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19 REF:

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

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19 REF:

	1	2	3	4	5	6	7	8	9	10
3								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, C)] 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 meanings (A = F, B = G etc.).

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19 REF: 1402/4

	1	2	3	4	5	6	7	8	9	10
	BGA.	60' [illegible] High 60'	1' [illegible] High 1' 1:100 '	1' [illegible] High 1:10⁴	1/100 60' 1:100 60'	1/10,000 60' 10⁻⁴ 60'				
		A	B	C	D	E				
plate	20'	4	0	0	~~15~~ 0	0				
	84'	52	28	~~not~~ spread	15	0		Plate recomb.		
			~2%/1½						Pen	BGA
								10^{-2}	2	1
	Penassay.							10^{-4}	0	0
		F	G	H	I	J				
	20	8	9	9	7	2				
	84	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} dilu 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.)

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

PULSE 1

(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 rough 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 3050, + .25 30x conc 3054 (0.r.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

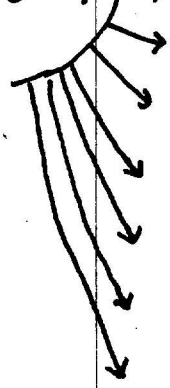
DATE: March 25 REF: Effect of manipul.

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 Pt. (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
3064: 1/50 → .05
} plate at 0' time.

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	0' 2 col.
.02 ml. plated:	122	378	93	~800	197	480	238	436	
Lac+/tot ⊕	3/41	22/40	3/35 → 8.2% → 4/50	12/26 → 50.0 26/50	3/24	76/124	2/44	16/41	
	7.3%	55.0%			12.5	61.3	~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_1^1/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'	60'	0.1 ml pipette 10'	60'	chilled broth 10'	60'	Blending 10'→50'	Streptom. 10'→50'	

⊕ includes "weaks".

DATE: PLATING BY FLOODING. REF: 1403-3

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

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

After 10' since dilution:

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

1/100 → .05 " }

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

1/1000 → .05 from B_1 }

DW → 1 ml flooded plate } 1/1000

1/10 → 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 incubated 5-10 minutes after preparation - Same liquid till pouring

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 $\begin{cases} 0.1 \\ 0.05 \end{cases}$ 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 lyogenized.

{ $F^+ \times F^-$ { Cross-infection
{ Hfr $\times F^-$ } again.

1404 A.

DATE: March 24, 1958 REF: 1322; AKC 4.1 9/56

If fertility of F^+ depends on the mutation to Hfr, $F^+ \times F^-$ should not be uniformly F^- except by reinfection. Expt. to determine whether the recombinants from Hfr $\times F^-$ are infected in presence of $F^+ \times F^-$. Use excess F^- and measure incidence of F^+ conversions in it.

Lucca letter 3/26/58.

Basic setup Hfr $V_6^r M^-$ = W3273 (cf. AKC as best linked to Lac).

$F^+ V_1^r M^-$ = Y40 (Use S^r - can terminate mating by sm ?)

W3089 = $Lac^- Mal^- S^r$.

(Review history of Lac linkage)

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

P23 Broth cultures rotate 37°. N24 Broth 1:10 in penassay rotate. [Better not to rotate??]

(A)

1^{15} PM (Est dens. ~5×10⁸/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 (too low!)
6	W3089	1 + W3273 .1 + Y40 .1	22, 27

Incubate standing.

2^{45} PM. Add .1 ml of sm (2 mg/ml) per tube → 200γ/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 dilution of 1. This tests plate recombinants.

Plate out #6 on EMB Mal for infection 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.

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 ⓧ progeny!

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F^+, Hfr × F^-.

1404B

4 May 1958 REF:

Use excess F^-! Should get timing of Lac, M, etc. Try $Lac^- S^r F^-$ × 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, then dilute + plate at intervals .... Should also isolate prototrophic Hfr S^s...

4 May. 10^30 PM.

ORC W1979 Hfr MV_1
W1632 F^+ MV_6
W3089 F^- Lac Mal S

Refresh 2 hours 1:10 on rotator.

Harvest 10 ml → 0.5 ml. in BGA. 37° standing in WB.

Dilute F^+ 1:10
Dilute Hfr 1:1000

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

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

also check samples of separate parents samples.

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

Platings on M Lac; sm added to inoculum.

6 May: Recombinants are still sparse + small. also control crosses W1979, W1895 × W3089 are very poorly grown 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?

Hfr. M V₁
F+ M V₆
F⁻ Lac Mal S.

SEGREGATIONS.

S. cols Lac + protos isol on streaks to mal broth
a) Spotted B mal
b) x br T6
c) replic. to check

DATE: 9 May 58

REF: 1404 B. EML

	A17 Mal	T1	T6	M mal x 3132	B57 Mal	T1	T6	M mal x 3132	6	D28 Mal	T1	T6	M mal 3132	9	10
1	−	S	R	+	+	S	S	•		−	S	S	++		
2	−	↓	S	+	−	S	↓	−		−	R.	↓	+		
3	−		S	++	+	S		•		+	S		•		
4	−		R	∓	−	S		−		−	R.		++		
5	+		R	•	+	S.		•		−	S		+		
6	−		R	++	+	S		•		−	R.		+		
7	−		S	+	−	S		∓		−	R.		+		
8	−		S	+	−	S		+		−	S	?	•		
9	−		S	−	−	S		+		+	R.	↑	•		
10	+		R	•	−	R.		+		+	S	S	•		
11	+		S	•	−	S		+		+	S	S	•		
12	−		S	∓	−	R.		∓		−	S	S	−		
13	−		R	∓	+	R.		•		+	R	R	•		
14	−		R	+	−	R.		+		−	S	S	∓		
15	−		S	+	−	S		+		−	S	↓	±		
16	+	↑	S	•	−	S		+		+	S		•		
17	+	S	S	•	+	S		•		−	S		−		
18	C36				−	S		∓		+	S		•		
19					−	S		−		−	S		+		
20					+	S		•		−	S		++		
21	−	R.	S	+	−	S		∓		−	S		+		
22	−	S	↓	−	+	R.		•		+	S		•		
23	−	S		+	−	S		∓		−	R		−		
24	−	S		∓	−	R.		+		+	S	↑	•		
25	+	R.		•	+	S		•		+	R.	S	•		
26	−	S		+	−	S		+		−	S	S	∓		
27	−	↓		+	+	S		•		−	R	↓	∓		
28	−			+	−	R.		−		+	S		•		
29	−		↑	++	+	S		•		−	S		+		
30	+		S	•	(+)	S		•		+	R.		•		
31	−		S	∓	+	R.		•		−	R		++		
32	+		S	•	−	S		∓		−	R.		+		
33	+		S	o	−	S		+		−	S		+		
34	−		S	−	−	S		+		−	R.		∓		
35	+		S	•	−	S		+		+	S		•		
36	−		R	+	+	S		•		−	S		+		
37	−		S	+	+	S		•		−	S	↑	++		
38	−		S	++	−	S		−		+	S	S	•		
39	−		S^P	+	+	S		•		−	S	R	±		
40	o				−	S		∓		+	S	S	•		
41	−		S	+	+	S		•		o					
42	−		↓	+	+	R.		•		−	S	S	++		
43	−	↑		+	−	S		−		−	R.	S	+		
44	−			∓	−	S		+		−	S	S	+		
45	+	S		•	−	S		+		−	S	R	+		
46	−	R.		+	−	S		+		−	R.	S	+		
47	−	R.		+++	−	S		+		−	S	R	+		
48	−	S		+	−	S		+		−	S	S	+		
49	−	R.		+	−	R.		+							
50	−	S		+	−	S	↑	++							
51	−	S		∓	−	S	S	+							
52	+	S		•	3820 +	S	S								
53	−	S		−	3064 −	R	R								
54	+	S		•	C +	S	S								
55	−	S	↑	+											
56	−	R.	S	+	2817 −			+							

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

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5/13 1404.8.

Working Summary sheet

D rewritten 19

REF:

	Mal	T1	T6	
1	−	S	S	#
2	−	R	↓	+
3	+	S		•
4	−	R	↑	#
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	R	±
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	S	•
9	−	S	R	±
0	+	S	S	•
1	O			.
2	−	S	S	#
3	−	R	S	+
4	−	S	S	+
5	−	S	R	+
6	−	R	S	+
7	−	S	R	+
8	−	S	S	+
3143	S^R +	S		##
0	2817 2860			#

		A	B	C	D
			51	36	47
Mal	+	5	17	8	15
	−	12	34	27	32
V1	R	0	10	6	14
	S	17	44	29	33
V6	R	2	0	1	4
	S	9	36	34	43
sickety 12	H	4	1	2	6
	M, L	4	>27	>22	>22
	0	4	6	3	3
					(13)

#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

~~All three cases~~ Pick colonies from 9, 10, ~~[illegible]~~ 11, 14, 15, 16.

9	10	11	14	15	16
39	33	50	20+	3	2
30	23		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-brushing from small broth against W3132 ($M^- S^S F^-$ Mal+) on EM-Mal medium.

Use a $Mal^- F^+$ TL^- or TL^+ control. ? 2817,

(results?)

140(4B): Pick ≯ 50 colonies each from A, B, C, D + restreak Blac. test by above "procedure"

	# cols	Mlac
A 1	4	all picked
2	13	
B 1	~~4~~ 5	
2	96	
C1	110 + clump	
C2	150	
D1	216 + sev'l clumps	
D2	258.	

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14: 1-84.85
86 #6
87 #9
88-90 #10
91-100 #11

1404c

by Emil

19 REF:

	1	2	3	4	5	6	7	8	9	10
	6.1	T1 T6								
		all R	G:							
1	2	R								
2	3	Mal+ S								
3	9.1	sucrose ↓								
4	2									
5	3		d1							
6	4		d2.							
7	5									
8	6									
9	"									
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: S S R S S S S S S S S S S S S S S S S R S S)

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Hfr, F⁺, F⁻

1404C

5 May 1958 REF:

Repetition: Hfr mixed variable proportions of F⁺ : F⁻ : Hfr. Use .1 ml of 20x F⁻ as standard. Add various proportions as follows. (in terms of 20X suspensions)

.1 ml 20XF⁻ plus .1 ml each of

~~1. Hfr conc.~~
~~2. 1/10~~
~~3. 1/100~~
~~4. 1/1000~~
~~5~~

				5/8 # + cols
1.	Hfr 1:100	F⁺	0	188
2	"	F⁺	1/2	235
3	"	F⁺	1:10	261
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

For plating, after 15 minutes add 2 ml of BGA - sm 5000 u/ml and plate 0.1 ml on MLac.

13 Hfr 1:100 ~~F⁺~~ 273
14 Hfr 1:1000 ~~F⁺~~ 20
15 F⁺ 1/2 3
16 F⁺ 1:10 2

} mixed after diluting ~~plate at once~~ plate at once (chilled) — not warmed at all

Harvest from refreshed ORC — rotated pressure 1:10 2 hours, hence into BGA.

Time by chilling in ice-water.

Probably not enough fertility of F⁺!

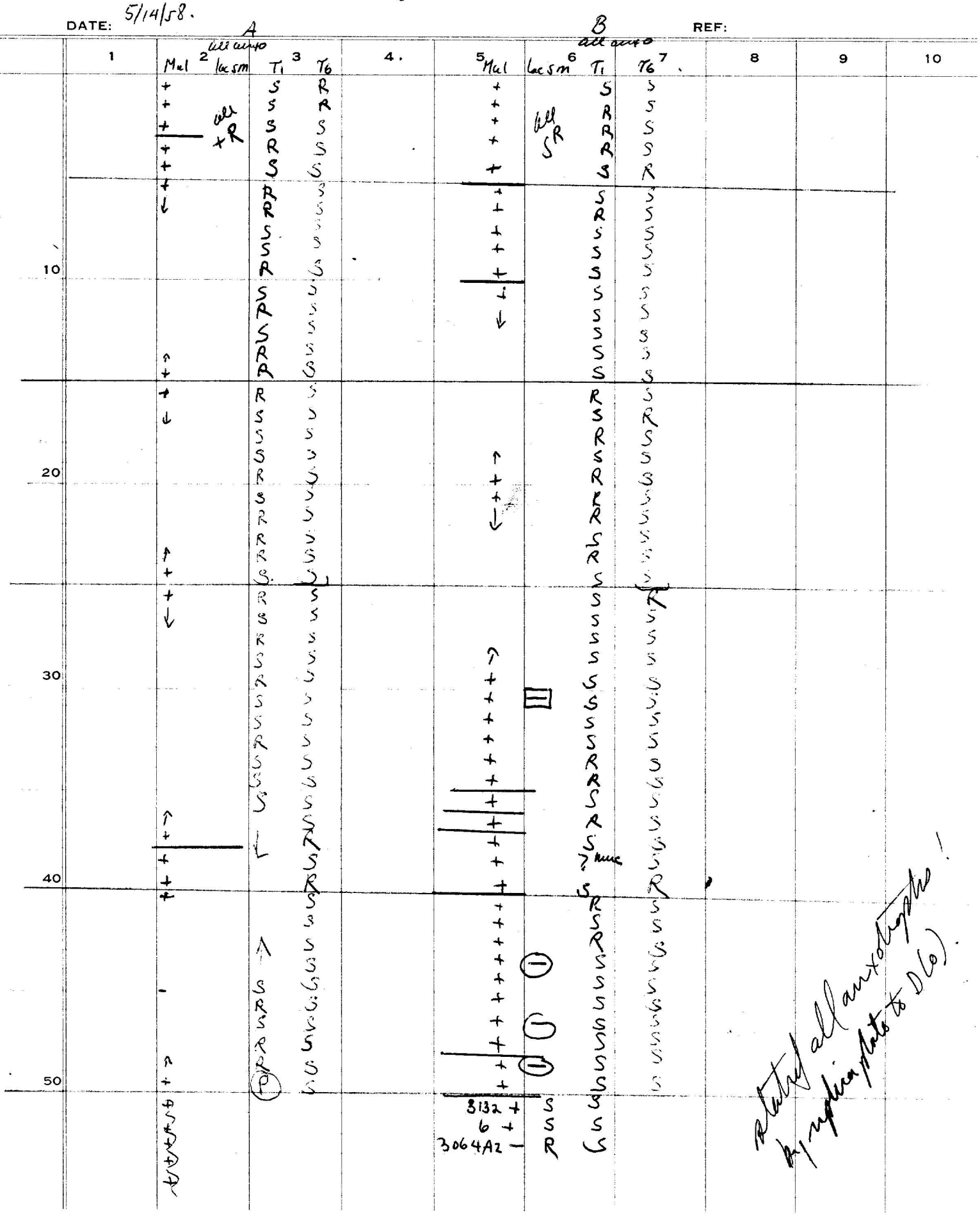
fin. 6¹⁰ PM

AM			
1	++	11	—
2	++		+
3	++		++
4	++		+
5	—		—
6	—		—
7	—		
8	—		
9	+		
0	—		

F⁺ relatively very infertile. (~10⁻⁴ of Hfr!) also mainly plate recombinants in any case! (despite addition of streptomycin and limited 3 dilution). Stated to be all auxotrophs!

Reading. 1404 C.

DATE: 5/14/58.



U.W.
MICROBIAL
GENETICS

(F+, Hfr) x F−

Reconstructions or reinfection of F− progeny in F+ x F− Hfr x F− crosses.

summary.
1404
WBCD

28 May 1958

REF:

24 March

A. W3273 = Hfr V6 Y40 = F+ V1 W3089 = F− Mal Lac S. Select lac+ S^R prototrophs.
But Hfr was 4x10^4 as fertile as F+. abandoned for lack of proper proportions

4 May.

B. Use excess F−. W3089, W1879 = Hfr, MV1 ; W1632 = F+ MV6.
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 x F− remain uninfected by neighboring F+ cells. Crosses can be done in both or on plates.

DATE: March 25, 1958 REF: PROTOPL. TIMING

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

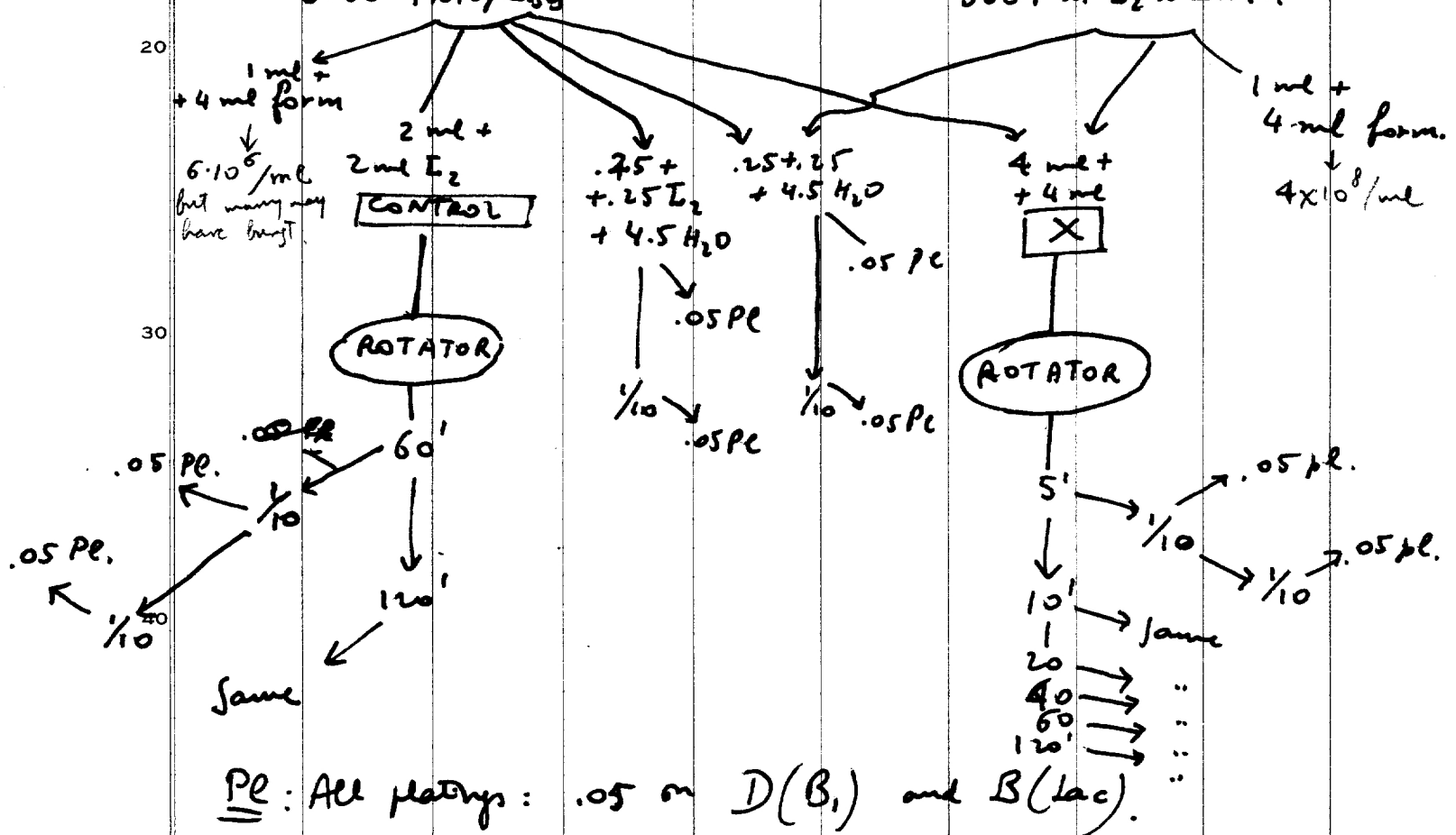
11.50 → L_2 medium : .5 / 1.0 / 1.5 / 2.0 } ml of semisatu. rated culture + 8 ml L_2 medium 10^3 u/pen./ml.

14.10 protoplasts well formed. agglutination in the higher concs. of sulf. 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



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

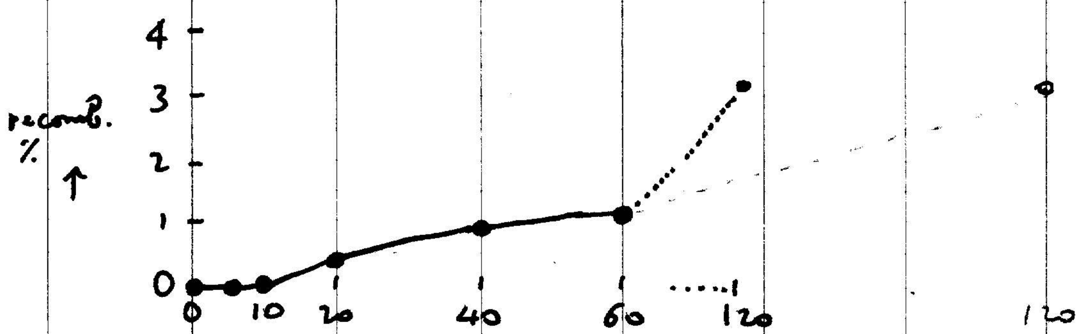
Microscope counts : 3060 protoplasts : 6×10^6/ml. But many may have lysed in formalin.
Rods : 3% or less (badly preserved).

3064 in L_2 medium : 4×10^8/ml

PROTOPLASTS

DATE: REF: 1405 b

COUNTS on min B1	0'	5'	10'	20'	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 Blac									
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 $6 \cdot 10^6$: $\sim 10^{-4}$.
Recombinants per Hfr protoplast after 60' : 2.3×10^4/ml out of $6 \cdot 10^6$: $3 \cdot 10^{-3}$.

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

At time 20' : on proportion exp. 13% (Hfr par / total "recomb" on cross plates)
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				(67% ?).	
5'		2/3	} very likely to be parental.				
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.