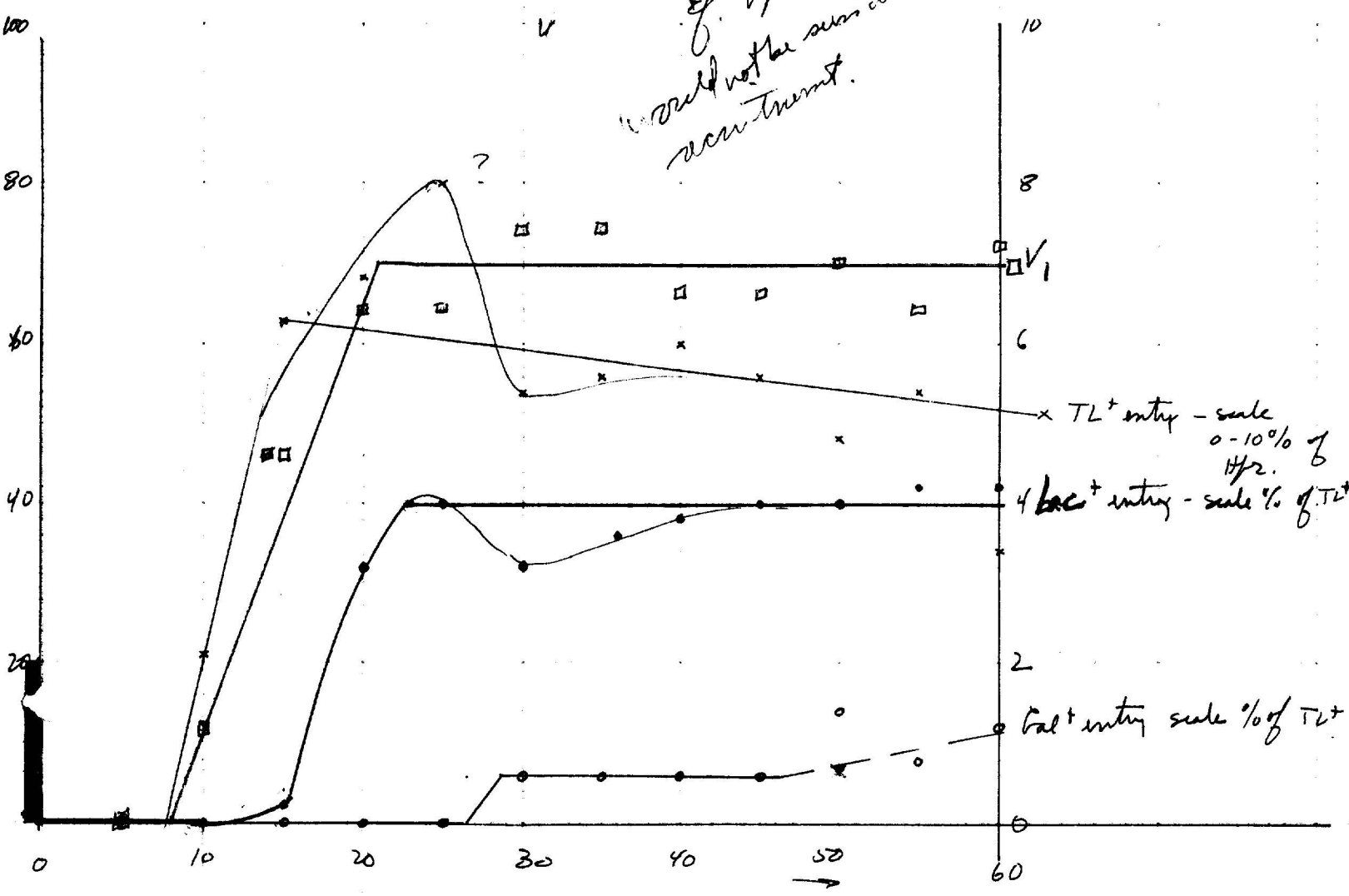




% of Lac, Gal, T<sub>1</sub>  
 based on first samples of  
 50 colonies each.

? Is this a real reflection?  
 - lethal effect of  $hps^+$  in  $hps^+$ ?  
 of  $hps^{sd} \times hps^s$ .  
 would not be seen with continuous  
 recruitment.



x TL+ entry - scale 0-10% of 1/2

• Lac+ entry - scale % of TL+

□ Gal+ entry scale % of TL+

1401-2

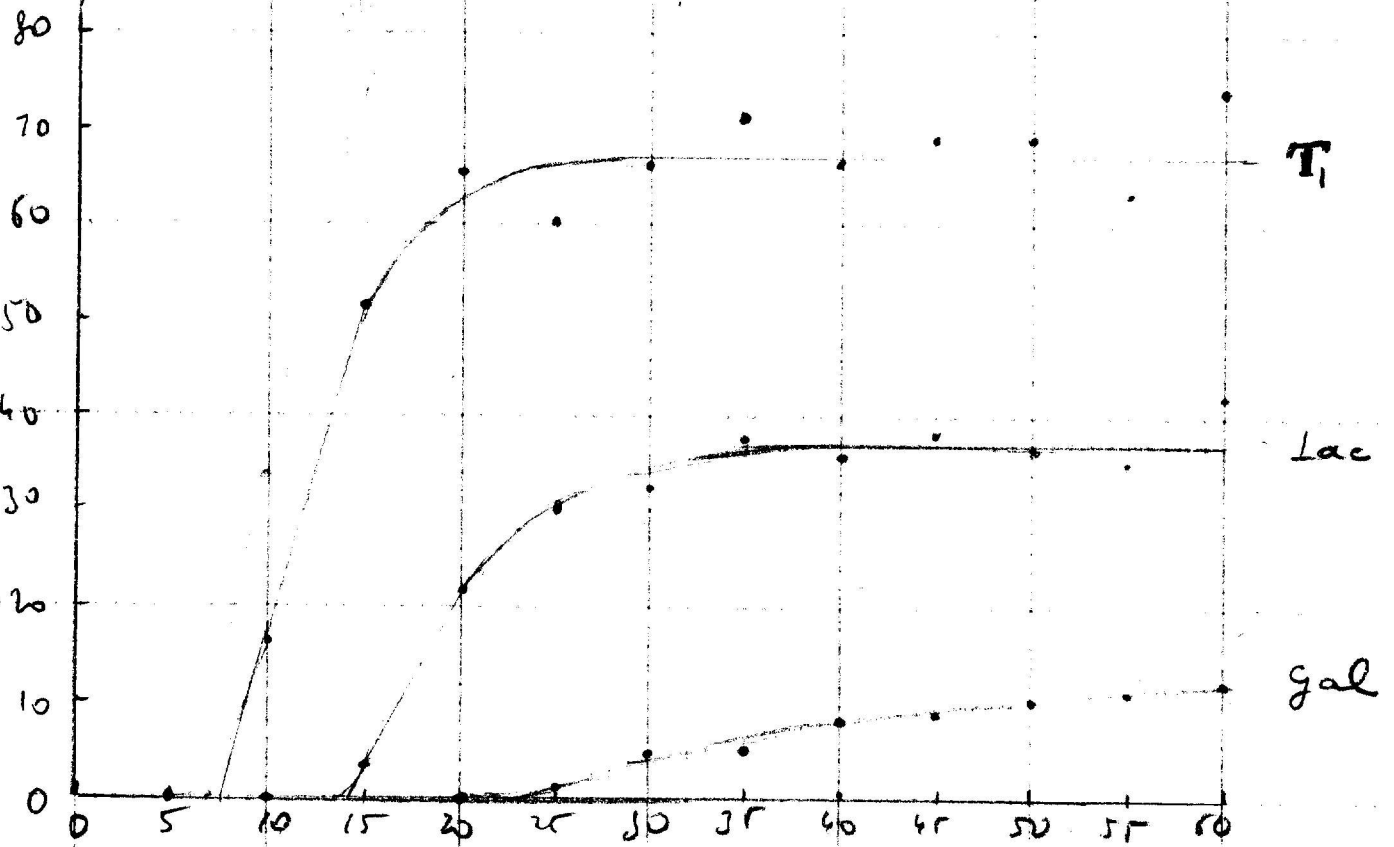
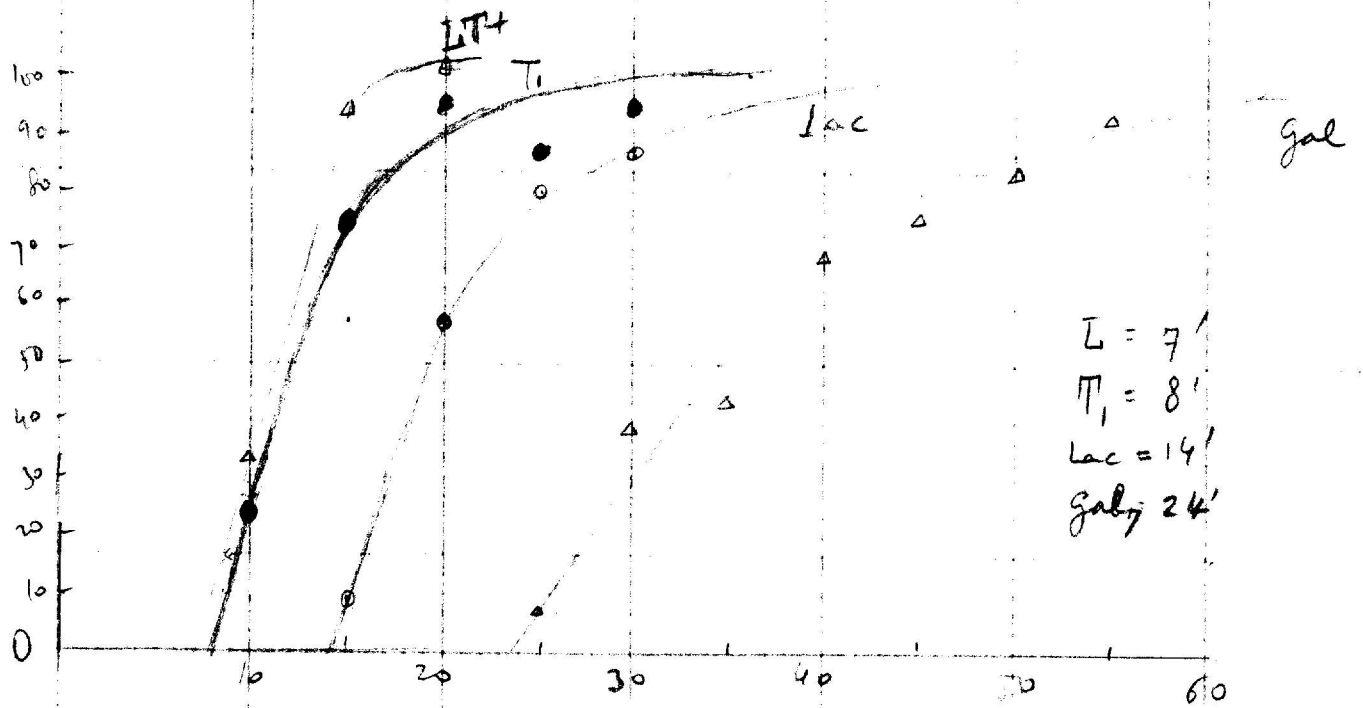
DATE:

REF:

Time	Lac + 3			5	Gal + 7		8	Tp		10
	first	next	total		first	next		total	first	
0										
5										
10	0/50	0/12	0/62		0/12		6	4/12	10/62	
15	1	6/142	7/192		0/142		23	76/142	99/192	
20	16	20/92	32/142	0	0/88		32	59/88	91/138	
25	20	28/110	48/160	0	1/108	1/108	32	63/108	95/158	
30	16	18/56	34/106	3	2/57	5/107	37	33/56	70/106	
35	18	19/49	37/99	3	2/49	5/99	37	33/49	70/99	
40	19	33/95	52/145	3	9/95	12/145	33	63/95	96/145	
45	20	18/50	38/100	3	6/50	9/100	33	36/50	69/100	
50	20	12/38	32/88	7	2/38	9/88	35	25/37	50/87	
55	21	16/56	37/106	4	1/57	12/107	32	35/56	67/106	
60	21			6			37			

Time	% of T <sub>1</sub>	% of all alleles Lac	% of gal	% of T <sub>1</sub>	% of asymptote Lac	% of gal
0	1					
5						
10	16.1	0	0	10'	23.2	0
15	51.6	3.6	0		74.5	9.6
20	65.8	21.5	0	20'	95.0	57.5
25	60.1	30.0	0.93		86.5	80.2
30	66.0	32.1	4.67	30'	95.0	86.0
35	70.8	37.3	5.1			99.9
40	66.2	35.8	8.3			
45	69.0	38.0	9.0			
50	68.9	36.4	10.2			
55	63.1	34.9	11.2			
60	74.0	42.0	12			
		44.4				
Asympt	69.5	37.4	12?			



Total data 140 1/2



19

May 26, 1958

REF:

1401/3

	1	2	3	4	5	6	7	8	9	10
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										
1										
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3										
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1										
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3										
4										
5										
6										
7										
8										
9										
0										
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										

W 3060, W 3052, 1<sup>h</sup> cultures -  
 spun, resusp. 4x & 2x resp. (♂ less turbid).  
 Mixture: 1 ml ♂ + 4 ml ♀.  
 After times: 10', 15', 20', 25', 30', 40'  
 sampled .2 ml and diluted in 10 ml  $\frac{1}{20}$  (shaded).  
 Blended, and plate .05 and .05  $\frac{1}{10}$ . (called 1, 2 resp.)  
 O': Plate recomb. control, .05 ♂  $\frac{1}{50}$  and .05 ♀  $\frac{1}{50}$ .  
 Also, dilutions  $\frac{1}{10}$  from such suspensions (Aug 1, 2) -  
 Media: - D(B, Gen)  
 1 D(Pool L B, Gen)  
 2 D(Pool B, Gen).  
 Controls, .05 ml of dil  $\frac{1}{50}$  ♂, ♀ on media 1, 2.



19

May 23<sup>rd</sup>, 1958

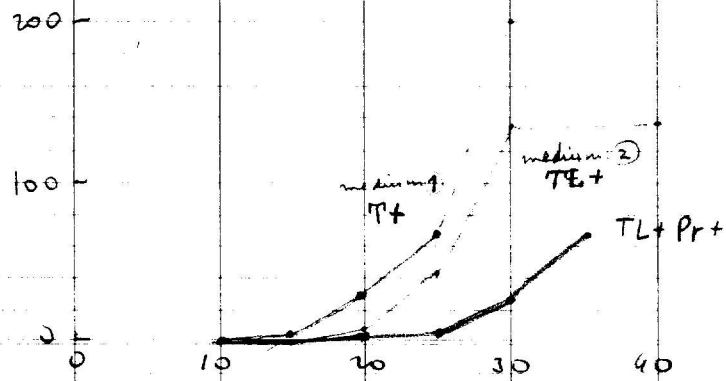
REF:

1402/5.

	1	2	3	4	5	6	7	8	9	10
		Medium								
		0		1		2				
		dl 1	dl 2	dl 1	dl 2	dl 1	dl 2	dl 3		
1										
2										
3										
4	10'	0	0	1	0	0	0			
5										
6	15'	0	0	5	0	1	0			
7										
8	20'	3	0	31	4	5	3			
9										
0	25'	6	1	67	9	47	5			
1	30'	28	4		21	134	7			
2										
3	40'	66	9			135	11	1		
4										
5		dl 3:								
6										
7	0'	0	0	0	0	0	0			
8										
9										
0										
1		Control medium 1:		3	4	2	3 colonies			
2							1 "			
3										
4				Media	0: D(B, fm)					
5					1: D(B, Pool Len fm)					
6					2: D(B, Pool fm)					
7										
8										
9										
0										
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										

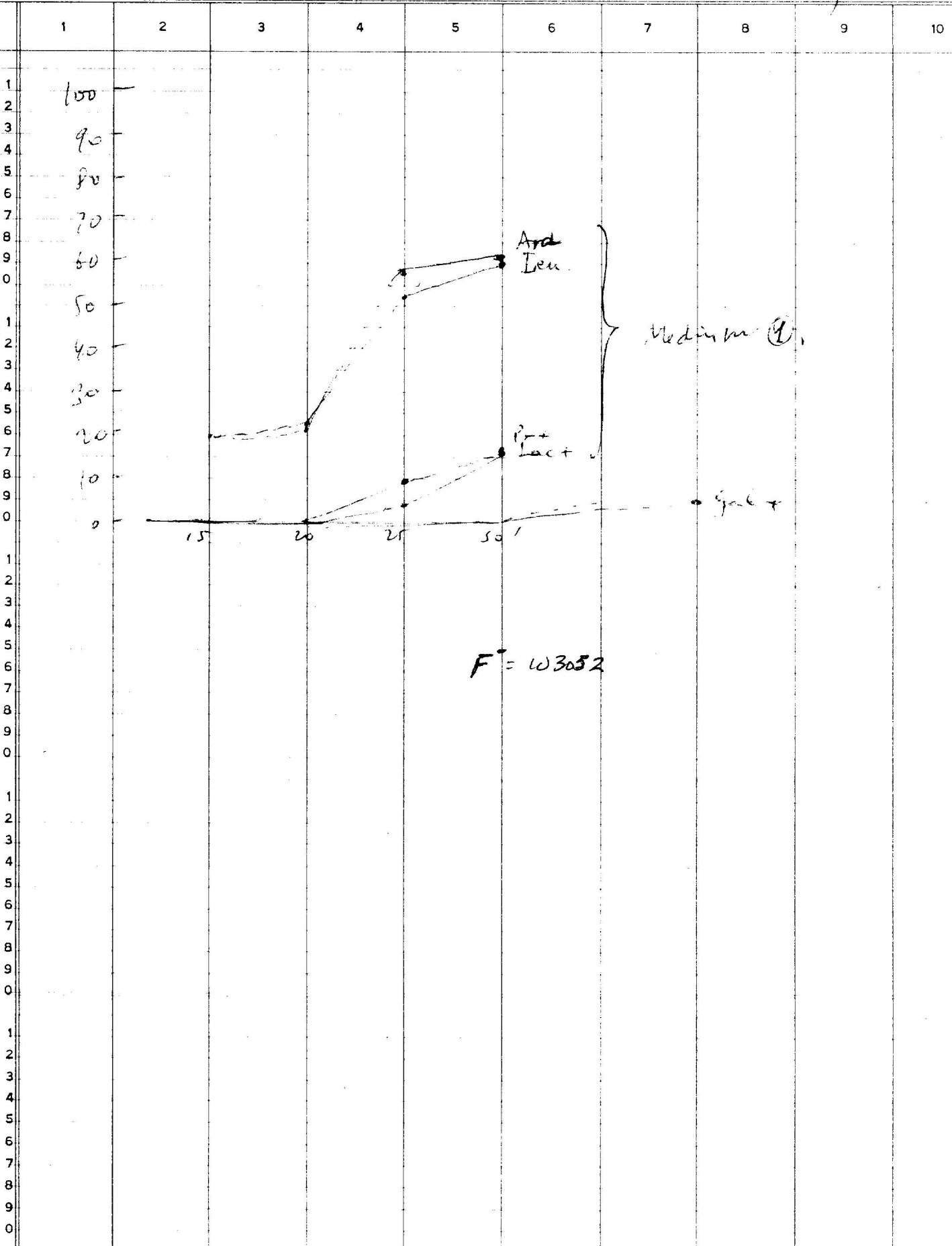
Control medium 1: 3 4 2 3 colonies  
 1 "

Media  
 0: D(B, fm)  
 1: D(B, Pool Len fm)  
 2: D(B, Pool fm)



W 3052 is descendant from W 945  
 3064 W 583 -









19

June 2, 1958

REF:

1401/4

1 2 3 4 5 6 7 8 9 10

Comparison between W3052, W3064.

ORC cultures of W3060, W3064, W3052. Same, resusp. at same conc.

A: 2 ml W3060, + 4 ml W3052

B: " " " " W3064

C: " " " + 2 ml W3052 + 2 ml W3064.

At 10', 20', 30', 40', 50', 60'

0.01 sample diluted in 10 ml chilled water, blended,

and: 10', 20', 30', 40', 50' → 0.05 plated on  $\frac{1}{2}$  Galferol B,  
30', 40', 50', 60' →  $\frac{1}{10}$  → 0.05 "

1  
2  
3  
4  
5  
6  
7  
8  
9  
0  
1  
2  
3  
4  
5  
6  
7  
8  
9  
0  
1  
2  
3  
4  
5  
6  
7  
8  
9  
0  
1  
2  
3  
4  
5  
6  
7  
8  
9  
0





June 24

1401/5

1 2 3 4 5 6 7 8 9 10

Hfr<sub>1</sub> and Hfr<sub>2</sub> timing X 3052

♀ # 3052 T<sup>R</sup>

♂♂: 3936 (1895 Az<sup>R</sup>), 3948 (3870 Az<sup>R</sup>)

ORC, spun, resusp. in Penallay, 1 x conc ♂♂, 1/2 x conc ♀.

Mating mixtures: 1 ml ♂ : 9 ml ♀ (conc ratio 1:5)

Mating in flask. Samples of 0.5 ml dil in 4.5 chilled water, blunted, → A plated .05 on various media, B diluted 1/10 and plate. B dilutions only for times ≥ 15'.

Times: Hfr<sub>1</sub> cross: 0', 5', 10', 15', 20', 25', 30', 35', 40', 50'  
Hfr<sub>2</sub> " : also 7 1/2' and 13'.

Media: For Hfr<sub>1</sub> media 1 Sm Me B, L T  
(all D(-)) 2 Sm Me B,  
3 Sm Me

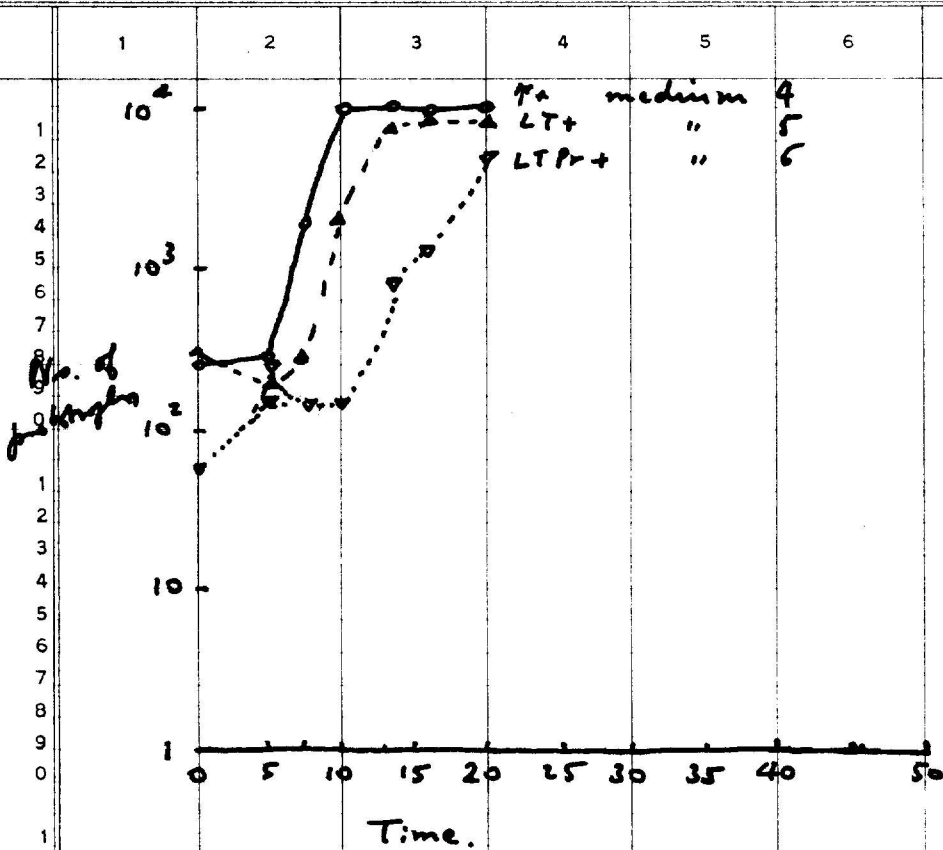
For Hfr<sub>2</sub> media 4 Sm B, Prol Len  
5 Sm B, Prol  
6 Sm B,

1  
2  
3  
4  
5  
6  
7  
8  
9  
0

	1	2	3	4	5	6	7	8	9	10
Turns	0	5	7 1/2	10	13	15	20	25		30
2										
3										
4										
1A	105	146		253	<i>100-200?</i> <i>small</i> <i>large</i> <i>1000 small</i> <i>63</i>	0	$N.5 \cdot 10^3$	(N 200 large) ( $10^3$ small)		$\infty$
1B								(9 large 320 small)	(49 large N 500 small)	(53 large N 500 small)
2A	88	34		52			155	N 1000		N 1000
2B							13	85		137 large
3A	8	6		9		26	40	18		44
3B						5	2	5		6
4A	245	320	N 2000	N 10,000	N 10,000	N $10^4$ *	N $10^4$	t.m.t.c		$\infty$
4B						N 800*	N 2000*	"		N 2000 large & small
5A	355	172	296	N 3500	N 8000	N $10^4$ *	N $10^4$	"		$\infty$
5B						N 1000	N 1000	"		N 2000
6A	58	276	171	165	798	N 1300	N $6 \cdot 10^3$ *	"		$\infty$
6B						143	494	N 1200		N 2000
7										
8										
9										
0										
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										

\* large & small: small in first preponderance  
 1A-20': large or of numbers as large as in 2A 20'





Hfr<sub>2</sub>

T-L : 2 1/2 minutes 5' Pr  
 L-Pr : 5 minutes 7 1/2 L  
 12 1/2 Pr

Hfr<sub>1</sub> : medium 1

Entry of Pr between 10' and 20'

Repeat 1:

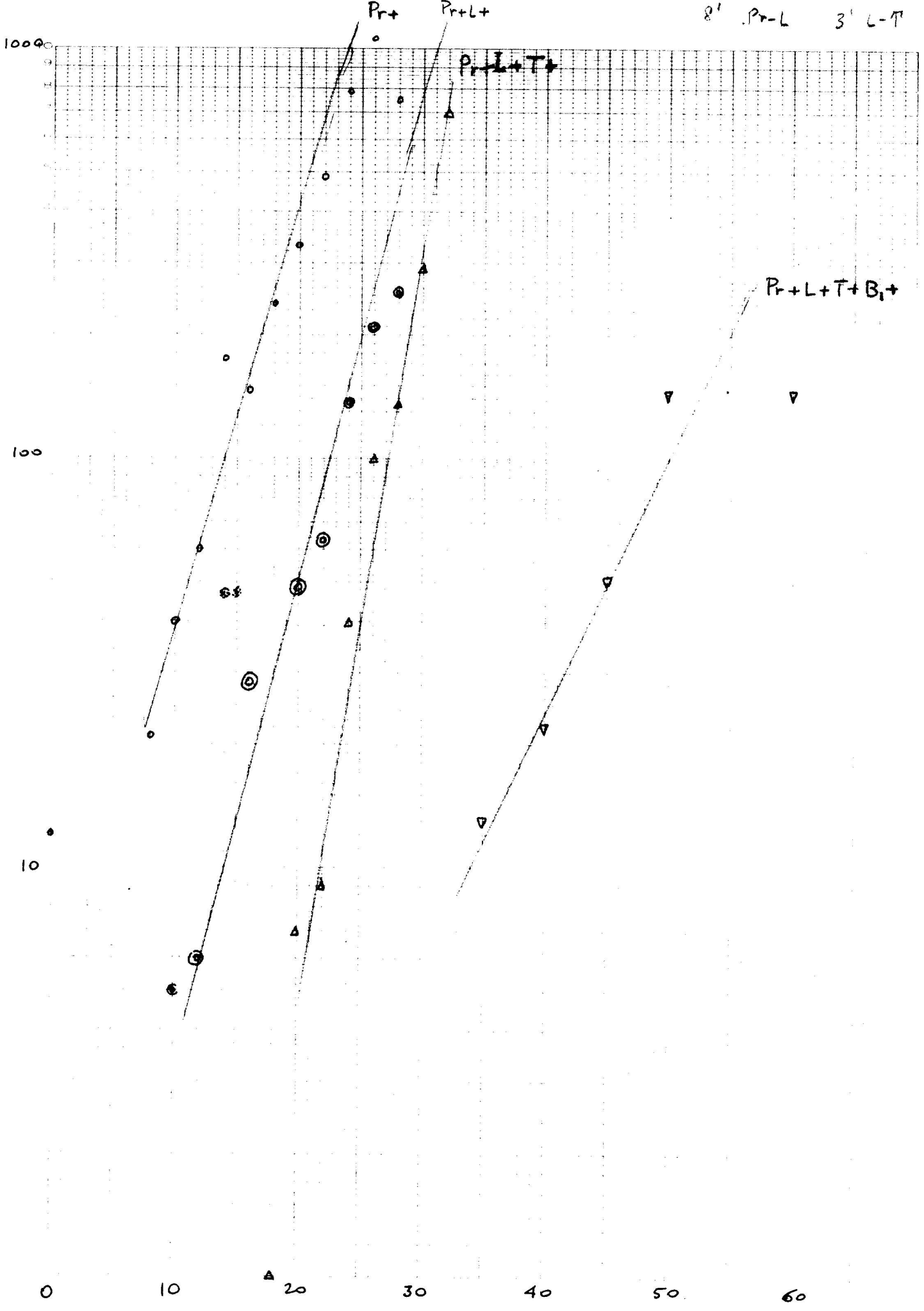
medium 2

Entry of LT probably between 15 or 20 + 25'. But explore 10-30' again

medium 3

Entry of B<sub>1</sub> between 35' and 40'  
 test for Xyl Meth.

2' Pr-L 3' L-T



Saturday, June 28, 1958

1401/6

TIMED MATING, Hfr<sub>1</sub> and Hfr<sub>2</sub>

ORC Cultures of W 3936 (Hfr<sub>1</sub>), W 3948, (Hfr<sub>2</sub>) W 3052.

Spun, resuspended in equal amount of Penassay, and mating mixtures prepared in flasks with 1.5 ml ♂ and 6 ml ♀ and 2 ml fresh broth. Samplings: 0.2 ml at every time, dilution in 9.9 ml chilled water, .04 spread on plated (A) and the same for dilution (B) of 1/10 for Hfr<sub>1</sub> and 1/50 for Hfr<sub>2</sub>.

Media:	1.	D-0	+	Sm	B <sub>1</sub>	M	L	T	
	2.			Sm	B <sub>1</sub>	M		T	for Hfr <sub>1</sub>
	3.			Sm	B <sub>1</sub>	M			
	4.			Sm		M			
	5.	B Lac		Sm					
	6.	D-0	+	Sm	B <sub>1</sub>		L	Prol	for Hfr <sub>2</sub>
	7.		+	Sm	B <sub>1</sub>			Prol	
			+	Sm	B <sub>1</sub>				



DATE:

June  
Sat. 28, 1958

REF:

1401/6

1 2 3 4 5 6 7 8 9 10

TIMED MATING, Hfr<sub>1</sub> x Hfr<sub>2</sub>.

O.K.C. Cultures of W 3936 (Hfr<sub>1</sub>), W 3948, (Hfr<sub>2</sub>) W 3052.

Spun, resuspended in equal amount of Penellay, and watery mixtures prepared in flasks with 1.5 ml ♂ + 5 ml ♀ + 2 ml fresh broth - samplings: 0.2 ml at every time, dilution in 9.9 ml chilled

water, .04 spread on plates (A) and (B) of 1/10 for Hfr<sub>1</sub>

and 1/50 for Hfr<sub>2</sub> -

Media:

- |    |                         |      |     |       |   |  |
|----|-------------------------|------|-----|-------|---|--|
|    |                         |      | M   | L     | T |  |
| 1. | D-0 + Sm B <sub>1</sub> | Meth | ten | three |   | } sel for R <sub>1</sub><br>for Hfr <sub>1</sub><br>for Hfr <sub>2</sub><br>(copies) |
| 2. | Sm B <sub>1</sub>       | Meth |     | three |   |  |
| 3. | Sm B <sub>1</sub>       | Meth |     |       |   |  |
| 4. | Sm                      | Meth |     |       |   |  |
| 5. | B Lac Sm                |      |     |       |   | } for Hfr <sub>2</sub>   |
| 5  | D-0 + Sm B <sub>1</sub> |      | ten | Prod  |   |  |
| 6. | + Sm B <sub>1</sub>     |      |     | Prod  |   |  |
| 7. | + Sm B <sub>1</sub>     |      |     |       |   |  |

10

20

30

40

50

COUNTS

(same for counts)

1401/6

Medium 1

each column: +, -, ±

July 12 19 58

REF:

1	2	3	4	5	6	7	8	9	10
TIME	Ara	T <sub>1</sub>	Az	MTL	(M) MB <sub>1</sub>	MLB <sub>1</sub>	TLB <sub>1</sub>	MTLB <sub>1</sub>	Σ
<del>1</del>	<del>13, 76, 0</del>	<del>76, 10, 3</del>	<del>13, 76, 0</del>	<del>1, 79, 9</del>	<del>9, 79, 1</del>	<del>9, 80, 0</del>	<del>14, 0, 75</del>	<del>10, 0, 79</del>	<del>89</del>
2	13, 76, 0	76, 10, 3	13, 76, 0	1, 79, 9	9, 79, 1	9, 80, 0	14, 0, 75	10, 0, 79	89
3	15, 52, 0	45, 16, 6	15, 51, 1	1, 54, 12	14, 53, 0	12, 54, 1	15, 0, 52	15, 0, 52	67
4	15, 52, 0	45, 16, 6	15, 51, 1	1, 54, 12	14, 53, 0	12, 54, 1	15, 0, 52	15, 0, 52	67
5	6, 76, 0	66, 6, 10	12, 69, 1	6, 76, 0	6, 76, 0	6, 76, 0	6, 76, 0	6, 76, 0	82
6	6, 76, 0	66, 6, 10	12, 69, 1	6, 76, 0	6, 76, 0	6, 76, 0	6, 76, 0	6, 76, 0	82
7	6, 54, 0	48, 11, 1	6, 54, 0	0, 54, 6	6, 54, 0	6, 54, 0	6, 0, 54	5, 0, 55	60
8	6, 54, 0	48, 11, 1	6, 54, 0	0, 54, 6	6, 54, 0	6, 54, 0	6, 0, 54	5, 0, 55	60
9	6, 59, 1	49, 11, 6	12, 54, 0	1, 63, 2	4, 56, 6	3, 63, 0	3, 0, 63	3, 0, 63	66
0	6, 59, 1	49, 11, 6	12, 54, 0	1, 63, 2	4, 56, 6	3, 63, 0	3, 0, 63	3, 0, 63	66
1	12, 84, 0	73, 16, 7	17, 74, 0	0, 90, 6	8, 84, 4	6, 90, 0	5, 0, 91	5, 0, 91	96
2	12, 84, 0	73, 16, 7	17, 74, 0	0, 90, 6	8, 84, 4	6, 90, 0	5, 0, 91	5, 0, 91	96
3	5, 65, 0	55, 8, 7	8, 61, 1	0, 67, 3	4, 64, 2	3, 67, 0	2, 0, 68	3, 0, 67	70
4	5, 65, 0	55, 8, 7	8, 61, 1	0, 67, 3	4, 64, 2	3, 67, 0	2, 0, 68	3, 0, 67	70
5									
6									
7									
8									
9									
0									
1									
2									
3									
4									
5									
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7									
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6									
7									
8									
9									
0									

Medium 2

sm B, MT Hfu

	Ara	T <sub>1</sub>	Az	MB <sub>1</sub>	MB <sub>1</sub> T	Σ
20	42, 5, 0	42, 5, 0	4, 3, 40	25, 22, 0	47, 0, 0	47
22	43, 17, 0	3, 6, 51	51, 8, 1	18, 42, 0	60, 0, 0	60
24	71, 25, 4	6, 0, 94	98, 1, 1	37, 63, 0	100, 0, 0	100
26	92, 15, 0	13, 2, 92	104, 3, 0	48, 59, 0	107, 0, 0	107
28	80, 15, 5	4, 94, 0	99, 1, 0	58, 42, 0	100, 0, 0	100

Medium 3

sm B, M Hfu

	Xyl	Mil	B <sub>1</sub>	M	MB <sub>1</sub>	Σ
0	0, 3, 0	0, 3, 0	3, 0, 0	1, 2, 0	3, 0, 0	3
10	0, 1, 0	0, 1, 0	1, 0, 0	0, 1, 0	1, 0, 0	1
14	0, 9, 0	0, 9, 0	7, 2, 0	3, 6, 0	9, 0, 0	9
16	0, 1, 0	0, 1, 0	1, 0, 0	0, 1, 0	1, 0, 0	1
18	0, 1, 0	0, 1, 0	1, 0, 0	0, 1, 0	1, 0, 0	1
20	0, 8, 0	0, 8, 0	8, 0, 0	0, 8, 0	8, 0, 0	8
22	0, 13, 0	0, 13, 0	11, 2, 0	0, 3, 0	13, 0, 0	13
24	1, 42, 0	0, 43, 0	41, 2, 0	2, 41, 0	43, 0, 0	43
26	0, 100, 0	0, 100, 0	100, 0, 0	1, 99, 0	100, 0, 0	100
28	0, 99, 0	0, 99, 0	99, 0, 0	0, 99, 0	99, 0, 0	99
30	0, 100, 0	0, 100, 0	100, 0, 0	0, 100, 0	100, 0, 0	100
35	0, 70, 0	0, 70, 0	70, 0, 0	0, 70, 0	70, 0, 0	70



COUNTS

1401/6

Each Column: +, -, ±

July 12, 1958

REF:

	1	2	3	4 <u>Medium 6</u>	5	6 sm B, P	7	8 Hfr	9	10
		Gal	Lac	T <sub>1</sub>	Az	B <sub>1</sub>	B <sub>1</sub> P <sub>1</sub>	Σ		
1										
2										
3	0	0, 1, 0	0, 1, 0	1, 0, 0	1, 0, 0	0, 1, 0	1, 0, 0	1		
4										
5	5	0, 2, 1	0, 2, 1	3, 0, 0	2, 1, 0	1, 3, 0	3, 0, 0	3		
6										
7	6	0, 1, 0	0, 1, 0	0, 0, 1	1, 0, 0	1, 0, 0	1, 0, 0	1		
8										
9	7	0, 8, 0	3, 5, 0	3, 2, 3	6, 2, 0	5, 3, 0	8, 0, 0	8		
0										
1	8	0, 2, 0	0, 2, 0	1, 0, 1	2, 0, 0	0, 2, 0	2, 0, 0	2		
2										
3	9	0, 10, 0	9, 1, 0	0, 2, 8	10, 0, 0	9, 1, 0	10, 0, 0	10		
4										
5	10	0, 8, 0	0, 7, 1	7, 1, 0	3, 5, 0	1, 7, 0	8, 0, 0	8		
6										
7	11	0, 2, 0	2, 0, 0	0, 0, 2	2, 0, 0	2, 0, 0	2, 0, 0	2		
8										
9	12	0, 67, 0	1, 66, 0	56, 6, 4	37, 29, 1	2, 65, 0	67, 0, 0	67		
0										
1	14	0, 30, 0	11, 19, 0	15, 6, 9	27, 3, 0	11, 19, 0	30, 0, 0	30		
2										
3	16	0, 99, 0	1, 98, 0	68, 21, 10	72, 27, 0	6, 93, 0	99, 0, 0	99		
4										
5	18	0, 6, 0	0, 6, 0	5, 1, 0	3, 2, 1	0, 6, 0	6, 0, 0	6		
6										
7										
8										
9										
0										
1										
2										
3										
4										
5										
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8										
9										
0										

Medium 7 sm B, Hfr

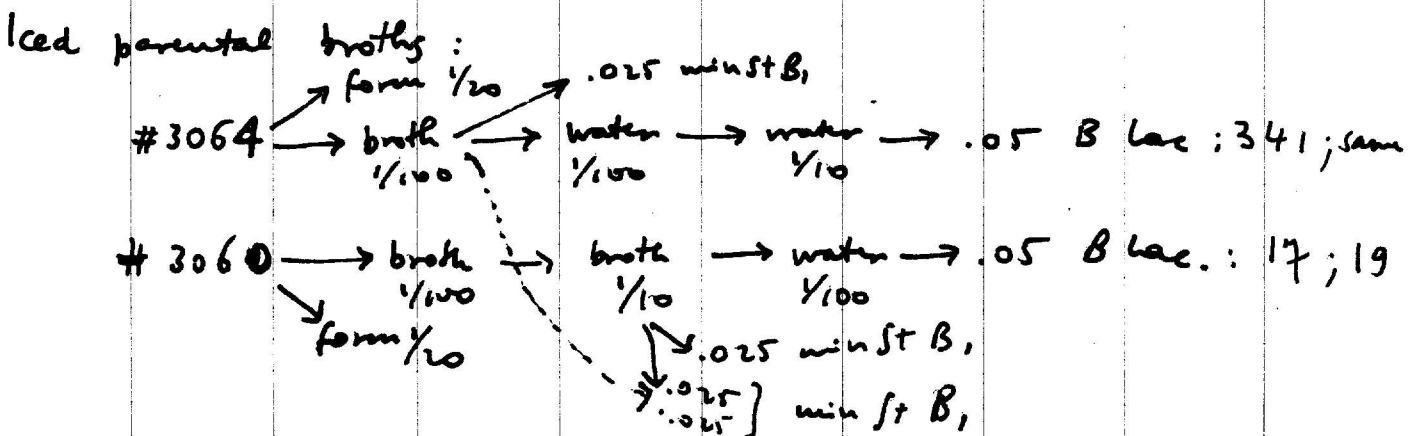
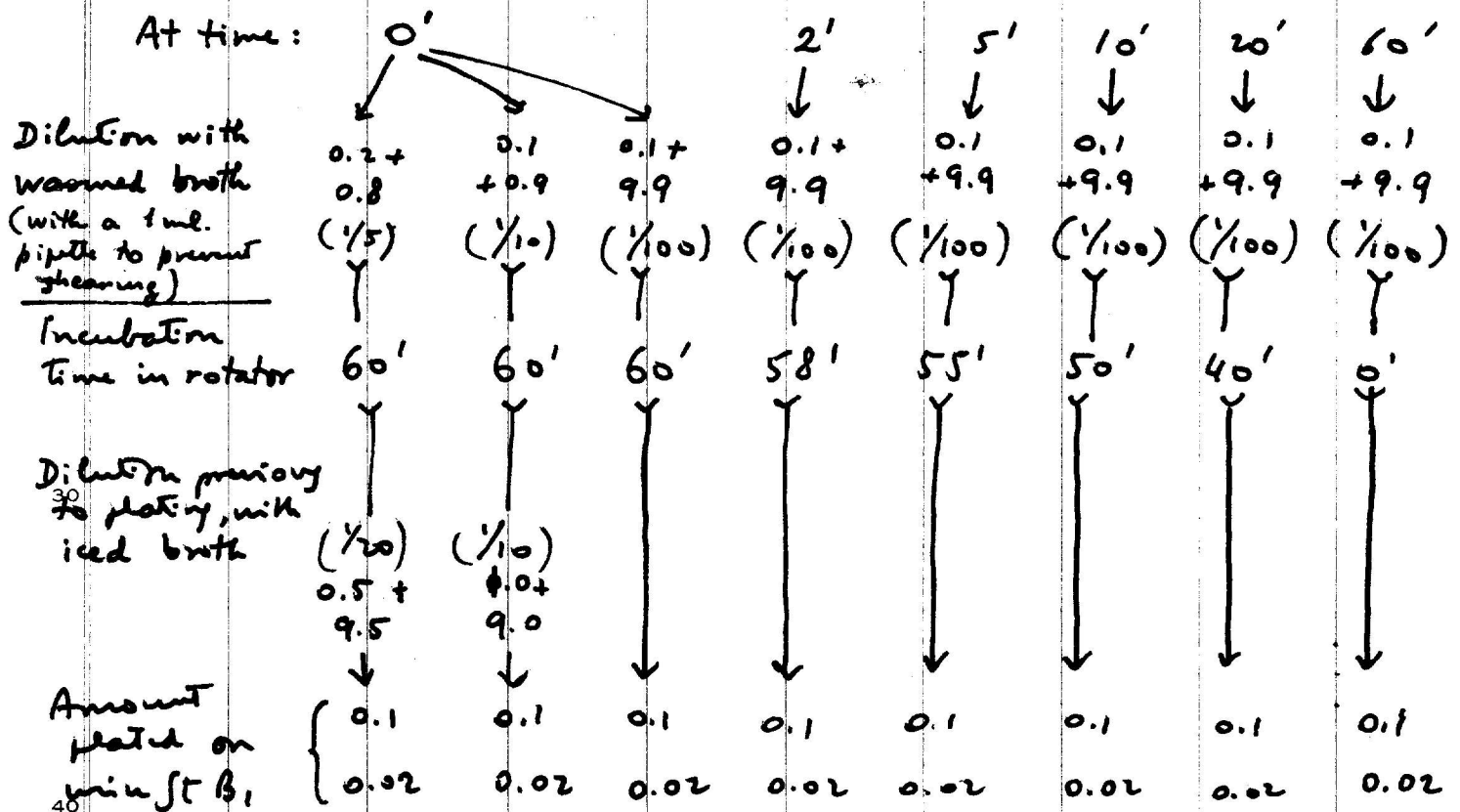
	Gal	Lac	T <sub>1</sub>	Az	Σ
0	1, 0, 0	1, 0, 0	1, 0, 0	1, 0, 0	1
10	1, 0, 0	1, 0, 0	0, 0, ±	1, 0, 0	1
12	0, 1, 0	0, 1, 0	0, 0, 1	1, 0, 0	1
14	0, 5, 0	5, 0, 0	4, 1, 0	5, 0, 0	5
16	0, 30, 0	8, 22, 0	0, 2, 28	30, 0, 0	30
18	0, 28, 0	7, 21, 0	5, 1, 22	28, 0, 0	28
20	0, 81, 0	23, 57, 1	3, 49, 29	81, 0, 0	81
22	0, 101, 0	41, 59, 1	7, 54, 40	101, 0, 0	101
24	0, 11, 0	5, 6, 0	0, 7, 4	10, 1, 0	11
26	0, 100, 0	57, 43, 0	7, 17, 76	100, 0, 0	100
30	0, 8, 0	4, 4, 0	0, 4, 4	8, 0, 0	8

DATE: March 24

REF: OPTIM. PULSE

13.15 : Broth cultures 3060, 3064 from water bath, saturated since 2 hrs, inoculated into warmed Penassay 0.75 + 7.5 ml → givello.

10 14.35 : 0.5 ml. # 3060 } → prewarmed, → givello and also  
 5 ml # 3064 }  
 5 ml broth } samples from parental culture viced.



DATE: March 28, 1957 - Summary

REF: 1402: PULSE

TIME	Gal+/tot. 2	Lae+/tot 4 (including w)	T <sub>1</sub> <sup>+</sup> /tot. 6	7	8	9	10
0'	4/31	12.9	4/31	21/31	68.0		
2'	5/57	8.8	(9/31) 21/57	29.0	39/57	68.4	
5'	12/81	14.7	(28/57) <del>40/81</del>	49.2	68/81	84.0	
10' 10	13/79	16.4	(42/81) 33/79	52.0	61/79	77.2	
20'	11/65	16.9	(36/79) 31/65	45.5	46/65	70.8	
60'	5/50	10.0	(31/65) 15/50	47.7	37/50	74.0	
			(18/50)	36.0			
0' 1/5	5/70	7.2	15/69	21.7	48/70	68.6	
0' 1/8	6/52	11.5	(20/69) 19/52	36.5	36/52	69.2	
			(19/52)				

Conclusions. 5'-10' Pulse is an efficient procedure to raise No. of recombinants.

Dilution of 1/100 at least after pulse if further increase must be avoided - segregation ratio unaffected, provided dilution is carried out with 1 ml. plates. 0.1 ml. plates not attempted. Interruption on simple dilution in exp. 1401 probably due to chilling, or plating.

Experiment 1403, not successful because of relation of F+ isolate from Hfr, must be repeated

1402 b

DATE: March 24.

REF: PULSE.

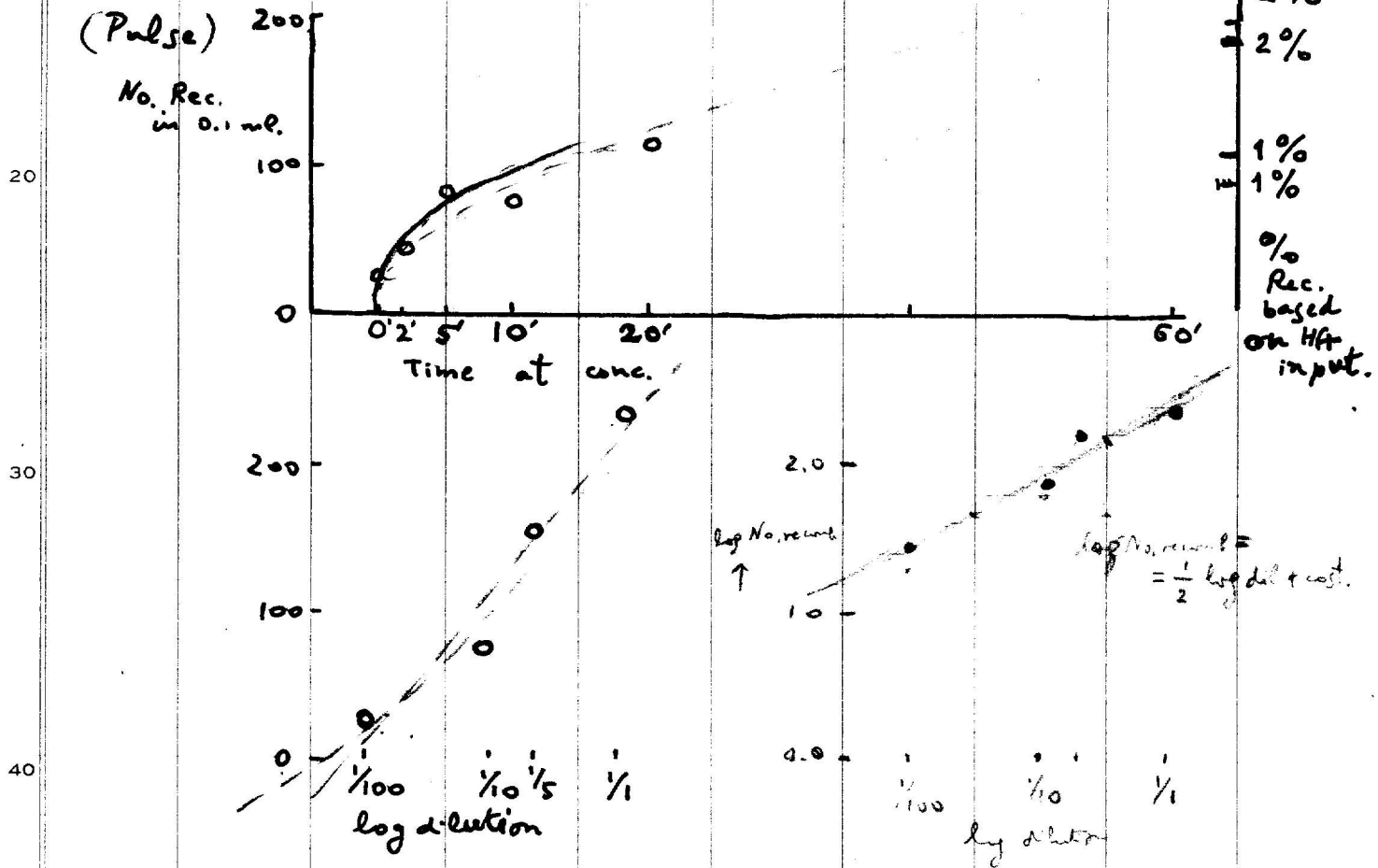
Plate counts.

Amount plated	Time	dilution 1/100					Time of d.e.			
		0'	2'	5'	10'	20'	1/100	1/10	1/5	1/1
0.1		28 <sup>†</sup>	47	85	77	118	236 <sup>‡</sup>	74	167	236 <sup>‡</sup>
0.02		2 <sup>†</sup>	10	15	13	25	31 <sup>‡</sup>	11	23	31 <sup>‡</sup>

†, ‡ : same values, repeated

(Pulse)

No. Rec. in 0.1 ml.



MICROSC. COUNTS OF PARENTAL BROTHS:

# 3060 :  $230 \times 10^6/\text{ml}$

# 3064 :  $1.4 \times 10^9/\text{ml}$

INPUTS per ml : 3060  $11.5 \cdot 10^6/\text{ml}$  Ratio Hfr/F- = 1:60  
 3064  $700 \cdot 10^6/\text{ml}$

REF: 1402-2

DATE: 22/4/58.

6 7 8 9 10

3<sup>h</sup> (1+10) to approx. saturation.  
for morphology & thr-ness.  
20 x negatively:

ml in 50 ml flask in water bath  
0.05 to 50 ml prewarmed broth.  
in plating (Hfr: F- = 1:2)  
conc. susp. dil 1:3; exp. repeated  
ove.

conc. suspensions dil. 1:33; exp.  
ctly as above.

and kept at 0° until used.  
broth } 0.1 & 0.02 ml  
broth } with StB<sub>2</sub>.

recombination: 1X suspensions  
and plated 0.05 + 0.05

difficult by smearing (wet plates used)  
counts: 1 colony.  
0.1 0.02 ml plated  
250 44 Note:  
400 63 counts unreliable  
1200 148

1 2 3 4 5

3060, 3064, cultures rotated  
New 3060 isolate tested  
Concentrated 10 x, and

10 x Exp: 0.2 ml + 0.2  
for 1', then  
30' inc. the  
3 x Exp 10 x & 20 x  
exactly as ab

1 x Exp. 3 x & 6 x  
repeated 2 x

All 1:1000 dilutions chilled  
10 x : 1/10 in  
3 x : 1/3.3 in  
1 x plated

For estimation of plate  
diluted 1:1000 in brot

Plate counts: made d  
Control of plate recomb

Conc 1 x  
3 x  
10 x

10  
20  
30  
40  
50





	1	2	3	4	5	6	7	8	9	10
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*mm mm mm mm*

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