Experiments on Interrupted Mating
of E. coli.

Collaboration with Luca Cavalli April – June 1958.

5·3·58

E.COLI Specific Projects 1400

P 1. Mathematical Theory of mating

P 2. Entry vs. Pairing

DIPLOIDS ; RETRIEVAL OF UNP. MARKERS; BLENDING S/ INTERRUPTION; ABORTIVE RECMB'TS ; DNA LEAKAGE AND VIABILITY ; DIFF'L INHIB'n (AZIDE RESISTANCE)

P 3 PARAMETRIC DETAILS : PULSE EXPTS. VARIATION IN ENTRY; DIFF'L TEMP. EFFECTS ON MATING-VS. ENTRY; INTEGRATION; POST-EL. MARKERS ; SPONT. INTERRUPTION; Lp$^+$ TRANSFER.

P 4. MAPPING : LAC ; GAL ; Lp ; Gal exogenote ; OTHER HFR'S; RAPID METH

P 5. OTHER ISSUES: (Hfr × F$^-$) F$^+$; Holoclonal F$^+$; Enumerate Hfr mutants ; complementary crossing-over;

P 6. Nutrition of W3060... Use of M$^-$ × TL$^-$ for pulse (s sm!).

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19 INDEX-1 REF:

1401

TIMED MATING WITH $11/T_2$.

(1) Non pulsed. Scored approx every 10'. TL+. Gal+, T_1
3000 × 3084

(2) Pulsed. Scored every 5'. TL+ Gal+ but T_1
3000 × 3084

(3) Non pulsed. Scored every 5'. T+, TL+, Pr+. other markers Gal+ as
3050 × 3052 for T_1

1402

(4) PULSE

(1) 3000 × 3084 - Various relations tested by pairing 4a with 1
Each of 4 strains was treated 2x every 1' with
and 30' interval

(2) 3000 × 3084 10x, 1x, 1x, 4 testing - Effect noted may be due to first 30' incubation

(3) 3000, 1895 × 3084. pulse with 5bn ion, 2d, Decrease in colony and zone
min delete mating negative effect of pulse.

(4) 3000 × 3084 room. 3x, 1a. conc. kept at 1' one zone, then put for
after 1' pulse. No effect of pulse.

(5) 3000 × 3084 - 3rd, same as 1402, 2. pulse 1' → some decrease in
pulse effective

(5) 3000 × 3084. same as 1402, 1895 pulse 1' then transf to Pen
and comparison with unrelated. Very small effect of pulse. B.A

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INDEX-2

19

REF:

1403 MUTATIONS

(1) Negative: $ff^- \rightarrow F^+$

(2) Effect of pipetting, Oil, 10 min Nutrient, shaking, blending, sonifying, plating & blending effects on interrupters. Sonic, Vibrating

(3) Test by spreading vs plating by blending, only the former interrupts

(4) Stability of pairs to plating - No recombinants formed - Cause of failure unknown

1405 PROTOPLASTS.

(1)

Detailed theory of mating; pulses etc. 1400 Pl. 4-30-58

A Assume linear mating. B Pulse mating

1. Variable time + rate of entry
2. Spontaneous breakage
3. Age-dependent integration (Poissonian c-o.)
4. Minor killing.
5.

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1958. March — REF: 1400

HISTORY OF # 3060.

When first used (3/21)

DATE: REF:

1	2	3	4	5	6	7	8	9	10
W 2945 —		$Hfr_4 Lp_5 TL O_1 S^R Mal_6$.							
W 2752		$Hfr_4 Lp^S$.							
3752		$Lp^S Hfr_4 Gal_6$							
3024		$Hfr_4 Gal_2$							
3023		$Hfr_4 Gal_2 Lp^S$							
3308		$Lp^S Hfr_4$ $\bar{I}_{W677}$.							
2324		$Hfr_2 B_1$							
2323		$M-St^R Az^R$							
3051		$F-Lp^+ TL Th$ Lac, Gal, Xyl, Ara, $V_1 V_6 S^R$							
3064		$F-Lp^+ TL Th$ Lac, Gal, Xyl, Ara, $V_1 V_6 S^R \lambda_2$ Mal							
3060		Hfr_2 B_1 + from 2324 —					Az^S		
3774		Yudkin's E. coli M2							
3514		E. coli 36							
3515		E. coli R1.							
3133		F- Lac-							
2735		Lp^+ ~~Gal_2~~ F- Het $TL B_1$ Lac, V_1 (Gal,) Az^S							
2323		$M- Hfr_2 S^R Az^R$.							
3908		Lp^+ F- tryp $Gal_6 S^R$.							
3801		$T-S^R$ Fl.							

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REF:

	1	2	3	4	5	6	7	8	9	10
1	W	3052	-	F- Lp^+ Pr- TL Th Gal_2 Lac, Xyl_2 Ara_2 Mtl Sf^R.						
2										
3										
4				(W945)-						
5										
6										
7	W	2914	-	F- Pr. TL Th Lac, Gal_2 Ara_2 Xyl Mtl.						
8										
9										
0										
1	W	3934	-	Hfr_2 Gal- (from 2323 x 945)-						
2										
3										
4	W	3960	-	F- Try- S^R Gal_4- Gal 6-						
5										
6										
7	W	3974		F- Lp^+ Gal-						
8										
9										
0	Lac	Sm-4								
1										
2	W	3975		F- Lp^+ Gal-						
3										
4	W	3953	-	F- Lp^+ Gal_2 Sf^R						
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										

j1480 C

DATE: 4/22/58 REF:

In previous exp, an alledged auxotroph (I, B,⁻) tested prototrophically. Now retest this same inoculum (= #18 from A) and also 13A, 14A, 16A, and slant. ~~made from orig~~

			1 DO	2 I, B,	3 I	4 B,
A	slant		−	±	±	+
B	13A		+++	+++	+++	+++
C	14A		+++	+++	+++	+++
D	16A		+++	+++	+++	+++
E	18A		+++	+++	+++	+++
F	8		−	−	−	−

Control: old Isoleucine −

~~A~~B: Slant + aa : −
+ Lys −
Arg −
M −
C
M, L +

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Selection of new 3060.
Cross W2523 x W945 on D(o)
Streaks on β Lac.

May 10 19 58

REF:

	1	2	3	4	5	6	7	8	9	10
	DO	Hfr	Gal	Lac	Ara	Xyl	Man	Mtl		
1	+	−	+	−	+		−	−		
2	+	−	−	−	+	−	−	−		
3	+	−	−	−	+	−	−	−		
4	+	−	+	−	−	−	−	−		
5	+	−	+	−	+	−	−	−		
6	+	−	−	−	+	−	−	−		
7	+	−	+	−	+	−	−	−		
8	+	−	−	−	+	−	−	−		
9	+	−	−	−	+	−	−	−		
0	+	−	−	−	+	−	−	−		
1	+	(+)	−	−	+	−	−w	−		
2	+	− (al)	+	−	+	−	−	−		
3	+	−	+	−	+	−	−	−		
4	+	−	+	−	+	−	−	−		
5	−	−	−	−	+	−	−	−		
6	+	−	+	−	+	−	−	−		
7	+	−	+	−	+	−	−	−		
8	+	−	+	−	+	−	−	−		
9	+	−	−	−	+	−	−	−		
0	−	−	−	−	+ −	−	−	−		
1	+	−	+	−	+	−	−	−		
2	+	−	+	−	+	−	+	w		
3	+	−	−	−	+	−	−	−		
4	+	−	−	−	+	−	−	−		
5	+	−	−	−	+	−	−	−		
6	+	−	−	−	+	−	−	−		
7	+	−	+	−	+	−	−	−		
8	+	−	−	−	+	−	−	−		
9	+	−	−	−	+	−	−	−		
0	+	−	+	−	+	−	−	−		
1	+	−	−	−	−	−	−	−		
2	+	−	−	−	+	−	−	−		
3	+	(+)	+	+	+	−	−	−		
4	+	+al	+	+	+	−	−	−		
5	+	−	+	+	+	−	−	−		
6	+	+	−	+	+	−	−	−	←	
7	+	−	−	+	+	−	−	−		
8	+	+al	+	+	+	−	−	−		
9	−	− al	+	+	+	+	w	w		
0	+	−	+	+	+	−	−	−		
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										

(+) = confluent growth

Sm R

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10^{-6} dil.

3060
Tempra lure - Plates

May 12, 1958

REF:

	1	2	3	4 @ 30	5	6 @ 37	7	8	9	10
1	Sm 30			~ 300		~500				
2										
3	Sm 30 + B_1			~ 250		~ 150 ?				
4	" Lac			~ 250		~ 500				
5	Lg 30			~ 20		0				
6										
7	Lg 30 + B_1			~ 200		~ 200				
8	" Lac			~ 200		~ 20				
9	Sm 37			~ 300		~ 150				
0										
	Sm 37 + B_1			~ 100		~ 100 ?				
1	" Lac			~ 300		~ 300				
2	Lg 37			~ 300		~ 100				
3										
4	Lg 37 + B_1			~ 200		~ 200				
5	" Lac			~ 300		~ 300				
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										

B_1 inhibition ?

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2323 X 945 X's

May 16 1958

REF:

	1	2	3	4	5	6	7	8	9	10
	DO	Hfr	Gal	Lac	ara	Xyl	Mal	M+l		
1	+	+	—	+	+	—	+	—		
2	+	+	—	—	+	±	+ (±?)	+		
3	+	+	+	+	/	+	+	—		
4										
5		↓								
6										
7		NO!								
8		Sm^R!								

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3060's
Temp. Conf Effect

May 9 19 58

REF:

2 days ago, sparse growth from dilute streakes on DO (No B1) were suspended in little water (i.e., from lg + sm colony streakes) & streaked ☐ "same" amount of inoculum on DO & incubated @ 30 & 37 C.

	30 C	37 C
@ 20 Hrs:		
Large	Sparse growth in brush	ea
Small	"	few (~10) sm. col.
@ 2 Days:		
Large	Good growth	Good growth
Small	Good growth	Few lg col, + few tiny satellites

1 lg colony from each of above streaks was picked + put in 9 ml saline on rotator @ 37 C. End of day:

10⁻⁶ dilutions of each spread on B lac and DO & incubated @ 30 + 37. Ditto in DO + DO + B1 (ie, @ 30 + 37).

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3060's

May 5 1958

REF:

"Prototrophic" 3060 (from Goffhil) plated on B lac to ~ 100 col/plate, which was replicated to DO and to DO B1, Sm & W3064 for Hfr testing. All colonies were prototrophic, and about 2/3 were Hfr. Next pick + streak one Hfr and one non-Hfr to single colony test for Hfr + prototrophy.

8 Hfr −'s → NOIN Hfr and prototrophic (on agar)

8 Hfr +'s → Hfr and prototrophic

but dilute inocula grew on DO·B1 and not on DO unsupplemented.

Also: streak of 3060 (water suspension from same slant as broth for above was prepared from) grew very poorly - few large colonies plus few satellites. Both streaked on B lac (no morphological difference). Both made from 5 c of 10 cm. Now test on DO + DO B1, both liquid + agar D.Ho for 3870 (difference on DO not so pronounced).

		Liquid		Agar		Dilute	
		DO	DO+B1	DO	DO+B1	DO	DO + B1
3870	Large	.400	.630	+	+	+	goodish
"	Small	.454	.620	+	+	+	sl
3060	Large	.527	.530	+	+	+	poorish
"	Small	.408	.590	+	+	+	sl

all Hfr

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Selection for Hfr prototrophic
"3060"

May 9 19 58

REF:

Crosses of 2323 X 945 (by Luca) on DO single colony streaked (total of 40). 1 colony from each streak was put in little penassay; at end of day each was streaked against 3064 on DO for ~~Sm~~ Str test B, + little water dilutions were streaked on DO for prototrophy test, ~~+ B lac for lactose ferm.~~

Also DO B, for auxotrophy.

Also spot on Lac, Gal, ~~Mal~~, Xyl, ~~Mtl~~, ~~Gly~~a.

Of late weeks [illegible] both

(+) count before -ypd.

1401

must be done in selecting primaries

DATE: REF:

	1	2	B Gal	Lac	Ts 1	6	7	8	9	10
*	0A *	16	all −							
*	Picked									
*	0C *	7	all −							
	5A *	26	all −							
10	10A *		all −							
	10B *	6	all −							
	10C *	23	all −							
	10D *	6	all −							
	20A R	(Replica plated to Gal)	1+							
20	20B *	5	−	"Lac−"? scanty						
	20C R	399	all −							
	20D *	38	−	−	ng.					
	30B	(65)	1+	15 (trace Gal+) (a)	? but all Lac+ are V_1^S					
30	30C R	(551)	7+							
	30A		8+							
	30D	(48)	1+ (very slow)	16 (B)	~19	all Lac+ are V_1^S				
	45A		47+							
	45B	(72)	8+	[15++ 12±]	3	Lac+ are V_1~		all Gal+ are Lac+		
40	45C	10×58	47+							
	45D	(58)	2+							
	60A	10×94	47+							
	60B *1	(47)	1+ → 1+		1.3	$4V_1^R$				
	60C *2	(10×52)	56+							
50	60D		2+ → 2+							

α: true Lac phenotypes: 9++ 6± 5++ 11±
(mixed 8 Gal+ 1 Gal+)

DATE:

REF:

1	2	Bbal	Lac	5	6	7	8	9	10
120 B	(257)	19+							
120 D	248	25+							
120 C	10x150	~100+							
120 B**	40 picked	6+ and +/-							

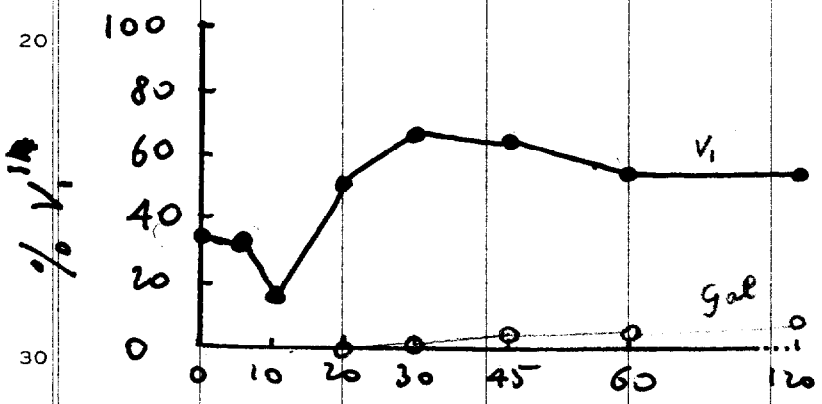
Notes on Lac: two batches of B Lac. - blue + yellow. Man F ai
on blue, only Gal+Lac+ shows
negative at 1' reading.

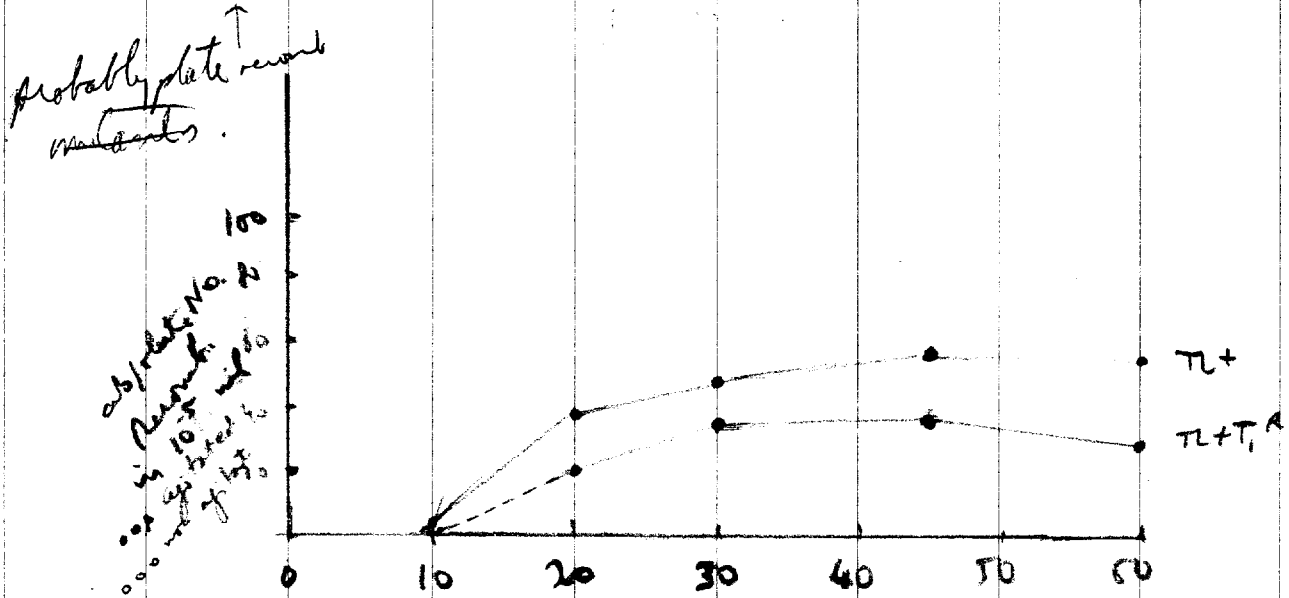
1/total V_1 segregation

DATE:

REF: 1401

	0	5	10	20	30	45	60	120	9	10
A	5/16	8/24	11/53							
B			2/6	26/53	44/65	42/72	25/48	47/92		
C	3/7		1/24							
D			0/6	20/38	31/48	41/58	19/33	31/49		
tot.	8/23	8/24	14/89	46/91	75/113	83/130	44/81	78/141		
	34.7%	33.3%	15.7%	50.6%	66.2%	63.8%	54.5%	55.2%		





1401a

DATE: March 21st, 1958 REF: TIMED MATING

Hfr 2

Overnight broth cultures:

3060 Hfr 2 ; # 3064 : F- lac+ T1B1 Lac, Gal, Xyl, Ara, V1 V6 S^R λ2 Mal

1.25 p.m.. Overnight broth cultures 1 ml + 10 ml warm broth. Pencillin broth throughout. ↓ girello.

Note: 3064 is 3-4X more dense (by inspection) than 3060.

2.55 p.m.

0.2 ml 3060 broth + 10 ml 3064 broth + 10 ml warm broth. 2×10^8. 2×10^6/ml Hfr

Distributed in 2 ml amounts in 8 tubes (7) → girello (1) → ice (time 0)

Also: 0.1 broth culture + 0.9 ml formalin 1% for count.

Tubes iced at: 0'; 5'; 10'; 20'; 30'; 45'; 60'; 120'

From each 2 ml tube: → 1 ml, blended 1'. → 1 ml, kept in ice.

then dilution with chilled waters, plating (minSt B1)

0'	: nondiluted,	: →	0.1 ml;	0.01 c in plate
5'	diluted 1/10		0.2 ;	0.02
10'	"		0.1 ;	0.02
20'			0.1 ;	0.01
30'			0.1 ;	0.01
45'			0.1 ;	0.01
60'			0.1 ;	0.01
120'			0.1 ;	0.01

0', 5': agitation before dilution: too small volume - Foam

≥ 10': agitation after dilution -

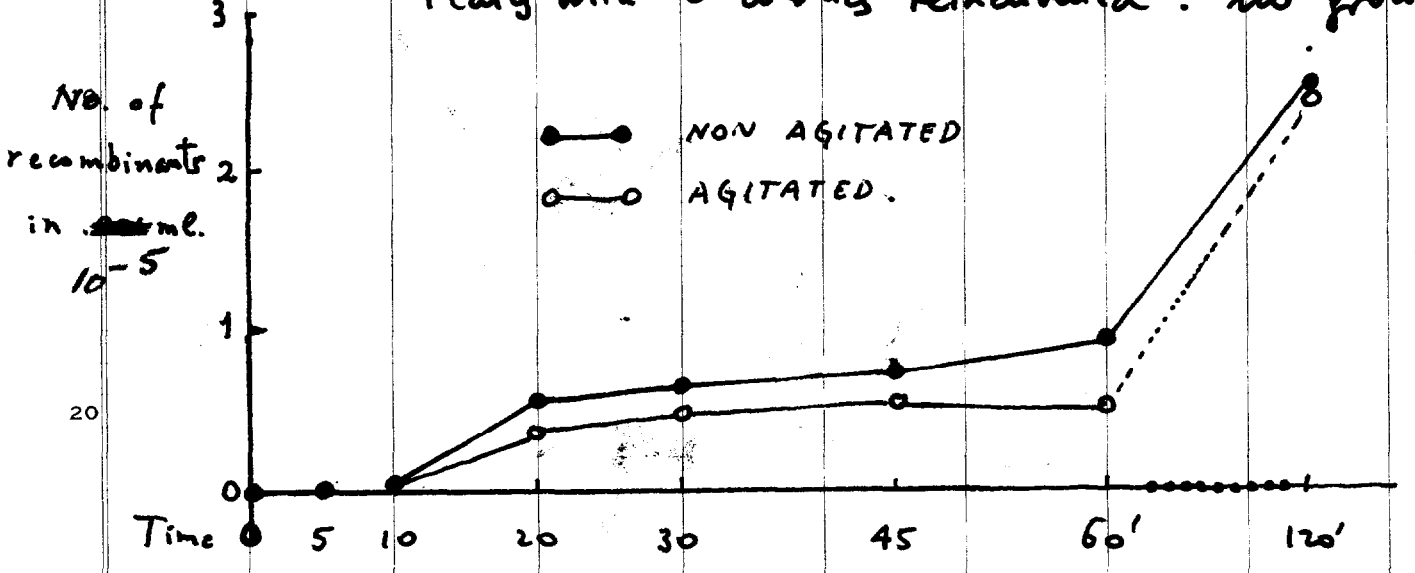
1401 b

[519].

DATE: March 23, 1958. REF: TIMED MATING

Time	0'	5'	10'	20'	30'	45'	60'	120'
NON AGIT. A	.1 : 17	.02 : 29	.01 : 63	.01 [265]	[370]			
B	.01 0	.002 : 0	.002 : 6	.001 : 57	: 65	: 72	: 94	: 257
AGITATED C	.1	.02 : 0	.01 : 26	.01 : ∞ 399	551			
D	.01 0	.002 : 0	.002 : 6	.001 : 39	: 48	: 58	: 52	: 248.

Plates with 0 colonies reincubated : no growth.



Parent cultures tested for purity : lac, gal, mal, xyl

Segregation : 20 B : 54 gal-
20 D : 38 gal-
60 B : 46 gal- 1 gal+ Σ = 47
60 D : 30 gal- 2 gal+ = 32

Hfr initial input : 2×10^6/ml. At 60' : 5% recombinants in respect of Hfr initial input.

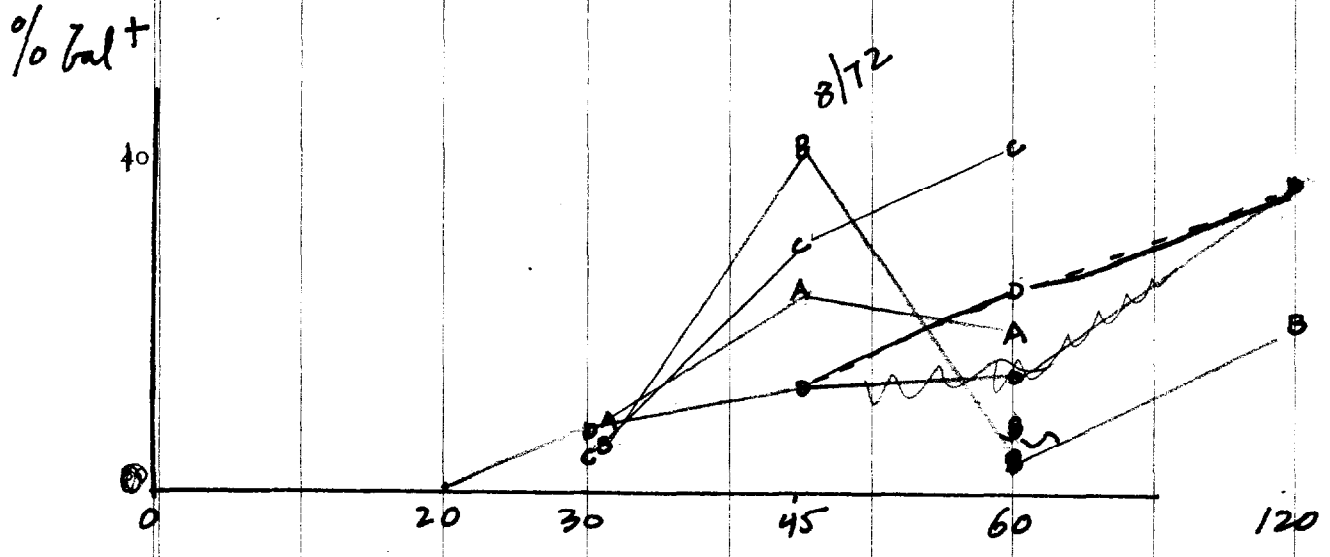
1401c

DATE:

Gal+/total.

REF:

	1	2	3 Gal+	4 = %+	5	A	B	C	D	B+D
0	A	0 ×	16	—						
	C	0 ×	7	—						
5	A	0 ×	26	—						Gal+/to
10	A	0 ×	63	—			0/63			
	B	0 ×	6	—			0/6			
	C	0 ×	23	—						
	D	0 ×	6	—						
20	B	0 ×	54	—			0/54			0/92
Sic.	C	0 R	400	—						
	D	0 ×	38	—						
30	A	~~55~~ 370 R	8	2		8/370	1/65	7/550	1/48	2/113
	B	65 R	1	1						
	C	550 R	7	1						
	D	48 R	1	2						
45	A	10. R	47	6.5		47/72.	8/72	47/58.	2/58	10/130
	B	72 R	8	11						
	C	10. R	47	8						
	D	58 R	2	3.5 ~~4~~						
60	A	10.94 R	47	5		47/94.	1/47	56/52.	2/32	3/79
	B	47 ~~R~~ ×	1	2						
	C	10.56 R	56	11						
	D	~~32~~ ~~5~~ ×	2	~~3~~ 6.5						
120							19/257	100/25..	25/248	44/505
	B	257 R	19	7.4						
	C	10. R	~100							
	D	248 R	25	10						



DATE: KINETICS 4/24/58 REF: 1401/2.

3060 , 3064 4 o.r.c. of each inoculated

↓ conc. 3x ↘ conc. 30x – Ratio Hfr : F– = 1 : 10.

0.5 ml + 0.5 ml : 1' pulse in 50 ml flask in water bath, under manual agitation.

After 1' add 20 ml of broth using two 10 ml pipettes operated at the same time ; then transfer immediately 0.5 ml to 9.5 ml of prewarmed broth, mix with 10 ml pipette and transfer 0.8 ml to 9.2 ml of prewarmed broth.

Total dilution = 1/5.000 . Time required for the operation of dilution : 63 sec.

Keep in water bath at 37°, and every 5' sample 0.5 ml, dilute with 4.5 ml of chilled DW*, ~~exactly~~ on blend with Virtis 30", plate 0.05 on 4 plates of minSt B, each for the first 7 samplings and 2 plats for the later samplings (from 0, 25', 30', 35', 40', 45', 50', 60').

Control of plate recombination and viable counts :

1) Dilute 1/5000 in broth each parental culture, then 1/10 in DW, and plate 0.025 of each parent on 4 minSt-B, pl.

2) Dilute further : 3060 : → 1/100 DW, plate 0.05 on B Lac 3 pl.

3064 : → 1/100 → 1/10 DW, plate 0.05 on B Lac. 3 pl.

* 5' sample : 1 ml + 4.5 ml

Also : microscopic counts of either parent (3060) & 1/5000 (3064) from first 1/100 dil, to which 4% of 10% formalin added.

Microscopic counts : 3060 $4.4 \cdot 10^9$/ml, 3064 $102 \cdot 10^9$/ml

DATE: 4/25/58 REF: 1401-2.

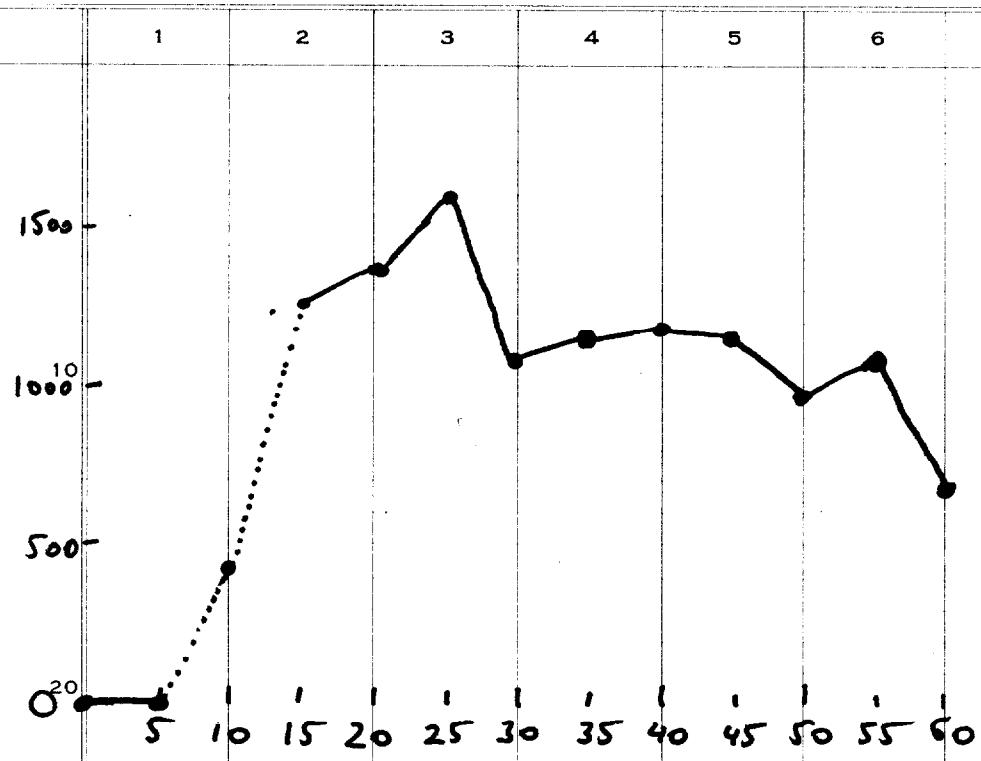
	1	2	3	4	5	6	7	8	9	10

Plate counts (28 hrs)

Time	StB.	~~Avg~~ Sum	No/ml	% Hfr input.	
0′	0,0,0	0	0		Same counts after 56 h.
5′	0,0,0	0	40	0	
10′	15,21,28	64	427	2.1%	
15′	~~57~~,68,64	189	1260	6.3%	
20′	62,75	137	1370	6.8	
25′	72,88	160	1600	8.0	
30′	47,61	108	1080	5.4	
35′	57,56	113	1130	5.6	
40	69,50	119	1190	6.0	
45′	52,63	115	1150	5.6	
50′	59,37	96	960	4.8	
55′	72,37*	109	1090	5.4	
60′	26,42	68	680	3.4	
	Blue				
3060	18,18,24	60	400		
3064	71,87,97:	255	1700		

DATE:

REF: 1401.2



30

40

50

DATE:

samples of 50 col

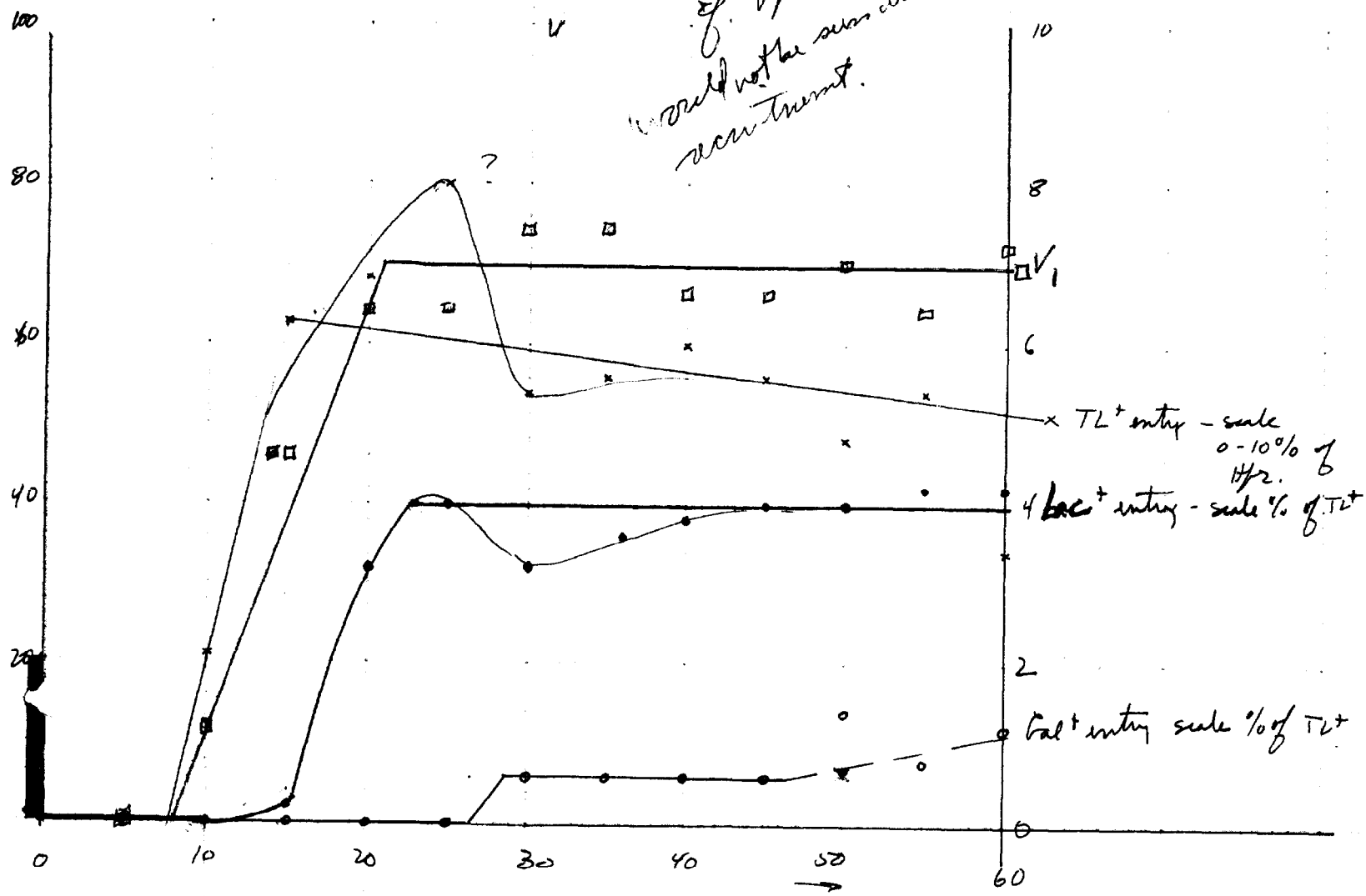
REF: 1401/2

	1	2	3	4	5	6	7	8	9	10
	Time	TL+ % (Hfr)	T' (=) % (TL+)	Lac° % (TL+)	Gal (2 TL+)					
	0	0	-	-	-					
	5	0	-	-	-					
10	10	2.1	12	0	0					
	15	6.3	46	2	0					
	20	6.8	64	32	0					
	25	8.0	64	40	0					
	30	5.4	74	32	6					
20	35	5.6	74	36	6					
	40	6.0	66	38	6					
	45	5.6	66	40	6					
	50	4.8	70	40	14					
	55	5.4	64	42	8					
30	60	3.4	72	42	12					
40										
50										

1401/2

% of Lac, Gal, T_1
based on first samples of
50 colonies each.

? Is this a real inflection?
- Lethal effect of hy^+ on hy^+?
of hy^s + hy^s.
would not be seen with continuous recruitment.

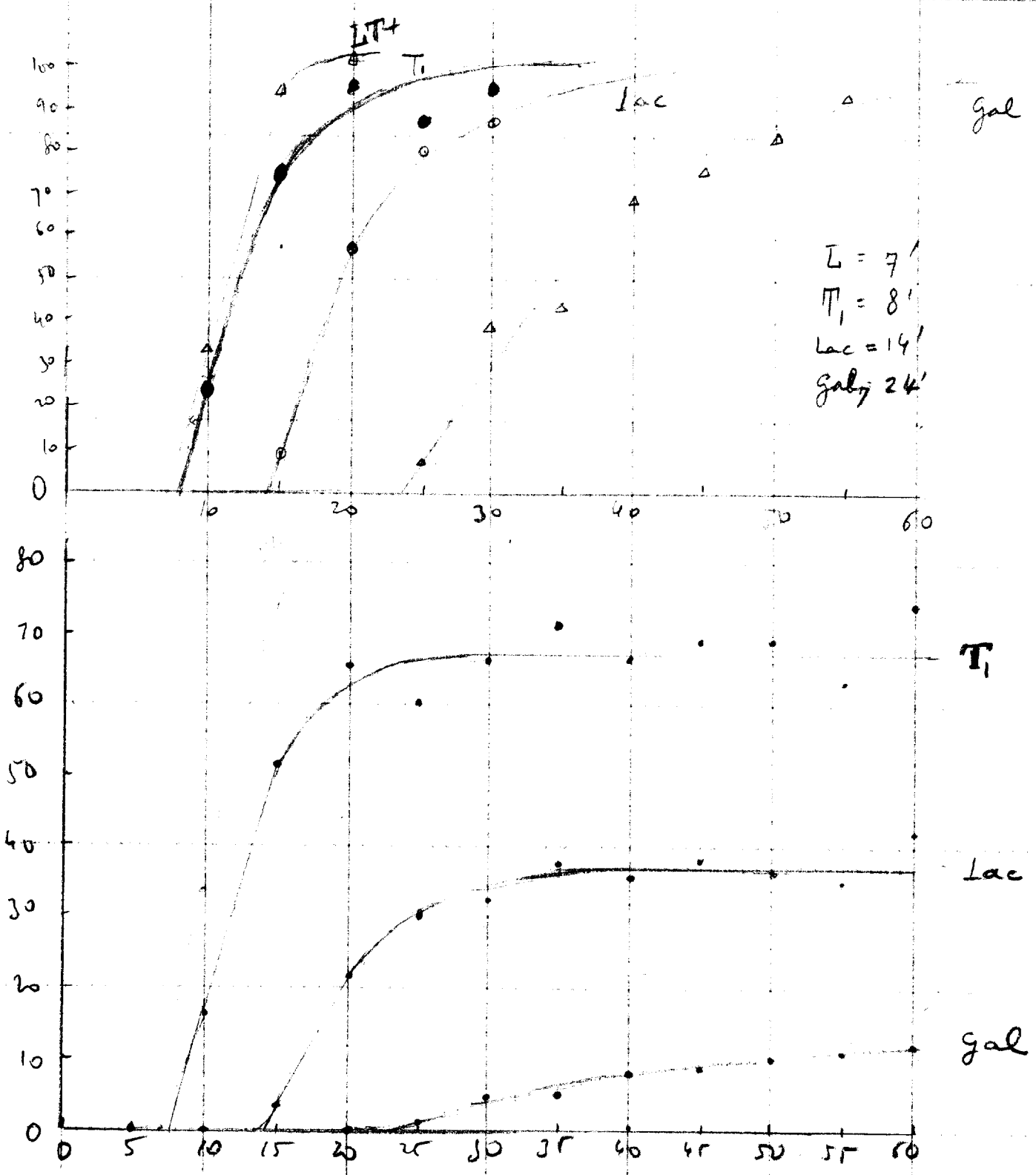


1401-2

DATE: REF:

time	Lac + first 50	Lac + next	Lac + total	Gal + first 50	Gal + next	Gal + total	T_9 first	T_9 next	T_9 total
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	8/57	12/107	32	35/56	67/106
60	21			6			37		

Time	% T_1	1-allele Lac	Gal		% of asymptote T_1	Lac	Gal
0	1 -						
5	-						
10	16.1	0	0	10'	23.2	0	0
15	51.6	3.6	0		74.5	9.6	0
20	65.8	21.5	0	20'	95.0	57.5	0
25	60.1	30.0	0.93		86.5	80.2	7.7
30	66.0	32.1	4.67	30'	95.0	86.0	39.0
35	70.8	37.3	5.1			99.9	42.5
40	66.2	35.8	8.3				69.2
45	69.0	38.0	9.0				75.0
50	68.9	36.4	10.2				83.5
55	63.1	34.9	15.2				93
60	74.0	42.0	12				100
		114.4					
Asymptote	69.5	37.4	12?				



Total data 1401/2

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19 May 26, 1958 REF: 1401/3

W3060, W3052, 1h cultures -
Spun, resusp. 4× & 2× resp. (♂ less turbid).
Mixture : 1 ml ♂ + 4 ml ♀.
After times : 10', 15', 20', 25', 30', 40'
sampled .2 ml and diluted in 10 ml $\overline{H_2O}$ (chilled).
Blended, and plated .05 and .05 1/10. (called 1, 2 resp.)
0' : Plate recomb. control, .04 ♂ 1/50 and .04 ♀ 1/50.
Also, dilutions 1/10 from such suspensions (Exp. 1, 2) -

Media : - D(B, Strep)
1 D(Proc L B, Strep)
2 D(Proc B, Strep).

Controls : .05 ml ♂ & ♀ of 1/50 ♂, ♀ on media 1, 2.

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19 May 25th, 1958 REF: 1402/5.

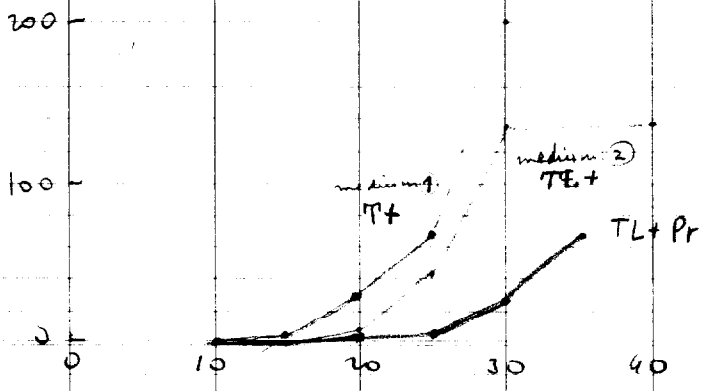
	Medium 0		Medium 1		Medium 2		
	dil 1	dil 2	dil 1	dil 2	dil 1	dil 2	dil 3
10'	0	0	1	0	0	0	
15'	0	0	5	0	1	0	
20'	3	0	31	4	5	3	
25'	6	1	67	9	47	5	
30'	28.	4.		21	134.	37.	
40'	66	9			130	11	1
dil 3:							
0'	0	0	0	0	0	0	

Control, medium 1: 5 4 7 → 1 "

Media 0. D(B, fm)

1 : D(B, Prol Leu fm)

2 : D(B, Prol fm)



W 3052 is descendant from W 945
3064 W 583

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19

REF: 1401/3

	1	2	3	4	5	6	7	8	9	10
1	Medium (sel)	Time	Gal$^+$	Lac$^+$	Ara$^+_2$					
2										
3										
4										
5	-	20'	0/4	1/4	4/4					
6	(TL Pro$^+$)									
7		25'	0/8	4/8	8/8					
8										
9		30'	0/32	21/32	31/32					
0										
		40'	8/75	39/50	72/75					
1						L$^+$	P$_r^+$			
2										
3	1	15'	0/5	0/5	1/5	1/5				
4										
5	(T+)	20'	0/40	0/40	8/40	3/40				
6										
7		25'	0/87	3/87	51/87	00/87	6/87			
8										
9		30'	0/24	4/24	14/24	12/20	4/24			
0										
1	2	15'	0/1	0/1	1/1		0/1			
2										
3	(TL+)	20'	0/9	0/9	7/9		0/9			
4										
5		25'	0/51	5/51	49/51		7/51			
6										
7		30'	0/50	14/50	49/50		18/50			
8										
9		40'	2/50	18/50	48/50		22/50			
0										
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										

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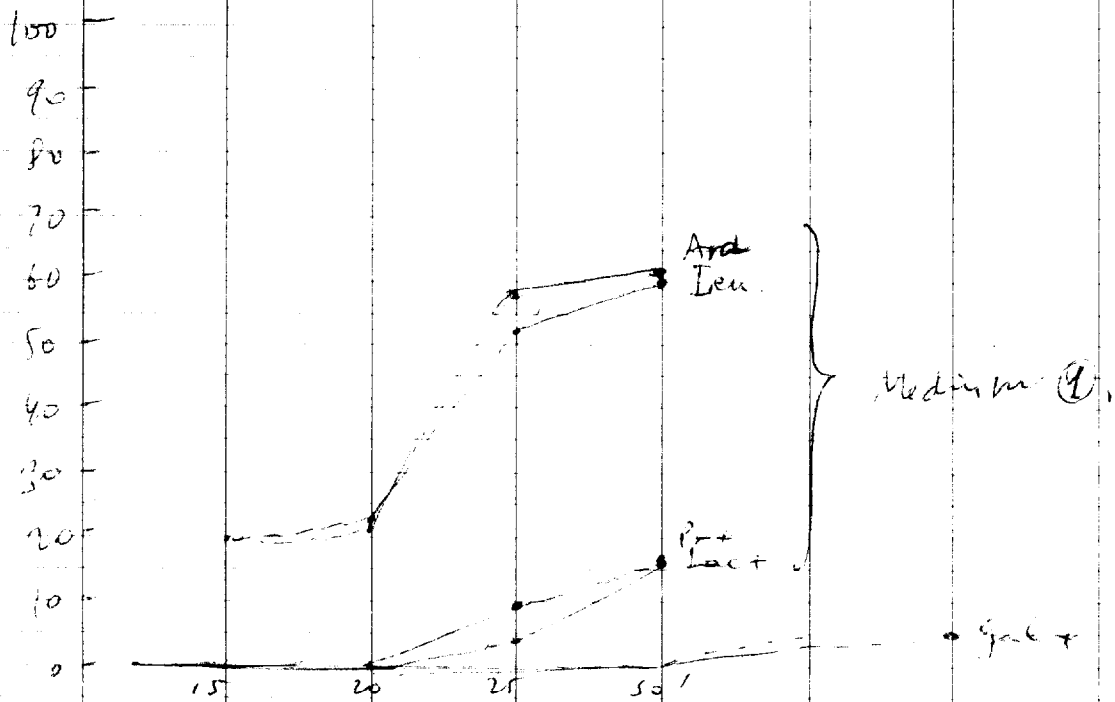
REF: 1401/3



F' = W3052

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19 June 2, 1958 REF: 1401/4

Comparison between W3052, W3064.

ORC cultures of W 3060, W3064, W3052. Sperm, resuspp. 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 SG Gal Sm Strep B,

30', 40', 50', 60' → 1/10 → 0.05 "

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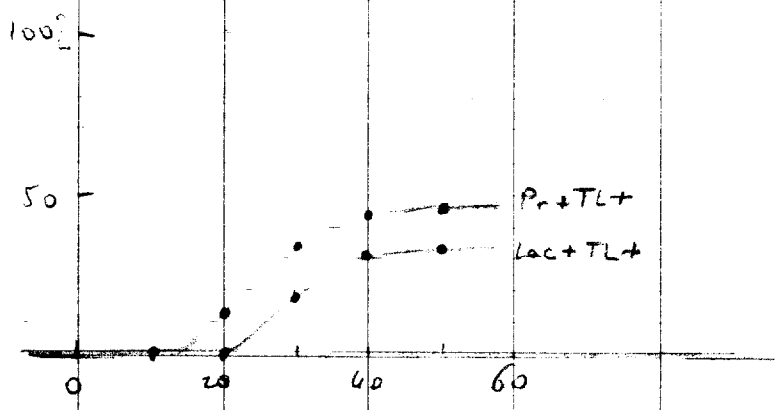
19

REF: 1401/4

	A (all Ara+)*			B (all Ara−)#		Plate counts		
	Gal+	Lac+	Pr+	Gal−	Lac−	A	B	C
						dil	dil	dil
10	−	−	−	−	−			0
20	0/50	0/50	6/50	0/50	0/50	252	112	82
30				0/50	5/50	33	310 4	294 13
40	0/50	20/50	23/50	0/50	11/50	353 47	108 7	111 21
50	2/50	19/50	23/50	0/50	9/50	224 84	222 18	133 9
60						80	9	14

C

	Ara+			Ara−		Ara+/Ara−
	Gal+	Lac+	Pr+	Gal−	Lac−	
20	0/25	0/25	3/25	0/25	0/25	25/25
30	0/27	5/27	9/27	0/23	3/23	27/23
40	0/23	7/23	10/23	1/27	2/27	23/27
50	1/22	10/22	14/22	0/22	6/22	28/22



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19 June 2 REF: 1401/5

Hfr_1 and Hfr_2 timing X 3052.

♀ # 3052 T_1^R.

♂♂: 3936 (1895 Az^R), 3948 (3870 Az^R).

ORC, spun, rsusp. in Penassay, 1 × conc ♂, ½ × conc ♀.

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

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

Times: Hfr_1 cross: 0', 5', 10', 15', 20', 25', 30', 35', 40', 50'
Hfr_2 " : also 7½' and 13'.

Media: (all D(-))

For Hfr_1 media 1 Sm Me B, L T
2 Sm Me B,
3 Sm Me

For Hfr_2 media 4 Sm B, Prol Leu
5 Sm B, Prol
6 Sm B,

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REF: 1401/5

	1	2	3	4	5	6	7	8	9	10
Temp	0	5	7½	10	13	15	20	25		30
1A	105	146		253		0	N 6.10³ *	(N 200 large 10³ small)		∞
1B						[100-200 small]?	[9 large 320 small]	[49 large N 500 small]		(53 large N 500 small)
2A	88	34		52		[70 large N 1000 small]	155	N 1000 large		N 1000 large
2B						63	13	85		139 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 10⁴ *	N 10⁴	t.m.t.c		∞
4B						N 800 *	N 2000 *	//		N 2000 large & small
5A	355	172	296	N 3500	N 8000	N 10⁴ *	N 10⁴	//		∞
5B						N 1000	N 1000	//		N 2000
6A	58	276	171	165	798	N 1300	N 6.10³ *	//		∞
6B						143	494	N 1200		N 2000

* large & small : small in great preponderance -
1A-20' : large are of numbers as large as in 2A 20'.

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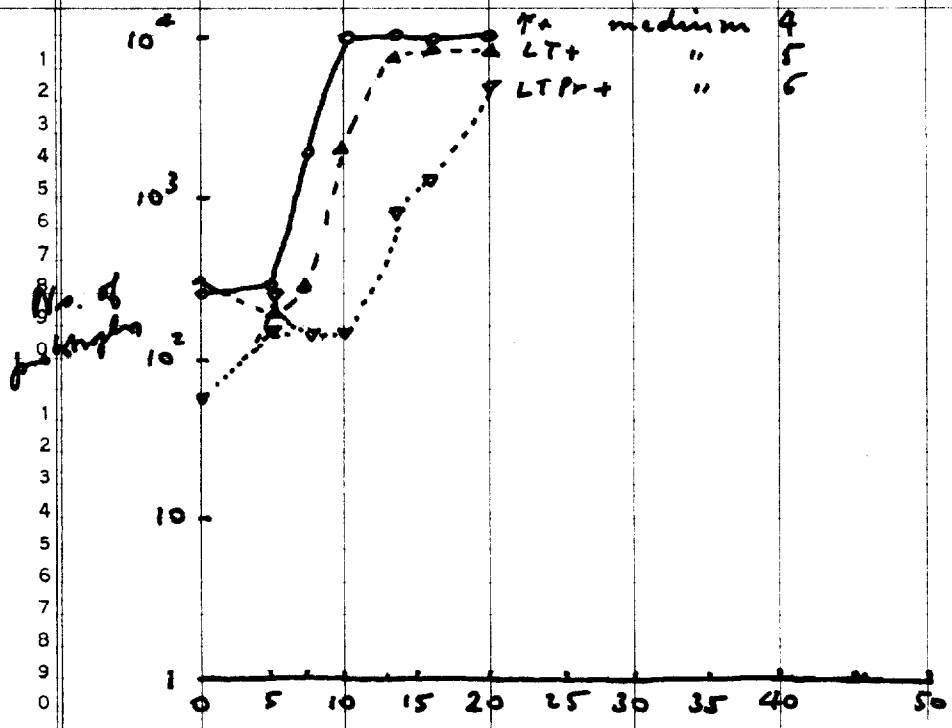
REF: 1401/5.

	1	2	3	4	5	6	7	8	9	10
	35'	40'	50'	control	♀					
1A	∞	∞	∞		0,0					
1B	(large ~100 small ~500)	(large ~10 small ~150)	(large 200 small ~)		-					
2A	∞	∞	∞		-					
2B	~600	~600	~600		-					
3A	79	231	~492		0					
3B	7	14	79		-					
4A	∞	∞	∞		0					
4B	~2000	~2000	~2000		-					
5A	60	∞	∞		0					
5B	~2000	~2000	~2000		-					
6A	∞	∞	∞		0					
6B	~2000	~2000	~2000		-					



Hfr_2

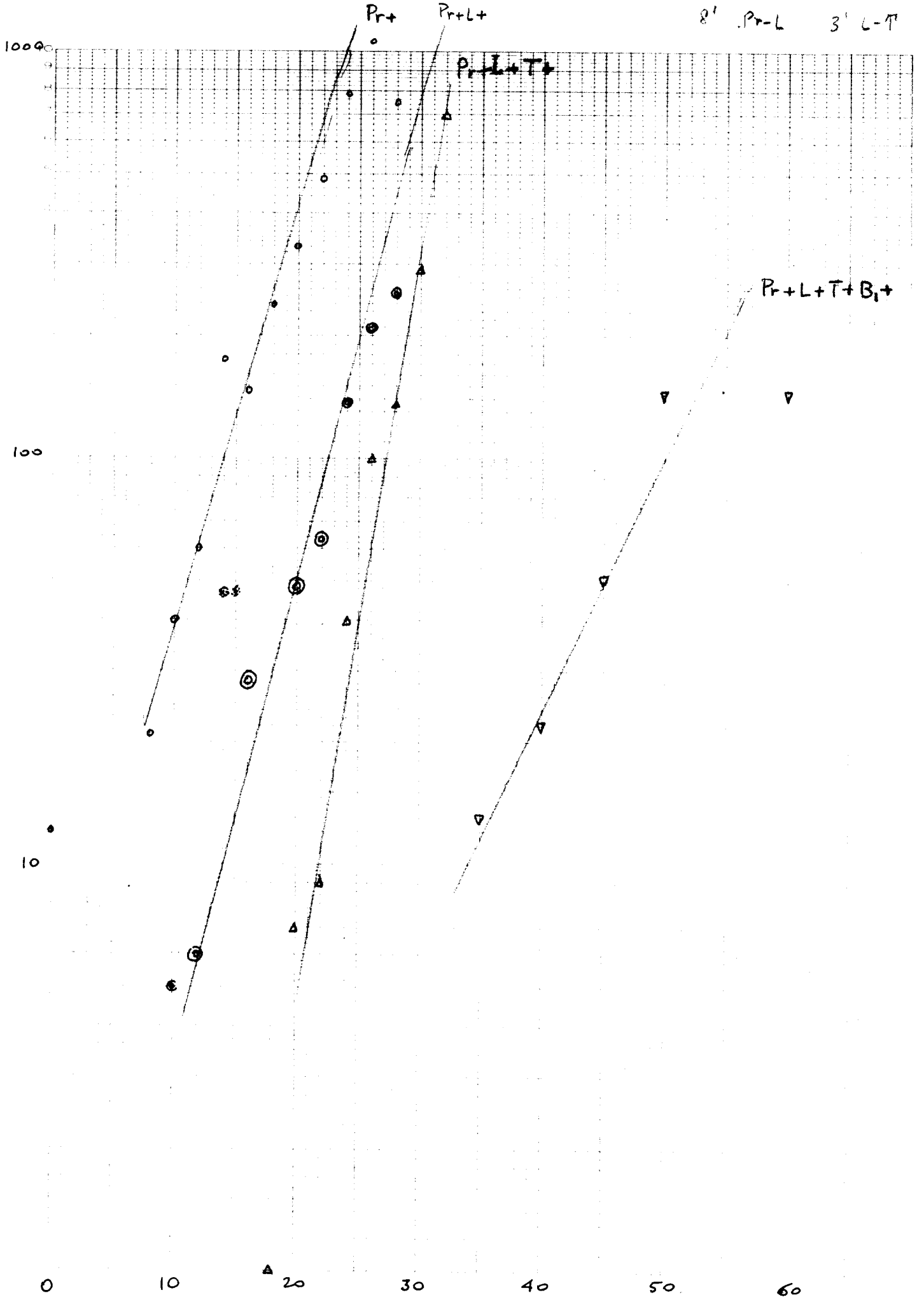
T-L : 2½ minutes 5'T 7½L

L-Pr : 5' minutes 12½Pr

Hfr_1 : medium 1 Entry of Pr between 10' and 20'
Repeat !:

medium 2 Entry of LT probably between 15 or 20 & 25'. But explore 10-30' again

medium 3 Entry of B_1 between 35' and 40'
test for Xyl Mch.


Pr+
Pr+L+
8' Pr-L
3' L-T
Pr+L+T+
Pr+L+T+B1+
1000
100
10
0
10
20
30
40
50
60

TIMED MATING, Hfr_1 and Hfr_2

ORC Cultures of W 3936 (Hfr_1), W 3948, (Hfr_2) 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_1 and 1/50 for Hfr_2.

Media:	1.	D-O	+	Sm	B_1	M	L	T	
	2.			Sm	B_1	M		T	for Hfr_1
	3.			Sm	B_1	M			
	4.			Sm		M			
	5.	B Lac		Sm					
	6.	D-O	+	Sm	B_1		L	Prol	for Hfr_2
	7.		+	Sm	B_1			Prol	
			+	Sm	B_1				

DATE: Sat. June 28, 1958 REF: 1401/6

TIMED MATING, Hfr_1 & Hfr_2.

ORC Cultures of W 3936 (Hfr_1), W 3948, (Hfr_2) W 3052. Spun, resuspended in equal amount of Penallay, and mating mixtures prepared in flasks with 1.5 ml ♂ + 5/5 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 (the same for) dilution (B) of 1/10 for Hfr_1 and 1/50 for Hfr_2.

		M	L	T	
Media:	1. D-0 + Sm B_1	Meth	Leu	Thre	sel for L for Hfr_1
	2. Sm B_1	Meth		Thre	
	3. Sm B_1	Meth			
	4. Sm	Meth			
	0. B Lac Sm			P	
	5 D-0 + Sm B_1		Leu	Prol	for Hfr_2.
	6. + Sm B_1			Prol	
	7. + Sm B_1				

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(space for counts)

1401/6

COUNTS

Medium 1 — each column: +, −, ±

July 12, 19 58

REF:

1 TIME	2 Ara	3 Ti	4 Az	5 MTL	6 (M) MTB	7 ML B1	8 TL B1	9 MTL B1	10 Σ
~~1~~	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~	~~22, 85, 6~~	~~[illegible]~~	~~144, 0, 19~~	~~[illegible]~~	~~163~~
16	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
18	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
20	6, 76, 0	66, 6, 10	12, 69, 1	6, 76, 0	6, 76, 0	6, 76, 0	6, 0, 76	6, 76, 0	82
22	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
24	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
26	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
28	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

Medium 2 — sm B, MT — Hfr 1

	Ara	Ti	Az	MB1	MB. T	Σ
20	42, 5, 0	42, 5, 0	43, 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, 96, 0	99, 1, 0	58, 42, 0	100, 0, 0	100

Medium 3 — sm B, M — Hfr 1

	Xyl	Mal	B1	M	MB1	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, 13, 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

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COUNTS

Each Column: +, −, ±

140116

July 12 1958 REF:

	1	2	3	4	5	6	7	8	9	10
			Medium 4		sm M		Hfr 1			
1		Ara	Gal	Lac	T1	Az	M	−(Do)	Σ	
2										
3	20	2, 0, 0	0, 2, 0	1, 1, 0	0, 2, 0	2, 0, 0	2, 0, 0	1, 1, 0	2	
4										
5	30	2, 0, 0	0, 2, 0	2, 0, 0	0, 2, 0	1, 0, 1	2, 0, 0	0, 2, 0	2	
6										
7	35	15, 0, 0	0, 15, 0	13, 2, 0	0, 15, 0	15, 0, 0	15, 0, 0	9, 6, 0	15	
8										
9	40	25, 0, 0	~~23, 2, 0~~ 0, 25, 0	~~23, 2, 0~~ 23, 2, 0	~~0, 25, 0~~ 0, 25, 0	25, 0, 0	25, 0, 0	17, 8, 0	25	
0										
	45	47, 0, 0	0, 47, 0	40, 6, 1	0, 47, 0	47, 0, 0	47, 0, 0	31, 16, 0	47	
1										
2	50	100, 0, 0	0, 100, 0	93, 6, 1	3, 97, 0	100, 0, 0	100, 0, 0	36, 61, 3	100	
3										
4	60	100, 0, 0	0, 100, 0	89, 10, 1	1, 99, 0	100, 0, 0	100, 0, 0	41, 56, 3	100	
5										
6										
7			~~Medium~~ 5		sm B, LP		Hfr 2			
8										
9		Gal	Ara	T1	Az	L B1	Pro1 B1	L P B1	Σ	
0										
	0	0, 1, 0	0, 1, 0	1, 0, 0	0, 1, 0	0, 1, 0	0, 1, 0	1, 0, 0	1	
1										
2	4	2, 5, 0	3, 4, 0	7, 0, 0	3, 4, 0	2, 5, 0	3, 4, 0	7, 0, 0	7	
3										
4	5	0, 16, 0	9, 7, 0	16, 0, 0	6, 10, 0	4, 12, 0	7, 9, 0	16, 0, 0	16	
5										
6	7	0, 10, 0	2, 8, 0	10, 0, 0	2, 8, 0	2, 8, 0	2, 8, 0	10, 0, 0	10	
7										
8	8	0, 6, 0	1, 5, 0	6, 0, 0	1, 5, 0	0, 6, 0	1, 5, 0	6, 0, 0	6	
9										
0	9	0, 3, 0	3, 0, 0	3, 0, 0	3, 0, 0	3, 0, 0	3, 0, 0	3, 0, 0	3	
1	10	0, 82, 0	7, 75, 0	82, 0, 0	3, 79, 0	2, 80, 0	4, 78, 0	82, 0, 0	82	
2										
3	11	0, 7, 0	5, 2, 0	7, 0, 0	4, 3, 0	0, 7, 0	5, 2, 0	7, 0, 0	7	
4										
5	12	0, 99, 0	14, 84, 1	99, 0, 0	5, 94, 0	0, 99, 0	17, 82, 0	49, 0, 0	99	
6										
7	13	0, 98, 0	26, 72, 0	98, 0, 0	15, 83, 0	0, 98, 0	35, 63, 0	98, 0, 0	98	
8										
9	14	0, 20, 0	13, 7, 0	20, 0, 0	8, 12, 0	6, 14, 0	11, 9, 0	19, 1, 0	20	
0										
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										

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COUNTS

Each Column +, −, ±

1401/6

July 12 19 58 REF:

	1	2	3	4 Medium 6	5	6 sm B, P	7	8 Hfr 2	9	10
1		Gal	Lac	T1	Az	B1	B1 Rel	Σ		
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										
	8	0, 2, 0	0, 2, 0	1, 0, 1	2, 0, 0	0, 2, 0	2, 0, 0	2		
1										
2	9	0, 10, 0	9, 1, 0	0, 2, 8	10, 0, 0	9, 1, 0	10, 0, 0	10		
3										
4	10	0, 8, 0	0, 7, 1	7, 1, 0	3, 5, 0	1, 7, 0	8, 0, 0	8		
5										
6	11	0, 2, 0	2, 0, 0	0, 0, 2	2, 0, 0	2, 0, 0	2, 0, 0	2		
7										
8	12	0, 67, 0	1, 66, 0	56, 6, 4	37, 29, 1	2, 65, 0	67, 0, 0	67		
9										
0	14	0, 30, 0	11, 19, 0	15, 6, 9	27, 3, 0	11, 19, 0	30, 0, 0	30		
1	16	0, 99, 0	1, 98, 0	68, 21, 10	72, 27, 0	6, 93, 0	99, 0, 0	99		
2										
3	18	0, 6, 0	0, 6, 0	5, 1, 0	3, 2, 1	0, 6, 0	6, 0, 0	6		
4										
5										
6				Medium 7		sm B,		Hfr 2		
7										
8		Gal	Lac	T1	Az	Σ				
9										
0	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				
1	12	0, 1, 0	0, 1, 0	0, 0, 1	1, 0, 0	1				
2	14	0, 5, 0	5, 0, 0	4, 1, 0	5, 0, 0	5				
3	16	0, 30, 0	8, 22, 0	0, 2, 28	30, 0, 0	30				
4										
5	18	0, 28, 0	7, 21, 0	5, 1, 22	28, 0, 0	28				
6										
7	20	0, 81, 0	23, 57, 1	3, 49, 29	81, 0, 0	81				
8										
9	22	0, 101, 0	41, 59, 1	7, 54, 40	101, 0, 0	101				
0										
	24	0, 11, 0	5, 6, 0	0, 7, 4	10, 1, 0	11				
1										
2	26	0, 100, 0	57, 43, 0	7, 17, 76	100, 0, 0	100				
3										
4	30	0, 8, 0	4, 4, 0	0, 4, 4	8, 0, 0	8				
5										
6										
7										
8										
9										
0										

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 → girello.

14.35 : 0.5 ml. # 3060 } → prewarmed → girello and also
5 ml # 3064 } samples from parental cultures iced.
5 ml broth

At time: 0' 2' 5' 10' 20' 60'

Dilution with warmed broth (with a 1 ml. pipette to prevent shearing)	0.2 + 0.8 (1/5)	0.1 + 0.9 (1/10)	0.1 + 9.9 (1/100)	0.1 + 9.9 (1/100)	0.1 + 9.9 (1/100)	0.1 + 9.9 (1/100)	0.1 + 9.9 (1/100)	0.1 + 9.9 (1/100)
Incubation Time in rotator	60'	60'	60'	58'	55'	50'	40'	0'
Dilution previous to plating, with iced broth	(1/20) 0.5 + 9.5	(1/10) 1.0 + 9.0						
Amount plated on min St B_1	0.1 / 0.02	0.1 / 0.02	0.1 / 0.02	0.1 / 0.02	0.1 / 0.02	0.1 / 0.02	0.1 / 0.02	0.1 / 0.02

Iced parental broths :

#3064 → broth 1/100 → .025 min St B_1 (from 1/20)
#3064 → broth 1/100 → water 1/100 → water 1/10 → .05 B Lac : 341 ; sam
#3060 → broth 1/100 → broth 1/10 → water 1/100 → .05 B Lac. : 17 ; 19
#3060 → .025 min St B_1 (from 1/10)
#3060 → .025 min St B_1 (from 1/20)

DATE: March 28, 1957 – Summary REF: 1402 : PULSE

TIME	Gal+/tot. 2		Lac+/tot (including W) 4		T1+/tot. 5	6	7	8	9	10
0'	4/31	12.9	4/31 (9/31)	29.0	21/31	68.0				
2'	5/57	8.8	21/57 (28/57)	49.2	39/57	68.4				
5'	12/81	14.7	40/81 (42/81)	52.0	68/81	84.0				
10'	13/79	16.4	33/79 (36/79)	45.5	61/79	77.2				
10 20'	11/65	16.9	31/65 (31/65)	47.7	46/65	70.8				
60'	5/50	10.0	15/50 (18/50)	36.0	37/50	74.0				
0' 1/5	5/70	7.2	15/69 (20/69)	21.7	48/70	68.6				
0' 1/20	6/52	11.5	19/52 (19/52)	36.5	36/52	69.2				

Conclusions. 5'-10' Pulse is an efficient procedure for raising No. of recombinants.

Dilution of 1/100 at least after pulse if further damage must be avoided. Segregation ratios unaffected, provided dilution is carried out with 1 ml. pipettes. 0.1 ml pipettes unreliable. Interruption on simple dilution in Exp. 1401 probably due to chilling, or plating.

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

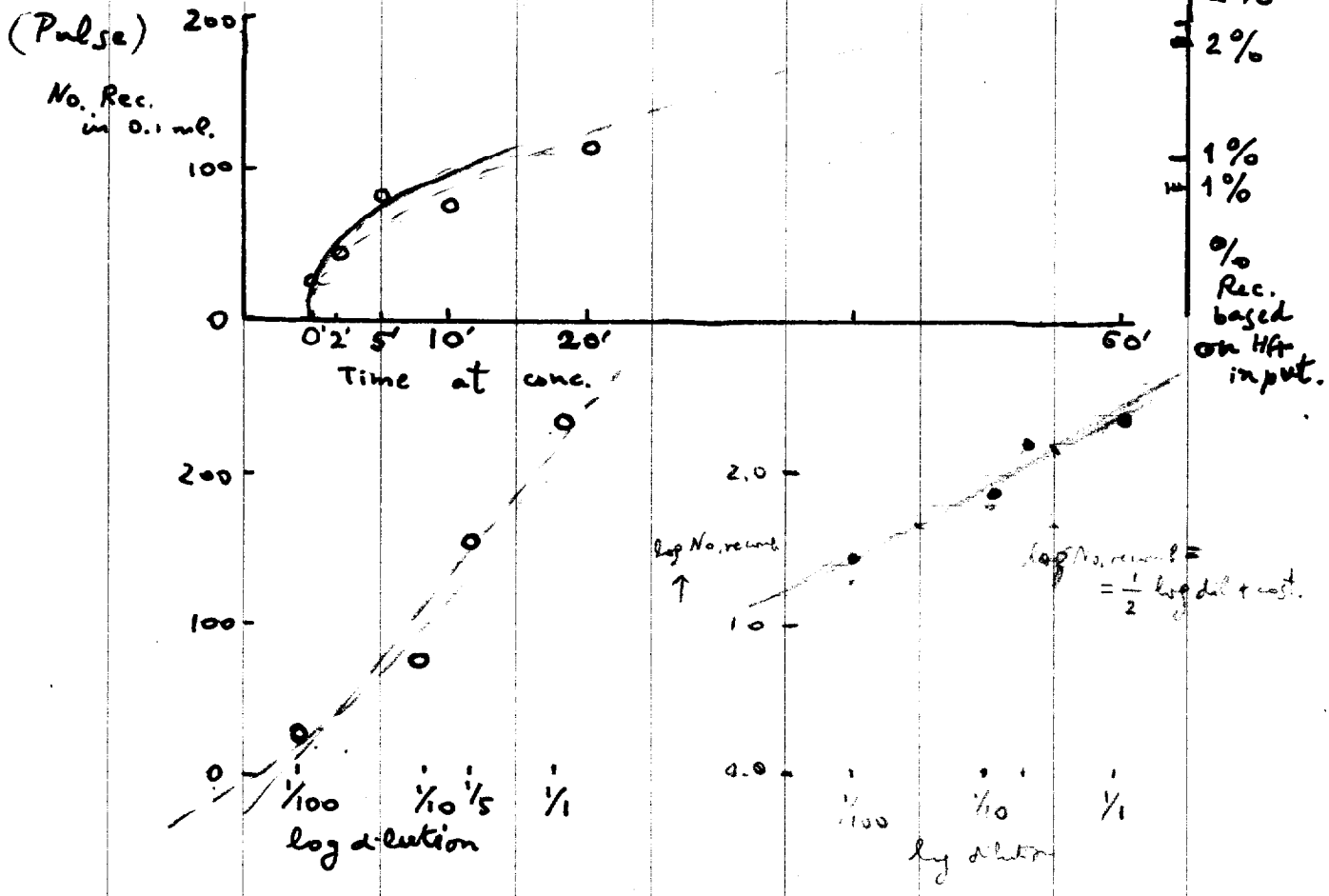
1402 b

DATE: March 24. REF: PULSE.

Plate counts.

Amount plated	Time	dilution 1/100 0'	2'	5'	10'	20'	60'	Time 0' dil. 1/100	1/10	1/5	1/1
0.1		28 +	47	85	77	118	236 ≠	28 +	74	167	236 ≠
0.02		2 +	10	15	13	25	31 ≠	2 +	11	23	31 ≠

+, ≠ : same values, repeated



MICROSC. COUNTS OF PARENTAL BROTHS:

3060 : 230×10^6/ml

3064 : 1.4×10^9/ml

INPUTS per ml : 3060 $11.5 \cdot 10^6$/ml Ratio Hfr/F- = 1:50

3064 $700 \cdot 10^6$/ml

REF: 1402-2

DATE: 22/4/58.

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 exp

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

Control of plate recomb

Conc 1 x

3 x

10 x

3^h (1+10) to approx. saturation.

for phototrophy & Hfr-ness.

20 x respectively :

ml in 50 ml flask in water bath

0.05 to 50 ml prewarmed broth.

n plating (Hfr : F- = 1:2)

nc. susp. dil 1:3 ; exp. repeated

ove.

conc. suspensions dil. 1:3.3 ; exp.

ctly as above.

and kept at 0° until used.

broth } 0.1 & 0.02 ml

broth } with tB_1.

recombinations : 1x suspensions

& and plated 0.05 + 0.05

fficult by smearing (wet plates used)

counts : 1 colony.

0,1 0,02 ml plated

250 44

400 63

1200 148

Note:
counts unreliable

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19

REF:

	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										
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7										
8										
9										
40										
10 1										
2										
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9										
0										

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19

REF:

	1	2	3	4	5	6	7	8	9	10
1										
2										
3										
4				84%						
5										
6				86%						
7										
8				82%						
9										
0										
1										
2										
3										
4										
5										
6										
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0										

19 REF:

1	2	3	4	5	6	7	8	9	10
							Plate recombination control		

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19 5/21/58 REF: 1402-4

RECHECK ON PULSE

1hr exponential cultures of W3060, W3064 - (1 + 7.5 ml Pen).
Spun, resuspended in 3 ml for ♂, 0.3 ml for ♀. Kept ice cold until warmed for use.

For resuspension: 1) BGA old type.
2) Penassay.

Mating mixtures equal amounts of suspensions, concentrated and diluted 1/100, 1/10.000. From concentrated; dilute 1/100, 1/10.000 in same medium after 1'.

A conc. mixture D 1/100 dil. E 1/10.000 dil.
↓ 1' purge
B 1/100
↓
C 1/10.000

Plating: after 20' from A dil 1/10.000 0.05
[for D(from B,)] B dil 1/100 0.05
C ~~dil~~ → 0.05
D dil 1/100 0.1
E → 0.05

same after 80' for BGA and

A-E letters refer to BGA
similarly F, G, H, I, J for Penassay, with equal mix. meanings (A=F, B=G etc.).

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19 REF: 1402/4

	1	2	3	4	5	6	7	8	9	10
		60' broken Highs 60'	1' Pen → ½' Highs 1' 1:100 60'	½' Pen → ¼' Highs 1' 1:10⁴	½' 60' 1:100 60'	½' 60' 10⁻² 10⁻⁴ 60'				
	BGA.	A	B	C	D	E				
plate	20'	4	0	0	~~15~~ 0	0		Plate counts		
	~~86~~	52	28	++ not spread	15	0			Pen	BGA
			~2%/1½					10^{-2}	2	1
	Penallay.							10^{-4}	0	0
		F	G	H	I	J				
	20	8	9	9	7	2				
	~~86~~	28	55	43	51	29				

10^{-4} in BGA might work for pulsing: 1' pulse followed by 10^{-4} in BGA gave 2% yield. But cross may be delayed.

10^{-2} in BGA not enough to limit mating (D)
No valid test of 10^{-4} dilution in BGA. This is adequate predilution (E) to prevent further mating, ~~and~~ but there is no measure of pulse : post pulse mating (a/c C being n.g.)

19 May 26th, 1958. REF: 1402/5

PULSE I

(P).

Experiment identical to 1401/2, except for dilutions employed and times.

Samplings every 20', samples chilled, blended, plated (0.05) and also 1/10 dilutions plated (0.05) on D(from B_1).

Note: 3064 strain is extremely soupy and very difficult to resuspend in Penassay.

(D). From same suspensions as P, diluted each 1/5000, a mixture of equal parts of 1/5000 dilutions incubated, sampled and plated exactly as for P.

		dil 1/1	1/10
Pulsed	P20	56	5
	P40	95	5
	~~P50~~	~~~~	~~~~
	P60	251	9
Diluted	D20	0	2
	D40	16	0
	~~D50~~	~~~~	~~~~
	D60	54	4

19 May 29th, 1958. REF: 1402/6

Transfer from pulse to BGA

(P) Experiment identical to 1401/2 and 1402/5 with following differences

.25 3x conc 1050, + .25 30x conc 3054 (0.1 c.) in flask; then add two tubes Penallay (1/40) and dilute

{ 0.1 + 10 BGA (PB)
{ 0.1 + 10 Pen (PP).

sampled at 20'; 40'; 0.05 on min/ft B_1.

(D). conc. suspension diluted 0.2 + 10 → 0.125 + 10 { Pen (DP) / BGA (DB) } and mixed in equal amounts ♂ + ♀.

sampled at 20'; 40'; 0.05 on min/ft B_1

	20'	40'
PB	4	21
PP	8	19
DP	1	11
DB	0	23

1403

DATE: March 25 REF: Effect of manipul.

14.25 1 ml + 10 ml of broth → Rotator.

3060, 3064 Cultures from fresh single colony isolates.

15.30 Time 0 : 0.5 ml 3060 + 5 ml 3064, + 5 ml broth → ROTATOR

15.40 10' : → dil 1/100 with 1 ml pipette → .1 ml to poured St B1 agar

dil 1/100 " " → 50' Waterbath → .1 ml Pl. (B)

" " " → 50' Rotator → " (C)

" " " in chilled broth → 50' Rotator → " (D)

" " " 60" VIRTIS → " → " (E)

" " " 60" VIBRATOR → " → " (F)

" with 0.1 ml pipette → 50' Rotator → " (A)

Also : from C, at end of 50' time in Rotator : .1 ml to poured D (St B1) agar.

All platings : 0.1 ml in D (St B1).

VIRTIS : Speed 45.

Parents used at time 0 : Also 1/10 dilution in formalin 1% for microscopic count.

3060 : 320×10^6 /ml

3064 : 470×10^6 /ml

Results : A : 1,0 colony

B 0,0

C 0,0

D 0,0

E 0,2

F 0,1

Conclusion.

Single colonies selected from 3060 is Ft reversion.

To be repeated.

min St B1 after 10' and 60' : 0 colonies.

DATE: 4/8/58. REF: 1403 - II.

Strains 3060 3064. 3060 reselected for Hfr. Now kept on agar / stroke

Broths aerated overnight: 1 ml + 9 ml → rotated. 1½ hours.

0.5 ml 3060, + 5 ml 3064 + 5 ml warmed broth → rotator

and also: 3060: 1/50 → 1/10 → .05 } plate at 0' time.
3064: 1/50 → .05 }

After 10' rotation:

A) 1/100 dil. with 1 ml pipette in warmed broth: → 10' plating: 0.1, 0.02 (A1)
→ 50' rotator, then plating as above (A2)

B) Same with 0.1 ml pipette – plating. (B1), (B2)

C) Same as A using chilled broth for dilution, then rotator as above (C1), (C2)

D) 1/100 dil with 1 ml pipette, blending, → rotator 50' → plating

E) 1/100 dil with 1 ml pipette, in warmed broth + 10^4 γ/ml Streptomycin → rotator 50' → plating

Plating on min Stk. B_1.

Counts: .1 plate always too many.

	A_1	A_2	B_1	B_2	C_1	C_2	D	E
.02 ml. plated:	122	378	93	~800	197	480	238	436
Lac+/tot ⊕	3/41	22/40	3/35	12/26	3/24	76/124	2/44	16/41
	7.3%	55.0%	4/50	26/50	12.5	61.3	4.5	39.0%
Gal+/tot	1/41	5/40	0/85	18/80	1/24	23/124	0/44	3/41
	2.4%	12.5%	0%	13.7	4.2	18.5	0	7.3
T_1^r/tot	10/41	26/41	34/86	60/76	12/24	82/126	18/44	30/41
	24.4%	63.4%	39.5%	78.9%	50.0	65.1%	40.9	73.1
plating at	1 ml pipette 10'	1 ml pipette 60'	0.1 ml pipette 10'	0.1 ml pipette 60'	chilled broth 10'	chilled broth 60'	Blending 10'→50'	Strptom 10'→50'

⊕ includes "weaks". 8.2% → 50.0

2 col.

DATE: PLATING BY FLOODING. REF: 1403-3

12.30, 1 ml + 10 ml — rotation, # 3060, 3064.

13.30, 2 ml + 2 ml of exponential cultures mixed, incubated in water bath for 5', then diluted in warmed broth ~~from~~ 1/100, and kept in water bath.

After 10' since dilution:

1/100 Broth → .05 on B_1 plate } undil.

1/100 → .05 "

DW → 1 ml flooded plate on B_1 } 1/100

1/1000 → .05 on B_1

DW → 1 ml flooded plate } 1/1000

1/10.000 → 1 ml flooded plate } 1/10.000

Dilutions: first with 0.2 ml pipette, then 1 ml.

Mixing after dilution, by transfer to dry tube and back

After 20' same, except that first dilution in DW is 1/10 instead of 1/100; rest unchanged.

After 60' same.

Flooded plates have been inverted 5-10 minutes after preparation - Some liquid still floating

Read also 1423B (agar poured)

DATE: REF: 1403.3

	1	2	3	4	5	6	7	8	9	10
	Plate counts.									
		undil.	.05 1/10	1 ml 1/10	.05 1/100	1 ml 1/100	.05 1/1000	1 ml 1/1000	.05 1/10000	1 ml 1/10000
	10'									
	Spread	∞ (10^3-10^4)			29		4			
	flooded					~700		85		8
	20'									
	Spread	∞ (10^3-10^4)	397		52					
	flooded			∞		~900		82		
	60'									
	Spread	∞	~600		42					
	flooded			∞		~1600		162		

Red Circles around plates that were picked.

Summary of segregations.

		spread	flooded
10'	Gal+	0/27	3/50
	Lac+	0/27	14/50
	T_1'	9/27	27/50
20'	Gal	0/50	3/50
	Lac	13/50	14/50
	T_1	36/50	31/50
60'	Gal	4/50	10?/50
	Lac	15/50	26/50
	T_1	26/50	35/50

DATE: ?

REF: 1403/4.

STABILITY OF PAIRS TO PLATING.

To check if 2323 or 2735 are more resistant than 3060 x 3064 to interruption by plating.

Saturated o.r.c. of 2323, 2735, 3060, 3064 mixed in equal amounts:

After 20' ~~plated~~ diluted 1/1000 and { 0.1 / 0.05 } plated on min/ft B,

		Counts:	0.1	0.02
A	3060 x 3064		5	0
B	3060 x 2735		0	0
C	2323 x 3064		0	0
D	2323 x 2735		6	0

Not picked for Lac segregation -

What's wrong with this experiment?

5 May 1958 REF: ~~1401-2~~.

C -O area mess. Mal⁻ doesn't allow for decent scoring of F character! Will have to use another system, perhaps Hfr₂ ara⁻.

E.G. Hfr₂ ara⁻ T⁺L⁺ S^s
F⁺ ara⁺ M⁻ S^s × T⁻L⁻ S^R gal⁻ or lac⁻ ? F⁻

and discriminate on basis of ara character which is closely linked to T⁺⁺.

Wait for W4062 to be lysogenized.

{ F+ x F−
{ Hfr x F− } Cross infertile mogh.

1404 A.

DATE: March 24, 1958 REF: 1322, AKC 4.1

9/56

If futility of F+ depends on the mutation to Hfr, F+ x F− should not be uniformly F− except by reinfection. Expt. to determine whether the recombinants from Hfr x F− are inherited in presence of F+ x F−. Use excess F− and measure incidence of F+ conversions via it.

Basic setup Hfr V_6^2 M− = W3273 (cf. AKC as best linked to lac).

F+ V_1^2 M− = Y40

W3089 = Lac− Mal− S^R. (Use S^R — can terminate mating by sm ?)

Previous history of Lac linkage

Also retrieve stocks of W1979 = W1895 V_1^2; W3262 (but V_6^2 not linked to lac); W1632: F+ V_6^2.

P23 (A) Sm cultures. N24 Sm 1:10 is necessary rotate 37° rotate.

Better not to rotate ??

1:15 PM (Est dens. ~ 5×10^8/ml) Make following mixtures.

			Counts P26
1	W3089	1 ml	0, 0
2	W3273	1 ml · dil 10^{-3} (↓)	
3	Y40	1 ml	
4	W3089	1 + W3273 .1	41, 43
5	W3089	1 + Y40 .1	1, 1
6	W3089	1 + W3273.1 + Y40 .1	22, 27

too low!

Incubate standing.

2:45 PM. Add .1 ml of sm (2 mg/ml) per tube → 200 r/ml.

Add 10 ml water (room temp.) to dilute

Plate 0.1 ml samples on M lac. 1 = W3089 + .1 ml(2) + .1 ml(3) added after the dilutions of 1. This tests plate recombinants.

Plate out #6 on EMB Mal for infertile rate of W3089. — test only the Mal−

Crosses n.g. — not enough from F+. Repeat c/ new 3089 also.

1404A W1632 ($V_1^S V_6^R$ × W3089)

A: 5/17 Mal+. All V_1^S — 7/17 V_6^R. (2 of these Mal+)

B: W1979 × 3089 all V_6^S. 10 V_1^R/51.

~~B~~ A
F+ × F−: 12 tested × W3132. 11 F+ 1 ?F−. several F± weak reactions.

~~#~~ A all 5 V_6^R were F+.

B
also gave variable futility reactions! 18 Mal+.

C. NoRR. $V_1^R V_6^S$: 6: 1 Mal+ other 5: all F+!

$V_1^S V_6^R$: 1 F+!

D. RS: 11 : 3 Mal+ 8: all F+
SR: 3: all F+
1 RR indicated! — isolate.

? Note F status of W1979 (x) progeny!

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F^+, Hfr x F^-.

1404B

4 May 1958

REF:

Use excess F^-! Should get tuning of Lac, M, etc. Try Lac$^-$ S^r F^- x F^+, Hfr. Yield of prototrophs S^r will go down with time.

W3089 = Lac$_{1b}$ Mal S F^-.

W1979 = Hfr, M V_1^R

W1632 = F^+ M V_6^R

plate on M lac. add sm at time of plating.

Use pulse of mating, thin dilute + plate at intervals .... Should also isolate prototrophic Hfr S^S...

4 May. 10^{30} PM.

ORC W1979 Hfr M V_1
W1632 F^+ M V_6
W3089 F^- Lac Mal S

Repush 2 hours 1:10 on rotator.
Harvest 10 ml → 0.5 ml. Dilute F^+ 1:10
in BBA. 37° Dilute Hfr 1:1000
standing in WB.

at t = 0, mix 0.1 ml of W3089 ± 0.1 ml F^+ ± 0.1 ml Hfr; make up to 0.3 ml with BBA. at t = 15 minutes, add 2 ml BBA* to dilute and plate 0.1 ml samples.

A F^- ; F^+
B F^- ; Hfr
C F^- ; Hfr ; F^+
D. dilute, after dilution. Plate immediately.

also check samples of separate parents.

* containing sm, 5000 r/ml to limit plate recomb.

Platings on M Lac ; sm added to medium.

5 May: Recombinants are still sparse & small. also control diarrhea

W1979, W1895 x W3089 are very prototrophic on M lac (no sm!) Medium?

7 May
A ~5/plate
B ~20/plate
C ~35/plate
D ~100/plate.

Why should D have most?

SEGREGATIONS

Hfr M V1
F+ M V6
F- Lac Mal S.

S. cols Lac + protos isol on streaks to ime broth of spotted Bmal
b) x'ln T6
c) negative to Ch, R

DATE: 9 May 58

REF: 1404 B. &4x

A17

B5

D8

All confirmed as Lac + S^R Gal + protos.

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5/13

1404.5

Working Summary Sheet

Recombination 19

REF:

	1 Hab	T1	T6	2	3	4 A	B	6 C	7 D	8	9	10
						Hab + 5	17	8	15			
						− 12	34	27	32			
						V1 R 0	10	6	14			
						S 17	46	29	33			
						T6 R 2	0	1	4			
						S 9	36	34	43			
						recombs H 4	1	2	6			
						12 M 5	27	22	22			
						L 5						
						0 4	6	3	3 (1?)			
1	+	S	S	#								
2	−	R	↓	+								
3	+	S		•								
4	−	R	1	#								
5	−	S	S	+								
6	−	R	S	+								
7	−	R	↓	+								
8	−	S		•								
9	+	R	↑	•								
10	+	S	S	•								
1	+	S	R	•								
2	−	S	S	−								
3	+	R	S	•								
4	−	S	S	#								
5	−	S	S	±								
6	+	S	S	•								
7	−	S	↓	−								
8	+	S		•								
9	−	S		+								
20	−	S		#								
1	−	S		+								
2	+	S		•								
3	+	R	↑	—								
4	+	S		•								
5	+	R	S	•								
6	−	S	S	•								
7	−	R	↓	#								
8	+	S		•								
9	−	S		+								
30	+	R		•								
1	−	S		#								
2	−	R		+								
3	−	S		+								
4	−	R	⇑	#								
5	+	S	S	•								
6	−	S	S	+								
7	−	S	S	#								
8	+	S	R	•								
9	−	S	S	±								
0	+	S	S	•								
1	−	O	S	#								
2	−	S	S	+								
3	−	R	S	+								
4	−	S	R	+								
5	−	S	S	+								
6	−	R	S	+								
7	−	S	S	+								
8	−	S	S	+								
3143	S^R	+	S	+								
0	2817		2886	±								

#1 188
2 235
3 261
4 - 201
12 - 49
13 273

140(4C): Reincubate to Thursday. Count all plates
Pick any colonies which appear on 5, 6, 7, 8. 0 0 0 4
~~of the class~~ Pick colonies from 9, 10, [redacted] 11, 14, 15, 16.
39 33 50 20+ 3 2
30 4-

Procedure: isolate on EMB Lac. Test Lac$^+$X$^+$ for V_1 and V_6 and Mal. All or most should be Mal$^-$S^R. Test for F by cross-streaking from small broth ~~to~~ against W3132 ($M^-_{Mal^+}$ S^s F$^-$) on M-Mal medium.
Use a Mal$^-$F$^+$ TL$^-$ or TL$^+$ control.
? 2817, results?

140(4B)' Pick ≠ 50 colonies each from A, B, C, D + recheck B/c.
test by above "procedure" ± tolo Mlac

A: 4 acc picked
2 13

B: 1 ~~45~~ 5
2 ~~95~~

C1 110 + clump
C2 150

D1 216 + several clumps
D2 ~~258~~.

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14: 1 = ~~44~~.85
86 #6
87 #9
86-90 #10
91-100 #11

1404c

by [illegible]

19 REF:

	1	2	3	4	5	6	7	8	9	10
	6.1	all T1 T6 R	C9:							
1	2	R								
2	3	Mal+ S								
3	9.1	acufos. ↓								
4	2									
5	3		d1							
6	4		d2.							
7	5									
8	6									
9	7									
0	8									
	9	↑								
1	10									
2	11	S								
3	12	S								
4	13	↓								
5	14									
6	15									
7	16									
8	17									
9	18									
0	19									
	20	↑								
21										
22		S								
23		S								
24		↓								
25										
26										
27										
28										
29										
30										
31										
32										
33										
34	10-1									
35	2									
36	3									
37	4									
38	5									
39	6									
40	.									
41	.									
42	.									
43										
44	.									
45										
46										
47	.									
48	.									
49	.									
50	.									
9	.									
0	.									

(Column 2, written vertically beside rows 30–50: SSSSSSSSSSSSSSSSSSSSSSSSS with R entries, [illegible])

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Hfr, F+, F−

1404C

5 May 1958

REF:

Repetition: May need variable proportions of F+ : F− : Hfr. Use .1 ml of 20× F− as standard. add various proportions as follows. (in terms of 20× suspensions)

.1 ml 20×F− plus .1 ml each of

				5/8 # + cells	
Hfr alone.	1. Hfr 1:100	F+	0	188	For plating, after 15 minutes add
1/10	2 "	F+	1/2	235	2 ml of BOA - 8m 5000 u/ml and plate
1/100	3 "	F+	1:10	261	0.1 ml on Mlac.
1/1000	4 "	F+	1:100	201	
	5 —	F+	0	0	
	6 —	"	1/2	4	
	7 —	"	1:10	0	
	8 —	"	1:100	0	
	9 Hfr 1:1000	F+	0	39	
	10 "	"	1/2	33	
	11 "	"	1:10	50	
	12 "	"	1:100	49	
	13 Hfr 1:100 ~~F+ 1/2~~			273	mixed after diluting plate at once
	14 Hfr 1:100 ~~F+ 1:10~~			20	(chilled) — not warmed at all
	15 F+ 1/2			3	
	16 F+ 1:10			2	Harvest from refrushed ORC — rotated previously 1:10 2 hours, harvest into BOA.

Time by chilling in ice-water.

Probably not enough fertility of F+!

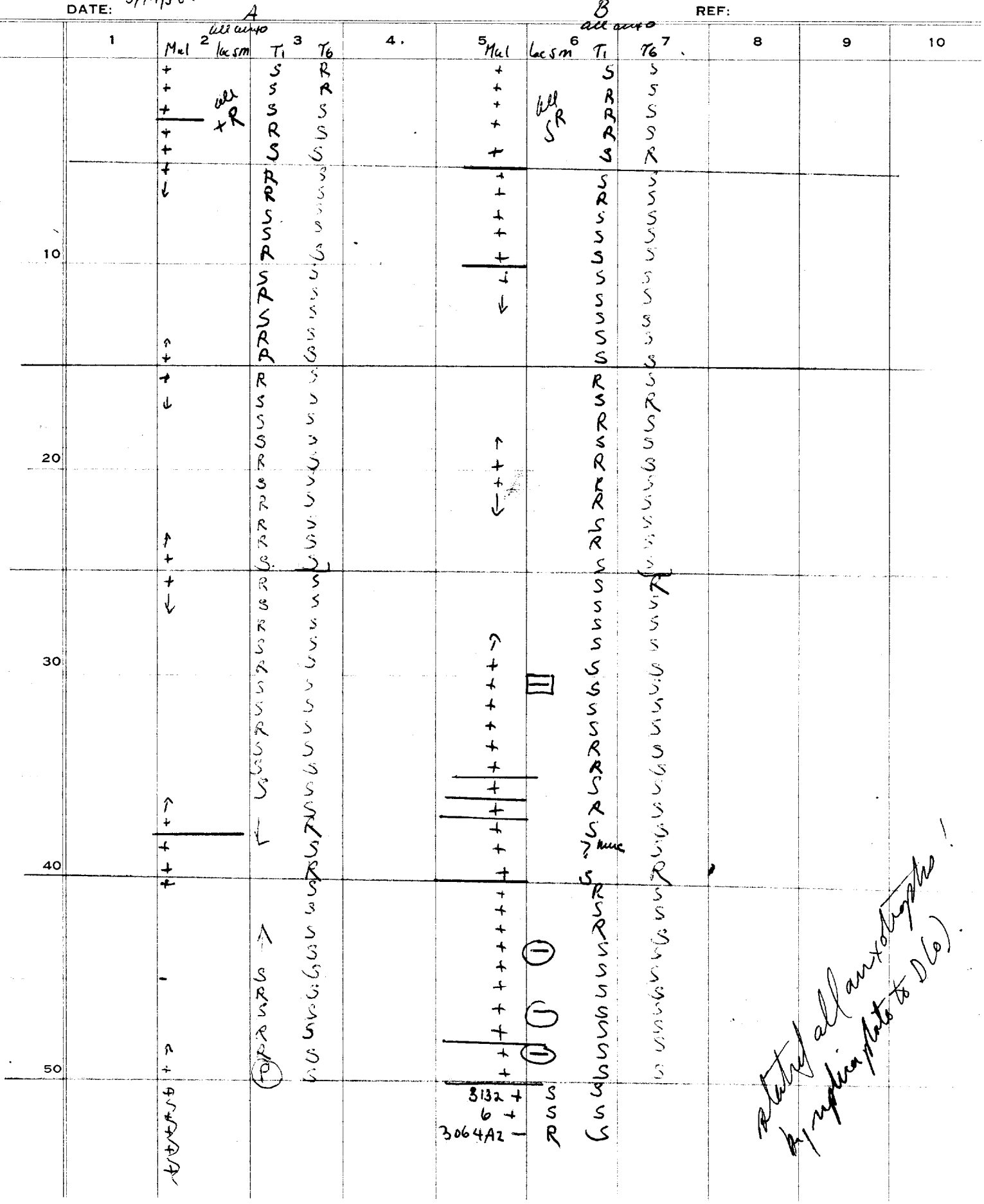
fri. 6^10 PM

A1	1	++	
	2	++	
	3	++	
	4	++	
		−	
		−	
		−	
		−	
		+	
		−	
	1	−	
		+	
		++	
		+	
		−	
		−	

F+ relatively very infertile. (~10^{-4} of Hfr!) also mainly plate recombinants in any case! (despite addition of streptomycin and [illegible] dilutions). Status to be allanstrophs!

Reading. 1404 C.

DATE: 5/14/58.



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(F⁺, Hfr) × F⁻

Reconstruction and reinfection of F⁻ progeny in F⁺ × F⁻, Hfr × F⁻ crosses.

summary.
1404
WBCD

28 May 1958

REF:

24 March

A. W3273 = Hfr V_6 Y40 = $F^+ V_1$ W3089 = F^- Mal Lac S. Select $lac^+ S^R$ prototrophs.
But Hfr was 4×10^{-4} as fertile as F^+. abandoned for lack of proper proportions

4 May.

B. Use excess F^-. W3089, W1879 = Hfr, MV_1 ; W1632 = $F^+ MV_6$.
Found plate recombinants at least equal to those mixed in broth 15 minutes.
Do 15 minutes too short a time ?

5 May

C. Use excess F^-, various ratios

Use a marked Hfr to determine whether its progeny × F^- remain uninfected by neighboring F^+ cells. Crosses can be done in broth or on plates.

DATE: March 25, 1958 REF: PROTOPL. TIMING

9.30 #3060 overnight broth culture 5 ml + 5 ml broth → rotator →

11.50 → L_2 medium : .5 / 1.0 / 1.5 / 2.0 } ml of semisatn. rated culture + 8 ml L_2 medium 10^3 U/pen./ml.

14.10 protoplasts well formed. agglutination in the higher concns. of cells. 0.5 ml conc. used

#3064 : 2 ml overnight broth + 10 ml broth → rotator for 45'.

Cultures of 3060, 3064 centrifuged, resuspended with 8 ml. warmed L_2 medium. 5' water bath.

14.45 3060 Protoplasts — 3064 in L_2 medium

1 ml + 4 ml form → $6 \cdot 10^6$/ml but many may have burst

2 ml + 2 ml L_2 CONTROL → ROTATOR → 60' → 120' → same

.25 + .25 L_2 + 4.5 H_2O → .05 Pe → 1/10 → .05 Pe

.25 + .25 + 4.5 H_2O → .05 Pe → 1/10 → .05 Pe

4 ml + 4 ml X → ROTATOR → 5' → 10' → 20' → 40' → 60' → 120' → same

5' → 1/10 → .05 pl. ; 1/10 → .05 pl.

1 ml + 4 ml form. → 4×10^8/ml

.05 Pe. ← 1/10 ← .05 Pe. ← 1/10

Pe : All platings : .05 on D(B_1) and B(lac).

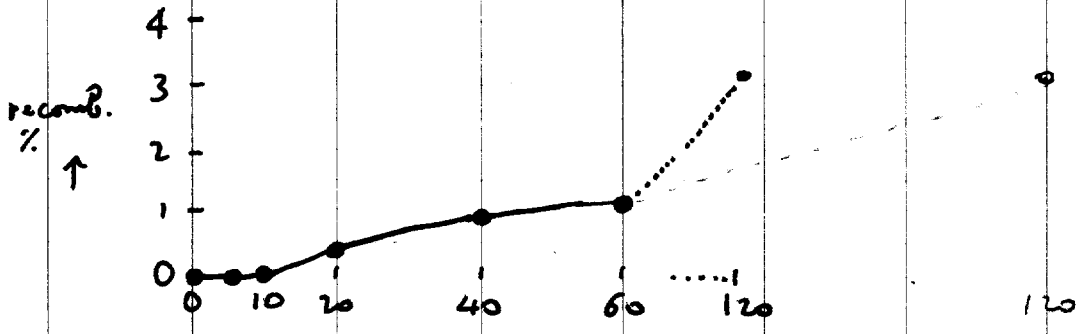
Microscope counts : 3060 protoplasts : 6×10^6/ml. But many may have lysed in formalin. Rods : 3% or less & badly fragmented.

3064 in L_2 medium : 4×10^8/ml

PROTOPLASTS

DATE: COUNTS REF: 1405 b

on min. B_1	0'	5'	10'	25'	40'	60'	120'	9	10
1/10 Protoplasts	3					20	184		
1/100 Control	0					0			
1/10 Cross	2	2	3	30	89	116	∞		
1/100	0	1	0	1	10	11	32		
on B Lac									
1/10 Protop	2					22	233		
1/100 "	0						16		
1/10 Cross	1	1	0	3	16	18	122		
1/100 "	0	0	0	0	0	2	8 + a clump.		



Viable protoplasts after shocking : 500/ml out of 6.10^6 : $\sim 10^{-4}$.

Recombinants per Hfr protoplast after 60' : 2.3×10^4/ml out of 6.10^6 : 3.10^{-3}.

Note : all "recombinants" at time 0, 5, 10 could be viable protoplasts.

At time 20' : on minimum Gal. 13% (Hfr par / total "recomb" on complete)

40' 10.4%

60' 18.1%

120' 65%

this accounts for the high Gal ratios observed

DATE: 3/28/58. 5 REF: 1405

1	2 Gal+	3	4	5	6	7	8 T₁ among Gal+
120'	:	13/46	+ 18/43	= 31/89		34.6	35/58 =
60'	:	9/73	+ 6/38	= 15/111		13.5	54/96 =
40'		4/23	+ 5/62	= 9/85		10.6	39/76 =
20'		3/28				10.7	8/25 =
10'		2/3	} very likely to be parentals.			(67% ?).	
5'		2/3					
0'		2/2.					

Note: 1) there is linkage between Gal+/smooth, small & Gal- rough large: it accounts for the long +++ & --- runs.

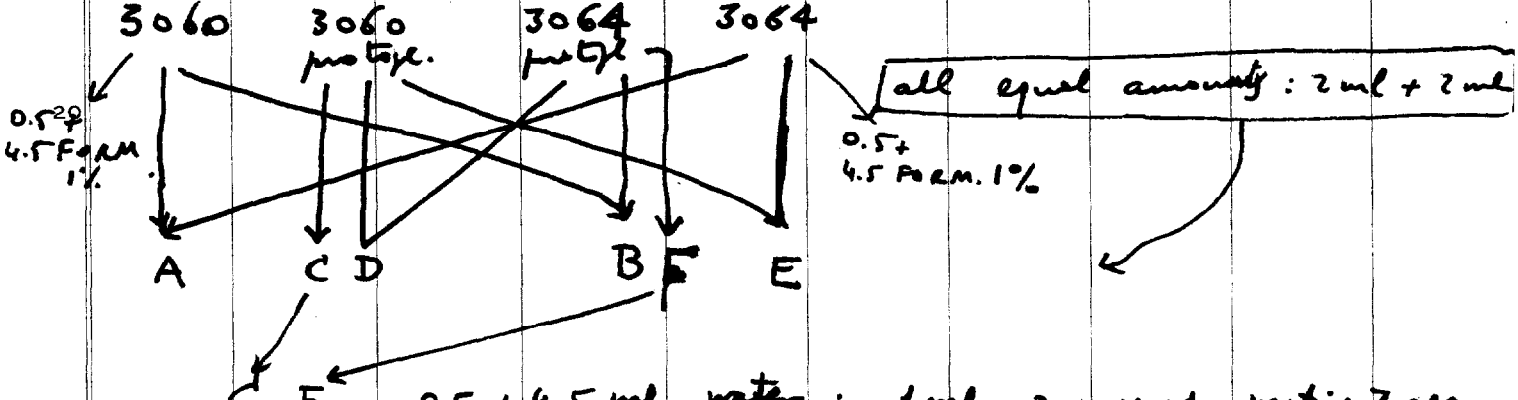
2) there are still too many viable protoplasts in the Hfr preparation, which can account for the high Gal+ ratio, especially at 0 time.

1405-II

DATE: March 26. REF: PROTOPL. 9'

12.20 3060, 3064 cult. → rotator 1+10 ml (from rotated cult.)
Note: same culturing used as for exp. 1403: F+ reversion of 3060.
13.20 1 ml + 9 ml L_2 + 10^4 penicillin → protoplasts (5 ml.
15.50 Centrifuge, resuspend protoplasts in L2 medium without penicillin

Also 14.50 3060, 3064, 1+10 ml to rotator.
15.50 Centrif., resuspend in 5 ml L_2 medium.



C, F: 0.5 + 4.5 ml water; 1 ml → poured nutrient agar.

16.40 A, B, D, E: 30' rotator → 0.5 + 4.5 ml water → minSt B_1
→ 0.5 + 4.5 ml Sucrose → agar Sucr/c M_1.
60' " → Same.

0.5 ml. residual of protoplast susp: 0.05 ml Form 10% added.

Microscope counts:
3060 : 400×10^6/ml
3064 : 940×10^6/ml
3060 protoplasts: 2.2×10^7/ml - no rods.
3064 " 2.7×10^7/ml "

3060 protoplasts: agglutinated before centrif. After resusp centrif., pipetting up and down releases agglutination.

DATE: 3/29/58 REF: 1405-II

Counts:	30'		60'	
	Sucro/e	B,St	Sucrose	B,St
Exp. A	15	33	54	66
B	0	0	0	0
D	0	0	0	0
E	0	1	1	1

Poured agar plates from C' (3060): 278.
F (3064): 72.

Conclusions: 3060 has reverted to F+. The same single colony isolate has was used as in Exp. 1403. Hence low numbers of recombinants.

Proportion of viable cells in protoplast preparations:

3060: 1.3×10^{-5}.

3064: 0.3×10^{-5}.

DATE: 4/14/58. REF: 1405 - III

PROTOPLASTS.

Exp 1405-I is repeated, but after bursting in DW, mixtures are either plated or kept in the waterbath until completion of 60' since the beginning of the expt. In addition, a pulse of 10' is given to the mixture.

9.05 am. 1 ml 3060 overnight aerated + 9 ml broth → Rot.
10.30 1 ml 3060 expon. + 9 ml I_2
12.10 coarse granular growth. Agitated, 10^3 Pen u/ml added.
14.40 centrifuged.

Algo: 14.20 3064 2 ml + 10 ml Broth.
14.40 Centrifuged.

3060 ♂, 3064 resuspended in 1.2 + 1 ml I_2; = 10 ×
0.3 ml in water bath.
3060 ♂ 10 × → 0.1 ml + 0.9 ml DW → 0.1 on B Lac.
→ 0.1 + 1.9 ml formalin 1% → Count (all burst out?)
3064 10 × → 0.5 } X
→ 0.5 }
→ 0.1 + 1.9 ml formalin 1% → Count = 9×10^8/ml

X and ♂ ♀ (diff. in I_2 of 3060 ♂) incubated in water bath for 10', then: diluted 20 × with I_2 medium warmed.

(X) → 0': 1 ml + 9 ml DW → plate 0.05 and 0.01 on minST B_1.
↓ + 10 ml broth, water bath. (= 0/60') → plate 0.05 / 0.01
10' water bath: same as above → = 10
20' " " = 10/50.
30' " "
45' " "
60' " 1/10 DW → = X-60 (pl. 0.05, 0.01)
→ + 10 ml broth → = 60/0 (pl. 0.05, 0.01)

DATE: REF: 1405-III

1 2 3 4 5 6 7 8 9 10

3 - Counts of viable cells in protoplast suspension:

From suspension obtained after centrif. -

0.1 + 0.9 DW, → 0.1 EMB bac plate. 246 col.

From same susp. dil 40× in I_2, → 0.1 EMB bac. 4 col.

↓

30' in cub w. b. → " (dried up)

↓

60' " → " . 67 col.

The initial No. of cells which were protoplasted is unknown. but is of the order $2-5 \cdot 10^8$/ml. Hence fraction surviving is of the order 10^{-5}.

—

	0	10	20	30	45	60	minutes
	X-0	X-10	X-20	X-30	X-45	X-60	
0.05 ml	0	191	∞	∞	∞	∞	
0.01	0	41	362	~800	~~920~~	760	
	0/60	10/50	20/40	30/30	45/15	60/0.	
0.05	11	178	∞	∞	∞	∞	
0.01	0	21	244	434	~900	609	← should be multiplied ×2 to give figures comparable to X

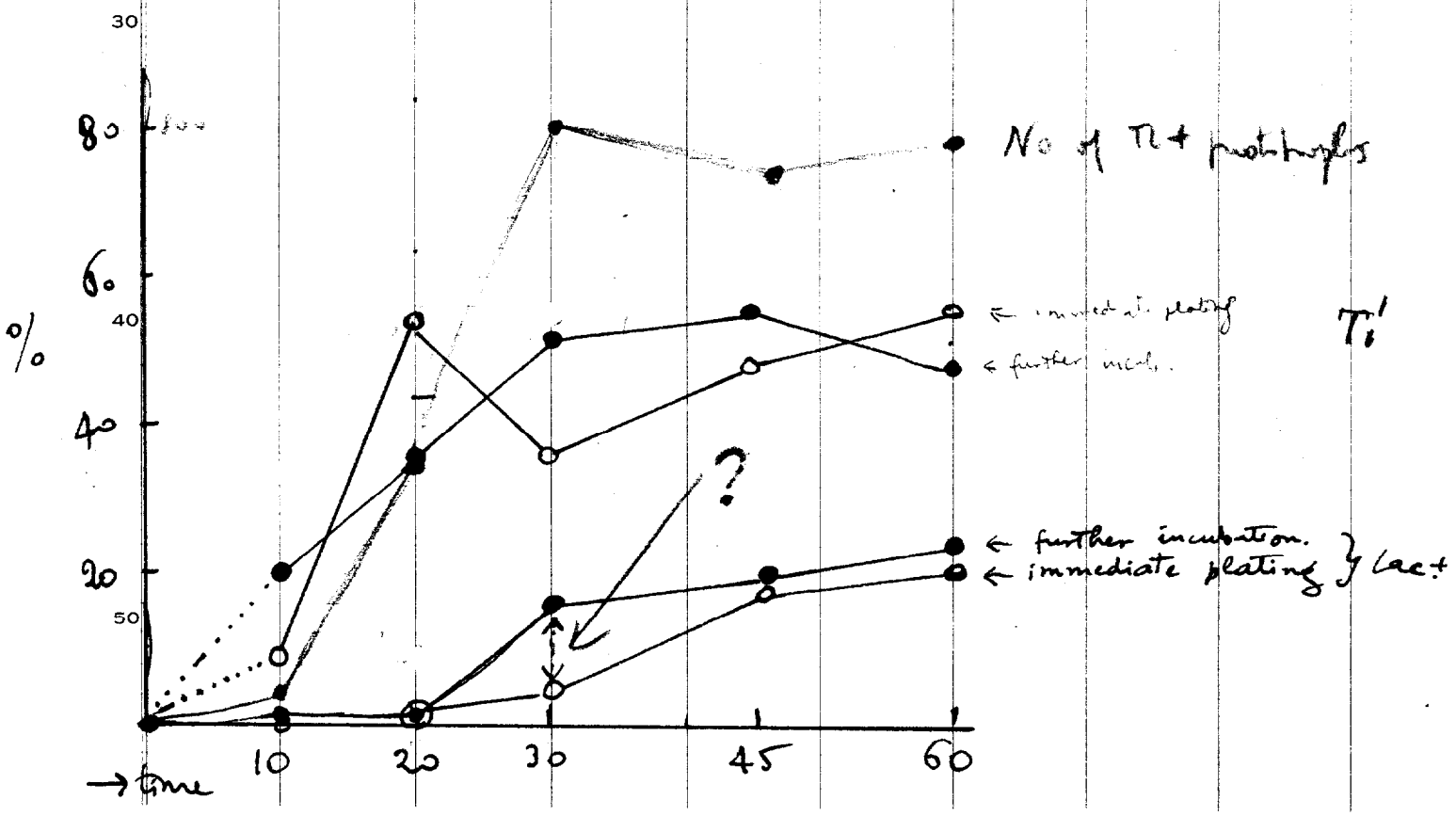
addition of water

Before | After 4/17/58.

REF: 1405-3.

TIME →	0/60	10/50	20/40	30/30	45/15	60/0
Gal+	0/12	0/71	0/50	0/50	1/50	1/50
Lac+	4/12	2/71	1/50	8/50	10/50	12/50
T_1'	8/12	7/71	18/50	26/50	28/50	22/50

	X-10	X-20	X-30	X-45	X-60.
Gal+	0/90	0/50	0/50	2/50	0/50
Lac+	0/90	1/50	2/50	9/50	10/50
T_1'	4/90	27/50	14/50	24/50	29/50.



DATE: April 14. REF: 1405 - IV

Repeat of 1405-II: Crossings between protoplasts and non protoplasts.

10.30 a.m. 1 ml 3060, 3064 + 10 ml L_2.

11.50 { 3060 poorly grown, agglutinated.
3064 well grown, 3-500 $\cdot 10^6$/ml.

Penicillin 10^4 units added.

13.35 cultures of 3060, 3064 started: 1 + 9 ml broth

15.00 3060, 3064 cultures and 3060, 3064 protoplasts centrifuged, resusp in L_2: 3064 + 3064 δ 10 ml, 3060 δ 2 ml 3060 5 ml.

15.35 Cross:

	A	B	D	E
3060	0.5	0.5	-	-
3060 δ	[illegible]	-	0.5	0.5
3064 δ	-	0.5	0.5	-
3064	0.5	-	-	0.5

- water bath. Algo:

Viability Test { C: 3060 δ: 0.1 ml + 0.9 water → 0.05 Blue → 0.01 Blue
E 3064 δ " " "

16.40. From water bath, A, B, D, E: 0.5 + 4.5 ml water → .05 → 1/10 DW → .05 resp (1/10 inst B,

Algo, on agar succ/e: A, B, E:
0.05 from test tube
0.05 1/10 dil. in L_2
0.05 1/100 dil in L_2.

D: 0.05 from test tube.

DATE: REF: 1405 - IV.

Viability tests: C 0.05 44 col 3060 ♂:
0.01 13 "
F 0.05 168 3064 ♂.
0.01 145. ?.

Microscopy: C 2-3 protoplasts per small square, many of which ghosts. = $5 \cdot 10^7$/ml.
F 8-9 $160 \cdot 10^6$/ml.

3060 ♂: Viable $2 \cdot 10^{-4}$;
3064 ♂: " $2 \cdot 10^{-4}$.

		♀♂	♀♂, ♂♂.	♂♂
	A	B	D	E
Sucrose B_1				
1/1	∞	∞	~1000, small	∞
1/10	∞	∞ irreg	not met	∞
1/100	2-5000	∞ irreg	v.v.	~500
Suc B_1				
1/1	∞	32	2	~2000
1/10.	~2000	0	0	162

Note. 3060 is not prototrophic on Suc B_1 or B_1, therefore crosses on Suc B_1 are valid

DATE: 4/19/58 REF: 1405-4

Summary of Segregations.

♂	♀		min Sm B_1 gal+	min Sm B_1 lac+	min Sm B_1 T_1^r	sucrose B_1 gal+	sucrose B_1 lac+	sucrose B_1 T_1^r
–	–	A	0/50	8/50	24/50	26/50	22/50 ⊖	
–	♂	B	2/50	10/50	27/50	~~24/50~~	–	
♂	♂	D	–	–		24/50 ⊖	28/50 ⊖	
♂	–	E	2/100	13/100	58/100	0/50	9/50	26/50

⊖ many mixed.

Also:

From D: Streaks on EMB Lac.

Later note: W3060 showed excessive growth on B_1 mediums. ? question of a usable auxotrophic marker in this strain. Should be surveyed in the fall.

6.29.58

DATE: PROTOPLAST CROSSES. REF: 1405-5

Repetition of 1405-4.

3060, 3064 ♂ as in experiment 1409-2, but diluted 5× with L_2. 3060, 3064 rods in L_2, 2× concentrated, from overnight rotated cultures.

	♂	♀	Plated on Suc B_1	Suc B_1 St	B_1	B_1 St
M	♂	♂	1/1 0.02 ; 1/10 0.02	1/1 0.02	1/1 0.02	1/1 0.02
N	♂	Normal	1/100 0.02	1/100 0.02	1/100 0.02	1/100 0.02
O	Norm	♂	1/1 0.05	1/1 0.05	1/1 0.05	1/1 0.05
P	Norm	Norm.	1/100 0.02	—	1/100 0.02	1/100 0.02

Mixtures for crosses set up in equal amounts at 12.20. After 60' incubation in water bath, dilutions (with L_2) and platings.

Viability test of protoplasts: suspensions employed in cross diluted 1+10 ml H_2O, → 0.01 / 0.05 B lac.
(2 hrs after preparation of suspensions)

← Drop of flamed alcohol

Counts: 0.01 # 3060: 70 col
0.01 # 3064: 136 col.

Microscope counts on protoplasts: (after stay at room temp.): ♂ 3060: $52 \cdot 10^6$/ml (but many rods!)
♂ 3064: $392 \cdot 10^6$/ml no rods.

Viability of protoplasts: $1.6 \cdot 10^{-3}$ for # 3060 ♂
$0.3 \cdot 10^{-3}$ for # 3064 ♂.

(after a 2 hr stay at room temperature; rods may have increased)

DATE:

REF: 1405-5

	1	2	3	4	5	6	7	8	9	10
		Plate counts.								
♂ ♀		Sucrose B_1			suc B_1 St		min B_1		min St B_1	
♂ ♂	(M)	0.02 : ~2000			0.02 : ~800		0.02 : many small and 158 large		0.02 : many small col.	
		$\frac{1}{10}$ 0.02 : 172 + many smaller colonies.								
♂ normal	(N)	302			374		255		314	
Norm ♂	(O)	10^4 ?			10^4 ?		~1500		~500 large + many small ones.	
Norm Norm	(P)	~3000					~3000		~2000.	
Controls:	3060 ♂	many small ones (~2000).								
	3064 ♂	no growth. except for 1 colony								

Picking

DATE: 4/19/58. REF: 1405-6

Repeat of Exp 1405-3 to test further difference between immediate and non immediate plating on Loc at 30' (see question marks in previous graph).

3060 δ, resuspended in L_2 10× conc.; pour with 3060 overnight rotated culture.

Mixture in equal amounts. Also, viability test of 3060 δ: DW 1/10 → 0.02 on EMB Loc (465 colonies counted).

From 3060 δ × 3064, 10' pulse then dilution 1/100 in prewarmed L_2, and further incubation in W.B. for 25, 30, 35'.

After such times dilution in DW 1+10, incubation for 10', then addition of 10 ml Penassay 2X and:

A) immediate plating

B) further incubation for until completion of 65' from end of pulse, then plating.

Platings: 0.1 & 0.02 from tube on mini St B_1.

By mistake, 35' A has been kept at room temperature.

	25'		30'		35'	
	A NON INC.	B INC.	A NON INC	B INC.	A NON INC.	B INC.
0.1	141	88	194	146	261	232
0.02	23	10	27	19	46	35

DATE: 23/4/58. REF: 1405-6

SEGREGATIONS.

	25'		30'		35'	
	NON	INC.	NON	INC	NON	INC.
gal+	0/100	0/100	0/100	0/100	0/100	0/100
lac+	2/100	0/100	3/100	4/100	7/100	8/99
T_1'	25/100	38/99	33/100	37/100	39/100	54/100

DATE: 4/23/58. REF: 1405-7

OSMOTIC SHOCK ON RODS

3060, 3064 O.r.c. resuspended in 5× conc. with T_2 medium.

Mix in equal amounts for a 5' pulse in water bath.

Dilute 1/100 in T_2 medium, warmed.

After 10'; 20'; 30' dilute 1/10 in DW, incubate 5', add 2× Penassay in equal amounts and:

A) plate 0.05 on min StB_1

B) incubate further until completion of 50' since dilution in T_2 1/100, plate 0.05

Plate counts:

1-2000 colonies in each except for 10' A (~100) and 20' A (~300).

	Gal	Lac	T_1
10' A		1/50	
B		22/50	
20' A		0/50	
B		11/50	
30' A		4/50	
B		13/50	

DATE: REF: 1405-8

CROSSES ♂ × ♂.

2323, 3064 protoplasts 2h30' incub. (1 ml + 9 ml from o.r.c.).
Resuspended in L_2 medium at 10× conc.
~~0.5~~ Mixed equal amounts, then diluted 1/100 after 10' pulse in L_2 medium. Incubated 50', then spread on min B_1 & sucrose B_1:
0.05 from undil. & 1/10, 1/100, 1/1000 and 1/10.000 dilutions in L_2 medium

From parental suspensions: → 1/10 dist. water → .05 B lac
→ 1/100 L_2 → .05 on Suc B_1
↓ 1/100 L_2
↓ 1/10 L_2 → .05 sucrose Penal/lay
↓ 1/10 L_2 → same.
and microscopic counts

Microscope counts: 2323 ♂: 4.2×10^9/ml.
3064 ♂: 3.2×10^9/ml

	10^{-5}	10^{-6}	
3064	107	15	$2 \cdot 10^8$/ml viable
2323	320	10	$= 6 \cdot 10^8$/ml viable

Viability tests: 3064 ~ 10.000
2323 ~ 3600

Crosses: Controls: 2323: clean
3064: many small colonies.

Crosses: as control 3064.
100 bigger colonies in undil.
10 " " 1/10.

Discarded.

DATE: 4/15 CULTURE PHASE. REF: 1406

1	2	3	4	5	6	7	8	9	10

Photometer, 650 mμ

9 a.m. 3060, 1 ml + 10 ml. broth 0.29

3064, 1 ml + 10 " 0.37
0.5 " 0.32
0.2 " 0.20.

10 a.m. photometer readings as above.

Algo: Saturated (overnight aerated) broth cultures:

3060 Sat 0.51

3064 Sat. 0.80.

All broths ~~taken~~ reduced to density equal to that of 3060 exponential, by dilution with broth. (3064 ~~Exp.~~ 0.5 ml + 10, dil 8 ml + 2 ml);
3060 Sat 5 ml + 6 ml broth, 3064 Sat 3 ml + 15 broth).

Mixtures in equal amounts, 2 ml + 2 ml to waterbath:

	3060 Sat	3060 Exp.	3064 Sat	3064 Exp.
A	x		x	
B	x			x
C		x	x	
D		x		x

10' pulse, then dilute 1/100 warm broth; → .05 min St B,

↓ stay in water bath 50' → 1/10 Dw, 1 ml flooded St B, plates.

0.05 min St B,

1/10 Dw → 0.05 "

0.01 except for A.

DATE: 4/17/58. REF: 1406

After 10':

		A	B	C	D
0.05		482	293	482	320
flooded with 1 ml of 1/10 sil.		~2000	~2000	~2000	~2000

After 60'

0.05		many	many	many	many
1/10	0.05	288	~300	~500	~500
"	0.01	not done	59	91	113

used for picking and typing.

DATE: CULTURE PHASE 4.19.58 REF: 1406

		A	B	C	D
	SUMMARY				
	♂	1	1	exp	exp
	♀	1	exp	1	exp.
10'	Mal+	0/50	0/50	0/50	0/50
	Gal+	0/50	0/50	0/50	0/50
	Lac+	1/50	0/50	1/50	0/50
	T_1'	12/50	9/50	10/50	8/50
60'	Mal+	0/50	0/50	0/50	0/50
	Gal+	6/50	5/50	2/50	6/50
	Lac+	20/50	16/50	12/50	21/50
	T_1'	30/50	30/50	22/50	29/50

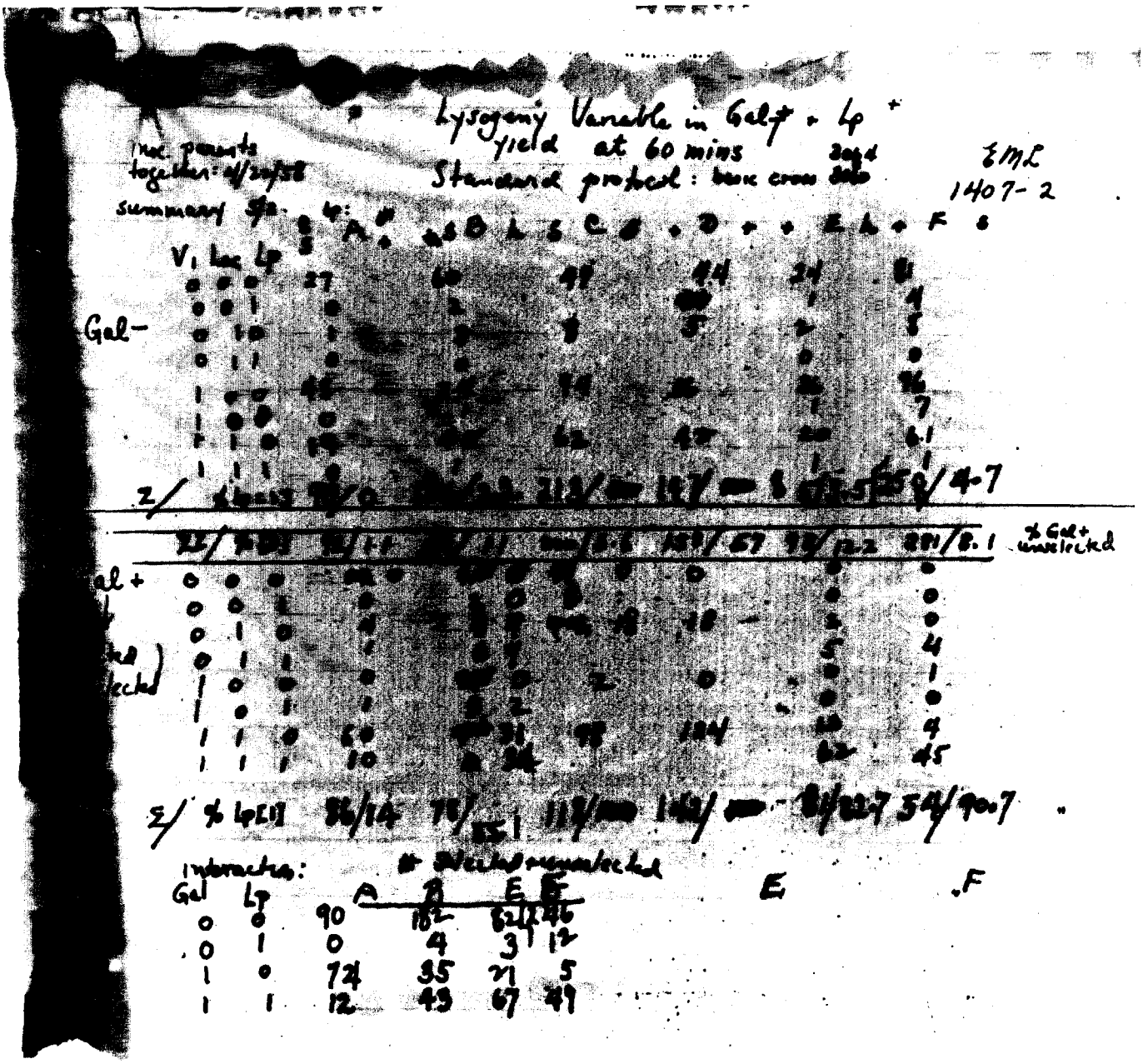
DAT

9 10

LEDERBERG, ESTHER M., University of Wisconsin, Madison, Wis.--Fine structure of the Gal loci in Escherichia coli K-12.---A distinctive recon (one of a group of closely linked loci separable by recombination) has been assigned to each of ten independently occurring Gal$^-$ (galactose nonfermenting) mutants. All the mutants were obtained after UV irradiation except Gal$_3$ which was spontaneous. Allelism was tested by large scale matings and by transduction analysis.---Prophage-linked transduction via Hfr λ, where practically every phage particle may result in a transductional event, produced heterogenotic clones when donor and recipient bore distinctive recons. The heterogenotes were unstable, segregating the two input and two crossover classes (Morse, Lederberg and Lederberg 1956). Two cistrons (cis-trans position effect groups) had been demonstrated: trans heterogenotes with members of the same cistron are phenotypically mutant. Galactokinase was missing in mutants of one cistron (Gal 2,8) while UDP transferase activity was absent in the other (Gal 1, 4, 6, 7), a defect corresponding to human congenital galactosemia, (Kalckar, Kurahashi 1957). Gal$_3^-$ and Gal$_9^-$ are genetically cistronic with <u>both</u> of the foregoing groups; they have not yet been successfully analysed for their enzymatic defect. Among two hundred new mutants, no recons identical with the first ten have recurred. Most have been assigned to either of the first two cistronic groups, but a small number which would represent a fourth genetic group have given only normal galactose-positive heterogenotes with every standard Gal$^-$ tested. A few mutants were not transformable by λ but were found to carry other modifiers including that of hexose metabolism which obscure their relationship to the Gal$^-$ group.---An attempt was made to map the Gal markers in linear sequence by their relationship to the Lp locus. Intercrosses of Hfr M$^-$ Gal$_x^-$ x F$^-$ Gal$_y^-$ were made on a medium which selected efficiently for M$^+$Gal$_x^+$Gal$_y^+$ recombinants, and these were then scored for the segregation of an Lp marker. However, the linkage of Gal-Lp proved to be not close enough to compensate for the perturbations of segmental loss from the Hfr parent, and the data were indecisive.

10th International Congress of Genetics, Montreal, 1958.

1407-2
EML


Lysogeny Variable in Gal+ λ+
yield at 60 mins
Standard protocol: base cross data
EML
1407-2
summary
Gal-
Gal+
Interaction:

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19 May 7th, 1958 REF: 1408/2

W3908 (F- Lp^+ tryp Gal6 S^R)
W3870 Hfr_2 $B_1^?$ Lp^R.

o.r.c. : ml: 0.5 ♂ + 1.0 ♀ – incubated for 1 hr.
Plated on S^r Gal Sm B_1 ; 0.05 undil, 1/10, 1/100
1/1000, 1/10.000

Controls : 0.05 of undiluted cultures.

After 48 hrs : undil : > 1000, Gal+ 4 Gal-
1/10 : 56 Gal+ 70 Gal-
1/100 : 4 Gal+ 3 Gal-
1/1000 : 0

Controls : W3908 : 3 col.
W3870 : 0 col.

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19 May 10th 1958. REF: 1408/3

Gal - Try linkage.

♀ 3908 s^R Gal_6 try - $4p^+$.

♂'s: 1 W3367 $4p^R$ M-

2A: W3060 + . Th?

2B: W3870 R - Th?

3 : W3848 s P Mal_5

4 : W3752 s

13 : W3782 s M-

21 : W3898 + L_6 (481 M).

Media:

M Gal tryp B_1 Sm

S Gal Sm B_1.

Cross:

.5 ♂ + 1.0 ♀, 1^h 37°

.05 of 10^0, 10^{-1}, 10^{-2}.

	yield 10^0	10^1	10^2	Select try⁺ Gal⁺/total	$4p^+/Gal^+$	$4p^+/Gal^0$
1	0	0	0	-		
2A	∞	154	10	32%		
2B	∞	63	-	25%?	85%	7%.
3	14	0	0	18%		
4	39	0	0	16%		
13	36	mss	0	4%		
21	17	0	0	2,5%		

DATE: 5/15/58 REF: 1408/4

1 2 3 4 5 6 7 8 9 10

Gal TIMING - Hfr 2

2 ml o/n c w 3370 + 7.5 ml Pen; 1 ml 3908 + 7.5 ml P—, 1 hr not

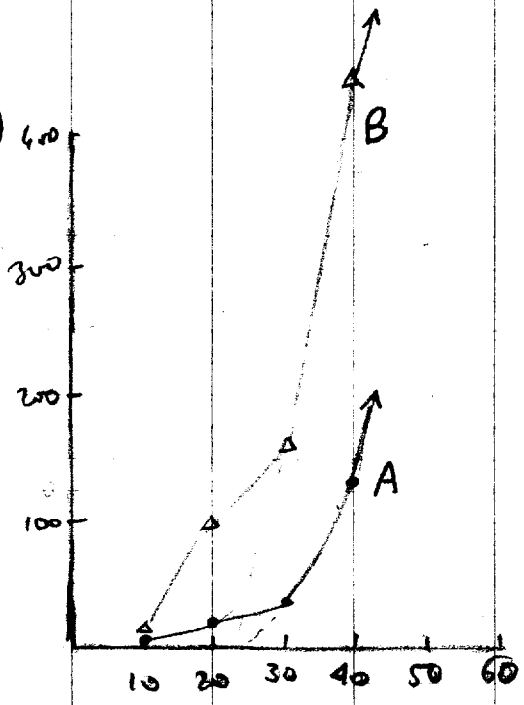
(A)

Spin ♂ & ♀, resuspend in Pen 3.0, 0.3 ml tube respectively
Mix 0.1 + 0.1, at 37°, after 1' add 10 ml warmed broth - (1/50 dil)
After 10', 20', 30', 40', 50' withdraw 2 ml/sample, chill
blend, plate on Gal Sm B, D(Sm B) - M Gal Sm B, Try.

(B) Spin ♂ & ♀, resuspend in fresh broth, to same conc as before spinning
Rest: same. ♂ .5 + ♀ .5 + 5 Pen

Plate counts D(Sm B)

	A	Gal Sm	B	
10	7	4	33	12
20	18	—	91	116
30	31	31	174	149
40	140	112	418	554
50	(1200)	823	∞	
60	(2500)		∞	



Entrance of Try at 20'?
Probably data insufficient to answer the point

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REF: 1408/4.

	1	2	3	4	5	6	7	8	9	10
		5' Gal fm B_1	Counts.							
			A			B				
			Gal+/Gal-	Tot	Gal+%	Gal+/Gal-	Tot	Gal+%	Gal+ / Gal-	%
		10'	1/3	4		2/10	12		2 / 1	9.1
		20'	missing			17/99	116	12%	17 / 88	5.7
		30'	0/31	31	0%	5/146	149	3.3%	5 / 105	1.8
		40'	18/94	112	16%	114/410	554	20%	70 / 98.6	4.2
		50'	447/376	823		too many				
		60'	too many			"				

Gal+ / Gal- — B %

A 4.8 — 1.8

B 4.2

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19 May 16th, 1958 REF: 1408/5

W 3870 : rchg culture, 2 ml + 7.5 ml broth → 1 hr rot.
W 3908 " 1 ml + 7.5 → " rot.

Spun, resuspended, warmed — Mating mixture.
2 ml ♂ + 10 ml ♀ + 10 ml broth in flask.

Controls : ♂, 1 ml + 10 ml broth → 1 ml + 1.5 water →
→ .05 & .1 plated
♀, .5 ml + .5 ml broth → + 1.5 water → .05 & .1

Plate record : .05 diluted ♂ + .05 diluted ♀, as above. .1 + .1 also.

Sampling from mating mixture by means of warmed 1 ml pipette, to 1.5 ml chilled water, then blended, and amount from .1 to .05 plated on
D (for B, Met+)
B (Gal for B, Met+)

Times : 10', 15', 20', 22', 24', 26', 28', 30', 32', 34', 35', 38', 40', 43', 46', 50'.

Note : 32' is actually 33'10"
35'~~35"~~ " 34'30"
36'~~50"~~ 36'10"
50' 50'35"

From 34th or 36th minute (possibly also 38th), contamination from waterbath through the pipette — Exp. discarded

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19 May 16, 1958 REF: 1408/5

GAL PREUM TIMING: Hfr3, Hfr4.

1 hr rotated cultures of ♂4 and ♂13, ♀ 3908.
♂'s same as in exp 1408/3. - Cultures started with 2 ml ea. ♂, 1 ml ♀
Centrifuged, resuspended in 4 ml (2×), and mixed in flask.
4 ml ♂2× + 8 ml ♀2×.
Sampled at 10' intervals, chilled, blended, plated .05 & .1 on 5th Gal fin Meth Prot.

Controls: .03 of ♂ + .09 of ♀

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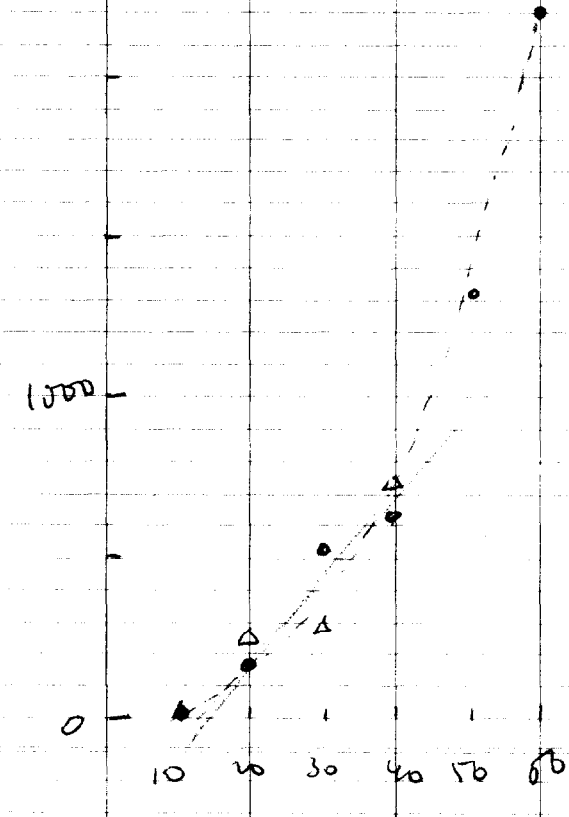
19

REF: 1408/6

	D (fm A,)		Sgal	
Hfr 13	A	B	A	B
	.05	.1		
10	22	104	21	
20	173	301 ~~304~~	271	
30	522		297	
40	637		725	
50	~320			
60	~200			



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copy + send

May 19, 1958 REF: 1408/7

Hfr_{13} - Gal

Hfr_{13}, W3908. - 1 ml + 7.5 ml Pen, 1 hr rotation.

Two timed incubs. A. non pulsed in Penassay

B. Pulsed in Penassay, diluted in BGA.

B. 8 ml ♂ conc 2x, + 12 ml ♀ conc. 2x, in flask in waterbath. 2 ml samples taken at 15, 20, 25, 30, 35, 40' — Chilled, blended, plated .05 on 5 Gal Bifco (some with Mg)

A. ♂ & ♀ conc 30 x in chilled Penassay (0.3 ml) then 0.2 of each mixed, incubated ½ 1'30", diluted to warmed BGA 1/100 (.2 to 20 ml).

Samples taken at one	15'	20'	25'	30'	35'	40'
amount	2	2	2	1	1	1
chilled diluent	–	–	1	1	2	3.

Blended, plated .05 on same medium as before.

Plate recombinants. .01 of 30 x conc. resp. + .9 water, of each parent .025 + .025, and .05 + .05 —

Plate counts	0	15	20	25	30	35	40
(A)	1	56	118	174	386	490	589
	2	83	126	176	208	497	541
	3		153	183	291	578	553
				213	317	521	571
		69.5	136	186	300	521	588
	x dilution	69.5	136	279	600	1563	2352

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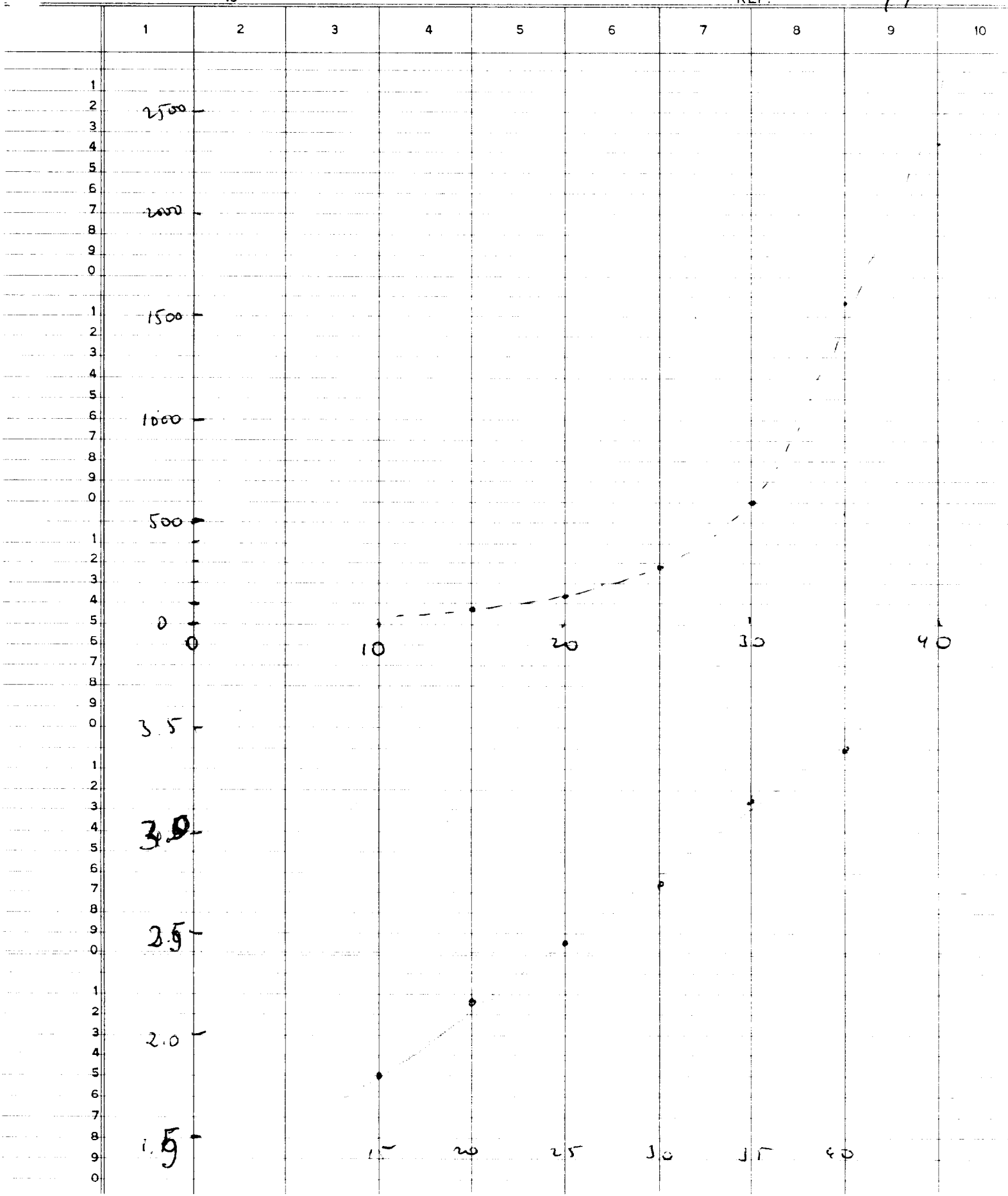
19

REF: 1408/7



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19 May 21, 1958 REF: 1408/8

Gal timing with Hfr_2

W 3870 from frz

W 3908 from recently isolated colony.

1 + 7.5 ml Pen, rotated 1 hr, spun, resuspended to same vol.

Mating mixture 5 ml ♂ + 15 ml ♀ at 37° in flask

Sample 1 ml every time, dilute with 1.5 ml chilled ~~water~~ broth & blend (A). and also further dilution (B).

times	17'	20'	23'	26'	29'	32'	35'	38'	41'
dilution B	1+1	1+2	1+3	1+4	1+5	1+6	1+7	1+8	1+9

(A) + H_2O

Plate .05 on minimum B_1 -

Plate recombination: suspensions diluted 1 + 1.5 water, .025 of each parent on plate.

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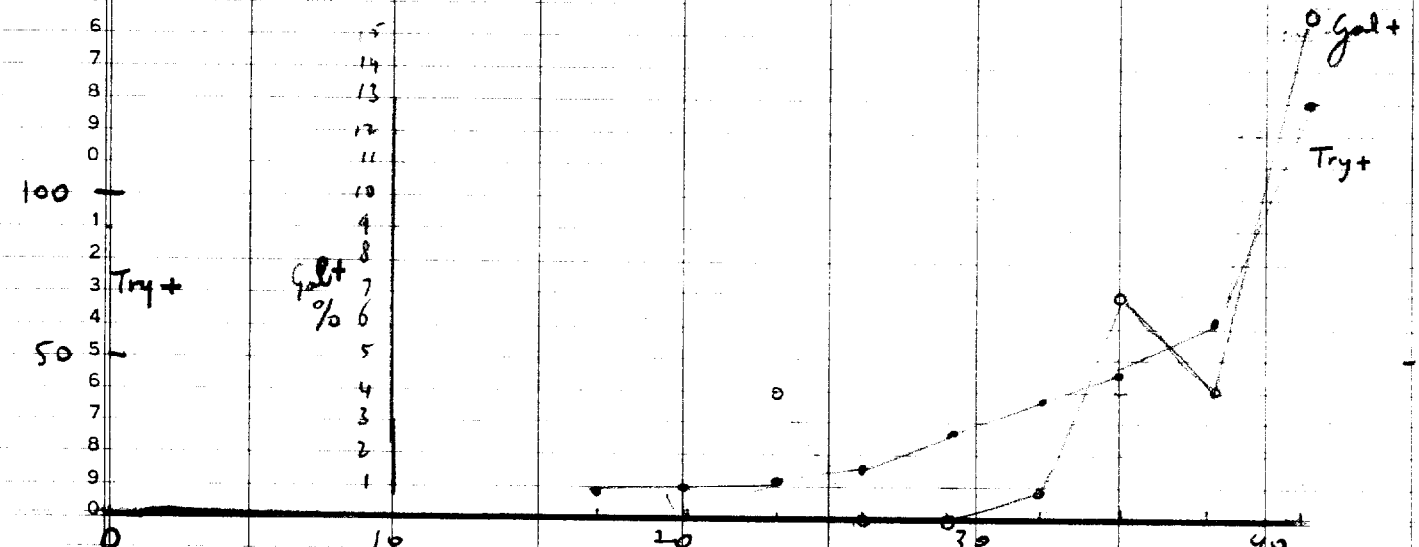
F2

23/5 19 53 REF: 1408/8

D (Sm B,

		A	B	ave	Gal A Gal+	Gal A TOT	Gal A %
17	10, 9	0, 4	9.5	–	–		
20	13, 12, 12	0, 3	10	0	3		
23	8	0, 0	11	1	25	4%	
26	19	1, 0	16.6	0	31	0	
29	31, 26	1, 0	27.2	0	52	0	
32	34, 39	0, 3	36.6	1	110	0.9	
35	39, 62	5	45.2	9	130	6.9	
38	51, 70	0, 1	60.7	5	122	4.1	
41	138	2, 5	130	43	275	15.6	



Two modes of entry ?

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19 May 24, 1958. REF: 1408/9

Gal timing with Hfr2

Identical to 1408/8 except that series B has been limited as follows:

Series B:	44'	47'	50'	
	1+1	1+2	1+3	(A) + H_2O
	1/2	1/3	1/4	dilution.

and timing: 10', 44', 47', 50' have been added

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19 May 26, 1958 — REF: 1408/9

Counts (5 4 9) —

	Time	D				S	
1							
2							
3							
4	0	43, 28,	Dilution 1/2.5: 13 colonies				
5							
6	10	10, 15, 21, 15				20, 26, 27, 19	
7							
8	17	33, 44, 30, 50				19, 33, 81, 81	
9							
10	20	53, 51, 50 51				33 26 29	
1	23	58, 56, 66, 56				45 42 52 31	
2							
3	26	72, 98, 82, 92				58 60 61 43	
4							
5	29	89, 97, 92,				47 45 45	
6							
7	32	168, 117, 121				55 106 85	
8							
9	35	123, 141, 138, (110)‡				94 138 115	
10							
	38	150 216, 200				152 161 150	
1							
2	41	322, 335, 360, 320				284/47 234/46 290/58 288/36	284/47 | 360/256
3		(*)					
4	44 B	253, 278. (1/2)	44 A : 443, 514			A. 386, 411	
5							
6	47 B	361, 500	47 A			B : 287, 262	
7							
8	50 B	580, 620				B 492, 525	
9							
10							

‡ agar surface scratched

*) inversion of A and B

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REF:

	1	2	3		4	5	6		7	8	9	10
		D				Sgal						
	Time	Σx	n	dil	$\bar{x} \times dil$	Σx	n	dil	$\bar{x} \times dil$			
8	10	61	4	1	15.25	90	4	1	< 22.50			
10	17	157	4	1	39.25	114	4	1	< 28.50			
11	20	205	4	1	51.25	88	3	1	< 29.33			
13	23	236	4	1	59.00	170	4	1	< 42.50			
15	26	345	4	1	86.25	222	4	1	< 55.50			
17	29	278	3	1	92.67	137	3	1	< 45.66			
19	32	346	3	1	115.33	246	3	1	< 82.00			
20	35	512	4	1	128.00	347	3	1	< 115.67			
22	38	566	3	1	188.67	467	3	1	< 155.67			
24	41	1337	4	1	334.25	1360	5	1	< 272.00			
26	44	531	2	2	531.00	797	2	1	< 398.5			
28	47	861	2	3	1291.5	549	2	3	< 823.5			
30	50	1200	2	4	2400.00	1017	2	4	< 2034			

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H 98-9

19

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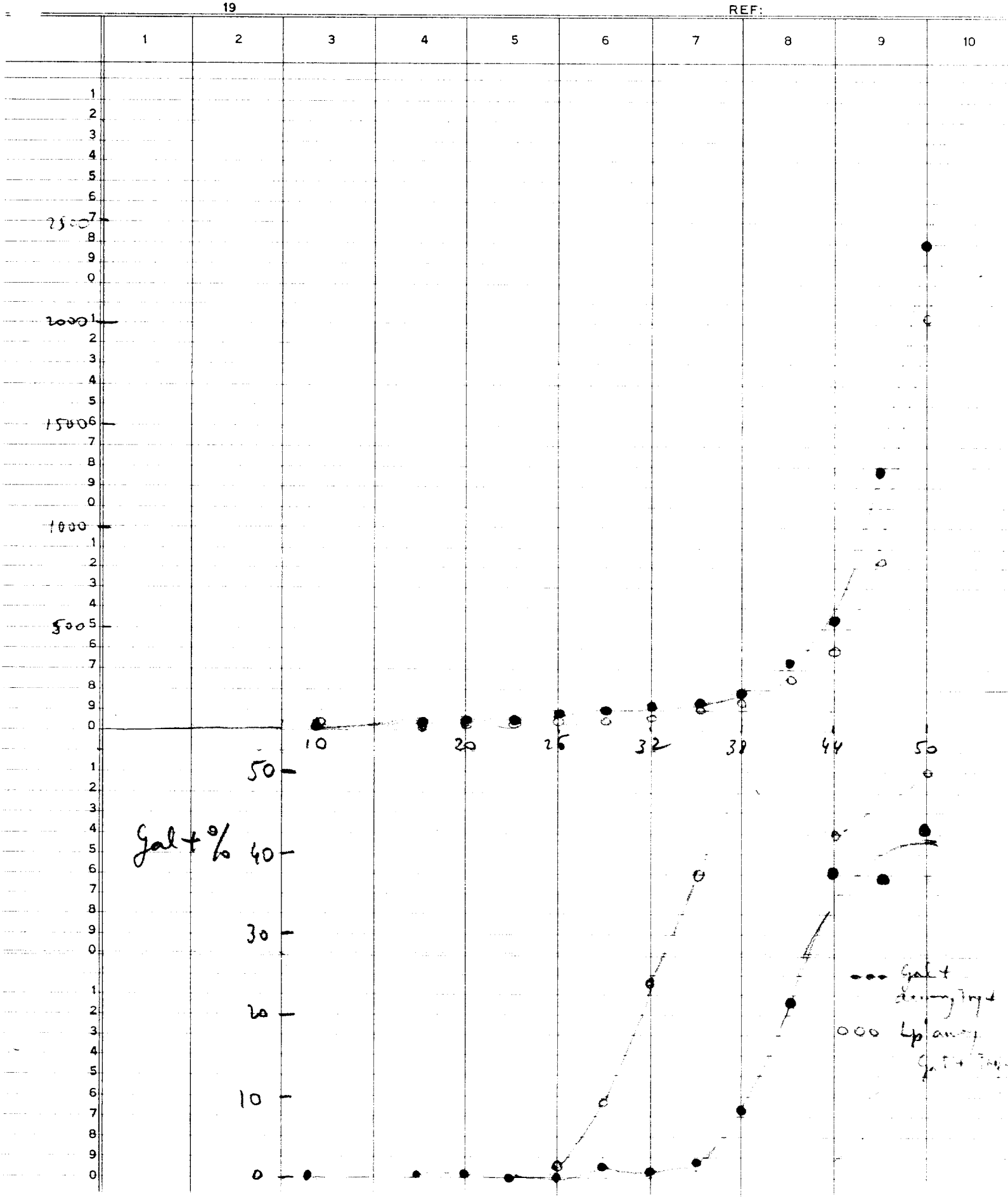

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19 REF: 1608/9

1	2	3	4	5	6	7	8	9	10
	20	29	32	35	38	41	44	47	50
Gal + on D red	tr –	–	–	1.8	87	17.4	37.9	36.4	43
Lp' among gal + on M try	1.9	9.6	24.1	37.5	60	42.5	50	47.5	50

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1958 May 31st REF: 1408/10.

Gal$_2$ timing - Hfr$_2$

W 3870 & W 3959 - (Gal$_2$ Try) -

Same as 1408/8 and 1408/9 -

1^{h}15' cultures (1 ml + 7.5 ml) - W 3870 from old broth in frig. - W 3959 recent (Rotated overnight) -

Spun, resuspended to same conc., mixed 5 ml ♂ + 18 ml ♀ in flask -

Sampled 1 ml at times: 23, 26, ... 59. + 1.5 ml chilled water

From 41' onwards also dilutions prepared (B Series).

	41	44	47	50	53	56	59	
Series A	1/2.5	1/2.5	1/2.5	1/2.5	1/2.5	1/2.5	1/2.5	blended → plated undiluted →
B*	2	3	6	12	20	50	100	

* dilution with respect to A

Platings: .05 on { D-0 B, Ser
S Gal " "
M Gal " " Try.

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♀ 3959 Gal_2^- Tr^- 4^+

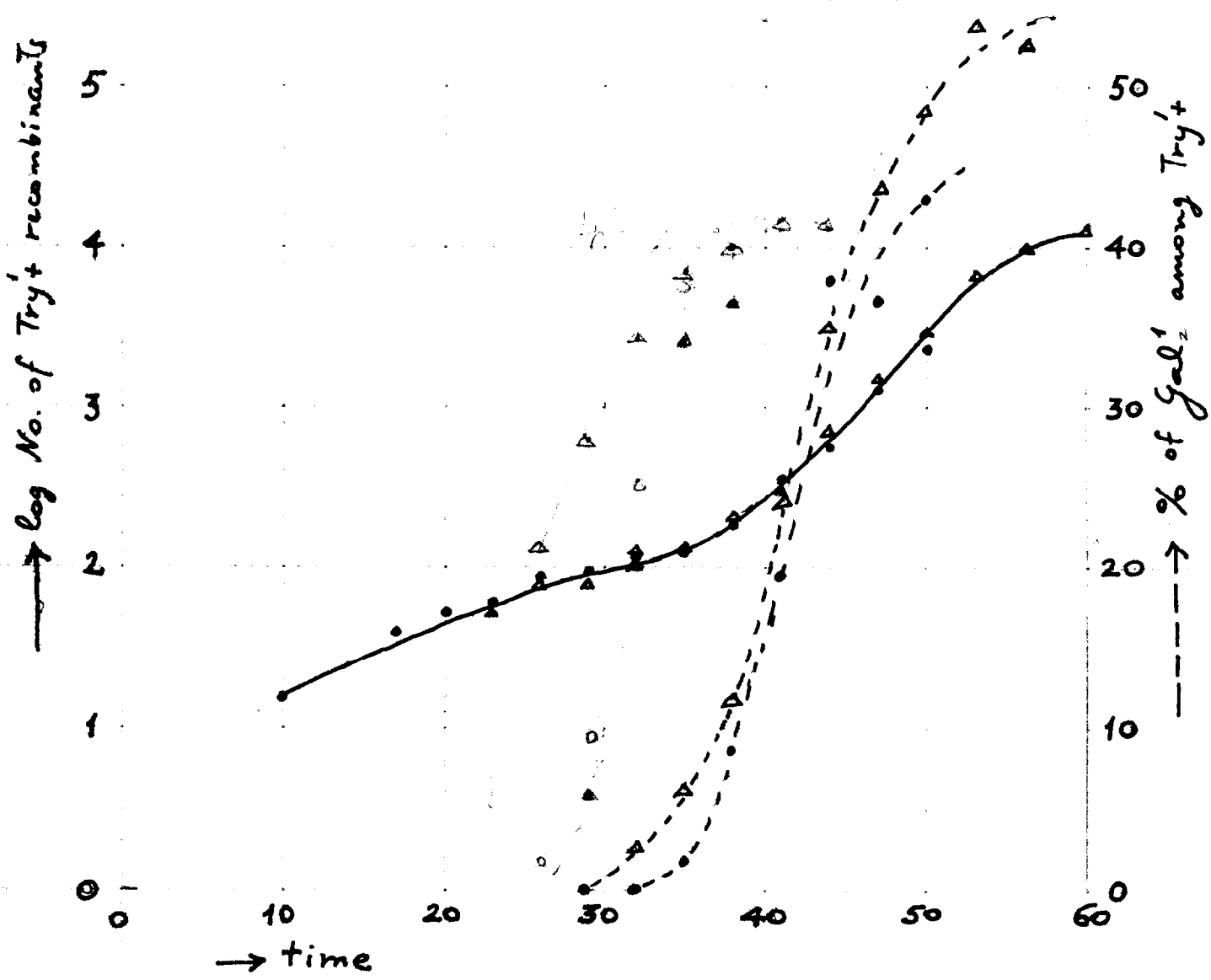
♂ 3870 2^+ Tr^+ 4h

2 June 1958 REF: 1408/10

	1	2	3	4	5	6	7	8	9	10
		D(B,fam)		Average x all	5' Gal fam B, Gal+	5' Gal fam B, tot	Sum	Ave	Gal %	
	0	8,(39)			3					
	23	61, 56, 40		52.3	1	32	32	32	3.12	
	26	62, 96, 79,		79.0	3, 9, 2	83, 90, 57	230	76.7	6.08	
	29	97, 63,		80.0	0	46	46	46	0.	
	32	108, 146		127	4, 3	55, 93	148	74	2.7	
	35	128 (162*), 115		135	2, 9	91, 90	181	90.5	6.1	
	38	230, 220, 165		205.0	24, 11	150, 148	298	149	11.7	
	41 B	139,	x 2	278	27, 19, 36	130, 106, 105	341	227	24.0	
	44 B	273, 217,	x 3	735	57	162	162	486	35.1	
	47 B	255	x 6	1530	88, 95	200, 219	419	1257	43.6	
	50 B	251	x 12	3012	81, 69	162, 147	309	1854	48.5	
	53 B	(333)*, 317	x 20	6500	121, 117	217, 225	442	4420	53.8	
	56 B	164, 236	x 50	10 000	47, 72	98, 130	228	5700	52.1	
	59 B	136,	x 100	13600	92, 72	143, 141	284	14200	57.7	

( ) clumps.

( )* " contaminated agar



Expts: 1408/9 •••• Gal_6
1408/10 ▲▲▲▲ Gal_2

Final yield of $\begin{matrix} Gal^+ Try^- \\ Gal^- Try^+ \end{matrix}$ } 3 × 10⁻⁶ of the input

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19 REF: 1408/10

	1	2	3	4	5	6	7	8	9	10
1										
2		Mgal Try fm 3/11	counts.		(after 2½ days, incub.)					
3										
4										
5		On the early	plates,	only	small	red gal+.				
6										
7		" "	late	"	also	big colonies	observed.			
8										
9										
10	Time		Gal+ (small)	(large) only	small (20 hour) colonies				9 Sq. under 6X objective 10 fields, 6 squares each	
11	23		11 *3							
12	26		58, 122 *50							
13	29		502,	1		49, 67, 40			33, 43, 39	11, 11, 5
14	32			3, 0		179, 147			198, 162	56, 33
15	35			4 2		345				67, 120
16	38			19, 26						175, 82
17	41 B			39, 33						104, 13
18	44 B			82, 62						52, 79
19	47 B			100 **						77, 113
20	50 B		*	128						49, 60
21	53 B		*	160, 95					146	43 29
22	56 B		*	61, 112					50, 29	14 5
23	59 B		*	105, 90					28, 30	10 8
24			* real, 50 taken							
25	0'		12,							
26										
27	** From 47 B	onwards	counts of "big gal+"s	are	different	because	of			
28	different	appearance	from	smaller	ones,	due	to	overcrowding.		
29	Comparison	between	various	methods	of	counting	small	colonies:		
30										
31		Total count	20 holes (×12)	10 fields 3a (×18)	10 fields 6a (×36)					
32	time									
33	29	502	624	738	684					
34	32	—	1955	3420	3382					
35	56 B	—	—	8[illegible]	723					
36	59 B	—	—	550	684					
37										
38										

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19

REF: 1408/10

	1	2	3	4	5	6	7	8	9	10
		(Average of duplicates)		Dilution factor						
1	Time	Gal + Strep	Gal + Trp			Ratio Gal + Trp + / Gal + Strep +				
2										
3										
4										
5										
6	0	12	-							
7										
8	25	14	-							
9										
10	30	115	-			-				
11	32	702	1			0.4				
12										
13	35	2719	1.5			3.4				
14										
15	35	7055	3			8.2				
16										
17	38	9728	23			24.0				
18										
19	40	18240	39	x 2		66.7				
20										
	44	14934	210	< 3		258				
21										
22	47	43320	600	x 6		667				
23										
24	50	31008	1536	x 12		1460				
25										
26	53	57700	2550	x 20		3497				
27										
28	56	36100	4300	x 50		5210				
29										
30	59	58400	7700	x 100		7847				
31										
32			* Interpolated from: (Trp +) x (50 Gal + strep +)							
33										
34										
35										
36										
37										
38										
39										
40										
41										
42										
43										
44										
45										
46										
47										
48										
49										
50										

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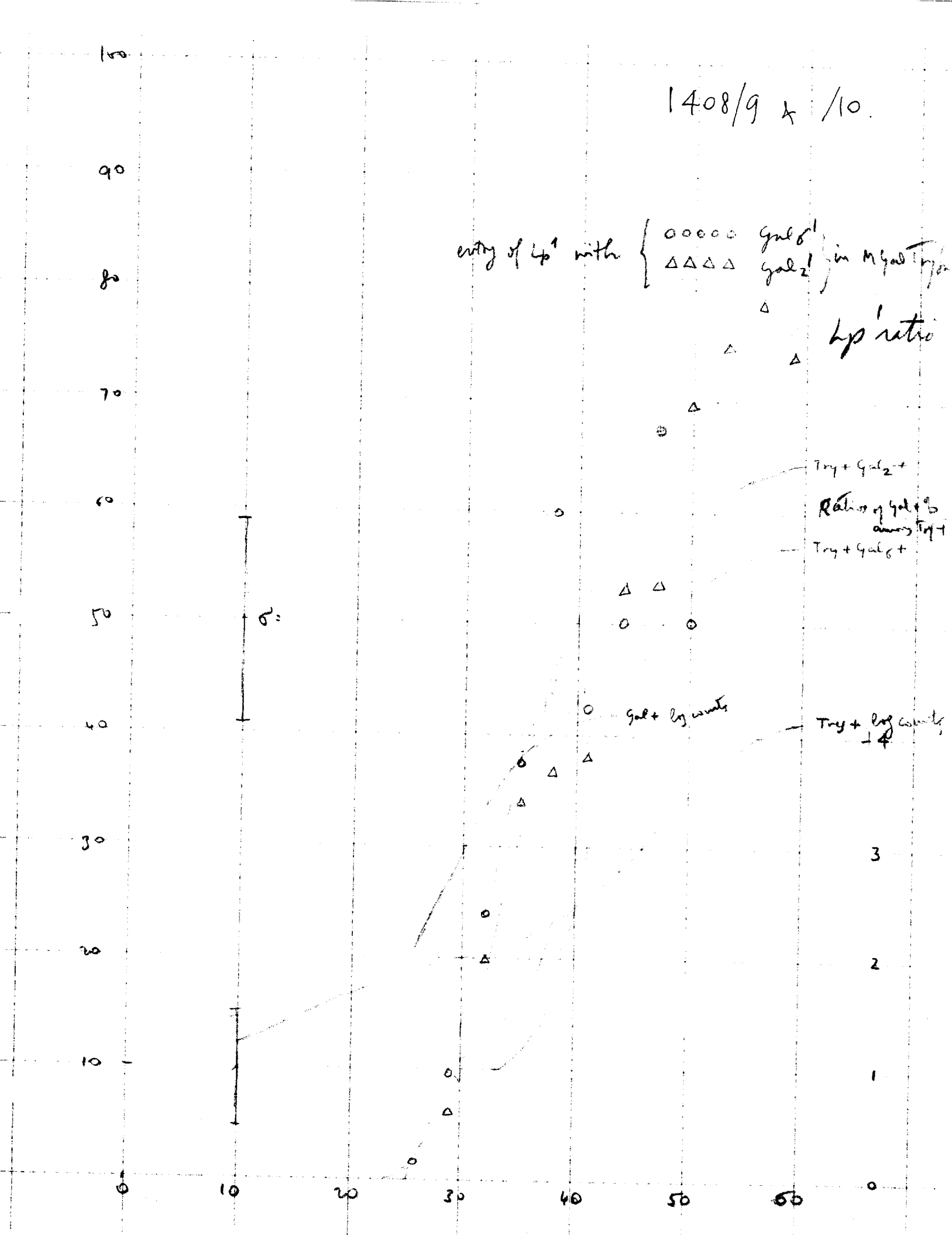
19 June 3 19[illegible] REF: 1401/10

	1	2	3	4	5	6	7	8	9	10
		4p⁻ 1R+		4p⁻ Tr t			4p⁻ Tyl + Try-			
1	T1 ax	many Sect-		many Sat+						
2										
3	23	-		33.	5/15		12/50			
4										
5	20	-			3/3	20.0	10/50			
6										
7	29	-				0.0	3/50			
8					5/6					
9	32	-				20.0	8/40			
0										
	3[illegible]	-			14/15	34.0	17/50			
1										
2	32	1.0 %	1/99		47/50	36.6	15/41			
3										
4	41	3.4 %	3/78		128/138	38.4	10/30			
5										
6	21	3.0 %	4/132		50/81	53.0	14/34			
7										
8	[illegible]	2.0 %	2/99		90/92	53.3	16/30			
9										
0	5	2 %	2/96		19/19	59.5	25/30			
1	3	4.2 %	4/90		23/31	74.5	35/47			
2										
3	[illegible]	[illegible] %	0.[illegible]		[illegible]	77.2	34/44			
4										
5	[illegible]		[illegible]		81/93	74.0	37/50			
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										


1408/9 & /10.
entry of Lp' with { ○○○○○ Gal6' / △△△△ Gal2' } in M Gal Try
Lp' ratio
Try + Gal2 +
Ratio of Gal + 6 assays Try +
Try + Gal6 +
σ
Gal + log counts
Try + log counts
14
100
90
80
70
60
50
40
30
20
10
0
10
20
30
40
50
60
3
2
1
0

19 June 10, 1958. REF: 1408/11

Hfr, TIMING OF GAL, LP, Try-

ORC W3953, W1895: 1 ml + 7.5 ml Penn 1^h vol.,
spun, resusp. each in 2 ml = 4 × conc.
Mixture: ♂ : ♀ = 1 : 1.

Sampled 1 ml at times: 10', 15', 20', 25', 30', 35', 40', 50'
and diluted 1/2.5 by addition of 1.5 chilled water.
Blended and plated .05 ml. on 3 media.
Sampled .5 ml at times: 60', 70', 80', 90', 100', 120', 150'
and diluted 1/3 by addition of 1 ml chilled water.
Blended and plated.

Media: M Gal Sm Meth Try
S' Gal Sm Meth
D Glu Sm Meth

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19 June 12 1958. REF: 1408/11

Time	S Gal from B, Meth. total count	S Gal from B, Meth. Gal+	D from B,	M Gal from B, Meth Try only Gal+
0	200	1	100, 139	0
10	183	0	310	0
15	213	0	N 320	0
20	172	0	Sm, 233	0
25	178	0	237, 234	0
30	192	1	N400, 209	0
35	269	0	275, 230	1
40	154	1	262, 230	contam 1, 1
50	142	1, 3	284, 140	3, 3
60	144	2, 0	milling	4, 1
70	Sm	0, 4	122, 181	3, 0
80	116, 126	3, 1	Sm, 196	1, 4
90	165	6, 6	191, 198	4, 4
100	138	5, 4	176, 150	7, 0
110	140, 80	7, 6	N350, 208	6, 6
120	140, 143	6, 5	223	2, 3
150	576, 599	68, 80	410	73, 64

From D from B, related on B Gal:

	total	Gal+	tot
0	45, 27	0, 0	0/72
30	51, 59	0, 0	0/110
70	32, 25	1, 1	2/57
90	57, 69	5, 2	7/128
120	48, 32	4, 0	4/80
150	85, 77	13, 11	24/162

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19 June 14, 1958. REF: 1408/12

Hfr timing of gal, Try.

ORC cultures, W 1895, W 3953. 1 ml + 7.5 ml 1% 3/4 nut. Spun, resusp. in chilled broth 2 ml each tube (4x), mixed 6+6 ml (A) and 2+2 ml in another tube (B).

(A): sampled at 0, 30, 60, 90, 120, 120, 140, 150, 160, 170, 180, 210, 240
0.5 ml + 2 ml chilled broth, blended, plated .05 on
M Gal Sm Meth Try.
D Sm Meth
S Gal Sm Meth
and also .05 on D Sm B, Meth powered.

(B) blended at 60', 120', 180' and sampled at 120', 150', 180', 210', 240'

0': From mixture in ice bath, dilute 1:5 in water as the others.

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1408-12

1895 ♂ M
3953 ♀ T-

19 June 15, 1958. REF: 1408/12

Time	D fm B_1 non blended	D fm B_1 blended	Pooled D fm B_1, mated not to B	Pooled D fm B_1, mated to B	B4 not B together Gal+	Gal-
0	0, 0		1, 0		0	
30	7, 6		4, 5			1
60	7, 9		1, 4			1
90	9, 10		6, 4		1	5, 1
120	7, 10	15, 9	5, 4	4, 2	1, 3, 3	4, 7, 10
130	6, 6		0,	3,		
140	10, 5	8	1,		0,	3
150	8, 4	6, 8	4,	5, 10, 6		
160	15, 15		5, 9			
170	11, 18		6, 14			
180	7, 9	19, 17	2, 13	10, 4	2, 0	7, 3
210	15, 11	9, 11	~~5~~, 18	~~7~~, 7		
240	24, 23	11, 29	not made.		4, 1, 2	8?, 7, 14?
W1895	0				0	
3953	0				1	

Counts too low — Experiment discarded without further testing.

Lack of late entry due to what?

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19 June 16 REF: 1408/13

	1	2	3	4	5	6	7	8	9	10
			Gal+	Mgal Sm Bl		Gal−				
			3-911			3172				
	Time									
	0		1			1				
	22		1			10				
	24		2			6				
	26		7			16				
	28		10			11				
	30		23			38				
	32		30			59				
	34		23			75				
	36		110			63				
	38		137			69				
	40		153			138				
	42		193			203				
	44		281			72				
	♀		0			1				
	♂		0							

Partial reversion to Gal+ 1/3870

2 Sm+ from set of 12 on indicator tube

19 June 16, 1958. REF: 1408/14

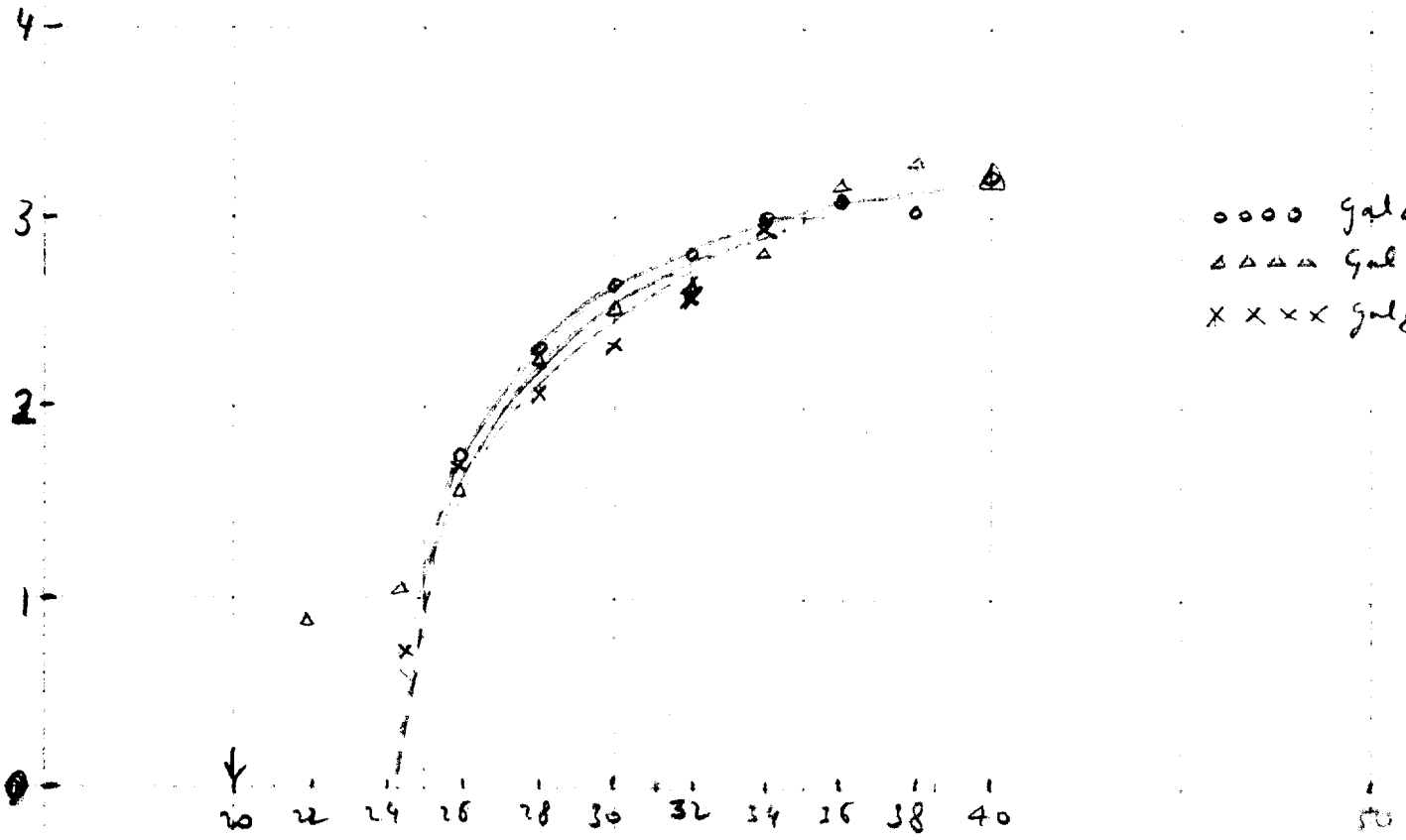
Gal timing, Hfr 2 / Gal 7, 4, 8.

ORC of W 3994, 3997, 3998 φ^R. W3870 from slant
~~Sp~~ 1 ml + 7.5 ml Pen, 1 h 30' rotation. Spun, resusp to same conc.
Mating mixture: 15 ml φ + 5 ml σ in flask.
(prewarmed)
Samples of 1 ml diluted with 1.5 ml of chilled DW, taken
at times: 0, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 50, 60'.
Blended and plated, .05 on M Gal B, Sm.

Algo: controls of parents, same conc. as for cross.

Note: 34' for Gal 7, diluted in 3 ml instead of 1.5.

1408/14


4
3
2
1
0
20
22
24
26
28
30
32
34
36
38
40
50
oooo Gal4 (cl. II)
ΔΔΔΔ Gal7 (cl. III)
×××× Gal8 (cl. I)

CISTRONS

I — II — (III) — IV ?

Kin → Transf. → Epim.

Gal_1

Gal_2

Gal_3

Gal_4

Gal_6

Gal_7

Gal_8

Gal_9

22

Lp $Gal_{1,6}$ Gal_2 →

Experiments :

1408/9 : Try Lp Gal_6 ← ; Gal_6 Lp ← } Gal_6 closer to Lp.

1408/10 Try Lp Gal_2 ; Gal_2 Lp ←

Lp Gal_2 Gal_6 ?

1408/13 1,2 Gal_2 Gal_1 ←

1408/14 4,7,8 Gal_4 Gal_7 Gal_8 ←

Lp (Gal_6 Gal_1)(Gal_4 Gal_7)// Gal_8 Gal_2 →

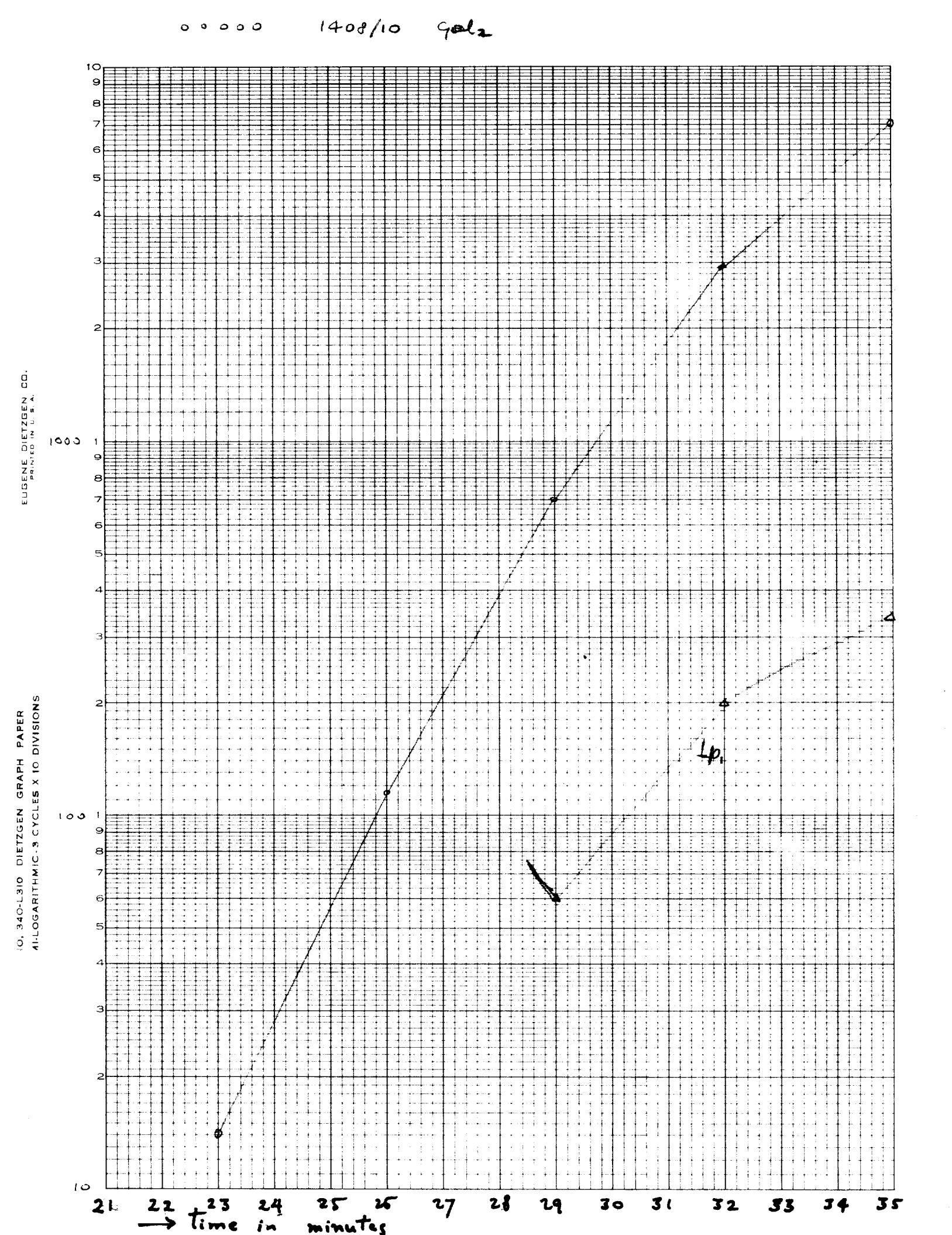
1408/15 2,3,4

1408/16 1,2,3,5

1408/17 1,2,8.

1408/18 2,4,6,9

/19 2,5,7,8.


00 000 1408/10 Gal2
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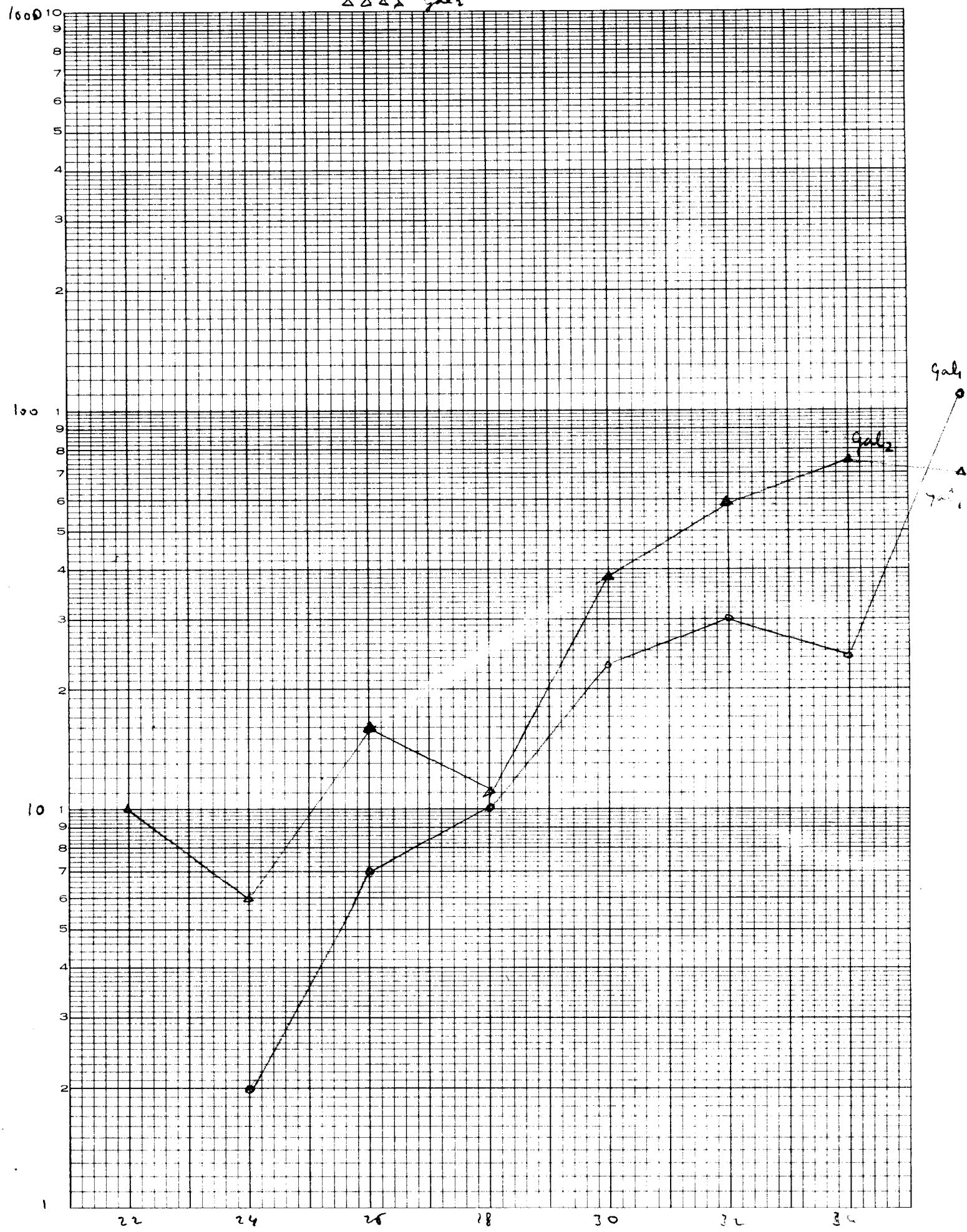
(O, 340-L310 DIETZGEN GRAPH PAPER
41-LOGARITHMIC-3 CYCLES X 10 DIVISIONS
Δp1
21 22 23 24 25 26 27 28 29 30 31 32 33 34 35
→ time in minutes

1408/13 ○○○○ Gal$_1$ △△△△ Gal$_2$ partial reversion F F+ of 3870.



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41-LOGARITHMIC-3 CYCLES X 10 DIVISIONS

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1412

B1

B2 and C

C

May 1 19 58

REF:

	1	2	3	4	5	6	7	8	9	10
	GAL	LAC	T1	GAL	LAC	T1	GAL	LAC	T1	
1	−	−	+	all −	−	+	−	−	+	
2	−	−	+		−	−	−	−	−	
3	−	−	+		−	+	−	−	+	
4	−	+	−		−	−	−	−	−	
5	−	−	+		−	+	−	−	±	
6	−	−	+		−	+	−	−	−	
7	−	−	+		− ↑.05	+	−	−	+	
8	−	−	+		− .1	+	−	−	+	
9	−	−	−		− ↓	+	−	−	+	
0	−	−	−			+	−	+	+	
1	−	−	−		−	−	−	−	+	
2	−	−	+		−	−	−	−	+	
3	−	−	+		−	+	−	−	−	
4	−	− ↑.05 0.1 ↓	+		−	+	−	−	−	
5	−	−	+		−	+	−	−	+	
6	−	−	+		−	−	−	−	−	
7	−	−	+		−	−	−	−	+	
8	−	−	+		−	−	−	−	−	
9	−	−	+		−	+	−	−	+	
0	−	−	+		−	+	+	+	+	
1	−	−	+		−	−	−	−	+	
2	−	−	−		−	+	−	−	+	
3	−	−	−		−	−	−	−	−	
4	−	−	−		+	−	−	−	−	
5	−	−	−		−	+	−	−	−	
6	−	−	−		−	−	−	−	−	
7	−	−	−		−	+	−	−	−	
8	−	−	−		−	+	−	−	−	
9	−	+	−		−	−	−	−	+	
0	−	−	+		−	+	−	−	−	
1	−	−	±		−	−	−	−	+	
2	−	−	+		−	+	−	−	+	
3	−	−	+		−	−	−	−	−	
4	−	−	−		−	+	−	−	+	
5	−	−	+		−	−	−	−	+	
6	−	−	−		+	−	−	−	+	
7	−	−	−		− ↑.05	+	−	−	−	
8	−	+	−		− .1 ↓	+	−	−	+	
9	−	−	+		−	+	−	W	+	
0	−	−	−		−	−	−	−	+	
1	−	−	+		−	−	−	−	+	
2	−	−	+		−	+	−	−	−	
3	−/w	−	+		−	−				
4	−	−	+		−	−				
5	−	−	+		−	+				
6	−	−	+		−	+				
7	−	−	±		−	−				
8	−	−			−	+				
9					−	+				
0					−	−				

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19 REF: 1408/14

A: Total count (binocular)

B: C. from 10 3×fields (× empirical factor 14) exp. factor 21).

Time	Gal4		Gal7		Gal8	
	A	B	A	B	A	B
0						
22	0		8		0	
24	0		11		6	
26	57		37		55	
28	210	(9)	195		128	(11)
30	471	(28)	326	(21)	224	(28)
32	630	45	420	30	427	(27)
34	994	71	630	45	1190	85
36	1246	89	1610	115	1176	84
38	1064	76	2310	165	1666	119
40	1890	135	1974	141	3080	220
50	7334	524	6496	464	8596	614
60	6930	495	6748	482	3430	245

( ) between brackets: values used for calculation of empirical factor; total plate count given in A

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19 June 18, 1957 REF: 1408/15.

Hfr₂ Timing of Gal₄, Gal₂, Gal₃.

Exactly as 1408/14, but:

1) ♀ parents were Gal₄, Gal₂, Gal₃, and they were seeded in this order.

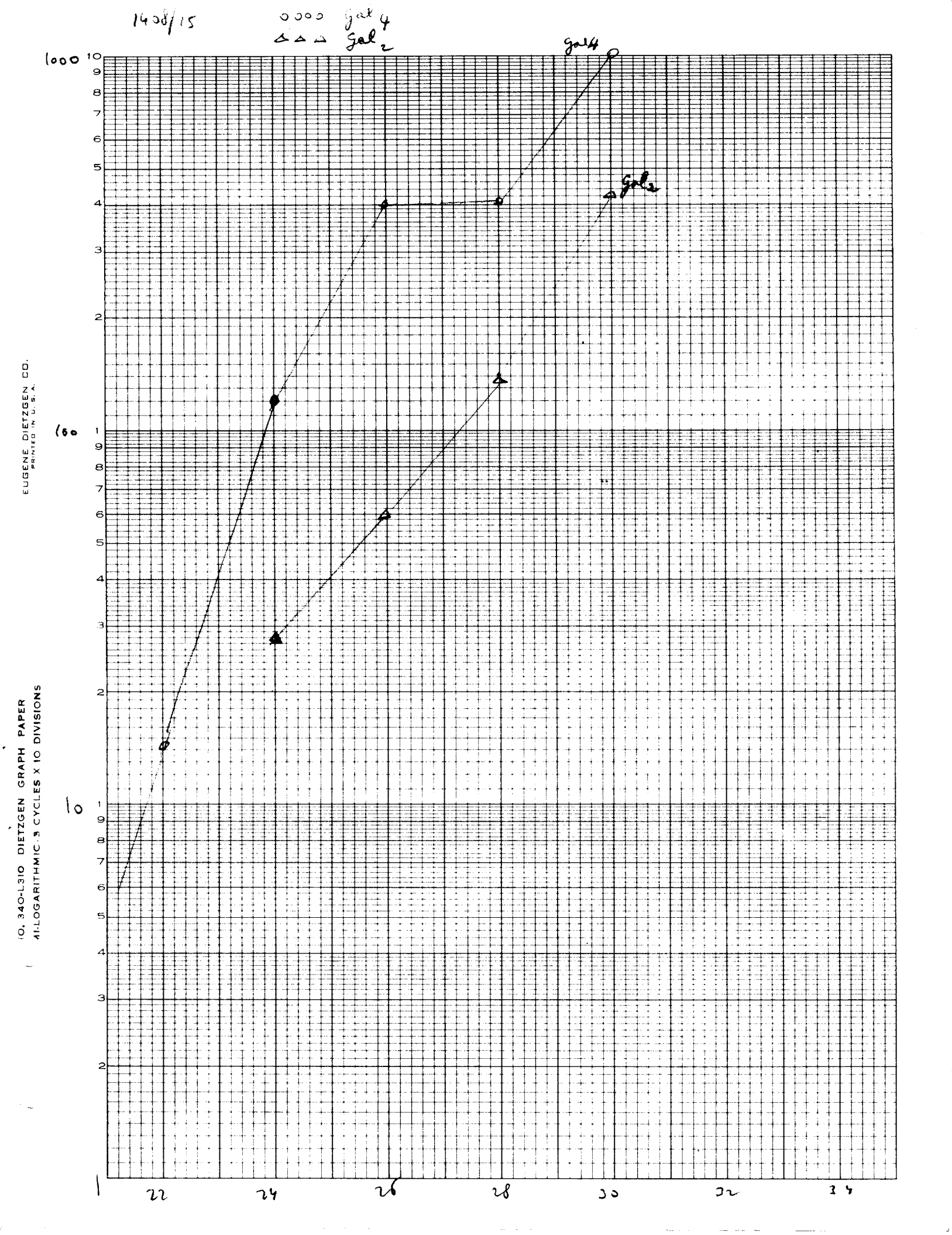
2) ♀ 3 was grown after Gal₄ and Gal₂ had been harvested, as a substitute for Gal₄ which was supposed to be tested today and was found to be St^s. ♀ 3 was found to be slightly less concentrated than the other two females and these two were collected into two, thus reaching 1.5 × conc. for this ♀.

3) Mating mixture: .5 ml ♂ + 12 ml ♀.

Plate counts. (48 hrs) (72 hrs)

Time	Gal₄	Gal₃		Gal₂
0	(35), 7	2, 0	13,	0, 0
15	7, 1	0, 3	11,	0, 0
18	2, 9	3.	27, 27	0, 1
20	3, 8	1, 0	23, 15	5, 2
22	18, 13	2, 3	18,	1, 10
24	138, 112	1, 3	16, 17	32, 24
26	451, 348	1, 1	8, 17	68, 51
28	410, 448	0, 1	11, 17	160, 110
30	t.m.t.b.c.	3, 12	13, 27	420,
35	↓	28, 25	38, 34	
40		55, 69	60,	
50		205, 198		
60		391, 356		

Note: Gal₃, all large colonies; in addition, few (<20 on plate) small ones and 1 or 2 extremely small.
Gal₂, Gal₄: all colonies small, as in former exptl.


1408/15
○○○○ Gal 4
△△△ Gal 2
Gal 4
Gal 2
1000
100
10
1
22
24
26
28
30
32
34
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19 June 20, 1950. REF: 1408/16

Hfr$_2$ timing of Gal$_{5,2,1,3}$.

Source of expts 13, 14, 15.

Mating mixtures: ♂ 4 ml + ♀ 12 ml.

Order of seeding flasks: Gal$_5$, Gal$_2$, Gal$_1$, Gal$_3$.

Times: 0', 18', 22', 24', 26', 28', 30', 32', 34', 40'.
120'

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1408/16

19 REF:

	1	2	3	4	5	6	7	8	9	10
		3991 Gal 1		3992 Gal 2		3993 Gal 3		3995 Gal 22		
4	0	1		4		2		2		
6	18	2		16		20,		3		
8	20	3,6	14	28, 14	63	17, 13	45	2, 0	3	
0	~~24~~	31, 30, 32	93	61, 91, 67	219	15, 14, 14	43	24, 40, 69	133	
1	22	23, 9, 30 D	62	22, 59, 54,	135	13, 12, 21	46	7, 17, 12,	36	
3	26	198, 174, 177	549	212, 252, 235	699	16, 21,	55	97, 69, 137	303	
5	28	193, 231, 101	525	313, 296, 264	873	19, 34, 18	71	227, 260, 818	731.	
7	30					11, 18, 13				
9	32					26, 41				
	34					54, 23 D				
2	40					191,				
	parent a	1		0		17		1		
6	parent b	light								

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19 June 22, 1958. REF: 1408/17

Hfr_2 timing of $Gal_{1,2,8}$

Same as expts 13, 14, 15, 16.

Mating mixtures: ♂ 4 ml + ♀ 12 ml.

Order of seeding flasks: Gal_8, Gal_1, Gal_2.

Times 0, 18, 20 (22), 24, 28, 28, 30, 32, 35'.

Note: Gal_1 at 22' is actually 23'.

19 June 23, 1958. REF: 1408/18

Hfr$_2$ timing of Gal$_2$, Gal$_4$, Gal$_6$, Gal$_9$

same as expts 13, → 17.

Mating mixtures: 15 mil ♀ + 5 mil ♂,

sampling at times: 0', 20', 22', 24', 26', 28', 30';
for Gal$_9$ also 35', 40', 50'.

Order of seeding: 2, 4, 6, 9

Note: Suspension of Gal$_9$ is granular, and slightly less conc. than others in spite of using 4 tubes resusp. to 24 mil. (4/3 conc.)

time 22' of Gal$_2$ seems very thin, possibly amount measured out of flask was spilled in ice bath?

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19 June 25, 1958 REF: 1408/19

Hfr$_2$ timing of Gal$_2$, Gal$_5$, Gal$_7$, Gal$_8$.

Same as exp^t 13-18. 1^{h}10' rotation. Gal$_5$ slightly less turbid than others.

Mating mixtures: 5 ml ♂ + 15 ml ♀.

Sampling at times: 0', 20', 22', 24', 26', 28', 30'.

Order of seeding 2, 5, 7, 8.

7 2/8 and 24/2 may have been exchanged at mating?

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19 June 27 REF: 1408/20

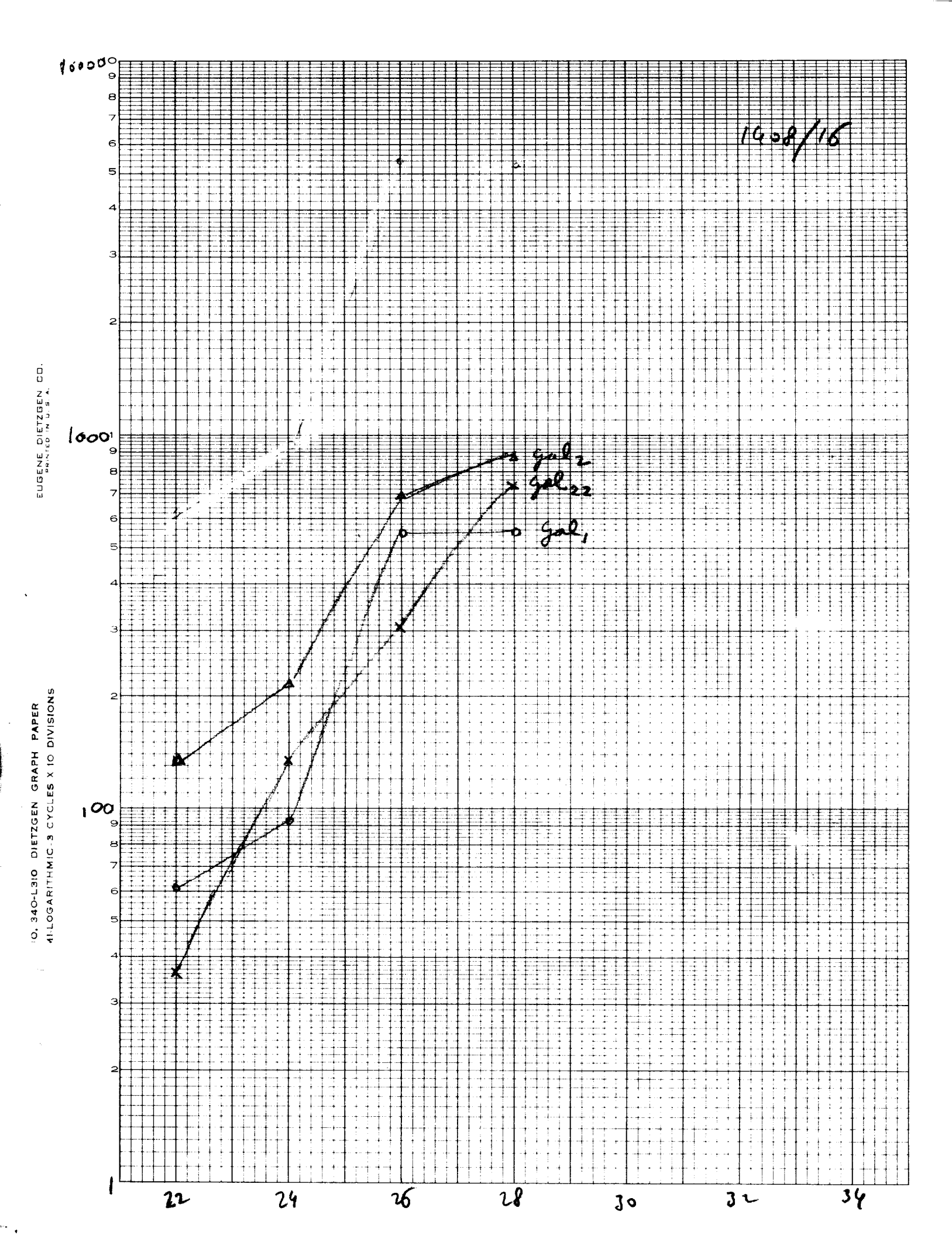
Hfr$_2$ Timing of Gal, Try.

ORC cultures of W 3870, W 3908, W 4066/1 (Try-S^{R}Gal-).
Refreshed, 1 ml + 7.5 ml for 1 hr I: indol 2 pm, T: tryptophan resusp. to 2 ml
(4 x conc). Crosses: 3870 x 3908 ; 3870 x 4066 - UV Gal+ W4076
Mating mixtures, in flasks: 20 ml ♂ + 6 ml ♀ = 1:3.
Samples: 0.2 ml + 1.8 chilled H_2O ; sound blended at every time: series (A)
if plated directly (.05 ml), if further diluted: series (B) (C)
Schedule:

Time ♀ Control	D(SmB$_1$) & S Gal SmB$_1$ A	B	M Gal SmB$_1$ Try A	& C
0	1/10		1/10	
10'				
20'				
22				
24				
26			26'	1/20 = A 1+1
28			28'	1/40 1+3
30			30'	1/80 1+7
33			33'	1/160 .5+9.5
36				
39		1/20 = A 1+1		
42		1/40 1+3	42'	1/500 .2+9.8
45		1/100 1+9		
48		1/200 .5+9.5		
51		1/400 .25+9.75	51'	1/1000 .1+9.9
54		1/800 .125+9.9		
57		1/1000 .1+9.9		
60'		1/1000 .1+9.9	60'	1/1000 .1+9.9
0/60 ♯		—		

0/60 : Control of plate recombination with parents kept in waterbath throughout the exp. (60').

Note: 51' missed. 33' was 40" late


100000
1000
100
1
22
24
26
28
30
32
34
Gal 2
Gal 22
Gal 1
1408/16
EUGENE DIETZGEN CO.
PRINTED IN U.S.A.
NO. 340-L310 DIETZGEN GRAPH PAPER
4 1 LOGARITHMIC 3 CYCLES X 10 DIVISIONS

DATE: 4.16.54. REF: 1409-I.

ENZYMES ON PROTOPLASTS.

1 and 2 ml of 3060 + 10 ml L_2 + 10^4 U/Penicillin
0.5 and 1 ml of 3064 + "
Centrifuged, resuspended in half the amount of L_2.
Mixed in equal volumes with:

A RN-ase 2 mg/ml in L_2
B Chymotrypsin "
C Lysozyme "
D DN-ase "

N : = control + L_2 medium,
F Versene 4% 0.25 ml added, + 0.25 ml L_2 + 0.25 ml ♂
G Lysozyme 0.25 ml + Versene 0.25 + ♂ 0.25.

After 15-30': Protoplast counts in Petroff chamber. (millions/ml).
(20 small squares: sum, multiply $\times 10^6$).

N	A	B	C	D	F	G
63	70	60	54	81	82	59.

After 2 hours:

44	27*	41	44	42	63	not done.

* many empties.

Conclusions:
Only RNA-ase seems to affect, and only to a moderate degree, the protoplasts count.

This is test of lysis of protoplasts by total count.

DATE: 4/18/58. REF: 1409-2

ACTION OF ENZYMES ON MATING ♂♂ × ♀.

3060 ♂ (2 ml + 10 ml L_2, 2'30' in veb.), centrifuged, concentrated 10× in L_2 + 3064 ♀ conc. 30× in L_2.

0.3 + 0.3 ml in waterbath for 20', then 0.1 ml. added to 1 ml of:

A Chymotrypsin 1 mg/ml in L_2
B Lysozyme, "
C RN-ase "
D DN-ase
L control, L_2 medium.

40' further incubation after addition to enzyme, then

~~0,02 ml~~ 0.1 + 10.0 DW, → { 0.1 ml / 0.01 ml } on mix St B,

Protoplast suspensions: same as those used for exp. 1405-5 before diluting 5×.

Plate counts:

	A	B	C	D	L
0.1	too many, ∞	∞	∞	∞	∞
0.01	113	43	62	74	93
Gal+	0/48	2/43	0/47	0/42	1/50
Lac+	7/48	5/43	5/47	1/42	6/50
T_1'	19/48	18/43	31/47	16/42	23/50

1410

DATE: 4/18/58 — REF: 1410

INTERRUPTION AND DIPLOIDS.

2323, 2735, ~~are~~ overnight rotated cultures, mixed in equal amounts, pulse of 8', then diluted 1/200 in warm broth, further incubation: 20'; 40'; 60' in water bath. After such times:

0.1 → min B₁ } Hyphiated 1': Stac NC!
1/10 0.1 → " } 2': Stac, Stac B₁ (P21)
1/10 0.01 → "
→ B Lac.

N.B. Numbers meaningless ~~on account~~ of smearing and poor scores on lac. ~~Colonies~~ as can.

	20' [A]	40' [B]	60' [C]	
D(B₁) total	288	412	181	($\frac{1}{10}$ .1 ml).
Stac B₁	9…29	—	52…80	Lac+
Stal B₁	34…42	—	38…42	… dyad-synthesis

Lac + Isol ✓ checked 4/22

2(2)

interrupted only by plating.

DATE: REF: 1410

Irradiation of JO60 to obtain Ara-

6 B Ara plates irradiated with 9 and 10 secs.
3 positive Ara- mutants found
streaked to B Ara

DATE: 22/4 REF: 1410/2

Experiment 1410/1 repeated.

Platings of $\frac{1}{10}$ dil in DW of 0.1 ; 0.02 on min B_1

0.1 on S Lac B_1, S Gal B_1

Note: Is plating interruption sufficient with 2323 ?

Frequency of Gal+ seems unaffected by plating time. in exp 1410-1.

S Gal milling for 40'.

Counts:			
$D(B_1)$	20'	40'	60'
$\frac{1}{10}$ 0.1	26	111	35
0.02	2	4	6
S Lac B_1	10 Lac+		

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19 30th April 1958. REF: 1410/3

1217 × 3064. ORC.

3 × conc. in saline / .05 on plate of min B_1.
3,5 ml.

1217 Control: 0 colonies.

Colonies streaked on EMB Lac.

DATE: 4/21/58 REF: 1411

COLCHICINE - Effect on mating

Solution of colchicine 1% in distilled water, non sterile, stored at -20°.

3060, 3064 overnight rotated cult:

0.1 ml to 0.8 ml Penassay + 0.1 H_2O (Control)*

" " + 0.1 colchicine solution 1%.

1h rotation -

CO: mixed equal amounts of colchicined broths, + 0.1 ml. colchicine/ml.

I: mixed equal amounts of (Control)* broths, 0.1 ml colchicine water added per ml.

C: mixed equal amounts of (Control)* broths 0.1 ml water added per ml.

10' incubation in water bath, then dilution 1/100 in broth, incubation 20', then:

1/10 DW → 0.05 mixSt B1

↓

1/10 DW → same

↓

1/100 DW → 0.05 B lac.

	CROSSES 1/10	1/100	B Lac Lac+	Lac-
CO	152	6	6	17
I	69	7	6	14
C	146	14	6	15

Conclusions: There is perhaps a small decrease in No of prototrophs adding colchicine to the mating mixture (but not adding it in advance to the cultures: adaptive enzyme destroying colchicine?) -

Freezing mating cultures

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April 26 1958 REF: 1401-2.

ORF = overnight aerated cultures.

W3060 (overnight) 3X 10ml → 3
W3064S (rotated) 30X 10ml → 0.3 ml. in fresh prewarmed } .2 .2 mixed.

Prewarmed cultures mixed in a 125 ml flask. After 60 seconds added 19.6 ml prewarmed broth for 1:20 dilution. Mix gently in flask.

[A2] Add 1 ml samples to prechilled tubes in CO_2-acetone bath.

[A1] Dilute 1 + 3 ml 20% glycerol and freeze. = glycerol-freeze.

Dilute 1:250 in broth, incubate 15 minutes. Chill in ice bath.

[B] Sample 1+3 ml glycerol & freeze.

Blend 30 seconds.

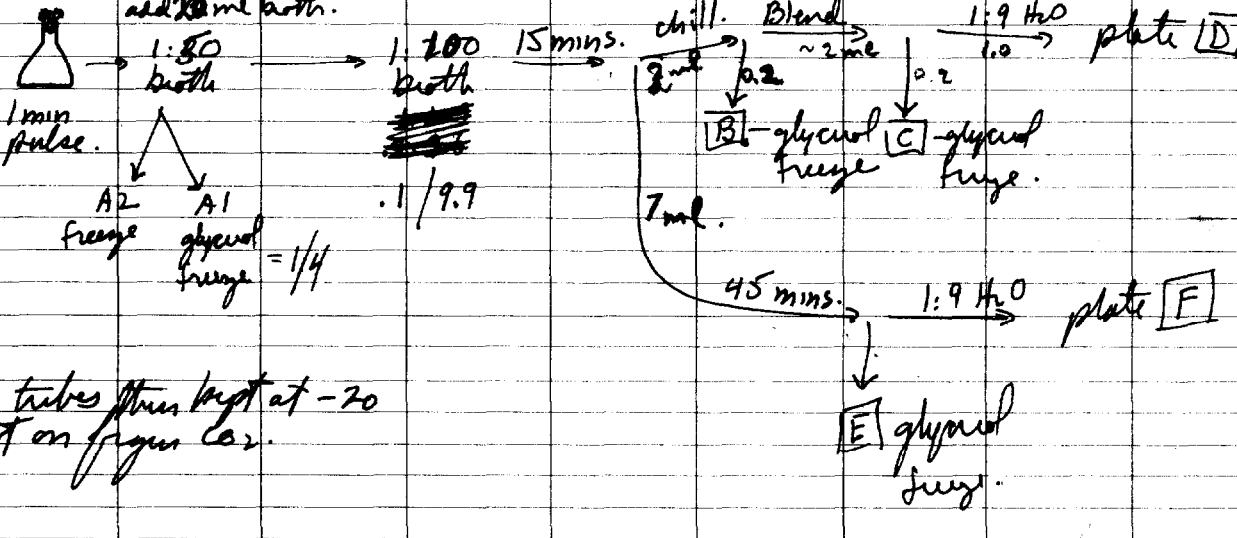
[C] glycerol-freeze

Dilute 1/9 in water and plate [D].

Incubate 45 minutes further

[E] glycerol freeze [F] dilute 1/9 in water and plate.

D and F plated .05 and .1 ml on DB, sm and B bac.



frozen tubes stored at -70 not on frozen CO_2.

addendum: Drosophila eggs and larvae in agar/10% glycerol. Chill in ice water & freeze quickly in CO_2 acetone. — proved workable.

See Luca's notes: he finds that freezing interrupts pairs; yields of recombinants are about 50% of non-frozen controls. Technique is usable subject to some possible bias; may be good way to interrupt (contra first diluting in chilled saline.)

(over)

3060 x 3064.

(1412)

5 1 ml samples.

1 ml. A.

Pulse 1 minute. 1:20 ~~dilution~~ dilution = 20 ml.

fuge 1 sample.

incubate 1:~~200~~ dilution

→ 15 minutes

- fuge 1 sample – 1 ml. B
- ~~dilute 1:10~~ blend dilute 1:10 → fuge 1 sample C → count
- ~~dilute 1:10~~ blend dilute 1:10 → plate 1 sample D → count
- incubate 45 m. → fuge 1 sample E → count
- incubate 45 m. → plate 1 sample F. → count

---

1. – F. interruption by fusing as TL+ count.

B – F interruption by bac ratio.

~~B~~ –

C – D viability of zygotes.

C – E interruption by bac ratio

B, CE vs D, F viability of zygotes as TL count.

C, E vs DF viability of input.

storage; methods of thawing

FREEZE

DATE: 4/26/58, REF: 1412

3060 0.2 c.c. conc. 3 x broth 0.2 ml } in 50 ml flasks warm
3064 30 x 0.2 ml }

After 1' pulse add 19.6 ml broth from test tube. (1/50 dil)

sample for further dilution (0.1 ml + 9.9 prewarmed broth) (1/5000)

(A1) glycerol freeze 0.2 + 0.6 glycerol 20%.

(A2) freez

15' incubation

chill in icebath 2 ml

incubate further 45'

(B) glycerol freeze 0.2+0.6

Blend

1+9 H2O

(C) glycerol freeze 0.2+0.6

(D) plate

(E) glycerol freeze 0.2+0.6

1+9 H2O

(F) plate

Immediate platings: D, F on twin ft B, 0.05 & 0.1

counts:

	0.1	0.05
D	12,13	7,9
F	45,30	16,25

DATE: REF: 1412

C, E plated on 4/28/58 after thawing in water bath without further dilution (they are 2.5 × more concentrated than comparable lab, 5, F). Plate on mist B_1 of 0.1 and 0.05 ml.

B thawed in water bath on 4/28/58, divided into B_1 & B_2

B_1 : incubated 45', plate 0.1 & 0.05 on mist B_1

B_2 : plated at once " "

Plate counts :

	B_1	B_2	C	E
0.1	19, 15	2, 11*	26, 27	30, 47
0.05	6, 8	4, 2	8, 9	31, 12

Comparison between C & D : C, total 70 col. ÷ 2.5 = 28

D, total 41

survival $\frac{28}{41}$ = 67%.

E & F : E total 120 col ÷ 2.5 = 48

F 116 col

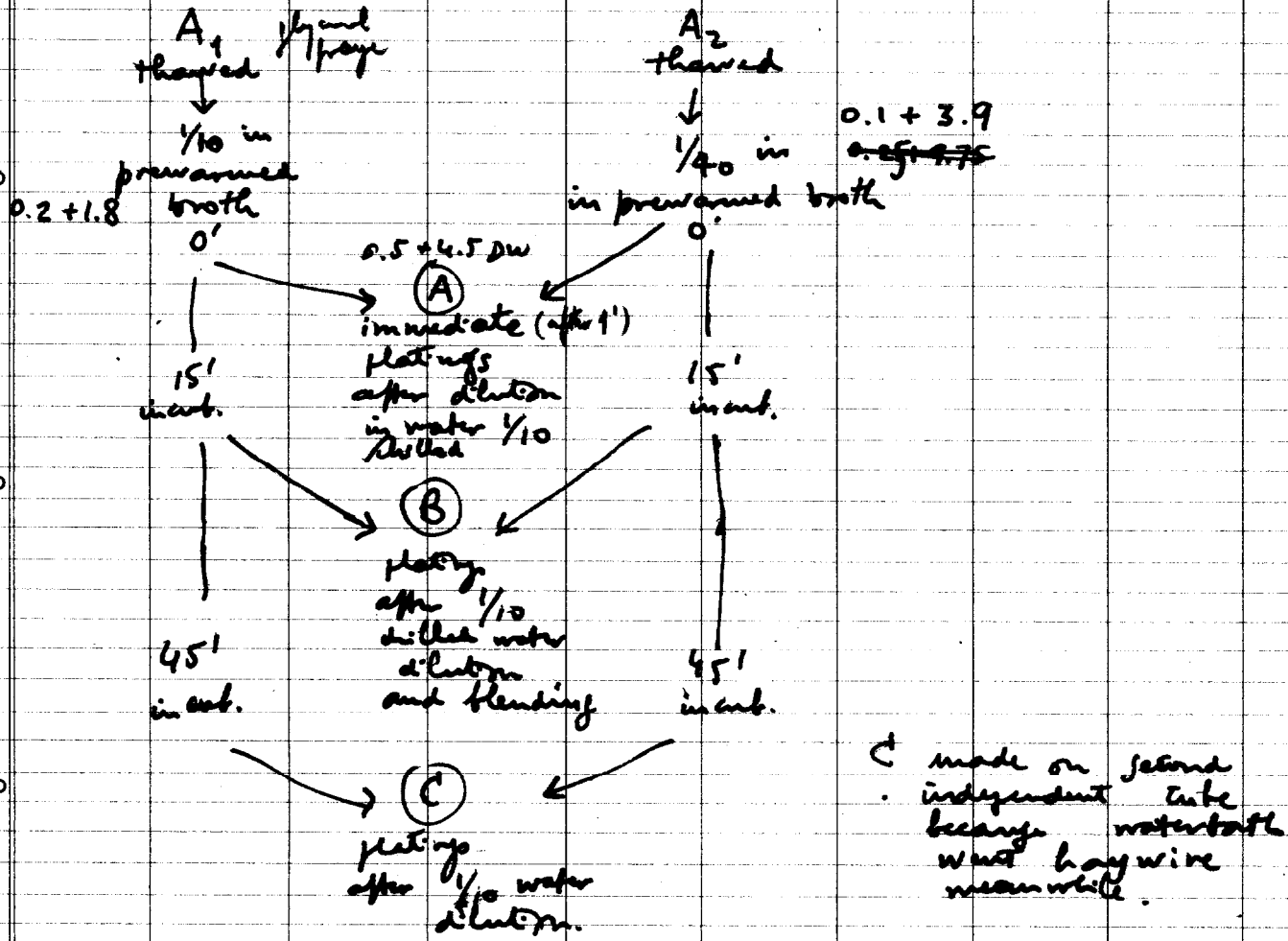
survival $\frac{48}{116}$ = 40%.

B_1 & C B_1 48 col.

C 70 col.

DATE: 4/29/58 REF: 1412

A_1 and A_2 tested for ability to resume mating.



Platings: .1 + .05 ml on minSt B_1.

Plate recombination controls.

Frozen parents (see exp 1412/2):

3060 [0.1 ml + 9.9 ml broth] → [0.5 + 9.5 ml] → 1/10 DW
30 x conc. from expon. culture, considered 20% of saturation.

3064 [0.1 ml + 9.9 ml broth] → [.5 + 9.5 ml] → 1/10 DW
30 x conc saturated culture.

From dilutions in water: plates with 0.05 + 0.05
0.01 + 0.01

DATE: 4/31/58 REF: 1412

Plate counts:

Plate recombination controls:

	0.1	0.05
A_1-A	3	0
A_2-A	0	0
A_1-B	4	3
A_2-B	0	0
A_1-C	42	14
A_2-C	0	0

0.1 1 colony
0.02 0 "

Conclusions:

- glycerol necessary.

Probably use of frozen pairs is OK, but only about 40-50% survival.

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19 29/4/58 REF: 1412/2

FROZEN PARENTS

3060 broth culture; 4 cult: 1 ml + 9 ml fresh broth, rotated. After 1 h, spun, concentrated 30 x in broth-glycerol * frozen in dry ice + acetone in 0.1 ml amounts.

* 100 gms glycerol + 100 ml Penassay 2 x strength

3064 : 2 saturated broth cultures used.

3060 } collected in 0.7 ml per tube.
3064 }

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1412

B1 B2 and C C

May 1 19 58

REF:

	1	2	3	4	5	6	7	8	9	10
	GAL	LAC	T1	GAL	LAC	T1	GAL	LAC	T1	
1	−	−	+	all −	−	+	−	−	+	
2	−	−	+		−	−	−	−	−	
3	−	−	+		−	+	−	−	+	
4	−	+	−		−	−	−	−	−	
5	−	−	+		−	+	−	−	±	
6	−	−	+		−	+	−	−	−	
7	−	−	+		−	+	−	−	+	
8	−	−	+		− .05	+	−	−	+	
9	−	−	−		− .1	+	−	−	+	
0	−	−	−		− ↓	+	−	+	+	
1	−	−	−		−	−	−	−	+	
2	−	−	+		−	−	−	−	+	
3	−	−	+		−	+	−	−	−	
4	−	− .05 .1 ↓	+		−	+	−	−	−	
5	−	−	+		−	+	−	−	+	
6	−	−	+		−	−	−	−	−	
7	−	−	+		−	−	−	−	+	
8	−	−	+		−	−	−	−	−	
9	−	−	+		−	+	−	−	+	
0	−	−	+		−	+	+	+	+	
1	−	−	+	C	−	−	−	−	+	
2	−	−	−		−	+	−	−	+	
3	−	−	−		−	−	−	−	+	
4	−	−	−		+	−	−	−	−	
5	−	−	−		−	+	−	−	−	
6	−	−	−		−	−	−	−	−	
7	−	−	−		−	+	−	−	−	
8	−	−	−		−	+	−	−	−	
9	−	+	−		−	−	−	−	+	
0	−	−	+		−	+	−	−	−	
1	−	−	±		−	−	−	−	+	
2	−	−	+		−	+	−	−	+	
3	−	−	+		−	−	−	−	−	
4	−	−	−		−	+	−	−	+	
5	−	−	+		−	−	−	−	+	
6	−	−	−		+	−	−	−	+	
7	−	−	−		−	+	−	−	−	
8	−	+	−		− .05	+	−	−	+	
9	−	−	+		− .1 ↓	+	−	w	+	
0	−	−	−		−	−	−	−	+	
1	−	−	+		−	−	−	−	+	
2	−	−	+		−	+	−	−	−	
3	−/w	−	+		−	−				
4	−	−	+		−	−				
5	−	−	+		−	+				
6	−	−	+		−	+				
7	−	−	±		−	−				
8	−	−			−	+				
9					−	+				
0					−	−				

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REF:

	1	2	3	4	5	6	7	8	9	10
	Gal	LAC	Li	GAL	LAC	T1	GAL	LAC	T1	
1	all −	−	−				−	−	−	
2		−	+				− (w)	−	+	
3		−	+	+	+	-.105	−	−	−	
4		−	−	w	−	- ↓	+	+	−	
5		−	−	+	+	−	−	−	+	
6		−	−	−	+	−	−	−	+	
7		−	−	−	−	−	−	−	+	
8		−	−	−	−	−	+	+	−	
9		−	−	−	+	−	−	+	−	
0		−	−	−	+	−	−	−	+	
1		−	+				−	−	+	
2		−	+				−	−	−	
3		−	+				−	−	−	
4		−	−				−	−	+	
5		−	+				−	−	−	
6		w .05	−				−	+	−	
7		− .1 ↓	−				−	−	−	
8		−	−				−	−	+	
9		−	+				−	−	+	
0		−	−				−	−	−	
1		−	−				−	−	−	
2		−	−				−	+	−	
3		−	−				−	+	−	
4		−	−				−	−	−	
5		−	−				−	−	−	
6		−	+				−	−	−	
7		−	+				−	−	−	
8		−	+				−	+	−	
9		−	+				−	+	−	
0		−	+				−	+	−	
1		−	−				−	−	−	
2		−	+				+/−	−	+	
3		−	+				− (w)	−	−	
4		−	−				−	+ .05	−	
5		−	+				−	− .1 ↓	−	
6		−	±				−	−	−	
7		−	+				−	−	−	
8		−	−				−	+	−	
9		−	−				−	+	−	
0		−	+				−	+	−	
1							+	+	−	
2							+	+	−	
3							−	+	−	
4							+	+	−	
5							−	−	−	
6							−	−	+	
7							-(w)	−	+	
8							−	−	−	
9							−	−	+	
0							−	+	−	

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E a E b F a

May 1 1958 REF:

	1	2	3	4	5	6	7	8	9	10
	GAL	LAC	TI	GAL	LAC	TI	GAL	LAC	TI	
1	−	−	+	−	+	−	+	+	−	
2	−	−	+	−	−	−	−	−	+	
3	+	+	−	−	−	+	−	−	+	
4	−	+	−	−	−	+	−	−	+	
5	+/−	−(tw?)	−	−	−	−	−	−	−	
6	−	−	−	−	+	−	−	−	+	
7	−	−	−	−	−	−	−	−	−	
8	−	−	+	−	−	+	−	−	−	
9	−	−	−	−	+	−	−	−	−	
0	−	−	+	−	+	−	−	−	−	
1	−	−	−	−	−	−	−	+	+	
2	−	−	−	−	+	−	−	−	+	
3	−	+	−	−	−	+	−	−	+	
4	−	+	−	+	+	−	−	+	−	
5	−	−	+	−	−	+	−	−	+	
6	−	+	−				−	−	+	
7	−	+	−				−	−	−	
8	−	−	−				−	+	−	
9	−	−	+				−	−	−	
0	−	−	+				−	−	−	
1	W(−?)	−	+				−	+	−	
2	−	−	−				−	−	+	
3	−	−	−				−	−	+	
4	−	−	−				−	−	+	
5	−	−	−				−	−	−	
6	−	−	−				−	−	+	
7	−	−	−				−	−	+	
8	−	−	−				−	+	−	
9	−	−					−	−	−	
0	−	−	+				−	−	−	
1	−	−	+				+/−	+/−	−	
2	+/−	+	+				−	−	−	
3	−	−	+				−	−	+	
4	−	−	−				+	+	−	
5	−	+	−				−	+	−	
6	−	−	+				−	−	−	
7	−	−	+				−	−	−	
8	−	W	−				−	+	−	
9	−	−	+				−	+	−	
0	+	+	−				−	+	−	
1	+	+	−				−	−	+	
2	−	−	−				−	−	−	
3	−	−	+				−	+	−	
4	−	−	+				−	−	+	
5	+	+	−				−	−	+	
6	−	−	+				−	+	−	
7	−	−	+				−	+	−	
8	−	−	+				−(w)	−	−	
9	−	−	−				+	+	−	
0	+	+	−				−	−	−	

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F+ F-

May 1 1958

REF:

	1	2	3	4	5	6	7	8	9	10
	GAL	LAC	TI	GAL	LAC	TI				
1	−	−	−	−	−	−				
2	−	−	+	−	+	−				
3	+	+	−	+	+	−				
4	−	−	+	−	−	−				
5	−	−	+	−	+	−				
6	−	−	+	−	−	−				
7	−	−	+	−	−	+				
8	−	+	−	−	−	−				
9	+	+	−	+	+	−				
0	−	+	−	−	−	−				
1	−	−	+	−	−	−				
2	−	+	−	−	−	+				
3	−	−	−	−	−	+				
4	−	−	+	−	+	−				
5	−	+	−	+	+	−				
6	−	+	−	−	−	+				
7	−	−	−	−	−	−				
8	−	−	+	−	+(w)	+				
9	−	−	−							
0	−	−	−							
1	−	−	+							
2	−	+	−							
3	−	−	+							
4	−	−	−							
5	−	−	+							
6	- or w	−	−							
7	−	+	−							
8	−	−	+							
9	−	−	−							
0	−	+	−							
1	−	−	−							
2	−	−	−							
3	−	−	−							
4	−	−	−							
5	−	−	−							
6	−	+	−							
7	w	−	−							
8	−	−	−							
9	+	+	+							
0	−	+	−							
1	−	−	−							
2	−	+	−							
3	−	−	+							
4	−	−	+							
5	−	+	−							
6	−	−	+							
7	−	+	−							
8	−	−	+							
9	−	+	−							
0	+	+	−							

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GENETICS

1412

A1 B and A1 C

A1 C0.1

May 3 1958

REF:

	1	2	3	4	5	6	7	8	9	10
	GAL	LAC	T1			GAL	LAC	T1		
A1B 0.05	1 —	—	+			—	—	+		
	2 —	—	—			—	—	—		
	3 —	—	+			+	+	—		
.1	4 —	—	—			—	+	—		
	5 —	—	+			—	—	—		
	6 —	—	—			—	—	—		
	7 —	—	+			—	+	—		
	8					+	+	—		
	9					—	+	—		
	0					+	+	—		
A1C .05	1 —	+	—			—	+	—		
	2 —	+	—			—	+/-	—		
	3 —	—	+			+	+	—		
	4 —	+	—			—	+	+		
	5 +	+	—			—	w	—		
	6 +	+	+			-	-	—		
	7 —	+	—			+	+	—		
	8 —	—	—			-	+	—		
	9 —	—(w)	+							
	0 —	—	+							
	1 —	+	—							
	2 —	—	+							
	3 —	—	+							
	4 —	—	+							
	5 —	—	—							
.1	6 —	—(w)	+							
	7 +	+	+							
	8 —	—	—							
	9 —	+	—							
	0 +	+	—							
	1 w	—	+							
	2 —	-/+	+							
	3 -	—	—							
	4 +	+	+							
	5 —	—	+							
	6 —	+	+							
	7 —	+	—							
	8 —	—	+							
	9 —	—	+							
	0 +	+	—							
	1 +	+	—							
	2 +	+	—							
	3 w	w								
	4 —	+	+							
	5 —	+	—							
	6 —	-/+	—							
	7 +	+	+							
	8 —	-/+	—							
	9 —	+	—							
	0 —	—	+							

Note: most of the Lac +'s may be weaks.

BLE-BLE-BLE-BLENDING -
ING-ING-

DATE: 4/26/58. REF: 1413/1 ING

1 2 3 4 5 6 7 8 9 10 ING

Saturated cultures of 3960, 3968 – Mixed in equal amounts. warm cultures

A) Blended 30" at once after mixing – incubated 30', plated.

B) 5' waterbath, blending, 5' waterbath, blending until 30' → plated. 5' waterbath, blending

C) Incubated 30', plated.

Platings: 0.05 from ~~undil.~~ undil, 1/100, ~~1/1000~~ 1/10.000

on min St B1

Counts;

	undil	1/100	1/10.000
A	∞	N1000	12
B	∞*	45	0
C	∞	N1000	17

* >4800. Probably many plate recombinants.

From B 1/100: 1 gal+? /50
8 lac+ /51
24 T1+ /50

DATE: 4/26/58 REF: 1413/2

TWO STAGE TRANSFER

3060 3 x conc x 2735 30 x conc. 0.2 + 0.2, 1 minute pulse.

↓

1 minute pulse in water bath

↓

dilute 1/500

↓

Incubate 15'

↓

Blend 30"

↓

2 ml + 2 ml 3064 10^9/ml.

↓

45' incubation

↓

Plate 0.05 undil, 1/10, 1/100, 1/1000 on B Lac/m

	undil	1/10	1/100	1/1000
Lac+	many	78	6	2
Lac-	∞	∞	∞	~ 10^4

40 Lac+ streaked, Lac+ isolated, stored and replicated on Bstar, Bgal

Thin B_1, thin B_1 T1, thin B_1 T, thin B_1 L, thin Penassay T_1.

All Lac+ were Gal+

Growth on B_1	T_1 1	T_1 0	
+	17	3	20
-	7	14	21
	24	17	41

F-type

Differential Recruitment.

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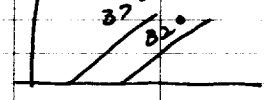
1413 D
-4

2 May 1958 REF:

Each tube received .05 ml H_2O (or DNP M/200), .05 ml W3060, .20 ml W3064, all precilled, innocated at 40x from [ORC → 1 hour $\frac{1}{4}$, $\frac{1}{10}$ in prinassay]. Resuspended in BOA* after elapsed time (10, 60 mins.) ~~add 10 ml warm BOA~~ dilute 1:100, 1:100 in warm BOA and incubate 30 minutes longer before plating. D Thom.

		1	2	
A	Melting ice 0°	10 minutes	60 m.	1:100 BOA
B	running water 12°			
C	water bath 25°			1:100 BOA inc. 37°
D	water bath 37°			for 30 minutes &
F	water bath 37° + DNP M/200	10 minutes	60 m.	plate.
E	after dilution 37°.			

Purpose: do zygotes form at lower temperatures or in presence of DNP? (in absence of progressive injection). Hayes finding of parallel 32° curve suggests that only time of density is changed, not rate of recruitment.)



Results: no recruitment at 0°. Same plate

	.005 10'	60'
A	13	18
B	7	19
C	23	36
D	183	~350
E	8,7	---
F	1	0

= plate recombinants plus dilute recruitment

DATE: 4/30/58 REF: 1413/3

Exhaustion of Hfr activity

3060, 4 exponential cultures of 1 hr: 2 ml + 7.5 ml Penassay, stated.
3064, 0.8 + 7.5 ml Penassay, 10 cultures as before.
Spun, resuspended in "optimal medium" *, 0,2 ml per tube (about 40-45 × conc.) - Optical density identical for 3060, 3064.

Mixture, in 150 ml flask prewarmed, of 0.07 ml 3060 & 1.4 ml 3064
Keep at 37°.
Take 0.1 ml samples, dilute in 10 ml prewarmed "optimal m.", then again 1/100 in O.M., ~~plate~~ at the following times:
1', 2½', 5', 10', 15', 20', 25', 30', 40', 50', 60'.

After dilution Keep at 37° until completion of 1 hr since mixing.

Platings: From 10^{-4} → 0.05 min St B_1
↓ 1/10 → { 0.05 min St B_1
{ 0.05 min.

Plate recombination controls (0'):
Parents diluted 1/10.000 → 0.025 of 3064 } on min St B_1
→ 0.025 of 3060
↓ 1/10 → 0.05 on { Bloc min (3060).
→ 0.025

* OPTIMAL MEDIUM. Called BGA later.

4 g	NaCl
0.2 g	$MgSO_4 \cdot 7H_2O$
1 g	Na_2HPO_4
9 g	KH_2PO_4
0.2 g	asparagine

dissolve in 93 ml of water and autoclave

Also: glucose 20% autoclaved separately.

MIX: { 1 ml glucose sol.
10 ml salt solution
90 ml water.

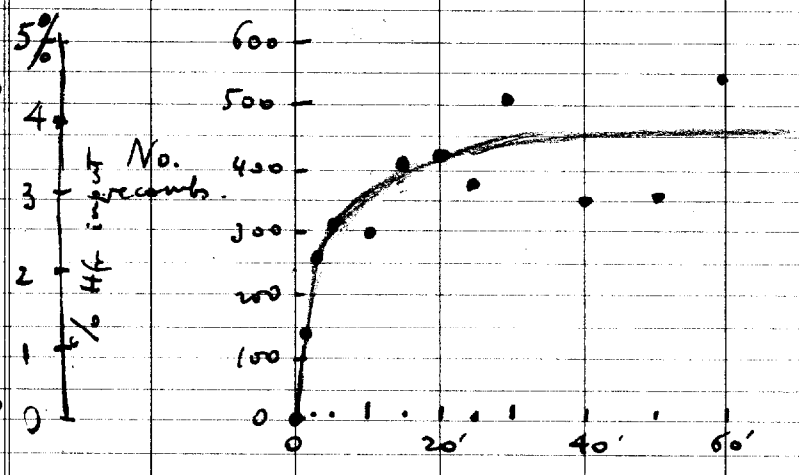
DATE: REF: 1413/3

	1	2	3	4	5	6	7	8	9	10
	Plate counts									
	#3060		10^{-5}	0.05 ml	B Lac.	1187 col.				
	3				min		4 col.!			
10	#3064			11		about 2000 col.				
20										
30										
40										
50										

DATE: May 1ST 1958 REF: 1443/3

Plate counts

Time	min β_1 St 1/1	min β_1 St 1/10	min 1/10	
0'	0,0,0		28	
1'	139	8	26	
2 1/2	259	29	22	
5'	307	35	30	
10'	290	33	21	
15'	~~402~~ 2	52	32	
20'	417	46	34	
25'	372	43	6	?
30'	510	53	37	
40'	345	55	5	!
50'	356	53	not done	
60'	540	43	"	



Note: 0' is not an accurate definition as it merely measures plate recombinants. A better 0' would have been 60' incubation of parents diluted 1/10.000 and mixed.

Conclusions.

t_{37} about 5'; to have 95% mated 15' are necessary. this time is too long.* In order to reduce it to about 1/3 try:

1. temperature decrease
2. DNP.

* as confronted with rate of entry of chromosomes. It is desired to have this time %

smaller than the difference, in time of entry, between T1 and last which is about 8'.

19 REF: 1413/3

	Gal+	lac+	T_1'
1'	7/100	30/102	55/100
60'	0/100	13/100	53/100

Why is the 60' experiment interrupted?

DATE: May 1st, 1958 REF: 1413/4

RECRUITMENT RATE

ORC W 3060, W 3064 —

Exponential culture: 2 ml + 7.5 ml Pen 3060 : 6 cultures.

0.8 " " 3064 : 10 "

1h rotation. Spin, resuspend in 0.2 ml BGA* per tube.

Chill parental suspension and prepare:

4 tubes with .05 ml conc 3060 + .05 ml water (A–D)

1 " + .05 ml Dinitrophenol M/100 (F)

Add .2 ml conc. 3064 to each tube, and transfer to following temperatures

A: 0° (melting ice)

B: 18° (running tap water)

C: 25° (waterbath)

D, F: 37° (water bath)

After 10' and 60' take 0.1 ml/sample, dilute 1/100 in water and then 1/100 in warmed BGA, keep 10^{-4} dilution at 37° for 30' plate 0.05 and 0.01 as usual B, —

(E): 3060 dil. 1/20.000 in 0.5 ml BGA
3064 " 1/10.000 in 1.0 ml BGA
} for 30' at 37°, plate 0.05 and 0.01 —

(A) Kept at 0° for a total of 20 hrs. dilute 1/100 in water → 1/100 in warmed BGA. Plate 0.05 at once, and after 30' incubation at 37°.
↓ A3 ↓ A4

* BGA = "optimal medium.

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1413/4

19 REF

	10′		60′	
	0.05	0.01	0.05	0.01
A	13	0	18	5
B	7	1	19	1
C	23	1	36	4
D	183	44	~350	100
E	8,7	1		
F	1	0	0	0

→ Control of recombination at 10^{-4} 60′.

3060 B Lac 10^{-6} 0.05 : 172, 216.
min 114 delayed

3064 B Lac 10^{-6} 0.05 626

Initial conc 4×10^9 / ml ♂
12×10^9 / ml ♀

A_3 : 0,2 ml
A_4 39,14 ml.

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19 May 6th, 1958. REF. 1413/5

1 2 3 4 5 6 7 8 9 1

melt of W3060 2 ml + 7.5 ml Pen rotated 2½ hrs.
10 " of W945 & 3064 1 ml + 7.5 ml Pen.

Resuspended after spinning in 0.1 ml of BGA per tube.

Mix W3060, W945 in proportion .05 + 1.00 in 37° waterbath for 15', - 25'.

A. At 15': .1 sample taken, diluted 1/10, BGA blended;
addition to .2 ml of .2 ml 3064
incubate in water bath.
Take samples of 0.1 after 10', 20', 40'. Dilute 1/10 ml chilled water, and further dil 1/100, 1/1000 (.1 + 9.9)².
From each dilution plate on .05 | Gal Sm (B)
Lac Sm (B)
min B,ST.

B. at 25'. same.

C. 3060 conc susp. 1/2000, 0.2 ml
0.05 + 10
0.2 ml + final water; take blend,
take 1.0 ml /sample, add + .2 ml 3064. Incubate
Samples of 0.1 after 10', 20', 40'. Dilute and plate as above:

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19 May 7th 1958 REF: 1413/5

Plate count on min/+B, :

		A	B	C
10	1/1	0	0	19
	1/10	0	0	0
	1/100	0	0	0
20	1/1	0	0	18
	1/10	0	0	1
	1/100	0	0	0
40	1/1	0	4	10
	1/10	0	0	0
	1/100	0	0	0

B lac/+, B gal/+ : no positive colonies.

Conclusion : 3060 has reverted to F+.

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19 May 7, 1957. REF: 1413/6

Testing W3870 (Hfr_2 $B_1^?$ $4p^h$)
for mating to 3064 in B Lac fm, B Gal fm.

0.5 W3870 o.r.c + 1.0 W3064 o.r.c.
1 hr at 37°. water bath.
dilute 1/100, 1/10.000, plate on B Lac fm B Gal fm

Same 3870 culture stored in frig to inoculate fresh cultures on the next day—

40 hrs readings:

	B Gal fm	B Lac fm	
1/100	9 *	6 *	Gal+ colonies
1/10.000	0	0	—

*: all $TL^r B_1^-$ Ara- Gal+ Lac+

Most likely explanation: 3870 reverted to F+ and other Hfr's than Hfr_2 are giving Tl- recombinants. Possibility that 3870 is behaving differently from 3050 should also be kept in mind.

5/12/58

1413/7

B - Lac - Sm		B - Gal - Sm	
A 1/10	~ 300 +	A 1/10	~ 200 +
	lawn -		> 1000 -
A ?	~ 400 +	A ?	~ 400 +
	lawn -		lawn -
A 1/100	55 +	A 1/100	6 +
	~ 1000 -		~ 700 -
A 1/100	9 +	A 1/100	64 +
	~ 400 -		~ 1000 -

Single colonies from streaks were stroked on Gal or Lac; to be tested for:

May 12

1413 / 7 (More interested in Gal's) · [3000 x 3004]

Lick, steak, check for : Lac, Mal, etc.

Pick more of 2343 x 9475

1306 M Lac, V_1^r V_6^r

streaked on B lac → Lac +!! (all +)

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19 May 9th, 1958 REF 1413/7

Control of efficiency of Gal+ and Lac+ selection.

3060, 3064 suspensions 80× conc. from exp. 1415/3 — Pulsed at high conc. for 15' then diluted 1/100, incubated for 45'. Plate on Gal fm and B Lac fm: 0.05 from

(A) → undil., 1/10, 1/100, 1/1000.

Also: Dilute parents kept meanwhile at high conc (for 1 hr) 1/100, plate .025 of each parent undil (= 1/100), 1/10 (= 1/1000), 1/100 (= 10⁻⁴), 1/1000

(B) → { on B Gal fm. (Plate recombination control)
and B Lac fm

		B Lac fm	B Gal fm
A	1/1	~10^3 Lac+	~10^3 Gal+ (& not blue very small)
	1/10	~200 Lac+	~100 Gal+ (")
	1/100	16 Lac+	10 Gal+ (")
	1/1000	5 Lac+	0
B	1/1	0 Lac+	0 Gal+
	1/10	0	0
	1/100	0	0

After another 40 hrs more Gal+ and Lac+ in the A series. None in the B series.

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19 May 9th 1958. REF: 1413/8

Reconstruction experiment.

10^3 cells 2323, alone and with 10^8 3064
on B lac fus, B gal fus.

No evidence of suppression by 3064 of 2323
in these conditions.

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19 5/13/1958 REF: 1413/9

REBLENDING REPEATED

Cultures of W3050, W3064 kept in frig 1 ml + 7.5 ml Pen. & 0.5 ml + 7.5 ml Pen. respectively. Rotated 3 hrs.

Mixed in water both in equal amounts (1.5 ml + 1.5 ml) in 2 tubes A, B. Also, parental suspensions incubated similarly for plate recombination control C.

A. Blended every 6' for 30" each time.
B. Left undisturbed

After 42', dilution of A, B, and parents 1/6, → 1/36 → 1/216 → 1/1296. Plate 0.05 of each dilution for A, .05 of last dilution for B, and .025* of either parent at each dilution for C (Plate recombination control) - All on minST B1

* By mistake, 0.05.

All plates prepared same day (6 days old).

C repeated on plates of 5 days with right amount

Dilution	A	B	C	C 5 days old
1	38	-	80	-
2	1	-	9	-
3	0	-	0	0
4	0	14	0	0

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May 14, 1958 REF: 1413/10

TWO STAGE TRANSFER.

W3060, culture in the frig. - 2 ml + 7.5 ml Pen 1 tube
W3064, W445 - 1 ml + 7.5 ml Pen 3 & 2 tubes -
Rotated 1 hr 15' -

Spun, resuspended in Penassay: W3060 1x, 0.05 ml
W3064, W445 } 30x } 0.3 ml.

A. 0.1 W3060 + 0.1 W445, 20' incubation, then 0.1 ml from this mixture + 0.2 ml W3064 - incubated 8', then 2 ml broth added, chilled + blended, reincubated, samples for plating 10', 20', 30' after reincubation.

B. 0.1 W3060 + 0.1 W445, 5' pulse, then 0.1 + 10 ml broth for 15' - From mixture, 0.1 ml + 0.2 W3064, 8' incubation, then 2 ml broth added, chilled + blended, reincubated, sampled for plating 10', 20', 30'

C 0.1 W3060 + 0.2 W3064, incub 8', 2 ml broth added, chilled + blended, reincubated and sampled at 10', 24', 30' -

D. 0.1 W3060 + 10 ml broth, from mixture, 0.1 + 0.2 W3064 incubated 8', 2 ml broth added, chilled + blended, sampled after 10', 24', 30'.

All times plating of 0.05 on B Gal Sm, (Sm B,) - Not enough B Gal Sm plating for C 20, D 20.

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19

REF: 1413/10

Counts on D(fm B_1)

	A	B	C	D
10	170	0	112	3
20	~5000	21	~10,000	67
30	~12000	128	~$2 \cdot 10^4$	167

B(Gal, fm)

	A	B	C	D
Gal+ 10			0	0
20			85	0
30				

Gal+ 3 unlisted were sifted

% from D(fm B_1)

	Gal+	Lac+ T_1
A 10	0/100	12/100
B 20	0/25	0/25
B 30	1/100	19/100
C 10	1/101	7/100
D 10	0/3	0/3
D 20	0/75	0/75
D 30	0/100	18/100

Gal$^+$ + Fus^R colonies from A30 and C30:
all Gal$^+$ Lac$^+$ (with one only exception).

	A30	C30
T_1^1	20/30	36/45
I_{1p}^1	16/30	36/45

No clear-cut difference in contribution from ♂ parent between A and C, i.e. genes pretreated and non-pretreated with Φ's.
distance cent of the

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Protective Blending

11414

May 1 1958

REF

Purpose: Can one blend in media so supplemented that pairing within the F^- mate will not be interrupted. (Hyp. of progressive pairing vs. progressive entry).

System: W3060 ♂ → W3064 ♀. Look for recovery of bac.

Pulse: 1 minute. ~~to~~ Dilute Incubate for 12 minutes post pulse.
Chill in cooled water, L-2, or serum broth. Blend in Waring grinder.

3PM. ORC: W3060(3x) + W3064(30x) ~~Blend~~ .1 ml each 1 minute 37°.
Add 10 ml warmed broth*, 1/10 in broth, incubate 12 minutes. (several tubes)
A. Add DNP to 10^{-3}M B. Add 1/10 chilled broth C Add 1/10 chilled 20% serum
(1/100 : .05)
Blend all 3 cultures.

~~A~~ A: ~~Dilute~~ Dilute (1/100) + plate	BA. D+p.	CA D+p.
AB: Incubate 20 minutes, d.+p.	BB. Chill hours, warm 20 minutes, plate	CB Chill hours warm 20m. plate
~~AC. Incubate 20 minutes, dilute, incubate 20 minutes, plate~~		CC ~~Incubate~~ Incubate 20 minutes, d.p.
AD. Incubate hours, dilute, incubate 20 minutes, plate.		

D. Add DNP 10^{-3}M. Do not blend.

DA. Incubate 20 minutes, d+p. (blend?)

DB Incubate 20 minutes, d., Inc. 20 min., plate.

6.30 – use uv!

Need: M bac B, for scoring

*broth = salts at pH 6.2
glucose - asparagine
per Fisher + Hayes.

19 May 1st 1958 REF: 1414

Protective blending.

3060, 3064, conc. 3 × and 30 × resp. : .1 ml each.
1 minute pulse at 37°, add 10 ml BGA warmed, dilution ~~factor~~ 1/50 in BGA, incubate 12 minutes.

A. dilute 0.1 + 9.9 chilled, blend & plate 0.05 on min ft B_1.
B. " " BGA warm, incubate 30', blend & plate.
C. add DNP (dinitrophenol) 1/50,* incubate 5', blend :
C_1 : remove 1 sample of 0.1, dilute blend & plate.
C_2 : incubate 30', dilute, blend & plate.
C_3 incubate 30', dilute, incubate 30', blend and plate

D. add DNP 1/50, incubate 30', dilute in ~~optonic~~ warm BGA
D_1 : blend & plate 1 sample
D_2 : incubate 30', blend & plate.

* from M/20 master solution. Final dil. wanted M/1000

Plate counts :

A	B	C_1	C_2	C_3	D_1	D_2
1,0	0,1	0,1	0,0	0,0	1,0	1,0

(too low. Need to do again!

Segregation by F+

14/14C.

Plan 6 May 1958. REF: B

Pulse W3064 x W3870 4/minutes (to t=4). Dilute 4:100. Incubate in BOA
.2ml (40x) .2ml
at 37° to t = 12m. Parallel (for C) W3064 only.

at t=12
A. Blend sample and plate (plate = 1:100 dilns. in chilled water, spread .0.1 ml DTham (many unity of true plate-ct.)
B. Reincubate and plate at t=60. (successful recomb.)

C. To W3064 dil. add W1306 Plate at t=60 W1306 = F+ M V, V6. melac s.r.i. 6/May/58.
D. after blending A, add W1306 Plate immediately
E. → " at t = 24
F. " at t = 60
G. —— " at t = 60. (continued mating control)

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Re entry stimulated by $F^+ \circ \rightarrow$?

1414B

3 May 1958

REF: 1414 see C

? Might reentry be stimulated by adding $F^+ \circ \rightarrow$

Pulse W3064 + W3870 (ORC) 4 minutes (to t=4). Dilute 4:100 in (30 × culture) .2 ml .2 ml

BBA to t=12 m. 37°

A. Blend sample and plate (plate = 1:100 in chilled water and spread 0.1 ml on DB, 5 m.) at t=12. (check non-entry of Lac; vs. plate recontamination; at time).

B. Unblended sample. Plate at t=35 m. (successful mating)

C. ~~W3870 s~~ W3064 s/ $\circ$ ~~W3870~~. Add W6 at t=12. Plate at t=~~24~~ 22, ~~t=32~~.
(check W6 × W3064 crossing)

D. A + W6 (= .5 ml 10× ORC W6 per 10 ml sample). Plate at t=12 (D0); intended t=~~22~~ but did C instead; t=32 (D20).

If D20 shows an effect of W6 addn. it is open to objection of further effect F⁻ mating from t=12 for 20 mins., allowing of some injection of Lac. alternatively (more interesting) (a) W6 facilitates re-pairing of preinjected genome; (b) pretreatment facilitates mating of W6!

Intention: look for Lac reentry by replica plating

Re-read 7 May

A	35, 30	No Lac⁺	No Lac⁺
B	~200;	42 Lac⁺/400	✓
C2:	no prototrophs.		✓
D0:	34, 37	No Lac⁺	
D20:	59, +	1 Lac⁺⁺; 13 probable Lac⁺ Cal⁻.	✓

∴ A shows early TL⁺ B full entry D is effect of reentry of Lac.
Replica plate of Stock B, : only ~10% Lac⁺ ? May be counting Cal⁺.
Remember. an effect is indicated but the objection may still hold that further mating has taken place. Lac scoring wrg. Strike out! of A, B, D0, D20, .

(over)

Summary - from individual strokes on Blac:

A: 0 lac^+/60

B 5 lac^+/70

C - no revertants

D-0: 0/30

D-20 8/120 lac^+

The addition of W6 and further incubation give lac^+ recombinants.

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14 14 B

A

A

B = 5/

May 7 19 58

REF:

	1	2	3	4	5	6	7	8	9	10
		LAC			LAC			LAC		
1								—		
2								—		
3								—		
4								—		
5								—		
6								—		
7								—		
8								—		
9								—		
0								—		
1								+		
2										
3										
4								—		
5								w		
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								+		

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1414 B

B 1

D 0 = all Lac-

D 20 = 8/120 Lac +

May 7 1958

REF:

	1	2	3	4	5	6	7	8	9	10
		LAC			LAC all -			a LAC		
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								−		
1								−		
2								−		
3								−		
4								−		
5								−		
6								−		
7								−		
8								−		
9								−		
0								−		

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D20

19

REF:

	1	2	3	4	5	6	7	8	9	10
1		c –		d –						
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		w								
6		–								
7		+								
8		–								
9		–								
0		–								
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										

1414B

D0 - versees D20 shows lacenty. Is this due to

(1) reverting of Hfr × F⁻ despite blending + dilution
(2) F⁺ × F⁺ owing to preferentiation of F⁻
(3) Resumption of pairing is interrupted Hfr × F⁻ under influence of F⁺.

suggest: use W1306 ($H^- F^+ V_1^r V_6^r$) to distinguish (2).
control: reincubate after blending + dilution to distinguish (1).

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19 May 9th, 1958. REF: 1415/3.

W 3060 (Esther's transfer) : 1 ml + 7.5 ml Penassay, 8 cult.
W 3064 " : 0.5 ml + " 10 " .
3 hrs rotation. Spun, resusp. in water 1 ml, pooled different cultures, distributed each strain in equal amounts in two tubes, spun again, of each strain one tube resusp in 0.5 ml. (Concentration about 80 x) of D(aspartic). *, and the other in D(aspatic + azide 10^{-4}).

*: to 10 ml of D(M): .1 of 20% glucose and .02 of 10 mg/ml aspartic acid. pH 7.0.

Azide and non azide parents incubated at 37° for 5', then:

(AB) Parents in non-azide D(asp) mixed, 0.2 ml + 0.2 ml and pulsed for 2½ minutes, then diluted as follows:
A. .1 ml in 10 ml D(asp)
B. same + azide .1 of 1% solution.

(CD) Parents in D(asp) azide 10^{-4}, 0.2 ml + 0.2 ml, and pulsed for 2½ minutes, then diluted as follows:
C. .1 ml in 10 ml D(asp)
D. same + azide .1 ml of 1% solution.

(E_1) Unpulsed recombination control 10^{-2}: .05 of each parents, 80 x conc. to 10 ml of D(asp) at 37°, 40'. Dilute 1/100, plate .05.

(E_2) Unpulsed recomb. control 10^{-4}: .05 of 1/100 dilution of each parent to 10 ml of D(asp at 37° for 40'. plate .05

A and C : after 40' from pulse, dilute 1/100 in water, plate .05 in min/sm B_1

B and D :

B_1, D_1 : .1 ml to 10 ml water, plate as above

B_2, D_2 : .1 ml to D(appo) 10 ml, 40' in water bath, plate .05 on min/sm B_1.

Plate recombination

10^{-4} dilution of parental suspensions, .025 of each to min/sm B_1 plate

Counts : photometry at 650 mμ of 1/100 dilution of concentrated parents :

3060	50%	= 240 × 10^6/ml
3064	52%	= 320 × 10^6/ml

10^{-7} dilution, .05 on B Lac :

3060 : 100, 139

3064 : 159, 164

Original conc. suspensions : 24 × 10^9/ml ; 32 × 10^9/ml resp.

Plate counts: Numbers were too high for accurate counts.

A_1 : ~800

B_1 : same

B_2 : same

C_1 : ~500

D_1 : ~500

D_2 : ~800

E_1 : 250

E_2 : 140.

Plate records: 0, 0 -

Conclusions: too many recombinants in the unpulsed controls for any valid conclusions to be drawn. Due to use of D (aspartic) instead of BGA? Azide is however almost ineffective. Try higher concentrations.

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19 May 15 1958 — REF: 1415/4

AZIDE DOSAGE

W 3060 from fridge: 2 ml + 7.5 ml Pen 1h not ; spun, conc 3x in Pen

W 3062 1 ml + 7.5 ml Pen " conc 30x "

Mating mixture: 0.2 ml + 0.2 ml, 1' pulse in Penassay Dilution in BGA new (pH 6) 0.1 + 10 & again 0.1 + 10, and the same in Penassay - incubated for another 12', then stopped by chilling.

1 ml added from melted { chilled tube / Pen / BGA } to 0.1 ml of Azide soln:

	0	1/10 000	1/3000	1/1000	1/300	1/100	final conc 1/10 of that indicated
final	0	10^{-5}	$3\cdot10^{-5}$	10^{-4}	$3\cdot10^{-4}$	10^{-3}	
called	0	5	+	3	2	1	for

then incubation resumed for 60' - Plated, 0.05 on new B, Pen

			Counts	10^{-3}	$3\cdot10^{-4}$	10^{-4}	$3\cdot10^{-5}$	10^{-5}	
			0	1	2	3	4	5	
P (Penassay)			>100	29	49	76	>100	>100	
B (BGA)			9	1	2	13	14	14	colonies
Pen	gal+		18%	3%	8%	20%	23%	23%	
	lac+		56%	31%	38%	43%	52%	48%	
BGA	gal+		20%	0/1	0/2	0/13	0/14	2/15	
	lac+		50%	0/1	0/2	3/13	2/14	5/14	

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May 23/58. REF: 14/5/5

AZIDE, DNP

W 3060 1 ml (big) + 7.5 ml Phos } 1½ 30' Rotation
W 3064 0.5 ml + 7.5 ml Phos

Mating mixture : 1:1 ratio ♂ : ♀.

Incubate 12', Chill for 10' → plate ⅒ 1/100 1/1000 0.05
and add to 0.9 ml of mating mixture:

A	:	0.1 ml of DNP M/20	=	M/200
B		0.03 "		M/600
C		0.1 " M/200		M/2000
D		0.1 of azide 10%		Azide 1%
E		0.03 "		.3%
F		0.1 " 1%		.1%
G		0.1 water		Control

(D, E, F: high solution)

Incubate 30'

then dilute Sample and plate .05 of dilutions ⅒, 1/100, 1/1000 of all.
Also : incubate for another 30' after 1/100 dilution in warmed broth. then plate undil, and ⅒ –

X : mating mixture incubated until the end for a total of 60' then plated : .05 of ⅒ 1/1000 1/100

All plates on medium B,

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19 May 27th 1958 REF: 1415/5

	1	2	3	4	5	6	7	8	9	10
1										
2		Gal+/lot	Lac+/lot	T1+/lot						
3										
4										
5	A	0/50	1/50	21/50	} DNP					
6										
7	B	0/50	9/50	33/50						
8										
9	C	1/50	15/51	30/50						
0										
	D	0/50	0/50	6/50	} Azide					
1										
2	E	0/50	0/50	11/50						
3										
4	F	0/50	6/50	21/50						
5										
6	G	2/50	14/50	31/50						
7										
8	O	0/50	0/50	16/50	plating after 12					
9										
0	X	9/50	19/50	26/50						
1										
2	A2	6/50	20/50	31/50	} DNP					
3										
4	B2	5/50	17/50	33/50						
5										
6	C2	10/50	21/51	30/50						
7										
8	D2	1/51	12/50	34/50	} Azide					
9										
0	E2	0/51	17/50	32/50						
1	F2	5/50	23/50	36/50						
2										
3	G2	9/50	28/50	34/50						
4										
5										
6										
7										
8										
9										
0										
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										

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19 5/23/1958 REF: 1415/5

	1	2	3	4	5	6	7	8	9	10
			1st time			2nd time				
		1/10	1/100	1/1000		1/1	1/10			
	A	∞	~500	57		~1000	92	DNP	M/200	
	B	∞	1000	118		"	137	DNP	M/700	
	C		∞	212		"	251	DNP	M/2000	
	D	~1000 245*	19	2		~	81	Azide	1%	
	E	∞	103	7		"	67	Azide	3‰	
	F	∞	~500	54		"	132	Azide	1‰	
	G	∞	∞	154		"	166			
	X	∞	~1000	135						
	O	∞	112	29						

* big & small, plate record

Conclusions

Matings probably stopped by Az 3%, DNP M/200 or more. Action apparently reversible, but new recruitment may have taken place (although G 1/1000 and G2 1/10 show equal numbers): nos. of prototrophs have considerably increased and it is unlikely that they were all blocked before TL at the 12'.

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19 5/28/58 REF: 1415/6

AZIDE RESISTANTS.

W 3060 ; W3947 (=3060 Az^R)_
W 3064 ; W3935 = (3064 Az^R)_

1hr exptg, Spun, resusp 2x conc.

Mating mixtures : 2 ml + 2 ml. using iced suspensions_

A ♂ s + ♀ s
B ♂ s + ♀ r
C ♂ r + ♀ s
D ♂ r + ♀ r

Each mixture distributed to 4 tubes, 0.9 ml each.

Additions : tube 1 0.1 ml water
2 : Azide 3% 0.1 ml, at 0'
3 : " at 5'
4 : " at 12'

At 40', dilutions 1/5 in chilled water, blending, plating of .05 on D(fm B,). Same for 1/10 dilution from the latter_

5. Plate recombinants

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19 May 31st, 1958. REF: 1415/6

	1	2	3	4	5	6	7	8	9	10
		Plate count		Aged 0'	Aged 5'	Aged 12'				
1										
2		1	2	3	4	5				
3										
4	A	∞	1	45	211	(24)				
5										
6	B	∞	3	73	271	18				
7										
8	C	∞	7	112	359	2				
9										
0	D	∞	16	254	~440	3				
1										
2										
3										
4	A''	(78)	0	0	19	mucoid				
5										
6	B''	(140)	0	2	-	"				
7										
8	C''	(140)	0	13	13					
9										
0	D''	(96)	0	8	26					
1										
2		↑ x factor 10...								
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										

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19 REF:

	Bact. \ Ph	λ	λh	$\lambda V_1 \lambda_2$	$\lambda V_2 h$	
	Lp^s	S	S	S	S	
	Lp^t	R	R	S	S	
	Lp^λ	R	R	S	S	
	$Lp(H)^s$	R	S	R	S	isolated once only
	$Lp(H)^h$	R	R	R	S	
Lp^d	Lp_{Ri}^g	R	R	S	S	= ~~S~~ source of defective [illegible] phage.
	Nal_r	R	R	R	R	$= Lp_2^R$

DATE: 1 ml 3514/ℓ^R Rotated cult., exponential. + 10 ml ~~medium~~ -

1	2	3	4	5	6	7	8	9	10
	1) 100 u/ml			2) 300 u/ml				3) 1000 u/ml	
Double strength Lg		1/1 protoplasts and rods. Turbidity higher than ~~in~~ 2 and 3.			at all protoplasts almost no rods. Turbidity higher than in 3			all protoplasts. turbidity ~~low~~ ~~high~~	
10									
Single strength Lg		4) Several ghosts, also protoplasts visible -			5) Many swollen protoplasts - No rods - Turbidity low			6) Many ghosts almost no protoplasts except two or three swollen only - Turbidity ~~high~~ low -	
20									

Ilg conditions between 2, 3, 5. But not 6.

30

40

50

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19 5/29/58 REF: 1415/7

AZIDE RES-: OPTIMAL AZIDE CONC.

O.r.c. cultures of W3050, 3064, 3947, 3935 - spun and resuspended in diluted ~~fol~~ ~~ma~~ Broth-

Mixtures as in exp 1415/6, then 0.9 of mixture per tube, to which 0.1 of the following conc. of azide in Penassay were added:

3%, 1%, 0.3%, 0.1%, 0.

Called 1 2 3 4 5 -

45' incubation, then dilution 1/1000, plating of 0.05 on D(str B,)-

	1	2	3	4	5
A	0	0	7	57	38
B	0	1	6	18	44
C	0	1	43	108	~400
D	1	10	41	118	318

No discrimination.

DNP.

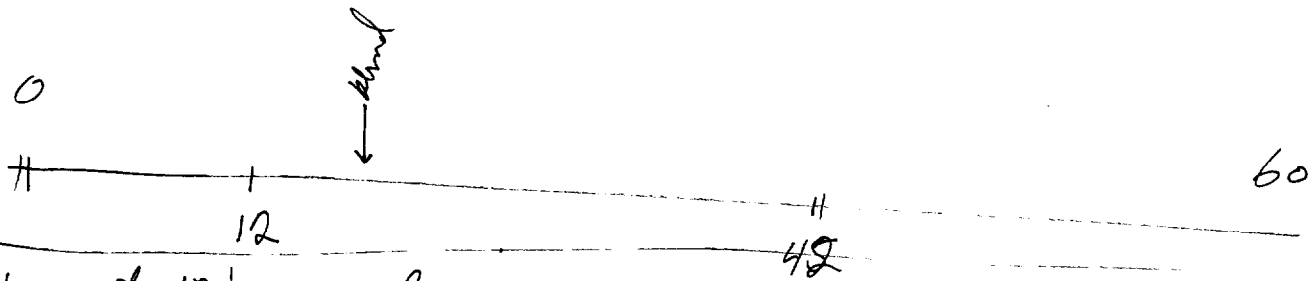
14/15

Pulse — 12 minutes.

1. Blend & plate.
2. Blend in DNP
3. DNP reversibility.

4. Incubate + 30 minutes. Blend + plate.

(2) add DNP. Blend. Incubate (in DNP) 1 hour and overnight.



1. at 12' d + pl.

2: at 12' add DNP. Incubate 30 minutes in DNP. → 3

3. Blend + plate. 4. Dilute (to remove DNP), Incubate 30 minutes.

↳ 5 after 5 minutes, blend. → incubate in DNP {30 minutes / overnight}

↳ dilute
incubate 30 minutes

(4/15)

I Effect on mating

plate after 60 m: = I A - B - C - D. at 10^{55}

II Effect on injection: $10^{15} - 11^{15}$ dilute 1:100 in B & A.

↳ plate A

plate B at 12^{15}

IV.

---

5 May.

14/15

3060 - 3064. ~ ½ ml 100x. chilled.

NaN_3 - now have 10 mg/ml. Use at 100γ/ml.
Need to dilute 100x at least.

avoiding orange.

---

(1) Do these compounds inhibit mating?
(2) Do they inhibit injection once started? 12 minutes.
(3) are they reversible.

(A) mating: add .1ml cells each parent + .2ml inhibitor.
pulse 1 minute. dilute ~~+ 1000 in~~ 1:10,000 in BOA.
warm BOA. 95° Plate on DSMB, at 60 minutes.

(B). Also dilute (C) sample 1:100 in BOA. at 12 minutes add inhibitor. Incubate 30 minutes ~~& plate~~ dilute 1:100 blend & plate. (Another sample chilled 30 mins.). Enzyme A chilled.

(C) after 30 minute dilute 1:100, incubate 30 minutes +, blend & plate.

B(A - D)
4 tubes. 1 ml each. $10^{0.3}$ PM warmed.
at 10½ chilled and add 1 ml of inhibitors. - Warm and incubate ~~+2 minutes~~.

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1415 A

May 8 19 58 REF:

Plates from 3 replicated from DC to 13 Gal and B Lac.

I-B, 0.05 = all Gal-
4 Gal+, 13 Lac-

I-C, 0.05 = 2 Gal+, 28 Gal-
12 Lac+, 18 Lac-

I-D = 1 Gal+, 22 Gal-
6 Lac+, 17 Lac-

all Gal+s are Lac+

Background overgrown.

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19

REF: 1415 B 3

1	2 Count	3 Gal +	4 Lac +	5 T1	6	7	8	9	10
A	88	2/88	15/88	51/88	} Control				
Ao	7	1/7	2/7	4/7					
2A	92	3/92	17/92	57/92					
B	25	0/25	2/25	8/25	} Azide				
2B	6	0/6	0/6	1/6					
C	38	1/41	9/41	26/41	} Acridine orange.				
2C	40	1/40	2/40	20/40					
D	9	0/9	3/9	6/9	} DNP				
2D	1	0/1	0/1	0/1					

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1415 B.3

May 9 2A 19 58

2A

B (and 2B)

REF:

	1	2	3	4	5	6	7	8	9	10
	GAL	LAC	T1	GAL	LAC	T1	GAL	LAC	T1	
1	−	−	+	all −	−	+	all −	−	+	
2	−	−	+		−	+		−	+	
3	−	−	−		w	+		−	−	
4	−	−	−		−	−		−	−	
5	−	−	+		−	−		−	+	
6	−	−	+		−	−		−	+	
7	−	−	−		−	−		−	+	
8	−	−	−		−	+		−	−	
9	−	−	−		−	+		−	−	
0	−	−	+		−	+		−	+	
1	−	w	+		−	−		−	+	
2	+	+	−		−	+		−	+	
3	−	−	−		−	+		−	+	
4	−	−	−		−	−		−	−	
5	−	−	−		−	+		−	+	
6	−	−	−		−	−		−	+	
7	−	−	+		−	−		+ / −	+	
8	−	−	+		+	−		−	−	
9	−	−	−		+	−		+	−	
0	−	−	−		−	−		−	+	
1	−	+	−		−	−		−	−	
2	−	+	−		−	+		−	+	
3	−	−	+		−	−		−	+	
4	−	−	+		−	−		−	+	
5	−	+	−		−	−				
6	−	+ / w	+		−	−				
7	−	−	+		−	−				
8	−	−	−		+	−				
9	−	−	+		−	−				
0	−	−	+		−					
								2B		
1	−	−	+		−	−		−	+	
2	−	−	+		−	+		−	+	
3	−	−	−		+	−		−	+	
4	−	−	−		−	+		−	−	
5	−	−	−		−	+		−	+	
6	−	−	+		+	−		−	+	
7	−	−	+		−	−				
8	−	−	+		−	−				
9	−	+	−		−	+				
0	−	−	−		−					
1	−	−	−		−	+				
2	+	+	−		+	−				
3	−	−	−							
4	−	−	−							
5	+	+	−							
6	−	−	−							
7	−	+	−							
8	−	−	−							
9	−	−	−							
0		−	−							

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~~1415 B 3~~
~~HFR 2~~

A

May 9
~~April 28~~ 1958

A

AO

REF:

	1	2	3	4	5	6	7	8	9	10
	GAL	LAC	TI	GAL	LAC	TI	GAL	LAC	TI	
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	−	−	−	−	w	+				
6	−	−	+	−	−	−				
7	−	−	−	−	+	−				
8	−	−	−	−	−	−				
9	−	−	−							
0		-/w	−							
1	−	−	−							
2	−	−	−							
3	−	−	+							
4	−	−	−							
5	−	−	−							
6	−	+	−							
7	−	−	−							
8	−	+	−							
9	−	−	+							
0	−	−	+							

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1415 B3

C 2C D (and 2D)

May 9 1958

REF:

	1	2	3	4	5	6	7	8	9	10
	GAL	LAC	T1	GAL	LAC	T1	GAL	LAC	T1	
1	−	−	+	−	−	+	all −	−	+	
2	−	−	−	−	−	+	↓	−	−	
3	−	−	−	+	+	−		+	−	
4	−	−	−	−	−	−		+	−	
5	−	−	−	−	−	+		−	−	
6	−	−	+	−	−	±		−	+	
7	−	−	−	−	−	−		−	−	
8	−	+	−	−	−	+		+	−	
9	+	+	+	−	−	−		−	+	
0	−	−	−	−	−	+				
1	−	−	−	−	−	−				
2	−	+	−	−	−	+				
3	−	+	−	−	−	−				
4	−	−	−	−	−	+				
5	−	−	−	−	−	+		2D		
6	−/w	−	+	−	−	+				
7	−	−	+	−	−	−	−	−	+	
8	−	−	+	−	−	+				
9	−	−	+	−	−	−				
0	−	−	+	−	−	−				
1	−	−	−	−	−	+				
2	−	−	−	−	−	−				
3	−	−	+	−	w	−				
4	−	−	−	−	−	−				
5	−	−	−	−	−	−				
6	−	−	+	−	−	−				
7	−	−	−	−	−	+				
8	−	−	+	−	−	+				
9	−	+	−	−	−	+				
0	−	−	−	−	+	−				
1	−	w/−	−	−	−	−				
2	−	−	−	−	−	+				
3	−	+	−	−	−	−				
4	−	+	−	−	−	−				
5	−	−	+	−	−	+				
6	−	−	−	−	−	+				
7	−	−	+	−	−	−				
8	−	−	−	−	−	+				
9	−	+/−	−	−	−	+				
0	−	−	+	−	−	−				
	−	−	−							
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										

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Azide effects on mating

14154

5 May 1958 REF:

Ultimate purpose: compare azide effects with Az^+ O^+ v O^-.

Below: A = —; B = Azide 100 r/ml C = acridine orange 60 r/ml D = DNP $\frac{M}{1000}$
[Note: agglutination of 0550 in C.] Make up 2 x in BOA.

Design: Effects on mating (during pulse) I; effects on entry (after pulse) II; Reversibility III.

I Pretreat each parent before and during mixing; dilute, incubate & plate.

II Dilute untreated pulse into inhibitor soln. Incubate.

III Redilute these and incubate further.

Pulse 1 min. | Dilute & Incubate

Treated → BOA 1 hour. Plate: I

Untreated → (12 min.) treatment 1 hour → Plate: II

A / B / C / D chilled

Dilute into BOA → Incubate 1 hour. Plate IV

→ Plate III.

Reversibility of inhibited entry is tested after 12 minutes.

8 PM ff. W3060, W3064 refreshed from ORC 5 tubes each harvested into 0.5 ml (ice) in BOA. Chilled until used. Timing by chilling in ice water.

M1. But Mergers present

I Add 0.1 ml each parent + .1 ml inhibitor (2X). Mix and incubate 1 minute (pulse)
Dilute 1/10,000 in warm BOA & plate at 60 m. (10^{55} AM). : I: A, B, C, D.

II. Tube A at t_1 (1:100) and dispense 1 ml to each of 5 tubes. Chill at t_{12}. (Plate III)
AO - keep chilled. A, B, C, D add equal vol. inhibitors and incubate 10^{20} - 11^{15}

III ~~Plate after~~ 1:50 dilution at 11^{05} → plate II
Incubate 1 hour + plate III.

Note: water bath went to 43° at 11 PM.

Counts: IA - 0 prototrophs. II and III zeros all 0!

IB { 25 prototrophs
36
36

Faulty IA? Are the "inhibitors" encourage mating? If they do not inhibit mating they do appear to inhibit entry and according to B reversibly.

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Azide at. on entry.

1415 B.

6 May 1958

REF:

Repeat 1415 in view of 43° incident. Change designation to measure entry of T2.

Use same cells as yesterday (chilled at 100X).

Pulse (1:1:2ml fresh BOA) 7 minutes at 37°. Chill and dilute 1:100 in BOA

disperse 1 ml volumes of dilutions. Add 1 ml of inhibitor. AO = chilled A (not inc.

Incubate 30 minutes. (Plate semis at at 1:50 dilu.) Dilute all samples 1:100 in

warm BOA and incubate 60 mn. Plate 0.1 ml.

.05 ml samples plated.

1 Plate A only at t_0

2 Plate AO, A, B, C, D at t = 50.

3 Plate AOABCD at t = 110

4

Needs reincubation, counting, and full scoring. Gal as T_1

semis 2 shows inhibition of entry

3 its reversibility.

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1415-6

28 May 1958. O = 5 45 PM

REF: about 3000 - since formulate 11 AM:

	1 ♂	2	3	4 ♀	5	6	8	9	10
1	W3060	Az^S		W3064	Az^R				
2									
3	W3947	Az^R		W3935	Az^R				
4									
5	A = ♂S	♀ S							
6									
7	B = ♂S	♀ R							
8									
9	C = ♂R	♀ S							
0									
	D = ♂R	♀ R.							

Tubes: 1 water at 13'
1.0 ml gate. 2 azide - 0'
add .03 ml azide. 3 azide 5'
4 azide 12'
5 kept chilled.

Harvest dilute exp. cultures to chilled pipettes. Mix + pulled 2× concentrates (at about 3×10^8/ml). Time by warming in temperature bath.

at 4'10' chill + blend. (after mixing, before warming). Add 4 ml chilled water. Plate .05 ml and .05 of 1:10 dilu.

Plate for plate recombinants. (at 2 30 minutes) dil .2 ml in .8 ml water and plate .05 ml). Replica plating for interruption.

	1	2	3	4	5
A	10^4	0	< 10	2	0
B	"	0	< 10	9	2
C	"	1	20	100+	0
D	"	6	200+	12	1

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1415-7

29 May 1958 REF: -6

-6 suggests that concentration of ~~azide~~ azide used here was too high for activity even of A_2^R phenotype. Try column #2 of yesterday's expt with range of concentrations added at t = 0. Train keychilling. ORC cultures resuspended in presaay. and chilled. 2.5 ml each mixed for four crosses. Same as 1415-6.

Note: use of ORC cultures today. Various concentrations of azide, added to .9 ml each cross while chilled. Make up $(\sqrt{10})\%$ $\times 10^{-\frac{n}{2}}$ sers.

i.e. ~~3.16%~~ .316% .1% .03$\frac{1}{2}$% .01% 0 final conc.
(1) (2) (3) (4) (5)

from 10X these conc. store solns.
(A1-2-3-5 are halved in volume).

Dilute 1/1000 at 45' incubation and plate .05 ml on Oxm B. (No blending)

O = 640 PM

But A5, B5 << C, D.
conclude W3060 n.u.g.

Counts. 3PM 31 May

decreasing azide →

	1	2	3	4	5
A SS	0	0	7	51	38
B SR	0	1	6	18	44
C RS	0	1	43	108	~~400~~ ~300
D RR	1	10	41	118	388

↑ effective concentration ↑ threshold

No indication of a differential effect of azide. ~~Conc~~ S·S ~~was~~ shows same yield reduction as R×R. Must to reconsider plan

19 May 3rd 1958 REF: 1416

DNA - LEAKAGE.

W3060, W3064 rotated to saturation or almost (2 x log) 9 cultures each.

Suspend in BGA 15 x after one washing.

NBC : not blended controls
BC : blended controls
M : mixture, blended

Sample at times indicated, 0.5 ml into 3.5 ml of chilled DW to be spun.

(M): 1.5 ♂ + 1.5 ml ♀ ; incub 37° for 10' → blended → incub. →
↓ time 0 ↓ 20' ↓ 40' ↓ 60

(BC) 1.1 ml ♂, blend → incub 37°
↓ time 0 ↓ time 60'

Same for ♀

1.1 ml ♂ + 1.1 ♀, both blended before : → incub.
↓ time 0 ↓ 20' ↓ 40' ↓ 60

(NBC) Same as BC, but unblended suspensions.

All blendings : Virtis 30" —

Notes. Caldwell & Hinshelwood find amount of DNA in Bact lactis aerog as : 2 mg DNA-P per 10^{12} cells.

P. content of DNA is 9%. Hence 220 γ per 10^{10} cells.

Sensitivity of method : 259 mμ : E(P) = 6650.
MW of a nucleotide : 354.
5 γ/ml should give E = 0.10.

Ceriolli : 2.5 - 15 μg/ml.

Tube Numbers:

1 BC, ♂ Time 0
2 " ♀ "
3 " ♂+♀ "
4 NBC ♂ "
5 " ♀ "
6 " ♂+♀ "
7 M after blending
8 BC ♂ + ♀ time 20'
9 NBC " " "
10 M Time 20' after blending.] mixed by mistake.
11 BC ♂ + ♀ Time 40'.
12 NBC " " " "
13 M Time 40'
14 BC Time 60' ♂ + ♀ (0.2 instead of 0.5).
15 BC " ♂ ♀
16 BC " ♀ ♂.
17 NBC " ♂ + ♀
18 NBC " ♀ ♀
19 NBC " ♀ ♂
20 M Time 60'

At the end after dilution: cooled, centrifuged twice, stored at -20° until read in the spectrophotometer.

19 REF: 1416

1	2	3	4	5	6	7	8	9	10

Difference in E between 260 & 240.
BGA = -13. Correction applied to all

	Time	BC Blended controls	NBC Non blended controls	Blended Mating
♂	0'	11	19	
	60'	39	23	
♀	0'	16	16	
	60'	28	–	
♂+♀	0'	11	4	–
	20'	13	–	–
	40'	11	12	43
	60'	18	20	25

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Slit 0.1

19

REF:

	1 220	2 230	3 240	4 250	5 260	6 270	7 280	8 290	9 300	10
BGA	~~.057~~	.059	.042	.035	.029	.025	.023	.018	.015	
1	~~.220~~	.175	.117	.118	.115	.098	.063	.045	.034	
2	~~.560~~	.262	.162	.162	.165	.138	.092	.058	.040	
3			194	187	192	163	122	.092	.062	
4			132	142	141	120	87	35	.044	
5										
6			.180	170	.171	147	105	73	.058	
7										
8	~~480~~	270	175	175	175	148	098	65	45	
9+10	~~500~~	275	170	170	170	150	98	65	45	
11	~~520~~	315	220	215	218	185	118	78	55	
12		235	143	~~138~~	~~142~~	120	88	~~45~~	30	
13		250	138	~~150~~	~~165~~	134	85	35	20	
14		215	50	~~55~~	~~35~~	45	39	20	05	
~~15~~										
15		238	132	142	147	110	65			
~~16~~		~~168~~	~~88-90~~	105-108	115-116	85-88	45			
16		242	148	150	155	125	85			
~~17~~ 20		172	130	140	142	120	77			
~~20~~ 18		235	155	155	165	140	94			
9				No. 13		No. 16				
0	Tube No 13		λ							
			230	240						
1	Slit .05		232	200						
2			4	180						
3			6	155						
4			8	154						
5			240	140						
6			2	142						
7			4	145						
8			6	148						
9			8	152						
0			250	152						
			2	160						
1			4	162						
2			6	162						
3			8	163						
4			260	170						
5			2	166						
6			4	167						
7			6	158						
8			8	143						
9			270	138						
0			2	122						
			4	110						
1			6	110						
2			8	90						
3			280	72						
4										
5										
6										
7										
8										
9										
0										

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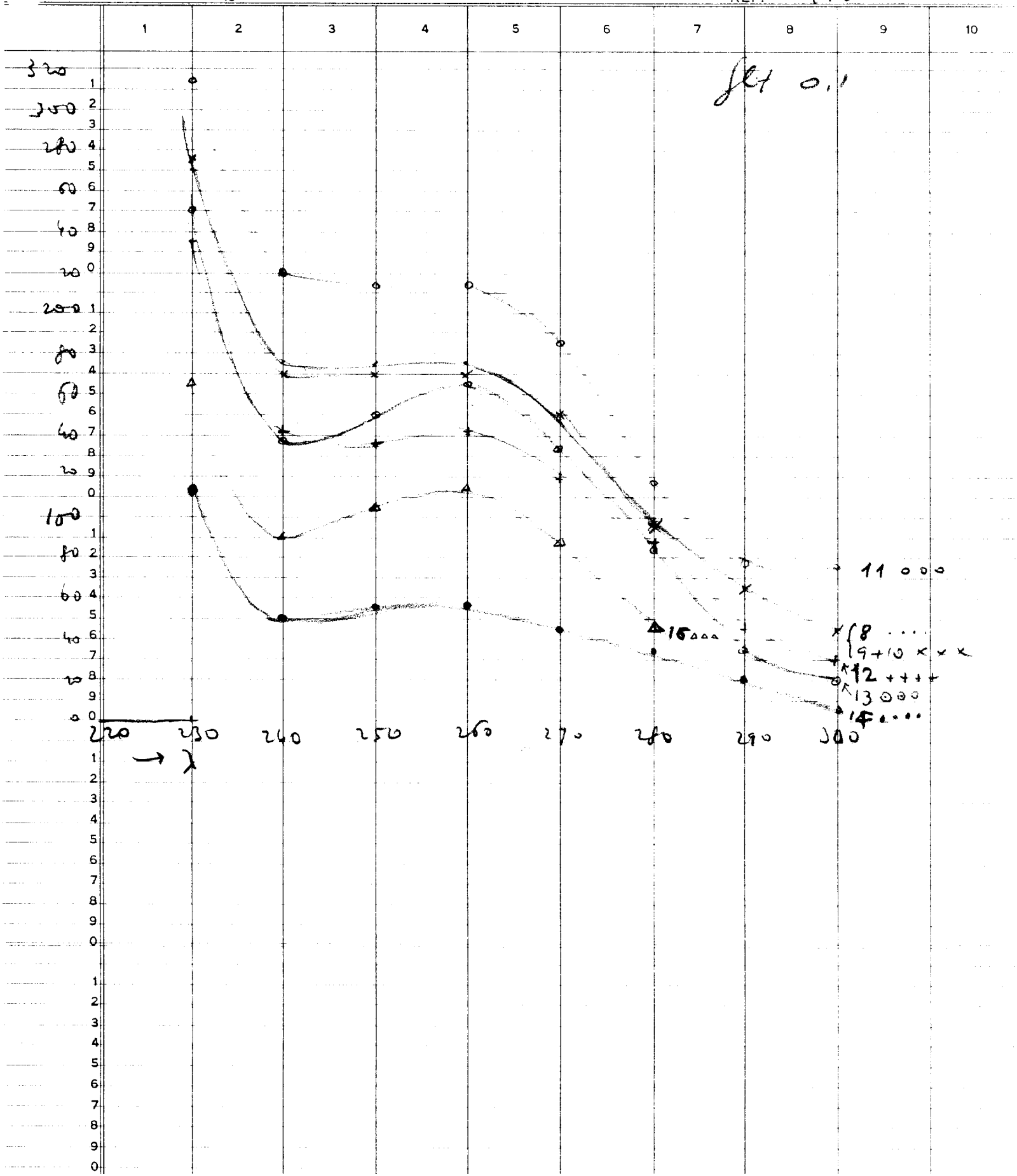
19

REF: 1416



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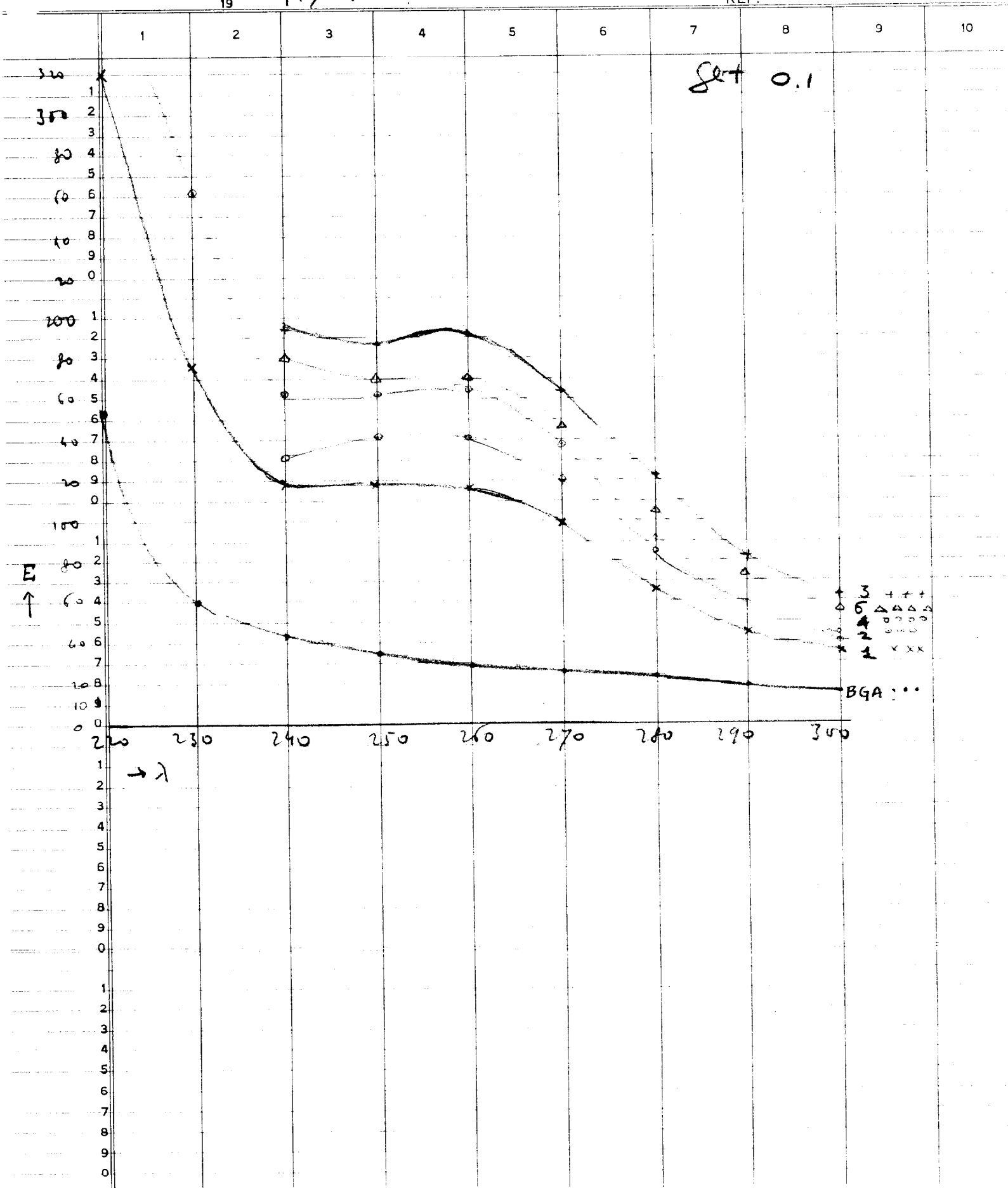
19 May 7, 1958 REF: 1415.



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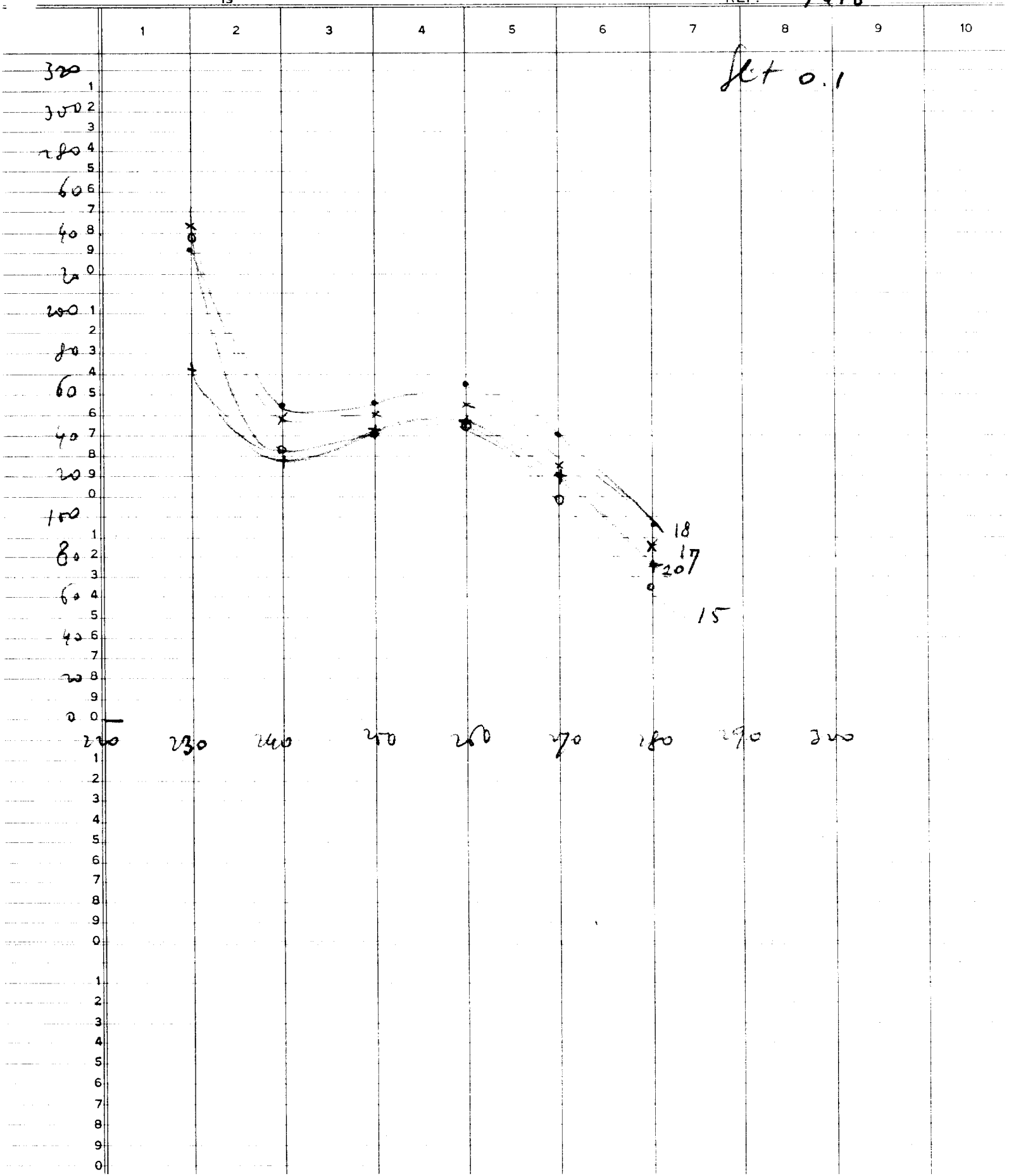
19

REF: 1616



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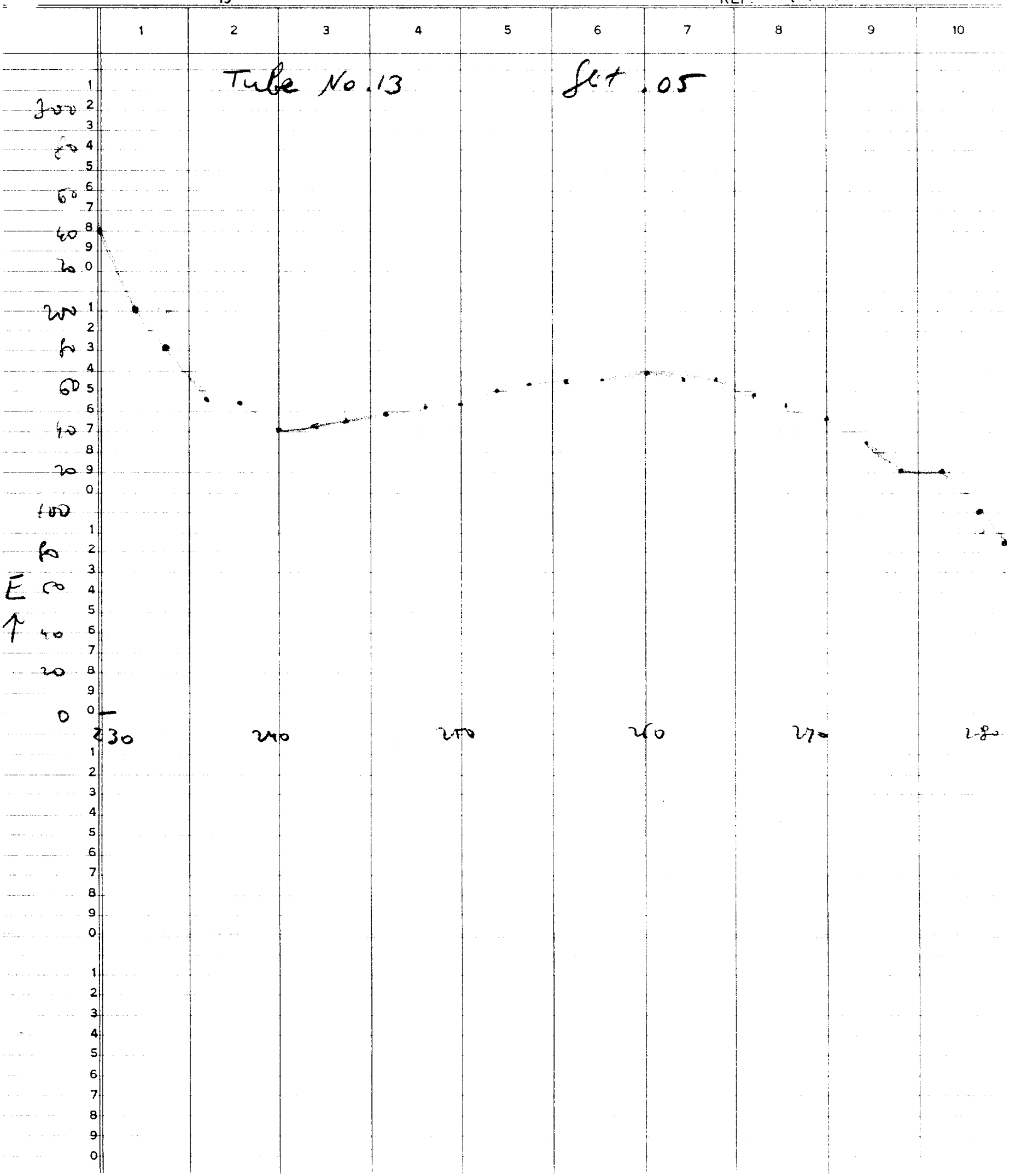
REF: 1416



1958, May 8th REF: 1417.

W1895. exp. culture *, 1 ml + 7.5 Penassay, 2½ h rotation
W3064 24h cult. same.

Spun, resuspended in Penassay 0.2 ml per tube. (50 x conc)

♂ 0.1 ml + ♀ 1 ml. ♀ pulse in water bath.
Dilution 1/1000 in broth.

At 10', 20', 30', 60', ~~90'~~, 60' blend and plate 0.05 of undil., ~~1/10~~, ~~1/100~~. on B Lac Sm, and of ~~1/10~~ undil on D(B,St) and D(0).

Plate recombination control: C
Control of recombination in diluted mixture: D

D: dilute W1895 & W3064 conc. susp. 1/1000 in broth, mix 1:10, incubate 60'. plate undil, ~~1/10~~ B Lac Sm, and ~~1/100~~ on B,St, D(0)

C: same: plate from 1/1000 dilutions W3064, 0.05 ml and 0.01 ml of 1/2000 dil of W1895. on B Lac Sm, D(♂+B,), D(0)

* 3 parallel cultures kept in frig for reuse.

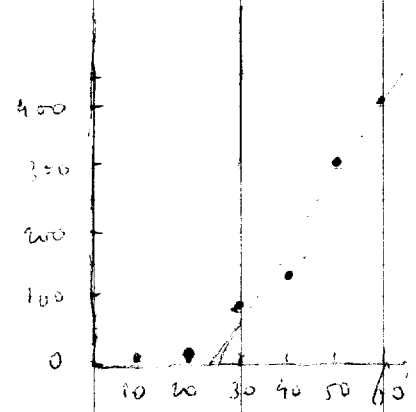
Photometry of 1/100 dil. in water of conc. suspensions:
♂ 68% } λ : 650 mμ
♀ 75%

Plate counts.

B Lac $5m$: growth entirely negative.

D(0) : all plates including C and D have approximately the same number of recombinants : about 1000 - All plate recombinations - mini St B,

C	10
D	~800
10'	12
20'	19
30'	86
40'	138
50'	320
60'	410



Pulse useless with Hfr_4 ?
May be due to use of Penassay for pulsing.
However there is a high recombination rate in D
Time pattern likely to be indicative of zygote recruitment rather than of anything else.

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19 REF: 1-717/2

W 3936 (1895 Hfr, Hz^R) 1 ml + 7.5 ml Pen 1^h rotation
W 3064 " "

Spun, resuspended in fresh Penassay at double conc.
Mixture: 1 ml ♂ + 9 ml ♀ in water bath
Sampled: .5 every 10' diluted in chilled DW 4.5 ml.
and then further dilutions and platings on the
following media: (.05)-
3) D-G B_1 threonine leucine methionine fm
4) " B_1 methionine fm
5) " Methionine fm
6) MLac - threonine leucine B_1 meth. Proline, fm.

Dilution	0'	10'	20'	30'	40'	50'	60'
A 1/10	4,5	3,6,4,5	4,5	5	5		
B 1/100	3,6,4,5	4,5	3,6,4,5	3,6,4,5	4,5	5	
C 1/1000	3,6,4,5	3,6,4,5	3,6.	3,6,4	3,6,4	3,6,4,5	4,5
D 1/10.000	3,6	(3,6) ↑ by mistake			3,6	3,6,4	3,6

40' is actually 43'-

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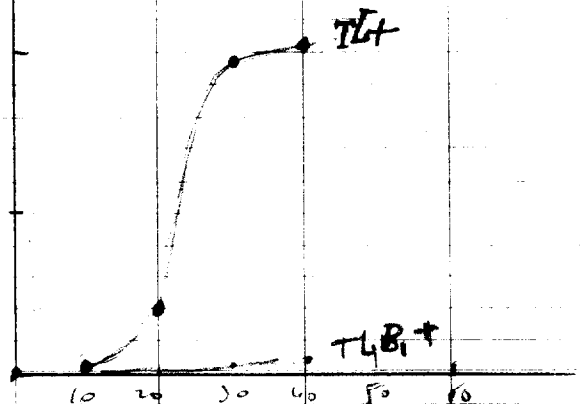
1417B

19 5/23 REF: 1417/2

Time	Dilution	3 D(MTLB, sm)	4 D(MB, sm) [TL+]	5 D(M sm) TLB, +	6 MLac (TLB, MP sm) (6 (growth only after 48 hrs))
	Medium		4 plus growth		
0'	A		0	0	—
	B	∞	0	0	20, 1 sm +, plus background
	C	~6000	0	0	~2000 mostly +
	D	~700			
10'	A	∞	34	0	24 +, many neg papillae, plus background
	B		2	1	
	C	∞	0	0	~300 mostly −
	D	~3000			227 mostly +
20'	A		394	2	
	B	∞	34	0	~100 neg small & background
	C	∞			~200 mostly +
30'	A			3	
	B		198	0	25 + background
	C	∞	20		~2000, sm +
40'	A			7	
	B		201	6	
	C	∞	8		~120 + and −
	D	1500			600 all +
50'	B			0	
	C	∞	7	0	~5000, 15 sm +
	D	2500	1		~600
60'	C		24	0	
	D	2000			~200 mostly +



Note on medium 6.

On most plates, there is a/pigment into bl + & Lac − . Due to gal+?

1. The slow transfer of ... due to ... with ... and ... of ...

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Hfr,
W2150 × W3064 ; chloramphenicol

1417C

26 June 1958. REF:

Timing of Xyl, Mtl among T1+ S° recombinants. (also among Lac+ S+ ?).
Abbar reported a zero incidence of Mal+ etc. in both crosses. These might be very late markers, and as such might be most influenced by the inhibition of replication.
Try timing and effect of 20γ/ml chloramphenicol on the Xyl rate, etc.
W2150 : W3064 chilled; 20 × ORC ; .1 : .1 : 5 ml. ~~Plate~~ Dilute 1:100 and plate .05 ml D sm B1.
at times indicated. t = 00, 0, ~~15~~ 16, ~~22~~ 22, 30, 40, 50, 60, 90, 90C [chloramphenicol from beginning].
undil.

Counts (Inc. 2 days; at room temp to 29 June).

P29.

t mins	SRP-D sm B1	B	Gal sm B1	M	Lac	Gal	Mal	Xyl
00	~10^4				50% ?	0	½ ?	13 ?
0	1							
16	32				33%	0	1 ?	0
22	65				66%	0	0	2
30	351							
40	~500				50%	0	0	0
60	10^3	0		0	66%	0	0	3
90	10^3	0		0	66%	0	1	8
60C	10^3			0	66%	0	1	2
90C	10^3-			0	50%	0	0	0

note: it is unrelated to early entry indicated TL

16 indicated

90C < 90 but too many to count. repeat., not 1/10 dilns

What is Xyl ratio ?, etc.

July 1. – To refrigerator to await scoring for late markers.

19 May 19, 1958. REF: 1418

TRANSFORMATION

Inserting DNA by making holes with F+ ~~or Hfr~~ and partial osmotic shock of recipient protoplasted ♀.

DNA: source W 3064, protoplasts, concentrated to 10^9/ml and shocked in DW. Kept at +20° until used; thawed, 4 vols. of alcohol added, filaments removed by glass rod to .6% saline. Partial redissolution, cell walls not entirely eliminated. Blending to resuspend.

Recipient: W 1827 (= W6 F-). Exponential → protoplasted 2½ hrs, spun, concentrated from 10 tubes into 5 ml (= 20x)

Hole makers: W6 expon. conc. 15x (to 0.7)
W 1895. expon. " (to 0.7).

(A). W6 + W1827 ♂: 0.6 + 0.4. 3' waterbath, then add, under blending, 2 ml of DNA prep. - incubate 65', then plate .05 on B gal Sm, 5' Mal (for lack of X⁰)

(B) same with W 1895, but 10' waterbath

(C) same with 0.6 lambda prep., 5' waterbath

(D) Control of 3064: .05 on B gal Sm, 5' Mal

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19 May 24th 1958. REF: 1419

DNP resistance.

Broth cultures drops W 3060, W 3064 spread on Plates

per with

			W 3060	W 3064
DNP	M/20	1 ml	–	—
"		.2 ml	growth	growth + ~~5~~ papillae
"		.04 ml	growth	growth.

W 3064 papillae, isolated on ~~Pen~~ B Lac and on DNP M/20 1 ml plate – No growth on the latter. Streaks on B lac replicated on .5, .4, .3 ml of DNP · growth of almost all – Slants made –

May 26th

.2 ; .3 ; .4 ; .5 ; .7 ml of M/20 DNP per plate and Spread (broth drop of W 3060, W 3064) again

		.2	.3	.4	.5	.7 ml of DNP
48 hrs	3060	growth	scanty growth papillae	~40 small colonies	5 colonies	no growth
	3064	growth and darkening of medium, one papillae	irregular growth.		1 colony	"

After ~~one~~ more days of growth at room temperature, there are about 100 resistant colonies on W 3060 on .4 and 21 on .5; 1 colony on W 3064 on .4 and 3 colonies on .5 –

Colony from W 3060 on .5 streaked on B(Lac) for further testing.

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19 May 31st, 1958 REF: 1419

	1	2	3	4	5	6	7	8	9	10
			Testing DNP. resistance.							
			.2	.3	.4	.5	.7			
1	# 3060		+	±	−	−	−			
2	3064		+	∓	−	−	∓			
3	3064 DNP/1		+	+	−	−	−			
4		/2	+	+ (+)	−	−	−			
5		/3	+	+	∓	−	−			
6		/4	+	+	−	−	−			
7		/5	+	+	−	−	−			
8	3947 : W 3060 Ar^R		+	∓	−	−	−			

(+) determines - slight brown coloration of the medium.
(formation of ?)

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19 June 1ST, 1958 REF: 1420

1	2	3	4	5	6	7	8	9	10

PULSATION

Orc. W 3060, w 3064, spun, resusp. in cold Penassay. *

2.5 ml + 2.5 ml in a big flask — 5' pulse. Mix at 37°

0.1 ml transferred to 0.9 of the following. Chill at 5'. Dispense .1 ml to .9 ml fractions

			No put	Put
D(0)	pH 4.5		##	#
	5.5		##	#
	5.9		##	#
	6.4		##	#
	6.9	± .5 % glucose	##	#
	7.3		##	#
	7.9	some caramel formed	#	#
	8.6		#	#
	9.0		#	#
D(0)	10 ml			
D(0)	thioglycolate .1% (10 ml)		#	#
D(0)	pivalate (in pH 6.9) M/100.		0	0
"		M/1000	#	#
"		M/10000	#	#
D(0)	sine Mg		#	#
w945 exp.	resusp 4x, Killed by boiling few seconds		#	#
"	living, in Penassay		#	#
Penassay			#	#
5' control	: dil 1/1000 → .05 S'Gal fm S,		0	0

4:30 PM. (1-19): Incubation 40' — Dil 1/100, plate .05 on S'Gal fm B,

* Cultures are viscous

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19 June 5, 1958 REF: 1420 A

	1	2	3	4	5	6	7	8	9	10
1										
2		Plate		Gal +	Lac +	T_1^r				
3		counts								
4										
5	1	310		1/50	5/50	39/50				
6	2	340		3/45	13/45	35/45				
7	3	298		5/50	8/48	30/50				
8	4	380		4/50	17/49	36/50				
9	5	310		4/50	14/48	36/50				
0	6	298		3/50	14/50	35/50				
	7	320		3/50	17/48	36/48				
1	8	280		2/50	11/45	25/45				
2	9	355		2/50	10/48	31/50				
3										
4	11	474		28/100	45/100	70/100				
5	12	365		14/100	39/100	64/100				
6	13	0								
7	14	127		0/80	3/80	35/80				
8	15	390		3/100	19/100	67/99				
9	16	370		6/50	23/50	35/50				
0	17	400		13/50	23/50	37/50				
	18	148		0/50	1/50	18/50	← interrupted			
1	19	430		6/50	15/48	33/50				
2	20	0								
3										
4										
5										
6										
7	Conclusions:									
8										
9	pH has practically no effect on transfer; some also on									
0	mating mixes, no recombinant takes place in these									
1	controls. Range 4.5 – 9.									
2										
3	Thioglycolate has practically no effect at 1/100									
4										
5	Iodoacetate M/1000 shows partial interruption									
6										
7	Addition of living F- cells interrupts, that of killed									
8										
9	F- cells does not									
0										
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										

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PULSATION

design 1420A

June 1 1958 REF:

Kinetic experiments based on pulsed mating have been unsatisfactory until now on account of an unsatisfactory separation of recruitment and entry phases. In previous control experiments, extent of recruitment varied as approximately the 1/2 power of cell density (instead of expected square), so that subsequent recruitment accounted for 5-25% of total yields. This is quite unsatisfactory.

In this experiment, screen for reagents which may impede mating without preventing further entry.

Following are suggested:

1. pH range in .5 units from 4.5 to 9 Adjust D(0) by HCl or NaOH
2. tris
3. versene
4. thioglycollate
5. ethanolamine
6. dead and live S^s F^-
7. fluorouracil deoxyriboside ↓ not available today
8. periodate M/100 M/1000 M/10.000
9. D(0) ∅ Mg

Experimental design: Mate ORC W-3060 and W-3064 (washed and resuspended in cold penassay) at sat'd cell density for 5 minutes at 37°. Dilute 1:10 into indicated solutions (made up in D(0) unless indicated, and incubate to 60'. Dilute 1:100 for plating .05 ml on SGalThSm. The pulse matings should gave a baseline count of ~~prem~~ SRP including some 10% Gal+.

Toxicity will be indicated by a loss of SRP

Inhibition of entry will be indicated by failure of Gal+ among SRP

No inhibition will be indicated by marked rise in SRP

Inhibition of mating ∅ inh. of entry will be indicated by stabilization of SRP with establishment of Gal+.

Controls needed: Plate recombinants (plate mixture after dilution))
Early interruption: plate mating at 5';)
1:1000 dilution at 5'; plate at 5' 15' and 60') Dilute in D(0).....
1:10 dilution: plate at 5' 15' and 60')

Dilution only 1:10 from penassay!

Criticism: may have already been pulsed by high density. Luca's conclusions: pH 5 to 8.5 no effect. 4.5 gradual reduction or slight 4 no effect. 2,3 not tried

D(0)-Mg no effect. periodate negative (partial interruption and null-recruitment)

6B no effect! But 6A

19 June 4, 1958. REF: 1420 B

PULSATION WITH PERIODATE

Parents: W3060, W3064

ORC cultures, 4pm, susp in chilled water 20 x until used then diluted in: Penassay, 1/20,

DM (D(M) + asparagine 1‰)

DO (D(O) + " + glucose .5%)

α1 Pulse 2' in Penassay, mixing equal amounts of parents: 1 ml each in a flask, starting from chilled susp mixed while chilled. Dilute after 2' in DM periodate M/2000, 1/100, incubate and sample every 5' until 40', dilute sample of 0.2 ml in 1.8 ml, plate and plate on D(Sm B_1) 0.05 ml.; for 20' amounts also further 1/5 dil. plated

α10 From pulse dilute in D(M) 1/100, without periodate; sample only after 40' and plate 1/5 and 1/25 .05 → D(Sm B_1)

There were no colonies except in α10 (where there were 4 colonies).

F+ reversion of W3060 ?

Moreover while this part of the experiment showed contamination with staph, the second part of this same exp., which used the same cultures, showed no or less contamination, but was also sterile.

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periodate pulse.

1420B

June 4 1958

REF:

	1	2	3	4	5	6	7	8	9	10
	Matings. Premix chilled matings (ORC W3060, W3064) in chilled presassay resuspended 1x. Pulse = time									
2	α Pulse 2 min in presassay / ml each with. held at 37°. α									
3	β Pulse 0 " " " Dilute 1:200 each parent ~~(500 ml thromboplastin)~~ in 10 ml tubes.									
4	γ " 2 " " D(o) .5 ml in tube									
5	Δ " 2 " " D(m) " "									
6	ε " (α) o kept chilled : for plate recombinants									
7										
8	add periodate to chilled ~~=~~ D(o, m) tubes.									
9										
0										
				Dilute in M/ Periodate						
sample @ 5 min. all others are plated at 40 (6 min)	α			D(m)	2000 → time series.					
2	β			"	0					
3	β			"	500					
4	β			"	1000					
5	β			"	2000					
Dilute 1:10 in chilled water. 6	β			D(o)	0					
7	β			"	500					
8	β			"	1000					
9	β			"	2000					
10	α			D(m)	0					
11	β add presassay just			D(m) + P_{10}	0					
12	β prior to cells.				500					
13	β				1000					
14	β				2000					
To stop reaction 15	β thioglycollate 0.1%			+	500					
16	γ 7 pulse			D(o)	0					
17	Δ 5 pulse			D(m)	0					
18	ε Mixed at 0 ; spun out & to 4° ; plated 1:1000									
19	ε - mix post dilutions 1:100 plated									
20	" 1:1000 plated.									
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										

Does dilution into periodate give a pulse? Possibility that M/2000 periodate is toxic if not neutralized? Should try adding thioglycollate at various times, providing thioglycollate does neutralize. M/2000 periodate was not toxic in 15 minutes with a heavier cell suspension (1420B). Again will neutralize periodate. Q questions.

No colonies! Presumably reacted Fx!

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19 June 9, 1958. REF: 1420 C.

Pulsation with periodate.

3060 (?); 3064. Sheet giving details of cultures missing.

Pulsed 2', diluted 1/100 in

P. DM + asparagin 1/1000 + glucose 1/1000 + periodate M/2500

C. " " " —

B. Penassay broth

Samples taken at 20', 30', 40', 50', 60' (or parts), diluted and blended and plated on D Sm B1.

1/5,

Plate counts. (Large colonies; also some minute colonies).

	P	C	B	
20'	3,9	7,5	12,8	911 stroked on B kc 6/11
30	1,4	10,3	8,14	
40	6,4	2,2		
50	8,5		6,9	
60	7,10	8,12	28,24	

1420 C - Lac strokes

P	20	4+, 9−
P	30	1+, 4−
P	40	1+, 9−
P	50	3+, 9−, 2 w
P	60	6+, 10−, 1 w
C	20	7+, 6−
C	30	6+, 7−
C	40	4−
C	60	9+, 11− (some w:)
B	20	6+, 14−, 1 w
B	30	10+, 15−, 1 w
B	50	7+, 9−
B	60	21+, 34−

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19 June 11, 1958. REF: 1420 D

INHIBITION & INTERRUPTION BY LIVING CELLS.

Cell suspensions 10 × (same as in exp. 1422)

A C. W 3870 0.1 ml
W 3064 0.1 ml
Broth 1.0 ml
} 10' incub → 0.1 + 0.5 ml W945 + 0.9 broth (A)
↘ 0.1 + 1.4 broth (C)

B. W 3870 0.1 ml
W 945 0.1 ml
Broth 1.0 ml
} 10' incub → 0.1 + 0.1 W3064 + 0.9 broth (B)

A, B, C : at 5', 15', 30' 0.2 ml + 1.8 chilled water.
0.05 D (from B,)

Plate counts.

	A	B	C
5'	91,72	–	164,128
15'	162,182	77,85	456,410
30'	160,155	343,510	460,486

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19 REF: 1420 D

	Gal⁺/Int	Lac⁺/Int	T₁⁺/Int	
A 5	0/50	1/50		Addition of W945 cells to the cross inhibits further mating, interrupts. Compare with control C.
A 15	0/50	1/50		
A 30	0/50	2/50		
B 15	0/50	0/50		No accelerated Gal detected in the second mating.
B 30	0/50	5/50		
C 5	0/50	0/50		
C 15	0/50	12/50		
C 30	0/50	12/50		

Note. W945 is added in some excess over physiological conc. of cells. Interruption may be due to transfer to ~~over~~saturated ~~plugged~~ cell concentration. Retest with normal cell conc.

19 June 25, 1958 REF: 1420 E

1	2	3	4	5	6	7	8	9	10

8 INTERRUPTION BY LIVING FEMALES.

3 hrs. R.C. W 3064, W 3870, W 945.

Sperm, resusp. in fresh broth.

A ♂ : ♀ = 10:1 ratio.

2 ml 3870 + 0.2 ml 3064, incubated 20'

then:

AC: 0.9 ml transferred with 1 ml warmed pipette to empty, warmed tube, incubated 20', diluted 1/20 & 1/100 (chilled) plated on ♀ Gal Sm B_1 (.05).

AD: 0.1 ml transferred to 0.9 ml W 945 warmed suspension. and incubated 20', diluted 1/2 & 1/10 chilled. plated as above

B ♂ : ♀ = 1 : 1 ratio

1 ml 3870 + 1 ml 3064, incub 20'

then:

BC: 1 ml. transferred with 1 ml. warmed pipette to warmed tube, incubated 20', diluted 1/100 & 1/500 chilled, plated on ♀ Gal Sm B_1 (.05).

BD: 0.1 ml transferred to 0.9 ml warmed W 945 susp. incubated 20', diluted 1/10 & 1/50 chilled, plated on ♀ Gal Sm B_1.

Purpose: to test if addition of living ♀♀ interrupts even when the whole experiment is kept at the same total conc. of cells throughout, and this concentration is within theoretical limits (i.e.

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19 June 28 1958 REF: 1420 E

1	2	3	4	5	6	7	8	9	10
Dilution 1 : too many colonies.									
Dilution 2 only counted.									
AC2	Gal + 9 ; 7		Gal-	about	1000	per plate,	two plates.		
AD2		53 ; 60		"	2000				
BC2		3 ; 2		"	400				
BD2		21 ; 11		"	1000				
	The effect of interruption is reversed. What happened ?								

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Periodate

1421A

June 1 19 58 REF:

1	2	3	4	5	6	7	8	9	10

W3062 ♀ × W3064 ♂

Same cells as 1420. Harvest 5 ml into .5 ml water.

.1 ml cells + .4 ml periodate : M/200; M/2000; M/20,000 and H_2O.

after 10 minutes of treatment at 37° add 4.5 ml presoaq + 1% glycerol.

Mate equal volumes 15' at 37°, dilute 1:100 and plate on D-13, 0.5%.

Cross

♂ treatment	♀ treatment	8P2 · Pulmi. A3	Count
0	0 *	++	149
2000	2000	−	2
200	200	−	0
20	20 *	−	0
2000	0	−	2
200	0	−	0
20	0	−	0
0	2000	+	45
0	200	÷	1
0	20	−	0

Tests by shifting

(1-6 diluted, via 0(m) rather than H_2O.

at M/2000, inactivation maybe differential.

Preliminary results are very promising (for periodate as a de-sorting agent). Could the aeration phenomena have been oxidation of surface receptors? If so should reverse Hpoise.

See 1420 for origin of this experiment. Periodate is of course known to affect the receptors of animal cells for influenza virus. (Will flu agglutinate any bacteria?).

Try other oxidants? Ferricyanide?

Conclusion (within limit of test): sexual competence is destroyed by periodate, ♂♂ more sensitive than ♀♀. [Viral kinetic and viability controls.] 1421B shows M/200 periodate is toxic; M/2000 is not.

If Hpoise inactive, will periodate/Protein deactivate ♂♂?

June 3 19 58 REF: 1420;1421

Harvest CSC W-3060, W-3064 3x7 ml each into 1 ml water. (Keep chilled during day before beginning experiment about 7PM. 1421 suggested differential effect of periodate. This is essentially a repetition with some added controls for viability and reproducibility.

In each case, add 0.1 ml cell suspension to 0.5 ml treatment solution (in D(m)). Treat 10 mins. at 37; neutralise 5 mins. by addition of 4.5 ml penassay+1% glycerol. Mate for 15' by mixing .5 ml each suspension. Then chill, dilute 1/100 and plate 0.1 ml on D-B$_1$sm agar: duplicate plates. Also dilute 10^{-6} and plate mk 0.1 ml on EMB Lac for viability.

	Treatment: (periodate conc.)		Viability		SRP counts	
	♂	♀	Lac+	-		
1	-	-	28,29	60,38	27	27
2	M/10000	-	49	78	20	15
3	5000	-	32	85	7	8
4	2000	-	30	71	0	0
5	200	-	2	104	0	0
6	-	10000	107	84	25	60sm.
7	-	5000	30	62	47	23
8	-	2000	36	52	38	37
9	-	200	53	1	0	0
10	2000P	-	43	68	102	55
11	-	2000P	22	71	50 sm	23

(A few SRP counts were slightly distorted by smearing)

These counts reflect a substantial pipetting error, but not enough to weaken the experiment.

Tubes #0-11 (P) had periodate neutralised by penassay-glycerol/prior for 5' to addition of the cells

CONCLUSIONS: M/200 periodate is toxic; M/2000 and less is not.

M/2000 periodate differentially inactivates fertility of ♂♂, with no perceptible effect on fertility of ♀♀ or viability. M/5000 has a partial inhibitory effect; M/10.000 is doubtful. Must explore possibility that mild treatment of ♀♀ enhances fertility; try especially neutralised M/2000 or M/10000. This might be explained if there is a phase of periodate treatment with slows the separation of the pairs.

Also need a more careful study now of effects on interruption and on speed of effects.

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Periodate

1421-B

June 3 19 58 REF: 1420;1421

Harvest ORC W-3060, W-3064 3x7 ml each into 1 ml water. (Keep chilled during day before beginning experiment about 7PM. 1421 suggested differential effect of periodate. This is essentially a repetition with some added controls for viability and reproducibility.

In each case, add 0.1 ml cell suspension to 0.5 ml treatment solution (in D(m)). Treat 10 mins. at 37; neutralize 5 mins. by addition of 4.5 ml penassay+1% glycerol. Mate for 15' by mixing .5 ml each suspension. Then chill, dilute 1/100 and plate 0.1 ml on D-B_1sm agar: duplicate plates. Also dilute 10^{-6} and plate 0.1 ml on EMB Lac for viability.

Treatment: (periodate conc.)

	♂	♀	Viability Lac+	Viability Lac−	SRP counts	
1	−	−	28,29	60,38	27	27
2	M/10000	−	49	78	20	15
3	5000	−	32	85	7	8
4	2000	−	30	71	0	0
5	200	−	2	104	0	0
6	−	10000	107	84	25	60sm.
7	−	5000	30	62	47	23
8	−	2000	36	52	38	37
9	−	200	53	1	0	0
10	2000P	−	43	68	102	55
11	−	2000P	22	71	50sm	23

Hfr tests on Lac+
0/10 from 1
0/2 from 2.
Valid tests? cultures attenuated of Hfr.

(A few SRP counts were slightly distorted by smearing)

These counts reflect a substantial pipetting error, but not enough to weaken the experiment.

Tubes 10-11 (P) had periodate neutralized by penassay-glycerol for 5' prior to addition of the cells

CONCLUSIONS: M/200 periodate is toxic; M/2000 and less is not.

M/2000 periodate differentially inactivates fertility of ♂♂, with no perceptible effect on fertility of ♀♀ or viability. M/5000 has a partial inhibitory effect; M/10.000 is doubtful. Must explore possibility that mild treatment of ♀♀ enhances fertility; try especially neutralized M/2000 or M/10000. This might be explained if there is a phase of periodate treatment with slows the separation of the pairs.

Also need a more careful study now of effects on interruption and on speed of effects.

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Periodate. Effects on F⁺.

1421C

5 June 1958. REF: 1421

ORC W6, W3064 prenssay → water. Chill 4°.

α: .2 ml + .8 ml D(m) + aspargine + M/2000 periodate } W6
β: .2 " " D(m) + " "

Incubate 10 minutes. Add .2 ml W3064 and :

Plate .05 ml samples on DB, sm.

1 α Crowded
2 β Crowded

dilute 1+4 in prenssay glycerol, rotate 15 minutes, chill :
dilute and plate for F transfer. add 5mm 200u to aliquot of 4 → 8.

3 α	~0 uncountable	.04 .5 −, 58+
4 β	200−, 130+	84−, 64+
5 Bsm.	290−, 2+	120−, 0+

Spin and resuspend in 1v. Plate .05 ml samples on DB, sm

6 α	18, 23 large
7 β	107 large, —

8 Bsm (Bonby) add periodate to M/2000, incubate 10 minutes and
9 β : plate .05 ml 26, 30 large,

20× cells. → .2 + .8 D(m) → β; .2 + .8 D(m) periodate → α

10 mins inc., add 306 4 .2 ml → 1 + 4 prenssay glyc. → .05 ml 1 α 2 β

15 mins. inc. Rot. Chill → Spin Resp. D(m) in 1 vol. → 10 ml EMBLac 3 α 4 β 8/Bsm

add 1v. periodate M/1000 (β) → inc. 10m. → .05 ml Dsm B, 7 β.

→ .05 ml 5 α 6 β D sm B,

β aliquot with 5mm 200u/ml.

Pretreat F⁺ with various concentrations of periodate.
Incubate 20× W6 1:1000 ins D(m). , 1:100 in treatment tubes (10 m.)

plate .05 ml, dessicate and 1:100 (duplicate). Viability

12	—
13	M/2000
14	M/1000
15	M/500
16	M/200
17	M/100.
18	— onto plates prepared with 0.1 ml M/100

Conclusions: 1 & 2 shows that periodated F⁺ still gives overwhelming yield of plate recombinants (by segmentation of receptors ?)

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19 June 5, 1958. REF: 1421 C

1	2	3	4	5	6	7	8	SECOND PART

PERIODATE OVER F+ INHERITANCE

W6, conc 20x, same as 1421 C 1st part, diluted 1/1000 —

0.1 ml to 10 ml DM + asparagine 1‰ + 0 periodate 11
M/2500 " 12
/1250 13
/625 14
/310 15
/150 16

After 10' treatment at 37°, undil → B(0)
↓
1/100 → B(0) in duplicate.

Alg 0 17. spread over B(0) and B(Lac) .1/50 periodate, 0.05, then 1/100 dilution of exp. 11. (untreated cells) —

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1421 C

June 6 19 58

REF:

	1	2	3	4	5	6	7	8	9	10
1										
2	Counts	–	on	B-O	:	1/100			Penicillase conc.	
3										
4										
5	11	:	~1000		234		192		0	
6										
7										
8	12		~1000		121		132		M/2500	
9										
0										
	13		683		32		46		M/1250	
1										
2										
3	14		128		3		3		M/625	
4										
5										
6	15		12		0		0		M/3125	
7										
8										
9	16		1		0		0		M/15625	
0										
1	17		~1000		183		207		periodate after	
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										

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19 June 5, 1958 REF: 1421 C

PERIODATE ON F+ TRANSFER

ORC culture of W6 ~~3-0-5~~ 3064 - Spun, resusp in water, chilled (2 ml of W6)

α. .2 ml + .8 ml of DM + asparagin + m/2000 periodate

β. .2 ml + .8 ml " "

Incubate 10 minutes. chill ~~rotate~~ rotate ~~samples for titering~~ Add .2 ml W 3064 and plate .05 ml samples on D(B,Sm).

1 = α
2 = β (Plate recombinations).

Dilute 1+4 in prewarmed (penassay glycerol, rotate 15 minutes, chill; dilute and plate for F transfer (Dilution $\frac{1}{2} 10^6$, 1 ml = $.04$ Bac

3 = α
4 = β.

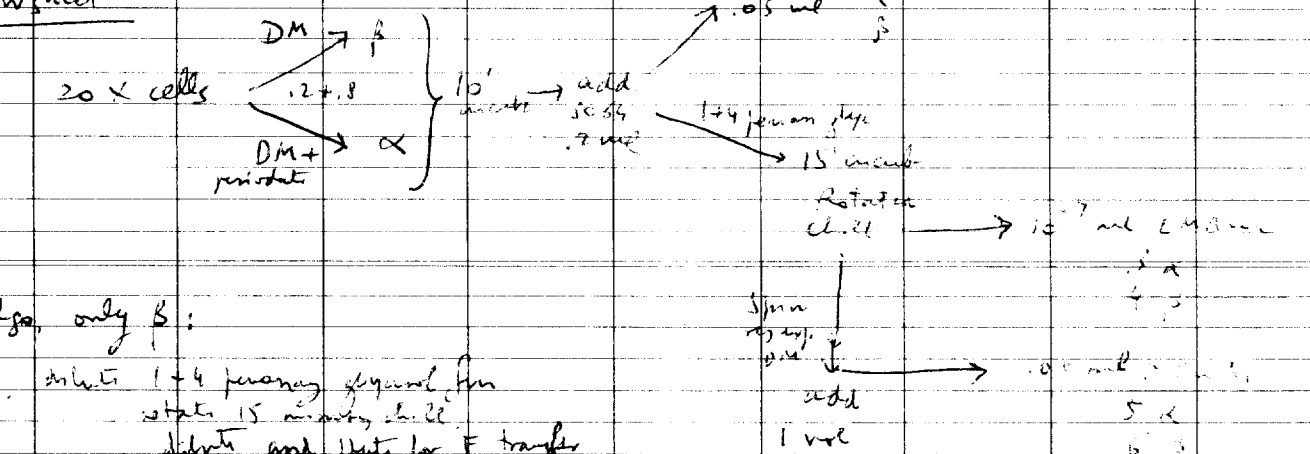
Spin and resuspend in ½ volume DM. Plate .05 ml samples on D(B,Sm)

5 = α
6 = β (F+ fertility).

β only: add periodate to m/2000, incubate 10' and

7 = β plate .05 ml on D(B,Sm)

Flow sheet



* also, only β:

8 = β. dilute 1+4 penassay glycerol, rotate 15 minutes, chill, dilute and plate for F transfer

$ ≠ Penassay glycerol 1%

.25 ml DgmBS

-3-

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Periodate and other oxidant effect on Hfs. Regeneration of receptors

1421 D

5 June 1958 RE 1421

As 1421A. — Pretreatments of W3060.

Incubate at 37 C unless otherwise stated

20x cells diluted .1 + .4 ml into treatments, Neutralize by pronase - glyc 10 m. 5 m.

Mate + Equal volume W3060 / 15 min., dilute 1:200 and plate .05 ml. (3B 1:1000)

				Counts	
Periodate	M/2000	37	10m.	~~46~~	0
"	"	at 0°C.	10m.	0	4
"	M/200	37°	5 m.	43	43 : [19, 37, ~45] should be 1/5
"	M/2000	37°	5 m.	~~83~~	2
Ferricyanide	M/10000	37	5m.		36
	M/1000		10		24
	M/100	"			22
Dichromate	M/10000				15
	1,000			~~20~~	
	100	"			2
Bubble oxygen		37	10 m.		2
		37	30 m.		4
		0°	30 m.		9

(.1 ml 20x oxalate + 2 ml D(m) — these 2.5 ml pronase equiv. to 1 ml in other.)

Regeneration: Use samples of ① after neutralization.

This is now at .1/5 = 1/50 x 20x = .4x (i.e. .4 of saturating concentration).

Dilute further 1:10 in pronase. F(t) take 0.2 ml + .75 ml of 1x Bg, incubate 12 m. (test titration) dilute dilute 1:10 + plate

0 minutes — postpone.

Control counts not very satisfactory and no control on plate count titration.

Conclude: periodate effect complete; incomplete at 0° and < 10 minutes.

Ferricyanide has no effect on fertility at M/100. (tests for viability!) (May generate a useful indicator). Dichromate effective only at M/100! (presumably viability).

Bubbling oxygen for 10 minutes reduces F phenocopy! Should also be regenerated.

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19 June 13 1958 REF: 1421 E

DIFFERENTIAL EFFECT OF VARIOUS AGENTS ON MATING ABILITIES.

ORC W 3870, W 3064, 3 pm, conc. 20 x in chilled water.

	A	B
1. DM	1 3	1
2. Nutrient Broth + $CaCl_2$ 1‰	4 4	1 0 3
3. RDE + $CaCl_2$ 1‰	3 1	4 0
4. Lysozyme .5%	2 0	2 0
5. Dichromate M/1000	2 2	0 3
6. Ferricyanide M/100.	1 0	2 4
	1: 0	

0.4 ml of each reagent added to
A. : .1 ml of ♂ susp. 20x conc.
B. : .1 ml of ♀ " " .

Treat for 10' at 37°. Chill, add 4.5 chilled broth to every tube.

From tube A_1 (control ♂), .5 in new tubes A_1, B_1-B_6; from tube B_1 (control ♀) .5 ml in A_1-A_6, B_1.

20' incubation then dilution 1/100, plating .05 Dfm B_1

7. Plate recombination control. Parental susp. diluted 1/100 → 1/50 dilutions mixed, .05 Dfm B_1 -

Cultures not enough recombinants! Culture no.?

19 June 11, 1958 REF: 1422

RDE - inhib. of ♂♂ x mating.

ORC, 18hrs, resusp in 1/10 x, chilled. ♂ = 3870, ♀ = W3064.

1. RDE 1 ml + 0.1 ml ♂, 10' incub. → 0.1 ml + 1 ml broth + 0.1 ♀: 20' incub. → 1/100 dilution → .05 ml D (Sm B_1).

2. RDE 1 ml + 0.1 ml ♀, 10' incub → 0.1 ml + 1 ml broth + 0.1 ♂, 20' incub. → 1/100 dil. → .05 ml D (Sm B_1)

3. Same as 1. but broth used instead of RDE

4. Same as 2. " " " " " -

Plate counts:

1.	201, 181	Sm	382
2.	185	164	349
3.	277	215	492
4.	171	216	387

5: 2 ml broth + 0.1 ♂ + 0.1 ♀, 25' incub → 5A / 5B

5A → 0.1 in 1 ml RDE for 15' → 1/10 → .05 D Sm B_1

5B 0.1 in 1 ml broth " " "

About 400 colonies in every plate of the 5A, 5B series.

Conclusions:

No effect of RDE detected on mating ability of cells.

Note: no Ca^{++} added.

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19 June 12, 1958 REF: 1423A

DELAYED ENTRY -

Chloramphenicol.

Testing possible delay of spontaneous interruption.

ORC W 3870, W 3064, spun and resusp. in water 20× concentrated. Chloramphenicol solution 1‰.

A. Penassay 1 ml + ♂ 0.1 ml + ♀ 0.1 ml + 0.05 Chloramph. (= 40 γ/ml)

B. " " " + —— (control)

C. " ♂ dil 1/50 0.1 ml + ♀ dil 1/50 0.1 ml + 0.05 chloramph.

D. " " " —— (control)

A, B: at 30', 60', 90' and 135' dilution 1/500 (0.02 in 10 ml), plating of .05 on D5m B1.

C, D: at same times dilution 1/10 (.1 in .9 water) and plating.

E.

Control of plate recombination at various concen. of cells.

E. From same mother suspension, .025 ♂ + .025 ♀ at 10 ml chilled water; → .05 in D5m B1 = E1

↘ 1 ml diluted serially → 2.15 chilled water = E2-E7

↓

Plate counts:

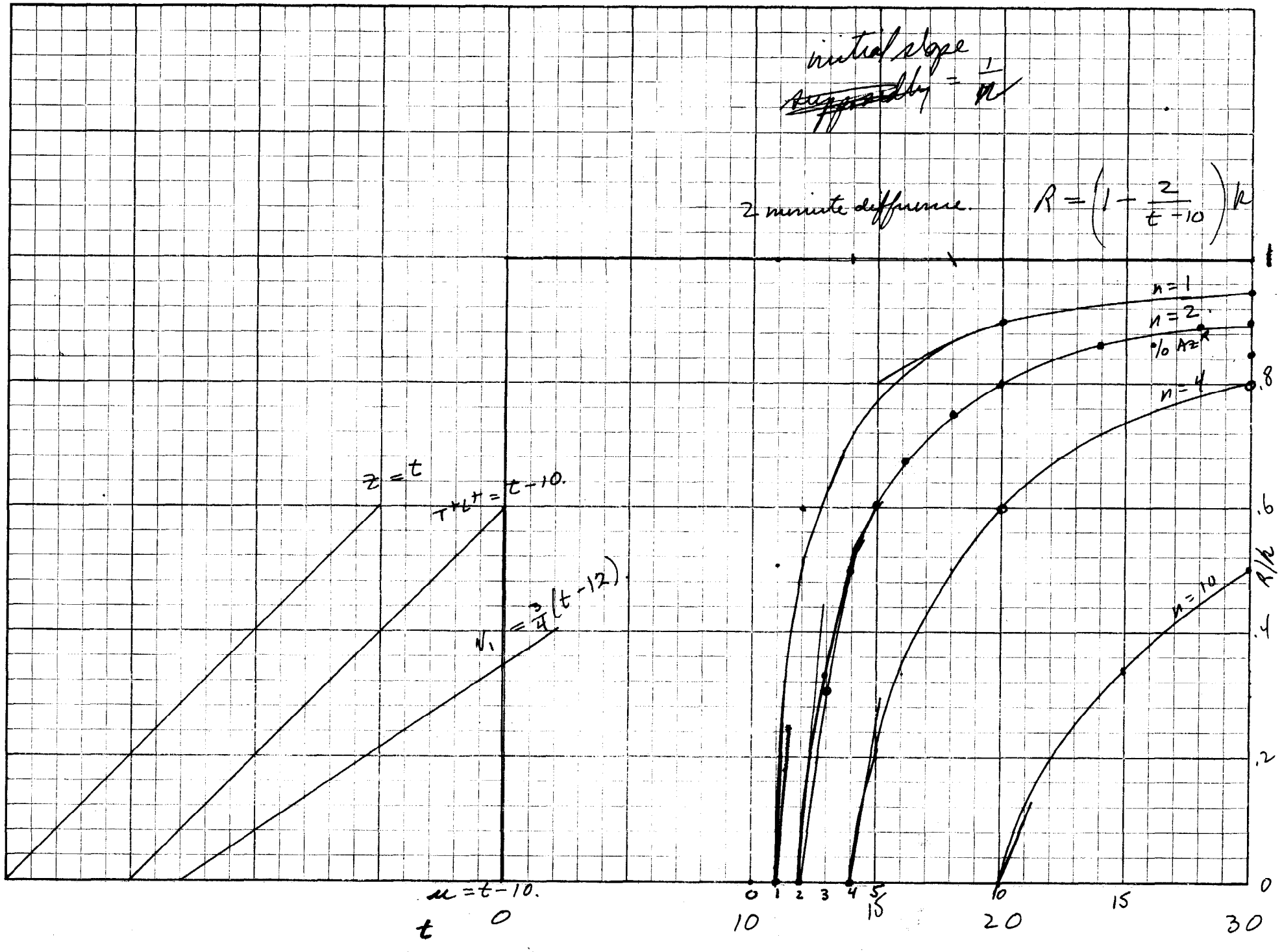
E 1	2,9
E 2	1,0
E 3	1,0
E 4	0,0
E 5	0,0
E 6	0,0
E 7	0,0

	Gal	Lac
A 30	0/7	2/7
A 60	0/5	1/5
A 90	0/9	0/9
A 135	0/6	0/6
B 30	0/13	0/13
B 60	0/10	1/10
B 90	1/10	2/10
B 135	1/25	2/25
C 30	0/23	7/23
C 60	1/20	11/20
C 90	1/10	5/10
C 135	0/7	4/7
D 30	2/50	16/51
D 60	4/50	19/50
D 90	2/50	22/50
D 135	9/50	22/50

Plate counts.

	30	50	90	135
A	7	5	9	6
B	13	10	10	25
C	23	20	10	7
D	65	218	320	520.

Conclusions.: A, B in unphysiological condition, low viability (excess of cells or poisoned culture?). In C, chloramphenicol may have slight bactericidal effect; at any rate does not permit multiplication, as judged from prototroph count. Chromosome entry possibly undisturbed.


initial slope = 1/n
2 minute diffusion.
R = (1 − 2/(t+10)) I∞
n = 1
n = 2
n = 4
n = 10
% Az R
z = t
T + L I = t − 10.
I₁ = ¾ (t − 12).
u = t − 10.
t
0
10
15
20
30
0
1
2
3
4
5
10
15
.8
.6
.4
.2
0
R/I∞

$Z = t \cdot n_a \cdot n_q$

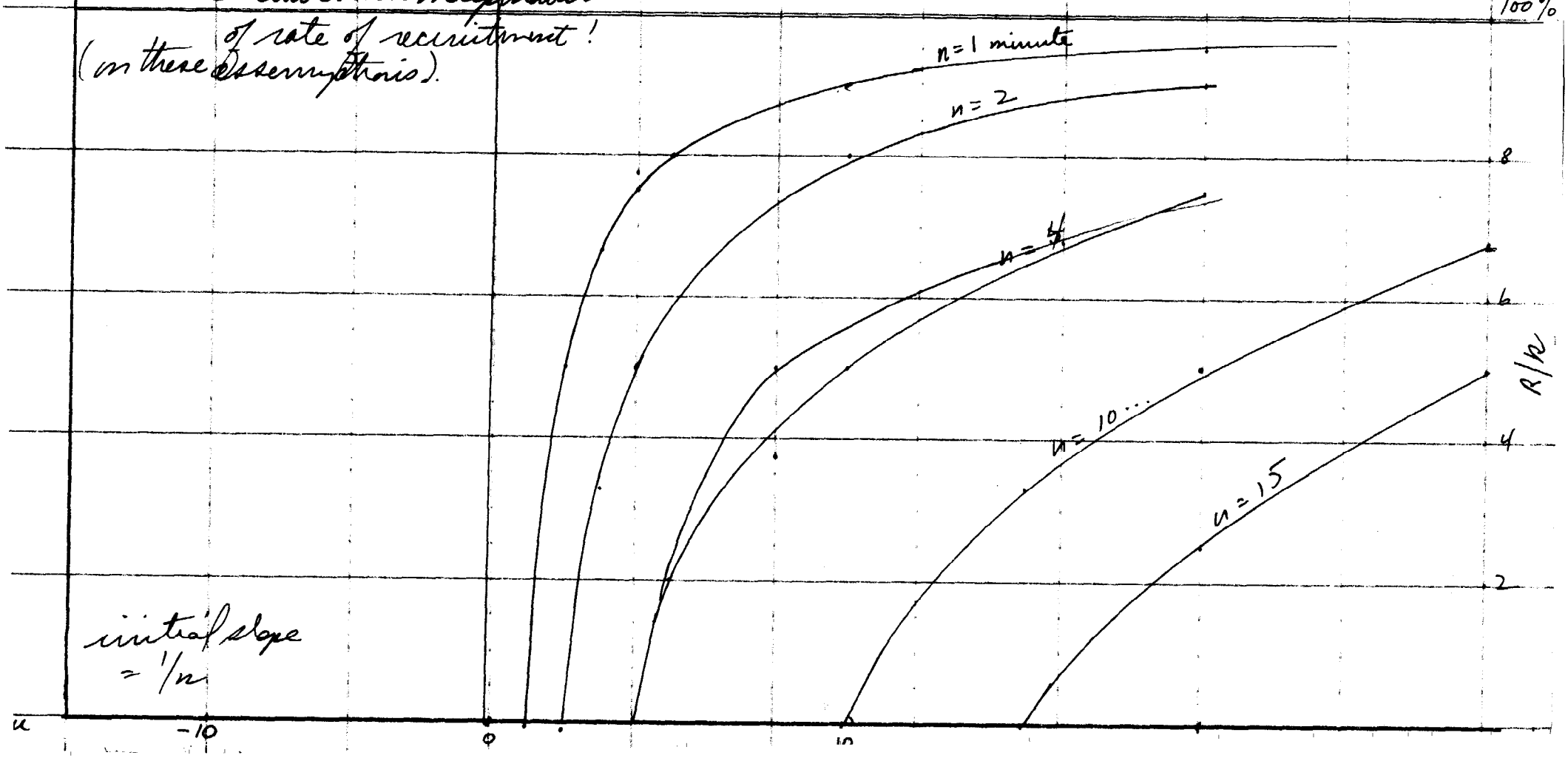
$TL^+ = \frac{t-10}{t}$

$\frac{\text{etc.}}{\frac{Lac^+}{TL^+}} = k\frac{t-10-n}{t-10} = \frac{u-n}{u}$

Note: slopes have to be multiplied by k.

Nota bene: curves are independent of rate of recruitment! (on these assumptions).

initial slope $= 1/n$

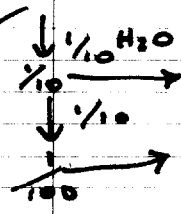


19 June 13, 1958. REF: 1423 B

POURED AGAR AS A MEANS OF DELAYING INTERRUPTION AND AVOIDING PLATE RECOMB.

ORC W3870, W3064, Spun, resusp in chilled water 20x. ♀ Susp. had to be blended because of coarse agglutination.

0': plate recombination. 0.1 ml ♂ + 0.1 ml ♀ + 4 ml broth, in ice bath → .05 plated on DSMB$_1$, surface and poured agar.



incubated in waterbath and* sampled at:

13', 40', 120', 240'.

0.1 ml samples diluted 1/100 in chilled water and a fraction blended. Unblended and blended fractions plated, .05 in DSMB$_1$, on surface and poured.

	Spread	Pour	B spr.	B pour
13	0 0	(14) (11)	1 0	0 0
40	41 49	40, 60	40 49	40 35
120	104		(115)	
240	223		223 (sic)	

Spread equivalent to blend!

	Spread	Complete	
	(196, 212)	~~292~~	44, 64
0 1/10	1, 0	0, 1	
0 1/100	1, 0	0, 0	

∴ pour plating gives some diminution in plate recombinants (possibly convergent) but not breaking up nonspecific agglutinations) but not a great deal. This is not a serious problem with these particular crosses. (of 1/100 dilutions of 13' stk)

Note linear increase of recombinants per ml

Further dilution of Sp. (Blend) Sp. (Blend) Pour Pour

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1423B

19 REF:

	1	2	3	4	5	6	7	8	9	10
	Blended t'	Sr. P	SRP	Cal+	Ratio	Lac+/amongCal+		Lac+/among Cal-		
	13	S	0	—	—					
			01	0/1	—					
	13	P	0	—	—					
			0	—	—					
	40	S	40	2/Σ	.011					
			49	0/Σ						
	40	P	40	0/9	.014	1/1		13/66		
			35	1/67						
	120	S	115	5/Σ	.036	5/5		~~21/94~~		
			81	2/Σ		5/5				
	120	P	90	6/180	.033	6/6		21/94		
			90							
	240	S	223	33/Σ	.137	27/29				
			216	27/Σ						
	240	P	237	16/94	.17	14/16		28/79		
			256							

Conclusions: the 4 treatments (blend or not) · (pour vs. surface) are concordant for SRP and Cal ratio except:

① : SRP, pour, unblended is consistently ≧, esp at 120 minutes.

② The Cal ratio of this class at 13' is exceptionally high

③ at 13' only this class shows a SRP – others presumably interrupted very nearly at "first entry" of Ta++.

④ Plate recombinants show Cal ratio about equal to 120' and << 240'

⑤ Plate recombination is negligible at $\frac{1}{10}$ and $\frac{1}{100}$ dilutions ($\frac{1}{100}$ was expt'l plating dilution)

⑥ Non-visible effect of plating on increasing the Cal ratio, e.g. at 40' or at 0'. ∴ no evidence of prolonged entry

⑦ High (expected) prevalence of Lac+ among Cal+ crossed class of alternate interr

The following were also n.p. tested for Mal, Xyl, Mtl^{-} and were all $^{-}$ $^{-}$ $^{+}$ ...

Psmic:	#tested
0	18
40	1
120	3
120B	5
240	20
240	8
240B	32

These markers are very low (>0?) even with prolonged entry, under conditions of SP selection.

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Comparison of power plating and spreading
? perception of mating ?

1423B

June 13 1958

REF:

	1	2	3	4	5	6	7	8	9	10
	Not Blended				Lac+/among Cal+		Lac+/Cal−			
	t'	Sr.P	SRP	Cal+	Ratio					
1	0	S	196	9/Σ	.055	17/18	26/34			
2			292	18/Σ		12/16				
3	0	P	44;75	0/21	.041					
4			64;137	5/100		4/5	28/95			
5	13	S	0	—	—					
6			1	— 0/1	—					
7	13	P	11	2/25	.08	2/2	5/23			
8			14							
9	40	S	42	1/Σ	.022	1/1	2/2			
10			49	1/Σ		1/1				
11	40	P	40	0/9 1/83	.011	1/1	17/72			
12			60							
13	120	S	107	3/Σ	.032	3/3	6/6			
14			100	4/Σ		3/3				
15	120	P	157	3/95	.032	2/3	17/91			
16			178							
17	240	S	223	32/Σ	.147	23/31	38/51			
18			192	29/Σ		15/20				
19	240	P	266	14/100	.14	13/14	18/94			
20			227							

unclustered = plate records.

Σ = replica test of surface plates to B Cal B, Sm. Others individually streaked to B Cal. From these, only Cal+ tested for Lac. Replica test better for Lac.

For further study:

reality of: (1) High Cal compared to 13' pour plate compared to 0'
(2) continued increase of Cal+ (reduction?)
(3) true function of Lac ratio among Cal+. 0S and 240S both have
(4) other markers, especially 0 and 240. a rather lower Lac ratio than, say, 240P.

SO 14/17

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14238

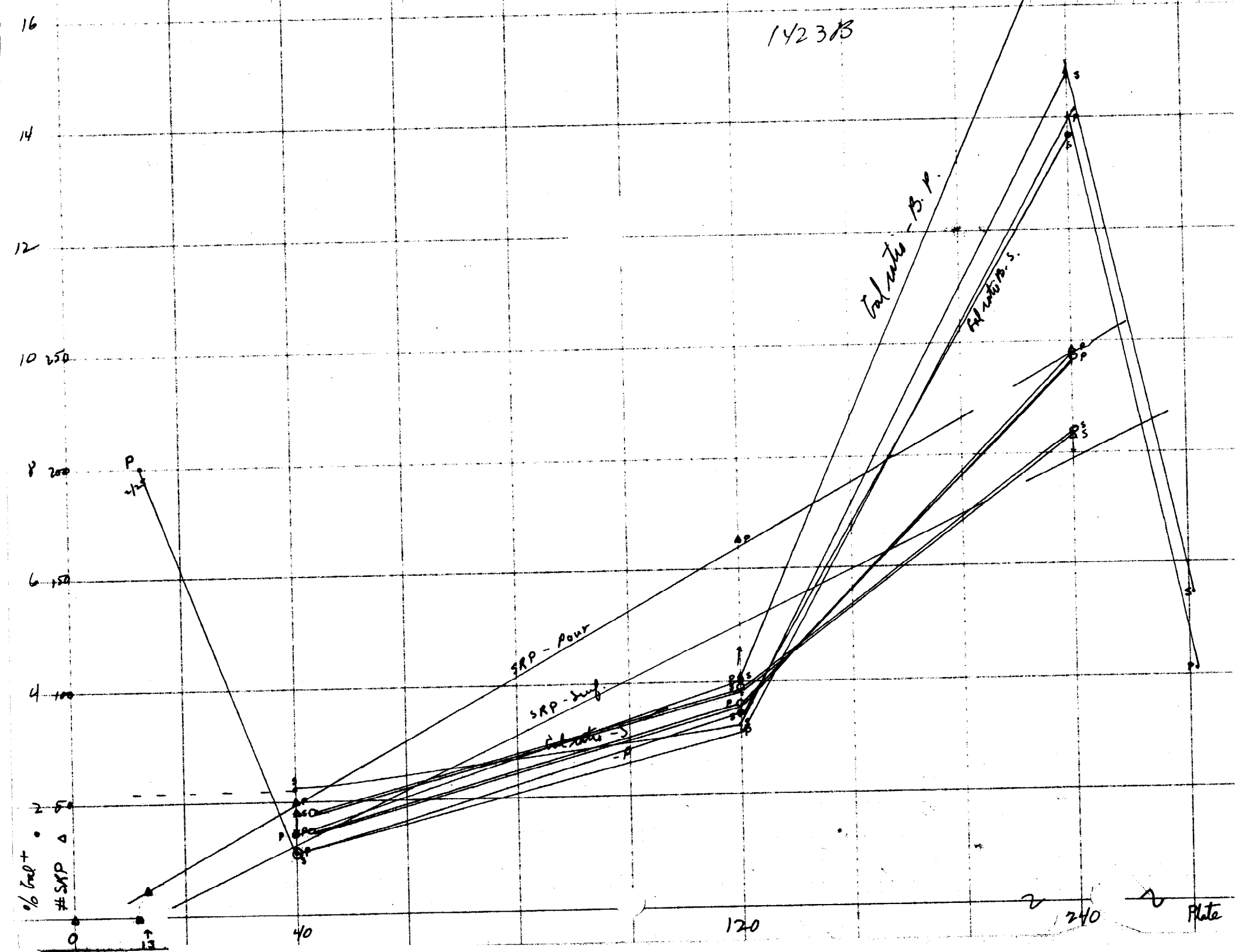
19

REF:

	1	2	3	4	5	6	7	8	9	10
	lac+ nitro among Cal+.						Cal−			
OR1 P3	1/2 3/3	} 4/5	✓				15/48 13/49	} 28/95 + 1 Cells lac±		
40	1/1 0	1/1	✓				14/~~49~~ 3/2 3	17/72	✓	
40B	1/1 0	1/1	✓				9/49 4/17	} 13/66	✓	
12	2/3 0	2/3					6/41 11/50	} 17/91		
120B	3/3 3/3	(sic) 1 mw+ 6/6	✓				10/47 11/47	} 21/94	✓	
240	8/8 5/6	} 13/14					10/50 18/44	} 18/94		
240B	5/8 9/9	14/17	✓				15/39 13/40	} 28/79	✓	

P.

S.


14238
16
14
12
10
8
6
4
2
0
250
200
150
100
50
% Engl
#SRP
40
120
240
Plate
SRP - pour
SRP - surf
Calculate - B.P.
Calculate - S.
-S
-A

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Screen Hfrs.

1424A

June 20 1958

REF:

	1	2	3	4	5	6	7	8	9	10
	available strains:			F⁻ = ~~W3044~~ W3991 × Hfr ×.				(all are H⁻ S^s)		
1	Hfr	W		0'	10'	20'	30'			
2	2	3870		+±	±	±	+			
3	8	3889		±	0	+	0			
5	13	3200		±	0	0	±			
6	15	3885		±	1	0	2			
7	16	3886		± +	++	+	++			
8	18	3887		0	± (many back)	0	++			
2	7	3888		±	++	3	4			
4	11	3890		1	+±	0	0			
0										

parents ORC prepassage, 20× shell of .1 + .1 + 4 ml. dispense 1 ml to each of 4 tubes. Time by transfer 0° → 37° → 0° (1 hr at each temp.: 0', 10', 20', 30'. 0' is undiluted (plate use.) 10-20-30 dilute 1:100 and plate .05 ml on H Cal B, 5mm (as on hand).

\+ > 10 ++ ~ 100 Recombinants as rather small papillae in most cases.

#11,0 #16 and #7 appear most promising on this screening: note that both show a considerable decrease after 10 minutes. This might be due to the segregation of H⁻ in absence of methionine. Spreading and elimination of late recombinants may have been important in this crude screening trial.

Purpose: for mapping Cal it would be best to have an Hfr that shows early entry of Cal. W3991 gives very weak Cal⁺. Try W3119

Note - Cattus says W2945 (Hfr 6) is high on all Cal × except Cal 3 !

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Recheck timing of Hfrs.
× W3119

1424B

26 Jan 19 58 REF: A.

1	2	3	4	5	6	7	8	9	10

.1 + .1 + 5 ml

OZC, chilled, 20× ~~.05 + .0~~ ml ~~[illegible]~~ ml penassay. Time by diluting into cold water .1 ml/10. Plate .05 ml on MCal B, sur...

0, 5, 10, 20 minutes. Count Cal+

	0	5	10	15			00
A W3945 = Hfr 6	~30	1	6	13			~10⁴
B W3886	0	1	0	0			10²
C 3888	0	0	8 (BCal)	0			15
D 3890	0	0	6 (BCal)	0			26.

Note high "0" for W2945.

This is not a very satisfactory experiment, as it does not indicate any particular timing for the Cal's and the Lysids are very low. The high "0" may be real — possible significance? —; Cal-S linkage perhaps. A and B should be repeated to longer times.

Inconclusive but Hfr 6 and W3886 should be studied further.

Cross Bernstein's on single colony isolates. All colonies homogeneous. 20 hrs 24 hours, W3945 gave very high response! (This is Alan's Hfr 6 and is Lymphotrop Cal) (DT is roughly complementary to Hfr 3).

	W3119 MCal	W3064 DmB
A	++++ (sic)	—
B	++	++
C	+	±
D	+	±

3945 3890 3888 3886

to F+

No evidence of reversion among any of these (20 tests each)

3886

Main purpose: to validate a better Hfr timing system for Cal markers Hfr 2 + is rather late for Cal.

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Hfr 4 (pulsed 10 m.)

1424C

June 27 1958

REF:

	1	2	3	4	5	6	7	8	9	10
1	W3752 = Hfr 4 X+ S5		x	W2961						
2										
3	ORC M1 x .1 : .1 : 2ml D(0);			~~.5ml to 10ml test tubes~~			t' = 0, 10, 22 ...			
4										
5	at t = 10 minutes, add 10ml warm D(0) and plate samples from time to time									
6										
7	P = predilutes (choices on the pulse). (dilute after ~10 seconds, mild agitation).									
8										
9										
0		SRP DsmB1	MValsmB	Gal	Mal all +	Xyl				
00		~ 200	0			?				
2										
10		0, 0								
22		1, 0								
30		0, 0								
35		0, 0								
40		2, 1	0							
50		6, 5	0							
60		24,	0	3		1				
75		35, 46	0	~8, 4		1, 2				
	P10	4								
	P60	1, 1	0							
	P75	2	0	½ - ⅔						
	24 C					0				
5										
6	P29. Save for tests of other markers: B1 ; Mal ; Xyl ; Lac ; ara; Gal assumed 0!									
7										
8										
9										
0										
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										

? Does Hfr go to Gal end? Any markers very late? TL entry is at about 60 minutes! (Could test chloramphenicol hive smigby by SRP count if desired) Gal ratio at this time must be very low, or 0.

Note: use of D(0) may have reduced yields.

notes send ✓ Phrases Thermofax 1-2. June 30 1958

1401-2 Best pulse for T, L, V, Lac. Good test of interruptors.

-3 P^+ selection; also delay in T, L. ?Different F^- different patterns. These were one-hour cultures! Cannot be ascribed to non-freshness. [discussion of Ag^+ in temperature equilibrium and initiation of male fertility

-4 Compared 3052 v. 3064 as F^-: no difference in time. Separately and a differential (ara, $^-$) marker test

-5 Hfr_1 v. Hfr_2 x W3052. Preliminary. P-I-T comparisons. But the 15' time for Hfr_1 was irregular + rejected. t(T)=5' t(L)=7' t(P)=12-13'. Leucine-sensitivity?

-6 Repetition.

1402- Pulsing

1. Various concentrations of cells. Reducing "0" is predilution. Unselected markers dilution and incubation does not interrupt at 1:100 dilution, mating was only 10^{-1} less. No emphasis with product.

2. Try to concentrate above "standard". Some increase noted but not product. 10x conc. → 4x recombinants.

5/14 3. Pulse in various media (various cultures). Buffers, penassay, predilution controls .... (many experiments were not properly controlled.) Hfr_1: very few recombinants – expt. n.g. Pulsing gave lower yields. Overcast conditions are inhibitory. Cells were conc'd into penassay and resp'd. Too high conc. (?) makes this a poor test of optimum medium.

5/21 4. Repeat, keeping cells cold. Compared penassay BBA (buffer, glucose, asparagine) and ~~Hfr~~ etc. No great difference. But too many recombinants were observed even at 10^{-4} dilution for accurate pulsing. Clarification of ABCDE on record. No difference possible for BBA. penassay failure at 10^{-4}!

1402-5 Attempted repetition of pulse for step of Tc^+. But 5x increase of X^+ 20'-60'! Was under misconception of pulse in 140(2-F1.) 140(2-F1 may have been pulsed by saturation.

5/29. -6. Trial improvement by using BFA for entry after necessary mating. No evident effect on step 20'-40'.

1403- 1 Manipulations. $Hfr \rightarrow F^+$ N.C.

2 See protocol

3 How to plate s/ interruptions. See also 1423B

4 Do strains vary in stability to plating? See 1410-1. ~~Thanks~~

1404 F^+. Hfr. F^- Test for cross infection of F^- progeny on plates. So far mechanisms a/c too low for stability of F^+ and poor design of F testing.

1405 Protoplasts ♂ and ♀

1. Interruption by lysis of ♂ protoplasts. Preliminary
2. 4 combinations of (♂ ♀)(P. B). $Hfr \rightarrow F^+$ n.g.

4.14. 3 Repeat 1. Best experiment! Do protoplasts show delay in Cal (and Lac) or spontaneous interruptions and lysis before Cal? Cal remained 0 to 60' See 1405-6

4/11 4. protoplast × protoplast. Based on auxotrophy of W3060. An interesting result: crosses on sucrose B, gave very high male medians (poor conditions of selection). Worry again about nutrition of W3060.

1405-5. Protoplast crosses. Repeat 1405-4. High residual viability. Still selecting on sucrose B$_1$. No conclusions

4.19.58 -6 Repeat -3. Effect of incubating shocked suspensions. No effect on enlarged scale. Good for interruption by lysis

-7 Osmotic shocks on rods. No interruptions of rods e.g. (rods only).

-8 repeat -4

1406. +/B1 Conditions of mating: age of cells.

4.16. No difference between fresh and old (3 yield; not obvious for timing either.

1407 EML Transfer of Hp$^+$

1408 -1 EML JL. Cal timing. -1 washout.

-2 Showed linkage of try - gal. (because know of Hfr - try linkage).

3 Various Hfr's — intensity of linkage of Cal - try.

4 Cal timing. Hfr$_2$ Preliminary only.

5 Hfr$_2$. Close timing. Discarded a/c contamination

6 Hfr$_{4,13}$. Preliminary. Try enters at 15'

7 Hfr$_{13}$ More accurate. " " " 13'. Exponential kinetics

8 Hfr$_2$ Try - Cal mapping. ? Two modes of entry.

9 Early rise, later more rapid.

10 Hfr$_2$ Cal$_2$. See disc. of two modes of entry.

11 Hfr$_1$: Cal timing. Very late entry.

12. Repeat: no effect

1408-13 Hfr 2 – compare two Cals. Low numbers: F+ rev. Cal 2 < Cal 1.

..... Current Caltimings in EML hands

-20 No evidence of early slow rise in titre. Probably was due to growth of plate recomb.

1409-1 Enzymes on protoplasts.

-2 " " mating. (?? slight effect of lysozyme).

1410 – Diploids send interpretative summary when ready.

1411 – Colchicine no effect.

1412 – Freezing. Parents OK.
Need to summarize and compare B1-B2.

1413-1 Reblending — differential F⁻
See record. Plate Rec. probably occt. for the protoplasts seen.
But unselected markers showed interruption.

-2 Two stage transfer.

-3 Exhaust males by excess F⁻? (to use them for 2' stage transfer to a new batch.) Required 20' to exhaust, which is too long to use most convenient available markers

-4 Increase recruitment s/entry. Temperature. 37° optimal. zero rate at 0°.

-5 } Crosses were altogether – N.C. W3060 → F+.

-6 } N.C. " " "

-7 Check selection for Lac+ S^R; Gal+ S^R on W2323 Blackson. Rather few!

-8. Test for suppression. (Reconstruction).

-9 Repeat -1. No reblend recombinants.

-10 Repeat 2. Two stage transfer. Negative.

1415 – Need to evaluate notes Azide

General conclusion: no differential effect.

No definite verification of reversible inhibition.

–3 concentration too low, no effect. Azide .2% in Difco gelatole

–4 necessary conc. to inhibit. Some effect at 10^{-4} – 10^{-3}. Still no prevention of F^+ entry. Cal excluded. (slow action?)

–5 Azide + DNP – 3% inhibits mating. DNP – M/200. maybe reversible (no evidence new recruitment).

–6 R x S; S x R. Inconclusive. Graded effect. Counts too high in controls

–7 Differential concentration lost on F^-. Stratified fertility of crosses!

– Nothing mean Azide-resistance!

DNP^R — See record. – low degree of resistance. .2–.5 ml/2 cm loop of M/20 DNP see 1419.

1416 – DNA leakage. – some leakage in every case.

240–260 mμ difference.

1417 – Timing of Hfr_1. TL: ~20'

–2 TL ~<20' Th very late ~ 60' Cf 1401–5. Thr 35–40'. In Progress

–3 (JZ) – Time entry of Mal, Xyl...; effect of chloramphenicol

1418 – DNA → F^+ mating with F^- prototrophs.

1419 – DNP-resistance

1420 – Pulsation.

–B – periodate "pulse" } e.g. $Hfr \rightarrow F^+$

–C " " }

–D. Effect of F^- cells. (High density!) Apparent interruptions

–E " " (lower density). Confused?? Cells does not interrupt, pipetting control does!

- E. Cal counted on Stalson B's. Good internal consistency - Luca repeat!

1421 - Periodate I.S.

C - will be finished by I.S. Collate protocols
- treatment of F^+; and effect on F infection etc. - look to plates

D. - Other oxidants. Rather low yields

E. - RDE. No Ca^{++} used see 1422
RDE may still be open question. Not yet used Eric Frank's.

F. -

1422. RDE + Ca^{++} No yields: inconclusive

1423 Delay interruption c/ chloramphenicol.
A. 50r/ml. Maybe bactericidal; no prolifération. No evidence of any effect on Cal and Lac entry. Part II OK.
B. Over plating to delay interruption. No interruptions; no prolongation

1424

Yudkin's E. coli: to make protoplasts L2, double strength; 300 - 1000 u/ml pc.
Burst more readily. (to prepare DNA from W3514 S^R).
→ W___.

Experiments Still Pending July 1958.

See 1410 deploids for bulk of experiments of May - June 1958. Then 1426 1427.

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Analysis of Gal$^+$ × Gal$^-$ diploid

Relationship of two modes of segregation

Segregation of 1410F48.

[1410C] [1426 ←]

1 July 1958

REF: 1410C1T(1-7)

7 Gal$^+$ heterogenotes have been isolated from 1410C1. Label these, 1426 A-G. Each derives from an Ara^v colony picked to D(Ara B,) and retrospectively verified as Gal$^+$. From the same streaks, Gal$^-$ were picked to EMB ara to verify (qualitatively) independence of Gal and Ara segregation. Results: (of Gal$^-$ tested)

B 5/5 ara$^-$ . . Ara^v Gal$^{t\,v}$ → Ara^v Gal^{t-}

C 4/6 → Ara$^-$ Gal^{t-}

D 3/6

E 1/3

F 5/9.

Ara$^-$ Gal$^{t\,v}$ presumed from consequences of earlier streaking.

The new labels (1426 (A-G)) now refer to the D(ara) broths mentioned above. all are still Gal$^+$ Ara^v

1 July: plate out A and B on Bara.

2 July: Streak out individual Ara^v on Bara for examination of single, discrete segments.

Pick 4 groups of 4 ara$^-$ from each ara^v streak out → Bgal. (see next page (1 July))

also from first plating, ara$^-$ pick to Bgal and Blac.

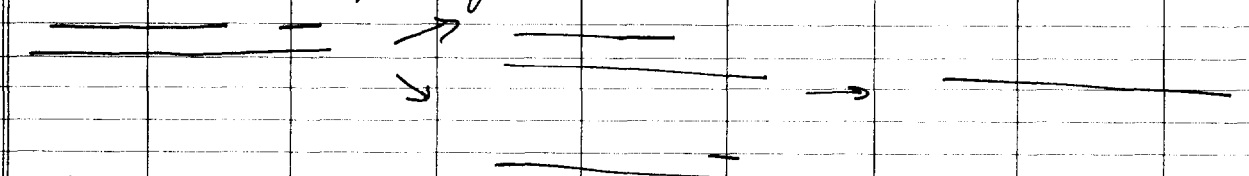
	G	L		G	L		G	L
A 1	+	−	B	−	−	8 B	+	−
2	+	−		+	+	9	−	−
3	+	−		+	−	10	−	−
4	+	−		+	v	11	−	−
5	+	v → lac^v Ara^v		+	−	12	−	−
6	+	−		+	−	13	+	v → lac$^+$ ara$^-$
7	+	v → lac$^-$ ara^v		+	−	14	+	v → lac^v Ara^v

? - partials for purity.

Three most Ara$^-$ lines are lac$^-$

Ara^v are these lac^v rarely ara$^-$? On streak out, 1 was ara$^-$ lac$^+$ 3 were ara^v lac^v and had been simply misjudged as ara$^-$. There may be some position effect as some Ara^v have microscopic central spots.

Conclusions (two pp.) Segregation of heterozygote + segregate occur independently, as if



Data are not sufficient to establish statistical independence. Among Ara$^-$ incidence of Gal$^-$ was 3/144 and 12/184 respectively in segregations of single Ara^v colonies. No data for the Ara^v pairs in same colonies. Much higher incidence of Gal$^-$ Ara^v in several both segregations (2/13; 13/32). Defer this problem to a more suitable marked diploid + subtract a Gal$^-$ hemi 2340 etc!

analysis of a heterogenotic heterozygote:

$\frac{\text{ara}^{TLP}}{\text{Gal}_2\ S}$ / $\frac{+}{+}$

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1426.

July 4 1958. REF:

Analysis of A and B. Is there linkage of the Gal factors to lac or ara?

A. 13 Ara^V / B. 32 Ara^V Stock cultures were plated on B ara. Single ara^V were streaked out on B Ara, and 4 ara^- isolates picked from each streak out to B Gal, together with mass bunch.

A: 2/13 ara^V were pure Gal^- (already segregated). Of the other 11, there was one $3^+:1^-$ and one $2^+:2^-$ split among the 4-sets of ara^-. ∴ Total incidence of Gal^- among Ara^- (recently derived from Ara^V) was 3/44.

B. 11/32 ara^V were pure Gal^-. Where 4-set derived from $ara^V Gal^+$ (21 sets), the following splits:

$4^+ 0^-$	$3^+ 1^-$	$2^+ 2^-$	$1^+ 3^-$	$0^+ 4^-$
10	10	1	0	0

and total incidence of $^-$ here is 12/84. This suggests that B is somewhat less stable a heterogenote than A (or that A gives significant Gal^+ segregants) but needs this may be selection. It is evident that $Ara^V Gal^V$ $\overset{V-}{\nearrow}\searrow$ $\searrow\nearrow$ $\overset{-+}{\longrightarrow}$ — at least qualitatively, independently. Replica plate to lac to determine if there is any linkage of Gal^+ to lac (viz. interaction in segregation when both have segregated. This tells little about linkage of the exogenote to Gal since all viable cells presumably have the same Gal factor.

Replica plate to EMB Lac. A

Gal^-: 24-sets pure Gal^- are pure lac^-; $Gal^- ara^V$ is $lac^\pm$

Each Gal^-: 3 all lac^- $Gal^+ ara^-$: All but 3 are also lac^-. These 3 are $(Ara^- Lac^+ Gal^+)$ two from a 2:2 split; 1 from a 1:3. ∴ The A-sets are

2 $Ara^V Gal^- Lac^\pm$: 4 sets $Ara^- Gal^- Lac^-$

1 $Ara^V Gal^V Lac^V$: 3 $Ara^- Gal^+ Lac^-$: 1 $Ara^- Gal^+ Lac^+$

1 " : 2 $Ara^- Gal^+ Lac^-$: 2 $Ara^- Gal^+ Lac^+$

7 $Ara^V Gal^V Lac^V$: 4 $Ara^- Gal^+ Lac^-$: 0 ——

(over)

B sets: all but 1 4-set are pure lac⁻ This one has following elements
(Gal⁺ara⁺lac⁺); 1 ara⁻gal⁻lac⁻; 1 ara⁻gal⁺lac⁻; 2 ara⁻gal⁺lac⁺
see below. one of these is ara⁺!

∴ again almost all ara⁻ are lac⁻.

These diploids are not optimal to study segregation in any more detail; wait for development of more suitable markers (V_1; V_6; AZ) But it might be worthwhile saving a set of sibling lac⁺ and lac⁻, ara⁻ for typing for ara. Spot these on B ara.

∴ lac ratio among ara⁻ (among all abscissates from ara⁺ colonies)

is	corrected (see below)
A) 3⁺/41⁻	~~2⁺/44⁻~~ 2⁺/41⁻
B) 2⁺/128⁻	1⁺/126⁻

→ But on recheck, The 4-set from A was ara⁺lac⁺: ara⁻lac⁺ (1); 2 ara⁻lac⁻ (2, 3)
B: ara⁻lac⁺ (1); ara⁺lac⁺ (2); ara⁻lac⁻gal⁺ (3); ara⁻lac⁻gal⁻

See 1410K for typing:
The two lac⁺ are ara_3^-
The four lac⁻ are ara_2^-.
This further confirms the structure of this diploid $\frac{ara_2^- lac^-}{ara_3^- lac^+}$.
no special bearing on Gal.

1410 X
2 July 58

Plans for further diploids:

1. additional markers should be segregating: V_1, V_6 and perhaps Az. Possibly leave P free to segregate? These will help rapid screening of heterozygosity.
2. Include possibility of selecting for Gal^V? (Introduce Gal^- into a parent).
3. Time more precisely: perhaps pulse. Measure bac and Gal concurrently
4. Select some Hfr diploids? (Maynard B, $^+$ isolate!) — should cross to get me!

5. Set up for Ara ristin preliminaries
6. Effect of streptomycin in disturbing appearance of Gal ek.?

Plans for present diploids:

11 ~~#~~. Reexamine 2 stated pure bac^+, say F26 and F31.
Reexamine the 2 $Gal^+ bac^V$ F5, F12 | G10, 3, 13
Examine zygosity of Gal, then bac in C2, F21, F22, F23, 27, 28, 30; G1, 2, 8, 21, 24 * *
Defer linkage + segregation studies. Complete F37, 38. Trace F30

12. Get an $Ara^- bac^V Gal^-$ autoinict to use as F^- for secondary nondisjunction: Or use $Ara^V bac^-$, selecting bac^+? This is messy with Gal^-, and bac not balanced. In any case, polarity of bac^+ would encourage formation of bac^+ prototrophs.

13. Other Ara tests? What to save from 1410H?

14 In C, examine for bp^h.

Also H plans.

* to D (and B,)!

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Segregation of 1410F48

14/26 C

July 11 1958. REF:

1	2	3	4	5	6	7	8	9	10

F48 is an unusual dyploid insofar as it rarely segregates, and hardly ever ara^+. Pick 1 ara^- from streaks of individual Ara^{++} colonies and purify, $\frac{1}{2}$ colonies to broth, cross back (XB) with Hfr_2 ara testers on Mara B, as follows:

$\frac{1}{2}$ — 2 — with $\{(1 \text{ or } 2) + (3 \text{ or } 4)\}$

	W4068 ara_3	W4069 ara_4 [$=2$]	Bara	Diagnosis
1	+	—		2
2	—	++		3
3	—	++		3
4	—	++		3
5	+	—		2
6	—	++		3
7	+	—		2
8	+	—		2
9	+	—		2
10	+	—		2
	+	—		2
	+	—		2
	—	+		3
	—	++		3
	—	++		3
	—	—		? 2,3
	+	—		2
17	+	—		2
18	+	—		2
19	—	++		3
20	+	—		2
	+	—		2
21	—	++		3
22	—	++		3
23	—	+		3
24	—	++		3
25	—	++		3
26	—	++		3
27	+	—		2
28	+	—		2
29	+	—		2
30	—	++		3
31	±	++	(±)	— + 3,(+)
32	+	—		2
33	+	—		2
34	+	—		2
35	+	—		2

Testers:

	4018	4069
2979	++	+
W4176 (K3)	—	++
4177 (K4)	++	—
4178 (K5)	—	—
mermaid text	—	++ 3
diaomi man $_A$ +++	++	
	+++	+++

Score: W4069 serves pro tem as tester for ara_2 pending isolation of a better one.

2^- :	24
3^- :	18
possible 2^-3^- :	1

The ara^+ are mic - picks.

The 2^-3^- should be confirmed.

Save a 2^- 1426 c2

3^- 1426 C3

putative 2^-3^- 1426 C1

~~Original~~ Repurify.

Also 2 gave ara^+ rep. (not this series but noting in these isolations.)

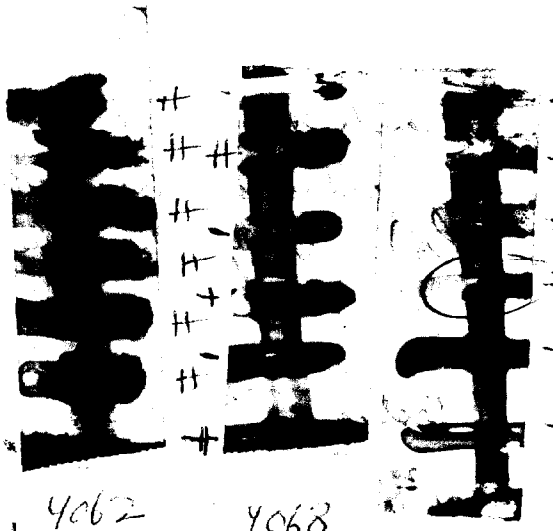
~~The so called~~ C1 might be infertile or F^+, etc., and should be checked, say, with ara^+ Hfr.

Needed for automation of bacterial techniques: a nutrient tape. (Cf. Reynis's ideas). This would be a marked advance over the copying to tape illustrated on this page.

July 15, 1958.

Retubed on C1-2-3

(W4163 only now available as Hfr, Ara2

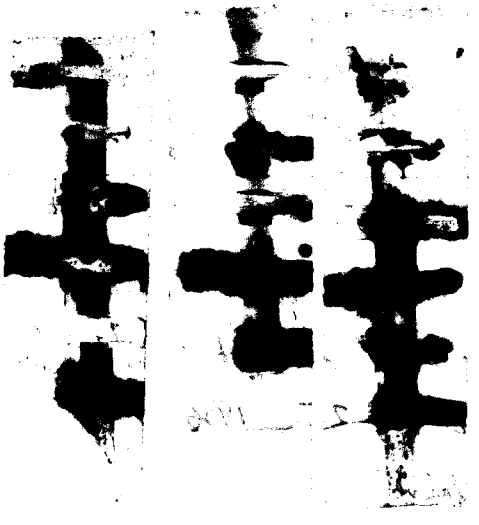


			- C1	Ara2, Ara3 ← confirmed.
			- C2	Ara2
			+ C3	Ara3
			= 2979	Ara2
			+ 4176	Ara3
			- 4177	Ara4
			~~10H10A~~	~~mix~~
4062	4068	4069		
Ara5	Ara3	Ara4		
Hfr1	Hfr2	Hfr2		

do + with a W4062

Note 2979 does have higher rate of + than W4177!

7/19:



	W4274	
1426C1	∴ Ara2⁻ Ara3⁻	(no recomb. seen here with Ara4 but v.s. ✱
1426C2	Ara2	same as C2, C3
1426C3	Ara3	
10H10A✱	Ara1 (rather reversible)	
W2979	Ara2 ✓	
W4176	Ara3 ✓	

W4163	W4068	W4069
Hfr1, Ara2	Ara3	Ara4
	Hfr2	

JULY 20, 1958. terminal notes for resumption of work in the fall

It is now clear that Hfr_2 crosses tend to give both Lac^v and Lac^- hemizygotes. It is probably not profitable to attempt to categorize more of the Lac^- from the 1410 series; it may be worthwhile doing a time sequence experiment for the production of diploids, though it is reasonably clear that the occurrence of Lac^+ is related to the production of Lac^v, while the Lac^- are hemizygous, in supp ort of the progressive entry hypothesis. But the nonoccurrence of Gal^v (compared to Gal^+ is enigmatic, and probably some more Gal^v or $^+$ should be looked for

However, in orddr to distinguish Gal^+ hemizgous from homozygous, it will be necessayy to use complementary Gal mutants, allowing either Gal^1 or Gal^0 to be analysed by reversion. This will also afford the opportunity of using Gal/Gal selection for Gal heterozygosity.

Therefore the main programs are:.

1. Time sequence on heterozygote isolations, to complete that picture. Include segregation of Az, V1 and V6 as now available.
2. Gal x Gal selections of diploids a) are there any Galv; b)Ara2,4 pos.eff.

3. Complementary to 2: crosses of diploid x haploid

4. Nay be worthwhile to look for automictic derivatives of Lac^v diploids as a Lac^- homozygotes would probably be a preferable ^{0}.

5. Time exogenote entry in crosses of Hfr heterogenotes.

Probably first item will be the review the accumulated Gal^- stocks for identity and complementarity to Gal_2.

other sugars for diploid selection: Rel?

Retesting of Cavalli Ara⁻ strains
With F⁻ Ara's x Hfr Ara's as Controls

1/28

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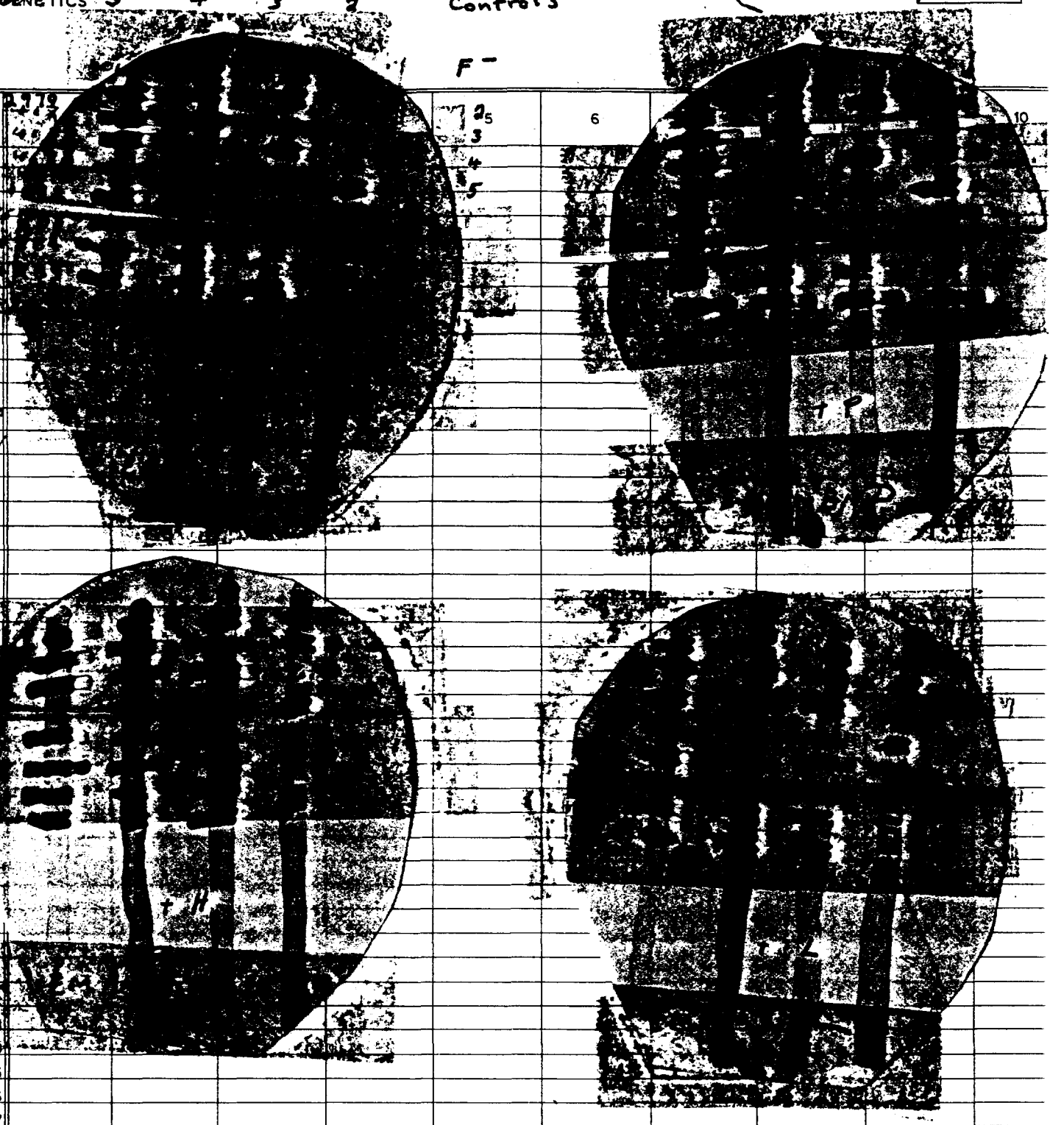
9/1/58

Hfr 5 4 3 2

F⁻



Medium = E M Ara B1 c/s indicated supplements.

Histidine affects Hfr transfer into W 4283.

Others are unaffected.

SUMMARY: (A) In these tests, ara⁻ W4178 as F⁻ gave no ara⁺ with either Hfr's tested. c/f 1410 H - where only ± 4 ara⁺/10⁸ were seen. Should have a control for ara⁺ segregation. Need a genotype ara⁺ Hfr.

(B) W4284-5 are distinct from all others; relatively "close" with W4163, perhaps c/c this is Hfr1.

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See EML notes and JStC work book.

E 50

♂ = W4358 ⇒ 4362

Dec. 1 1958

♀ = W4265

REF:

	1	2	3	6	7	8	9	10
	Below = Ara V's from streaks from original ⊗ plates (from EM-Ara-B1)							
		2 day	2 day	1 day	2 day	1 day	2 day	
		Ara	Lac	Gal		Xyl		
♂ W4358	1 - 5	V	- (or weak V?)	— —		— —		
	58 - 7	V	- and V (weak)	— —		— —		
	- 10	V	V and - (weak)	— —		— —		
	← 14	V	V and - probably V from pap	+ +		— —		
	- 15	V	V and -	— —	2 colony types	— —	2 colony types	
	- 17	V	-, highly pap * possibly noV from pap	+ +		—		
	- 18	V	- sl. pap after 4 days	+ +		— —		
	- 20	V	V? (weak?)	— —		— —		
	2 - 2	V	V and -	— —		— —		
♂ W4359	7	+	-					
	9	V	-	— —		— —		
	11	V (segregates poorly)	V and - (weak)	— —		— —		
	12	V	- (and weak V?) possibly V from pap	— —		— —		
	16	V	-	— —		— —		
segregate but no V col.	← 24	V ⊗;	highly pap possibly noV from pap	+ +		— —		
	← 28	V ⊗	-	— —		— —		
	34	V	highly pap * possibly no V from pap	+ +		— —		
	38	V	- (and V?) pro	— —		— —		
♂ W4360	3 - 5	V	-, sl pap (or V?)	— —		— —		
	10	V ⊗	- or weak	— —		—		
segregate with two V colonies	11	V	V and - V from pap	— (also 2 tiny col) — (large col segregating some thing)		—		
	14	V	-	— —		—		
	16	V	V and - V from pap	+ +		—		
	← 18	V ⊗	-	— —		—		
	22	—	-					
	23	V	V and - possibly V from pap	— 2 colony types		—		

test all #5's

X HFV tester

Get

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E 50 cont.

Dec. 1 19 58 REF:

	1	2	3	4	5	6	7	8	9	10
		2 day Ara	2 day Lac			1 day Gal		1 day Xyl		
1	4- 1	V	-, highly pap *		prob Gal+ No V from pap	+	+	-	-	
2	3	V	V and - (weak)		probably V from pap	-	-	-	- (*)	= "+"s from #'s possibly not V but simply mixed → restreaked and proved full +.
3	6	V	V and		prob Gal +	+	+	-	-	
4	12	V	-, highly pap *		probably V in pap	+	+	-	-	
5	13	V	-			-	-	-	-	
6	14	V	-			-	-	-	-	
7	17	V	- and V (weak)		V from pap	-	-	-	-	
8	19	V	- and V (weak)		probably V from pap	-	-	-	-	
9	22	-	- (V?)		too young	///				
10	23	V	-			-	-	-	-	
	30	V	- (and V?)		possibly V from pap	-	-	-	-	
1	34	V	- and V (weak)		V from pap.	-	-	-	-	
2	35	V (*)	-			-	-	-	-	
3	36	V	- and V? (weak)		probably V from pap	-	-	-	-	
4	41	V	-		V from pap.	-	-	-	-	
5	5- 5	V	-		sl. pap. after 4 days	+	+	-	-	
6	11	V	-, sl pap *		?	-	-	-	-	
7	13	V	-			-	-	-	-	
8	14	V	-			-	-	-	-	
9	15	V	-, sl pap *		too young	-	-	-	-	
10	20	V	-		sl. pap. after 4 days	+	+	-	-	
	28	V	-		too young	-	-	-	-	
1	27	V	-, some pap *		= Lac V	-	-	-	-	
2	10	+	-			-	-	-	-	
3	16	V	-		sl. pap	-	-	-	-	
4	34	V	-		(see circled col.)	-	-	-	-	

* - Ara + "is weak"

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E 50

19 REF:

	1	2	3	4	5	6	7	8	9	10
1	More # 5's									
2										
3		Gra	Lac	2 days			Gal		Xyl	
4										
5	5-52	+	−	sl. papp.			−	~~white~~	−	−
6										−
7	53	−	−	1 Rev.			+		−	
8										dim
9	see ~~57~~ 57	V	− 2 col types		hard weak ↓ papp		− 2 col. types		− 2 col. types	
0	50R									
	60	V	−	−			−		−	−
1										
2	61	V V	−	−			−	−		
3										
4	72	− −	−	−			−	−		
5										
6	77	+ +	−	−			+	+		
7										
8										
9										
0					Φ.					
1										
2										
3										
4										
5										
6										
7										
8										
9										
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6										
7										
8										
9										
0										
1										
2										
3										
4										
5										
6										
7										
8										
9	50C = straight Lac's from pap. streaks on Lac + Ara									
0										

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E 50

= Parents on Sugars

Dec, 2 1958

REF:

	1	2	3	4	5	6	7	8	9	10
		1 day 2 day								
		Ara		Gal		Lac		Xyl		
	♀									
	W4265	−	−	−	−	−	−	−	−	
	♂									
	1	−	−	+	+ all strong	−	blue −, sl pap	+	+ all strong	
	2	−	−	+	+	−	bright orange	+	+	
	3	−	−	+	+	−	blue −	+	+	
	4	−	−	+	+	−	− very sl pap	+	+	
	5	−	−	+	+	−	blue −	+	+	

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E50-A

Dec. 3 1958

REF:

Papillated areas from Lac- colonies 3 days old were streaked on B Lac to look for V vs [redacted] straight +.

1-14 probably V
17 possibly V
2-11
-12 possibly V
-24 = Ara + !
-34 probably not V
3-11 V
-16 V
-23 possibly V
4-1 Not V
-3 probably V
-12 probably V
-17 V
-19 probably V
-22
-30 possibly V
-34 V
-36 probably V
-41 V
5-11
-15 [redacted] see next ppt.
-21
-27

See next expt = E50B

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E50 A & B
cont

Dec. 3 19 58

REF:

Interest centers in Gal+ Lac- papillating, so of the questionable V's from such E50 A, several suspect colonies were restreaked on Mac.

		Lac	
	1-17	+, −, +, −	
	~~2-34~~	~~+, +, +, +~~	1 → 4 probably + pap
	2-34	+, +, + slow, + +	# 5 V pap
	4-12	+, +, +, +	
not Gal+	5-11	? V	
not Gal+	5-27	V V	

E50 C = + pap.

	Ara				Lac			
1-17	−	−	−	−	+	+	+	+
2-34	−	−	−	−	+	+	+	+
5-11	−	−	V	V	−	−	V,	V
5-27	−	−	−	−	+	+	+	+
4-12	−	−			+	+		

reinubate

(test both kinds of lac- from Ara V & Lac Rev.)

5-27 V = Ara V, Lac V

5-11 V = " "

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E50 D

Lac Reactions of Original E50's.

Dec. 4 1958

REF:

	1	2	3	4	5	6	7	8	9	10

Streaks on B Lac from DM-Ara-B, liquid of original E50's (only those circled in red by J L).

	1 DAY (all — except :)	2 DAYS
1 - 14	very few dark	> ½ V or pap? ; original darks now look full +
2 - 34	all —	bluish + pinkish —'s, larger col papillated. Few Rev+ (in brush area. E—)
3 - 16	very few dark	3 days - all pap original darks now look full +; < ½ of rest papillated ± +.
4 - 6	very few dark	> ½ pap. ± + (possibly V?) orig. dark seem full +
5 - 5	—	—
5 - 20	—	—

E50 E From 1st 4 of above pick and streak on Lac and Ara:

a = early +
b = V or pap.
c = — (control)

type ~~m~~ 2 Hfr Lac's ~~se~~ AC ~~...~~ to F' Hfr

Frankly +

2 days ~~...~~ Ara → get Ara ~~negs~~ ~~...~~

W4265 F⁻ TL Th P Lac1 Xyl2 Gal2 Ara2 V1 V6 S

W4008 Hfr 2 Ara3 Lac⁻ Th. a, 2 ?

(4538) = Hfr 13 Hfr = 3836 F⁻ = 3139

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E 50 E

19 REF:

1	2	3	4	5	6	7	8	9	10
From original E 50's ~~Lac~~ early + , 2nd day V or papillated) and —									
			B Lac	2 days		B Ara		2 days	
1-14	early +	+		+		—		—	
	"V"	+, —, V		+, —, V		V, —		V, —	
	—	—		— papillating		—		—	
2-34	blue V	+, —		+ and — (— papillating)		premature — also very small — (1 Rev)			
	pink V	+, —, V		+ and weak + V		—			
	— blue	— and few + !!!		several +, — papillated		—	(4 Rev)		
3-16	early +	+		+		—	—		
	"V"	+, —, V		+, —, V		V	V, —		
	—	—		— (2 Rev)		small col (as above) and also V ? —, sev. rev			
4-6	early +	+		+		—	—		
	"V"	+, —, V		+, —, V		V	V, —		
	—	—	(1 + = probably accident)	— , sl. papillating		—		—	

"V" in this run is definitely V, not papillated

5-57 Ara v ch [illegible]

destroy on Lac + Ara (from Ara) ; a. b. c - [illegible]

20 hrs = normal -

	Ara	Lac
48 hrs ← (a) (didn't grow in Ara broth)	-, +, V	dark + light - ?
b	+, -, V	-, + (or light -), and butterfly
c	+, -, V, butterfly	all butterfly, 1 large + normal

50 ⊕

= several Lacs (1 c [illegible]) not [illegible] Lac and Ara

20 hrs all normal -

	Ara	Lac
Pure light	-	-
Dark, pepperlating	+, -, V	+, -, V + butterfly
mixed dark & light	+, -, V, butterfly	+, -, V, butterfly

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5011

Dec 8 1958 REF:

1	2	3	4	5	6	7	8	9	10

= 5-3 S1

From 50 F ⇒ Lac + Ara :

A b : from Lac plate : −, +, butterfly

B from Ara : −, +, ∛

C c : from Lac plate : −, + (only one), butterfly

D " Ara " : −, +, butterfly

From 50 G : E From dark f.p. from Ara : −, +, V

F From " " Lac : −, +, V, butterfly

G From dark + light mixed from Ara : −, +, light V, dark V

H " " " Lac : −, +, light V, dark V

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50H

Dec. 9 1958 REF:

	1	2	3	4	5	6	7	8	9	10
			1 Day B Ara		2 Days		1 Day B Lac		2 Days	
A	5-57b from Lac plate		−		−		−		−	
			− (pinker)		−		+ weak		+ weak	
			V		V (+, −, "V") (−'s segregating?)		V		V (−, dark + lgt V) no pure +	
B	5-57b from Ara plate		−		−		−		−	
			+ (strong)		+		−		−	
			V		V				V (+ − and lightish V) no pure +	
C	5-57c from Lac plate		−	−			−		− (segregating?)	
			−	−			+		+	
			V	V			V		V (: V + −) no pure +	
D	5-57c from Ara plate		−	−			−		−	
			V	V (dark + lgt V)				lightish { V (− and V; no pure +)		
			V	V (no pure +)				V " " "		
E	5-57 dark papillated from Ara		−	−			−		−	
			−	−			+		+	
				− and light, small V and "+" (very light)			V		V (no pure +, −'s segregating)	
F	5-57 dark papillated from Lac		−	−			−		−	
			V	V			V		V (no pure −'s ?)	
			V	V			V		V no pure +	
			V	V (−'s segregating)					V	
G	5-57 dark + lgt from Ara plate		−	−			−		−	
			V	V (lgt + dark V)			V ?		segregating 2 + types ?	
			−	− and light V)			−		V (− + lgt V)	
			V	V (lgt + dark V)			V		No −'s; ase ↑ ?	
H	5-57 dark + lgt from Lac plate		−	−		(reverse ⟺ reading)	−		− ~~segregating~~	
			V	V			V		V	
			−	+ and mottled			+		− segregating	
			V	V, no pure +			V		V (2 types V)	

Note: V means all 3 types (+, −, varigated) unless otherwise noted.

50I

(couldn't pick clearly)

H 3 Ara : pick up lgt + dark sectors + streak on Lac + Ara

F 4 Ara : ditto not clean

G 2 Ara : lgt + dark V and V → Ara : Lac

E 3 Ara : "+" and − on tr Lac + Ara

E 3 Lac : ne H3 Ara

C 1 Lac : "

A 3 Lac : dark + lgt V → Ara, ~~Ara~~

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50I

Dec. 12 19 58 REF:

1	2	3	4	5	6	7	8	9	10

2-34 and 3-16 on B-Ara seemed to produce 2 types of − colonies, larger pink ones and smaller lighter (white) ones, all non-papillating. Representatives of each were restreaked on Lac and Ara to see if the Lac reaction differed and to see if the Ara difference were genetic or ~~the~~ environmentally produced.

2 day readings

		B Ara	B Lac
3-16 large	1	− , large	
	2		−
	3		− ~~orange and papillating~~
	4		− orange and papillating
	5		−
	6		−
	7		− orange and papillating
	8		−
	9		−
	10		−
	11		−
	12		− orange and papillating
3-16 small	1	−	−
	2		−
	3		− orange + papillating
	4		−
	5		−
	6		−
	7		−, +
	8		−
	9		−
	10		−
	11		−
	12		−
2-34 large	1	−	all colonies − + papillated
	2	−	(none not papillated)
	3	−	
	4	−	
2-34 small	1	−	
	2	−	
	3	−	
	4	−	

no real difference from above - perhaps el lighter color

12/13 Pick up non-pap'ted − 's from restreaks, ~~into~~ and test for Lac type. Also restreak papillated Lac+'s = 50J Differentiate 50K

Ara and Lac for Lac+ that test are Lac+ and Lac pap.

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50 J

Dec. 17 19 58 REF:

Ara and Lac typing of single colonies from 50 I. All picked from Ara.

	1	2	3	4	5	6	7	8	9	10
				EM-Ara-		B,	T1	EM-Lac		
				W4163	W4068	W4069		W3229	W3856	W4358
		3-16	large, non pap	−	+	+	−	7 col	−	−
			"	−	+	+	+	3 col	−	−
			"	−	+	+	+	−	−	−
			"	−	+	+	+	−	−	−
			"	−	+	+	−	1 col	−	−
			"	−	+	+	+	−	−	−
			"	−	+	+	+	−	−	−
			"	−	+	+	+	1 col	−	−
			"	−	+	+	+	−	−	−
			"	−	+	+	+	−	−	−
			Lac pap	−	+	+	+	−	−	−
			"	−	+	+	+	−	−	−
			"	−	+	+	+	−	−	−
			"	−	+	+	+	−	−	−
	Plate N ↑		"	−	+	+	+	−	−	−
			small (non pap)	+	−	+	?	3 col	−	−
			"	+	−	+	−	6 col	−	−
			"	+	−	+	−	−	−	−
			"	+	−	+	−	1 col	1 col	−
			"	+	−	+	−	1 col	−	−
			"	+	−	+	−	1 col	−	−
			Lac pap	+	−	+	−	1 col	−	−
			"	+	−	+	+	−	−	−
		2-34	large	−	+	+	−	1 col	−	−
			"	−	+	+	−	6 col	4 col	−
			"	−	+	+	+	2 col	−	−
			"	−	+	+	+	−	−	−
			small	+	−	+ (rather weak)	−	+ sev. col	5 col	few (+ reversal)
			"	+	−	+	−	+ sev. col	4 col	− ?
			"	+	−	+	−	−	−	−
			"	+	−	+	−	10 col	−	−
								+ +	−	

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50 K

19 REF:

Streaks from papillated Lac colonies from Lac plates of 50 I on B Lac.

2-34 large : mostly +
2-34 small : "
3-16 #4 large : mostly −, some pap = lgt
#4 small : mostly +
#8 large : +, −, V?
#11 large : +, −, V?

hard L used (too old)

Restreak a +, −, + V on Lac + Ara = 40 L

	B Ara	B Lac
#1	−	+
	−	−
	− Lara type?	+, −
#2 (2-34)	−	−
	−	+
#3	−	−
	− Lara type's?	+, − and V?
	−	+
	−	+
#4	−	+
	−	+, −(slimy)
	− (slimy)	− (slimy)
#5 (3-16)	−	+
	−	−, +
	−	+
#6	−	+
	−	−
	−	+

not = aggregat.

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50 L

Dec. 12 19 58

REF:

Testing of Lac test system.

	M Lac			M Lac Meth			M Lac B1			M Lac Meth B1		
	3229	3836	4358	3229	3836 (reverting)	4358	3229	3856	4358	3229	3836 (reverting)	4358
W4117 (F⁻ Lac⁻ prototroph)	+ (6 col)	−	+	+ (5 col)	−	+	+	−	+	−	−	+
50 F b #8 (Rec. ± 3229 and 3836 ?)	−	+ (8 col)	[illegible]	Syn grow[illegible]	+	−	grow[illegible]			grow[illegible]		
50 F b #7 (Rec ± neither)	+	−	−	+	−	−	grow[illegible]					
1-14 #23 (Rec. ± 3229 and not ± 3836)	−	−	−	−	−	−	grow[illegible]					
3-16 #40 as above but 1 instead of 3 col.	−	−	−	−	−	−	grow[illegible]			+ col grow[illegible]	−	+

reverted

Theoretically:

	W3229	W3836	W4358
♀ Lac (=13)	−	−	+
♂ Lac	+	+	−
Compd	−	−	−
W4117	+	−	+

W3836 = Hfr Lac⁻ M⁻ V₁⁻

W3229 = Hfr Lac 13 M 1a

W4358 = Hfr Lac⁻ Ara s Th

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E50 M

12/12 1958

REF:

	1	2	3	4	5	6	7	8	9	10
1			O = growth on EM Ara - B							
2										
3			EM - Ara - B₁			EM - Lac did not grow after 3 days				
4			4163	4068		3836	3229			
5			W ~~2994~~	W ~~4076~~		W ~~3229~~	W ~~3256~~			
6	1-14	# (1)	+	−		−	−			
7		(2)	+	−		−	−			
8		(3)	+	−		−	−			
9		4	−	+		−	−			
10		5	Ara +			−	−			
		6	−	+		−	−			
11		sputty (7)	− sl	+		−	−			
12		8	−	+		−	−			
13		(9)	−	+		−	−			
14		10	−	+		−	−			
15		11	−	+		−	−			
16		12	−	+		−	−			
17		13	Ara +			−	+ (1 col)			
18		14	−	+		−	−			
19		15	−	+		−	−			
20		16	−	+		−	−			
21		17	−	+		−	−			
22		18	−	+		−	−			
23		(19)	+	−		−	−			
24		20	−	+		−	−			
25		(21)	+	−		−	−			
26		(22)	+	−		−	−			
27		23	Ara +			−	+ (3 col)			
28		24	−	+		−	−			
29		25	−	+		−	−			
30		26	−	+		−	−			
31		27	−	+		−	−			
32		28	Ara +			−	−			
33		29	−	+		−	−			
34		(30)	+	−		−	−			
35		31	−	+		−	−			
36		32	Ara +			−	−			
37		33	−	+		−	−			
38		(34)	+	−		−	−			
39		35	−	+		−	−			
40		(36)	+	−		−	−			
41		(37)	+	−		−	−			
42		(38)	+	−		−	−			
43		(39)	+	−		+ (1 col)	+ (1 col)			
44		40	−	+		−	−			
45		41	−	+		−	−			
46		(42)	+	−		−	−			
47		43	−	+		−	−			
48		44	−	+		−	−			
49		45								
50										

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E 50 M

12/12 1958

REF:

	1	2	3	4	5	6	7	8	9	10
			EM - Ara - B,			EM - W3229	Lac W3836 didn't grow after 3 days		T,	
7	2-34	(1)	+	−			−		−	− =
8		(2)	+	−			−		−	NO
9		(3)	+	−			−		−	6 round
10		4	−	+			−		+	(Sensitiv
		(5)	+	−			−		−	
1		6	−	+			−		−	+ =
2		7	−	+			−		−	Res. stan
3		8	Ara +				−		+ (P)	P =
4		(9)	+	−					P	partiall
5		10	−	+					−	R
6		11	−	+					−	
7		(12)	+	−					−	
8		(13)	+	−					−	
9		(14)	+	−					−	
10		(15)	+	−					−	
		16	+	−					−	
1		(17)	+	−					−	
2		18	Ara +						+	
3		(19)	+	−					−	
4		(20)	+	−					−	
5		(21)	+	−					P	
6		22	−	+					+	
7		(23)	−	+					−	
8		24	Ara +						P	
9		25	−	+					+	
10		(26)	+	−					−	
		27	Ara + sl						+	
1		(28)	+	−					−	
2	sl	(29)	−	+					?	
3		(30)	+	−					−	
4		(31)	+	−					−	
5		32	−	+					+ (P)	
6	→	(33)	+	+					−	
7		(34)	+	−					−	
8		35	−	+					+	
9		(36)	+	−					−	
10	→	(37)	+	+					+	
		38	−	+					+	
1		(39)	+	−					−	
2		(40)	+	−					−	
3		(41)	+	−					−	
4		(42)	+	−					−	
5		(43)	+	−					−	
6		44	−	+					−	
7		45								
8										
9										
10										

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E50M

12/12 1958

REF:

	1	2	3	4	5	6	7	8	9	10
			EM - Ara - B1			EM - Lac 3229 (didn't grow 3 days) 3B3C				
6	3-16	(1)	+	−		−	−			
7		(2)	+	−		−	−			
8		(3)	+	−		−	−			
9		(4)	+	−		−	−			
10		(5)	+	−		−	−			
		(6)	+	−		−	−			
1		(7)	+	−		−	−			
2		(8)	+	−		−	−			
3	→	(9)	+	+		−	−			
4		10	−	+		−	−			
5		(11)	+	−		−	−			
6		(12)	+	−		−	−			
7		13	−	+		−	−			
8		14	+ sl	−		−	−			
9		(15)	+	−		−	−			
10		sp #7 (16)	−	+		−	−			
		17	Ara +			−	−			
1		(18)	+	−		−	−			
2		(19)	+	−		−	+ (1 col)			
3		20	−	+		−	−			
4		21	−	+		−	−			
5		(22)	+	−		−	+ (1 col)			
6		(23)	+	−		−	−			
7		24	−	+		−	−			
8		25	−	+		−	−			
9		26	−	+		−	−			
10		27	−	+		−	+ (1 col)			
		28	−	+		−	−			
1		29	−	+		−	−			
2		30	−	+		−	−			
3		31	−	+		−	−			
4		32	−	+		−	−			
5		33	−	+		−	−			
6		34	−	+		−	−			
7		35	−	+		−	−			
8		(36)	+	−		−	−			
9		(37)	+	−		−	−			
10		(38)	+	−		−	+ (1 col)			
		39	−	+		−	−			
1		(40)	+	−		−	+ (1 col)			
2		(41)	+	−		−	−			
3		(42)	+	−		−	−			
4		(43)	−	+		−	−			
5		(44)	+	−		−	−			
6		45	−	+		−	−			
7										
8										
9										
10										

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E 50M

19 REF:

	1	2	3	4	5	6	7	8	9	10
		EM-Ara-B1			EM-Lac		didn't grow after 5 days			
4	4- 1	Ara	+		3229	5836				
5	→ 2	+sl	+							
6	3	−	+sl							
7	→ 4	−	−							
8	5	−	+							
9	6	Ara	+sl							
0	7	−	+							
	8	−	+							
1	9	−	+							
2	10	−	+							
3	11	Ara	+							
4	(12)	+	−							
5	→ (13)	+	+sl							
6	→ 14	−	−							
7	15	−	+							
8	16	Ara	+							
9	17	−	+							
0	spotty (18)	−	+							
	→ 19	−(1 col)	−							
1	20	−	+							
2	21	−	+							
3	22	+	−							
4	23	−	+							
5	24	−	+							
6	25	−	+							
7	26	−	+							
8	(27)	+	−							
9	28	+sl	−							
0	29	−	+							
	30	+sl	−							
1	spotty (31)	+sl	+							
2	32	Ara	+							
3	33	−	+							
4	34	−	+							
5	→ (35)	+	+							
6	36	−	+							
7	37	−	+							
8	38	Ara	+							
9	39	Ara	+							
0	40	Ara	+							
	(41)	+	−							
1	42	−	+							
2	43	−	+							
3	44	Ara	+							
4	45	+(1 col)	−							
5										
6										
7										
8										
9										
0										

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E50M

19 REF:

	1	2	3	4	5	6	7	8	9	10
1										
2										
3			EM-Ara-B1			EM-Lac 3836 didn't grow after ten 3 days				
4	44-6 (1)		Ara +							
5	2		+ −							
6	3		− +							
7	4		+ −							
8	5		− +							
9	6		− +							
0	7		Ara +							
	8		− +							
1	9		− +							
2	10		− +							
3	(11)		− +							
4	12		+ −							
5	13		− +							
6	14		− +							
7	15		Ara +							
8	sl (16)		+ −							
9	17		− +							
0	18		− +							
	19		− +							
1	20		− +							
2	21		− +							
3	(22)		+ −							
4	23		− +							
5	(24)		+ −							
6	sl (25)		− +							
7	26		− +							
8	27		Ara +							
9	(28)		+ −							
0	29		+ −							
	30		− +							
1 →	31		+ sl +							
2	32		− +							
3	sl (33)		+ −							
4	34		− +							
5	35		− +							
6	spotty (36)		− +							
7	37		− +							
8	38		− +							
9	39		− +							
0	40		− +							
	41		− +							
1	42		− +							
2	(43)		+ −							
3	44		− +							
4	sl (45)		− +							
5										
6										
7										
8										
9										
0										

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E 50 M

19 REF:

	1	2 EM Ara B,	3	4	5 Em Lac	6	7	8	9	10
	5-20					didn't "grow"		I1		
1		Ara + sl				– after 3 days		– (P)		
2		+				–		–		
3		Ara + sl				–		– (P)		
4		Ara +				+(1 col)		P		
5		+	–			–		–		
6		+	–					–		
7		–	+					–		
8		–	+					–		
9		–	+					–		
0		+	–					–		
1		+						–		
2		+						+		
3		Ara + sl						P(–)		
4		Ara + sl						–		
5		+	–					–		
6		+	–					–		
7		Ara	+					+		
8		+	–					–		
9		Ara + sl						P		
0		Ara + sl						+		
1	→	+	+ sl			–		P		
2		Ara + sl						–		
3		+						–		
4		+						–		
5		–	+					P(–)		
6	→	+ sl	+					+		
7		–	+					–		
8		+	–					–		
9		+	–					–		
0	→	+ sl	+					+		
1		+	–					–		
2		–	+					P		
3		+	–					–		
4		+	–					–		
5		+	–					–		
6		–	+					+		
7		–	+					+		
8		+	–					–		
9		+	–					–		
0		Ara + sl						–		
1	→	+	+ sl					–		
2		+	+					–		
3		Ara + sl						+		
4		Ara + sl						– (P)		
5		Ara +						+ (P		
6										
7										
8										
9										
0										

50L: streaks of above for s's for retest.2

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○ = grew "well" ~~in~~ in whole stock

E50M

19 REF:

	1	2	3	4	5	6	7	8	9	10
1										
2										
3			EM Ara B1			EM Lac	3 days on EM Lac			
4										
5	50F6 (1)	+	−		1 col	−	streak dark			
6	= 2	+	−		−	−	"			
7	5-57 3	+	−		−	−	"			
8	4	+	−		−	−	"			
9	5	+	−		−	−	"			
10	6	+	−		−	−	"			
	~~dark~~ 7	+	−		−	−	~~v~~			
1	dark 8	+	−		+(4 col)	−+(sev col)	—			
2	9	+	−		~~+(4 col)~~	~~+(sev col)~~ 1 col				
3	10	+	−		−	−(				
4	11	+	−		−	−				
5	12	+	−		−	−1°				
6	13	+	−		−	−				
7	14	+	−		−	−				
8	15	+	−		−	−				
9	16	+	−		−	−				
10	17	+	−		−	−				
	18	Ara +	−		−	−				
1	19	+	−		−	−				
2	20	+	−		−	−				
3	21	+	−		−	−				
4	22	−	+		−	−	—			
5	23	−	+		−	−	—			
6	24	+	−		−	−				
7	25	+	−		−	−				
8	26	+	~~+~~		−(1 col)	+ sml	= LacRev?			
9	slight 27	−	+		−	−	—			
10	28	+	−		−	−	—			
	29	+	−		−	−				
1	30	+	−		−	−				
2	31	+	−		−	−	growing but light			
3	32	+	−		−	−				
4	33	+	−		−	−				
5	34	Ara +	−		−	−				
6	35	+	−		−	−				
7	36	+	−		−	−				
8	37	+	−		−	−				
9	38	+	−		−	−				
10	39	+	−		−	−				
	40	+	−		−	−				
1	41	+	−		−	−				
2	42	Ara +	−		−	−				
3	43	+	−		−	−				
4	44	+	−		−	−				
5	45	+	−		−	−				
6										
7										
8										
9										
10										

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50M
check

Dec. 16 19 58 REF:

From Ara plates

Plate 1:	2-34	#33	lgt	1-3	, dark 4-6 , pap 7-8
	2-34	#37	lgt	1-5	, dark 6-10
Plate 2	3-16	#9	lgt	1-5	, dark 6-10
	4-1	#13	lgt	1-5	, dark 6-10
Plate 3	4-1	#16	small	1-4	large 5-10
	4-1	#35	lgt	1-4	, dark 5-8 , v 9-10
Plate 4	4-6	#31	lgt	1-4	, dark 5-8 , pap 9-10
	5-20	#21	lgt	1-4	dark 5-6
Plate 5	5-20	#26	lgt	1-4	dark 5-8
	5-20	#41	lgt	1-4	, dark 5-9

Since some "single" colony isolates gave ambiguous results in Ara typing (see 50 M), they were restreaked from little penassay on B Ara. They manifested colonial differences, (see below), so representative types were retested for Lac and Ara types.

On B Ara (2 days)

2-34	#33	mostly orange -, few light -, 2 sl. pap.
	#37	as above
3-16	#9	as above
4-1	#13	as above
	#16	no good color dif; few small col, mostly larger
	#35	more light than orange, few v?
4-6	#31	mostly orange, some light, some pap (large center area)
5-20	#21	mostly lgt, very few dark
	#26	mostly orange, few lgt
	#41	mostly lgt, few dark ?

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50 M
check
(cont)

Dec, 18 1958 REF:

	1	2	3	4	5	6	7	8	9	10
1	= Typing of sing. colony isolates from B - Ara.									
2										
3										
4					EM- ~~Ara~~ - B				EM - Lac	
5										
6				W4163	W4068	W4069		W3229	W3836	W4358
7		2-34	# 33 lgt	+	−	+		−	−	−
8			"	+	−	+		↓	↓	↓
9			"	+	−	+				
0			dark	−	+	+				
			"	−	+	+	weak			
1			"	−	+	+	recomb.			
2			pap	−	+	+				
3			"	−	+	+				
4		2-34	# 37 lgt	+	−	+				
5			"	+	−	+				
6			"	+	−	+				
7			"	+	−	+				
8			"	+	−	+				
9			dark	−	+	+				
0			"	−	+	+		2 col		
			"	−	+	+	weak	−		
1			"	−	+	+				
2			"	−	+	+				
3										
4		3-16	# 9 lgt	+	−	+				
5			"	+	−	+				
6			"	+	−	+				
7			"	+	−	+				
8			"	+	−	+				
9			dark	−	+	+				
0			"	−	+	+	very			
			"	−	+	+	weak			
1			"	−	+	+				
2			"	−	+	+				
3										
4		4-1	# 13 lgt	+	−	+				
5			"	+	−	+				
6			"	+	−	+				
7			"	+	−	+				
8			"	+	−	+				
9			dark	−	+	+				
0			"	−	+	+	very			
			"	−	+	+	weak			
1			"	−	+	+				
2			"	−	+	+				
3			# 14 small	+	−	+				
4			"	+	−	+				
5			"	+	−	+	weak			
6	poor reading		"	+	−	+				
7	mold		large	+	−	+				
8			"	+	−	+	very			
9			"	+	−	+	weak			
0			"	+	−	+				

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50M
check
cont

Dec. 18 19 58 REF:

	1	2	3	4	5	6	7	8	9	10
				EM - Ara - Bi				EM - Lac		
1										
2				W4163	W4068	W4069		W3229	W3856	W4358
3		4-1	#14 large	+ } very weak	−	+		−	−	−
4			"	+	−	+				
5			#35 lgt	+	−	+				
6			"	+	−	+				
7			"	+	−	+				
8			"	+	−	+				
9			dark	−	+	+				
0			"	−	+	+				
			"	−	+	+ } weak				
1			"	−	+	+				
2			V	−	+	+				
3			"	−	+	+				
4										
5		4-6	#31 lgt	+	−	+				
6			"	+ } weak	−	+				
7			"	+	−	+				
8			"	−	+	+				
9			dark	−	+	+ } very				
0			"	−	+ } weak	+ } weak				
			"	−	+	+				
1			"	−	+	+				
2			pap	+	+	+				
3			"	+	+	+				
4										
5		5-20	#21 lgt	+	−	+				
6			"	+	−	+				
7			"	+	−	+				
8			"	+	−	+				
9			dark	−	+	+ } weak				
0			"	−	+	+				
			#26 lgt	+	−	+				
1			"	+	−	+				
2			"	+	−	+				
3			"	+	−	+				
4			dark	−	+	+				
5			"	−	+	+ } weak				
6			"	−	+	+				
7			"	−	+	+				
8			#41 lgt	+	−	+				
9			"	+	−	+				
0			"	+	−	+				
			"	+	−	+				
1			dark	−	+	+				
2			"	−	+	+				
3			"	−	+	+ } weak				
4			"	+ } weak	+	+				
5			"	+	+	+				
6										
7										
8										
9										
0										

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50 m

!

Dec. 26 1958 behaves as cmpd. Lac

REF:

1	2	3	4	5	6	7	8	9	10
Typing of 5-20			and 5-27	Ara-	Sgs.:	mBlac			
	w3836	w4358	w4362				w3836	w4358	w4362
w4265	−	+	+						
w4362	+	+ strong	−						
5-20, 1	−	+	−			5-57, 1	−	+ ?	+ ?
2	↓	↓	↓			2	↓	↓	↓
3						3			
4						4			
5						5			
etc						etc.			

?

[illegible] reacting
+ [illegible] ↳ [illegible] thing [illegible]

∴ most zygotes w --

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50 m

Dec 2C 19 58

REF:

	1	2	3	4	5	6	7	8	9	10
	2-34 and									
1	4-1	Lac typing on EMB Lac				4-1: Rather papillated; 2-34 very papp.				
2										
3			w3229	w3836	w4361		w3229	w3836	w4358	w4361
4	♀	w4265	−	−	+	w4265	−	−	+	+
5	♂	w4361	+	+	−	w4359	+	+	?	?
6		4-1 1	−	−	+	2-341	−	−	+	+
7		2				2				
8		3				3				
9		4				4				
10		5				5				
		6				6				
1		7				7				
2		8				8				
3		9				9				
4		10				10				
5		11				11				
6		12				12				
7		13				13				
8		14				14				
9		15				15				
10		16				16				
		17				17				
1		18				18				
2		19				19				
3		20				20				
4		21				21				
5		22				22				
6		23				23				
7		24				24				
8		25				25				
9		26				26				
10		27				27				
		28				28				
1		29				29				
2		30				30				
3		31				31				
4		32				32				
5		33				33				
6		34				34				
7		35				35				
8		36				36				
9		37				37				
10		38				38				
		39				39				
1		40				40				
2		41				41				
3		42				42				
4		43				43				
5		44				44				
6		45				45				
7										
8										
9										
10										

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5017

Dec. 24 19 58

REF:

	1	2	3	4	5	6	7	8	9	10
	5 - 5 recombinants				EMB - Lac			EM - Ara - B.		
			W3229	W3836	W4361	W4368		W4163	W4068	W4069
1		1	—	—	+	—		ara +		
2	5 - 5	2			+			— (few)	+	+ weak
3		3			+			+	—	+
4		4			+		very weak	+	—	+
5		5			+			+	—	+
6		6			+			— few	+	+ weak
7		7			+			+	—	+
8		8			+			—	+	+ very weak
9		9			+			+	—	+
10		10			+			—	+	+ very weak
11		11			+			+	—	+
12		12			+			+	—	+
13		13			+			— few	+	+ weak
14		14			+			+ weak	—	+
15		15			— (few pp)			—	very weak +	—
16		16			+			+	—	+
17		17						+	—	+
18		18						+	—	+
19		19						ara +		
20		20						+	—	+
21		21						+	—	+
22		22						+	—	+
23		23						ara +		
24		24						+	—	+
25		25						+	—	+
26		26						+	—	+
27		27						—	+	+ very weak
28		28						+	—	+
29		29						—	+	+ very weak
30		30						+	—	+
31		31						+	—	+
32		32						ara +		
33		33						—	+	+ very weak
34		34						—	+	+ very weak
35		35						—	+	+ very weak
36		36						ara +		
37		37						—	+	+ very weak
38		38						—	+	+ very weak
39		39						+	—	+
40		40						+	—	+
41		41						—	+	+ very weak
42		42						+	—	—
43		43						ara +		
44		44						+	—	+
45		45						—	+	— very weak
46										
47										
48		♀	—	—	+	+ ?				
49		♂	—	+	+	—				
50										

recombinants H+ (→ 15)

Triple exceptions

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Dec. 26 19 58

REF:

	1	2	3	4	5	6	7	8	9	10
	5-20 isolates from ~~Ora~~ Ara (see expt. 40 mchs. A)			his⁻ W3836	his⁺ W4358	his⁻ W4362				
5	5-20	#41	lg +	−	+	−		sand		
6			"		+	−				
7			"		+	−				
8			"		+	−				
9			dark		+	−				
0			"		+	−				
			"		+	−				
1			"		+	−				
2			"		+	−				
3		#21	lg +		+	−				
4			"		+	−				
5			"		+	−				
6			"		+	−				
7	didn't grow well		dark		no ~~+~~	~~+~~ −				
8			"		+	−				
9		#26	lg +		+	−				
0			"		+	−				
			"		+	−				
1			"		+	−				
2			dark		+	−				
3			"		+	−				
4			"		+	−				
5			"		+	−				
6										
7										
8										
9				all his⁰		mostly his¹				
0										
1										
2										
3										
4				! + mostly his¹ his⁰						
5										
6										
7				I erase his⁰ No! Above + was artefact.						
8										
9										
0										
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										

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50-0

Reversions and arrangements in 5-57.

19 REF:

Sing. col. of Lac Reversions of (5-57) were streaked on B Lac, B Ara

	B Lac	B Ara
5-57-1 light Lac Rev a	+	−
b	+, −, V	− but "segregating"
c	+	−
5-57-2 dark Lac Rev a	+ but "segregating"	+, −, V
b	+	−
c	+ but "segregating"	+, −, V
d	+	+, −, V

5-57 is Lac⁻ Ara^V, Gal⁻, which yields on Lac heavily mottled

of Lac^V → Lac⁺: (1) Ara^V pure Lac^V (mostly Lac⁺ & Lac⁻ sectors) but better

(2) Ara^V (Ara⁺ & Ara⁻ sectors), Lac⁺ mottled.

Ara^V Lac⁻ Gal⁻ —select Lac⁺→ Ara⁻ Lac^V Gal⁻ — confirmation mystery!

→ Ara^V Lac⁺ Gal⁻ — Lac is sectors Lac⁺/Lac± no Lac⁻

Expt 50 P = V from 5-57-1b and 5-57-2a on Gal, Lac, Ara
from Lac and from Ara

Restored Ara^V: two types found.

Ara^V Lac⁻^V (light on BAra).

Ara^V Lac^V (+/−)

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50 P

Dec. 22 19 58 REF:

	1	2	3	4	5	6	7	8	9	10
	5-57 isolates from E50-O									
1										
2		B - Gal			B - Lac			B - Ara		
3	1-b	-, 2 colony types,			+, -, v			-, 2 colony types,		
4	from Lac	smaller one "segregating" (darker)						darker one "segregating"		
5										
6	from Ara	ditto			ditto			ditto		
7										
8										
9										
0										
	2 a	as above; larger type			strong and weak +,			+ and 2 - types;		
1	from Lac	also "segregating"?			~~we~~ strong type			the + and darker -		
2					"segregating"			"segregate"		
3										
4	from Ara	ditto			+ "segregating"			ditto		
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										

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50 Q

Dec. 23, 58 REF:

	1	2	3	4	5	6	7	8	9	10
1	= Parents X Parents and Lac Testers on complete medium									
2	(B Lac)									
3				Lac^D			ignore =Lac±		ignore	
4				W3229	W3836	W4358	W4359	W4360	W4361	W4362
5										
6										
7	F-Lac Proto.		W4147	+ sl	−	+	−	+	+	+
8										
streak all 9	♀ Parent		W4265	−	−	+	−	+	+	(+ ?) * streak
0	♂ "		W4358	+	+	−	−	+	+	+
1		Lac$^+$	W4359	+	+	−	−			
2			W4360	+	+	+	−	−	−	+
3			W4361	+	+	+	−	−	−	+
4			W4362	−	+	+	−	+	+	−
5										
6										
7	W4359 is poorly readable as it ferments somewhat.									
8	W4360 and 61 behave as =.									
9										
0										
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										

W3279

from test { F− 4265 4362 ... ∴ only W4362 in this series may be ≅ 4265
H/2 3836

cross test

all these are ≠ 4265

5 - series

* Upon streaking of doubtful + areas, there are +'s (low proportion in 4265/4362).

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50 R

Dec. 28 19 58 REF:

Since the b isolate of 5-57 had segregated in liquid, the c isolate was streaked on Ara + Lac + Gal = Ara V, Lac peculiar (= - and ± papillating) and Gal - 2 colony types.

4 single colony V's were picked from Ara, streaked on Ara and Lac, and inoc. into Om-Ara-B1.

	B Ara	B-Lac
5-57c-1	-, +, V	- and weak papillated
-2	-, +, V	ditto
-3	ditto	-, "dark -", strong papillated
-4	ditto	ditto

Also 2 darker pap + 2 light col from Lac plates restreaked on Lac + Ara = R L

	B·Ara	B Lac
- (= light)	+	-
"	+	-
darker, pap.	+, -, V	- and medium dark papillated
"	+, -, V	-, "dark", strong + weaker papillated

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50 S

Dec. 26 1958 REF:

Ara V # 5's (= W4362 ♂ parent) tested for Lac types.

	W3229	W3836 (on EMB Lac)	W4358	W4360	W4362
I⁻ x ♂ parent					
I⁺ x ♂ parent					
WH265	−	−	+	+	+
WH362	sic! +	+	+ strong	+ strong	− ?
5-5		−	+	+	− ?
5-13			+		● −
-14			+ weak		
-15			+		
-20			+ weak		
-21			+		
-27			+		
-11			+ weak		
-34			+		
-57			+	strong	
5-20 Ara⁻ Sm⁻	−	−	+	+ strong	−
			+		
♀ I⁻ x⁺					
♂ I⁺ x⁻					

I assume these are hemizygotes for Lac chromosome.

Test on EMB Lac unreadable.