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19 REF: 1413/10

Counts on D(fun B_1)

	A	B	C	D
10	170	0	112	3
20	~5000	21	~10'000	67
30	~12000	128	~2·10⁴	167

B(Gal, fun)

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

Gal + is isolated and tested

% from D(fun 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	

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19 REF: 1413/10

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

	A30	C30
T_1^1	20/30	36/45
I_{ep}^1	16/30	35/45

No clearcut difference in contribution from ♂ parent between A and C, i.e. genes pretreated and non pretreated with ♀'s.
~~[illegible]~~

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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) ~~Dilute~~ .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~~: ~~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.

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19 May 1st 1958 REF: 1414

Protective blending.

3060, 3064, conc. 3 x and 30 x 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!

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Superfetation by F^+

1414C.

Plan 6 May 1958.

REF: B

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

at t=12 A. Blend sample and plate (plate = 1:100 dilns. in chilled water, spread .0.1 ml DTh mm (no entry of bac) plate-ice.)

B. Reincubate and plate at t=60. (successful recomb.)

C. To W3064 dil. add W1306 Plate at t=60 W1306 = F^+ M V_1 V_6. make s.c.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)

Re entry stimulated by $F^+ \sigma\sigma$?

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1414B

3 May 1958 REF: 1414 see C

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

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

BGA to t=12 m. 37°

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

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

C. ~~W3870 s~~ W3064 s/ σ W3870. Add W6 at t=12. Plate at ~~t=24~~ 22, ~~t=32~~.
(check W6 x W3064 crossing)

D. A + W6 (= .5 ml 10 x 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 Hfr x 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) prefertilization 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 protrophs.		✓
D0:	34, 37 No Lac+	D20: 59, +	1 Lac++; 13 probable Lac+ Gal−.

∴ A shows early TL+ B full entry D is effect of reentry of Lac.

Replica plate to S Lac B1: only ~10% Lac+? May be counting Gal+.

Remembrate. An effect is indicated but the objection may still hold that further mating has taken place. Lac scoring mg. Stroke out! cf 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 = 5/70

May 7 19 58

REF:

	1	2	3	4	5	6	7	8	9	10
		LAC			LAC			LAC		
1		all			all			—		
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

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			a LAC		
1		–			all –			–		
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 - versus D20 shows Lac entry. Is this due to

(1) remating of Hfr × F^- despite blending + dilution
(2) $F^+ \times F^-$ owing to prefertilization of F^-
(3) Resumption of pairing in interrupted Hfr × F^- under influence of F^+.

suggest: use W1306 ($M^- F^+ V_1^r V_6^r$) 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 record: 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 frig: 2 ml + 7.5 ml Pen 1h not ; spun, conc 3x in Pen
W 3064 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 each chilled tube (Pen / BGA) to 0.1 ml of Azide conc:

	0	1/10000	1/3000	1/1000	1/301	1/100	final conc 1/10 of that indicated
final	0	10^{-5}	10				
called	0	5	4	3	2	1	fr

then incubation resumed for 60'. Plated, 0.05 on mm B, Gen

Counts			10^{-3}	$3 \cdot 10^{4}$	10^{-4}	$3 \cdot 10^{-5}$	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

19

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
and add to 0.9 ml of mating mixture. 0.05

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	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+/tot	Lac+/tot	T1+/tot						
3										
4										
5	A	0/50	1/50	21/50						
6										
7	B	0/50	9/50	33/50	DNP					
8										
9	C	1/50	15/51	30/50						
10										
	D	0/50	0/50	6/50						
1										
2	E	0/50	0/50	11/50	Azide					
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										
10	X	9/50	19/50	26/50						
1										
2	A2	6/50	20/50	31/50						
3										
4	B2	5/50	17/50	33/50	DNP					
5										
6	C2	10/50	21/51	30/50						
7										
8	D2	1/51	12/50	34/50						
9										
10	E2	0/51	17/50	32/50	Azide					
1										
2	F2	5/50	23/50	31/50						
3	G2	9/50	28/50	31/50						
4										
5										
6										
7										
8										
9										
10										
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										

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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): no.s 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 cultures, 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(Sm B_1). Same for 1/10 dilution from the latter –

5. Plate recombinants

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

Plate count

	1	2	3	4	5	6
			Azide 0'	Azide 5'	Azide 12'	
		1	2	3	4	5
A	∞	1	45	211	(24)	
B	∞	3	73	271	18	
C	∞	7	112	359	2	
D	∞	16	254	~440	3	
A''	(78)	0	0	19	miss	
B''	(140)	0	2	-		
C''	(140)	0	13	13		
D''	(96)	0	8	26		

x factor 10..

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19 REF:

	1	2	3	4	5	6	7	8	9	10
	Bact. \ Ph	λ	λh	$\lambda \bar{v}_1 \lambda_2$	$\lambda \bar{v}_2 h$					
	Lp^s	S	S	S	S					
	Lp^r	R	R	S	S					
	Lp^h	R	R	S	S					
	$Lp(H)^s$	R	S	R	S		isolated once only			
	$Lp(H)^h$	R	R	R	S					
Lp^d	$Lp_{R_1}^c$	R	R	S	S	= ~~Lp~~ [illegible] of defect on lambda phage.				
	Nal_r	R	R	R	R	$= Lp_2^R$				

DATE: 1 ml 3514/ft^R Rotated cult., exponential. + 10 ml ~~[illegible]~~ -

	1	2	3	4	5	6	7	8	9	10
	Double strength L_2	1) 100 u/ml 1/1 protoplasts and rods. Turbidity higher than ~~at~~ 2 and 3.			2) 300 u/ml all protoplasts almost no rods. Turbidity higher than in 3			3) 1000 u/ml all protoplasts. turbidity low ~~[illegible]~~		
10	Single strength L_2	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 zero ~~[illegible]~~		
20										
		Use 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 W3060, 3064, 3947, 3935 -
Spun and resuspended in dilled ~~fed~~ ~~[illegible]~~ 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, platings of 0.05 on D(Sm B1) -

	1	2	3	4	5
A	0	0	7	51	38
B	0	1	6	18	44
C	0	1	43	108	~400
D	1	10	41	118	318

No discrimination.

DNP.

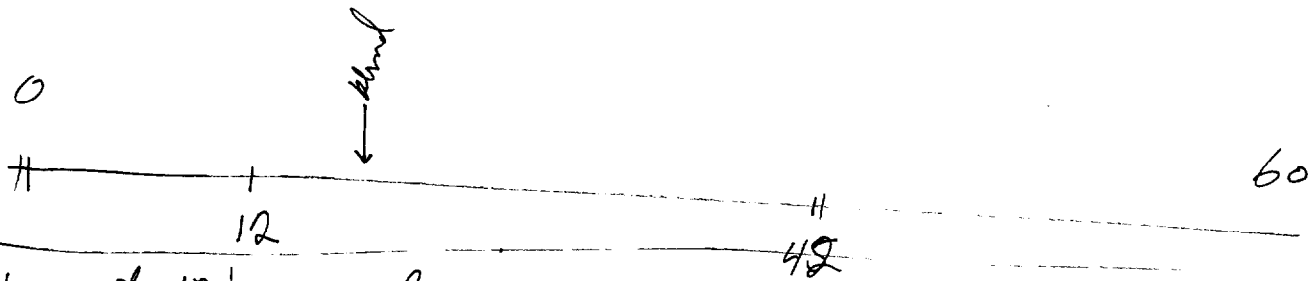
14/15

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 $10^{\underline{55}}$

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

plate A

plate B at $12^{\underline{15}}$

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. (One 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^{03} PM warmed.
at 10¹⁵ chilled and add 1 ml of inhibitors. - Warm and incubate ~~+2 minutes~~.