11/7/54

Recombination between lp^u to give λ

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

2. Proced. - culture grown in all combinations in pen assay. Starting from 0.1 ml of overnight culture. Cultures centrifuged after 24 hours and loopful of supernatant 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+ [c HFT]

1. W1321 (518 F-)

a. untreated

b. 0.3 ml ~~[illegible]~~ of overnight culture ~~[illegible]~~ } incubated 10' at 37, dil c 10 ml
1.0 ml of HFT 2 (2342) broth added

c. as b. only using 269-1 lysate - 2342 t K12 Hk 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 ♀ gal.
a, b, c: also spotted on S gal separately, 945 as well, as controls - no growth

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

1412 attempts to transduce c̄ λ-2

1. Internal malt^R of (Mal- λ^R) have interaction with λ-2 such that transduction might be obtained ?
2. 8 Mal +R obtained with varying degrees of +-ness
   1 = extreme (+) 8 = (-)
3. λ-2 prepared by growth on 1485. 0.1ml plated B gal

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

W2872

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

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

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

The transd these (-) came from ares

3. Analysis of segregants

	#	HFT 1	HF 2	HFT 4	
295-1	1.	+	−	+	found NFT
295-2	2.	−	+	−	
295-3	3.	+	−	+	
295-4	4.	+	−	+	found NFT
295-5	5.	−	+	−	
	6.	+	−	+	
	7.	+	−	+	
	8.	+	−	+	
	9.	+	−	+	
	10.	+	−	+	
W2953	11.	+	−	+	found NFT
295-12	12.	−	+	−	
	13.	+	−	+	
	14.	+	−	+	
	15.	+	−	+	
	16.	+	−	+	
	17.	+	−	+	
	18.	+	−	+	
Σ: 15 idi	1 19.	+	−	+	
3 allo	2 20	+	−	+	
	3 21	+	−	+	found NFT
	4 22	−	+	−	
	5 23	+	−	+	

	#	HFT 1		HF 2		HFT 4	
6	24	+		−		+	
7	25	+		−		+	
8	26	+		−		+	
9	27	−		+		−	found NFT
10	28	+	W	−		+	W
11	29	+	F	−	2-3	+	F
12	30	+	W	−		+	W
13	31	+	F	−		+	
14	32	+	F	−		+	
15	33	+		−		+	
control population 16	34 contam	−		+		−	possibly HFT or contam. Re ✓ indicates contamination
17	35	+	F	−		+	
18	36	+	F	−	2	+	found NFT
19	37	−		+		−	W
20	38	+	W	−		+	W
21	39	+	W	−	2	+	W
22	40	+	W	−		+	F
23	41	+	F	−		+	W
24	42	+	W	−		+	F
25	43	+	F	−		+	F
26	44	+	F	−		+	W
27	45	+	W	−		+	F
28	46	+	F	−		+	F
29	47	+	F	−		+	W
30	48	+	W	−		+	

W2853

* These vacuum for HFT required a day extra for full development suggesting that the [illegible] is not full (+)

(295A)

13 idio
14 allo
3 amphi (all 1-)

F = full
W = weak

1210 + 283-1 Continued.

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

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

Reg	HFT 1-	AFL-	AFT 9-	
(45) 63	+	−	+	
(46) 64	+	−	+	
(47) 65	+	−	+	
(48) 66	+	−	+	
(49) 67	−	+	−	found NFT
(50) 68	+	−	+	
(51) 69	+	−	+	

Batch II

	HFT 1-	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	+	−	+	

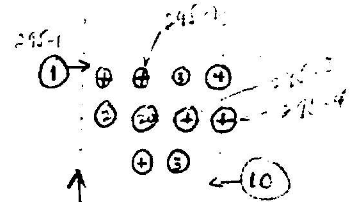
29	+	−	+	
30	+	−	+	
31	+	−	+	
32	+	−	+	
33	+	−	+	
34	−	+	+	[illegible] (−)
35	+	−	+	
✓ 36	+	−	+	
37	+	−	+	
38	+	−	+	
39	+	−	+	
40	+	−	+	
41	+	−	+	
42	+	−	+	
43	−	+	−	found NFT
44	+	−	+	
45	+	−	+	
46	+	−	+	
47	+	−	+	
48	+	−	+	
49	+	−	+	
50	+	−	+	
51	−	+	−	found NFT
52	+	[illegible]	+	
53	+	−	+	
54	+	−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

one of these found HFT in 2175

49 idio
4 allo
1 amphi

78 idio 7
5 allo
1 amphi

4/23/56
Retesting these stocks shows no HFT [illegible] extra days [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.	−	+	−
ok 2.	−	+	−
ok 3.	−	+	−
4.	−	+	−
5.	−	+	.
6.	−	+	−
7.	−	+	−
8.	−	+	−
9.	−	+	−
10.	−	+	−

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

1. β gal
no add. ½ = 41
HFT 2 (2342 pap/ for gal) ½ = 317

2. Batch I
24 papillae picked and streaked β gal (2) - all stable, all non lysogenic /518, are Mal+ (2) revert dont test λ^5 -

3. Batch II
22 papillae picked and streaked β gal (2) - all stable, all non lysogenic /518
~~23 Batch III papillae picked ..... (2) all~~

4. 24 papillae .. .. (2) 1 4th found, all non lysogenic /518 (296-1) W2881

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

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

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

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

Rpt experiment HFT 2	HFT 4
3	0
8	0
contamination 0	0
0	0

7. 296-1,2,3,4 gal+ seg used as indicators in plating with conc. (>10^9) 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 termination		#	Appearance of Gal+ on Mal	Reversion obtained	Reversion ϕ type	Gal transd. λ-2	Mal+R λ /518	
	1	1	pink	+	free +	resistant	S	n.d.
	2	2	blue	+	~~free~~ +	"	S	..
296-5	3	3	pink	+	full +	"	S	..
296-6	4	4	blue	+	full +	"	R	..
296-7	5	5	pink	+	full +	..	S	..
	6	6	pink	+	intermed (+)-	"	S (weak)	..
	7	7	pink	+	free +	"	S	..
		8	..	~~+~~	—	"	—	
	8	9	..	+	free +	"	S	..
	9	10	..	+	free +	"	S	..
	10	11	..	+	intermed (+)-	..	S (weak)	..
	11	12	..	+	free +	"	S	..
	12	13	..	+	free +	..	S	..
	13	14	..	~~+~~	int. (+) -	..	S (weak)	..
		15	..	—	—	..		
	14	16	..	+	free +	..	S	..
	15	17	..	+	free +	..	S	..
		18	blue	—	—	..		
		19	blue	—	—	..		
	16	20	pink	+	free +	..	S	
		21	pink	—	—	..		
	17	22	pink	+	free +	..	S	..
	18	23	pink	+	full +	..	S	..
	19	24	pink	+	free +	..	S	..

W2884
W2885
W2886

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

SEE BELOW

1210 transduced with 283-1 - same medium as ↑

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

SEE 297A

K12 lysate above - Test of layer plating method.

Trays. 0.1 ml lysate + 2.5 ml Bgal (0.6% agar) poured in Bgal plate

817.	① no add	38	
	② 0.1 ml	294	256
1210	① no add	4	
	② 0.1 ml	44 very small	40
750.	① no add	4	
	② 0.1 ml	346 (quite a few small	342
2289	① no add	7 c. 400 small plaques)	
	② 0.1 ml	1/2 = 162 ; 1196	

Contam streaked culture?

11/30/54

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

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

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

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

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

Attempted transduction of Ylemonald arabinose and also direct attempt at (Mal- Lp_2^R).

1. 2347 = 583 mal- ara- Lp_2^R Lp+ S^R ← obtain testable gal+ for S^r direction) (This reverts in drives of HFT
 control — 4 days many small } no transduction.
 on Broth 0.1ml — .. ..
 HFT 2⁻

2. 2307 = 583 mal + Lp_2^S ara- S^s
 HFT 2⁻ — 4 days
 on B ara — control — 0
 0.1ml — 0

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

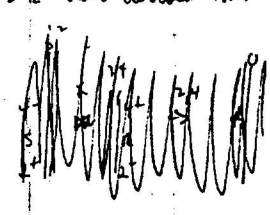
257C-6 - Continuation. see also 292
seg from dipl. (+)

	HFT 2⁻	HFT 4⁻	A. React.	Seg from rev I	Seg from rev II				Page 292	Total
1.	−	+	R	1 2⁻S	2⁻S	J dir type	4⁻ S	1	2	3
2.	−	+	S	—	—	allotypic	2⁻ R	6	16	22 Lp^R
3.	−	+	S	—	—	allotypic	2⁻ S	2	4	6
4.	−	+	R	2 2⁻S	2⁻R	ampli	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 ole 2⁻R	2⁻S					
10	+	−	S	(to include)						

298 (left margin, rows 1–7); 298-8 (row 8)

W2840 also got down.

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



S S × R R (1,4 / 3,4) → S R
4⁻ 4⁻ × 4⁺ 4⁺ → 4⁺ 4⁺
2⁺ 2⁺ × 2⁻ 2 → 2⁻ 2⁻
‖ 2⁻ R

Rev → S R / 4⁺ 4⁺ / 2⁻ 2⁺ ↓

S R / 4⁺ 4⁺ / 2⁺ 2⁻ not seen

S / 4⁺ / 2⁻

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

12/2/54

Spontaneous lambda from HFT stocks.

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

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

} poor assays. Redone (c. 2000 = 2 x 10^5; abs = 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 /200

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

241-14 making Ly_2^R by mal-selection
6 colonies tested

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

2431 - HFT gal₄ stock. Streaked out from 2937-2A and colonies tested. The broths given to H. Bernstein for immediate lyophile. All 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 colonies (^{10}) 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 sol. : <10^5 ⟩ this dil.
0.1 + 10 sol. : <10^3
1.0 + 10 sol. : = ~10^2

Assay against 750, 2229

Time		/750	/2229	colonies
10:24	0			10/0.1 ml
	5	Skip		
10:34	10			
10:39	15			
10:44	20			
10:49	25			
10:54	30			
10:59	35			
~~11:04~~	~~40~~			
~~11:09~~	~~45~~			
~~11:14~~	~~50~~			
~~11:19~~	~~55~~			
~~11:24~~	~~60~~			
~~11:29~~	~~65~~			
~~12:29~~	~~125~~			

failed — over diluted since 10 colonies gave plaques — suggesting non-inducible state

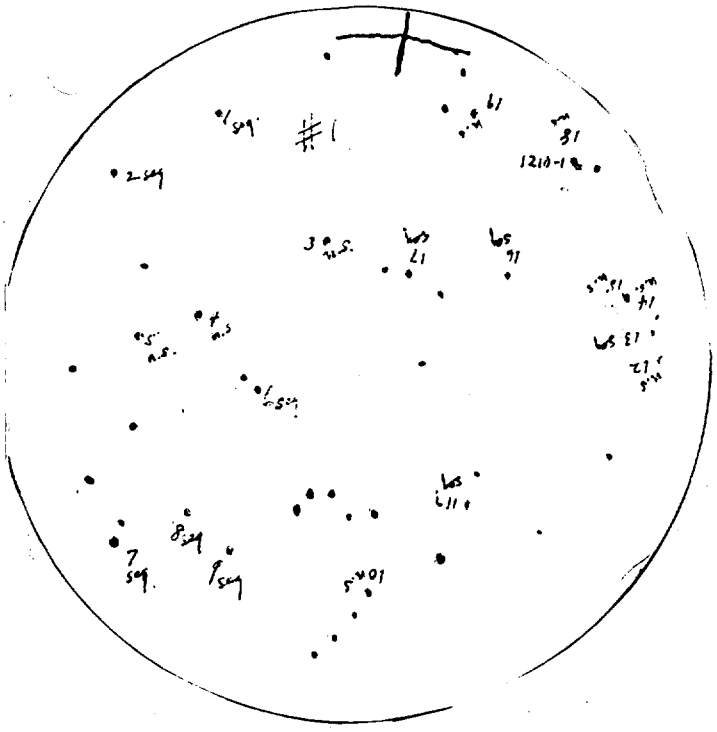
2. 241-14 - attempt to make Gp*.
Streaked out on λ-2 m. B mal.
1. Mostly Mal + seen -
2. ~~2~~ mal - col picked and streaked out
3. pure [illegible] mal - 6 col streaked on B gal - all gal (-)

4. Repeat — Supernat. broth of culture used to make these: 0X10^5 plaques / 2X10^5 transd.? (checked) ↑ Repeat assay (6)

5. On next run pure mal - obtained - tested for HFT all HFT except 1

6. Stock made called (241/14 Mal -)
1. Lysate 12/18/54 53 x 10^7 plaques / 2279 = 5.3X10^8
transd. assay 13 x 10^7 = 1.3x10^8

3. 202-1CH - (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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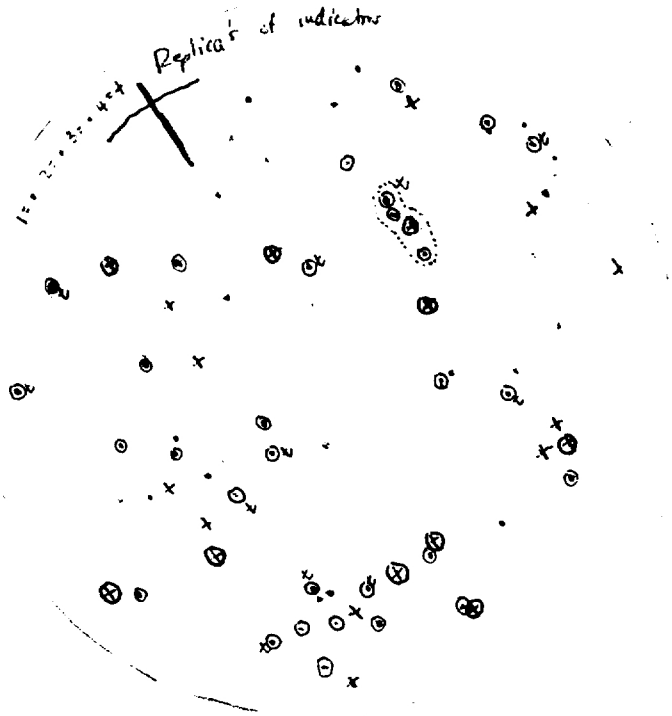
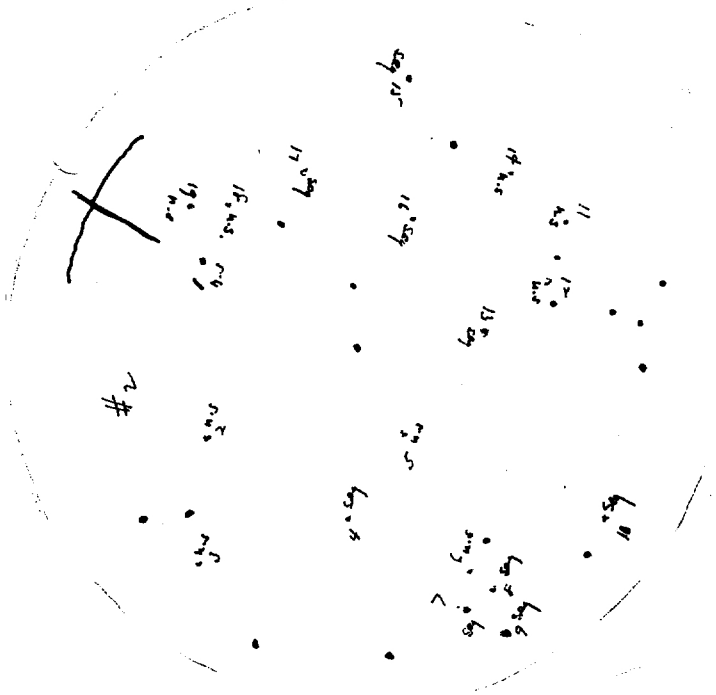

Replicas of indicators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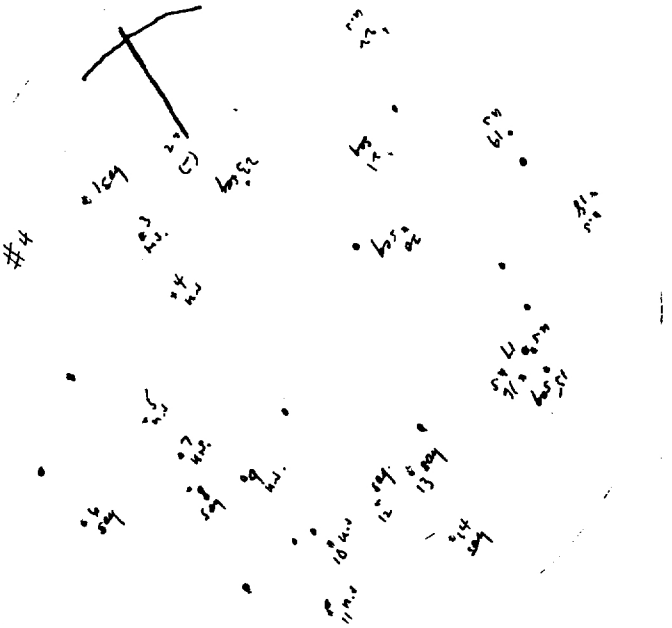
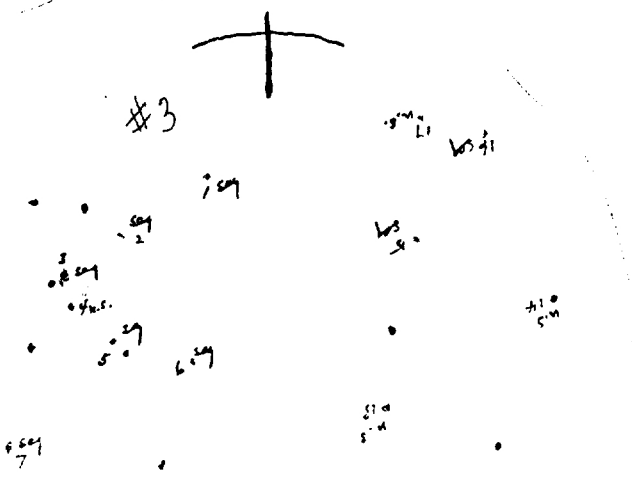

#4


#3

12/11/54

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

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

12/12/54

Observations on 292-3 = $4^+ 2^- 5$ (from 510 by 2 - x)
against 295A-4 = presumed HFT $1^- 4^-$ to see if initial complex
to phen. (+).

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

		After 3 days
1. control	= 3×10^3 (+) col.	c. 12 pap. on colonies representing c. 12 reversions
2. control	.. ..	c. 14 .. .. 14 ..
3. 295A-4	= 200-300 col	c. 12 .. .. .. representing ?
4. ..	.. ..	c. 8 .. .. ?

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

Observations on 2307 = 583 mal+ h_{p2}^s gal$_7^-$ h_p^+

W2874

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

3. Exposure

		2 days	3 days
1. broth	c. 400 (-) col.	c. 5 reversions →	
2. ..	.. (-) col.	c. 3 .. →	
3. HFT 1 -	c. 500 col (-)	0	c. 30 (+) - also most colonies papillating
4. ..	..		
5. HFT 2 -	..		c. 1 pap. - also at the end of 2 days all colonies papillating and reverting to (+)

302-1A = 54
302-1

The transductions

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

colonies almost stellate in appearance because of (+) growth. At the end of 3 days c. 9 % of colonies (+)

For further information See p 309

4. Examine $\frac{1^- 7^+}{1^+ 7^-}$ phenotype also

2308 = h_p^s 2307

1. Expt. interrupted for about a week - 2308/HFT (2) plates had after 3 days about 3150 papillating pale pink colonies of about 1000 colonies - Controls about 56 per the same number

302 A →
2. pick the presumed (+) and see if $\frac{1^- 7^+}{1^+ 7^-}$ phenotype can be discovered
 1. The phen.-s mixed clone streaked out, 24 clonal cell picked and streaked out to obtain discrete change to (+), 22 mixed streaks obtained, 2 pure (-) streaks
 2. Single (+) picked from each of the 22 streakings
 1st (+) streaking 7/22 show "slight segregation, i.e. few (-) col.
 3rd (+) .. 3 apparently stable, 3 "slightly" seg., one lost
 .. 1/15 seg., stable (+)

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

SEE PAGE 307

12/12/54

Observations on tp^s transductions and stable transduction

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

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

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

A

	combination		λ RR	Allele	~~Recessive [illegible]~~ RR
1.	+	−	S	1⁻	cultures mixed up
2.	+	−	S	1⁻	and partially
3.	+	−	S	1⁻	lost, in attempting
4.	+	−	S	1⁻	to recover,
5.	+	−	S	1⁻	tested against
6.	+	−	S	1⁻	λ, 4/4 (-) not
7.	+	−	S	1⁻	grow, λ^s
8.	−	+	R	4⁻	3/3 (-) λ^R(?)

Save one of the 1tps of 518 [?]

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

B

(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?	[illegible]
6.	R	yes	1⁻	S
7.	R	yes [illegible]	—	—
8.	R	yes	1⁻	S
9.	R	yes	parental	R
10.	R	yes	1⁻.	—
11.	S	no	—	—
12.	S	no.	—	—

298-8 - Gal$_4^-$ Gal$_c^-$ Ly^s from 257C-6

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

1. 298-8
- Not add. — 4 days (lysis here also) — 0
- K12 (11/13/54) — 0? (perhaps 50 very small)

A

a. This is odd since in .297, found 256/84, 40/1210, 342/750, c1300/2099. This lysate approximated at 150/0.1 ml

this lysate titered against 2360 ?
1/8 = c. 2000 = titer = 2000
control = 0

304A-

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

	Phenotype	2	4	λ RR
1.	-	-	-	R
2.	-	-	-	R
3.	-	-	-	R
4.	+	-	-	S
5.	+	-	-	R

N2892

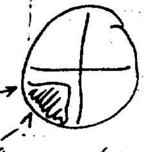
	2+	4+
Ly^R		
Ly^s	-	-

Stock: 304A-1

B

269-1 - because of EML's finding of unusual phage in lysate of this.
1 sep of 269-1 streaked and transduced with 10^{-5} dil. of 2478-1
un add = 10
0.1 2478-1 lys = 53

Plated against		269-1 lys	drop of 269-1 #2 lys	probably not sterile supernat. of 269-1	area control
on Bgal	1. 2297	cleared, c.60 col.	lysis, with film of contact, pink in color	contam. c (+), on the background are also about 100-200 plaques.	on
on Bgal	2. 750	c. 20 col remaining	no lysis, contam. c +	no lysis contam. c (+)	do
on B(1)	3. 269-1	c. 4 resist. col.	no change	no change	do
	4. 1412	c. 10 resist. col.	no lysis contam. c +	no change	



Clear for 269-1 lysate against all

1/9/54 Additional lysate made as in 1150, another in Pen, also using actinic glass tubes —

	Pen	NSP	
2279	λ^s	λ^s	← usual λ lysis
811	λ^R	λ^R	
1177	λ^R	λ^R	

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

12/29/54

2341 × wild type HFT to Arlam

0 s	z⁻
R	z⁻
+	z+

} and see segregants

1. 2341 fresh culture Pen, overnight cul/11 ml incubated in water bath, no air, for 3 hours, diluted to c. 10^8 cells/ml
2. 750 t K12 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′ (1.8×10^8 transd/ml)
4. control (broth) exposed cells $= (\frac{?}{plate}) 275 \times 4 \times 10^3 = 1100 \times 10^3 = \frac{1.1 \times 10^6 \text{ cells}}{5.8 \times 10^8 \lambda}$ = ~~[illegible]~~

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

5. expt. plates:

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

6. purified twice

	Stbl	/λ	/2279	single seg /λ	single seg /2279
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

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

283-1 Cont. 304.

lysate K12 12/18/54 on,

no cell = 0
0.1 ml K12 = 175

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

On to → 307

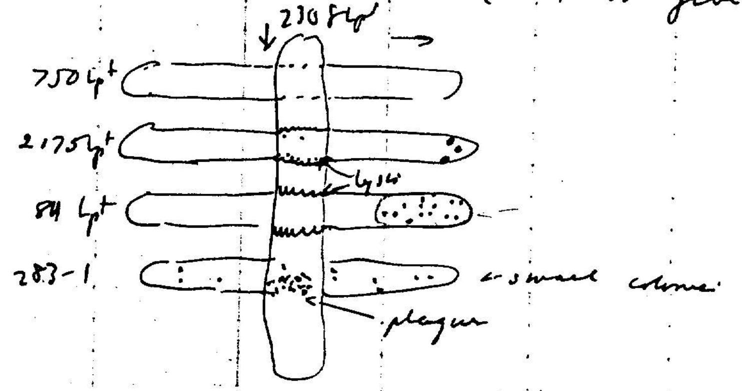
B

see pg. 304

/2279	c 1300
/750	342
/1200	40
84	256

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. So there a phenotypic interaction between 7 and 4 to give (+)?



202-11H wal – 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

0.1/10 ml Pen → Pen

Time	Post 0.1 ml /2279	0.1 ml /750	0 assay 0.1 ml plated
0	3	5 (1)	0
15	4	5 (1)	—
38	4 ave 3.5	3 0	—
45	4	5 1	—
60	1	5 1(9?)	—
75	5 (3 pap)	7 1.75	—
	Grad. = 25 sec.		
210	58 (6 transd)	14 10	
Burst size	c. 16 plaques	c. 3 transd. part	

→ 3 of these were also marked as plaques.

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

750 no. over = 3

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

10X above Eqt

Grad. = 25"

		P	pap	T	C
	0	15 (6)	8	6	9
2:15	60	21 (4)	16	6	—
2:35	80	33 (6)	8	10	—
2:55	100	165	0 2	13	—
3:15	120	145 (4)	6	13	—
Burst size		c. 10	4		

in the places marked for plaques from top to bottom 3, 8, 2, 2, 0

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

$1^- \to \times 7^-$

307

uc

307-1A, 1C

307A Continued.

Tried	segregation	Segregant 1	Segregant 2	ϕ		Rc ✓ /1	/7	Genotype
1	"slightly"	−	+	1^-, or 1^-7^-	all of these spotted on	−	+	1^-
2	"	parental di-heterozygote			2175	[illegible]		
3	"	−	+	1^-, or 1^-7^-	on	−	+	1^-
					5 gal			
4	"as usual"	parental di-het.		−	and			
5	"	+	+	7^-	used.	+	−	7^-
6.	"	+	+	7^-	none	+	−	7^-
7.	"	−	+	$1^-, 1^-7^-$	found	−	−	double
8.	"	−	+	$1^-, 1^-7^-$	HFT	−	+	1^-
9.	"	−	+	$1^-, 1^-7^-$		−	−	double
10.	"	−	+	$1^-, 1^-7^-$		−	+	1^-
11.	"	+	+	7^-		+	−	7^-
12.	"	+	+	7^-		+	−	7^-
13	"	−	+	$1^-, 1^-7^-$	307-1 →	−	−	double
14.	"	+	+	7^-		+	−	7^-
15.	"	parental di-heterozygote		−				
16.		+	+	7^-		+	−	7^-
17.		+	+	7^-		+	−	7^-
18.	"as usual" → stable (+)			−	~~7 7⁻ / 7 1⁻, 1⁻7⁻~~	all these 7⁻ are (minute) pap. after 4 days.		
19.	"slightly"	} → stable (+)						
20.	"							
21.	"							

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

W2854 = 307-1

Also for derivative W2855

Continued from 305 – the 2341 × (+) $\frac{1^-}{\frac{1^-}{+}}$

① # 4, 5, 11, 12 restreaked from original talking from "brush"
② single colonies (+) picked and tested / λ, 2279, streaked out 8 pol

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

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

Stocks 1/4/55 (307A)

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

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

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

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

7 4⁻ (6 7⁻)

W2792

?
50°

1/9/54

Position effect between Gal_1^- and Gal_6 (2070)

1. NA4pap-1 mixed with cells of 2070 with λ in excess (c. 10⁹ cells/10⁸ λ), incubated for 15 min at room temp., diluted and plated. Broth used as control

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

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

Stock gal+ → discarded Sept 1, 1964

2. Segregants from pap. (+) organisms tested

1.	0 pap	0 pap	papillating parental
2.	+	0	6−
3.	−	+	1−
4.	−	+	1− ← 308-4
5.	0 pap	0 pap	papillating parental
6	+	0	6−
7.	+	0	6−
8	0 pap.	0 pap.	papillating parental
9.	+	0	6−
10.	+	0	6−
11.	+	0	6−
12	0	0	pap. parental
13	0	0	1-6− some pap. all through
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 new higerats
8 6−
2 1−
3 1-6−

← W2856

+ −
− +
↓
+ + or − −
− − + +

← W2857 also lp derivat

+ −
+ +

1/9/54

Continuation of 302 -

302-1, 2, 3, 4, 5 - In search of HFT gal₂ -
1. Since gal₂- cultures when spotted on either 2, 5 and irradiated give "apparent" false positive reactions for HFT, experiment was done on S gal using prototroph 2175 gal₂ -

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

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

1/26/54
To test L.L. Cavalli's hypoth about crossing over between fragment and chromosome.
7. 309-1 Plated on B lac, xyl, gal, arabinose to obtain reversion and to test their stability
A. no reversions obtained B lac, xyl, several on B gal. B arab. react. appears to indicate mixed culture (how - this came from single colony above). Streaking out of the growth on the plate suggests segregation for gaal going on. Poss. of round the gaals?
B. 2307 streaked against HFT 1, 2, 4, 7 on B arab.
C. 309-1 gal+R should be checked (after purification) on B arab. - Apparently 309-1 is ara+ as 2307 has been on ✓. Streaks of 309-1 rather showing different colony type, do not show any (-)
D. Gal+R of 309-1 are unstable 6/6

Prob. segregates 2- 1- 4+
2+ 1+ 4-

309B-1

4-8- ← 1-

2350 × ← 1- See 272 for order of loci. Gather additional information on segregants.
2-4- × ← 1-

W2859 also 4+ der

$\frac{2}{1}{4}$ $\frac{-}{+}\frac{-}{+}$ × $\frac{+}{-}\frac{+}{+}$ → $\frac{1}{+}\frac{3}{+}$ (-) ... ; $\frac{1}{+}\frac{3}{-}$ (+) (2)(1-4-) ; $\frac{-}{+}\frac{+}{+}$ (+) (2)(1-)

309B-11
triple (-)
[illegible] 31 ... 2

B

Seg.	/1-	/2-	(10⁵ diluted of NA-[illegible]) /4-	Alleles
1.	mixed with + ?	ND → position effect		–
2.	+	– *	+	2-
3.	mixed cult + ?	Discarded →		
4.	+	– *	+	2-
5.	+	– *	+	2-
6.	–	– *	–	1-2-4-
7.	–	– *	+	1-2-
8.	+	+	–	4-
9.	+	– *	+	2-
10.	+	– *	+	2-
11.	+	– *	+	2-
12.	–	+	+	1-
13.	–	+	+	1-
14.	+	– *	+	2-
15.	+	– *	+	2-
16.	+	– *	+	2-
17.	+	– *	+	1-2-4-
18.	+	– *	+	2-
19.	+	– *	+	2-

(0/48 ; 7+ stb/47)

Seg.	/1-	/2-	/4-	Alleles
20	+	– *	+	2-
21	+	– *	+	2-
22	–	– *	–	1-2-4-
23	–	– *	–	1-2-4-
24	+	– *	+	2-
25	–	–	–	1-2-4 (thin spray)
26	–	+	+	1-
27	+	– *	+	2-
28	–	– *	–	1-2-4-
29	–	+	+	1-
30	+	– *	+	2-
31.	–	+	+	1-
32.	–	+	+	1-
33.	–	+	+	1-
34	–	+	+	1-
35.	+	– *	+	2-
36.	+	– *	+	2-
37.	+	– *	+	2-

* (2-4) [illegible] 2-

Classes

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

Previously
idio 2- 8
allo 1- 2

1/9/54

2433 x 4⁻ For order of loci - see 272 and also other comb. on 309, but my

Seg	no add ulg 2470-1 /1	0 17 /2	~~Segregant~~ - 2/17 stable, 1 lost, 14 tested /4	Comment
1	—	—	+ ?	heavily papillating after 3 days
2	—	—	+ ?	" " "
3	contam c̄	(+)		
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	—	—	+ ✗	as ①
14	—	—	+	no papillation

A

Rpt tests

confirm these as to allele, but no pap. fails

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

On making purification before retest, more specific (pap. forms were selected).
See if pap occur in 3 or B(0).
Also if this cult. occurs, in Negro?

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

B

old lysate: no add 2070λ+(0.1); 7 30 (lysate also)

27 picked - all appear stable (✗)

	/λ	/1321
1	R	lysogenic
2	↓	↓
3		
4		
5		
6		
7		
8		
9		
10		
11		
12	↓	↓
13	↓	↓
14	↓	↓

	/λ	/1321
15	R	lysogenic
16	↓	↓
17	S	non lys
18	S	" "
19	R	lysogenic
20		
21		
22		
23	↓	↓
24	S	non lys
25	R	lysogenic
26		
27	↓	↓

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

trans'd.	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 nonseg
~~24~~			14 seg

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

① no add 11

311-10 (old lysate) 0.1 2070λ+ 97 – This titer higher than on 2308 lp+, but again this may be indication of decay of lysates on standing (only measured
311-13 on lp+ cells) or a protection effect between 6– and 7–.

②. pick and streak to obtain HFT 6–

③ 13 neg tested /2175 on stol, 2 possible HFT, #10, #12

311-x ④ 17 " ~~tested~~ /2175 Bgal, 1 #x 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 [illegible]

A

Mood = 0

0.1 (1-100 dil)

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

~~[illegible]~~

2020 cross brushed with HFT drypals - Gal₆⁻ distinct from Gal₇⁻

/1	/2	/4	/7
++	solid ones	+++	++++

1/25/5

307-1 × 4⁻ to obtain order

① the 4⁻ (247R-1) lysate diluted 1-100 gave c. 5×10³ pap

② 24 papillae picked, streaked ③ (−) 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⁻

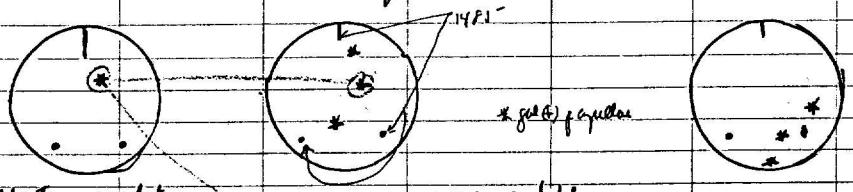
Contrast this with 1⁻4⁻ × 7⁻ where double crossovers are required in order ?

DATE: 1/26/54 REF:

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

1. Cultures of 1210 750
2. Overnight culture of W1210 replicated to 3 prewarmed B gal plates and incubated c. 45 minutes. At this time the three plates were irrad. 20" with U.V (50 cm). Reincubated count 30 minutes and then replicated to plate (previously spread c W750, 30 min) and the plates incubated 3 days.
3. Confirming of the replicas aided by marking original 1210 plate with 3 streaks of W1485 (gal+) thus.
4. Results:

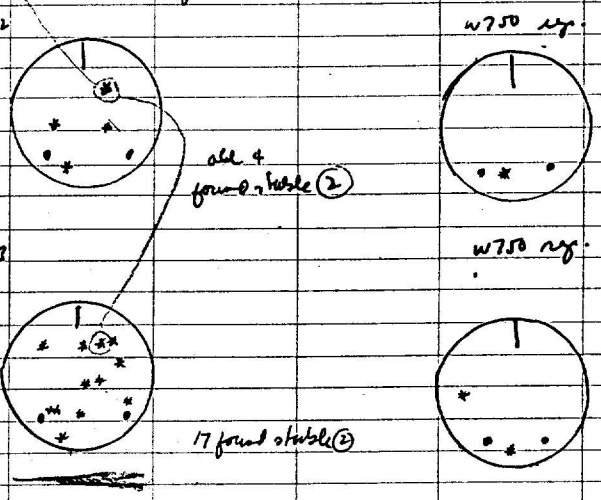
W1210-irradiated plate (Bottom view) W750 replica of 1210-1 plate



Master - 1210 plate

all 3 found stable upon (2)

all 3 found stable after (2) - on a wealth



Where are the (+) clones from?

315

DATE: 1/31/55 REF:

1	2	3	4	5	6	7	8	9	10

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

What are segregants? The result says yes

A

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

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

3 isolated (+)

29 2⁻
1 1⁻7⁻

1210 = gal8⁻ gal2⁻ not gal2⁻

W1210 = gal8⁻

315-12

W2861

750 C1C12-1 λ Prey 1/28/55
Lawd/750 = 2.6 × 10⁷
0.2 ml in Pen = Sterile

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

Dose	spont. = 3 pg/227 (dil 10)	spont. = 2 pg/759 (dil 10)	spont = 0 Plaques 2279 ↓ 10² dil	spont = 0 pg 2279 10² dil	Exam of Papillae stable/total	Seg	Lambda	Stable Reactions
0	28	67	155	1	1/1	— mottled (–) 1 lp^R		
15	54	140	151	15	15/15	s, rr	2 lp^+, 7 lp^s, 3 lp^R	
30	78	184	172	22	17/18	R	— , 14 lp^s, 2 lp^R	
45	108	293	66	25	18/18	—	— , 17 lp^s, 1 lp^R	
60	176	434	28	22	17/18	R	, 16 lp^s, 1 lp^R	
75	230	638	39	19	17/18	R	1 lp^+, 16 lp^s, —	
90	221	711	14	24	17/18	s ? stable (+)	— , 17 lp^s, —	
105	237	536	17	37	18/18			
120	252	958	12	10² 31	17/18	discarded		
135	184	798	10¹ = 45	10¹ 201	18/18			
150	163	699	und : 305	und 247 (¼)	18/18			
165	163	717	und : 231	ca. 4	18/18			
180	148	589	und = 141	ca. 11	17/18			
~~195~~								

0 dose

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

$1.55 \times 10^5 \times 10^{?} = 1.55 \times 10^8$/ml

1.6×10^8 plaques/ml

6.7×10^6 transd/ml

1-10 dil /ml = 15500 plaques
9000 transd

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

probably 2279

Multiplicity Aspect.

(1) 2497 + 0.1 ml 10^2 0 dose plated. To ½ of plate 0.1 ml 750-2 λ added (Per Plate) see above
plaques on control (no 758-2) = 37
papillae on 750-2 half = = 175
papillae on control = 2
2.4 > than the highest value for plaques or transd. given above in 0, 15, 30 (155, 151, 172)

(2) 0 above 10^2 dil
(1) 0.5 ml + 0.5 ml cells (c. 10^8) adsorbed 10'
(2) add 0.5 ml 750-2 (= ⅔ dil of original), shake 5'
(3) plate 0.1 ml. No. papillae - 188 × 3/2 = 282

DATE: 2/5/55 REF:

1	2	3	4	5	6	7	8	9	10

298-8 see that page and 304

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

2279 against HFT 6, 7 to obtain recip. test of prot. effect.
0.1 ml c. 10⁸ cells + 0.5 ml lysate diluted 1-100 ?/ml plated
1. 2279 control 3 plates c. 600 colonies/plate are (–)

2. 2279 × 6⁻

Failed no transd. observed. Much lysis. Killing was very high here

W2862 also type derivative

Lost ?

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

✓ 317C-6

317C-4

8 additional segregants from the same transduction

2175 against HFT 6⁻, HFT 7⁻ to make double (– –) for position gene order test

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

6⁻

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

7⁻

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

DATE: 2/8/55 REF:

1	2	3	4	5	6	7	8	9	10

Crosses to see distribution of (−)

~~1. 578 Gal− F+ × 902 Gal F− a) [illegible] b) cross plates~~

750E902-1 × 2274

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

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

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

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

~~2. 902 F+ Gal− × [illegible]~~

279-3 × 1321

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

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

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			
small gal (+)	11	s	R	non lys