Experiments on Interrupted Mating
of E. coli.

Collaboration with Bruce 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 REMB'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: $(Hfr \times F^-)F^+$; Holoclonal F^+; Enumerate Hfr mutants ; complementary crossing-over;

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

Index

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19 INDEX - 1 REF:

(1401) TIMED MATING WITH Hfr$_2$

(1). Non pulsed. Scored approx every 10'. TL+. Gal+, T$_1$
3000 x 3058

(2) Pulsed, scored every 5'. TL+ gal lac T$_1$
3000 x 3054

(3) Non pulsed. Scored every 5'. T+, TL+, Pr+, other markers. Gal lac [illegible]
3050 x 3052

(1402) PULSE

(1). 3050 x 3054 - Various dilutions [illegible] no [illegible] 50 [illegible] 1
Factor of decrease [illegible] 2x every 10 [illegible]

(2). 3000 x 3054 10x, 3x, 1x, dilution [illegible] after 1' pulse, then further [illegible] and 30' incub.
& plating - Effect noted may be due to [illegible] 30' [illegible]

(3) 3080, 1895 x 3004. pulse with 500 [illegible], 24, [illegible] and [illegible]
with diluted mating. negative effect of pulse.

(4) 3000 x [illegible] 3x, [illegible] [illegible] of 1' [illegible], [illegible]
after 4' pulse. No effect of pulse.

(5) 3000 x 3054 [illegible] same as 1401/2. Pulse 1' [illegible]
Pulse effective

(6) 3000 x 3004. Same as 1401/2, 1401/5 pulse [illegible] then transf. to 1' Pen
[illegible] comparison with undiluted. Very small effect of pulse. BSA

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INDEX-2

19 REF:

1	2	3	4	5	6	7	8	9	10

1403 ~~Mo3~~ MANIPULATIONS

(1) Negative: Hfr → F+.

(2) Effect of pipetting, 0.1 g 1.0 ml pipetting, [illegible], [illegible], [illegible] plating & flooding effective on interrupting. [illegible]

(3) Plating by spreading vs plating by flooding. [illegible] the former interrupts

(4) Stability of pairs to plating — no [illegible] recombinants obtained — 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	—	$Hfr_4\ Lp_S\ TL\ B_1\ S^R\ Mal_6$.							
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		$Lp^S\ Hfr_4\ I^-_{W677}$.							
2324		$Hfr_2\ B_1$							
2323		$M-St^R\ Az^R$							
3051		F- Lp^+ TLTh Lac, Gal, Xyl, Ara, $V_1 V_6 S^R$							
3064		F- Lp^+ TLTh 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- Hcl 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 F.							

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	1	2	3	4	5	6	7	8	9	10
1	W 3052			F- $4p^+$ Pr- TLTh Gal_2 Lac_1 Xyl_2 Ara_2 Mtl Sf^R.						
2				(W945)-						
3										
4										
5										
6										
7	W 2914			F- Pr. TLTh Lac_1 Gal_2 Ara_2 Xyl Mtl.						
8										
9										
0										
1	W 3934			Hfr_2 Gal_6 (from 2323 x 945)-						
2										
3										
4	W 3960			F- Try- S^R Gal_4- Gal_6-						
5										
6										
7	W	3994		F- $4p^+$ Gal_2						
8										
9										
0										
1	Lac	3994								
2										
3	W	3979		F- $4p^+$ Gal_2						
4										
5	W	3953		F- $4p^+$ Gal_2 Sf^R						
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										

1480 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 13 A, 14 A, 16 A, and slant. ~~made from agar~~

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

Control: old Isoleucine −

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

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Selection of new 3060.
Cross W2323 × 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										

(+) = complete

Sm R

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10^{-6} dil.

3060
Temperature — Plates

May 12, 1958

REF:

	1 (Strains)	2 (Plates)	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										
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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	Mtl		
1	+	+	—	+	+	—	+	—		
2	+	+	—	—	+	±	+ (±?)	+		
3	+	+	+	+	/	+	+	—		
4										
5		↓								
6										
7		NO!								
8										
9		Sm^R!								
0										

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3060's
Temp. Effect (cont)

May 9 19 58

REF:

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 ~ "same" amount of inoculum on DO & incubated @ 30 + 37 C.

	30 C	37 C
@ 20 Hrs:		
Large	Sparse growth in brush	aa
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 Lyophil) 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 for above was prepared from) grew very poorly - few large colonies plus few satellites. Both streaked on B lac (no morphological difference). Broth made from SC of each. Now test on DO + DO B1, both liquid + agar. Ditto 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	+	+	+	goodish
"	Small	.454	.620	+	+	+	sl
3060	Large	.527	.530	+	+	+	goodish
"	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, ~~+ B1 lac for lactose ferm.~~

Also DO B1 for auxotrophy.

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

plate means [illegible] long broth.

Count (?) before - ypd.

must be done on selective minimal

DATE: REF:

	1	2	B Gal	Lac	T5 1	6	7	8	9	10
* { Picked	0A *	16	all −							
	0C *	7	all −							
	5A *	26	all −							
	10A *		all −							
	10B *	6	all −							
	10C *	23	all −							
	10D *	6	all −							
	20A^R	(Replica plated to Gal) 1+								
	20B *	5~~5~~	−	all − ? [illegible]	scoring n.s.					
	20C^R	399	all −							
	20D *	38	−	−	ng.					
	30 B	(65)	1+	15 (incl Gal+) (α) ?	but all Lac+ are V_1^S					
	30 C^R	(551)	7+							
	30 A		8+							
	30 D	(48)	1+ also Lac+	16 (β)	~19 all Lac+ are V_1^S					
	45 A		47+							
	45 B	(72)	8+	[15++ 12±]	3 Lac+ are V_1^R			all Gal+ are Lac+		
	45 C	10×58	47+							
	45 D	(58)	2+							
	60 A	10×94	47+							
	60 B *1 }	(47)	1+ →	1+	1:34 V_1^R					
	60 C *2 }	(10×52)	56+							
	60 D		2+ →	2+						

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

DATE:

REF:

1	2	Bfal	Lac	5	6	7	8	9	10
120 B	(257)	19 +							
120 D	248	25+							
120 C	10x250	~100+							
120 B**	40 picked	6 + and +/-							

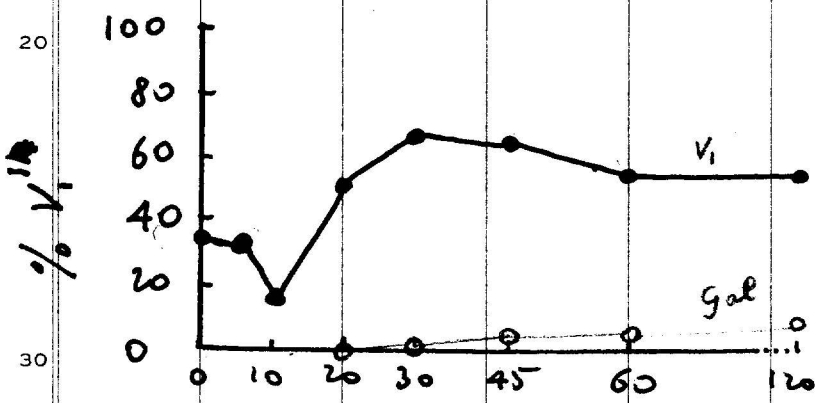
Notes on Lac: two batches of B Lac. - blue + yellow. Man F-ei
on blue, only Gal+ Lac+ show
no yellow at 1' reading.

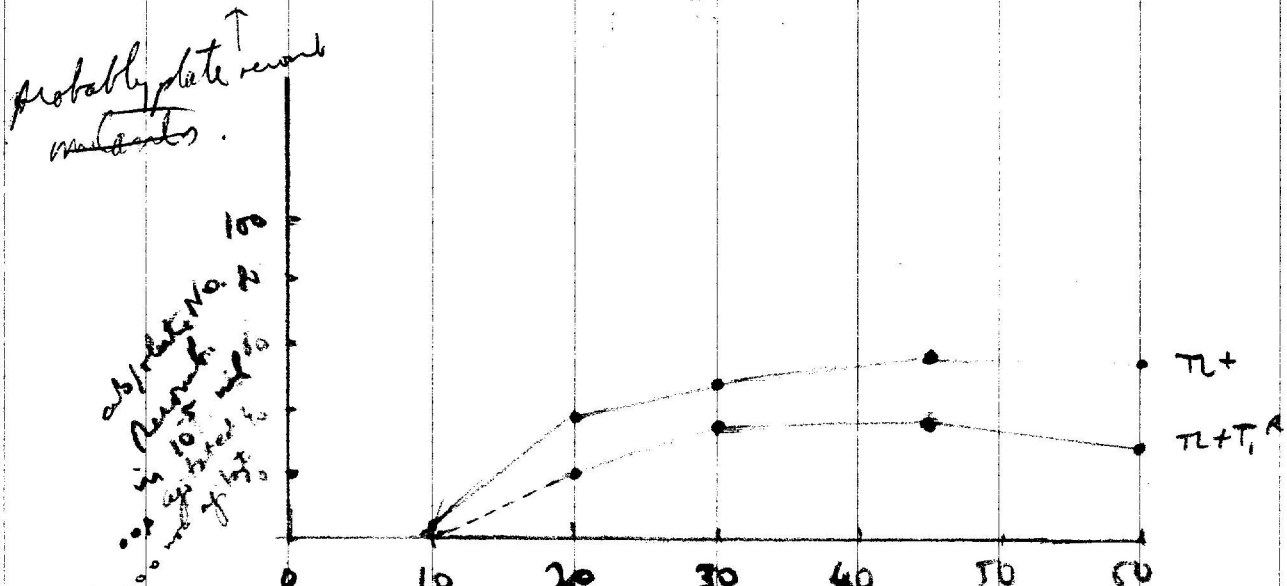
1/total V_1 segregation

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%		





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

Hfr₂

Overnight broth cultures:

\# 3060 Hfr₂ ; # 3064 : Falp⁺ TLB₁ Lac, Gal, Xyl, Ara, V₁V₆ S^R λ₂ 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.

2x10⁸
2x10⁶/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 B₁ c)

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 —

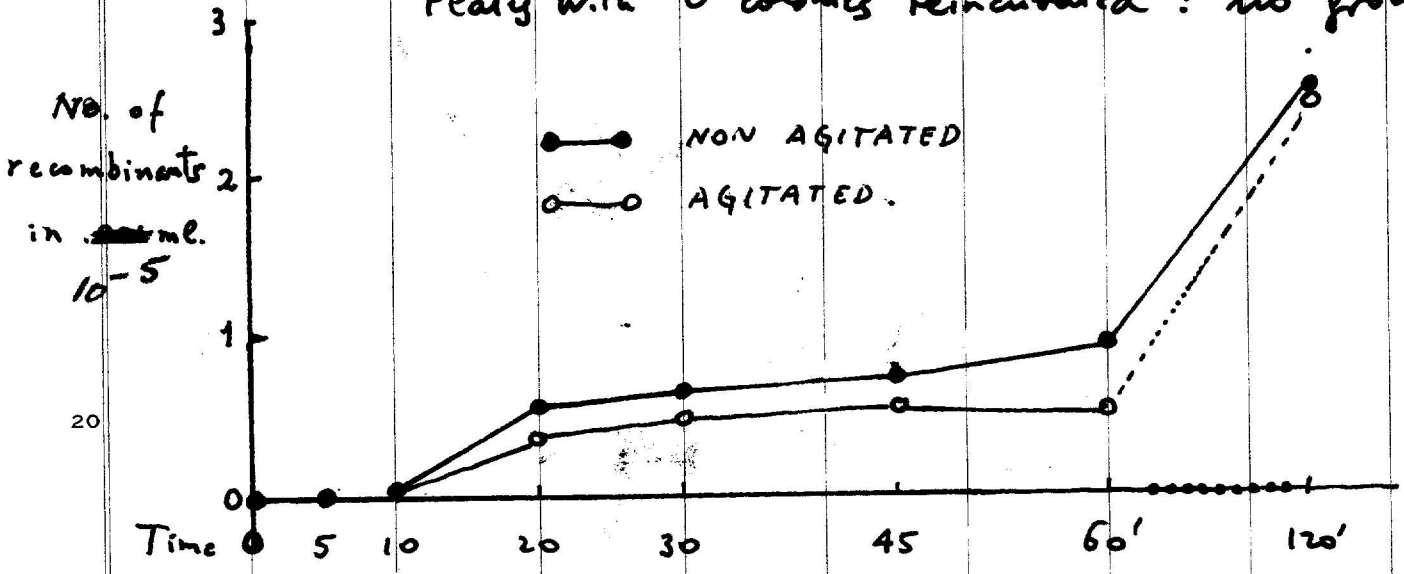
1401 b

[519].

DATE: March 23, 1958. REF: TIMED MATING

Time	0'	5'	10'	20'	30'	45'	60'	120'	9	10
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

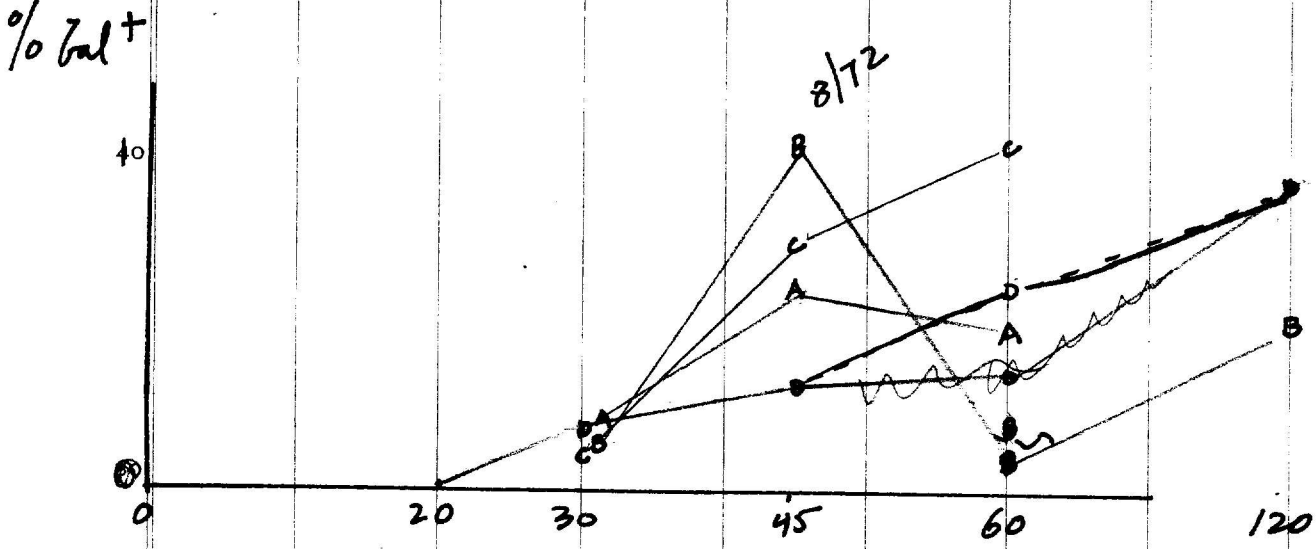
Hfr initial input : 2×10^6/ml. At 60' : 5% recombinants in respect of Hfr initial input.

1401c

DATE:

Gal+/total.
REF:

	1	2	3 Gal+/−%+	5	A	B	C	D	B+D
0	A	0 ×	16 —						
	C	0 ×	7 —						
5	A	0 ×	26 —						Gal+/to
10	A	0 ×	63 —			0/0.3			
	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	~~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						
60	A	10.9 R	47 5		47/94	1/47	56/52	2/32	3/79
	B	47 ×	1 2						
	C	10.5 R	56 11						
	D	~~32~~ ×	2 ~~3~~ 6.5						
120	B	257 R	19 7.4			19/257	100/250	25/248	44/505
	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 min St B, each for the first 7 samplings and 2 plates for the later samplings (from 20', 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 min St-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,	~~Avirulent~~ 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	
3060	Blue 18,18,24	60	400		
3064	71,87,97:	255	1700		

DATE:

REF: 1401.2