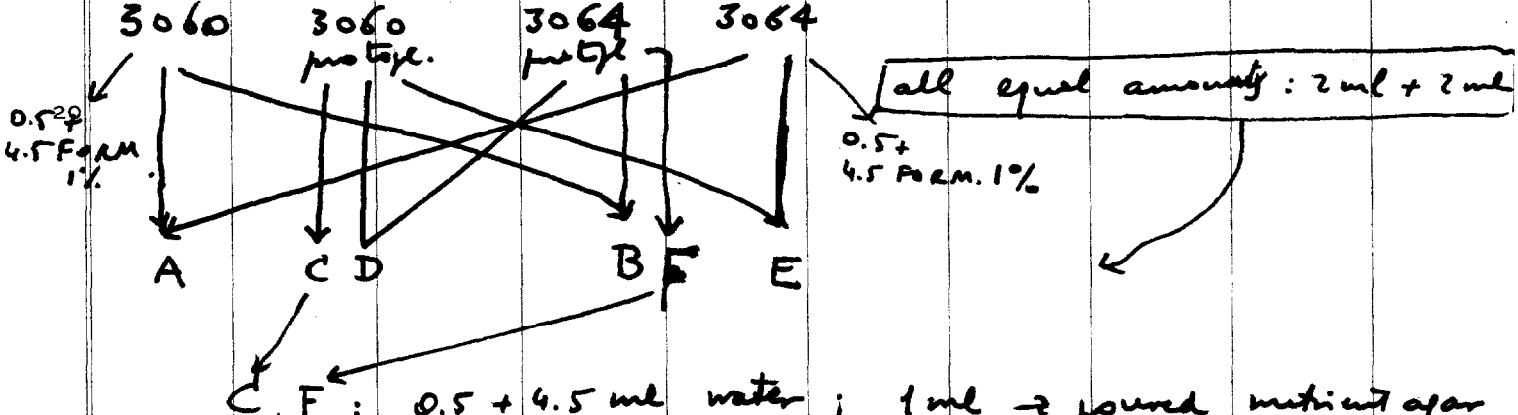
1405-II

DATE: March 26. REF: PROTOPL. 9'

12.20 3060, 3064 cult. → rotator 1+10 ml (from rotated cult.)
Note: same culturing used as for exp. 1403: F+ reversion of 3060.
13.20 1 ml + 9 ml L_2 + 10^4 penicillin → protoplasts
(5 ml.
15.50 Centrifuge, resuspend protoplasts in L2 medium without penicillin

Also 14.50 3060, 3064, 1+10 ml to rotator.
15.50 Centrif., resuspend in 5 ml L_2 medium.



C, F: 0.5 + 4.5 ml water; 1 ml → poured nutrient agar.

16.40 A, B, D, E: 30' rotator → 0.5 + 4.5 ml water → minSt B_1
→ 0.5 + 4.5 ml Sucrose → agar Sucr. M_1.
60' " → Same.

0.5 ml. residual of protoplast susp: 0.05 ml Form 10% added.

Microscope counts:
3060 : 400×10^6/ml
3064 : 940×10^6/ml
3060 protoplasts: 2.2×10^7/ml - no rods.
3064 " 2.7×10^7/ml "

3060 protoplasts: agglutinated before centrif. After resusp. centrif., pipetting up and down releases agglutination.

DATE: 3/29/58 REF: 1405-II

Counts:	30'		60'	
	Sucro/e	B,St	Sucrose	B,St
Exp. A	15	33	54	66
B	0	0	0	0
D	0	0	0	0
E	0	1	1	1

Pooled agar plates from C' (3060): 278.
F (3064): 72.

Conclusions: 3060 has reverted to F+. The same single colony isolate here was used as in Exp. 1403. Hence low numbers of recombinants.

Proportion of viable cells in protoplast preparations:

3060: 1.3×10^{-5}.

3064: 0.3×10^{-5}.

DATE: 4/14/58. REF: 1405 - III

PROTOPLASTS.

Exp 1405-I is repeated, but after bursting in DW, mixtures are either plated or kept in the waterbath until completion of 60' since the beginning of the expt. In addition, a pulse of 10' is given to the mixture.

9.05 am. 1 ml 3060 overnight aerated + 9 ml broth → Rot.
10.30 1 ml 3060 expon. + 9 ml I_2
12.10 coarse granular growth. Agitated, 10^3 Pen U/ml added.
14.40 centrifuged.

Also: 14.20 3064 2 ml + 10 ml Broth.
14.40 Centrifuged.

3060 ♂, 3064 resuspended in 1.2 + 1 ml I_2; = 10 ×
0.3 ml in water bath.

3060 ♂ 10 × → 0.1 ml + 0.9 ml DW → 0.1 on B Lac.
→ 0.1 + 1.9 ml formalin 1% → Count (all burst out?)

3064 10 × → 0.5 } X
→ 0.5 }
→ 0.1 + 1.9 ml formalin 1% → Count = 9×10^8/ml

X and ♂ (diff in I_2 of 3060 ♂) incubated in waterbath for 10', then: diluted 20 × with I_2 medium warmed.

(X) → 0': 1 ml + 9 ml DW → plate 0.05 and 0.01 on minSt B_1.
↓ + 10 ml broth, waterbath. (= 0/60') → plate 0.05 / 0.01
10' waterbath: same as above → = 10
20' " " = 10/50.
30' " "
45' " "
60' " 1/10 DW → = X-60 (pl. 0.05, 0.01)
→ + 10 ml broth → = 60/0 (pl. 0.05, 0.01)

DATE: REF: 1405-III

1 2 3 4 5 6 7 8 9 10

3 - Counts of viable cells in protoplast suspension:

From suspension obtained after centrif. -

0.1 + 0.9 DW, → 0.1 EMB lac plate. 246 col.

From same susp. dil 40× in I_2, → 0.1 EMB lac. 4 col.

↓

30' in cub w. b. → " (dried up)

↓

60' " → " . 67 col.

The initial No. of cells which were protoplasted is unknown. but is of the order $2-5 \cdot 10^8$/ml. Hence fraction surviving is of the order 10^{-5}.

—

	0	10	20	30	45	60	minutes
	X-0	X-10	X-20	X-30	X-45	X-60	
0.05 ml	0	191	∞	∞	∞	∞	
0.01 ml	0	41	362	~800	~~920~~	760	
	0/60	10/50	20/40	30/30	45/15	60/0.	
0.05 ml	11	178	∞	∞	∞	∞	
0.01 ml	0	21	244	434	~900	609	← should be multiplied ×2 to give figures comparable to X

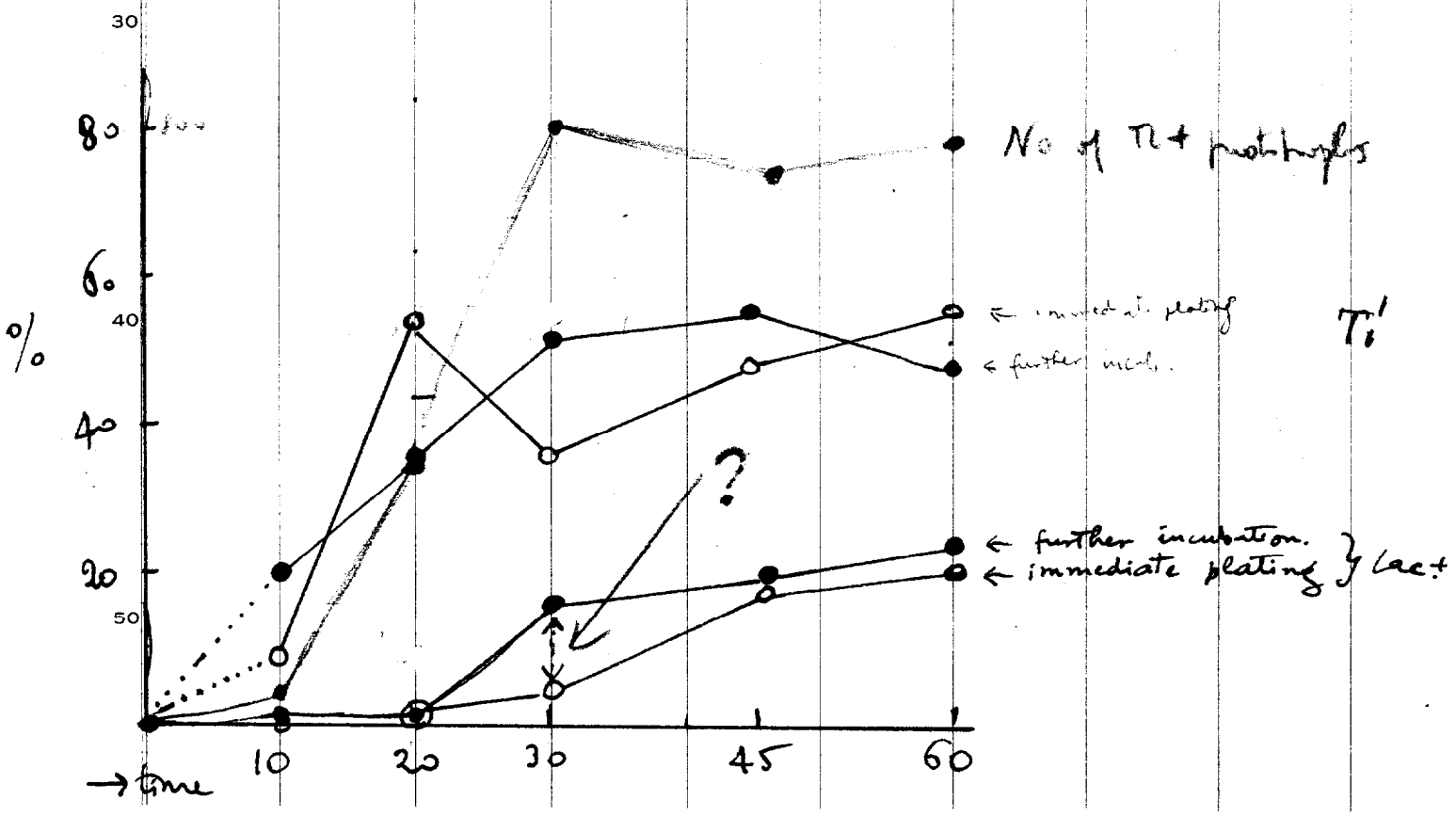
addition of water

Before | After 4/17/58.

REF: 1405-3.

TIME →	0/60	10/50	20/40	30/30	45/15	60/0
Gal+	0/12	0/71	0/50	0/50	1/50	1/50
Lac+	4/12	2/71	1/50	8/50	10/50	12/50
T_1'	8/12	7/71	18/50	26/50	28/50	22/50

	X-10	X-20	X-30	X-45	X-60.
Gal+	0/90	0/50	0/50	2/50	0/50
Lac+	0/90	1/50	2/50	9/50	10/50
T_1'	4/90	27/50	14/50	24/50	29/50.



DATE: April 14. REF: 1405 - IV

Repeat of 1405-II: Crossings between protoplasts and non protoplasts.

10.30 a.m. 1 ml 3060, 3064 + 10 ml L_2.

11.50 { 3060 poorly grown, agglutinated.
3064 well grown, 3-500·10^6/ml.

Penicillin 10^4 units added.

13.35 cultures of 3060, 3064 started: 1 + 9 ml broth

15.00 3060, 3064 cultures and 3060, 3064 protoplasts centrifuged, resusp in L_2: 3064 & 3064 ♂ 10 ml, 3060 ♂ 2 ml, 3060 5 ml.

15.35 Cross:

	A	B	D	E
3060	0.5	0.5	–	–
3060 ♂	~~[illegible]~~	–	0.5	0.5
3064 ♂	–	0.5	0.5	–
3064	0.5	~~[illegible]~~	–	0.5

– water bath. Algo:

Viability Test { C: 3060 ♂: 0.1 ml + 0.9 water → 0.05 B Lac, → 0.01 B Lac
F 3064 ♂ " " "

16.40. From water bath, A, B, D, E:
0.5 + 4.5 ml water → .05 → 1/10 DW → .05 pl (1/10
min St B,

Algo, on agar sucrose: A, B, E:
0.05 from test tube
0.05 1/10 dil. in L_2
0.05 1/100 dil in L_2.

D: 0.05 from test tube.

DATE: REF: 1405 - IV.

Viability tests: C 0.05 44 col 3060 ♂:
0.01 13 "
F 0.05 168 3064 ♂.
0.01 145. ?.

Microscopy: C 2-3 protoplasts per small square, many of which ghosts. = $5 \cdot 10^7$/ml.
F 8-9 $160 \cdot 10^6$/ml.

3060 ♂: Viable $2 \cdot 10^{-4}$;
3064 ♂: " $2 \cdot 10^{-4}$.

		♀♂	♀♂, ♂♂.	♂♂
	A	B	D	E
Sucrose B_1				
1/1	∞	∞	~1000, small	∞
1/10	∞	∞ irreg	not met	∞
1/100	2-5000	∞ irreg	v.v.	~500
fruc B_1				
1/1	∞	32	2	~2000
1/10.	~2000	0	0	162

Note. 3060 is not prototrophic on fruc B_1, therefore crosses on fruc B_1 are valid

DATE: 4/19/58 REF: 1405-4

Summary of Segregations.

♂	♀		min Sm B_1 gal+	min Sm B_1 lac+	min Sm B_1 T_1^r	sucrose B_1 gal+	sucrose B_1 lac+	sucrose B_1 T_1^r
–	–	A	0/50	8/50	24/50	26/50	22/50 ⊖	
–	♂	B	2/50	10/50	27/50	~~24/50~~	–	
♂	♂	D	–	–		24/50 ⊖	28/50 ⊖	
♂	–	E	2/100	13/100	58/100	0/50	9/50	26/50

⊖ many mixed.

Also:

From D: Streaks on EMB Lac.

Later note: W3060 showed erratic growth on B_1 mediums. ? question of a usable auxotrophic marker in this strain. Should be surveyed in the fall.

6.29.58

DATE: PROTOPLAST CROSSES. REF: 1405-5

Repetition of 1405-4.

3060, 3064 ♂ as in experiment 1409-2, but diluted 5× with L_2. 3060, 3064 rods in L_2, 2× concentrated, from overnight rotated cultures.

	♂	♀	Plated on Suc B_1	Suc B_1 St	B_1	B_1 St
M	♂	♂	1/1 0.02 ; 1/10 0.02	1/1 0.02	1/1 0.05	1/1 0.05
N	♂	Normal	1/100 0.02	1/100 0.02	1/100 0.02	1/100 0.02
O	Norm	♂	1/1 0.05	1/1 0.05	1/1 0.05	1/1 0.05
P	Norm	Norm.	1/100 0.02	—	1/100 0.02	1/100 0.02

Mixtures for crosses set up in equal amounts at 12.20. After 60' incubation in water bath, dilutions (with L_2) and platings.

Viability test of protoplasts: suspensions employed in cross diluted 1+10 ml H_2O, → 0.01 / 0.05 B lac.
(2 hrs after preparation of suspensions)

← Drop of flamed alcohol

Counts: 0.01 # 3060: 70 col
0.01 # 3064: 136 col.

Microscope counts on protoplasts: (after stay at room temp.): ♂ 3060: $52 \cdot 10^6$/ml (but many rods!)
♂ 3064: $392 \cdot 10^6$/ml no rods.

Viability of protoplasts: $1.6 \cdot 10^{-3}$ for # 3060 ♂
$0.3 \cdot 10^{-3}$ for # 3064 ♂.

(after a 2 hr stay at room temperature; rods may have increased)

DATE:

REF: 1405-5

	1	2	3	4	5	6	7	8	9	10
		Plate counts.								
♂ ♀		Sucrose B_1			suc B_1 St		min B_1		min St B_1	
♂ ♂	(M)	0.02 : ~2000			0.02 : ~800		0.02 : many small and 158 large		0.02 : many small col.	
		$\frac{1}{10}$ 0.02 : 172 + many smaller colonies.								
♂ normal	(N)	302			374		255		314	
Norm ♂	(O)	10^4 ?			10^4 ?		~1500		~500 large + many small ones.	
Norm Norm	(P)	~3000					~3000		~2000.	
Controls:	3060 ♂	many small ones (~2000).								
	3064 ♂	no growth. except for 1 colony								

Picking

DATE: 4/19/58. REF: 1405-6

Repeat of exp 1405-3 to test further difference between immediate and non immediate plating on Lac at 30' (see question mark in previous graph).

3060 σ, resuspended in L_2 10 x conc.; pure with 3060 overnight rotated culture.

Mixture in equal amounts. Also, viability test of 3060 σ: DW 1/10 → 0.02 on EMB Lac (465 colonies counted).

From 3060 σ x 3064, 10' pulse then dilution 1/100 in prewarmed L_2, and further incubation in w.b. for 25, 30, 35'.

After such times dilution in DW 1+10, incubation for 10', then addition of 10 ml Penassay 2X and:

A) immediate plating

B) further incubation for until completion of 65' from end of pulses, then plating.

Platings: 0.1 & 0.02 on min St B_1.

By mistake, 35' tube has been kept at room temperature.

	25'		30'		35'	
	A NON INC.	B INC.	A NON INC	B INC.	A NON INC.	B INC.
0.1	141	88	194	146	261	232
0.02	23	10	27	19	46	35

DATE: 23/4/58. REF: 1405-6

SEGREGATIONS.

	25'		30'		35'	
	NON	INC.	NON	INC	NON	INC.
gal+	0/100	0/100	0/100	0/100	0/100	0/100
lac+	2/100	0/100	3/100	4/100	7/100	8/99
T_1'	25/100	38/99	33/100	37/100	39/100	54/100

DATE: 4/23/58. REF: 1405-7

OSMOTIC SHOCK ON RODS

3060, 3064 O.r.c. resuspended in 5× conc. with T_2 medium.

Mix in equal amounts for a 5' pulse in water bath.

Dilute 1/100 in T_2 medium, warmed.

After 10'; 20'; 30' dilute 1/10 in DW, incubate 5', add 2× Penassay in equal amounts and:

A) plate 0.05 on min StB_1

B) incubate further until completion of 50' since dilution in T_2 1/100, plate 0.05

Plate counts:

1-2000 colonies in each except for 10' A (~100) and 20' A (~300).

	Gal	Lac	T_1
10' A		1/50	
B		22/50	
20' A		0/50	
B		11/50	
30' A		4/50	
B		13/50	

DATE: REF: 1405-8

CROSSES 8 × 8.

2323, 3064 protoplasts 2h30' incub. (1 ml + 9 ml from o.r.c.).
Resuspended in L_2 medium at 10 × conc.
~~0.5~~ Mixed equal amounts, then diluted 1/100 after 10' pulse in L_2 medium. Incubated 50', then spread on min B_1 & sucrose B_1:
0.05 from undil. & 1/10, 1/100, 1/1000 and 1/10.000 dilutions in L_2 medium

From parental suspensions: → 1/10 dist. water → .05 B lac
→ 1/100 L_2 → .05 on Suc B_1
↓ 1/100 L_2
↓ 1/10 L_2 → .05 sucrose Penassay
↓ 1/10 L_2 → same.
and microscopic counts

Microscope counts: 2323 ♂: 4.2×10^9/ml.
3064 ♀: 3.2×10^9/ml

	10^{-5}	10^{-6}	
3064	107	15	$2 \cdot 10^8$/ml viable
2323	320	10	$= 6 \cdot 10^8$/ml viable

Viability tests: 3064 ~ 10.000
2323 ~ 3600

Crosses: Controls: 2323: clean
3064: many small colonies.

Crosses: as control 3064.
100 bigger colonies in undil.
10 " " 1/10.

Discarded.

DATE: 4/15 CULTURE PHASE. REF: 1406

1	2	3	4	5	6	7	8	9	10

Photometer, 650 mμ

9 a.m. 3060, 1 ml + 10 ml. broth 0.29

3064, 1 ml + 10 " 0.37
0.5 " " 0.32
0.2 " " 0.20.

10 a.m. photometer readings as above.

Algo: Saturated (overnight aerated) broth cultures:

3060 Sat 0.51

3064 Sat. 0.80.

All broths taken reduced to density equal to that of 3060 exponential, by dilution with broth. (3064 Exp. 0.5 ml + 10, dil 8 ml + 2 ml);
3060 Sat 5 ml + 6 ml broth, 3064 Sat 3 ml + 15 broth).
Mixtures in equal amounts, 2 ml + 2 ml to waterbath:

	3060 Sat	3060 Exp.	3064 Sat	3064 Exp.
A	×		×	
B	×			×
C		×	×	
D		×		×

10' pulse, then dilute 1/100 warm broth; → .05 min St B,

↓ stay in waterbath 50' → 1/10 Dw, 1 ml flooded St B, plates.

0.05 min St B,

1/10 Dw → 0.05 "

0.01 except for A.

DATE: 4/17/58. REF: 1406

After 10':

		A	B	C	D
0.05		482	293	482	320
flooded with 1 ml of 1/10 sil.		~2000	~2000	~2000	~2000

After 60'

0.05		many	many	many	many
1/10	0.05	288	~300	~500	~500
"	0.01	not done	59	91	113

used for picking and typing.

DATE: CULTURE PHASE 4.19.58 REF: 1406

		A	B	C	D
	♂	1	1	exp	exp
	♀	1	exp	1	exp.
10'	Mal+	0/50	0/50	0/50	0/50
	Gal+	0/50	0/50	0/50	0/50
	Lac+	1/50	0/50	1/50	0/50
	T_i'	12/50	9/50	10/50	8/50
60'	Mal+	0/50	0/50	0/50	0/50
	Gal+	6/50	5/50	2/50	6/50
	Lac+	20/50	16/50	12/50	21/50
	T_i'	30/50	30/50	22/50	29/50

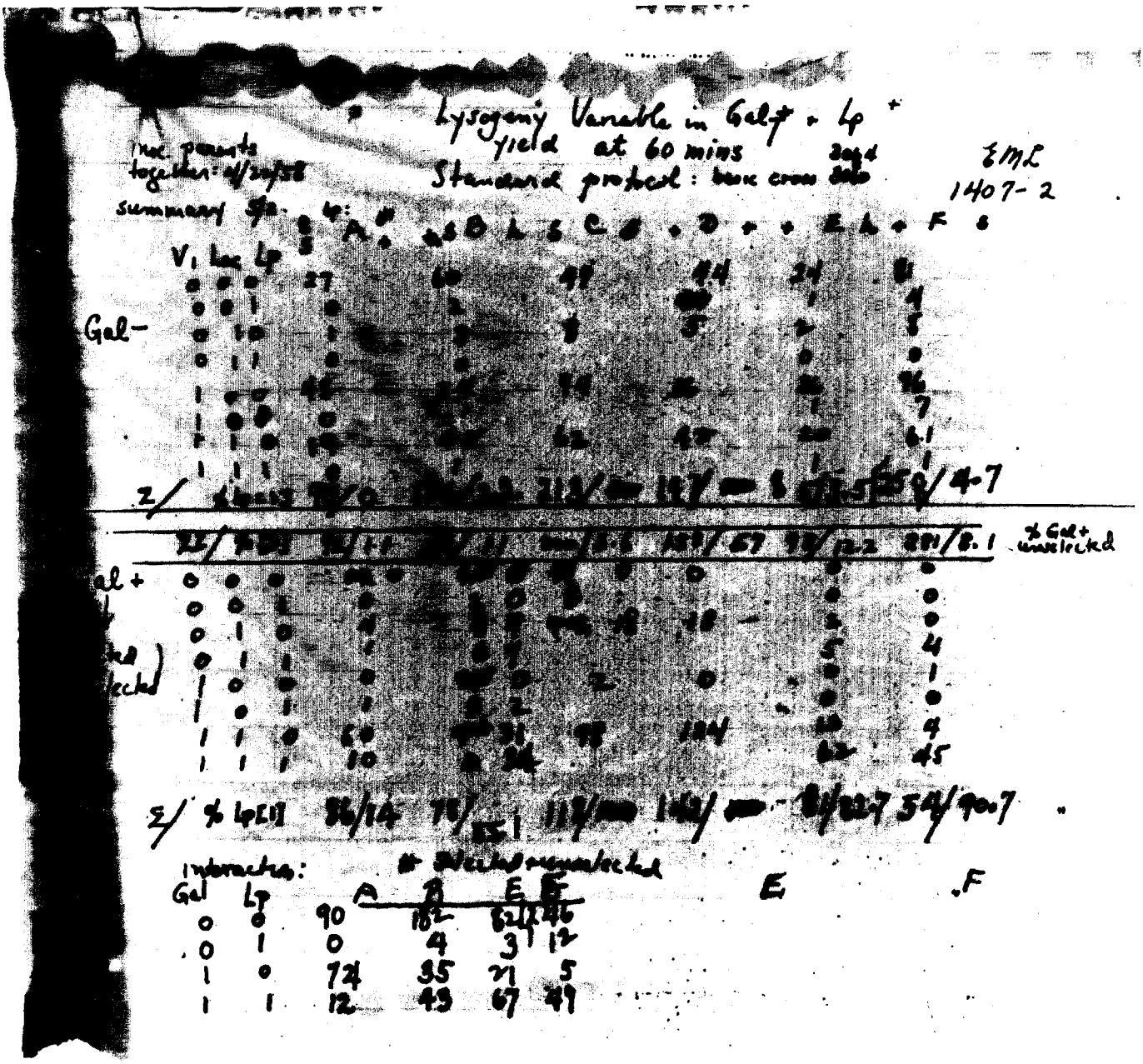
DAT

LEDERBERG, ESTHER M., University of Wisconsin, Madison, Wis.--Fine structure of the Gal loci in Escherichia coli K-12.--A distinctive recon (one of a group of closely linked loci separable by recombination) has been assigned to each of ten independently occurring Gal^- (galactose nonfermenting) mutants. All the mutants were obtained after UV irradiation except Gal_3 which was spontaneous. Allelism was tested by large scale matings and by transduction analysis.---Prophage-linked transduction via HFT λ, where practically every phage particle may result in a transductional event, produced heterogenotic clones when donor and recipient bore distinctive recons. The heterogenotes were unstable, segregating the two input and two crossover classes (Morse, Lederberg and Lederberg 1956). Two cistrons (cis-trans position effect groups) had been demonstrated: trans heterogenotes with members of the same cistron are phenotypically mutant. Galactokinase was missing in mutants of one cistron (Gal 2,8) while UDP transferase activity was absent in the other (Gal 1, 4, 6, 7), a defect corresponding to human congenital galactosemia, (Kalckar, Kurahashi 1957). Gal_3^- and Gal_9^- are genetically cistronic with both of the foregoing groups; they have not yet been successfully analysed for their enzymatic defect. Among two hundred new mutants, no recons identical with the first ten have recurred. Most have been assigned to either of the first two cistronic groups, but a small number which would represent a fourth genetic group have given only normal galactose-positive heterogenotes with every standard Gal^- tested. A few mutants were not transformable by λ but were found to carry other modifiers including that of hexose metabolism which obscure their relationship to the Gal^- group.---An attempt was made to map the Gal markers in linear sequence by their relationship to the Lp locus. Intercrosses of Hfr M^- Gal_x^- x F^- Gal_y^- were made on a medium which selected efficiently for $M^+Gal_x^+Gal_y^+$ recombinants, and these were then scored for the segregation of an Lp marker. However, the linkage of Gal-Lp proved to be not close enough to compensate for the perturbations of segmental loss from the Hfr parent, and the data were indecisive.

10th International Congress of Genetics, Montreal, 1958.

9 10

1407-2
EML



Lysogeny Variable in Gal+ + Lp+
yield at 60 mins
Standard protocol: basic cross 3064 3060

Inoc parents together: 4/22/58

EML
1407-2

summary 5/2 Lp: A B C D E F

	V1	Lac	Lp	A	B	C	D	E	F
Gal-	0	0	0	27	60	49	44	24	81
	0	0	1	0	2		[illegible]	1	4
	0	1	0	1	[illegible]	8	5	2	5
	0	1	1	0	0			0	0
	1	0	0	45	[illegible]	94	[illegible]	[illegible]	96
	1	0	1	0	[illegible]			1	7
	1	1	0	[illegible]	[illegible]	62	42	[illegible]	61
	1	1	1	[illegible]	[illegible]			1	1
Σ/	% Lp[illegible]			[illegible]/0	[illegible]	213/[illegible]	197/[illegible]	[illegible]	[illegible]/4.7

	Σ/	% [illegible]		[illegible]/1.1	[illegible]/11	[illegible]/3.6	159/57	98/12.2	281/8.1	% Gal+ unselected
Gal+	0	0	0	[illegible]	[illegible]	[illegible]	0	0	0	
	0	0	1	0	[illegible]	[illegible]			0	0
	0	1	0	4	[illegible]	[illegible]	18	18	2	0
[illegible]ted)	0	1	1	1	[illegible]				5	4
[illegible]lected	1	0	0	0	[illegible]	2	0		0	1
	1	0	1	1	[illegible]	2			0	0
	1	1	0	60	[illegible]	31	98	124	[illegible]	4
	1	1	1	10	[illegible]	34			62	45
Σ/	% Lp[1]			86/14	78/[illegible]	118/[illegible]	142/[illegible]	81/22.7	54/90.7	

interactions: # selected+unselected

Gal	Lp	A	B	E	F
0	0	90	182	82	46
0	1	0	4	3	12
1	0	74	35	21	5
1	1	12	43	67	49

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GENETICS

19 May 7th, 1958 REF: 1408/2

W3908 (F-Lp+ tryp Gal6 S^R)
W3870 Hfr2 B1? Lp^R.

o.r.c. : wl: 0.5 ♂ + 1.0 ♀ – incubated for 1hr.
Plated on S Gal Sm B1 ; 0.05 undil, 1/10, 1/100
1/1000, 1/10.000

Controls : 0.05 of undiluted cultures.

After 40hrs : undil : > 1000, Gal + 4 Gal –
1/10 : 56 Gal + 70 Gal –
1/100 : 4 Gal + 3 Gal –
1/1000 : 0

Controls : W3908 : 3 col.
W3870 : 0 col.

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19 May 10th 1958. REF: 1408/3

Gal - Try linkage.

♀ 3908 s^R Gal_6 try - $4p^+$.

♂'s: 1 W3367 $4p^R$ M-

2A: W3060 + . Th?

2B: W3870 R - Th?

3: W3848 s P Mal_5

4: W3752 s

13: W3782 s M-

21: W3898 + L_6 (481 M).

Media:

M Gal tryp B_1 Sm

S Gal Sm B_1.

Cross:

.5 ♂ + 1.0 ♀, 1^R 37°

.05 of 10^0, 10^{-1}, 10^{-2}.

	yield 10^0	10^1	10^2	Select try⁺ Gal⁺/total	$4p^+$/Gal⁺	$4p^+$/Gal°
1	0	0	0	-		
2A	∞	154	10	32%		
2B	∞	63	-	25%?	85%	7%.
3	14	0	0	18%		
4	39	0	0	16%		
13	36	miss	0	4%		
21	17	0	0	2,5%		

DATE: 5/15/58 REF: 1408/4

Gal TIMING – Hfr_2

2 ml O.r.c. w 3370 + 7.5 ml Pen; 1 ml 3908 + 7.5 ml Pen, 1 hr rot

(A) Spin ♂ & ♀, resuspend in Pen 3.0, 0.3 per tube respectively. Mix 0.1 + 0.1, at 37°; after 1' add 10 ml warmed broth – (1/50 dil)

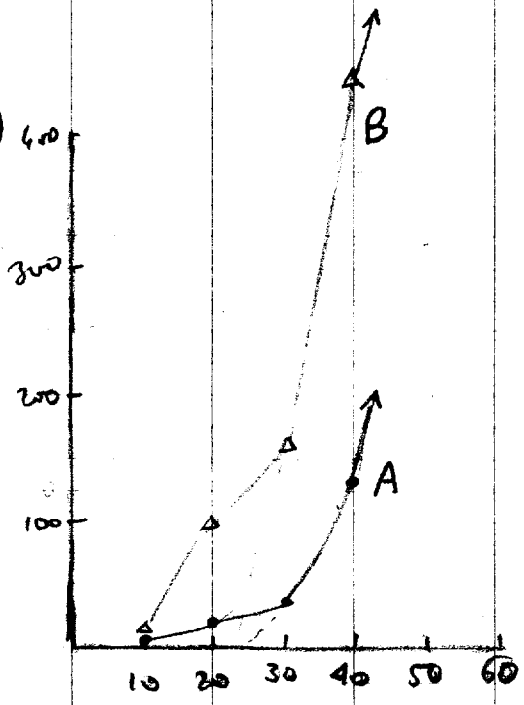
After 10', 20', 30', 40', 50' 60' withdraw 2 ml sample, chill, blend, plate on S Gal Sm B_1 D(Sm B_1) – M Gal Sm B_1 Tryp.

(B) Spin ♂ & ♀, resuspend in fresh broth, to same conc. as before spinning .5 ♂ + 5 ♀ + 5 Pen

Rest: same.

Plate counts D(Sm B_1)

	A	Gal Sm	B	
10	7	4	33	12
20	18	–	91	116
30	31	31	174	149
40	140	112	418	554
50	(1200)	823	∞	
60	(2500)		∞	



Entrance of Try at 20'?

Protoplasts data insufficient to answer the point

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19 REF: 1408/4.

	1	2	3	4	5	6	7	8	9	10
		5' Gal for B_1		Counts.						
			A			B				
			Gal+/Gal-	Tot	Gal+%	Gal+/Gal-	Tot	Gal+%	Gal+ / Gal-	%
		10'	1/3	4		2/10	12		2 / 1	9.1
		20'	missing			17/99	116	12%	17 / 88	5.7
		30'	0/31	31	0%	5/146	149	3.3%	5 / 105	1.8
		40'	18/94	112	16%	114/410	554	20%	70 / 98.6	41.2
		50'	447/376	823		too many				
		60'	too many			"				

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MICROBIAL
GENETICS

19 May 16th, 1958 REF: 1408/5

W 3870 : refrig culture, 2 ml + 7.5 ml broth → 1 hr not.
W 3908 " 1 ml + 7.5 → 1 hr not.

Spun, resuspended, warmed – Mating mixture.
2 ml ♂ + 10 ml ♀ + 10 ml broth in flask.

Controls : ♂, 1 ml + 10 ml broth → 1 ml + 1.5 water →
→ .05 & .1 plated
♀, .5 ml + .5 ml broth → + 1.5 water → .05 & .1
Plate recomb : .05 diluted ♂ + .05 diluted ♀, as above. .1 + .1 also.

Sampling from mating mixture : by means of warmed 1 ml pipette, to 1.5 ml chilled water, then blended, and amount from ~~.05~~ .1 to .05 plated on
D (Gm B, Meth)
S (Gal Gm B, Met).

Times : 10', 15', 20', 22', 24', 26', 28', 30', 32', 34', 36', 38', 40', 45', 50', 60'.

Note : 32' is actually 33'10"
34' ~~30"~~ " 34'30"
36' ~~10"~~ 36'10"
50' 50'35"

From 34th or 36th minute (possibly also 38th), contamination from waterbath through the pipette – Exp. discarded

U.W.
MICROBIAL
GENETICS

19 May 16, 1958 REF: 1408/6

GAL PREUM TIMING: Hfr13, Hfr4.

1 hr rotated cultures of ♂4 and ♂13, ♀ 3908.

♂'s same as in exp 1408/3. - Cultures started with 2 ml f. ♂, 1 & 8 ♀

Centrifuged, resuspended in 4 ml (2x), and mixed in flask.

4 ml ♂2x + 8 ml ♀2x.

Sampled at 10' intervals, chilled, blended, plated .05 & .1 on S Gal Sin Meth Prol.

Controls: .03 of ♂ ÷ .09 of ♀