11/7/54

Recombination between Lp^s to give λ

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

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

3. Set up

	A	B	C	D	E	F
	W2281 F+	W2373 F+	W518 F+	W2344 HFR	W1673 F+	W2280 F+
	Gal₂ -	Gal₁ -	Gal₄ -	Gal₂ -	Gal +	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 518 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 (518 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 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. ductions or HFT ductions

1412 attempts to transduce ē λ-2

1. Interrupted matⁿ of (Mal-4₂ᴿ) have interactions with λ-2 such that transductants might be obtained?
2. 8 Mal+R obtained with varying degrees of +-ness
   1 = extreme (+) 8 = (−)
3. λ-2 prepared by growth on 1405 0.1 ml plated 3 pfu

Rec'd	plaque type	Control area	Lysate area
1	(++)	8	19
2	(++)	6	18
3	+	5 (many small blu)	19
4	±	10 (many small blu)	29
5	±	10	29
6	±	8	16
7	(−)	14	25
8	(−)	20 *	26

* wrote the description of this plate and plate does evidence of lysis by the phage.

W2872

1210 transduction of each 1-4 lysate. ADDITIONAL RUN -297

1. lysate 283-1 (appears to be a good lysate, cleared promptly at 2 hours, virions −

2.

control	lysate
6	81

after 24 hours no signif. diff.
" 36 " lysate >> control
" 48 " >>>> "

The transd 3. Analysis of segregants

these (−) came from		HFT 1	HFT 2	HFT 4	
ones	295-1	1. +	−	+	
	295-2	2. −	+	−	found 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. +	−	+	
Σ: 15 identical	1	17. +	−	+	
3 allo	2	20 +	−	+	
	3	21 +	−	+	
	4	22 −	+	−	found NFT
	5	23 +	−	+	

		HFT 1	HFT 2	HFT 4	
	24	+	−	+	
	25	+	−	+	
	26	+	−	+	
	27	−	+	−	found NFT
	28	+	−	+	
	29	+ W	−	+ W	
	30	+ F	− 2-3	+ F	
	31	+ N	−	+ W	
	32	+ F	−	+	
control	33	+ P	−	+	
lysate	34	−	+	−	possibly HFT + contam. Re-✓ indicates contamination
	35	+ P	−	+	
	36	+ P	− 2	+	
	37	−	+	−	found NFT
	38	+ W	−	+ W	
	39	+ W	− 2	+ W	
	40	+ W	−	+ W	
	41	+ F	−	+ F	
	42	+ W	−	+ W	
	43	+ F	−	+ F	
	44	+ F	−	+ F	
	45	+ W	−	+ W	
	46	+ F	−	+ F	
	47	+ F	−	+ F	
	48	+ W	−	+ W	

W2853 ←

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

295A

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

13 idio
14 allo
3 amphi (all 1-)

F = full
W = weak

1210 + 283-1 Combined.

Reg		HFT1-		AFL-		AFT 9-	
31 49		+	F	−	4	+	F
32 50		−		+		+ found NFT	W
33 51		+	F	−	2	+	F
34 52		+	F	−	1	+	F
35 53	cont	+	F	−	3	+	F
Re ✓ 36 54		−		+		+ found NFT	W
Re ✓ 37 55	cont.	+	W	−		+	
38 56		−		+		− found NFT	
39 57		+	F	−		+	F
41 58	blue	+	W	−		+	W
41 59	blue	+	W	−		+	W
42 60		+	F	−	2	+	F
43 61	blue	+	W	−	blue	+	W
44 62	blue	+	W	−	blue	+	W

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

Reg	HFT1-	AFL-	AFT 9-	
(45) 63	+	−	+	
(46) 64	+	−	+	
(47) 65	+	−	+	
(48) 66	+	−	+	
(49) 67	−	+	−	found NFT
(50) 68	+	−	+	
(51) 69	+	−	+	

Batch II

	HFT1-	AFL-	AFT 9-	
1.	+	−	+	
2.	+	−	+	
3.	+	−	+	
4.	+	−	+	
5.	+	−	+	
6.	+	−	+	
✓ 7.	+	−	+	
✓ 8.	+	−	+	
9.	+	−	+	
10.	+	−	+	
11.	+	−	+	
12.	−	+	−	found NFT
13.	+	−	+	
✓ 14.	+	−	+	
15.	+	−	+	
16.	+	−	+	
17.	+	−	+	
18.	+	−	+	
19.	−	+	−	found NFT
20.	+	−	+	
21.	+	−	+	
22.	+	−	+	
23.	+	−	+	
24.	+	−	+	
25.	+	−	+	
26.	+	−	+	
27.	+	−	+	
28.	+	−	+	
29.	+	−	+	
30.	+	−	+	
31.	+	−	+	
32.	+	−	+	
33.	+	−	+	
34.	−	+	+	[illegible] ⊙
35.	+	−	+	
✓36.	+	−	+	
37.	+	−	+	
38.	+	−	+	
39.	+	−	+	
40.	+	−	+	
41.	+	−	+	
42.	+	−	+	
43.	−	+	−	found NFT
44.	+	−	+	
45.	+	−	+	
46.	+	−	+	
47.	+	−	+	
48.	+	−	+	
49.	+	−	+	
50.	+	−	+	
51.	−	+	−	found NFT
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 (to 54)

78 idio ?
5 allo
1 amphi

one of these found HFT in 2175

4/23/86
Retest of these stocks shows no HFT [illegible] after extra days digest [illegible]

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 transductions

B gal

1. no odd 1/2 = 41
HFT 2 (2342 pop for gal)$^{1/2}$ = 317

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

Batch II
3. 22 pupillar picked and streaked B gal ② - cell stable, all non lysogenic /518 — W2881
~~23 pupillar picked ② all~~

Batch III
4. 24 pupillar " " " ② 1 +" found, all non lysogenic /518 (296-1)

5. 24 pupillar picked " " " ② 3 +" found, all non lysogenic /518 (296-2, 3, 4)

W2882
W2883
W2883

6. tests of 296-1, 3, 4 - ~~[illegible]~~ gal - seq obtained from each and brushed against HFT 2, 4 - after 2 days

	HFT 2	HFT 4
1	4 pop	0
3	1 pop	0
4	3 pop	2 pop
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

Bpt experiment

HFT 2	HFT 4
3	0
8	0
contamination 0	0
0	0

7. 296-1, 2, 3, 4 gal+ seq 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 terms of sig	#	Appearance of gal+ on Mal	Reversion obtained	Reversion ∅ type	Gal transd. λ-2	Mal+ λ /518	
1	1	pink	+	full +	resistant	S	n.l.
2	2	blue	+	full +	"	S	"
3	3	pink	+	full +	"	S	"
4	4	blue	+	full +	"	R	"
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	"

296-5
296-6
296-7

W2884
W2885
W2886

K-12 Lysate 11/12/54 - transd. titer about 150/0.1 ml against 1210. Papillae show in development. 3 days required and small. Other 'duchin's with 1210 were also show at this time. 1210 bad medium?

SEE BELOW

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

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

SEE 297A

K12 Lysate above - Test of layer plating method.

0.1 ml lysate + 2.5 ml Bgal (0.6 % agar) poured on Bgal plate

Assays.

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 1/8 = 162, 1296

Contaminated culture?

11/30/54

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

① 3-1++ Gal- against HFT 1⁻,2⁻,4⁻ - cultures not gal-, no segregation of transductants

② Lysates of cultures TCN III-2 and TCN VII-94, also lysate of 583. Lysate additions = 0.1 ml, control = 0.1 ml buffer, spotted on film

Lysate	/350	/2175	/Pu	/2307	/3-1++
control	0	2	0	0	
583	0	49	9	0	not
III-2	1	34 *	1 *	0	* gal-
VII-94	c.120	c.150	c.120	c.100	

+ a little clearing from transferred to plate causing enhancement of growth

Attempted transduction of Himmicalis arabinose and also direct attempt at (Mal- $4p_2^R$).

1. 2347 = 583 mal- ara- $4p_2^r$ $4p^+$ 5^R <- obtain transferable gal+ for 5^R donor (This reverts during HFT
   control (4 days) many small
   or Basal 0.1 ml " " } no transductants.
   HFT 2⁻

2. 2307 = 583 mal+ $4p_2^S$ ara- 5^S
   HFT 2⁻ (4 days)
   Ara+ control 0
   0.1 ml 0

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

257C-6 - Combinations. See also 292

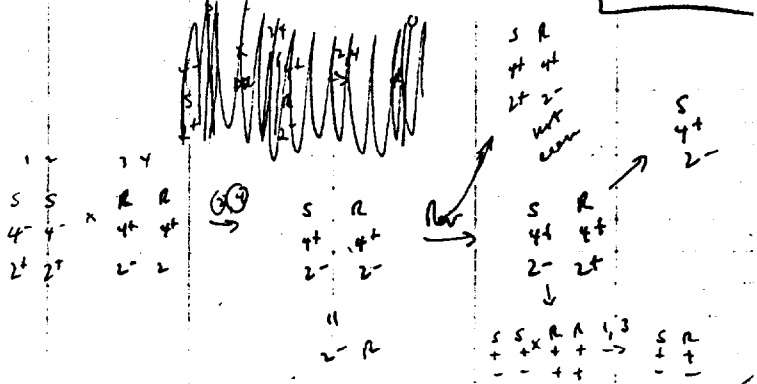
seg	from diploid (+) HFT 2⁻	HFT 4⁻	A. React.	Segs from mix 1	Segs from mix 4			Page 292	Total
1.	−	+	R	2⁻S	2⁻S	Tdis type	4⁻S	1	2	3
2.	−	+	S	—	—	allotypic	2⁻R	6	16	c. 32 $4p^R$
3.	−	+	S	—	—	allotypic	2⁻S	2	4	6
4.	−	+	R	2, 2⁻S	2⁻R	amplif	4⁻2⁻S	1 ?		1
5.	−	+	R	3	—	—				32
6.	−	+	R	4	2⁻S	2⁻S				
7.	−	+	R	5	2⁻S	—				
8.	−	−	S	(Rev)	—	—				
9.	−	+	R	1 or 2	2⁻R	2⁻S				
10.	+	−	S	(no trichoids)						

298

298-8

On the basis of the reversion study The 2⁻R were derived from

IW2890 also $4p^+$ donor.



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

Additive	2175	~~Naples~~ 578
none		0
0.1 1-100 sup.		~~100~~
0.1 1-10 "		~~HFT~~
0.1 mod. "		

} poor assays. Reduce { 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 />50

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

241-14 making Lp_2^R by mal-selection.
6 colonies tested

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

2431 - HFT gal_4 stock. Streaked out from 293-12A and colonies tested. The broths given to H. Bernstein for immediate lyophile. Cell ~~tests~~ colonies tested HFT. after about 2 weeks, 2 lyophile tubes opened and tested - all still HFT. Titers from lyophile suspensions not high

12/7/54

One Step c̄ HFT 241-14

1. 241-14 Streaked out single 10 colonies picked to broth and tested for HFT by mad. on 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 this dil.
0.1 + 10 sal. <10^3
1.0 + 10 [illegible] = <10^2

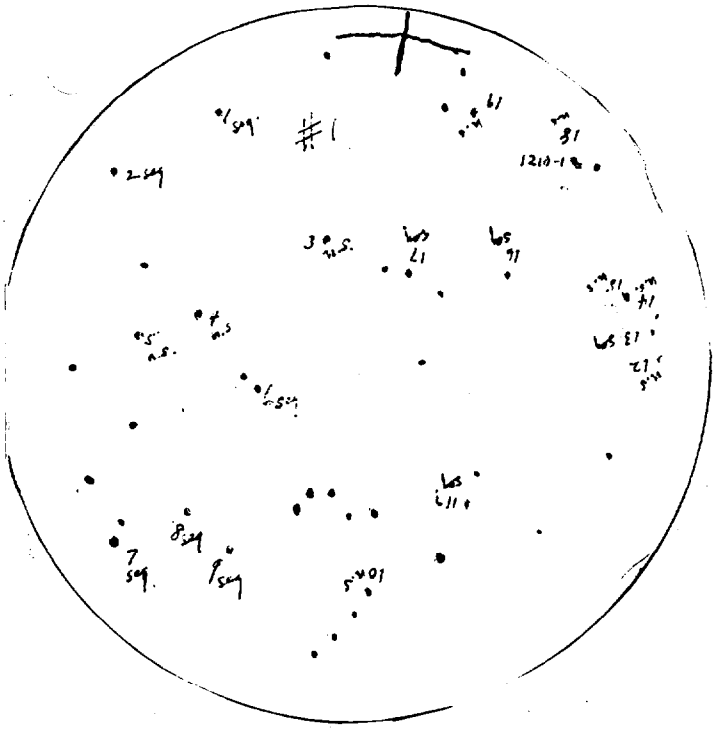
Assay against 750, 2279

Time		/750	/2279	colonies 10/0 ml
10:24	0			
	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 more 10 colonies gave plaques [illegible] suggesting non-inducible state

2. 241-14 - attempt to make lp*.
Streaked out on λ-2 m B mal.
1. Mostly Mal + seen -
2. 2 mal - col picked and streaked out
3. purified mal - 6 col streaked on B gal - all gal (-)
4. Repeat
Supernat. CHCl3 broth of culture used to make this: 0x10^5 plaques 2x10^5 transd.?
Repeat assay
5. On next run several mal - obtained - tested for HFT all HFT except 1 (6)
6. Stock made called (241-14 Mal)
1. Lysate 12/18/54 53x10^7 plaques/2279 = 5.3x10^8
transd. assay 13x10^7 = 1.3x10^8

3. 202-16H - 1 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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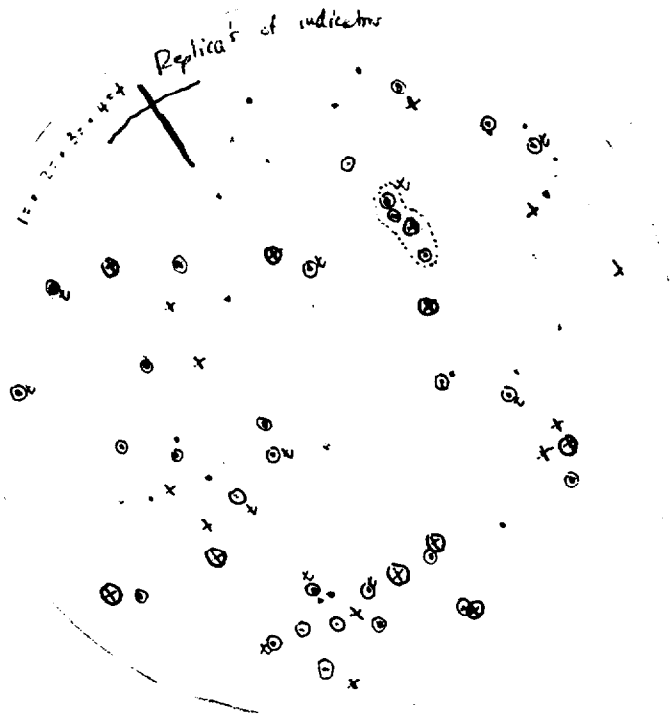
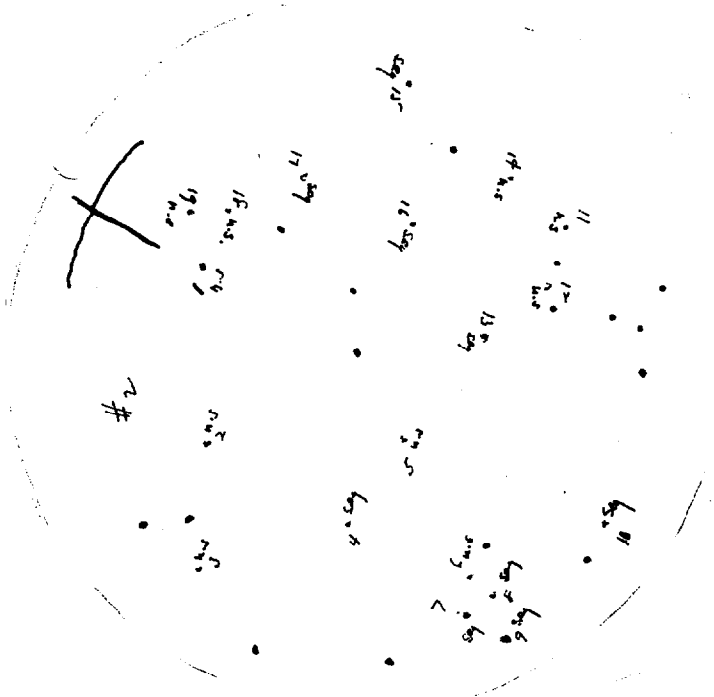

Replicas of indicators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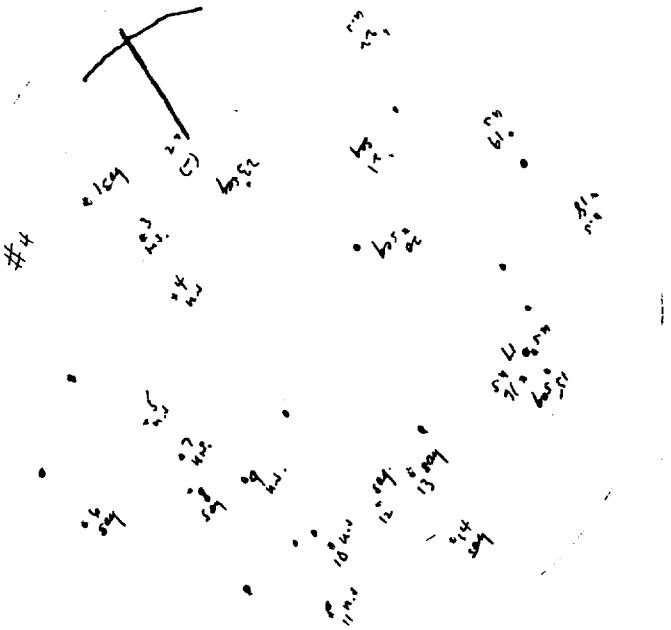
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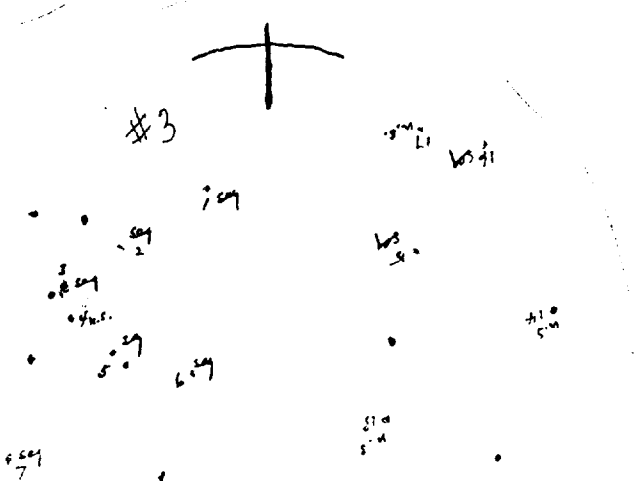

#3

12/11/54

Are the NFT cells giving ~~[illegible]~~ transducing phage clonally related?

1. W1210 - spread 0.1 on B gal from overnight culture.
2. Incubated about 12 hours ~~[illegible]~~
3. replica'd to 4 prewarmed B gal
4. after 30 min. 4 replica's irradiated 20" - 50 cm steri lamp, incubated 10'
5. Irradiated plates replica'd to B gal plates spread with W750 cells as indicator of transduction. W750 cells = 1/10 dilution, 0.1 ml of overnight culture, spread 1/2 hour before replication
6. Results of replica comparisons on attached figures.
7. All colonies on indicator plates picked, streaked out twice to see which are segregating

12/12/54

Observations on 292-3 = 4+ 2- 5 (from 5105y 2 -x)
against 295A-4 = presumed HFT 1-4- to see if mutual complex is phen. (+).

1. c. 10^3-10^4 cells exposed to lysate of 295A-4 (after centrifuge) 1.0 ml, 37°C. 15 minutes, 0.1 ml plated on B. gal. Both exposure used as control

	After 3 days
1. control = 3×10^3 (+) col.	c. 12 phage on colonies vegetative c. 12 revertants
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 c good lysate.

W2874

Observations on 2307. = 583 mut + $4p^s$ gal - 4p+

302 → 1, 2, 3, 4, 5

1. 2307 diluted to about 10^3 cells/ml
2. Exposure.
   a. 4 broth = control
   b. 1 " 2307
   c. 1.0 ml NA-4 prop

3 Exposure

			2 days	3 days
1. 6 broth	c. 400 (-) col.	c. 5 revertants	→	
2. " "	(-) col.	c. 3 "	→	
3. HFT 1-	c. 500 col (-)	0	c. 30 (+) - abs. most colonies papillating	
4. " "	"			
5. HFT 2-	5 "	0	c. 1 pap. - abs. at 1hr and 2 days; all colonies regularly papillating and reacting (+)	

For further experiments see p 309.

302-1 = 4p 302-4

Pick transductants

All checked - Seg. 1 not against HFT 2 all knob, or 7-. Spotted and read. on B11, apparently all HFT - B1 lysates and see

1. 1st lysate = 302A-1, not HFT (1, 2, 4, (1, 3)

4. Examine 1- 7+ / 1+ 7- phenotype also

2307 = 4p 2301

colonies picked

colonies almost stellate in appearance because of (+) growth. At the end of 3 days c. 1, 2 % colonies (+)

1. Spot. not incubated for about a week - 2307/HFT plate (2) read after 3 days; about 3:50 papillating pale pinpoint colonies / about 1000 cells - Controls abs. of 5-6 per the same number

302A →

2. pick the presumed (+) and see if 1- 7+ / 1+ 7- phenotype can be discovered

1. the phen. was tested clone otherwise not., 24 clonal cells picked and streaked out to obtain definite change to (+), 22 knobbed streaks obtained, 2 pure (-) streaks

2. Single (+) picked from each of the 22 streakings

1st (+) streaking 7/22 shows "slight" segregation, i.e. pure (-) col.
2nd (+) " 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 t_p^s transductions and stable transduction

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

285-2 Streaked out on B(s) - after 3 days, 20 colonies picked

B gal

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

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

A

Save one of the 1 t_p^s of 518

apparently 4- t_p^R lost somewhere during this procedure. Correct!

② Gal(+) λ | Gal Segregating? | Segregant | Seg. tp

1.	R	yes	1-	S
2.	S	no	—	—
3.	R	yes	1-	S
4.	R	yes	1-	S
5.	R	yes	parental?	—
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 - Gal$_q$ - Gal$_2$ - Ly^s from 257C-6

From the purpose of settling two problems, possibly 3
1. Ly^s transductants?
2. Stable transductants how many?
3. Size of the fragment.

1. 298-8

Strain	# Days	(lysis here also)
K12(11/12/54	0	
	0?	(perhaps 50 very small)

A

a. This is odd since on 297, appx concentrated at 150/0.1 ml

found	
	256/811
	40/1210
	842/750
	<1300/2099

this lysate titered against 2368 ?
1/8 = c. 3000 + titer. 2000
control = 0

304A-1

b. the unused pop., inhibited by something?
① 6 picked, 5 found to contain (1) in first streak
② 3 found segregating (one of these appears to be

N2892

Strain:	Ly^R 2+ 4+ / Ly^s - -
304A-1	

	phenotype	2-	4-	λ RK
1.	-	-	-	R
2.	-	-	-	Λ
3.	-	-	-	S
4.	+	—	-	R
5.	+	—	-	R

B

269-1 - because of EML's finding of unusual phage in lysate of this.

1 pkg of 269-1 strained and diluted with 10^{-5} dil. of 247B-1

un adsd	= 10
0.1 247B-1 Ly^s	= 53

Plated against		269-1 Ly^s	trop ? 269-1 Ly^s	probably not phage? expand. of 269-1	cross control	
on Bgal	1. 2297	dense, c 60 col.	lysis, solid plus of contact pinch in water	contam. c + ⊙, on the background also about 100 200 plaques.	ox	
on Bgal	2. 750	c. 20 col remaining	no lysis, contam. c +	no lysis contam. c (+)	de	
on B(1)	3. 269-1	c. ¼ about col.	no change	no change	ste	
	4. 1412	c. 10 remt. col.	no lysis contam. c +	no change		

Clear for 269-1 lysate against acc

11/9/54 Additional
lysate made on 11/8? against Bgal, also assay against Gluconate tubes —

	Pen	NSB	
2279	λ^s	λ^s	← usual λ lysis
811	λ^{11}	λ^{11}	
1177	λ^R	λ^R	

Presumably the new plaque is not in the original or the new lysate. In addition, pop. appears not stable

12/29/54

2341 x —— wild type HFT to obtain: 0 S 2⁻ / R 2⁻ / + 2+ } and see segregants

1. 2341 fresh culture Pen, overnight 2 ml/11 ml incubated in water bath, no air, for 3 hours, diluted to c. 10^6 cells/ml
2. 750 tK122 lysate = 5.8×10^8 plaques/ml (see pg 284)
3. 0.1 ml cell susp + 0.8 ml lysate incubated at 37C for 10' (1.8×10^7 transf/ml)
4. control (broth) exposed cells = (1/4) plate $275 \times 4 \times 10^3$ = 1100×10^3 = $\frac{1.1 \times 10^6 \text{ cells}}{5.8 \times 10^8 \lambda}$ = ~~[illegible]~~

3 plates about the same no of colonies, no (+) on any of them, no evidence of lytic activity.

5. expt. plates:
   1. 1/4 = 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
1.	u	R	n lys.	R	nl
2.	s	R	"	—	
3.	u	R	"	R	nl
4.	u	R	"	R	lys
5.	u	R	lys	s	nl
6.	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
13.	u	R	lys	R	lys
14.	u	R	n lys	s	nl.
15.	u	R	lys	R	lys
16.	u	R	lys	R	lys
17.	u	R	n lys	s	nl
18.	u.	R	n lys	s	nl

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,

no add = 0
0.1 ml K12 = 175

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

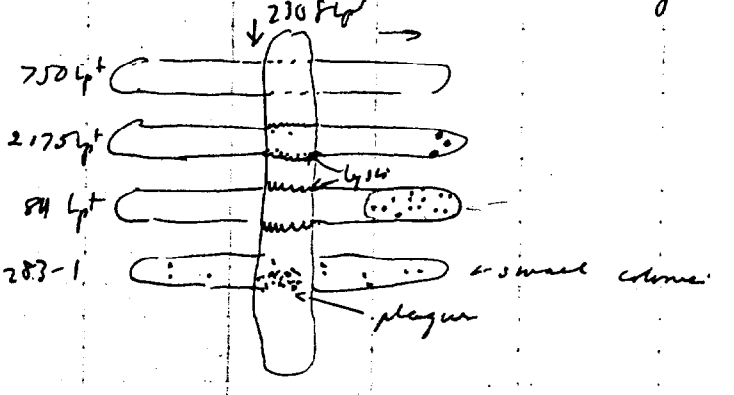
On to → 307

see pg. 304
/2279 c1300
/750 342
/1200 40
.84 256

B

2308

previous test of some 7 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 (+)?



202-16H mal – One step – Aerated culture from overnight Pen.

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

5 ml 5 ml 5 ml

0 assay 0.1+10 ml Pens → Pen

Time	Port 0.1 ml / 2279	0.1 ml / 750	0.1 ml plated
0	3	5 (1)	0
15	4	5 (1)	—
38	4	3 0	—
45	4	5 1	—
60	1	5 1 (9?)	—
75	5 (5 pp)	7 1.75	—
		Mod.	= 35 sec.
210	58 (6 trud)	14 10	

3 of these were also marked as plaques.

	0.1/2279	0.1/750	0.1/plated
—	0 (3 pp)	4	20

750 no. odd = 3

Burst size	c. 16 plaques	c. 3 trud. port	
	P pp	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 (4) 6	13	—

10X above Est

Mod. 35"

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

Burst size c. 10 4 in the places marked for plaques from top to bottom 3, 5, 2, 2, 0

$1^+ 7^-$ / $1^- 7^+$ ← position effect stocks

$1^- \times 7^-$

307

307-1A, 1C etc

307A Continued.

Trend	segregation	Segregant 1	Segregant 2	∅		Re ✓ 1	Re ✓ 7	Genotype
1	"slightly"	−	+	1⁻, w 1⁻7⁻	all of these spotted on 2175	−	+	1⁻
2	"	parental di-heterogenite				[illegible]		
3	"	−	+	1⁻, w 1⁻7⁻	on 5 gal	−	+	1⁻
4	"as usual"	parental di-het.		−	and			
5	"	+	+	7⁻	unsd.	+	−	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-heterogenite		−				
16.		+	+	7⁻		+	−	7⁻
17.		+	+	7⁻		+	−	7⁻
18.	"as usual" → stable (+)				~~7 7~~	all take 7⁻ ave. (minute)		7 7⁻
19.	"slightly"				7 1⁻, 1⁻7⁻	pap. after 4 days.		4 1⁻
20.	"	→ stable (+)						3 1⁻7⁻
21.	"							3 parental
								4 stable +

W2854 = 307-1

Also you derivative W2855

Continued from 305 - the 2341 × (+) 2⁻ 2⁻ ±

① # 4, 5, 11, 12 restreaked from original taking from "brush"

② single colonies (+) picked and tested / λ, 2279, streaked out 3 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 4' present

Stocks 1/4/55

307A

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

Sht HFT 7- (309-1) to obtain 4-7-
pop. removed from a cross brush.

1. 7 sep. seq. transd. /12 picked
single seq from each 3 7- <- stock of this crude M-galt-
4 4-
307A-1

2. Restreaked to obtain 7 more seq.
6 obtained, 3 7-
3 4-

14- (6 7)

W2792

55?

1/9/54

Position effect between Gal_1^- and Gal_6 (2070)

1. NA4prep-1 mixed with cells of 2070 with λ in 1 tube, incubated for 15 min at room temp., diluted and plated. Broth used as control (c. 10^7 cells / 10^8 λ)

	(−) col	orange col. after 2 days
broth	95	0
	82	0
	100	0
	277	0
CHF71 cysate	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 seg. (+) crossovers tested

	1	6	acute	
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− ? small pap and smooth	
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 from digenotes
8 6−
2 1−
3 1−6−

W2856

+ −
− +

+ + or − −
− − + +

W2857 also bp's derivat

+ −
+ +

1/9/54

Continuation of 302 -

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

1. Since gal - cultures when spotted on either + or - and irradiated gave "apparent" false positive reactions for HFT, experiment was done on S gal using portion of 2175 gal -

2. Above cultures streaked to 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 appar HFT	← 309-1

1/26/54

To test L.L. Cavalli's hypothesis about crossing over between fragment and chromosome.

1. 309-1 plated on B bac, tyl, gal, arabinose to obtain reversion and to test stability
   - A. no reversions obtained B bac, tyl, several on B gal. B arab. reac. appears identical mixed culture (here - rev. come from single colony above). Streaking out of the growth in the plate suggests segregation for arab going on. Pos. of round rev. glucose?
   - B. 2307 streaked against HFT 1, 2, 4, 7 on B arab.
   - C. 309-1 gal + to be checked (after purification) on B arab. — Apparently 309-1 is ara + as 2307 has been on streaks of 309-1 rather showing different colony type, do not show any (-)
   - D. Ozet + of 309-1 are unstable 1/6

Prototrophs 2- 1- 4+ / 2+ 1+ 4-

309B-1

4- 8- ← 1-

2350 ← 1- See 272 For order of loci. Gather additional segregants

2-4- ← 1- as segregants.

W2859 also 4+ dec

309B-11 triple (-) plaque 31

B Seg.	/1-	/2-	/4-	Alleles	7+ Stk /47	/1-	/2-	/4-	Alleles
1.	mixed with + ? ND → genuine appear -				20	+	— *	+	2-
2.	+	—	+	2-	21	+	— *	+	2-
3.	mixed with + ? Discarded →				22	—	— *	—	1-2-4-
4.	+	— *	+	2-	23	—	— *	—	1-2-4-
5.	+	— *	+	2-	24	+	— *	+	2-
6.	—	— *	—	1-2-4-	25	—	—	—	1-2-4 (thin 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.	+	— *	+	2-4-	35.	+	— *	+	2-
17.	+	— *	+	1-2-4-	36.	+	— *	+	2-
18.	+	— *	+	2-	37.	+	— *	+	2-
19.	+	— *	+	2-					

* plaque (2-4)

1 3	2 3	
+ −	+ −	+ +
− +	− +	− −
(2) (1-4-)	(2) (1-4-)	(2) (1-)

Clones

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

Previously
idio 2- 8
allo 1- 2

1/9/54

2433 λ— λ⁻ for order of loci - see 272 and also other comb. on 309, last exp

no add 0
dil of 2478-1 17 — 2/17 obvious, 1 less, 14 tested

A

Seq	/1	/2	/4	Comment
1	—	—	+ ?	heavily pegillating after 3 days
2	—	—	+ ?	.. ..
3	curlam 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 pegillation

Rpt tet

confirm these as to allele, but no pap. usually have thinner growth

Also segregating on 2nd day. Most some of the pegillating forms are λ⁻s or slow. λ but something. Pap. from

Reshark on tests at pap. against λ⁻s in these clone? except #3.

In making purification before next, more sectors (pap. forms were selected)

See if seg occur in 3 on B(0)
Also if thes cult. acts, in lysin?

2308 λ— 2070 λ - to see if Gal_6^- is distinct from Gal_7^-

B

no add 7
old lysate 2070 λ (0.1) 30 ($\frac{1}{10}$ $\frac{1}{10}$)

27 picked - all appear clotable (~~λ⁻~~)

	λ	1721
1	R	lysogenic
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12	↓	↓
13	↓	↓
14	↓	↓

	λ	1721
15	R	lysogenic
16	↓	↓
17	S	unlys
18	S	.. ..
19	R	lysogenic
20		
21		
22		
23	↓	↓
24	S	unlys
25	R	lysogenic
26	↓	↓
27	↓	↓

2360 t K12 – to see incidence of stables (+) and lysogenicity
transd. region 304 c2000/0

transd.	reactions. 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~~			+ = yes total 8 non seg
~~24~~			14 seg

2070 λ+ – for making HFT 6– ~~onto~~ 2175

311-10 (old lysate) ⑦ no add 11
0.1 2070 λ+ 97 – this titer higher than on 2308 tp's, but again this may be indication of decay of lysates on standing (only measured on tp's cells) or a pronounced effect between 6– and 7–.

311-13

② pick and streak to obtain HFT 6–

③ 13 seg tested / 2175 on stool, 2 possible HFT, #10, #12

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

1/26/54

283-1 against 7⁻ - If the order is 1⁻ 7⁺ 4⁻, then only double crossover will give (+)

#3 must be 1⁻ 7⁺ 4⁻ or ⁻ ⁺ ⁺ or ⁻ ⁺ ⁺

A

mood = 0

0.1 (1-100 bc)

Pap.	309-1 stable (2)	10 Streaks of (-)	/1	/4	/7		Genotype
1	+	—					—
2	single (+) →	(-)	—	2 small pap	—	—	1 pap	1⁻ ~~7⁻~~ or 1⁻4⁻?
3	c. 10 (-)	portion of colonies 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 (+)

~~[illegible]~~

2070 cross bushed with HFT sugars - Gal_6^- distinct from Gal_7^-

/1	/2	/4	/7
++	solid omnia	++++	+++++

1/25/~~34~~ 5

307-1 X — 4⁻ to obtain order

1 − − x + +
7 + − x + + !
4 + + x − −

− +
+ +, X − +
− +

① The 4⁻ (247B-1) lysate diluted 1-100 gave c. 5×10^3 pop ← Contact this c 1-4⁻ x — 7⁻ where double crossover are required on order 1/4

2nd add =

② 24 papillae picked, streaked ②
(−) 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⁻

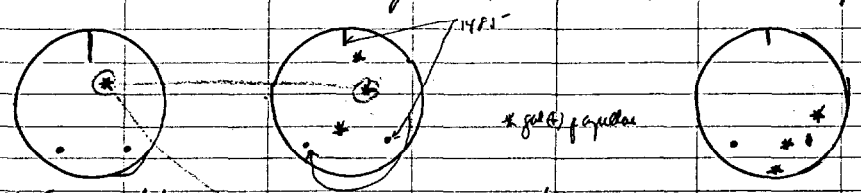
~~The results suggest the order is as above, but the absence of the 1,3 shared clears is still puzzling~~

DATE: 1/26/54 REF:

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

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 read. 20' with U.V. (stentary 50 cm). Reincubated about 30 minutes and then replicated to plate (previously spread c̄ 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.

w1210-1 irradiated plate (Bottom view) w750 replica'd c̄ 1210-1 plate



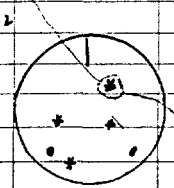
O——O related gal+

Master - 1210 plate

all 3 found stable after ②

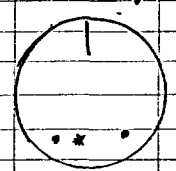
all 3 found stable after ② - one a weak(+)

w1210-2



all 4 found stable ②

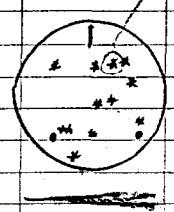
w750 rep.



found stable ②

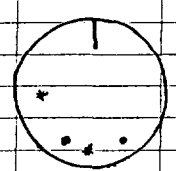
no suggestion of seg. colonies on streak ①

w1210-3



17 found stable ②

w750 rep.



2 found stable ②

Where are the (+) above from?

DATE: 1/31/55 REF:

1210 against lysate of 307-1. Completion of Positive Effect c. 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. 0.1 ml 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⁻
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⁻
3 stabs(+)				1 1⁻7⁻

save M-1⁻7⁻

1210 = galg⁻
not galg⁻

W1210 = galg⁻

315-12

W2861

750 E/C12-1 λ Prey 1/28/55
transd / 750 = 2.6 X 10⁷
0.1 ml in Pen & Sterile

1.43 x 100 (100 x 100 1.4 x 10^5)

(316)

DATE: 2/1/54 Inoc of 241-14 mal - Q Isola REF:

1	spot 3 Pap 2279 (dil 10^8)	spot 2 Pap 750 (dil 10^8)	spot 1 Morgan 2279 ↓ 10^2 dil	spot 0 Pap 2279 10^2 dil	6 Gram + Stable/Total	7. Capsules Sol	8 Cal Lambda	9 Rhodamine Neostomin	10
Dose									
0	28	67	155	1	1/1	—	2 Lp+, 7 Lp-, 3 Lp R		
15	54	140	151	15	0/15	S	— , 14 Lp-, 2 Lp R		
30	78	184	172	22	17/18	R	— , 17 Lp-, 1 Lp R		
45	108	293	66	25	18/18	—	, 16 Lp-, 1 Lp R		
60	176	434	28	22	17/18	R	1 Lp+, 16 Lp-, —		
75	230	638	39	19	17/18	R	— , 17 Lp-, —		
90	221	711	14	24	17/18	S: stable (+)			
105	237	536	17	37	18/18				
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 (4)	18/18				
165	163	717	und = 231	ca. 4	18/18				
180	148	589	und = 141	ca. 11	17/18				
~~195~~									

Stippled

1.6 x 10^8 phages/ml

0 dose

1 ml = 155 x 10^3 plaques
= 900 x 10^2 Neomin.

1.55 x 10^5 x 10^3 = 1.55 x 10^8/ml

6.7 x 10^6 transd/ml

1 - 10 dil fac = 15500 plaques
9000 transd.

10^4 x 10^3 = 1.

Multiplicity Aspect.

Pap 2279

(1) 2497 + 0.1 ml 10^2 0 dose plated. To plaques on control (no 750-2) = 37
pipettes on 750-2 half = = 175
pipettes on control = 2
21.4 > than the highest value for plaque or transd. given above in 0, 15, 30 (155, 151, 172)

½ of plate

0.1 ml 750-2 + control (Pur Plate) see abv

(2) 0 above 10^2 dil
(1) 0.5 ml + 0.5 ml cell (c. 10^8) adsorbed 10'
(2) add 0.5 ml 750-2 (= 2/3 dil of original), adsorb 5'
(3) plate 0.1 ml. Rec. pipettes. 188 x 3/2 = 282

DATE: 2/5/53 REF:

298-8 see last page and 304

1. 298-8 made lysogenic with 1 from 311
2. To complete this study of 8 colonies.
   a. 298-8 × — HFT 1
   b. 298-8 × — " 6
   c. " " " 7
   } undiluted 0.1 ml
3. Recall. No papillae on any of the plates. What goes?

2279 against HFT 6, 7 to obtain recip. test of prot. effect.
0.1 ml ca. 10^8 cells + 0.5 ml lysate diluted 1-100 — 5 ml plated
1. 2279 control 8 plates c. 600 colonies/plate are (−)

2. 2279 × — 6⁻

Failed to kill. Should be observed. Killing was very light here

W2862 also lysogenic for derivative

317C-4

	2	1	ϕ	Unit 7
1	0	+	2⁻	
2	0	+	2⁻	
3	0	+	2⁻	
4	0	+	2⁻	✓
5	0	+	2⁻	317C-6
6	0	0	2-7⁻	↙
7	0	+	2⁻	
8	0	+	2⁻	

8 additional say regular from the plates transductions

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

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

	2	6	ϕ	
1.	+	0	6⁻	8 like
2.	0	+	2⁻	1 alike
3.	0	+	2⁻	1 ampl
4.	0	0	2-6⁻	
5.	0	+	2⁻	
6.	0	+	2⁻	
7	0	+	2⁻	
8	0	+	2⁻	
9.	0	+	2⁻	
10.	0	+	2⁻	

6⁻

7⁻

	2	7	ϕ	
	0	+	2⁻	7 like
	0	+	2⁻	2 alike
	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. 518 gal - F+ x 902 gal F-~~
~~a. control streaks of each ok, 518 has a few (+)~~
~~b. cross plates~~

750E902-1 x 2274

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

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

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

	stable	λ	/2279		stable	λ	/2279
1	u	R	lys	11	s	s	non lys
2	u	R	lys	12	s	s	..
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	..				

~~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 bkgrd of (-) colony, irreg shaped about 20-30 weak (+) per plate

12 (+) 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			
full gal (+) 11	s	R	non lys