DATE: 5/11/55 REF:

HFT 7⁻ — × 6⁻ 342 D — Of 04 P.E. (−) picked for (+), 4 were true (−), 20 P.E. (−)

Procedure as in 343

	(+) Nt. seg.?	no. (−)	/6	Segregant /7	/4		
P.E.(−) #1	0	−	+	+	+	−	−
2	+	7	0 ✓	+	+		6⁻
3	+	10	~~0~~	~~+~~	~~+~~		~~lost~~
4	+	3	0 ✓	+	+		6-7⁻
5	0	−				~~P.E.?~~	−
6	+	12	0 ✓	0 ✓	+	~~P.E.~~	6-7⁻ ← 344-6
7	+	7	0	+	+	~~P.E.?~~	6⁻
8	+	4				P.E.(−)?	
9	0	−					−
10	+	4				P.E.(−)?	
11	0	−					−
12	+	9	+	+	+	P.E.(−)?	
13	0	−					−
14	0	−					−
15	0	−					−
16	+	4	0	+	+		6⁻
17	+	5	0	+	+		6⁻
18	+	730	0	+	+		6⁻
19	+	750	———	———	———		
20	+	10				P.E.(−)?	

13 seg. — one lost
7 non seg.

5 6⁻
2 6-7⁻
4 P.E.(−)?

344B = ... (+)

(A) 344-6 above, the 6-7⁻, × K12λ 7/27/54
No. add. = 0
0.1 ml λ = 44 see p. 325 for previous use of this lysate
many small pap.

Seg.	/6⁻	/7⁻	/4	Str. type
1	0	0	+	6-7⁻
2	0	0	+	"
3	0	0	+	"
4	0	0	+	"
5	0	0	+	"
6	0	0	+	"
7	0	0	+	"
8	0	0	+	"
9	0	0	+	"
10	0	0	+	"
11	0	0	+	"
12	0	0	+	"
13	0	0	+	"
14	0	0	+	"
15	0	0	+	"

(B) 344-6 × 2580
no. add. = 10
0.1 ml = 215

Segregants	/2	/6	/7	Gen.
1	0	+	+	2⁻
2	0	+	+	2⁻
3	0	+	+	2⁻
4	0	+	+	2⁻
5	0	+	+	2⁻
6	0	+	+	2⁻
7	0	+	+	2⁻
8	0	+	+	2⁻
9	0	+	+	2⁻
10	0	+	+	2⁻
11	0	+	+	2⁻
12	0	+	+	2⁻
344-13 → 13	+	0	0	6-7⁻
14	0	+	+	2⁻
15	0	+	+	2⁻
16	Nat+	0	0	6-7⁻?
17	0	+	+	2⁻
18	0 0	0 +?	0 0	2-7⁻?
19	0	+	+	2⁻
20	0	+	+	2⁻
21	0	+	+	2⁻

redo 9/18
344-18, 24

18 2⁻
2 6-7⁻
1 2-7⁻

DATE: 5/21/55 REF:

1	2		3	4	5	6	7	8	9	10
			342C1							
HFT 4⁻ → x 6⁻			24 P.E. (-)	picked	7 were	true (-)	17 P.E. (-)	to m	344, 343	
	(+obt. [illegible])	no. (-)	/4	/6	/+					
P.E.(-) #1.	+ yes	13	+	o	+					
2.	+	5	+	o	+					
3.	+	6	+	o	+					
4.	+	12	+	o	+					
~~5.~~	~~+~~	~~10~~								
5 ~~6~~.	o	–								
6 ~~7~~.	+	3	+	o	+					
7 ~~8~~.	+	24	+	o	+					
8 ~~9~~.	+	13	+	o	+					
9 ~~10~~.	+	11	+	o	+					
10 ~~11~~.	o	–								
11 ~~12~~.	+	14	+	o	+					
12 ~~13~~.	+	28	+	o	+					
13 ~~14~~.	+	11	+	o	+					
14 ~~15~~.	+	6	+	o	+					
15 ~~16~~.	+	9	+	o	+					
16 ~~17~~.	+	6	+	o	+					
17.	o	–								
	14 seg				14 6⁻					
	3 nonseg.									

DATE: 5/11/55 REF:

	1	2		3	4	5	6	7	8	9	10
				342E1							
	HFT 6⁻ — × 4⁻			24 P.E.(-) picked, 1 weak(-), 23 P.E.(-). On ice 3 Feb, 4 Feb, 4 Feb.							
		+ obl. seg?	mal + str	4	6	4+					
	P.E.(-) #1	0 = ww	-						Rpt. Expt. using culture		
	a	0	-						next to Expt		
	3	0	-								
	4	0	-								
	5	+ = yes	9	-	-	-	P.E.(-) Yp				
	6	0	-								
10	7	0	-							Theo is Yp C/S	
	8	0	-								
	9	0	-							Use Yp+	
	10	+	3	0	+	+	Yp³	Gal⁻			
	11	0	-								
	12	0	-								
	13	+	1	+	0	+	Yp³	Gal₆⁻	W2589		
	14	+	1	+	0	+	Yp¹	Gal₆⁻			
	15	0	-								
	16	0	-								
20	17	0	-								
	18	+	1	0	+	+	Yp³	Gal₄⁻			
	19	+	2	0	+	+	Yp^R	Gal₄⁻	<- 346-19		
	20	0	-							24p³	
	21	0	-						3 Idw	1 Yp^W	
	22	0	-								
	23	0	-						2 Gelv		
		6 neg		Strk II	Strk III						SEE 347
		17 non-neg		5/26/55							
	342E2	+ obl. seg	mal + str	Picked to Yp Pen - Cross tested and							
30	Yp^W 1	0	-	seg. Yp+ 4 6 4+			~Gal₆⁻	W3163		5/27/55	
	2	+	1	+ nd	inviable		Gal₆⁻	346.4 R ✓		Lysate of 346-4 — × 2180	
	3	+	7	- 0 nd	+	+	Gal₄⁻ I	5/16/56 found 4-6⁻7		W3088 - 14	
	4	+	1	- 0 nd	0	+	Gal₄⁻Gal₆⁻ II	<- 346-4		0.1ml = 71	
	5	0	-	+ nd	- - +		Gal₄⁻Gal₆⁻			Seg. /4⁻ /6⁻ /+ Gal	
	6	0	-	+ nd	- + +		Gal₄⁻			1 0 0 + Yp⁻	
	7	0	-	— —						2 0 0 + -	
	8	+	8	— 0 nd	+	+	Gal₄⁻ I	Wt results		3 0 0 + -	
	9	0	-	+ nd	- + +		Gal₄⁻	346 4		4 0 0 + -	
	10	+	1	— + nd	0	+	Gal₆⁻ I	+		5 0 0 + -	
40	11	+	1	+ 0 nd	+	+	Gal₄⁻ I	All apparently		6 0 0 + "	
	12	0	-	+ nd	+ - +		Gal₆⁻	stable + were		7-16 0 0 + "	
	13	0	-	+ nd	+ - +		Gal₆⁻	picked and		16 are Yp⁻	
	14	0	-	— —				restreaked on W2589 (Gal⁻ Mal+ S^R)			
	15	0	-	— —				on B gal streptomycin,	W2589 formed		
	16	0	-	— —				read 10 sec, mucus.	Gal⁻ Mal⁻, λ-2 Resist		
	17	0	-	— —				2 days. No. papillae at			
	18	0	-	+ nd	- - +		Gal₄⁻Gal₆⁻	spots observed —	WHITE MATURE SU		
	19	0	-	— +	+ - +		Gal₆⁻	indicating HFT			
	20	0	-	+ nd	+ - +		Gal₆⁻				
50	21	0	-	— —				5/19/55. After streaking III, remaining			
	22	0	-	— —				7 col (from brothes) were spotted on			
	23	0	-	— +	+ - +		Gal₆⁻	W2175 on S Gal + med. 10 sec. After			
								2 days all other papillae suggesting HFT			
		17 non-neg		13 16			8 Gal₆⁻				
		6 neg		Total tested			5 Gal₄⁻				
							? Gal₄⁻Gal₆⁻				

DATE: 5/25/55 REF:

1. Continuation of 346.

A. 346-4 X— K12 7/27/54 $\frac{++}{--}$ = + ?

no add = 0

0.1 mc = 46 cf. 344-6

B. results on P. 346 under 346-4 → x 2580

C. 346-4 → x 2580

1. 1st 24 segregants were 2-
2. Rpt. streaking of above yielded 16(-) = 15 gal_2^-, 1 gal_6^-
3. Rpt. " " " " 15(-) = 13 gal_2^-, 1 gal_6^-

} is this lysate of a Gal_6^- culture?

See 418 4 Rpt experiment using same lysate and applying to 2580 20- 19 Gal_2^-, 1 Gal_6^-

Retest 346-4

See 346 for retest

Note, this 6- required 2+ days to be classified, have the tests of 346-4 been allowed to go long enough?

DATE: 6/6/55 REF:

1	2	3	4	5	6	7	8	9	10
285-1	Rpt lkan of 4" x 1"								
	Cells removed from stab, streaked to obtain P.E. (-), restriked to give 24 P.E. (-), from these 24 Gal+ obtained								
Gal+	Seg.? (-)/Obt	/1	/4						
1	0								
2	0								
3	0								
4	+ 2	0	+	1"					
5	+ 5	0	+	1"					
6	0								
7	+ 8	0	+	1"					
8	0								
9	0								
10	0								
11	+ 6	0	+	1"					
12	+ 10	0	+	1"					
13	+ 5	0	+	1"					
14	+ 3	0	+	1"					
15	0								
16	+ 1	0	+	1"					
17	+ 5	dark	dark	?	P.E. (-) and Gal+				
18	+ 5	0	+	1"					
19	+ 4	0	+	1"					
20	0	0	+	1"					
21	+ 2								
22	0								
23	0								
24	0								
	+ = yes			11 1"					
	0 = no.								
	12 stable								
	12 seg.								

DATE: 6/13/55 REF:

1. Do mixtures of the Gal - ferment galactose?
NSB + 1.0% Gal + fermentation vials

			Reaction after 2 days
1.	~~[illegible]~~ W750	Gal_1^-	small bubble
2.	W811	Gal_4^-	no bubble
3.	W2580	Gal_2^-	small bubble
4.	W750+W2580	1+2-	small bubble
5.	W750+W811	1+4-	small bubble
6.	W811+W2580	4+2-	no bubble

Apparently no fermentation in mixtures by two's

2. Tested by Tro
293-12J - Gal_4^- HFT. Found equivalent or better than 247B-1

DATE: 6/20/55 REF:

1. Testing for segregation of bp in isolated heterogenotes.
Also effect of temperature shift on frequency of transduction.

A. Overnight cultures 2279, 2790, 518 at 37C in incubator.
1. Cultures divided and portion placed at rm temp for ca. half hour.
RM temp cells, 37C cells.

B. 1. Lysates
Gal_2 - λ for 2279 518 = 2342 3/20/54
Gal_1 - λ for 2790 = 2346λ
2. Lysates 0.4 ml ~~plated~~ in small tubes, caps off incubated at 37C for half hour.

C. Technique.

0.4 ml lysate + 0.1 ml cells ← overnight cult {0.1+10 Pen 2790 2279; 0.5+10 Pen 518}
↓
incubated 37C 10 min
↓
diluted
0.1 +10,
1.0 +10
↓
0.1 ml plated
Expt'l counts

↓
Control counts.
↓
0.1 +10,
0.1 +10,
↓
0.1 ml plated

37C Culture	Plate 1	fraction (+) 2	3		Culture	Control. RM temp	Counts.	37C	
W518	1/443	3/460	0/428	← those col. also plaqued.	W518 =	112, 103	(1.1×10^7)	119, 130	(1.3×10^7)
W2790	1/460	1/372	1/422	← also contam. with plaques	W2790	35, 42	(3.9×10^6)	173, 184	(1.8×10^7)
W2279	8/173	1/166	5/142	← Just about every colony has plaques - multiplicity too high.	W2279	180, 144.	(1.6×10^7)	338, 290	(3.1×10^7)

No gal + on these dates

RM temp. Cult.	Plate 1	fraction (+) 2	3	
W518	2/ca. 50	3	4	← these plates have contaminant - no count of total mode.
W2790	2/263	1/292	0/262	
W2279	1/55	3/56	0/50	← these colonies all are plaqued. mult. too high

The multiplicities in this experiment were too high to be used for the purpose of testing bp seg

DATE: 6/22/55 REF:

1 Observations on segregation rate ..

A. Culture = 348 edi type
B. Obtained wet and antigen pour cul. inoculated into Bou - Gaw overnight
C. Diluted $10^2 - 10^4 - 10^6 - 10^8$

Series 60 min log. Air every 30 min, from 9:30AM → 12:30 11:30
2h - leave until 1:00PM
2h = (256X)

5 0.1 ml samples plated B gal

1.	100
2.	108
3.	134
4.	136
5.	108
	586 = 117
	5

Therefore Ave. → 1.2 cells/0.1 ml = 3.6 cells/tube
1.0 ml
0.6 cell

10. 0.3 ml samples incubated 37C 8:30AM → 1:00PM
Plated 0.05 ml ea

1.	361
2.	61
3.	219
4.	122
5.	855
6.	1279
7.	441
8.	676
9.	319
10.	336

6/24/55

2. Repeat experiment. using 750tKR-1
A. Overnight culture from stock.
B. diluted $10^2 - 10^4 - 10^6 - 10^8$

4 0.1 ml samples incubated in horizontal 0.2 ml pipette at 37C. 9:15AM
20 in number
Plated 1:45PM
Read next morning

Assay 0.05 ml

1	98,	(2)
2.	112,	(5-)
3.	106,	(4-)
ave	106	(4-)

212 (B-) / 100 = 2 cells/0.1 inoculum, 0.08 Geo-

(1)

Samples incubated in pipettes	(-)	tube
1	*	*
2	6	1169
3	3	595
4	4	251
5	0	237
6	*	*
7	23	1252
8	* 103	* 2730
9	319	1622
10		
11		
12		
13		
14	15	1/4 = 141
15	9	1113
16	all (-)	
17	22	1966
18	15 (18)	1/4 = 479
19	est. 100	est. 2000-3000
20	19	897

*. These platings have ca. 3000 cells the no. (-) appears to vary from ca 10-200

(2) At the same time 5 0.1 ml ~~samples~~ plated on dry form on Bgal, respread after plateings ca. 2:15PM by adding a few bents + spreading

	(-)	(+)	
1.	0	89	Seg. colonies
2.	0	52	Seg. colonies
3.	7	1295	
4.	22	1222	
5.	52	730	

Calculations on next page - also notes -

$a = 0.602\,(\text{no. mutants})/N \log N)$

DATE: REF:

1	2	3	4	5	6	7	8	9	10
A. Incubation in pipette									
No. (–)	Total cells	N log N		0.602 R		5/3	Recalculated		
6	1169	1169 (3.06819) = 3580		3.612		1.0×10^{-3}	1.0×10^{-3}		
3	595	595 (2.77452) = 1650		1.806		1.1×10^{-3}	1.1×10^{-3}		
4	251	251 (2.39967) = 600		2.408		4.0×10^{-3}	4.0×10^{-3}		
23	1252	1252 (3.09760) = 3880		13.846		3.6×10^{-3}	3.6×10^{-3}		
9	1113	1113 (3.05308) = ~~3400~~ 3391		~~3.718~~ 5.418		~~1.1×10^{-3}~~	✓ 1.6×10^{-3}		
19	897	897 (2.95279) = 2650		11.438		4.5×10^{-3}	4.3×10^{-3}		
0	237	—	—	—		—			
10B. Resparading									
7	1295	1295 (3.11227) = 4030		4.214		1.0×10^{-3}			
22	1222	1222 (3.08707) = 3780		13.244		3.5×10^{-3}			
52	730	730 (2.86332) = 2085		31.304		1.5×10^{-2}			
0	89								
0	52								
103	2750	2750 (3.439) = 9430		62.00		6.6×10^{-3}	6.6×10^{-3}		
319	1622	1622 (3.21005) = 5200		~~192~~ 192.		3.7×10^{-3}	✓ 3.6×10^{-2}		
22	1966	1966 (3.29358) = 6460		13.2		2.0×10^{-3}	2.3×10^{-3}		

$\frac{32.0}{9} = 2.5 \times 10^{-3}$

Estimated P_0

using 200 $a = \frac{2.3}{200} \log \frac{1}{\frac{1}{12}} = (0.012)(\log 12) = (0.012)(1.08) = 1.3 \times 10^{-2}$

using 1024 $a = \frac{2.3}{1024} \log \frac{1}{\frac{1}{10}} = \frac{2.3}{1024} \log 10 = \frac{2.3}{1024}(1) = 2.2 \times 10^{-3}$

DATE: 6/22/55 REF:

Yield of HFT λ from heterogenote

1. Culture 241-14 (~~[illegible]~~) - streaked from stock and 10 colonies picked, tested for HFT against 2279 - Colonies stored Pen assay frig

1 0 0 0 0 0
0 0 0 0 0

2. Reaction HFT not apparent 24 hours - select most likely appearing col and proceed. Bottles incubated 34 hours - Estimated titer 10~~8~~9

3. Exptl.
a. dilute 1.0 ml pen to obtain ca. 1000 col/ml in Saline. Inad 35 seconds and plate 6 gal with 750 col, - Make pre mad. count
b. Inc. 2 hours - respread 3 plates

4. Results:

Pre mad count/0.1 ml = 67, 50, 46 } ave: 53

Post mad plaques/2279

				Pap.	PAP in plaques	352A 6 hrs
0.1 ml:		22	ave 16	5	3	(1) 3/5 +"
		12		8	4	(2) 5/8 +"
		14		5	2	(3) 2/5 +"

				352B	stk 1
Post. mad. pap /750	(1)	2	ave 3	1	0/2 +"
NOT SPREAD	(2)	5		2	1/5 +"
	(3)	2		3	1/2 +"

				352C	stk 1
	(1)	~~4~~ 3	ave 2	(1)	3/3 stk 1
SPREAD	(2)	2		(2)	1/2 stk 1 <-
after 2 hours	(3)	3		(3)	2/3 stk 1, 1(-)

DATE: 6/22/55 REF:

For multiplicity duction; - does λ segregate?

1. Culture - 2279 overnight
   λ - 24/-14 (10/12/54) titer 5.3×10⁸ plaques / 1.3×10⁸ round } see p 300

② 0.4 ml 2279 + 0.1 ml HKT 2⁻
   " " 0.1 ml buret

③ precipitate 37C 10 min.

④ dilute $10^3, 10^4, 10^5$ → plate 0.05 ml
   λ - 6 plates
   ⑤ control 3 plates — 53, 47, 49 ave Ctl - $50 \times 20 \times 10^6 = 1.0 \times 10^9$

⑥ Expt plates

(+)	Total
1	66
0	54
0	40
0	41
0	54
0	56
(1)	311

tot (-) from 5 cultures with λ as prod. lysed.

titered aqueous 2279 Cacl2 stored not by segregants

353-1

Expt.

1. Culture = 2344 JL's δ stock Cal2 - by S Hfr taken from his stock culture centrifuged δ
2. Lysate = 750 KH-1 1/28/55 titer = transd. titer - 2.6×10^7 see 315
3. Expt 0.1 ml lysate + 0.1 ml cells inc. 37C 15' - 10 ml Pen added; diluted $10^3, 10^4 \rightarrow 0.1$ ml

Results -

A. control plates - exposure to broth

Plate		
1	237	$282 \times 10 \times 10^4 \times 10^{-2} = 14 \times 10^8$ cells/ml overnight cult.
2	326	
3	283	

B. Lysate exposed

	+ *	(-)	(-) lysed (particle)	Total
1.	3	92	0	95
2.	2	84	4	90
3.	0	76	1	77
4.	3	71	4	78
5.	1	61	0	62
6.	0	94	1	95
7.	0	96	2	98
	9		12	595

750 KH-1 5.6×10^7 from Pasteur titer estimated

* the + appear to be in two categories: (1) appearing ca. 18 hours and represented by intact (not lysed) colonies that are almost all (+) (2) appearing in (-) colonies, λ contaminated and partially lysed, after ca. 24 hours. In the above there were 5 were early, 3 late.

This correlation was noted.

	Lysis sensitive	Fraction Cont.
Earlier early	2/5	predom. (+)
Later late	3/3	" (-)

} are transd. not predict - titer = 0

C. Plates - primary strikes of (+) given to EML.
   # 1-5 early - 2/5 ↑ as given
   6-8 late - 3/3 ↑

EML 900 gave titers A-H

DATE: 6/30/55 REF:

Crossover rate in P.E. heterogenote
342E2 6⁻ — x 4⁻
Expt'l as in 357 with 750CKR-1

1. Overnight culture from single colony
2. Dilute $10^2 - 10^4 - 10^6 - 10^8$ → 20 0.1ml samples

3 plates for estimate of ratio cells/0.1ml

	(+)	total	
1.	4	67	(8 bal-)
2.	4	78	(7 bal-)
3.	6	78	(8 bal-)
		Ave 71	= 0.71/0.1ml

Using null method and N = 5900 (from culture 14)

$a = \frac{2.3}{5900} \log \frac{1}{\frac{12}{18}}$

$a = (0.00039)(0.176) = 7.0 \times 10^{-5}$

$a = \frac{2.3}{2.9 \times 10^3} \; 0.176 = \frac{0.405}{2.9 \times 10^3} = \frac{4.05 \times 10^{-4}}{2.9} = 1.4 \times 10^{-4}$

incubated 9:30 → 2:30

These all 20-30% low because log values used

	(+)	total	Max Prob
1.	0	0	—
2.	0	1406	1.3×10^{-4}
3	0	ca. 3000	6.0×10^{-5}
4	0	1/8 = 193 1544	—
5	0	1/16 = 193 3088	6.0×10^{-5}
6	0	contaminated	—
7	ca 1000	ca 3000	—
8	0	ca. 150	—
9	4	1/8 = 643 5144	ca. #3 13×10^{-4}
10	0	ca 4000	ca. #3 4.1×10^{-5}
11	2	1/8 = 841 6728	5.3×10^{-5}
12	5	2266	4.0×10^{-4}
13	0	0	—
14	1	1/16 = 370 5925	3.0×10^{-5}
15	0	ca. 7000	2.5×10^{-5}
16	ca 1000	ca 3000	—
17	0	0	—
18	0	2245	—
19	0	748	—
20	0	34	—
	total	29128/10 =	2913

B Reciprocal 342C1 to above - except stab picked to lithic pen and this grown overnight

10:30 AM
1:45 PM

	(+)	total
Control 1.	0	577
2.	2	595
3	0	556
		583 = 5.8/0.1ml

Using null method, and N = 1200 (A19)

$a = \frac{2.3}{1200} \log \frac{1}{\frac{17}{20}} = (1.9 \times 10^{-3})(0.13)$

$= 2.6 \times 10^{-4}$

$a = \frac{2.3}{779} \log \frac{1}{17/20} = \frac{2.3}{7.79 \times 10^2} \log 1.2$

$= 2.4 \times 10^{-4}$

	(+)	total	cash	
1.	2	728	276 (1004)	4.0×10^{-4}
2.	0	818	62 (880)	
3	0	1/16 = 110	660	
4.	0	1/8 = 86	688	
5.	0	1/8 = 80	640	
6	0	1/8 = 49	392	
7.	0	1/8 = 80	640	
8	0	1/8 = 91	728	
9.	0	1/8 = 83	644	
10	0	1/8 = 149	1192	
11.	0	1/8 = 62	496	
12	0	1/8 = 83	664	
13	0	1/8 = 137	1096	
14	0	1/8 = 74	592	
15	0	1/8 = 90	720	
16.	0	1/8 = 152	1216	
17.	1	185	185	
18	0	1/8 = 87	696	
19.	1	1249	1.4×10^{-4}	
20.	0	1/8 = 62	496	
		20	15588	$\frac{1559}{2}$ = 779

DATE: 6/30/55 REF:

1924 x — 2346 λ to look at single heterogenates

1 0.1 ml overnight culture + 10 ml

0.1 ml + 0.1 ml 2346 λ 1/11/54

↓

incubated 37C - for 15'

↓

add 10 ml Pen → dil 10^2, 10^4 → 0.1 ml samples

2. Broth - control

2346 λ 1/12/55 - Expll.

DATE: 7/6/55 REF:

Segregation from W2730

1. Expt as before - see page 357

2. Overnight cult

$10^2 - 10^4 - 10^6 - 10^8 \rightarrow$ 20. 0.1 ml sample.

0.1 ml	(−)	total
1.	17	122
2.	19	168
3.	21	151
	19	$\frac{441}{3} = 147$

= 1.5 cells/0.1 ml

28/19

Using null fraction

$$a = \frac{2.3}{100}\left(\log \frac{1}{p_0}\right) = \frac{2.3}{100}\left(\log \frac{1}{0.5}\right) = 0.012$$

$= 1.2 \times 10^{-2}$

Estimated clone size ⇒ 40, 80, 200, 150, 40, 30, 150, 100, 30

ave est. 100

	(−)	total	
1.	11	323	8.1×10^{-3}
2.	2	176	3.0×10^{-3}
3.	0	0	8.9 —
4.	8	1669	$\cancel{7.6} \times 10^{-4}$
5.	3	317	2.2×10^{-3}
6.	52	1236	8.1×10^{-3}
7.	0	10	—
8.	0	0	—
9.	ca. 300	ca. 700	7 —
10.	36	1055	6.6×10^{-3}
11.	0	0	—
12.	0	0	—
13.	3	299	2.4×10^{-3}
14.	0	0	—
15.	0	0	—
16.	6	386	3.6×10^{-3}
17.	0	0	—
18.	0	0	—
19.	55	1965	5.1×10^{-3}
20.	0	0	

$\frac{46.2}{9} = 5.1$

8 failed to grow

1 contained only Gal+

10 contained both Gal+ & Gal−

DATE: 7/13/55 REF:

1. Rpt 2344M1 (SL stock) from ← HFT ↓
   Lysate = 293-125 –

2. 0.3 ml lysate + 0.3 ml overnight cult.
   ↓
   add 10 ml broth
   ↓
   $1-10^2 \cdot 10^4$
   ↓
   0.1 ml broth as control.

Plates bad
fro wet.

3. Result. A. Control. $6/10 \cdot 10^2 \cdot 10^2 \cdot 10 = 6 \times 10^7$
   1. 457
   2. 540 5.0×10^9 ? Inv (+)
   3. wet plate

B. Eight

	(+)	total (−)	contam. (−)	Descrip.
1.	2	322	2	●2 ●1
	1	ca 300	3	●3

Rest of plates (5) have run in center counts-
about the same as above - after 2 days
some (+) appeared - may be reversions - This
culture seems to revert more than other Gal₂ -

DATE: 8/10/55 REF:

Penassay broth test tubes – Blank = water – PC-4 filter

λ	OD	Aerated Cult ca. 10⁹/ml)
650	0.051	0.74
600	0.071	0.81
550	0.11	0.90
500	0.19	1.04
450	0.365	1.23
400	0.630	1.45

Lysates	1210	K12	2236
10:50 AM 0	0.58	0.73	0.80
1:10 PM 2:20	0.75	0.52	0.80
1:25 PM 2:35	0.69	0.44	0.64
1:45 PM 2:55	0.67	0.40	0.53
	0.67	0.40	0.48

$\frac{0.2}{20.0} \times 5$ 0.05% RNA

9/25/55.

750 tK12-1 grown on B gal + 0.05% RNA

1. no RNA 5 Gal – + ca 10 sec + ca 60 Gal +
2. RNA 6 Gal – + ca " + ca 70 Gal +

} No effect of RNA at this conc

1210 λ – x750 in search of HFT 8

no add 2
0.1 ml 18 poor lysate

HFT test	No. tests	against?	HFT?
1	10	750, 2341	possibly one/2341 – lysate made 358-1
2.	10	750, 1210	possibly one/750 358-2

] none HFT – reversion of both made – both stable

2279 + 750 ← K12 8/10/55

1. 750 0.1 ml = 142 x 4 = 568
2. 2279 0.1 ml = 426

Rather low titer in Lp³ – This lysate has been at room temp since it was made

no Control

DATE: 8/17/55 REF:

UV 309-1 = (HFT ?)

Assay of irradiated HFT lysate - Irradiated since time ago - Preliminary assay.

(359A) ← ← ordered at later

750 spontaneous + = 5 frequency ← 1.6×10⁷ purified 2790

UV Dose	1-100 dil (0.1 ml?) /750		0.05 ml /2307	0.05 ml 10⁴ 2580	0.05 ml 10⁴ 2790	plaques	0 dil/2790
0 min	21	0	13	257	37	ca. 500+	196
1 "	273	252	14	729	190	ca 500+	297
2 "	517	496	6		227	584	994 ca 1000
3 "	593	572	6		341	436	
4 "	885	864	10		413 (1st plate)	234	
5 "	950	929	11		175 (2nd plate)	16	
6 "	809	888	11		148	7	

spont = 8 sp = 22 sp 14 sp. 17

← at max 413×20×10⁴ = 8.2×10

← RPT = 359/2

1-100 dil for conc. frequency on 2790 - dil at least 10² more

these with prevaccinated plates

See 359A →

359A test — λ^{lysat} rev against 2279 →

ss seus r s rand

#	Spont (+) λ	/2790	0 λ	/2790	1 λ	/2790	2 λ	/2790	3 λ	/2279	4 λ	/2279	5 λ	/2279	6 λ	/2279
1.	s ns	0	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
2.	s	0	r s	0	r ? ns	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
3	s	0	r s	0	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
4	s	0	s ns	0	r ns	r+	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
5	s	0	s ns	0	r s	r+	s ns	0	s ns	0	r ns	0	s ns	0	s ns	0
6	s	0	r s	0	r s	0	s ns	0	s ns	0	r ns	+	s ns	0	s ns	0
7	s	0	r s	0	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
8	s	0	r s	r+	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
9	s	0	s ns	0	r s	0	s ns	0	r ns	0	s ns	0	s ns	0	s ns	0
10	s	0	r s	0	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
11	s	0	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
12	s	0	s ns	0	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
13			s ns	0	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
14			s ns	0	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
15			r s	0	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
16			s ns	0	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
17			r s	r+	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
18			r s	r+	r s	0	s ns	0	s ns	0	r s	0	s ns	0	s ns	0
19			s ns	0	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
20			r s	0	r s	0	s ns	0	s ns	0	s ns	0	s ns	0	s ns	0
21			r s	0	r s	0	s ns	0	s ns	0	r s	0	s ns	0	s ns	0
22			r s?	0	r s	0	s ns	0	r s	0	s ns	0	s ns	0	s ns	0
23			s ns	0	s ns	0	r s	0	s ns	0	s ns	0	s ns	0	s ns	0
24			r s	0	r s	0	—		s ns	0	s ns	0	s ns	0	s ns	0

λ/2280 + = lysogenic, 0 = nonlysogenic

ns = not segregating

↑ The surviving growth from 16 of these streaks, streaked on B gal - found pure Gal+ - adding λ does not restore stability

Spont	0	1	2
12/12 lp^s	9 lp^s	3 lp^s	22 lp^s
	3 lp^+	2 lp^+	1 lp^r
	12 lp^r	19 lp^r	

Spont	0	1	2	3	4	5	6
	-5.6 lp^s	-1.7 lp^s	-1.44 lp^s				
12 ns	9 lp^s ns	3 lp^s ns	22 lp^s ns	23 ns - 22 lp^s - 1 lp^r	22 ns - 20 lp^s - 1 lp^r	24 ns	24 ns
	3 lp^+ s	1 lp^+ ns	1 lp^r s	1 s lp^r	2 s lp^r		
	12 lp^r s	1 lp^r ns					
		1 lp^+ s					
		18 lp^r s					

359A'

DATE: 9/28/55 REF:

2915 used for 4

Rpt. of 359- 2nd dose 2750 48 papillae picked sheared out purple col tubes λ 1227Å sheared out on B agar for stability. This dose taken because the probability from nearly playmates - Early doses require more dilution & aeration

Reversion 10/3/55
0.1 ml 10⁴ dil / 27N⁻
C = 25 ca. Spl : 1t
359A0 0 = 355 ↓ co
359A1 1 = 615 ca 2 Pl : 1t
2 = 678 ca 1 Pl : 1t

Strong if Mem for post. sty, Lp^ax ots

Rev'd sensitivity		1	2	3	4	5	6	7	8	9	10
				359A0	359A1						
		359A2' Dose 2' 2750	Gen. rep. λ	Plate							
ws	1	s w o		r s +	r s +						
s	2	r s o	Lp^l	r s +	r s +						
ws	3	s w o		r s o	r s +						
ws	4	s w o		r w +	r s o						
ws	5	s ws o		r s o	r s o						
s	10	r s o	Lp^l	r s o	r s o						
w	7	s ws +		r s o	r s o						
s	8	r s o	Lp^r	r s o	r s o						
ws	9	s w o blgy		r s +	r w o						
ws	10	s ws o		r s o	r s +						
ws	11	s ws o		r s o	r s o						
w	12	s ws o		r s o	r s o						
w	13	s ws o		r s o	r s o						
ws	14	s ws +		r s +	r s o						
w	15	s ws o		r s o	r s o						
—	216	no growth		r s o	r s o						
w	17	s w +		r s o	r s o						
w	18	s ws o		r s o	r s o						
w	19	s ws +		r s o	r s o						
w	20	s ws o		r s o	r s o						
s	21	r s o	Lp^l	r s o	r s o						
w	22	s ws o		r s +	r s o						
w	23	s ws +		r s o	r s o						
w	24	s ws o		r s o	r s o						
s	25	r s o	Lp^l	r s o	r s o						
w	26	s ws o		r s +	r s o						
w	27	s ws o		r s +	r s o						
w	28	s ws o		r s +	r s +						
s	29	r s o	Lp^l	r s +	r s +						
w	30	s ws +		r s o	r s o						
w	31	s ws +		r s o	r s o						
w	32	s ws +		r s +	r s o						
w	33	s ws o		r ws +	r s o						
w	34	s ws o		r s +	r s o						
s	35	r s o	Lp^s	r s +	— —						
w	36	s ws +		r s +	— —						
w	37	s ws +									
w	38	s ws o		8 Lp^t	2 Lp^t						
w	39	s ws o		28 Lp^r	32 Lp^r						
w	40	s ws o									
w	41	s ws o		2 ws Lp^t	33 s Lp^t+r						
ws	42	s ws +		34 s Lp^t+r	1 ws Lp^r						
w	43	r s o	Lp^l								
s	44	r s +	Lp^s								
w	45	s ws +									
w	46	s ws o									
w	47	s ws o									
total w	48	s ws o									

Lp^t = 0
Lp^r s = 9
Lp^s w = 38

10 Lp^r s
60 Lp^l ws

3913

DATE: 10/6/55 REF:

Continuation of 359 359A

Platings of W Lead 309-1 by sole on 2580 (4pt) - 0.1 ml 10^5 dilutions of irradiated by sole.

	1	2	3	4	5	6	7	8	9	10
	UV dose control	Pequea 31		Assay/2341 (same dil.)						
	0	75	Not. 44	35					#10 streaked out - 10 col. V Agard 2220 all similar. 359B(4) 7m Seq #10	
	1	183	142							
	2	388	347							
	3	576	545							
10	4	710	689	253						
	5	563	532							
	6	412	351							359B1
	S= Sm ws= non ser +=hyperimm 0=non hyp	0 Dose / 2279	4' Dose / 2279 Sec Bkm	1st Seq	2nd Seq	3rd Seq	4th	5th	on N wet wash 6th	7th	Cla Pl 2 S/16
	1	wc +	r s +	2-4pt	1-4pt	2-4pt	1-4pt	1-4pt	2-4pt	1-4pt	
	2	s +	r s + x	2-4pt	2-4pt	2-4pt	2-4pt	2-7-4pt	2-4pt	2-4pt	
	3	a s +	r s + x	2-4pt	2-4pt	wet Obst.	2-4pt	2-4pt	wet Obst.	2-4pt wk	
	4	i s +	r s + x	2-4pt	2-4pt	2-4pt *	2-4pt	2-4pt	2-4pt	2-4pt	
	5	r s +	r s + x	2-4pt *	2-4pt	2-4pt *	2-4pt	2-4pt	2-4pt	2-4pt	
	6	o ws +	r s + x	2-4pt	2-4pt	more	2-4pt	2-4pt	2-4pt	2-4pt	
20	m s +	r s + x	2-4pt	Contam.	.2-4pt	2-4pt	2-4pt	2-4pt	2-4pt		
	n. c +	r s + x	2-4pt	2-4pt	2-4pt	2-4pt	2-4pt	2-4pt	2-4pt	359B10	
	a s +	r s +		2-4pt	2-4pt	2-4pt	2-4pt	2-4pt	2-4pt	L in S/16	
	t ws +	r s + x	2-4pt *	2-4pt	2-4pt	2-4pt	2-4pt	2-4pt	2-4pt Rp		
	e ws +	r s + x		more	more	more	more	more	more		
	q s +	r ws +									
	s +	r s + x	7-4pt	2-4pt	2-4pt	wet test	2-4pt	2-4pt	2-4pt wk	1	
	ws +	r ws +		more	more	more	more	more	more		
	s +	r ws +		more	more	more	more	more	more		
	ws +	r s + x	2-4pt *	2-4pt	2-4pt	2-4pt	2-4pt	wet Ant	2-4pt wk		
	s +	r ws +		more	more	more	more	more	more		
30	s +	r s + x	7-4pt	2-4pt	2-4pt	2-4pt	2-4pt	2-4pt	2-4pt	1	
	ws +	r s + x	2-4pt *	2-4pt	2-4pt	2-4pt	2-4pt	2-4pt	2-4pt		
	s +	r s +		wet Obtained	2-4pt	2-4pt	2-4pt	2-4pt	2-4pt		
	s +	r ws +		more	more	more	more	more	more		
	s +	r s + x	2-4pt	2-4pt	2-4pt	2-4pt	2-4pt	2-4pt	2-4pt		
	s +	r s + x	2-4pt *	2-4pt	2-4pt	2-4pt	2-4pt	2-4pt	2-4pt	<-359B-22	
	s +	r s +		contam.	more	more	more	2-4pt	more		
	s +	r s + x	2-4pt *	2-4pt	2-4pt	2-4pt	2-4pt	2-4pt	2-4pt		
	6 ws 24+	5 ws 24+									
	18 s	19 s									
40											
			* Contam Plate Many Obtained Pt								
			14 ends	14 ends	16 ends	16 ends	16 ends	17 ends	17 ends	10	
			2 etc	1 etc		1 etc	1 etc		1 etc	6	
				1 amphi			1 amphi			2	
										55 17	
										~ 1	
50										1 2	

DATE: 8/18/55 REF:

1924 X — HFT 1-6 to study lysogenization + crossing over
a-4a X — 1-4+

1. Transd. plates not recorded - ca. 300 colonies/plate
   No gals on control, lysate agreed.
2. After 2 days on the lysate plate several repelleting colonies
3. Results:
   Repelleting colonies streaked out and from the primary streaks 8 (-) and 8(+) tested for lysogenicity against 2279

	8 gal+ys?	Gal+ex?	Ex-Lys?	dry ± plate?
Rep. Col. 1	no lys	no agg	no lys	—
- 2	no lys	no agg.	no lys	yes.
- 3	no lys	very sl. agg.	no lys	—
4	no lys	no agg	no lys	—
5.	no lys	no agg	no lys	—
6.	no lys	yes	no lys	yes } discarded
7	no lys	yes	no lys	yes }

Cont'd 1964 Cult. 360-7 discard

4. 360-2 - 24 Gal+ colonies from 24 diff. ± colonies tested 2279 for lysogenicity and found that lysogenic.

	Stbl?	2279	Ex-2279	HFT /1	HFT /4	Genotype	Uninduced spotted /2279	Gal+ Stbl?
1	Stbl	non lys						
2								
3								
4								
5								
6			Not done				Uninduced cultures (overnight) incubated without air bubbles ca. 10⁸	
7								
8								
9								
10								
11								
12								
13	unstbl	non lys		0	0	1-4-	ca. 1000 plaques	— ← 360-3
14				0	0	1-4-	"	— 360-4
15				0	+	1-	"	(1) 1 found stbl
16				0	+	1-	"	(1) 1 found stbl
17				0	+	1-	"	(2) 2 found stbl
18				+	0	4-	"	(8) 8 found stbl
19				+	0	4-	"	(8) 7 found stbl
20				0	0	1 4-	"	—
21				0	+	1-	"	(0) -
22				— 1E (-) —			"	
23				+	0	4-	"	(6) 4 found stbl
24				+	0	4-	"	(9) 8 found stbl.

4 Prods
4 ess
3 Ampli

DATE: 8/21/58 REF:

Preliminary Expt. on segs. from $\frac{-}{+}$ 6hr $4p^+$ and $4p^R$'s

1. Culture of W2868 $\frac{4^- 2^+ 4p^S}{4^+ 2^- 4p^R}$

A. Overnight culture diluted $10^2 - 10^4 - 10^6 - 10^8 \rightarrow$ 10 0.1 ml sample

	(+)	(−)	total	
↓				
assay 0.1	1. 40	3	43	mean/sample
	2. 76	2	78	$= \frac{57}{100} = 0.6$
	3. 52	3	55	
	168 56	8/3 = 3	176/3 = 59	
	3			

B. Samples 10 (0.1 ml) plated after 5.25 hours

1.-6	no growth		
7.	5	1208	
8.	1	462	
9.	63	648	
10.	ca 19	1166 ca 185	

361-7 361-8 shared tube + 361-9 Phage check at 25° from

On a plate streaked from 10. This a colony - almost pure (+) noted = (1)

Analysis of this colony is on 362-B

Reversions - Clones (−) segs from 361-8, 361-9, 361B8 streaked on B gal rich + 4 4^R obtained from each - examined for Gal stability

1/4+	1/2−	1/4−	$4p^S$	4^R from each of 5 $4p^S$ obt.
+	−	+	S	1 3/4 intermix, not seg
+	−	+	R	2 3/4 int. (+) are not seg
+	−	+	S	3 3/4 int. (+) are not seg
+	−	+	S	4 3/4 " " " "
+	−	+	S	5 3/4 " " " "
+	−	+	R	
+	−	+	S	
+	−	+	S	2-S = 8
+	−	+	S	2-R = 2
+	−	+	S	1 3/4 int. (+) 1 (+) seg.

2 Culture of W2869 $\frac{4^- 2^+ 4p^+ ?}{4^+ 2^- 4p^+ ?}$

1/4+	1/2−	1/4−	
+	+	0	result = 4-

Tested + 0 + $4p^S$ = Gal$_2$ - $4p^S$

↑ 4 ^{R} obtained

3 full +, 1 intermed.

4/4 not segregating

A. Overnight cult. diluted $10^2 - 10^4 - 10^6 - 10^8 \rightarrow$ 10 0.1 ml sample

↓ 0.1 ml assay

	+	=	total	
1	18	7	21	
2	22	8	30	0.33/0.1 ml
3	22	20	42	
	62	31	97/3	
	3 = 21	3 12	33	

B. samples 10 (0.1) plated 5.25 hours

1.-5	no growth			
6-7	all (−)	(+) ca 2000 col	1 = ca 500	
8.	(+) 4	361 ← these	4 seg	
9.	1/4 = 66	ca 101		
10.	ca 1500	ca. 200		

361-138

1.	4/4 int. (+) mono seg
2.	3/4 int. (+) mono seg
3.	3/4 int. (+) mono seg
4.	4/4 int. (+) mono seg

SEE 362 ALSO

Using inocula from above - 0.1 ml of 10^6 cells in each case added to 7 ml Pen and incubated 20 hours at 42C

2868 medium rate 56+/3- 20 hour rates: 167+/10-, 155+/10-, 175+/12-

2869 " " 21+/12- " " " 214/176- +?-/ +?-/

DATE: 8/24/55 REF:

Repeat study of segregation from $\frac{2^{x}y^{-}}{+\ +}$ 4pt - as in 361 - W2869

Culture streaked out and single (+) colony selected. Overnight culture from this colony diluted $10^{2} - 10^{4} - 10^{6} - 10^{8} \rightarrow$ 20 0.1ml sample incubated 4.5 hours, plated 4pt washed with 0.1ml buffer

	Sample	(−)	(+Gal)	allele ✓	cell assay (Δ1)	made (−)	rest day (+Gal)
	1-5	no growth					
	6	0	354				
10	7	1/4=157	ca. 300K+	discarded		14	140
(Diploid)	8	19	1209	8 Gal+	2-4- 1 HFT + + 0 = 8(4-)	2	136
	9	2	464	6 Gal+	3	16	118
	10	2	540	Gal-		32 = 11	394 = 131
(Diploid)	11	19	1089	4 Gal+	HFT + 2 4- 1 3 + + 0 = 3 4-	3	3
	12	17	1357	discarded		HFT + ≤ 4-	
6(+)	13	297	933	10 picked	2 contam E(+)	8 + + 0	= 8(4-)
	14	4	428	4 Gal-			
	15	12	840	discarded			
	16	11	1525	"			
20	17	33	796	discarded	1 + 12- 14-		
12(−) picked 6(+)	18	441	690	4	12(-) + + 0 = 12 4-		
	19	494	1722	"			
	20	18	604	"			

3628 the ⊕ colony of 361-9

#1. the ⊕ colony streaked out gave almost all (−)

2. from #1 10 (−) colonies tested + ½ ¼- all 2- 4p^{3}

from #1 4 Gal+ colonies obtained and streaked out - 5 tetrad (−) segs from each to test for secondary non disjunction - 6 Gal+ patches from each of the 4 and streaked to obtain 6 separate segregational events

Gal+ #	Observation	Single cells	
1	4/6 all (−) segs	2/2 Gal$_2^-$ 4p^{R}	2 0
2	4/6 all (−) segs	5/5 Gal$_2^-$, 2/5 4p^{3}, 2/5 4p^{R}	2 2
3	1/6 "	5/5 Gal$_2^-$, 2/5 4p^{3}, 2/5 4p^{R}	2 2
4	4/6 "	4/6 Gal$_2^-$, 4/6 4p^{3}, 4/6 4p^{R}	2 2
		6 4p^{3} + 12 4p^{R}	12 6

DATE: 8/25/55 REF:

Observation of erotic induction

1895 ⊗ 2790 Aerated antifoam added cultures 3 hours from overnight

1.0 ml + 5.0 ml + 4.0 ml Pen - aerated no antifoam. (4.1×10⁸ Hfr) (2.0×10⁹ F⁻)

$10^2, 10^4, 10^6$ → 0.5 ml
1. 206
2. 206
206

Samples taken out
diluted $10^2, 10^4$
↓
0.05 ml plated
1. /2279 on B Gal
2. / D(o)

Optical Density Read / 10 ml Pen at 650

$10^2 - 10^4, 10^6$ → 0.05 ml
1. 209
2. 157
396 / 2 = 198

Time	O.D.	Plaques/2279	Proto. D(o)	Prototrophs /I	Prototrophs /Lps
0	0.95	—	—		
15	—	7 *	3	35	Contam with phage
30	1.10	19	1	15	
45	1.7	36	0	—	
60	2.2	69	3	35	
75	2.0	147	11	115	
90	2.2	ca 110	6	65	

* Since these plated on B gal estimation of 1895 Galt component can be made appear to be ca 500 at 0 and no reduction in number up to 90 min noted.

Ratio of Hfr/F⁻ = 20.6/99 = 1/5

$4.12 \times 10^7 - 4.1 \times 10^9$ 4.00×10^9

$7 \times 20 \times 10^4 =$
$140 \times 10^4 = 1.40 \times 10^6$ λ/ml at Zero
$\frac{150}{7} \times 1.4 = 29 \times 10^6 = 2.9 \times 10^7$
21 =
1.4
84
21
29.4

$\frac{2.9}{41} = 7\%$ Hfr

0.07
410 / 29.00

2341 ⊗ 2308 Mal - Inad. 2341 to look for transcocatus and seg behavior of exogenote

1. 2341 aerated wet. inad. 60 sec. - mixed equivalent volumes of Hfr & F - incubated 30 min 37C diluted 1200 and plated B gal, S Mal - 5 Mal plates discarded - difficult to Malt from Mal -
2. 20 Galt prototrophs on S Gal picked and tested /2279 for lysogenicity all non lysogenic

363 B → The 20 Galt are divided into several groups on degree of Galt-ness
12 full Galt - these do not segregate
3 intermed Galt
5 weak Galt - these contain a few full Galt
} all appear to be seg.

DATE: REF:

Segregant diploidy. Test by reverting segregant, v stability

Culture.			Rev. stbl	0 = no + = yes
1	518E1K12		0	
2	518E750		0 (wk +)	
3	518E902-1		0	
4	518E902-2		0	
5	2175E811-1		0 (wk +)	
6	2175E811-2		—	
7	750E1K12-1		0	
8	750E1K12-2		0 (wk +)	
9	750E902-1		—	
10	750E902-2		0	
11	811E902-1	all +	—	
12	811E902-2	all +	0 (wk +)	
13	286-1		—	
14	286-2	failed to grow	—	
15	2175E750-1		0 (wk +)	
16	2175E750-2		—	
17	290-1		—	
18	295-2		—	
19	302-2	7-x-2-	—	
20	3448	2-x-6-7-	0	

10/10 successful tests no seg = 10 seg not diploid

311-2 = HFT 6- =
sheared out 2 Gal+ reversions obtained
both segregating Gal+/Gal-

2 HFT seg obtained both /+ + /2 + /6 0 = 6-

HFT 6- +R

309-1 = HFT 7-
sheared out 8 Gal+ reversions obtained
2/8 segregating Gal+/Gal-

HFT 7-

2874 looking for Gal_2^- in 583 line
1. first 8 seg = Gal_7^-
2nd 6 " = "

DATE: 8/31/55 REF:

Segregation — non disjunction? Obtain clone with early seg and test the Gal+ component to see if it segregates 50% homozygotes.

7. Cuclone. W2867. from single (+) colony → overnight Pen. culture

$10^6 - 10^4 - 10^2$ (c. 133/plate)

Spot 0.01 ml samples B Gal incubate 3 hours and replica. - look for clones with many (-)

0.1 ml for Gal+ : Gal- ratio

	Gal+	Gal-	total
1.	89	28	117
2.	17	125	142
3.	19	120	139
	55/3 = 18	273/3	398/3 = 133/0.1 ml

1:3 / 0.01 ml

Reproddns
1. failed to grow on replicum - 19
2. ore gal+ - 32 (ca. 20 clone size)
3. ore gal- - 6
4. mixed Gal+ + Gal- - 15 / 72

Discarded

Gal+ $\bar{X}$ = 1.2
Gal- $\bar{X}$ = 0.18
$\bar{X}$ = 1.3
e^{-x} = 19.4
e^{-x} = 21.6
e^{-x} = 59.76

obs
19
(19+6) = 25
13 ε Gal-

Two used for any plate

1		1. (1-) : 13+	no other (-) on plate		1 +				Gal4-
20	46	2. 8- : 38+	(-)	+					
2	11	3. (2-) : 9+	no other (-) on plate		2 +	(2)	+	+ o	2 Gal4-
	45	4. 8- : 87+		+					
3	8	5. (1-) : 7+	same plate		3 +				
	39	6. 15- : 24+		+			+	+ o	Gal4-
	24	7. 10- : 14+	(-)	+					
	41	8. 10- : 31+	(-) same plate	+					
4	33	9. (1-) : 32+	(-)		4 +		+	+ o	Gal4-
5	11	10. (1-) : 10+	(-) same plate		5 +		+	+ o	Gal4-
	9	11. 7- : 2+	(-)	+					
30	36	12. 13- : 22	(-)	+					
6	12	13. (1-) : 11+	(-) same plate		6 +		+	0 +	Gal2-
	5	14. 4- : 1+	no other (-) on plate	+					
	one 12 (8)	15. 1- : 11+	no other (-) on plate	+					

TEST OF ORIGINAL SEGREGANT COLONY

2 Gal+ were chosen + selected from each all proved Gal stable

Localization of clone on plate and relationship to other Gal-, in case of spontaneous from one check to another

Segregants from three Gal+

#6 cl A = 1 (2-), 5 (4-)
B = 3 (2-), 3 (4-)
C = 2 (2-), 1 (4-) 1 no test
D = 1 (2-), 4 (4-) ≤

tabul against +, 2-, 4-

A1 (2-)
D4 (4-)
B1 (4-)
B2-4 (2-)
B5-6 (4-)
A repeat 6 seg analysis — 1-4 (4-) 3 4-; 5-6 (2-) 3 2-

C1 (4-)
C2-3 (2-)
D1-2 (4-)
D3- (2-)
D4-5 (4-)
C repeat 5 segregants — 1 = 4-; 2β = 2-, 4, 5 = 4- — 4 (4-); 4 (2-)

Segregants from #1 Gal+:
A 3 (2-) 5 (4-)
B 2 (2-) 3 (4-)
C 4 (4-)
D 5 (4-)

Gal+ Revertants (2) obtained from these and tested

2 Rev of A1 white ε 16 (+) Cul. tested / 750
10 (-) cul. tested / 1 HFT / 851
4 Centers (+) 5 NFT 7 HFT
Rl (-) sibs
½ Rl white — NFT segregants obtained ½ Gal2-
also B5-6)

tested for stability
½ sibs
10/16 other

DATE: 9/25/55 REF:

293-12 (which one) gave increased frequency of HFT Gal4⁻
Use to test for dyeing of distal markers as a result of C.O. between Gal's.

1. A mat obtained by λ^2 recombination in B mat.
2. 12 segs obtained, tested for HFT against 2580, 811

control	2580	811
○ ○ ⊙ ○	○ ○ ○ ⊕	○ ○ ⊕ ○
○ ○ ○ ○	○ ⊕ ○ ○	○ ○ ○ ○
○ ○ ○ ○	○ ⊕ ○ ⊕	○ ○ ○ ○

⊙ = control
⊕ = HFT

Total 13 HFT 4⁻ 37 + 17/#9 or 16/#9

+/12 HFT 4⁻ matt progeny

Often mat ♀ not allele

3. Streak HFT 4⁻ on B mat, ♂ mat ♀ strk from exp. colonies
#4 - 6/6 matt^2 strk, 3/3 Gal+^{R} strk; #6 6/6 matt^R strk, 3/3 Gal+^{R} strk; #10 6/6 matt+^{R} strk 3/3 Gal+^{R} strk; #12 6/6 matt^R strk 3/3 Gal+^{R} strk

A1

4. Tet Tem seg /2⁻ /4⁻ /+ (these are mat -)
A. Despite mat reaction - after 3 days at 37C, reactions obtained - are appear to be transduced by +, 2⁻, but not by 4⁻ - means all Gal4⁻ - Gal4⁻ is in progeny
B. Reversions of #4, 6, 10, 12 obtained to check HFT.

The HFT abnormal (A1) and 3 from A2 grown with 1177 24 hours and plated on SM at 5×10⁷ no growth - indicating these cultures not HFT.

Reversions above say cultures not homogenate why?

5. Rpt. on 12 additional seg.

control	2580	811
○ ○ ○ ○	○ ○ ⊕ ○	○ ○ ○ ○
○ ⊙ ○ ○	○ ⊕ ⊕ ○	○ ○ ○ ⊕
○ ○ ○ ○	○ ○ ○ ○	○ ○ ○ ○
Mat^R, Gal+^{R}	6/6 +^{R} strk #3; 5/6 " "	6/6 +^{R} strk #6; 5/6 strk	6/6 +^{R} strk #7; 6/6 " "	6/6 matt^R #8 strk; 2/2 strk 4

A2 the Gal+ are from mat ♀

CONTINUED PAGE 366

6. 293-12 introduced on Bzyl, Baer, Bact. - 1 Hyl⁻ obtained, 1 mat⁻ (?) not Gal+/Gal-

2852 = 365B = Gal1⁻ Gal4⁻ x — 2⁻ to obtain (- - -) for expt. to show crossing over between genes, as $\frac{+}{2} = \frac{1^+}{1^-}$ = not Gal+, C.O. between genes to give + yields Gal+.

7. 2852 streaked / HFT 2⁻
8. 10 streak mode from cross bact.
7 yielded 4p+ from 1.
3 " 4p^R/4p^S semi seg 1-4⁻ 4p^S
3. Obtain 1-4-3?4p^S JJ 1+ 4+ 2-4p+ (one)
a. seg. 11 1-4⁻ 4p^S
1 2⁻ 4p^S
1 2⁻ 4p+ → streaks Bgal → 7 seg obt. 6 = 1⁻ 2⁻ 4⁻ 4p^S, 1 1⁻ 4⁻ 4p^S

→ Reverse 365B-1 and 365B-2 mat

A5

(366)

DATE: 10/1/55 REF:

1 2 3 4 5 6 7 8 9 10

1. 1578 - H Asbai F⁻ 1⁻ 4⁻ and F⁻ 1⁻ for EMC

1578 X — 2346 X (Col,⁻) - Exposed to 0.3 ml 2346 - mult 10⁷
dilute 10^2 10^6 10^7 0.1 ml - No counts done.
/Plate

Segregation from H		(-)	2 segs pos
11 Isth 1 (H)	1.	14	0
12 segs	2.	15	2 (1 may be in square
Segregants Used	3.	17	1
1 1⁻ 4⁻	4.	12	1 Smt
6 4⁻	5	8	0
1 1⁻		66	4 (6) 4/70
2 P.E.			carrier = 3, no lysis

366-1

366-2 2 segs Stock gel 1/94 Used 11 segs

F⁻ Gal,⁻ Gal₄⁻ Lys⁺ = 366-3 W2994

K12 lysate 9/28/55

titer	10/1/55 =	$10^8 \times 10^8$	1.1×10^{10}
	10/4/55 =	$10^8 \times 10^8$	1.1×10^{10}

365A3 continued. 293-12 mal - → segregates for mal dependency, etc.

A3 9 additional segs.

	/2550	/51		/2550	/51
Phenotype of clone	○○●○	○○○○	Not isolated	○○●○	○○○○
streaks is different from	○⊕○○	○○○⊕20		○⊕○○	○○○⊙2
titer streak on Bgal →	⊕	○		⊕	○

THESE APPARENT HFT SEGREGANTS ARE OBSERVED WITH UV TESTING.

This group not found

These were not numbered. 4 were obtained from 3, 2 from one. Check?

A3 Gal⁺ ← of above (1) 2/4 +ve seg
(4) 1/1 +ve seg
(3) 3/4 +ve seg.

Mal⁺ from Gal+/-	Mal+ Gal+/-?	→ Gal⁻
#1 6/6 stbl	6/6 Gal⁻	6/6 stbl
#2 5/5 stbl	2/5 Gal+/-	6/6 stbl
#3 6/5 stbl	1/5 Gal+/-	6/6 stbl

Does mixed segregants have a genetic tendency to fall apart? Do medium changes affect results? Of 16 Mal⁺, only 2 were stable Gal+/-

Return to 293-12 - are the segregants really Gal₂⁻? 365 suggests no.

/2550	/51	
⊕ ○ ○ ⊕	○ ○ ○ ○	tested against HFT 2⁻, +⁻ all Gal₄⁻
○ ○ ● ○	○ ○ ○ ○	
○ ○ ⊕ ○	○ ○ ○ ○	
○ ○ ⊕ ○	○ ○ ○ ○	
○ ○	○ ○	

DATE: 10/8/55 REF:

2341 Lys^r/Lys^s $2^-/2^-$ X — (+) 750 CH12-1

1. 2 day cult. 2341 → 1-10
↓
0.1ml + 0.4ml 750CH12-1 → 10^2, 10^4 → 0.1ml
inc. 10' 37C

2. same with broth as control →

		(−)	(+)
Control	1.	150	0
	2.	164	0
Exptl.	1.	173	1
	2.	158	2
	3.	162	2
	4.	123	2
	5.	189	2

(2-4) several partially lysed colonies/plate

One streaked out of (+) cult. / 1, 2, 3, 4, 5

Gal+	Gal−	1st strike
1 Lys^R	Lys^r	→ Lys^{R+}, −s
2. Lys^r	Lys^r	→ Lys^{R+}, −s
3 Lys^R	Lys^r	→ Lys^{R+}, −s
4 Lys^R	Lys^r	→ Lys^{R+}, −s
5 Lys^R	Lys^r	→ Lys^{R+}, −s
6 Lys^{s+}	Lys^s	→ Lys^{s+}, −s
7 Lys^{s+}	Lys^s	→ Lys^{s+}, −s
8 Lys^{s+}	Lys^s	→ Lys^{s+}, −s
9. Lys^{s+}	Lys^s	→ Lys^{s+}, −s

(−), (+) pooled titered (λ 2171

[These discarded]

365B2 = H^- Gal_1^- Gal_4^- Gal_2^- Lys^s X — HFT 7 to see Guadenych (−)

1. Procedure as with 2341 above. 309-1 lysate used.

		(−)	per cal.
1. Control	1.	428	0
	2.	454	0
	3	503	0
2. Exptl	1.	171	15
	2.	189	27
	3.	196	20
	4.	206	33
	5.	200	46

These ab. low, perhaps 30% most (80%) colonies partially lysed

A

B

$N_0 = 43 \times 30 \times 10^3 = 86 \times 10^4$

$N_0 = 2 \times 30 \times 10 = 4 \times 10^2$

DATE: 10/10/55

REF: $\frac{N_0}{N_0} = \frac{4}{86} \times \frac{10^2}{10^4} = 0.5 \times 10^{-2} = 0.5\%$

1	2	3	4	5	6	7	8	9

241-14 - (a colony picked for HFT) HFT 1 yield

1. dilute to obtain 10^4-10^5/ml semi → 10^1, 10^2 → 0.05 ml plated
2. Incubate 60 seconds.
3. 1.0 ml + 10 ml Ber. ca 10^4/ml
4. 0.05 ml samples: /750 PAO /2279 PLAQUE /2279

Time	/750	PAO /2279	PLAQUE /2279	
0	52	79	>62	Plaques small
15	30	81	>168	" "
45	32	93	>107	" "
55	43	88	>280	" "
65	50	86	>334	wet plate
90	56	93	>374	" "

SEE RESPEAD → 253/6 = 42

N_0 =
1. 1 cul.
2. 3 cul
3. 3 cul / 1/3 = 2 all here!

1. 36 (1 gave 1)
2. 40
3. 53

129 / 3 = (43)

1. 33
5. 35
6. 34

Nonplaquers

102 / 3 = (34)

HOPELESS

Total Caryon Nonplaquers 1964

20 For EMC - A poly- w F-750 lysis.

2902 ← HFT 4

2 heterogenotes (P.E.-) obtained

2902 / 4 Gal+ 18/22 + stable

13 Gal+ Cal+ λ, 2229 3 $4p^s$, 10 $4p^+$

368-1 / 368-2 ← $4p^s/4p^+$ 4 day old. 2 $4p^s$ tol, 2 $4p^+$ tol

30 2915 ← HFT 7 - (309-1) - Do $4p^s/4p^+$ heterogenotes come from clones were there 11 also $(4p^+)\lambda^+$? Recovery of 4 strands.

1. Fresh med culture of 2915 ca. 10^8 dilute 0.1 ml + 0.4 ml 309-1 λ (prop.) incubated 10' 37C, dil $10^2, 10^3, 10^4$ → 0.1 ml plated B Gal

		(+)	(-)	(-)/+ ratio	total
1. Both control	1.	0	92	0	92
	2.	0	94	0	94
	3	0	96	0	96

2. Hybrids expected.

		hyst. colony			
1.	4 (3)	ca. 80 wet plate	12	ca. 16	
2.	3 (2)	ca 60 wet plate	14	ca. 21	
3.	6 (6)	65	10	16	
4.	2 (1)	41 wet plate	9	18	
5.	5 (3)	ca 60 wet plate	ca. 16	ca. 23	

CONTINUED →

40

3. Testing the Retrogenotes - streaked / 2902, streaked wet B Gal.

	2902 hybrid test			
1	+	Gal sec	11	+ Gal-
2.	+	"	12	+ Gal sec
3	+	"	13	+ "
4	+	"	14	+ "
5	+	"	15	+ "
6	+	Gal-	16	+ "
7	+	Gal sec		
8	+	"		
9	+	"		
10	+	"		

50 These last 4 came from wet col., the others from particularly picked colonies.

DATE: 10/18/55 REF: 368 A

Continuation of 368 - Do lyt/lyr transductions come from λ+ particles?

1. Examination of the Lutz genotypes - 6 gal+, 6 gal- taken from the 1st streaking out of the isolated (single colony) heteroscente, and streaked against λ, 2279

		Het # 1	2	3	4	5	6	7	8	9	10
Gal+	1	+	+	+	R	+	+	+	+		
	2	+	ut	+	R	+	+	+	+		
	3	+	+	+	R	+	+	+	+		
	4	r ?	+	+	R	+	+	+	+		
	5	+	ut	+	R	+	+	+	+		
	6	+	+	+	R	+	+	+	+		
Gal-	1	+	+	s	s	c	+	+	+		
	2	s	s	+	s	+	+	+	+		
	3	s contam	s	+	s	c	+	+	s		
	4	+	+	ut	s contam	ut	+	+	ut		
	5	ut	+	+	s	ut	+	+	+		
	6	s contam	s	+	s	ut	+	+	s contam		

+ = lyt, r = lyr c = lys ut = no test

Reason of note on which Dr. Lutz based methotrophous gene type

Re. #16, the plate showed all 3 and ca. plaque

		#10	#12	#13	#14	#15	#16	#18			
Gal+	1	+	+	+	+	+	R	+			
	2	+	+	+	+	+	R	+			
	3	+	+	+	+	+	R	+			
	4	+	+	+	+	+	R	+			
	5	+	+	+	+	+	R	+			
	6	+	+	+	+	+	R	+			
Gal-	1	s	s	+	ut	+	s	+			
	2	s	s	+	s	s	s	+			
	3	+	s	+	s contam	s	s	+			
	4	+	+	+	s	s	s	+			
	5	+	s	+	s contam	+	s	+			
	6	+	s	+	s	+	s	ut			

(See 371)

FOR #4 #16: Spot from the original isolate (colony from the first plate) grown up in broth and spotted (spot centrifugated) on 2279. One set of plates UV, one UV. both showed λ, the UV=0 UV=100. (3)

For λ tests

To AR. (W new Hfr 3, X — Gal2- (24-1) W

also Gal4- (24R-1) —X

From the Hfr -X 4 Gal+ Obtained 3 lyt+ 1 lyr+ were found lyt+

368A-3