3/28/54 (269)

p 184

518E750-1 (Look up origins) Nature of segregant.

Seg.	/HFT-1	/HFT-4	Locus
1	0	+	1⁻
2	0	+	"
3	0	+	"
4	0	+	"
5	0	+	"
6	0	+	"
7	0	+	"
8	0	+	"
9	0	+	"
10	0	+	"
11	0	+	"
12	0	+	"
13	0	+	"
14	0	+	"

what happened to 4⁻?

all λ^R

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

2341 transduction (for TCN) using his culture which was found $\frac{L}{F}$ 5) — Use in crosses of added segment as a test of elim. and pattern of segment in 5th crosses.

269-1 1. K12 lambda

1. mock = 4
2. 0.1 K12 (5/30?) = 32×58. 116 = 1856; 174/1856

2345 (above) 2. 902 lambda — (undate HFT Hfr) — Just done beyond [illegible]

1. no add — 2
2. 0.1 902 — 42

4/7/54

In Relation to 518E750-1 Above. Transduction of 511 with HFT 1⁻ 2346. Examination of stability — Is the (1⁻ 4⁻ / 1+ 1⁻) combination +? Does 1⁻ and 4⁻ segregate from such a complex? Are the observed (+) in 4⁻, 1⁻ transductions (+) after elim. of a (−) allele?

269-3

1. 8 papillae examined 0/8 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) (streak on plate possible (drop of added to indicator, plate incubated). On each case of 10 colonies 8 were HFT - From Each a NFT and HFT selected and plated 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 gal_2 - 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 gal_2 - HFT) as 2 above

against 750	○ ⊖ ⊖ ○ ○ ⊖ ○ ⊖ ○ ⊕
against 1210	○ ○ ○ ○ ○ ○ ○ ○ ○ ○

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

Cultures	% Reversion	Stability	Transduction analysis /+ HFT	/1- HFT	/2 HFT	/4 HFT	Allele
241-14 HFT	19 (Brown)	12/12 +ᵘ	(+)	+	0	(+)	2⁻
" NFT	12	12/12 stable	+	+	0	+	2⁻
241-19 HFT	12	12/12 +ᵘ	(+)	+	0	(+)	2⁻
NFT	0	—	+	0	0	+	1⁻2⁻
2342 HFT	62	12/12 +ᵘ					
2342 NFT	(0)	—	+	0	0	+	1⁻2⁻
2346 HFT	5	4/5 +ᵘ					
2346 NFT	8	8/8 stable	+	0	+	+	1⁻

4/5/54

Examination of HFT lysate:

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

dil	0.1 ml No. pap.	Assay cult	dil	0.1 ml No. pap.	Assay cult.	dil.	0.1 ml No. pap.	Assay cult
no	0	750	no	0	2438 (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. 750 t 902-2 4/8/54 (Lysate not worked)
Titered on U2 (sp. λ^s) 10^7 = 717 = 7.2×10^9 - Lysate tested stock · p. 267

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

Assay	(1⁻) 750	(2⁻) 1210	(1⁻2⁻) 2433
no add	0	3	0
0.1 ml of dil 10^2	+++	½ 1:200 1600	183
" 10^4	++	13	1
" 10^6	12	?	0
titer	1.2×10^8	1.0×10^6	1.83×10^5

suggests

4/10/54 ·

U2 - spont. Lp^s? from 2175 t 811 → (-) seg Lp^s gal₄ - Only useful marker for elim. possibility of contamination & pototrophy. This culture grows on EMS gal - Presumably this is a valid recurrence of Lp^s - No other gal₄ - Lp^s prototrophs about at time of isolation.

4/5/54

In search of triplet (→) (1⁻ 2⁻ 4⁻)

1. W2350 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	+

2⁻ +
1+ X⁻ −
4⁻ +

8 2⁻.
2 1⁻.

In these cases a few small papillae noted similar to those of 2350 against HFT 2⁻ significance?

2. TP8 = W2437 $gal_1^- gal_2^-$ exposed to HFT 4⁻ (2478-1) - Segregants examined

	seg #			
	1	0	0	+
	2	0	0	+
→	3	0	0	+
minute papillae in three-eighths of colony	4	0	0	+
	5	0	0	+
	6	0	0	+
	7	0	0	+
spots: →	8	0	0	+
	9	0	0	+
	10	0	0	+

all 1⁻ 2⁻

2⁻ +
1⁻ +
4+ −

272-2

4/7/54 - and several days earlier

Recovery of Lp_2^s from Lp_2^L culture

W902 -

1. Transduced c 2346 AFT 1- λ - Reaction suggest fairly large fraction of Lp_2^s present.
2. Streaked on B gal - seg +ˢ observed.
3. Pick for seg and (c.b.) all gal-
4. One tested for nutrition - D(0) no growth; + TZ growth 2 days; + LB no growth; + TB, no growth; + TLB, growth 24 hrs. } nutrition [illegible] — tested on B Mal = mal + — nearly sterile

5. Examination of [illegible] segregants

W2584

W945

1. Transduced c 2346 AFT 1- λ - React. also suggest fairly large no. Lp_2^s present.
2. Streaked B gal - +ˢ observed.
3. 6 seg tested /2046 λ /2342 λ - all gal₂-
4. Examination of the culture for sugar reactions.

Mal + Xyl - Mtl. -

3 additional colonies also found Mal +

W2580

4/9/54 On search of phage mutants.

Platings on Endo - all platings gave no plaques. With the following cultures.

1485, 578, 2281

Assays on NSA (hours)

	/10⁶ dil	/10⁵ dil
2281 =	274	10
1485	237	10
578	281	6

4/10/54 Repeated at lower dilution

	10³	10⁵
Spont. Lp^s → U2		
578	0	0

4/14/54

Is the complex $\frac{1^+ 4^+}{1^- 4^+}$ phen. (+)? All (+)" appear to be secondary - segregate clearly one (-)

SF × 2746 λ - Single exposure to phage - unaerated fresh, near sat. per cell.

(A)

Treat	SF cell. Dil	No. (+) papillated	No (+)	No. pure (-)	Total
1. broth	10^6	0	0	67	67
2. 2346 λ HFT 1⁻	"	9	2 (papillate)	41	52

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

1. Single (-) appearing colony (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	+ ?	Sens R?	Rc ✓	—
9.	intermed (+) turning dark after 3 days				—
10.	"	"	R?		—

Only segregants are 4⁻ Lp^S

to Lp^R presenting segregation of 1⁻ ?

parental

~~pale examine~~ from parental streaks

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 crated 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 Malt	No. pap. B gal + HFT λ	Stability of Gal pap. (6 picked)	Mal + Lp_2 character of unstable pap. (tested with blue)	Transd. of Segreg. ant. λ HFT	Transd. from 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 intermed 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(-))

	o/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) + 518 cells
2.4×10⁹
0.5 ml + 0.5 ml → Adsorption 10 min 37°C → Centrifuge 10 min → resuspend 0.5 ml pen. →

Total Cells	No. (+)
213 185 / 2 199 = 2.0×10⁸	16 (18)
dil 10² 10⁴ 10⁶ 425 16 · 3.8% (+) dilfd 1:10⁶	
401 451 / 2 = 4.25×10⁸	0

2. Control
0.5 ml Pen + 0.5 ml us → → →

24 hour count

JL reports no. hanied present in his sample of cells studied c̄ the microscope.

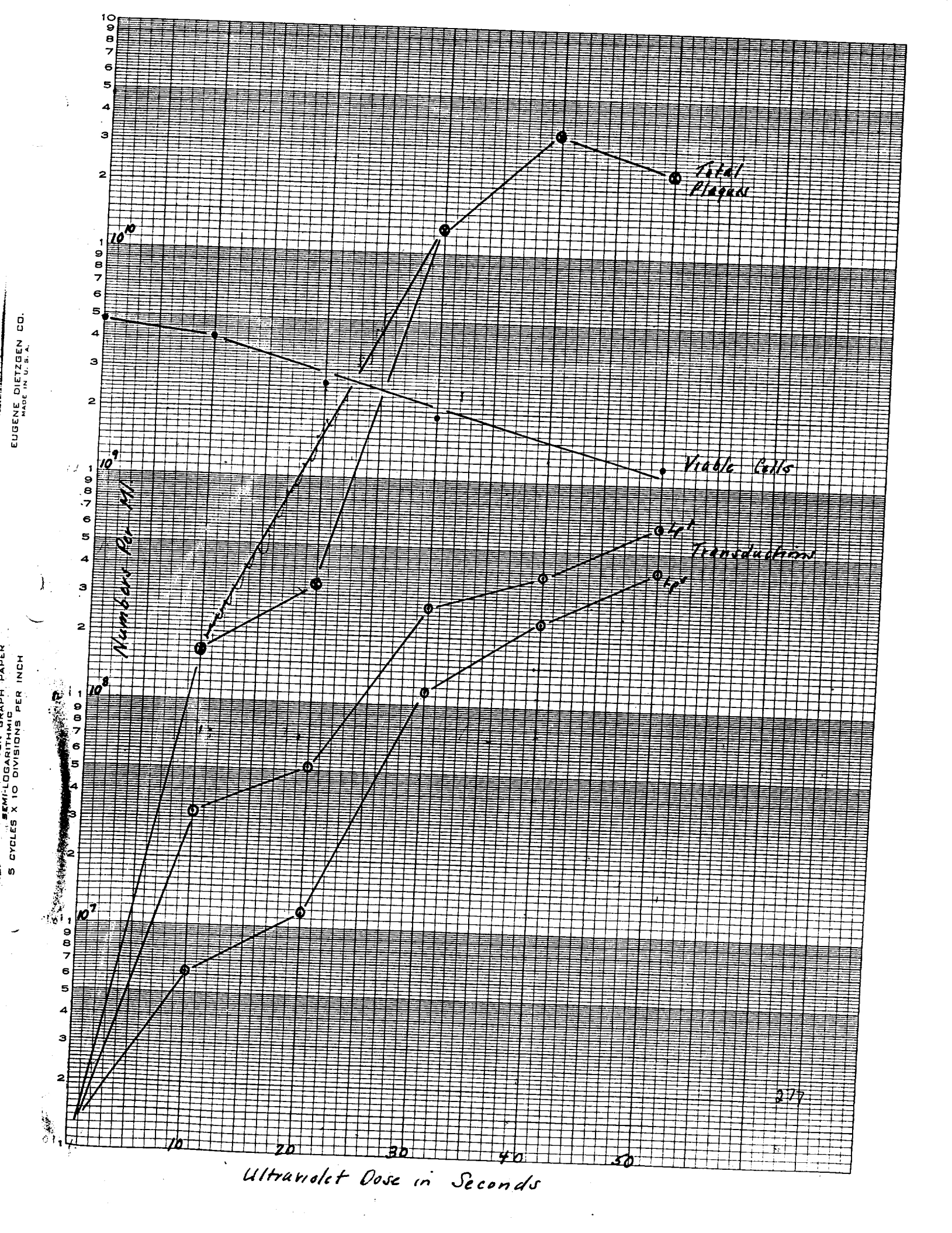
5/6/54

This day 2252 × — Hft gal₄⁻ (24/13/4 lyr. of) 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 piece in crosses. As sugg. by JL. Transf. 2252 (= Hft gal+ lp³) to +1/-+ and then cross to ++/-+ ⊗ +-

1. 2252 treated Hft gal₄⁻ (4/1/54) (W16) colonies examined for "phage contam" appearance - some found streaked out.

2. on second streaking, all +ˢᵗᵗᵏ


Total Plaques
Viable Cells
tp¹
tp²
Transduction
Number per ml.
Ultraviolet Dose in Seconds
10
20
30
40
50
277
EUGENE DIETZGEN CO.
MADE IN U. S. A.
SEMI-LOGARITHMIC
5 CYCLES X 10 DIVISIONS PER INCH

5/26/54

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

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

Irrad.			Single plate Count	N/N_0	Plaque (total) 1-100 dil	Transd./plate $\times 10^3$	Δ		Plaque/1-100 dil
0	0.1 ml added to 10 ml Pen incubated	dil 10^4 add. in NSB for cell Assay	477	1.0	0	50	0		0
10			417	0.88	103×16 = 1648×10^5	112	62	Δ $\times 10^3 \times 10^4$	~~1.65×10^8~~
20			263	0.55	c. 2×10^1	164	114		
30			194	0.41	1284×10^7 = 1.3×10^{10}	1200	1150		
40			(252) -	—	3456×10^5×10^2	2368	2318		
50			117	0.25	—	4160	4110		
	1-100	10^6							

Time	Viable Cell Present	"Induced" cells	Transd. produced	Plaque produced
0	4.77×10^9	0		
10	4.17×10^9	0.6×10^9	6.2×10^6	1.65×10^8
20	2.63×10^9	2.14×10^9	1.14×10^7	c3.3×10^8
30	1.94×10^9	2.83×10^9	1.15×10^8	1.28×10^{10}
40	est. 1.59×10^9	3.18×10^9	2.32×10^8	3.46×10^{10}
50	1.17×10^9	3.60×10^9	4.1×10^8	2.4×10^{10}

Transd. Assay on 811

			Plaque / Transd.	Ratio
no add.	17			
Irrad. Dose				
10′	16×200×10^4	= 3.2×10^7	16.5/3.2	5.2
20′	32×163×10^4	= 5.2×10^7	33/5.2	6.4
30′	169×16×10^5	= 2.7×10^8	103/2.7	38
40′	379×10^6	= 3.8×10^8	340/3.8	90
50′	654×10^6	= 6.5×10^8	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 c 2342 λ
   no add 40
   2342 λ 456

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

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

Mal pheno		Mal Reversion	Lp_2	Gal		Mal Rev	Lp_2^R of Mal Rev	λ	Mal Rev. 518	Gal Rev
intermed	+1.	+	R	seg		full	full sens.	R	non lys	+*
full +	2.	+	R	seg		"	" "	S	non lys	—
full +	3.	+	R	seg		"	" "	R	" "	+*
intermed	4.	+	R		?	intermed	intermed sens. R		" "	+*
~~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
1.	S	non lys	r	non lys	r	non lys
2.	S	"	"	"	r	"
3.	S	"	"	"	S	"
4.	r	"	"	"	v	
5.	v	"	"	"	—	—

Irradiate 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. stock indicates mixed for gal - lysate showed no evidence of plaques or transd. in 518 B gal.

10/21/54 - In testing for whether selection for (Mal-Lp^R) intermediate allele from absorbing λ selected by HFT expt above. Stocks found to be revertants of Mal and Lp_2^-. Two $gal_4^- Lp^R$ seg found from #3, #4. Revisions (3x repeat) of both found stable such that it is likely those Lp^R are not different

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

1. Titer - 209 × 10^8 = 2.1 × 10^{10}

518 t 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	$\frac{216}{3} \times 10^5$ = 7.2 × 10^6	
	② 2342 lysate (65'')	206	10	215	$\frac{215}{3} \times 10^5$ = 7.1 × 10^6	c. 5% (+)

WS 2877
2878
2879
2880

6/17/54

279-1 1412t2342 plate - the spontaneous (+) - Revert (18 picked for Mal) and examine lp₁ lp₂ 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 previously data 278)

1. All 12 mal - gal + were found to be lp_2^R
2. the Mal+ R (of varying degrees of + ness)

	518		λ_2	on Bgal		Rev	
1.	un lys	R	s wk	+			-
2.	"	R	s	seg			-
3.	"	R	s	seg			-
4.	"	s not confirmed →	s wk	seg?	rev	seg, + R	-
5.	"	s	s "	+			-
6.	"	s	s "	+			-
7.	"	R	s	seg			-
8.	"	R	s wk	+	rev	stab, + R	-
9.	"	s	s	(-)			-
10.	"	s R not confirmed →	s wk	+	rev	stab, s	-
11.	"	s	s	+			-
12.	"	R	s wk	+	rev	stab, R	-

Σ
4 spont + λ^s
3 transd. + λ^R
4 " $+^u$ λ^R } Refer to 278 (1, 8, 12)

free Mal+ revertants above not obtained

Mal + rev.

ABOVE 1321	λ	λ_2
1. unsly	sens.	sens.
2. "	"	"
3. "	"	"
4. "	"	"
5. "	"	"
6. "	"	"
7. "	"	"
8. "	"	"
9. "	"	"
10. "	"	"
11. "	"	"
12. "	"	"
13. "	"	"
14. "	"	"

Σ 14 spont (+) λ^s

General summary

transd. appear to be gal + λ^R
gal $+^u$ λ^R → seg gal- λ^R
gal $+^u$ 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 - Segregates gal - isolated opts (2)
6 segregants of this (+) tested against HFT 2⁻, 4⁻
all transduced by 4⁻, ∴ Gal₄⁻ from 2580tK12 F+

W 2896

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

	(+)		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⁻, HFT 4⁻ - all transd. by HFT 4⁻ ∴ Gal₄⁻ from 2580tK12 F⁻

W2897

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

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

W2898

Stock - 279-5

6/24/54

280-1 Are the transd. of Lp^+ really of Lp^R/Lp^+ nature? Examine on Lp^+ transd. W2570tKC2

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 (62.78) titer = 2.1×10^{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 - 7.6×10^9

③ Transduction w/t.
on D(o). 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 fairly turbidity in saline
Irrad. all 0.1 ml + 10 min

281-1 Production of HFT λ – Assay plates Bucket? 518

Time	Cell Assay	Infective Centers *		Transd. Centers (no ? = 49)		Transd. 1-100 (SP = 25)			Plaques 1/100	corrd	A x100	A/Inf Centers	HFT/NFT Assay of Colony of Excision
0	622	3	0	45	0	97	72		6	0	0	0	3/25 = 0.12 ← use data here ? others
10	381	193	190	51	2	47	22		~~12~~ 11	5	500	2.6	10/25 = 0.4
20	170	372	369	87	38	38	13		~~48~~ 47	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	32	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 ? infective centers about them.

↑ These values cannot be trusted since they represent some growth effects after irradiation ? ?

Estimate of fraction HFT

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

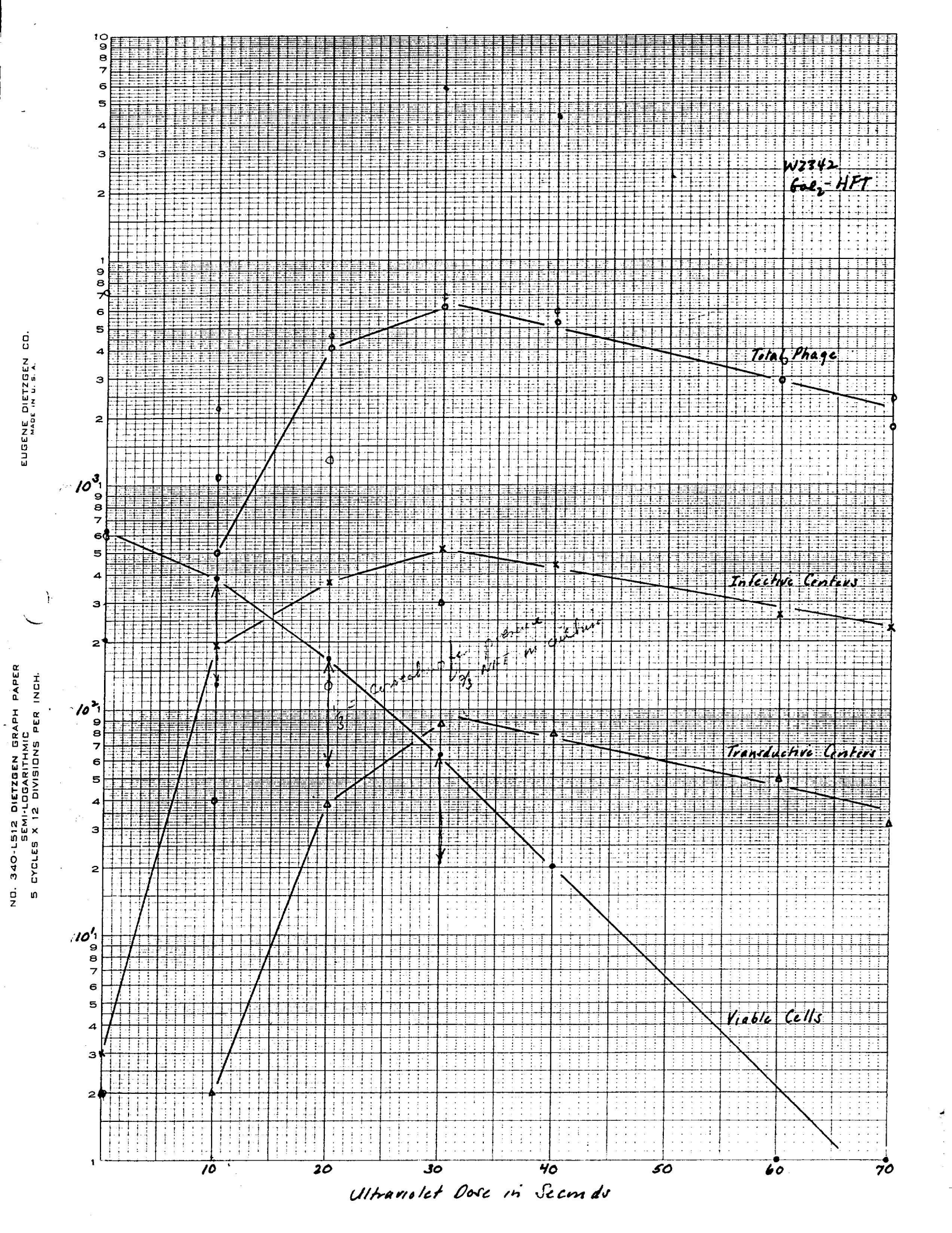
D.

	Total N/No	P/No	T/No	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

Corrected for LFT

$N_0 = 622 \times 0.29 = 180$

D	N/No	Inf x0.29	P/No
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	



282-1 In the 4" data so far suggests lysates of 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	112×4: 448	49 (small)	533×10⁵×4: 2.1×10⁸		2132/497 = 4.3	Higher titer in Gal₁⁻ than 2 suggest that fragment is 2⁻ or Gal₂⁺
lysate culture	2	~~281~~ 460	~~27~~ 26 (small)	307×4×10⁵: 1.2×10⁸	(about 2× as many minute plaques)	1228/486 = 2.5	
	3	~~33~~ 336	36 (")	136×4×10⁵: 5.4×10⁷	-	544/372 = 1.5	
	4	281	57 (")	217×4×10⁵: 8.7×10⁷	-	868/338 = 2.6	
lysate culture	5	382	34 (")	191×4×10⁵: 7.6×10⁷	-	764/416 = 1.8	

due to lower reg. level with 1210? Recessive in W2175

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

also low derivative ~~cultured~~

W~~2852~~ 2852 | W2851

Lysate = 282-2

282-2 Transd. of W518 with HFT 1⁻ (λ) 408 (-), 2 (±) Control 440 (-) c.6, 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 (+) peckers. 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 $\frac{1^- 4^+}{1^+ 4^-}$

(a) 1 discovered labelled #2. lysate prepared - Streak of culture before done 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

(4) 6/24 stable (+)

Remember these (5) (-) colonies from remainder tested for allele

(-) came from pure (+)

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

	/HFT 1⁻	/HFT 4⁻	λ^R	Comment	
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 against 1210 and found NFT

These are not derived from separate events

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 as cross broth - suggestion $\frac{1^-4^-2^+}{1^+4^+2^-}$ is not pure +?
ⓢ Streaking out - suggestion that (+) may be of two classes - one pure (+), other unlabeled.

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 stain (+) - this showed then segregate phenotypic (-) which revert rapidly - whose genetic constitution is $\frac{1^+4^-}{1^-4^+}$
① Transd. c̄ SRT+K12 [(#13) p. 267] - dil 10⁻ = solid smear, dil 10⁴ = c100, dil 10⁶ = 20 (loop full)
ⓒ 24 (+) seg streaked out - all appear to be stable (-) - all are 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 t^4 - after picking single colonies -
Check on lysate cell population:

			Sterility	Assay 10⁶ 0.1ml 750 c.c. 0		10⁶ dil 2175 c.c. 5		811 c=8	
1.	518t ~~750~~ -1	6(+) cd.	sterile	14	14	20	15	32	deletion too great
2.	518t902-1	neg, mostly +	"	0	0	17	17	23	
3.	518tK12	" "	~~not sterile~~ sterile	—		4	-	~~16~~	
4.	750t902-1	" "	sterile	2	2	9	4	26	
5.	750tK12-2	" "	"	5	5	11	6	26	
6.	811t902-1	" "	"	3	3	11	6	16	
7.	2175t~~750~~-1	" "	"	9	9	15	10	27	
8.	2175t811-1	" "	~~not sterile~~ sterile						

Lysates Repeat assay 10⁶ dilution 0.1ml

			518 for plaque		no cell 13	wet	811: 37		
→ 1.	518t750	(5)		148: 1.5×10⁸	88	75	111	74	— active on 1⁻, 2⁻, 4⁻
2.	518t902-1	0		62: 6.2×10⁷	18	5	45	8	—
3	518tK12	0		113: 1.1×10⁸	10	0	39	2	
4	750t902-1	1	~~3441: 3.4×10⁸~~	99: 9.9×10⁷	~~11~~	0	25	0	
→ 5	750tK12-2	large 0 4(+R)	~~99: 9.9×10⁷~~	577: 5.8×10⁸	32	18	50	13	— active on 2⁻, 4⁻
6	811t902-1	0	~~577: 5.8×10⁸~~	344: 3.4×10⁸	10	0	35	0	
→ 7	2175t750-1	3 large 24 (?)	~~616: 6.2×10⁸~~	615: 6.2×10⁸	160	147	170	133	— active on 1⁻, 2⁻, 4⁻
8	2175t811-1	6	~~116: 1.1×10⁸~~	116: 1.1×10⁸	12	0	30	0	

~~Above report chance errors intimate cont~~

Reassay 750 0.1ml
Control = 0
(1) 10⁴ = 501, 5×10⁷
(5) 10⁴ = 24 = 2.4×10⁶
(7) 10⁴: 433 = 4.3×10⁷

presumably colony 750 culture + altered such that assay suspect - other experiments about this time suggest this is true also

284-B

1. Cross 2487 × 2593 - Galy-Hfr × Gal₂-Het - to secure diploid? not plan. (+)?
2. Culture grown to gether c 3 hours. plated out EMS lac

10² dil - c 10³ prototrophs.
10⁴ dil - c. 50 " "
10⁶ dil - c. 20-70 " "

3. Streakings out of group c colonies - slightly positive in appearance, large. 14 in number

	EMS gal	S. lac
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

2279 (2299) = Gal, - tp^s - Repeat of 518 t AFT, using Gal, cells instead of Gal4

1. Actively growing culture of 2279 in Pen. c. 10^8 cells /ml. 0.5 ml cells + 0.5 ml NA-4 prep 2. Ad. inc 10', dil 10², 10⁴, 10⁶. Control cells both treated

(a). Control cells - one of three plates - 155 = 1.55x10² x10x10x10⁴ 1.6x10⁸
no (-), lysed, or phage exposed colonies observed, nor any weak (+) colonies

(b) exptl. cells - one of three plates - 118
on all 3 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.

285-1 (+) colony	stability	seg. (-) test 1⁻	14⁻	Allele	285-2 stbl	(-) Segs tested 4⁻	notation
1.	s = stabl	+	+	—	u	parental	
2.	u = unstbl	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	
8.	u	0	+	1⁻	s	—	Many of parental (-) show evidence of lysis-segregation so that this is an tp^R transduction, segregating (-) seg that are tp^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	

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

6 were parental
3 were gal, - tp^s

As in the case of the 1st

gal, - x gal4 - where the transduction was also tp^R and segregating tp^s homotype

no heterotype recovered. Crossing over apparently takes place with

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

7/22/54

286

Linearity of Gal₂⁻ to an Gal₁⁻ and Gal₄⁻?

3/17/54 902A	a purified 750		84	
Q	2	Δ₀	26	Δ₀
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 try to confirm earlier phenomena of reduced lysogenicity ~~with~~ in Gal tarstab. Examine segregants to see if these [illegible] are Ly^R or Ly^+. Also lyse one of each to see behavior of + mutants. Make stock.

286-1 = 750t902 | of the 12 picked - after 2 subl. 11/12 mottled (+)
286-2 = 84t902 | " " " " 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 84t902

286-1 titer against 518 10^7 AS = 1 plaque = c. 1×10^7

Assay

		10^2	0.1 ml of 10^4	10^6	titer
1	750 (control:0)	81	18	0	1.8×10^6
	2175 (cont.:11)	63	11	13	6.3×10^4

750t902

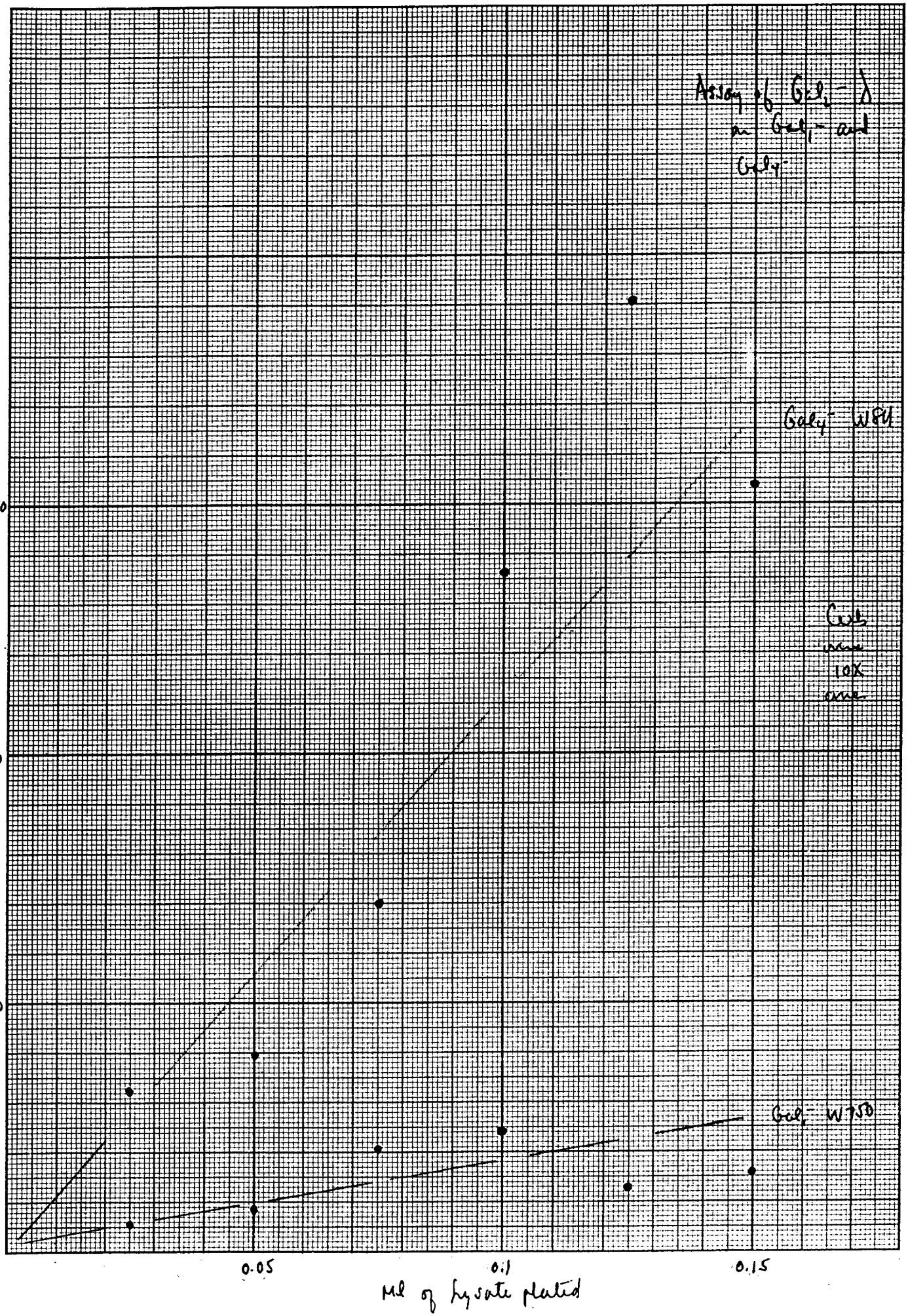
286-2 titer against 518 0.1 ml of 10^7 = 73 = 7.3×10^8

Assays

	10^2	0.1 ml of 10^4	10^6	
750 (control:1)	44	251	2	2.5×10^7
84 (control:11)	276	13	14	2.8×10^5

84t902

||| Assay culture



9/9/54

2341 Its constitution: Isolated as ~~F+~~ Hfr gal$_2$ - lp^R - TCN 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
 $\frac{2^-}{lp^R}$ × $\frac{+}{lp^S}$ → $\frac{+S}{-R}$ → $\frac{-S}{-R}$

9/10/54 7 col + 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 transductions

9/13/54

$1924EKR^{F+}$ - Recheck and cross $\bar{c}$ 1436^{F+} to see if fragment is $\pm R$ by recovery of $\frac{- \; s}{+ \; R}$

8 possible trans. obtained - all non lysogenic 1518
(1.) Crossed $\bar{c}$ 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 tested = 24 (3 appear either mixed or reverting / others mixed)
 21 were λ^R

Stock plate of one saved for further.

9/20/54

2341 · 2344

2344 is a V_1^R derivative of 2341. Since 2341 is $\frac{2-Lp^S}{\frac{2-}{Lp^E}}$
what is 2344 with regard to mu?

① testing of colonies of 2344 streaked out
17 colonies tested / λ - all Lp^V. Suggestion that only Lp^V seg. of 2341 can be made V_1^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.

~~Stk~~ 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 Gel so as to judge segregation

{811 - Making HFT by adding fragment?
{518 (9/17/54) -
- 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 ← WR870

289-1 → ① 518 against EMC'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 - Titered for transd. / 2297, 811

② 2070 λ - no + found of 24 tested

	no add	0.1ml of dil 10^7	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 c̄ revertants } to make gal - HFT by segregation from double duction

10 colonies tested against 2 and 4 HFT

① after 16 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 transductions:

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

(exp 2)

no's ↑ out stocks out 5/12/55

Stocks 290-1→8

1210 - transd. c 2070 ↓ to Blan 2-6+ → 2+6-

		lysate	Cont	0.1 ml		
290-5	1. 1210	2070 ↓+	1	17	9/12	+u

Single seg clone picked - lysate prepared

7 seg from seg. transd. tested against HFT 2 - all not transd. F 2.)

1810 t 811 for Cavalli Expt
291-A control 3
811λ 62

saved 5/13/55

[1, 2, 3 cultures made 3/1 for 1" λ] 291A1, 291A2, 291A3 (out)

1. 291A-1 ① 8 seg tested by u.v. irrad. spots on 811 - all NFT
8 spotted on 8 for to see if reversions stable ① c. 8 found stable
② 12 other. Tested a. to HFT - none HFT ② c. 5 reversions found stable
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 reassay. 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 contain:
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 = ~~0.5~~ 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 rebdy?)

10/10/54

① Reexamination of 257C-6 $\frac{4^- 2^+ s}{+ \; - R}$, its segregants to study crossing over - 29 colonies picked and streaked out from original streaking of stock. Single (-) seg selected.

	HFT 2	HFT 4	Lp	Genotype		Gal Red Stbl	2⁻	4⁻	Lp	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(!) 21	0	+	R	✓ 2-R
gal- lp [illegible]	7. 0	+	S	✓ 2 - S		S(!) 22.	0	+	partial S	2-seg? (292-22)
	8. 0	+	R	✓ 2 - R						
	9. 0	+	S	✓ 2 - S		S(!)				
	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						

			Gal
Idiotype	4⁻ S	= 2	1/1 +R stbl
Allotype	2 - R	= 15	11/12 +R unstbl
Allotype lp^s	2 - S	= 4	3/3 +R stbl
Allotype seg?	2 - R/S	= 1	? not done.

See also 298

CONTINUED 292A

② Transduction of Lp^R - 1924 → gal_2^- → $gal_2^- Lp^R$. Culture is W1 from Expt W. Is this culture heterogenic ~~[illegible]~~ for ~~gal~~ or Lp? Plated out on B gal and reversions 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 col examined for lambda reaction
1. seg +
2. pure +
3. pure (-)
/ 2279 All non lysogenic

Attempt to isolate $\frac{x^- Lp^r}{x^- Lp^r}$? and see character of phage

③ 1503 Lac B.E. - 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 ~~([illegible])~~ discarded.

~~Test of the gal [illegible]~~ 292-1 W16 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 segregation of papillation, (+)
- on streaking out for colony test to see if lp segregating - streak found mixed (+), (-). Pure (-) colonies tested against λ and 1 (+)

1. The (+) - weakly sensitive, (segregating probably lp^R/lp^s
2. 1 of 10 (-) colonies sensitive lp^R/lp^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 lp^R/lp^s
1. 10 (-) colonies tested 7 out of 10 lp^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

Apparently two cultures of 292A-1503 retained and none of the transd. gal of 292 original = W2733

1503 In search of gal- Het 1503
From the spotting of HFT 4 on 1503 a (-) obtained not transd. c
9-20 /54 HFT 1-, 2-, 4+ apparently sensitive to λ (less so than usually found)
Cross with 2279 - gal, - lp^s to see 1. non-gal, - 2. diploids 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.	~~4~~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 / 69.0 = 0.114 ; 592 ; 980 ; 592 ; 2880 = 11.4% (+)

12 (+) examined and found stable for gal

10/17/54

Search for HFT 4 - (After many unsuccessful attempts)
2175 EMB - 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 to 2175. Stocks made and also streak out for purification (results 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 inoculation.)

- unsterilized lysate streaked against

	lysate 12	13
2175	++	0
811	0	??

SEE 365

11/5/54 293-1, 2, 3 are transductions from which NFT seg were obtained. 9/13/55 Rpt 293 12
Behavior of transductions in giving HFT or NFT - Separate ability from dep. (+)

Seg. picked spread on 1210 811 and read 15 sec.

(811 has high bkgd)

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
2	0	c(30)	1	6	0	(18)	0	2	(50)	0 B	0	(c.18)	0	0
3	2	5	0	9	0	(13)	0	7	(55)	2 C	0	0	0	0
4	0	(18)	1	(15)	0	(23)	0	3	(55)	2 D	0	0	0	0
5	0	(14)	0	(55) A	0	11	0	3	0	(12)	0	0	0	0
6	0	~~(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	~~[illegible]~~ 1	1	(12)	0	4	0	3	(8)	6	0	(c.20)	0	0
9	0	~~[illegible]~~	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		~~2~~	0	0
12	—		—		—		—		(55)	0 G	—		0	0
	1 HFT 2		2 HFT 2				1 HFT 2 (poor plate)		7 HFT 4 3? HFT				0	
est. no. papillae out	0	2	0	3	0	6	0	5	0	3	0	3		

total 9 HFT 4 / 25

12/16/54 293-12A substituted for W2431 as HFT 4 stock

SS = solid smear

Repeat assay

	293-1 A /750	293-2A B /750	/750	293-11A /750	293-12 A-G	/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	—	2	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	—
	V	V	✓		ok		✓

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