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19 REF:

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

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19

REF:

	1	2	3	4	5	6	7	8	9	10
1										
2										
3								Plate recombination control		
4										
5										
6										
7										
8										
9										
0										
1										
2										
3										
4										
5										
6										
7										
8										
9										
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9										
0										

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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 fresh 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 0.05
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 nos. 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' [illegible]	[illegible]	[illegible]	1/100 60'	1/10,000 60'				
		High 60'	High 1'	High 1/100	1:100 60'	10^{-4} 60'				
	BGA.		1:100 [illegible]'	1:10^4						
		A	B	C	D	E				
plate	20'	4	0	0	~~15~~ 0	0		Plate	recomb.	
	~~86~~'	52	28	++ not spread	15	0			Pen	BGA
			~2% H/2					10^{-2}	2	1
	Penalty.							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} 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(for B_1).

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

(D). From same suspension 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
	P60	251	9
Diluted	D20	0	2
	D40	16	0
	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 St 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 St B_1

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

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 colonies

B 0,0

C 0,0

D 0,0

E 0,2

F 0,1

Conclusion.

Single colonies selected from 3060 is F+ revertion.

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 Str. B_1.

Counts : .1 plates always too many.

	A_1	A_2	B_1	B_2	C_1	C_2	D	E	● 2 col.
.02 ml. plates:	122	378	93	~800	197	480	238	436	

● includes "weaks".

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_1^1/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'	1 ml pipette 60'	0.1 ml pipette 10'	0.1 ml pipette 60'	chilled broth 10'	chilled broth 60'	Blending 10'→50'	Streptom. 10'→50'

DATE: PLATING BY FLOODING. REF: 1403-3

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

13.30, ~~2~~ 2 ml + 2 ml of exponentiae cultures mixed, incubated in water bath for 5', then diluted in warmed broth for ~~0.01~~ 1/100, and kept in water bath.

After 10' since dilution:

1/100 Broth → .05 Sm B1 plate } undil.

1/100 DW → .05 " } 1/100
→ 1 ml flooded plate Sm B1

1/10 DW → .05 Sm B1 } 1/1000
→ 1 ml flooded plate

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 inverted 5-10 minutes after preparation - some liquid still floating

See also 1423B (agar poured)

DATE: REF: 1403.3

	1	2	3	4	5	6	7	8	9	10
	Plate counts.									
		.05 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^r	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 / 0.02 plated on min St B_1

			0.1	0.02
A	3060 x 3064	Counts:	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 bal⁻ or lac⁻? F⁻

and discriminate on basis of ara character which is closely linked to T1^H.

Wait for W4062 to be lysogenized.

$\left\{ \begin{matrix} F^+ \times F^- \\ Hfr \times F^- \end{matrix} \right\}$ cross-infection on agar.

1404 A.

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

9/56

Lacca letter 3/26/56.

If futility 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.

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

Review history of Lac linkage

$F^+ V_1^r M^-$ = Y40

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

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

W1632: $F^+ V_6^r$.

P23 Inoc cultures. rotate 37° N24 Inoc 1:10 in penassay rotate.

Better not to rotate ??

(A)

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
too low! 5	W3089	1 + Y40 .1	1, 1
6	W3089	1 + W3273 .1 + Y40 .1	22, 27

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 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.

~~B~~ A
$F^+ \times F^-$: 12 tested × W3132. 11 F^+ 1 ? F^-. several $F^\pm$ weak reactions.

~~B~~ A all 5 V_6^r were F^+.

B also gave variable fertility reactions! 18 Mal$^+$.

C. No RR. $V_1^r V_6^s$: 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 x F^-.

1404B

4 May 1958

REF:

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

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

W 1979 = Hfr, M V_1^R

W 1632 = 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. Dilute F^+ 1:10
in BGA. 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 BGA. at t = 15 minutes, add 2 ml BGA* to dilute and plate 0.1 ml samples.

A F^- ; F^+
B F^- ; Hfr
C F^- ; Hfr ; F^+
D. ditto, 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 inoculum.

6 May: Recombinants are still spare + small. also control brushes

W1979, W1895 x W3089 are very pony 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?

SEGREGATIONS

Hfr, M V1
F+ M V6
F− Lac Mal S.

S. cols Lac + protos isol on streaks to ime broth
a) of spotted B mal
b) xln T6
c) mixture to ch. R

DATE: 9 May 58

REF: 1404 B. 6 4L

	1	2	3	4	5	6	7	8	9	10
	A17			B51			D			
	Mal	T1	T6	Mal	T1	T6	Mal	T1	T6	M mal x 3132
	−	S	R	+	S	S	−	S	S	++
	−	↓	S	−	S	↓	−	R.	↓	+
	+		S	+	S		+	S		•
	−		R	−	S		−	R.		++
	+		R	+	S.		−	S		+
	−		R	+	S		−	R.		+
	−		S	−	S		−	R.		+
	−		S	−	S		−	S		?
	−		S	−	S		+	R.	↑	•
10	+		R	−	R.		+	S	S	•
	+		S	−	S		+	S	S	•
	−		S	−	S		−	S	S	−
	−		R	+	R		+	R	R	•
	−		R	−	R.		−	S	S	±
	−		S	+	S		−	S	↓	±
	+	↑	S	−	S		+	S		•
	+	S	S	+	S		+	S		−
C 36				−	S		+	S		•
				−	S		+	S		+
20				+	S		+	S		++
	−	R.	S	−	S		+	S		+
	−	S	↓	+	R		+	S		•
	−	S		−	S		+	R		−
	−	S		+	R		+	S	↑	•
	+	R.		+	S		+	R.	S	•
	−	S		−	S.		−	S	S	++
	−	↓		+	S		−	S	↓	+
	−			−	R		−	R		•
30	+		S	+	S		+	S		+
	−		S	−	S		−	R		++
	+		S	+	S		−	S		++
	−		S	−	S		−	S		++
	+		S	−	S		+	R		•
	−		R	+	S		−	S	↑	+
	−		S	−	S		−	S	S	++
	−		S	+	S		+	R	S	•
40	−		S	+	S		+	R	S	±
	−		S	+	S		+	S		•
	−		S	+	R		−	S		S
	−	↑		+	S		−	R.		S
	+	S		+	S		−	S		++
	−	R.		+	S		−	R		++
	−	R.		+	S		−	S		++
	−	S		+	S		−	R		++
	−	R.		+	R		−	S		+
50	−	S		+	S					
	−	S		−	S	↑				
	+	S		+	S					
	−	S		3820 +	S	S				
	+	S		3064 −	R	R				
	−	S		6 +	S	S				
	−	S	↑	2817 −						
	−	R.	S							

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

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5/13

1404.8

Working Summary Sheet

D rewritten 19

REF:

	1 Hab	1 T_1	2 T_6	2	3	4 A	5 B	6 C	7 D	8	9	10
1	−	S	S	#		Mal +	5	17	8	15		
2	−	R	↓	+		−	12	34	27	32		
3	+	S		•		V_1 R	0	10	6	14		
4	−	R	↑	#		S	17	41	29	33		
5	−	S	S	+								
6	−	R	S	+		V_6 R	8	0	1	4		
7	−	R	↓	+		S	9	36	34	43		
8	−	S		•		Fechlet						
9	+	R	↑	•		12 H	4	1	2	6		
10	+	S	S	•		M	4	>27	>22	>22		
						L	4					
						O	4	6	3	3		
										(1?)		
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	+								
31 43	S^R +	S		#								
0	2817 3860			#								

#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

~~If there are~~ Pick colonies from 9, 10, ~~11, 12~~, 11, 14, 15, 16.

39 33 50 20+ 3 2

30 • 26 4- • 3

Procedure: isolate on EMB Lac. Test Lac+ X+ for V1 and V6 and Mal. All or most should be Mal- S^R. Test for F by cross-brushing from small broth ~~to~~ against W3132 (M- S^S F-) on M-Mal medium.
Mal+

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"

	# colo	Mlac
A1	4	
2	13	all picked
B1	45	
2	95	
C1	110 + clump	
C2	150	
D1	216 + sev'l clumps	
D2	258.	

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14: 1-~~44~~.85
86 #6
87 #9
88-90 #10
91-100 #11

1404c

19

REF: by Eric

	1	2	3	4	5	6	7	8	9	10
		T1 T6								
	6.1	all R	C9:							
1	2	R								
2	3	Mal + S								
3	9.1	acutes. ↓								
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 4	2									
36 5	3									
37	4									
38	5									
39	6									
40										
41										
42										
43										
44										
45										
46										
47										
48										
49										
50										
9										
0										

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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 x F^- as standard. Add various proportions as follows. (in terms of 20 x suspensions)

.1 ml 20 x F^- plus .1 ml each of

~~1. Hfr conc.~~		1. Hfr	1:100	F^+	0	5/8 # + cols 188
~~2.~~	~~1/10~~	2	"	F^+	1/2	235
~~3.~~	~~1/100~~	3	"	F^+	1:10	261
~~4.~~	~~1/1000~~	4	"	F^+	1:100	201
~~5~~		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			mixed after diluting plate at once 273
		14	Hfr 1:100			(chilled) 20 — not warmed at all
		15	F^+ 1/2			3
		16	F^+ 1:10			2

For plating, after 15 minutes add 2 ml of BOA - 8 m 5000 u/ml and plate 0.1 ml on M bac.

Harvest from refreshed ORC — rotated overnight 1:10 2 hours, low cent into BOA.

Time by chilling in ice-water.

Probably not enough fertility of F^+!

Fri. 6 10 PM

AM: 1	++	" —
2	++	+
3	++	++
4	++	+
	—	—
	—	—
	—	
	—	
	+	
	—	

F^+ relatively very infertile. (~ 10^{-4} of Hfr!) also mainly plate recombinants in any case! (despite addition of streptomycin and limited 3 dilutions). Stated to be allauxotrophs!

Reading. 1404 C.

DATE: 5/14/58.

REF:

	1	A 2 Mul	A 2 Loc sm (all aux o)	A 3 T_1	A 3 T_6	4.	B 5 Mul	B 6 Loc sm (all aux o)	B 7 T_1	B 7 T_6	8	9	10
		+		S	R		+		S	S			
		+		S	R		+		R	S			
		+	all +R	S	S		+	all S R	R	S			
		+		R	S		+		R	S			
		+		S	S		+		S	R			
		+		R	S		+		S	S			
		+		R	S		+		S	S			
		↓		R	S		+		S	S			
				S	S		+		S	S			
				S	S		+		S	S			
10				R	S		+		S	S			
				S	S		↓		S	S			
				R	S				S	S			
				S	S				S	S			
				R	S				S	S			
		↑		R	S				S	S			
		+		R	S				R	S			
		+		S	S				S	S			
		↓		R	S				S	S			
				S	S		↑		R	S			
20				R	S		+		S	S			
				S	S		+		R	S			
				R	S		↓		R	S			
				R	S				S	S			
		↑		R	S				R	S			
		+		S	S				S	S			
		+		R	S				S	S			
		↓		S	S				S	S			
				R	S				S	S			
				S	S		↑		S	S			
30				R	S		+		S	S			
				S	S		+		S	S			
				R	S		+		S	S			
		↑		S	S		+		R	S			
		+		S	S		+		R	S			
		+		S	S		+		S	S			
		+		S	S		+		R	S			
				S	S		+		S	S			
				S	S		+		S	S			
40				S	S		+		S	S			
				S	S		+		S	S			
				S	S		+		S	S			
				S	S		+		S	S			
				S	S		+		S	S			
				S	S		+		S	S			
				S	S		+		S	S			
				S	S		+		S	S			
50				S	S		+		S	S			
							8132 +		S	S			
							6 +		S	S			
							3064A2 −		R	S			

Plotted all aux depths by replication to D(6).

U.W.
MICROBIAL
GENETICS

$(F^+, Hfr) \times F^-$

Reconstructions or reinfection of F^- progeny in $F^+ \times F^-$, $Hfr \times F^-$ crosses.

summary.
1404
ABCD

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 $\times F^-$ remain uninfected by neighboring F^+ cells. Crosses can be done in broth or on plates.

DATE: March 25, 1958 REF: PROTOPL. TIMING

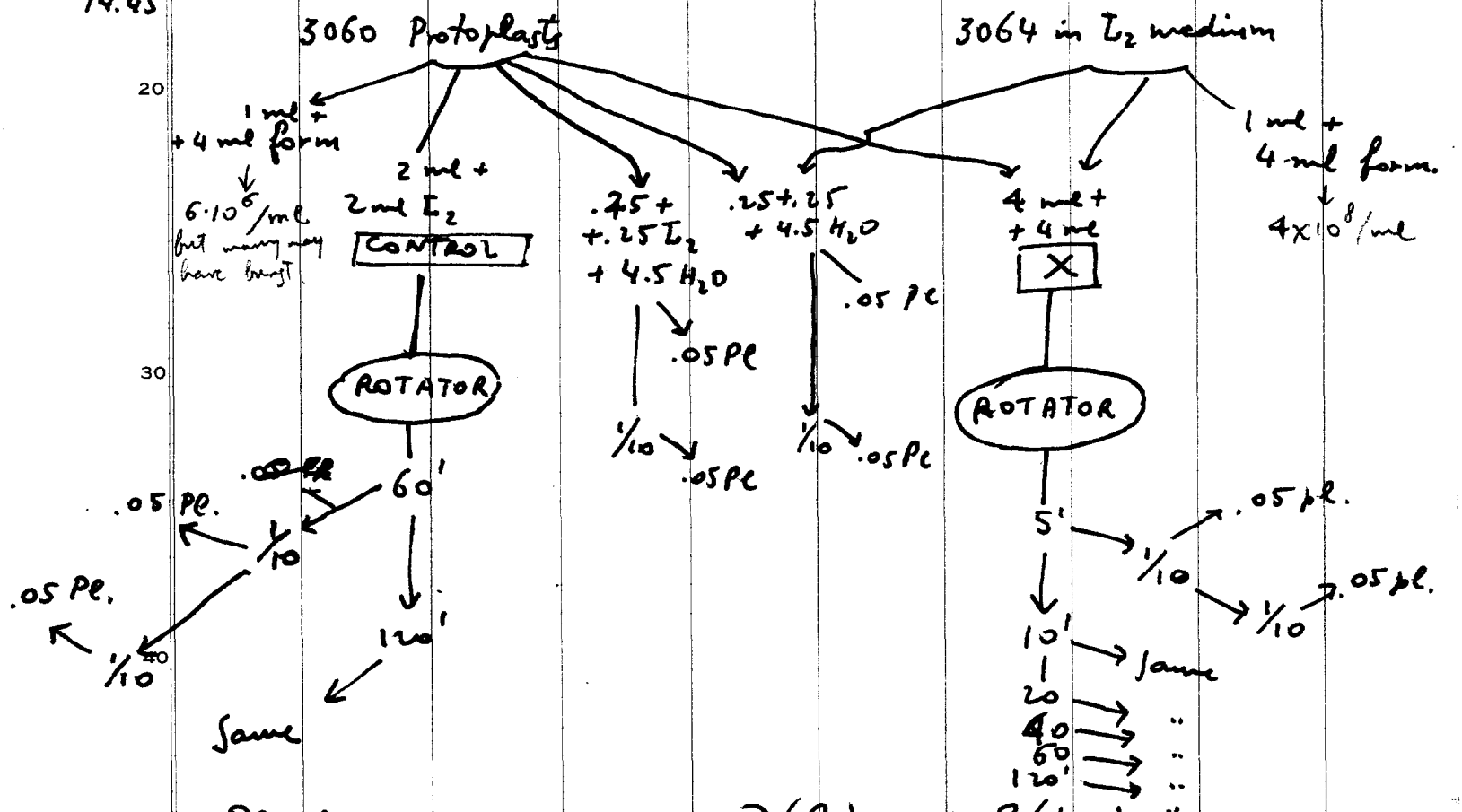
9.30 #3060 overnight broth culture 5 ml + 5 ml broth → rotator →

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

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

10 #3064 : 2 ml overnight broth + 10 ml broth → rotator for 45'.
Cultures of 3060, 3064 centrifuged, resuspended with 8 ml. warmed L_2 medium. ~~Res~~ 5' water bath.

14.45



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

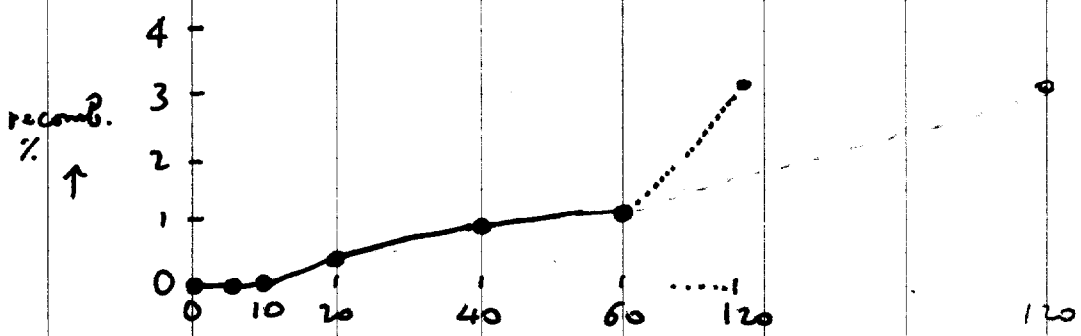
Microscope counts : 3060 protoplasts : 6×10^6/ml. But many may have lysed in formalin.
Rods : 3% or less (badly prepared).
3064 in L_2 medium: 4×10^8/ml

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	0		
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 $6 \cdot 10^6$: $\sim 10^{-4}$.

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 plating)

40' 10.4%

60' 18.1%

120' 65%

this accounts for the high Gal ratios observed

DATE: 3/28/58. G 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						
0'	2/2.						

} very likely to be parentals.

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.