37-X666 / serum and clones.

1245

DATE: APR 12 1955 REF:

A. Misc. tests on serum, diluents.
Water whether tap, this lab distilled, or enzyme institute double distilled appears to be suitable diluent (contra "spent broth"), to limit growth. Inocula c. 10-20 cells retained motility, did not flatten unduly and growth was limited. (Unavoid contamination with broth of isolation.)

B. 9, 27 ⊕ resp. were completely inhibited by 1: anti-b, 2 anti-i serum. Overnight in B1 (b) large clone of small cells, no motility except a single wriggling cell (planted out) A12 (N.G.) In B2, noted clone at center as above but at periphery, net and cords of long cells and filaments (somatic serum?) This was also noted later in i-serum.

C 12 Y 2 B pepsin. Freeze degas 12^{20} Spot ① to 2^{20} DCG pulsed (43) 2^{50} - 3^{30} Inc. to 5^{35} and plate in MGA 60%. Inc. 5^{35} PM - 8^{30} AM (15 hours).

35 (34 clones + 1 pure swarms) 9 blanks. (probably pulsed late) - hold to 5/31/55 for photo and counting.

serum inhibition of trails

1240.
1246

drops fused 12
(~~1245~~ pups)

DATE: APR 13 1955 REF:

A. Serum effect microscopic. Initials isolated from
B. see 1245 (c. 2 35)
FA11 → SW666; FA32 → SW666 and put in 1:100 i,
(A) (B)
serums in broth. b serums inactivated both very quickly; i after 30–90 seconds (usually).

Then tried plating. (by c. 3 45). Harvest c. 300/ml A; 500

(but middle broke + numbers in B are doubtful.

A – ~~#~~ .2 ml samples (est. count is 50 cells/plate). in
MGA60 + (1) 0 Plates ~~MGA~~ somewhat messy but profuse T + S.
(6) b 1:100 3 inh. swarms, rest are all singles.
(5) b 1:1000 5 swarms (sl. inh.); 75 singles; No trails.
(4) ~~b~~ i 1:100 Spread contam. But no trails
(3) i 1:1000 6 large 3 small ~~trails~~ swarms (b, i ?) } No tr
why two kinds here ? Q

B .1 ml samples (do.) (1–6).
1 swarms too messy; about 50% T;
2 (MGA) [5 swarms, 56 1's; 7 short trails]; [4S; 77 1's;
3 No T or S.
4 No T or S
5 3 inh. S., No T
6 2 ow (1 spread contam?) No T.

1:1000 is adequate to inhibit trails!
i serum inhibits b trails!

abandon i → b system for this study

DATE: APR 13 1955 REF:

37 X 666

	1	2	3	5	6	7	8	9

ca 50 plate

D. SW967 plate in MGA, MGA60 for minor trails in <24 hours — 5PM. but too crowded.

E Ore. continuously ~~to~~ ditto check SW666 MGA60 only — at 2 days, no minor tra[illegible] same diffusion: colonies are [illegible]

ca 10 plate

F 1237A+ for flares — incubate 5PM - 10 PM. MGA only note singles, not pure?

G control SW673 "

H 1 MGA
2 MGA 60 → excessive proportion of swarms. (? age of preparation ?. Do not use.

APR 14 1955 Platings of c. 50 initials each in MGA60 + .01 ml sec all show swarms.

	trails
a	+
b	−
c	+
d	+
i	−
k	+
r	+
12	+
B-O	±

a, c, ord would be quite good for further test.

(c. 5 30 PM)

D Replate P14 A15: excellent development of minor trail 60% MGA; intact only in MGA but will probably show later.

12 40 D1A for Photog. — Refrigerated 10 AM. 5/15. 60% MGA. Reinitiate sister plates

Needs to be done in my own expts.

APR ~~15~~ 13 1955

	Today	
Serum effects	✓	
Swarm sibs		*
flares	✓	
terminals	start	*
E.M.		
SW967 / MGA60	✓ ✓	
Paired subclones for multiplicity of "E" (Use ¿ TZ?)		*
Isolations in water?		
Viability pH 4		
Viscosity fx.		
Trails in Lp^+ x (viability of subclones multi, still unstable)		

APR 15 1955

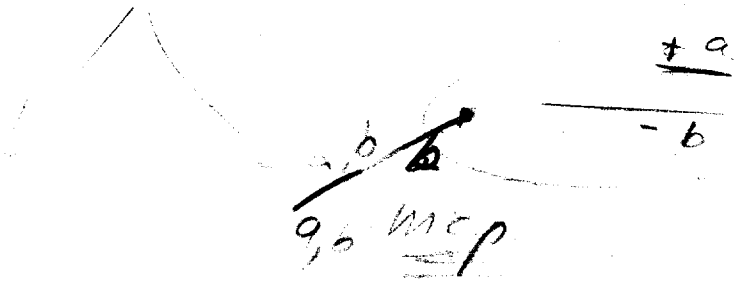
Notes: Phase Var - TH2 ph2 monoph?

Mention to Bruce b/i — cf. burgundof.

Fla^{+} $H.^{a}$

↓

Fla^{-} $H.^{b}$



Serum inhibition a → x

1248

DATE: APR 14 1955 REF:

APR 15 1955

14: n.g.

4/15: Frese degs, FA76a (S. miami a) → x SW666 11AM.
FA37 → x ...

Preliminaries
see 1250, 1252

not tabulated but results indicate that a does not inhibit b trails, part inh a/b trails. b inhibits all trails } at 1:100
as well a
1:100

Reviews # 4, 4

3 cells isolated

1131 ? ~~8 cells picked~~. ~~mit this expt~~.

~~1) pedigree to n_3~~

Flat: H_1^i (8^+:12

1) pedigree to n_3. 1/8 gave motile on transfer, found to be mixed +/- ~~is~~ H_1^b. ∴ segregation at n_{47}!

2) n_{13} : all −

3) n_{13} : all −

1132 2 cells. followed directly to about n_3:

1) showed 1 chain to n_3; n_{13}^-

2) 2 sibs both motile, catenated to n_3 ~~[illegible]~~ n_3, n_8 both. n_{13}^-.

11 3

1134 C3): → 3/22., each then catenated

Precis 11.

4/15,

1131) 1) 1 pedigree to n_3. 1/8 ~~still~~ gave swarm, mixed $Fla^+ H_1^i$ $Fla^- H_1^b$. Segr ≮ n_4.

2) 2 cells → n_{13}^-.

n_0 = (1 initial chain

1132) 1) 1 chain ~~$n_1 > n_3 < n_{13}$~~ $n_0 - n_3 < n_{13}$

2) 2 chains $(n_0 > n_1 - n_8 < n_{21})(n_0 > n_1 - n_8 < n_{21})$

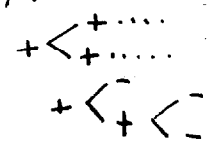
1133) 1) Swarm : pure (1/4 inviable; 3/4 → H_1^i) but late cool.

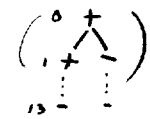
1) $n_0 > n_1 - n_1 > n_{13}$. (2 interm.)

1) $n_0 - n_1 > n_1$.

1) $n_0 - n_1 > n_{13}$.

initials only ↑





1134) E3) $n_4 > n_5$ < n_{15} / n_{15} — $n_{19} > n_{19}$. (3 interm.)

D4) $n_1 > n_4$. 1 interm — n_4 term.

E5) SW T+S from 1 cell. At n_5 — n_{17}. chain (F1) F2 = nm. E11 = swarm in summer

C5) 1 $n_0 - n_{23} > n_{33}$.

C4) 5 $n_1 > n_5$ ..... $n_5 > n_{10}$ [5 isolates $n_{10} > n_{18}$]. 16, 50 interm ed.

$n_{16} > n_{26}$

A3) 1 $n_1 - n_3 - n_8$? not contd.

B5) 10 $n_{13} > n_{15}$ < $n_{15} > n_{20}$ / (4) $n_{19} > n_{26}$

$n_{15} > n_{18}$ (6) < n_{28}. / n_{18}. / n_{4}.

Precis 4/15/37

1134 A5 7 $\left\{ \begin{array}{l} n_2^{2} > n_{12} - n_{12} > n_{28} \quad (3\text{ rol.}) \\ n_2^{+7?} > n_{12} \begin{cases} n_{12} < n_{19} \\ n_{12} > n_{19} \\ \vdots \\ n_{40}. \end{cases} \end{array} \right.$

(100⊕?)

(14 resolutions, 6 days [refr. overn.])

~~A4 $\left\{ \begin{array}{l} n_1' > n_{14} - n_{16}. \\ n_1' > n_{14} - n_{19}. \end{array} \right.$~~

A4$_2$ $n_1' > n_5 \begin{cases} n_{17}. \\ n_{20}. \end{cases}$

1138 most isol dried. 1 clone saved (B4) also had trouble drying

B4 -F22

$n_4 > n_{14} - n_{42}.$

$n_6 > n_{14} \begin{cases} n_{17} > n_{27} \\ n_{18} > n_{23} \\ n_{31} > n_{39} \\ n_{27}. \end{cases}$

$n_6^{3+} > n_{16} -$

19
22 > 27
17 > 30
32.
31.
32.
19 > 32
19 > 29
33 > 38
16
19 > 32
19 > 24
44.

∴ branching ended (use to 1 histo
not sooner than n_9
not later than n_{16}.

14 + 13

$n_{27} + n_{15}$ n_{45}

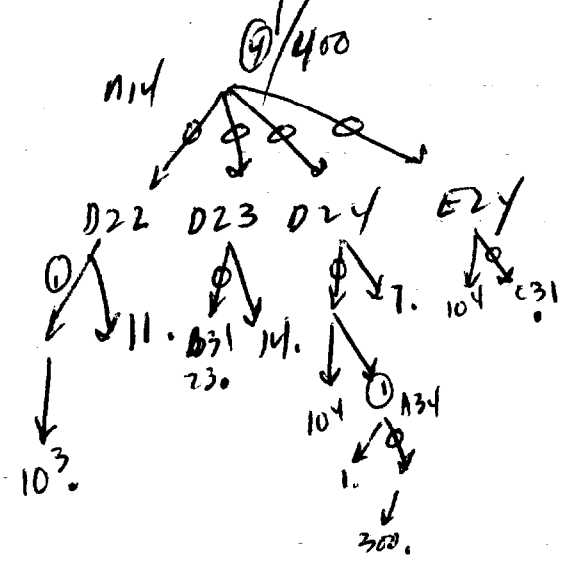
1138 addl chain ~~tot~~ of

n

B21

n6 B21

n14



B21

A21 3 C23 lost

22 2 C24. 4 C33. < 5

23 1. > 13

24 3 C25 13 A32. > 1

25 2 D25 ~~> 2 A33~~ 13 A33. > 2.

B22 3, 13 A31.

23 3. > 13

24 3. > 10

25 4 E23 13 B34. < 5

C21 . > 4

22 3. > 13

E21 13 B32. > 5

22 13 B33 2, 8 E31 1,

<

∴ total chain lengths

n16 —

A21 19.

A22 22. ~~> 27~~ > 27

A23 17 > ~~13~~. > 30

A24 32

A25 31

B22 32

23 19 > 32

24 19 > 29

25 33 > 38

C21 16.

22 19. > 32

E21 19 > 24

22 44.

H_1^a —x

1250

DATE: APR 20 1955 REF:

1	2	3	4	5	6	7	8	9

FA93 (SW940 4,5,12 a:—) —x SW666 . 11AM-12[15] — 12[40] Ref

FA37 " "

A) 93 —x 2[35] fuse drops. 5PM Harvest c. 5×10^3/10 ml.

B) Same dilute 500/ml. = 500/1ml .2 ml samples.
B5 = 1ml 1b.

C) 37—x (SW1257) 400/.4ml
1. 0.1ml MG-A60
2 ± "
3 .001 a
4 .001 b

Plate C 3[35] PM
5/31 The results here are not tabulated but cover i below.

APR 2 3 1955 Regurated.

		T			T.
A1	—	++	C 1} 2} —		++ >90%
3	a	+ (reduced in % + extent)	3	a	++ large trail no in
4	b	—	4	b.	—* . — dried out b any check
5	b	— (Recheck carefully — see over			

* some small late trails?

T 12/58

Conclusions: effect of anti-a serum is specific, as it works on H_1^a—x but not on H_1^b—x. The effect is, however, not complete and may be difficult to measure. Hold plates to photograph. It is possible that early chains are b and ~~later~~ are a or vice versa.

S. A1 2 3 B1 B3 tabulated photos 5/31.

5/31

from 5 plates of B5 and 2B4 looks for any trails or suspicions.

on 1B4 plate only, 3 susp. trails? or clusters.

B4/a

b

c

These may have had early ~~Bs~~ a phenotype with little enough b to swim in presence of anti b.
Plates to DCG to attempt reisolation of these trails.

6/4. DCG found (on replating isolates)

a: gave fuzz 1's and many clusters (ca 100 cols.) (after 3 hour incubation 37 then overnight at 23°. MG photographed.

b: pure 1's

c: mostly swarms – attempt to isolate any 1's (doubtless contain)

~~pat~~ partition of chains

DATE: APR 21 1955 REF:

1	2	3	4	5	6	7	8	9

See 1257 prepn.

3^05 fuse degs

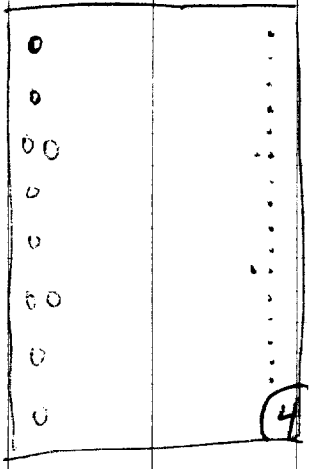
APR 21 1955

1252 prepn.

fuse degs 1^45 2^10 - 2^35 spot 56 (1)

D C B A

0
0
00
0
0
00
0
0 (4) ↑



A 1-15
D 1-11.

Examine 5:30 Most active without being handled are

A 2, 3, 4, 5, 7 B1 B7 B9 C 1 2 3 4 8 14
D 2, 5. Check for correlation c̄ swarm, trail.

5^15 P22 - Pick swarm clones + plate samples
est 10^4/~~ml~~ 1.0 ml = 1000/ml plate .02 ml
.1 ml samples in M

activity is jostling

DATE: REF:

	1	2	3	APR 22 1955 (+)	log10 (−)	6	7	8	9
A 1	(+) ~~+~~ uf			–	0				
2	(1) 1.			3	4				
3	(1) (1) F		v. active	10	4				
4	(1)			10	4				
5	(11) F			4	4				
	T 1.			1	4				
	(1) 1			0	4	Research for (+) by trap:			0
	11.			11	4				
	uf			–	0				
10	1.	vs		0	4		"		0
	F			–	0				
	+. 1. 1.			6	4				
	11.	dirty		14	~~0~~ 4				
	11.			1	4				
15	11.			0	3 pd				
B 1	(11) +.	v.a.		4	4				
	uf			–	0				
	1.			18	4				
	++ 1.			1	4				
25	TT +.			0	3+				
	1T.			12	4				
	(1) 1.			(1)	4				
	11.			3+	4				
	(+) +.			2	4				
	1. dirty			1	4				
	uf F			~~0~~	0				
	1 (1) rot			28	4				
	T (1) slow			1	4				
15	uf			3	4				
1	(1)			–	0				
C2	(1)			c. 30	4				
				swarm	20% – 3+.				
	(11)			–	0				
	1.			4	4				
	uf			–	0				
	T T			–	0				
	(11)			1	(200)				
	T 1.			0	~~100~~ 3				
40	+ dirty			20	4				
C11	+ 1.			1	4				
12	(1)			50% sw	4				
13	uf			2	3+				
15	(1)			–	0		4/23.		
18	1.	1" D1" →		3	3+				
D 1	(1)	2		40+	(500)		Proved to be swarm now		(10%)
2	1.	3		1	3+				
3	1.	4		swarm	<1	Inv. uf			
4	1.	5		3	4				
5	1. (1)	6		0	3 lys.				
	1.	7		8	4				
	1. T	8		swarm? 100	3		✓	10% mot	
	+.	9		sec. > 500%	4		✓		
	+	10		8	4				(over)
				10	4				

Save swarms

D1
D7
D8
C2
C11

① from samples directly to Massay
(plated precinc., over)

in stabs for whole clone
in MGA tubes for pooled motile
pool Pla⁻ cols. from MGA over for non

Replate

D1 - see infra

D8 - too crowded to count swarms
ca. 50%

to recover components at rate for later colony tests.

37 ~~xxxx~~ —x SW666
undivided clones

April 21, 1955

56 (1) isolates, grown to 2^{13} and summarily examined for motiles. Counts are underestimates. No tech losses

NG ~~12~~ 13 Swarms 4 No motiles 6 less than 9 22 10 or more 11

Maximum extimate: 40+. 5 clones were harvested for replating of the intermediate chains.

No.	Est motiles	harv.	+ nm.	mot left behind	Plate
B3	18	20		4	10 1's ~~only~~; 2 vs T. 8 ooo
B12	28	22	4	10)	11 + 1 vs T 1 clusters
C9	20	28	6	5	1 trail, def. multiple but compact 28 1's.
C1	30	37			4 v.s. trails Ooo. 31 1's
* D1	40	45	10	10)	34 swarms + 13 nm. No trails.

*Replate Residue

8 sw: 92 cols. (ooo)

Estimates on clones with many chains are therefore moderately low. Some of the serc may have had a motile but this was looked for. However, these drops were not searcl with a trap owing to shortness of time.

In addition, 4 drops had apparent swarms, but it was difficult to estimate incidence of non-motile elements. Therefore these were blind-picked and plated immediately. (picked to 10 ml, est. 60-70% recovery; plate .02 and .2 ml samples)(This will help evaluate estimate of clone size as 2^{13}.)

Swarm	% mot.	Found	clone size $\log_2$	Plate
C2	20	10%	11	.02 4 sw; 43 1's .2 33 sw — not cont
C11	50	20%	13	.02 4 sw 29 1's .2 51 sw — 250 1's
D6	100?	—	10	[No sw 1st pl. D6...]
D8	50+	10%	4	.02 3 1's .2 3 sw; ~~35~~

swarms are placed but not centered.

Repeats

The data may be grouped as follows:

mots.	clones	Initials active:
inv	A1 9 11 B2 11 15 C3 5 6 14 D 10 11 ; D3 D5	12+ snl
0	A10 15 B5 C8 D4 A7	6
1	A6 14 B4 7 10 12 C7 10 D2	9
2	B9 C12	2
3	A2 B8 14 C14 D2	5
4	A5 B1 C4	3
m6	A12	1
8	D6 D9	2
"10"	A3 A4 D10	3
11-12	A8 B6	2
14 18 20	B12 B3 C9	3
[illegible]	[illegible]	[illegible]

C15-D1 conf. in tally but not pltg

D/swarm: Semi 45/55? Flat was removed from DI before plating, the count on residue of 8 swarms: 92 colonies (in $\frac{.92\ ml}{10\ ml}$) is not fair estimate but the ratio must still have $\approx 10\%$. Late segregators?

April 21, 1955. Tue faire trails.

(1). Serum inhibition. of anti-a, b // b → x b
a → x b
i → x b.

(a) Since a does not inhibit b → x b, probably specific.
Is b effect specific? Would need a $Fla_1^- H_1^{st}$, e.g.
(Repeat c S. Heidelberg initials ?).

(b) Should also be tried on intermediates as early trails might all tend to be H_1^b and agglutinated.

(2). Late branching? Pedigree + platings of $\begin{cases} n_{10} \\ n_{13} \end{cases}$ isolates

(3). E-branching < platings of initial sibs
any large trails in sibs to swarms?

(4). Are all segregates H_1^b (a) trail ends
(b) swarm sibs — esp. if $H_1^b Fl_n^+$.
(c) look for b-resistant trails.

Today: (A) Replate a/. and of a/ i → x b.
(B) start (2.)

Tonight Review notes — summarize for (1).

Does b (minnesota) serum also inhibit $H_1^a Fla^+ \rightarrow x H_1^b$ trails.

any $Fla_1^- H_1^{non\,b}$?

125

DATE: APR 22 1955 REF: 1250

"b" New Prepn FA10 → x SW666 10^{40} – 12^{15} – 12^{40} Phif.

Freeze traps 240. Harvest 445 c. 800/ml.

mc. c. 5^{30} – 10^{00}

A. PA10 → x SW666 (.1 ml) B a → x. (.2 ml) c. 100 cells/plate

9A23: trails

			A b → x b	B. a → x b.
1. —		–	++	++. 10 swarms, none
2. b minnesota (Edwards)	1/100	b	–	– no trails
3. "	1/1000	b	–	– no trails
4. a	1/100	a	++	± } much the same.
5. a	1/1000	a	++	± } appearance now ≈ MGA dilution.

B prepn. may be late, segregated.

photo!

∴ b, minnesota also inhibits completely. Reaction may be specific for → x H_1^b but this cannot be verified unless a $Fla^- H_1$ can be isolated. (intent of 1250B5 platings?).

C Search for 1b trailets. 1252A2 ① ② 1252B2 ③ each show near edge (prob. serum not diff.)

2 1 3 } no nearby swarms.

1250B5 4 plates

See 1256.

5/31 ~~from 1250B4, B5 search carefully.~~

photos of 1252A

1253

APR 2 2 1955
APR 2 3 1955

Tetrazolium- formazan chains preliminary

.005%
P22. Prepare stained cultures. Add 1 ml overnight culture to 7 ml broth + TZ .0[illegible]% Incubate c. 3-4 hours. Also (A) add TZ (1/200 .5%) to 1 ml culture directly.

Best method of preparation appears to be growth for short interval with TZ. Probably only older nongrowing cells willstain.

Refr. to 1 P 23. Test isol. to agar, small liq. drops. Main traouble with agar is confusion from dirt even under oil. Probably better in fluid with a nonmotile culture.

1PM isol. 1) mot. W-2344 to A1

4 PM 3 more to A2

4PM 6 stained W-2802 to small drops near situs C. These were terminally marked. (Z = f[illegible] gra[illegible]
Hunt for rare medial marked= 7,8

2 W-1177,(dividing) 9 is terminal, 10 is medial.

of 10 4 clones found, 3/10 still located "Z". Fate limited - see protocol.

APR 2 5 1955
APR 2 3 1955 Try out agar block methods to immobilize. Mostly n.g. owing to dirty agar - hard to refind indiv. cells. Try methocel instead. (cf. note 1-2 yr[illegible]
Disadvantage of oil chamber is solubility of released Z in oil

But prel. observations above bear out earlier concept
(1) [illegible] → [illegible] as regular mode of division
(2) most Z ~~ter~~ unipolar
(3) Z dies out.

DATE: APR 2 5 1955 REF:

Standard system now: SW-940 (FA 93; H_1^a) —x SW 666.

37°

A. Overnight SW666. 1ml + 1 ml .01% TZ broth. Stained 9:00-10:30. Wash and add FA 93 to pellet. Inc 11 AM - 1 PM. (Cf. B); sediment pellet for harvesting motile :

A= cells prestained; 2hours+ phage/

B. 1:1 + FA93 9:30-11AM. Add = vol. TZ broth. Incubate till stained (1PM). Sedime to harvest pellet. (3i/2 hrs. + phage).

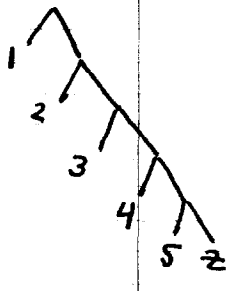
B= cells poststained.

Found: many motile initials in each, but almost no motile A were stained (overstai About 2% of motile B were labelled; c. 50% of parent population.

Summary: 28 isolates from B, 2 from A. 5 clones inviable. initially., only one Z (granule) chain died later. Z chains were followed for 4 to 6 fissions. 1 clone gave a swarm (c. 50% motile) = 31B/ E = preponderance of motiles (>10)

8 clones showed E. This appea

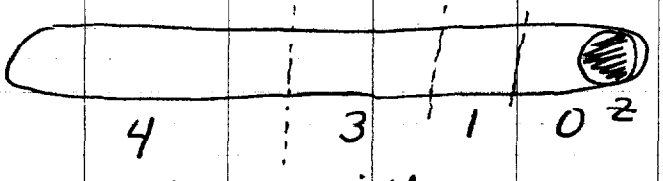
If clones are summarized :



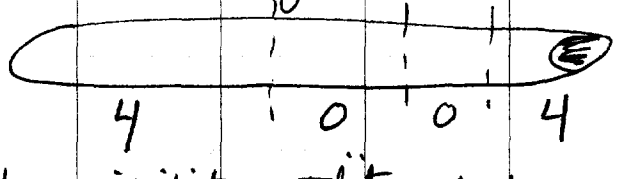
as follows :

(+) n	Found	Random expectation
1	4	4
2	3	2
3+	1	{1
Z	0	{1

or, if cell is



The result agrees with random expectation, but possibility of a negative correlation of Z (after n_3 or n_4) should be tested. Original notion if E were polar was expectation of :



if Z had random chance of marking the E or ~E pole initially. However, Z may either inhibit motility if it marks the E pole, or correlation may be one of selection (in terms of age correlation). Further study is made of Z-marked chains, in 2 senses. (over)

APR 25 1955

1254

does this mean notable 6/56

8PM.

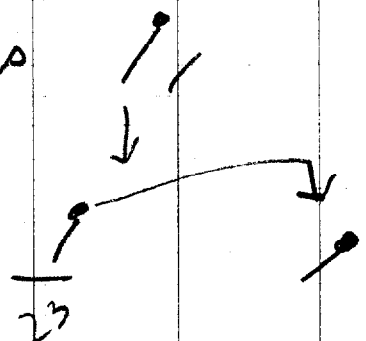
DATE: REF:

	1	app.	3	total	5	6	7	8	9
1.	5	sher. wlsm.			4				
	4				4				
	4				4				
	3	no transf.			3				
	wf z								
	8				4	1/2.			
	4				4				
	4				5				
	5 (2³)				5	b. hes 4/16 mot.		c. 0/8	d 1/4
	4				5				
	4				4				
15	4	(all 4 mot)			4				
	8	only mot in a.		b. hes 3/8	4				
18	z n.f.	2 mot in b.							
19	New	cool from old trap 9PM							
21	4				4				
22	5 2²		b. hes 8/32		4				
23	5 2²				5	8/2	(1⁺, 1⁻) . 6.		
24	8		3/8						
25	16 z nf.				4				
26	4				4				
27	8 z nf								
28	8 z nf.								
29									
30	4				6				
31	11 2³				3				
32	4				6 (1 var. already)				

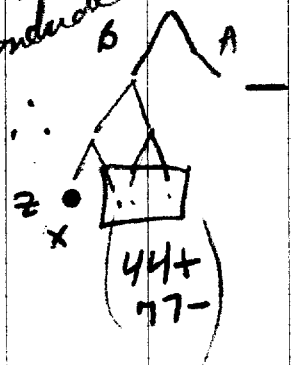
Reexamine at 8PM and isolate residual z from ind. # of non z. to rightmost drop.

St. RT.

E.G. 31 was



conclude 6/56

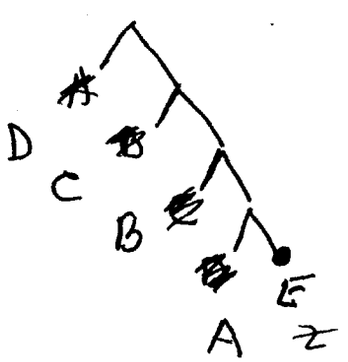


Reconstruction:

in general, rightmost is Z chain cell.

the others are successively later sibs of it.

e.g.



1254

DATE: APR 26 1955 10 30 AM. REF: 2

(+) Sign	A		B		C		D		E	Σ (+)	F	7	8	(+)	9	n
4 miles 3t. 1	0	4	4	4	(25)	4	0	–		29	22 → 3:05 PM.			2		4
2	0	3	1	4	1	4	0	4		2						4
3	0	–	~~2~~	~~4~~						–						0
4	2	4	(15)	4	1	4	0	4		18	too close to pits.			1		4
5	0	4	1	4	4	4				5						3
6	1		3	4	(12)					16				2		2
7		–								–						0
8	0		3'		0		0		0	3						6
9	1	4	1	3'	0	3	0	4.		2						4
10	0		(23)	3	0					23	23 → tube for plating			1		2
	1		(18)	4	0		2		1	22	18 →			1		5
	0		1		0		1		0	3	2					6
	0		3	3	0	3	0	3	0	3	3					5
	1		(33)	4	1	4	2	4		37	28 →			1		4
12 30 / 1 15 15	(44)	4	3	4	2	4	1	4		50	43 →			3-4		4
	0	–								–						0
	2	4	3	4	0	4	1	4		6						5
18	(17)	4	2	4	6	4				26	17 →	6 →				2
19	1.	100,	(3).													7
21	1		0		1		0			2						4
	0		4		0		0			0						4
	1		0	3'	(22)	4	0		0	23	1 4	21 →		2		6
	2		2		0	2+	0			4						5
25	3		1							4						1
	1		0	3	0	3	0	4		1		~~41~~				4
	(42)		1		2		2			47		41 →		Z nf		0 Z
	1		5		0					6				Z nf		
										–						0
30	0		2		0	3	0		0	2	0					5
	0	4	sw	3	0	•				sw no trails		Pick sw later - pure ? 1				4
32																
	×	↑	×	↑	×	↑	×	↑		total chains in whole clone.						×
35																

(+) is chain in which perpendicular motiles appeared.

n = number of fissions for which Z was followed. (not necessar isolated!

Save + Recover Fla⁻ sibs in 31B swarm. Plating of sample fra .01 ml. the clone gave (1 ml) 44 swarms : 77 colonies, no trails.

TZ; methocel; divided clones

1255

DATE: APR 28 1955 REF:

Preliminary expts 4/28 showed that 2% Methocel 4000 immobilized cells so they would stay together after fission and form nubbles. Use to test distribution of Z in a chain, and to reisolate after n_3 or n_4. Make up Methocel in Penassay. The methocel completely immobilizes the bacteria. ? What concentration allows Fla^+ to swim?

93 → x Sco 666 8:30 – 10 AM, add = vol TZ .01% to 12:05. Spin down and refr.

Abandoned.

B) Apr 29. 2 PM stained W-1177 (c. 2 hrs. mixture overnight + TZ broth). Plate out in methocel broth on c.g., over oil. Also isolate a few definite anpmalies.

Conc. Most cells stain unipolar. Rare (5% bipolar, subpolar). Most chains show terminal granule. Occ. cells lyse in random position.

Some exceptions with interstilial granules— probably from subpolar cells.

Need: observations at first division of subpolar and bipolar cells. should also spot a fair number of unipolar cpntrols.

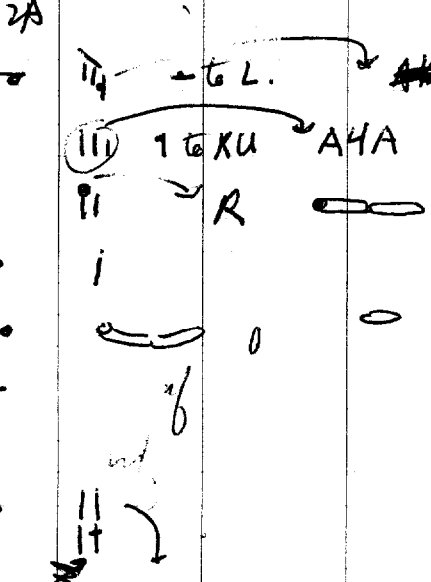
Z chains

1255

APR 2 8 1955

DATE: 2-3PM 5PM REF:

(+)	1	2	3	4	5	6	7	8	9
A1 A	to A2A								
B		II	to L.	~~A4B~~ A5A					
C		III	1 to KU	A4A					
D		II	R						
B1 A		I							
B			0						
C									
D									
C1 A		II							
B		II							
C									
D									
A2 A	moves.								
B									
C									
D									
A3 A									
B2 A									
B									
C									
D									
B3 A									
B									
C									
D									
C2 A									
B									



Exp. n.g. too much slippage perhaps fluid added
Methanol drop or motility present. Let go — at Ref.
c. 5P27

1255

DATE: REF:

(−) c.2 30M spotted | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9

E1 A (+)
B
C
D
E2 A
B
C
D
E3 A
B
C
D