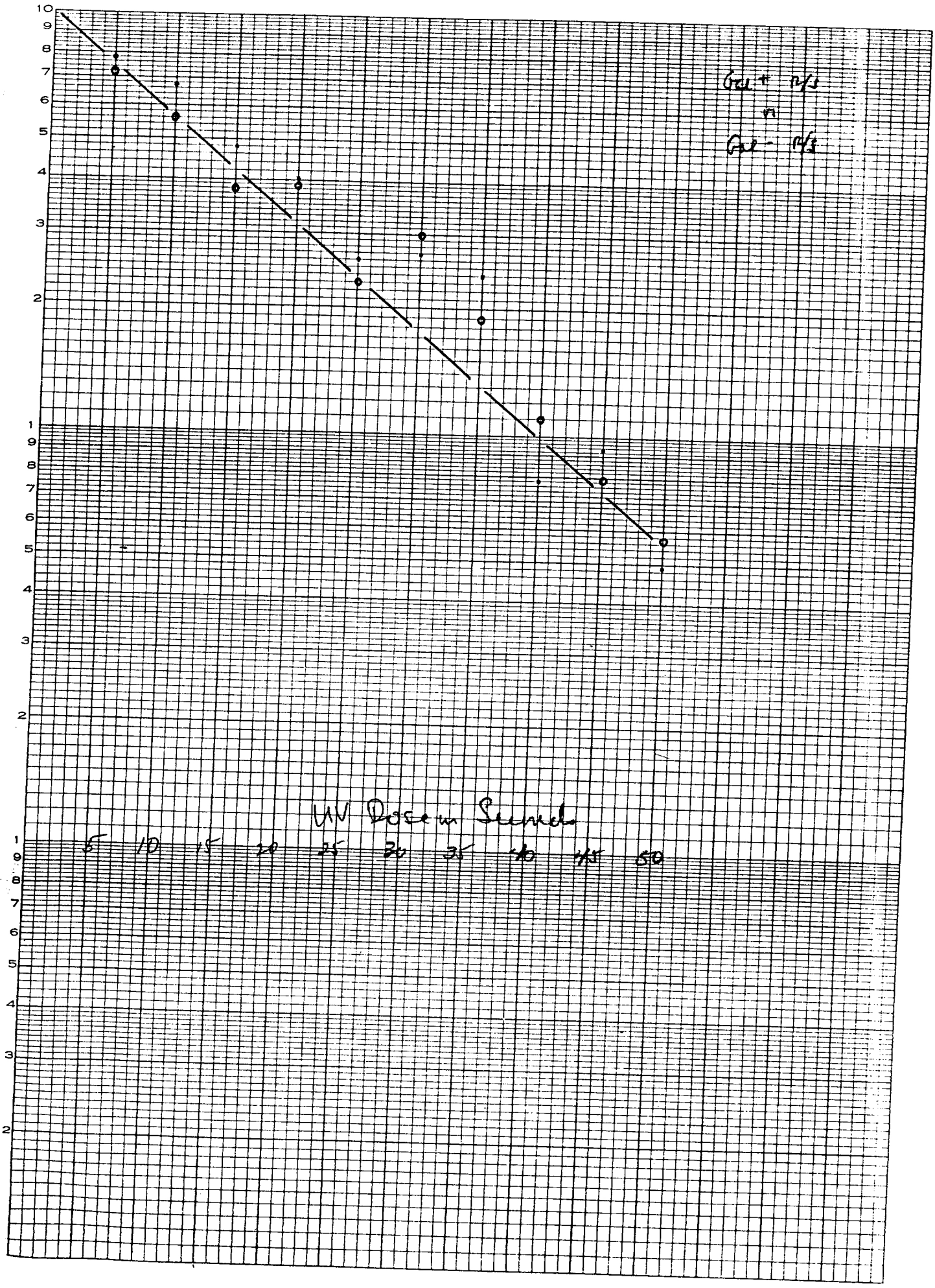
U.V. Resistance F/s heterogenote vs seg.
W2868 taken and a F/seg of it. On testing against λ, 4p' the segregant formed F/s as well as the heterogenote

Both cultures diluted to 1-10 incubated 2 hours, diluted 50 x 50 x 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 tube 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	0.65
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 in saline OD = .60
(~~Adsorb~~), irradiate 60 seconds, dilute 1-2 with 2X pen

Assay 1. Pre UV dilute $10^2, 10^4, 10^5$ → 0.05 ml

Cells	(1) 163	Bgal		175 = 2X10^6	350 X10^6 / 3.5 X10^8 cells/ml OD 0.6
	(2) 186				
	+ 3079				
Plaques & Transd	(3) 0 (ca 1000 plaques) Bgal SM				
and	(4) 0 (ca 1000 ")				

4.2×10^3 / 3.5×10^8 cells/ml

Post UV - dilute $10^2, 10^4, [10^5]$

Time		Pen incubate Bgal		Unincub. Incubated Bgal SM	
9:25 0 time Cells	(5) 0				
	(6) 0	(9) 104	103	(11) 1 ca 1000 plaques	
		(10) 101		(12) 0 " "	
λ ascents	(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 (10^4 plaques)	
10:40 75	(37) 53	(39) 88		(41) 2 confluent lysis	
	(38) 57	(40) 127		(42) 5	
10:55 ~~85~~ 90	(43) 54	(45) 80		(47) 31	
	(44) 64	(46) (67)		(48) 24	
11:05 ~~95~~ 105	(49) 99	(51) 71		(53) 29	
	(50) 143	(52) 118		(54) 46	
11:15 ~~100~~	(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	(70) 161		(72) 149	
	2379 : 1189				
12:25 180	(73) 1422	(75) 203		(77) 193	
	(74) 1196	(76) 174		(78) 208	

no cells induced to yield transd
ave = 210 X20 X2 =
0 = no growth
+ = growth

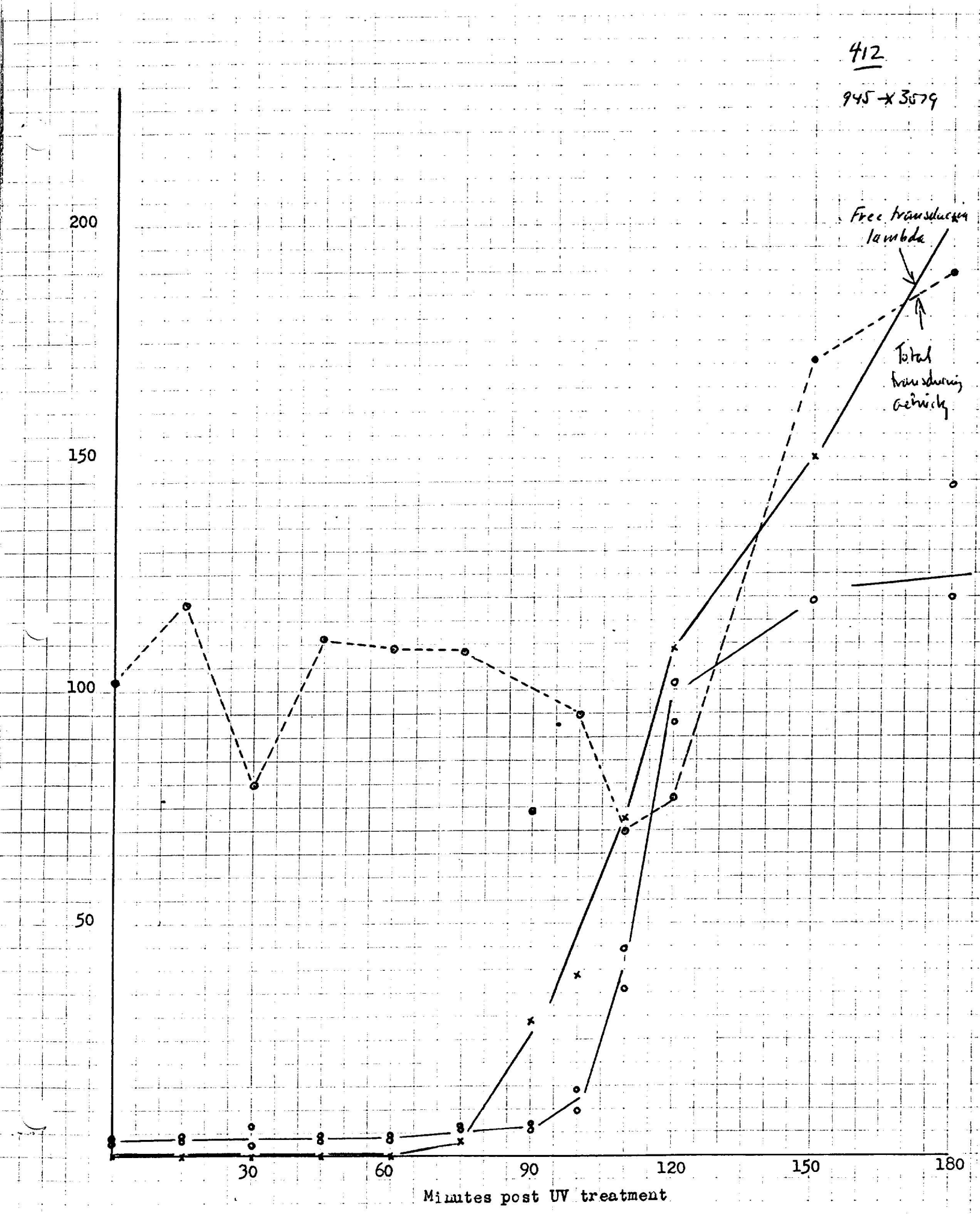
Some papillae checked

	Plate 46 Bgal	Bgal SM	45	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
Free transducing lambda
Total transducing activity
30
60
90
120
150
180
Minutes post UV treatment

DATE: 7/14/56 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. Cinder [illegible] made up to O.D. .60 in saline — diluted 1-2, [illegible] $\times 10^5$, irradiated UV standard dist, etc., assay for cells: B gal (pour method
for plaques: B gal + 3079, also pour method.

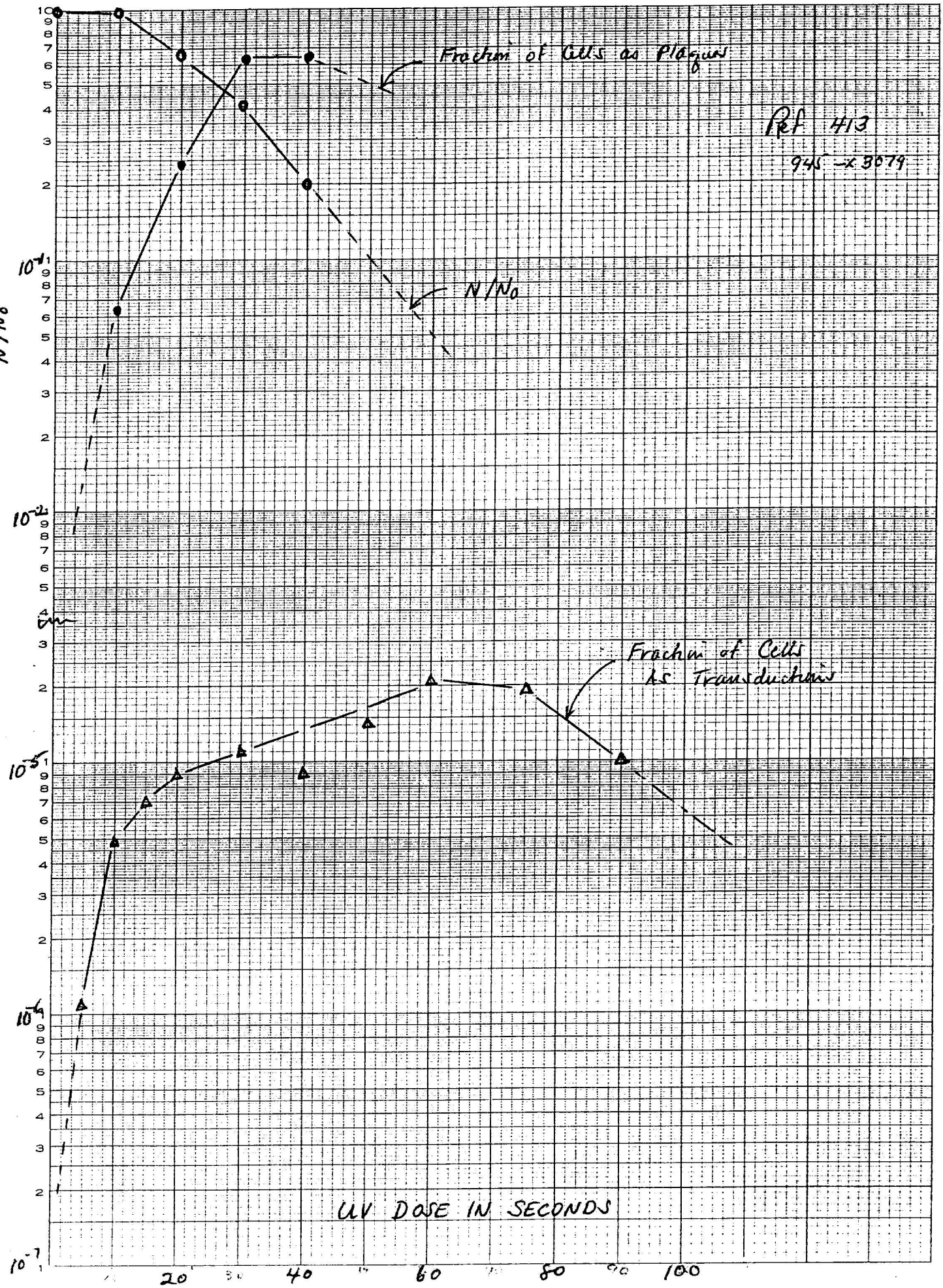
2.0×10^8

Dose	Plaques	Cells	Total cells + plaques	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 large clumps	42	172	0.20	1.3×10^8	0.64

2. Transduction induction. Cells 1-2 of OD=.60

2×10^3

Dose	Papillae	Δ	Transd/ml	Fraction Cells yielding transd.
0	23	0	0	0
5	45	22	2.2×10^2	1.1×10^{-6}
10	110	97	4.7×10^2	[illegible] $\times 10^{-6}$
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}



DATE:

REF: ~~413~~ ~~413A~~ 413-1

Isolation of Lys^R from 2307

1. Overnight cultures - diluted and plated to give ca. 100 colonies per plate
2. 9 plates - two counted
   1. 97 colonies
   2. 109 colonies
   } all colonies gal-
3. Replicated to B gal plate spread with 3079, UV'd 20 seconds 50 cm, etc.
4. On the mini plates 3 colonies found not to give a lysogenic reaction (2 on one plate, one on another)
5. The colonies cross streaked on λ, 3079 after purified found λ^R and non lysogenic
6. Lysate made (not as conc. as usual) - after 4 hours spotted on 3079 on B gal

   W2307 Lys^R = W3172

   1. ca. 20 small plaques
   2. ca. 17 " "
   3. ca. 9 " "

   45/0.3 = 15 x at. 0.01 = 1500 per ml = 1.5×10^3

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 tests (1) found Lys^S

   W2345 against - Lys^S = W3173

   (1) Ilfo with 945 after 3 hr growth in Pen and plated 1-100 on M gal, mod. on S gal

   (3) Titers against HFT 2^-, 1^-, + by 2^-, 0 by 1^-

   (4) gave no plaque when treated as above in lysate LFT

945- Are the ~~LFT~~ cells giving HFT lambda in LFT culture equivalent? Ca 2×10^3 945⁻ colonies per plate replicated to 3079 spread with Gal and UV'd 20 sec. Incubated 3 days, gal + looked for. Many observed, suggesting most 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~~ corresponding to the Gal+ on 3079 located and picked and restreaked by spotting on 3050 and incubating - 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 - Gal+ found LFT. None of the Gal+ areas on 3079 corresponding to them showed seg. clone.
2. To distinguish between gal+ due to reversion of 945⁻ plate replicated to B gal Str, since 3079 is Sr. From the Gal+ on Gal + Str reference made for (1) the original plate to obtain reference to original clone (2) the UV'd plate to see

418A

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	
A3	24	A12	33	
S3	11 4/6	S12	18	3/6 streaked gave mosaic 1/5 striking
Z5	9	Z4	11	
A5	ca 100	A4	40	
S5	58 3/6 gave mosaic 1/5 strike	S4	18	3/6 "

Theoretically Z + S = A, however many many Gal+ are papillae and may not replicate well

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 spread on B gal. Grown 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+ 1 to obtain lower limits of resolution of the method. Suggestion is that cells producing HFT λ are not [illegible] selected.

5/2/56

Preparing for Kalckar experiments

Obs that 1, 6, 7 all are defective for enzyme II (Gal transferase?)
Prepare gal₁⁻, gal₄⁻ stocks also 2734 (= non transducible gal⁻)

1. Because 3092 easily revertible, a new stock made by HFT₁⁻ × 3110
A. a sep. clone obtained.

2. 2734 crossed with 1895 - growth in broth, plated M gal⁻
A gal⁻ colony picked purified once, streaked on D(0) and tested against HFT₁(+)

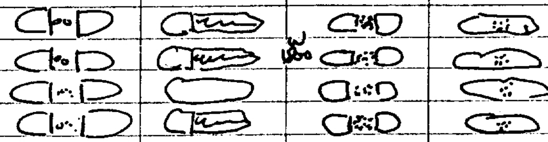
A. 6/6 colonies grew O(c) (labeled (413A-1) = W3142
B 6/6 colonies not transf. to + by HFT (+) all resist. to λ.

DATE: 4/20/56 REF: 414

1. Recheck on 269-1 lysates.

Rpt 269-1

	269-1	λ^2	10^2	10^4
3079				
3080				
945				
(#/3) 343-8				



↑ Resistant colonies picked — very tiny plaques

2. Examination of the results of 1.

After two purification streaks – 2 from each culture

Stocks — These tests in B gel – all cultures OK –

		269-1 (1-100)	Growth	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 4/27/56 – To show that fragment size is constant – titers of 2⁻ × 1⁻4⁻, 4⁻, 1⁻ are the same

Assay

(dil 10^4 → 0.05 ml surface plating)

① Preliminary – /3080 (1-4⁻) 101 hazed.
/3102 (1⁻) 4 hazed, ca 500 plaques.
/3104 (4⁻) 4 hazed, ca 500 plaques

② Rpt. same dilution
/3094 5 hazed.
/3091 8 hazed
/3080 96 hazed.

DATE: 5/1/56 REF: ~~415~~ 415

307-1A Segregation. 1⁻ — X7⁻ see 308A, 307

1. Overnight culture from a single colony B gal.

2. dil $10^2 - 10^4 - 10^2 - 10^5$ → 0.05 ml samples spotted on B gal, (8 plates) 6 per plate. Incubated 4.0 hours, 0.05 ml - 0.1 ml 1% NT added and the ~~plates~~ area removed.

$\frac{10}{100/2} = 0.05$ $e^{-.05} = 0.951$ $e^{-1.1} = 0.338$

3. Parent susp. ~~0.05~~ ml

			Gal+	Gal-pap	Gal-	Total			
10	Calculated								
	3 samples ~~had~~ 5? got a "+" cell	1.	10	209	10	229	230/100 x 2 =	2.3/2 =	1.1 cells/sample
		2.	10	212	9	231			
	3 samples had 4 samples got a (+) cell	Plate 1 1.	220	0	0	220			
		2.	0	222	0	222			
	16 samples got no cells.	3.	0	6	0	6			
		4.	0	201	1	212			
		5.	58	ca 100	ca 100	—			
20		6.	0	9	0	9			
		Plate 2 1.	0	265	1	266			
		2.	0	0	0	0	NG		
		3.	0	185	0	185		c.c.	
		4.	0	209	0	209	0.602r	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}
30		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			
40		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			
50		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

Continuation –

	Gal+	Gal-sey	Gal-	Total	
Plate 7 1.	0	0	0	0	NG
2.	0	153	0	153	
3.	0	68	0	68	
4.	1.	ca 50 ?		ca 500	
5.	0	450	1	451	
6.	125 (cont)	25	0	150	
Plate 8 1.	0	223	1	224	
2.	0	0	0.	0	NG
3.	0	72	0	~~257~~ 72	
4.	0	–	ca 100	467 –	
5.	0	272	5	277	
6.	0	223	2	225	

This het. is R/s (307-1C)

For comparison with. Lp+ [illegible] was 389 for 307-1A

III. Single Gal- sey obtained from as many clones as possible – 17 obtained from 17 clones.
Tested / HFT 6- , / HFT 7-
~~[illegible]~~

6-	Lp^{J}	= 8		
7-	Lp^{J}	= 8		
7-	$Lp^{R/s}$	= 1	=	415A-1

3057 – in search of Lp^{R}

1. ca 2000 colonies tested by replication / 2730 sey (Gal$_2$-) – None found [illegible]

DATE: 5/3/56 REF: 416

1	2	3	(4/13) 4	5	6	7	8	9	10

Redo a 945 experiment to see if "mutants" cells are responsible for transd. 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+ Gal2- prototrophs) on UV'd. Incubated 2 days and replicated to Bgal, Bgal SM — AMEND

This plate then replicated to M Gal

1. 113
2. 118
231

Example of Gal+ after 2 days

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

UV'd plate	300
Bgal replica	300
Bgal SM	10
Mgal replica	300

Overnight 389-1 $10^2 - 10^4 - 10^6$

2. This contains $231 \times 2/9 \times \frac{100}{10} = \frac{4620}{9} = 512$ 945 cells

2230 Sag

Replicates / [illegible] UV'd

0.05 ml = 108 [25 col LFT]
65 [11 col LFT]
173

$173 \times \frac{1}{12}$

Original plate (non UV'd) ca 10

This experiment abandoned - Replication to Bgal SM showed ca. 10 Gal+ per irradiated plate which presumably are transductions 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 previously.

W2872 (1210+ - 8/λ)

in search of HFT 8-

1. 17 seg tested against ~~[illegible]~~ 3094 - all 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

W7550 x W2854

no add	14
0.1 ml lys	54 (per 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 10^7 10^6 10^5 → 0.05 ml sample 15 gal - 10 spread 4 hours.
↓
assay 0.1 ml

	+	−	rep −	Total
1.	18	30	49	
2.	28	24	116	

Respread Clones	Colonies +	−	rep −
~~#'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

1	2	3	4	5	6	7	8	9	10

(2^{-3} x 7^{-})

Segregation from W2874 2⁻ 7⁻ heterozygote - It is full Gal+, does it still contain both Gal- alleles?

1.

/2-	/7-	Number
+	o	14
o	+	4

} both alleles present but segregation pattern reversed

2. 14/14 2- tested for HFT+ /3091 all LFT

4/4 7- tested /3092 all LFT

At this time W2308 x— HFT 2⁻
W2915 x— HFT 7⁻ } to compare reciprocal cross

FOR SEG. ANALYSIS SEE 420

made in the "standard" way 0.1ml of 1:10 dil overnight culture + 0.4ml HFT lysate - diluted to obtain ca 100-200 colonies / B gal plate (1/50 & 1/80)
Results not recorded but ca 200 colonies / plate, >50% show contact with phage (partial lysis)

W2308 - 2 ~~apparent~~ transduction clone apparent [418-1, 418-2]

W2915 - 1 trans. 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 80 seconds - This, to see if any homogenetic clone formed by transduction (all transduction clones found have been selected on the basis of plus phenotype, in the case of (-) recipient (-) or mosaic phenotype in "+" recipient. No evidence of HFT (by gal clone on M Gal) indicating no Gal₂ - homogenote formed.
The extent of the test: ca. 500 gal₂- colonies (W2915) } Planned to replicate
ca 300 gal₇- colonies

to Gal₂- indicator generated by reversion of recipient

5/14/56

1210 x— 308-5
8⁻ x— 1-6⁻ } for stock for recheck

no add -	0			
0.1ml 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 MGal found LFT	

1210 x— 344-6
8⁻ x— 6-7⁻

no add - 0			
(old lysate) 0.1ml 344-6	153 (small)	① 19/19 found 8⁻	18 tested /3091 and found LFT
		② 11/11 found 8⁻	10 tested /3091 MGal - and found LFT
		③ 16/16 "	

2580 x— 346-4

no add - ✓		① 20 any tested	19 Gal₂⁻ 1 Gal₆⁻ }	See 347
0.1ml 346-4	64	② 10 any tested		

DATE: 5/16/56 REF: 4/9

1. In the experiments to see if HFT+ cells are distributed as "mutants" in LFT clones (such as on 4/16) many galt clones possibly transductions, reversions or something else observed in the UV irradiated [illegible] — the number appears too high. Both cultures used in these later experiments were F- and no more crossing is probable. Are [illegible] 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+ in [illegible]; 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 segregants found 7- 4-

346-4 X K12
no add = 0 (some small) this culture very rough.
0.1 ml = 19
7. 21 seg found 4-6-

DATE: 5/23/56 REF: 420

1	2	3	4	5	6	7	8	9	10

Seg analysis of 418-1 $2^- \times 7^-$ This clone λ^R/s

Number	$/2^-$	$/7^-$		
16	+	0	$= 7^-$	all λ^s
2	0	+	$= 2^-$	both λ^s λ^R, probably λ^R/λ^s

Seg analysis 418-3 $7^- \times 2^-$

Number	$/2^-$	$/7^-$		
4	+	0	$= 7^-$	all λ^R, probably λ^R/s
8	0	+	$= 2^-$	all λ^s
1	0	0	$= 2^- 7^-$?	appears λ^s

2880X—2851 $2^- \times 1^- 4^-$ — [illegible]

	$/1^-$	$/2^-$	$/4^-$	
19	+	0	+	$= Gal_2^-$
2	0	+	+	$= Gal_1^-$

[illegible] 0.1ml 2851 50

Heterogenote for Photographic Illustration.

Lysate of K-12

Culture	undil.	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	$Gal_6^- Gal_7^-$