Experiments on Interrupted Mating
of E. coli.

Collaboration with Bruce Cavalli April – June 1958.

5.3.58

E.COLI Specific Projects 1400

P1. Mathematical Theory of mating

P2. Entry vs. Pairing
DIPLOIDS; RETRIEVAL OF UNP. MARKERS; BLENDING S/INTERRUPTION; ABORTIVE REMB'TS; DNA LEAKAGE AND VIABILITY; DIFF'L INHIB'n (AZIDE RESISTANCE)

P3 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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1424 Various Hfr for Gal entry

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19 INDEX - 1 REF:

1401 TIMED MATING WITH H/fr_2.

(1). Non pulsed. Scored approx every 10'. Tl+. Gal+, T_1
3060 × 3064

(2) Pulsed. Scored every 5'. Tl+ Gal Lac T_1
3060 × 3064

(3) Non pulsed. Scored every 5'. T+, Tl+, Pr+. other markers Gal Lac
3060 × 3052 [illegible]

1402 PULSE

(1). 3060 × 3064 - Various dilutions [illegible] No prototrophs [illegible]
Factor of [illegible] 2x every [illegible]

(2). 3060 × 3064 10x, 3x, 1x, dilution 1/100 and 30' incub. approx 1' pulse, then further dil. & plating. Effect noted may be due to ~~first~~ 30' incubation

(3) 3060, 1895 × 3064. pulse with [illegible] with dilutch mating. negative effect of pulse.

(4) 3060 × 3064 [illegible] 3x, 10x [illegible] Kept at 1' [illegible] approx 4' pulse. No effect of pulse.

(5) 3060 × 3064 [illegible] Same as 1401/2. Pulse 1' [illegible] pulse effective

(6) 3060 × 3064. Same as 1401/2, 1401/5 pulse [illegible] then transfer to [illegible] Pen B&A
~~Used~~ comparison with ~~undiluted~~. Very small effect of pulse.

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INDEX-2

19 REF:

1403 MANIPULATIONS

(1) Negative: Hfr → F+

(2) Effect of pipetting, 0.1 & 1.0 ml pipettes, chilling, blending, sheptoning. only plating & blending effective in interrupts. 1000, 1000

(3) Plating by spreading vs plating by flooding. only the former interrupts

(4) Stability of pairs to plating. almost no recombinants obtained. Cause of failure unknown

1405 PROTOPLASTS.

(1)

Detailed theory of mating; pulses etc. 1400 P1. 4-30-58

A Assume linear mating. B Pulse mating

1. Variable time + rate of entry
2. Spontaneous breakage
3. Age-dependent integration (Poisson 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_s TLB_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 $\bar{L}_{W677}$.							
2324		Hfr_2 B_1							
2323		M-St^R Az^R							
3051		F-Lp^+ TLTh Lac_1 Gal_7 Xyl_1 Ara_1 V_1 V_6 S^R							
3064		F-Lp^+ TLTh Lac_1 Gal_7 Xyl_1 Ara_1 V_1 V_6 S^R λ_2 Mal Az^s							
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 TLB_1 Lac_1 V_1 $\{Gal_1\}$ Az^s							
2323		M-Hfr_2 S^R Az^R.							
3908		Lp^+ F-tryp Gal_6 S^R.							
3801		T-S^R F.							

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1	2	3	4	5	6	7	8	9	10
W 3052		F-Lp$^+$ Pr-TLTh Gal$_2$ Lac$_1$ Xyl$_2$ Ara$_2$ Mtl St^R. (W945)							
W 2914		F-Pr-TLTh Lac$_1$ Gal$_2$ Ara$_2$ Xyl Mtl.							
W 3934		Hfr$_2$ Gal$_2$ (from 2323 x 945)							
W 3960		F-Try-S^R Gal$_4$-Gal$_6$							
W	3094	F-Lp$^+$ Gal$_2$							
W	3064								
W	3093	F-Lp$^+$ Gal$_2$							
W	3953	F-Lp$^+$ Gal$_2$ S^R Try							

1400 C

DATE: 4/22/58 REF:

In previous exp, an alledged auxotroph (I, B_1^-) tested prototrophically. Now retest this same inoculum (= #18 from A) and also 13 A, 14 A, 16 A, and slant. ~~made from origin~~

		1 DO	2 I, B_1	3 I	4 B_1
A	slant	–	±	±	+
B	13 A	++++	++++	++++	++++
C	14 A	++++	++++	++++	++++
D	16 A	++++	++++	++++	++++
E	18 A	++++	++++	++++	++++
F	8	–	–	–	–

Control: old Isoleucine –

Also: Slant + aa1 : –
+ Lys –
Arg –
M –
C –
M, L +

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Selection of new 3060.
Cross W2323 x W945 on D(0)
Streaks on B Lac.

May 10 1958

REF:

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

(+) = complete growth =

Sm R

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10^{-6} dil.

3060
Temptric lawn Plates

May 12, 1958

REF:

	1 (Strains / Plates)	2	3	4 @ 30	5	6 @ 37	7	8	9	10
1	Sm 30			~300		~500				
2										
3	Sm 30 + B1			~250		~150 ?				
4	"	Lac		~250		~500				
5	Lg 30			~20		0				
6										
7	Lg 30 + B1			~200		~200				
8	"	Lac		~200		~20				
9	Sm 37			~300		~150				
0										
	Sm 37 + B1			~100		~100 ?				
1	"	Lac		~300		~300				
2	Lg 37			~300		~400				
3										
4	Lg 37 + B1			~300		~300				
5	"	Lac		~300		~300				
6										
7										
8										
9										
0										
1										
2										
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B1 inhibition ?

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2323 x 945 x's

May 16 19 58

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		NO!								
7										
8		SmR!								
9										
0										
1										
2										
3										
4										
5										
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3060's
Temp. Cont Effect
REF:

May 9 19 58

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

	30 C	37 C
@ 20 Hrs:		
Large	Sparse growth in brush	↵
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 penassay 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 c w3064 for Hfr testing. ~~About 2/3~~ All colonies were prototrophic, and about 2/3 were Hfr. Now pick + streak one Hfr and one non-Hfr to single colony test for Hfr + prototrophy.

8 Hfr -'s → NON 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 was prepared from) grew very poorly - few large colonies plus few satellites. Both streaked on B lac (no morphological difference). Broth made from 5 c of each. Now test on DO + DO B1, both liquid + agar D. Ho for 3870 (difference on DO not so pronounced).

		Liquid DO	Liquid DO+B1	Agar DO	Agar DO+B1	Dilute DO	Dilute DO+B1
3870	Large	.400	.630	+	+	+	poorish
"	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 Sm B1 for Hfr test, + little water dilutions were streaked on DO for prototrophy test, ~~+ B lac for lactose ferm.~~

Also DO B1 for auxotrophy.

Also spot on Lac, Gal, Mal, Xyl, Mtl, Ara.

of plate reasons ~~[illegible]~~ long both

(-) count before-ypl.

must be done on selective minimal

1401

DATE: REF:

	1	2	B Gal	Lac	T1	6	7	8	9	10
* Picked	0A *	16	all −							
	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~~5~~	−	all − ? [illegible]	scoring n.g.					
	20C^R	399	all −							
	20D *	38	−	−	ng.					
	30B	(65)	1+	15 (mid Gal+) (α)	? but all Lac+ are V1^S					
30	30C^R	(551)	7+							
	30A		8+							
	30D	(48)	1+ also L+	16 (β)	~19 all Lac+ are V1^S					
	45A		47+							
	45B	(72)	8+	[15++ 12±]	3 Lac+ are V1^S			all Gal+ are Lac+		
40	45C	10×58	47+							
	45D	(58)	2+							
	60A	10×94	47+							
	60B *1	(47)	1+ →	1+	34 V1^R					
	*2									
	60C	(10×52)	56+							
50	60D		2+ →	2+						

α: two Lac phenotypes: 9++ 6± (mid 8 Gal− 1 Gal+) 5++ 11±

DATE:

REF:

1	2	3 Bgal	Lac	5	6	7	8	9	10
120 B	(257)	19 +							
120 D	248	25 +							
120 C	10x750	~100 +							
120 B*	40 picked	6 + and +/-							

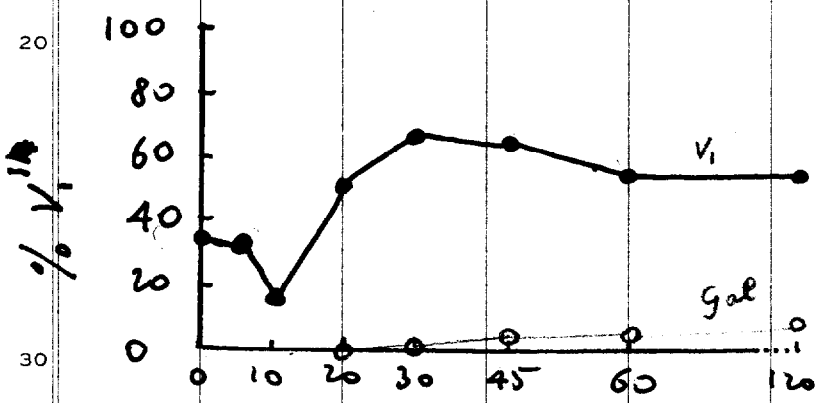
Note on Lac: two batches of B Lac. - blue + yellow. (Man, F-ri)
on blue, only Gal + Lac + show
on yellow at 1' reading.

1/total V_1 segregation

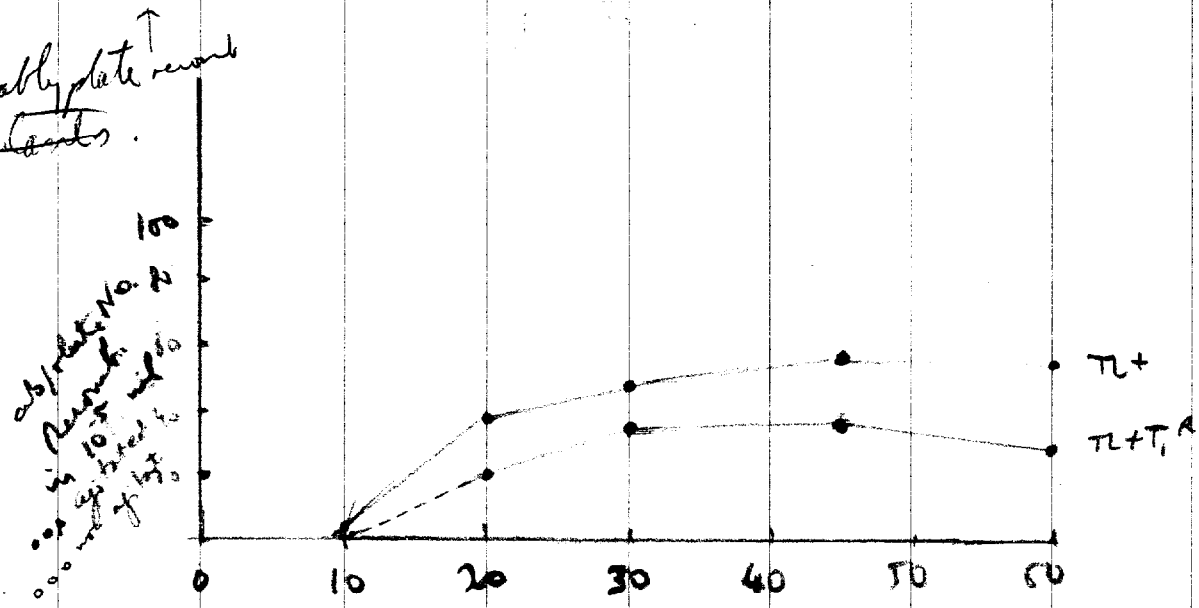
DATE:

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%		



probably plate records



DATE: March 21st, 1958 — REF: TIMED MATING

Hfr_2

Overnight broth cultures:

3060 Hfr_2 ; # 3064: $FaLp^+ TLB_1 Lac_1 Gal_7 Xyl_1 Ara_1 V_1 V_6 S^R \lambda_2 Mal$

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

Penassay broth throughout.

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

2×10^8 2×10^6/ml Hfr

2.55 p.m.

0.2 ml 3060 broth + 10 ml 3064 broth + 10 ml warm broth.

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 water, plating (min St B_1)

0'	nondiluted,	: → 0.1 ml;	0.01 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 —

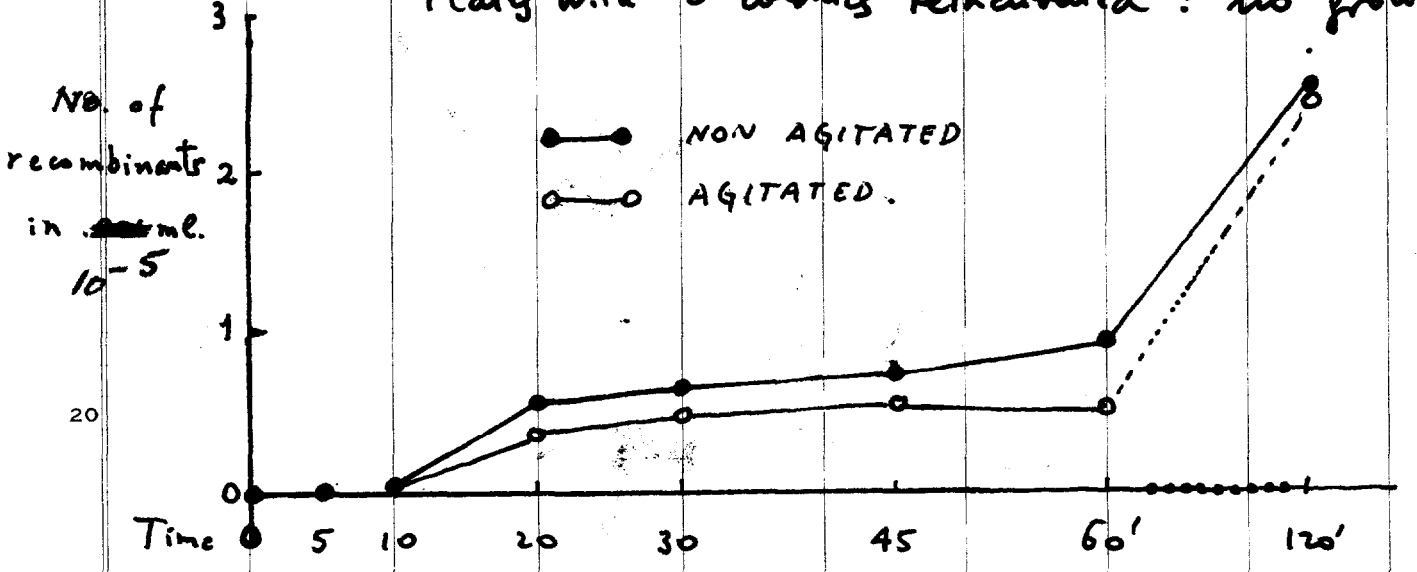
1401 b

[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.

Platy with 0 colonies reincubated : no growth.



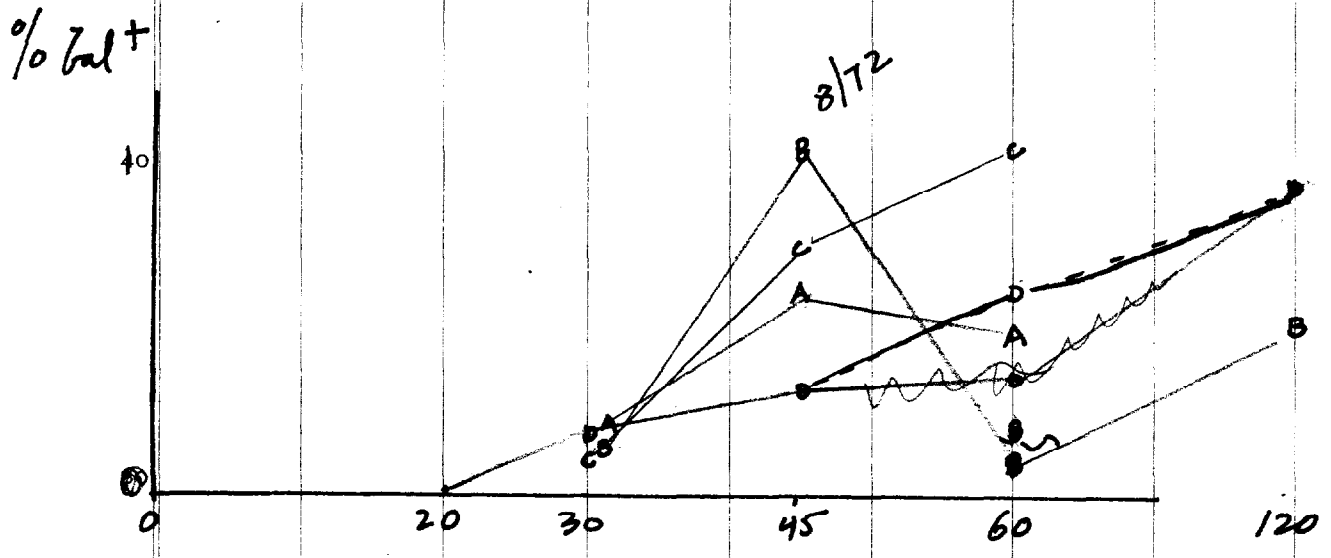
Parent cultures tested for purity : lac, Gal, Mal, Xyl

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 output of Hfr initial input.

DATE: REF: Gal+/total.

	1	2	3 Gal+	/ = % +	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 (10 sic.)	B	0 ×	54	—			0/54			0/92
	C	0 R	400	—						
	D	0 ×	38	—						
30	A	~~65~~ 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		47/720	8/72	47/580	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		47/940	1/47	56/520	2/32	3/79
	B	47 ~~[illegible]~~ ×	1	2						
	C	10.56 R	56	11						
	D	~~32~~ ~~[illegible]~~ ×	2	~~[illegible]~~ 6.5						
120							19/257	100/250	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~~ m blend with Virtis 30", plate 0.05 on 4 plates of minSt B, each for the first 3 samplings and 2 plates for the later samplings (from 10', 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

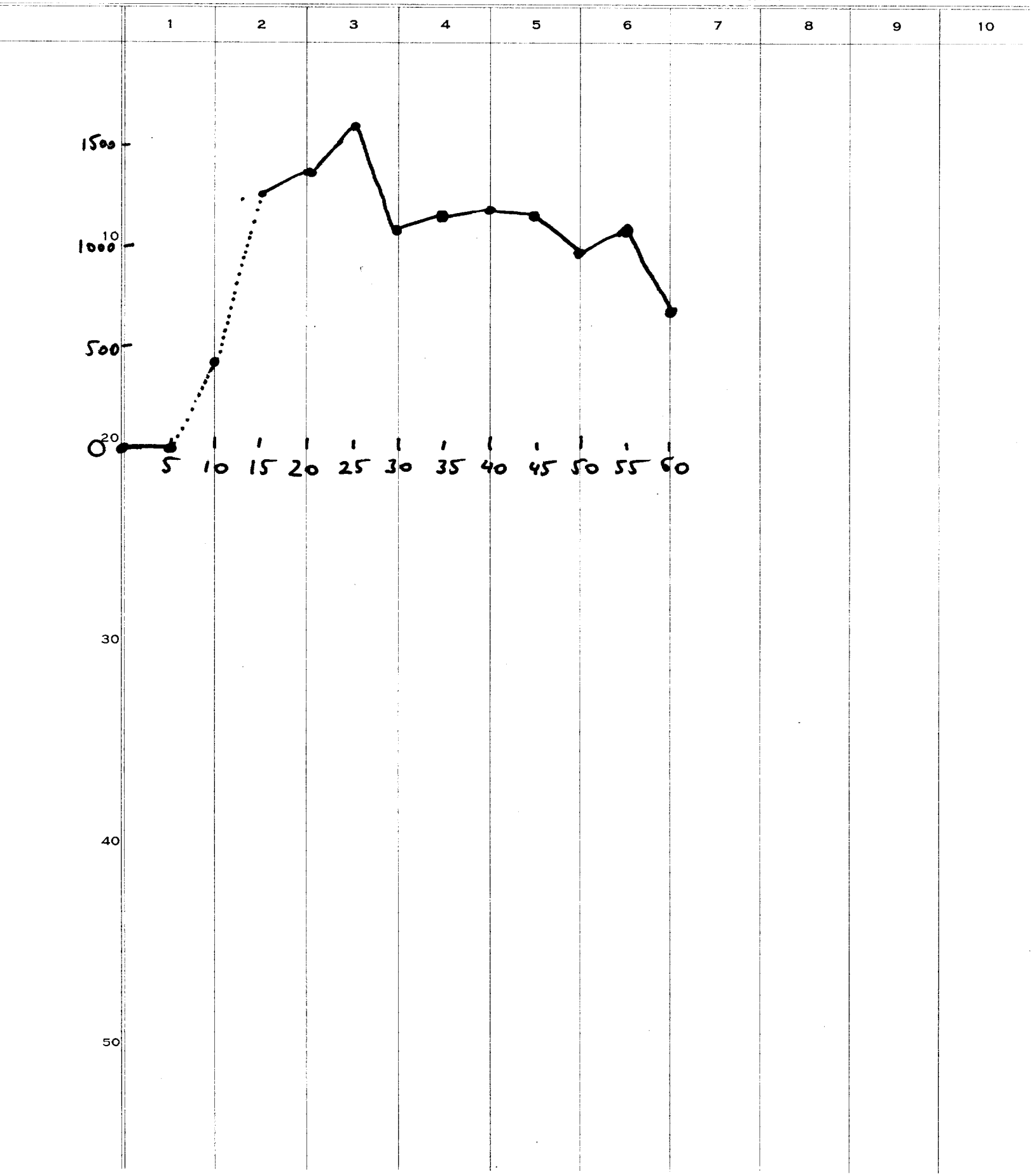
Also: microscopic counts of either parent (3060) & 1/5000 (3064) from first 1/100 dil, to which 4% of 10% formalin added.

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

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

Plate counts (28 hrs)

Time	StB.	~~Ave/ml.~~ Sum	No/ml	% Hfr input.	
0'	0,0,0	0	0		Same counts after 56h.
5'	0,0,0	0	0	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	
	Blac				
3060	18,18,24	60	400		
3064	71,87,97:	255	1700		

DATE:

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