Experiments on motile lines

1131 – 1151	Feb 15, 1954 – April 14, 1954
1212 – 1262	Jan 11 – June 2 1955
1272 (Leifson)	Sep 8 1955.

2/15/54.

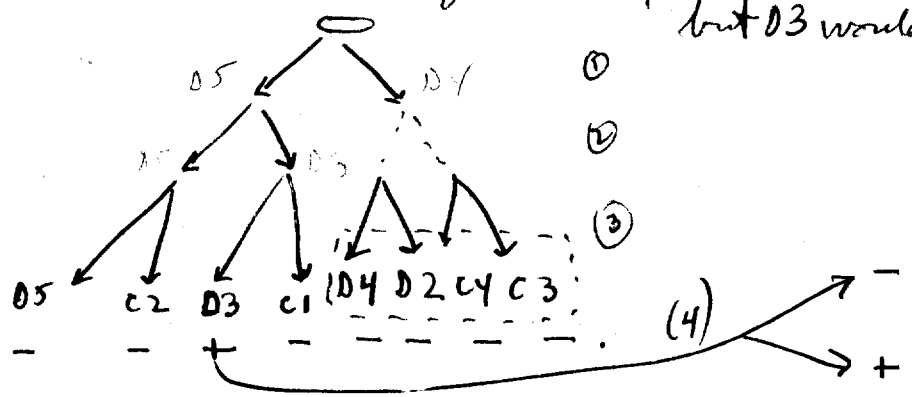
Mix FA12 (SW623) + SW666 + ca/vol. broth About 10^{30} Am — 33° etc. Search for motile cells in enc. droplets ~~Rare~~ Rare Fla$^+$ seen (none in FA22 ✕). Pick and set to allow clones as convenient.

Clone CD. Motile, but became immotile on transfer

Ca 5:15 First division: separate At 7:50 both daughters had divided, shortly thereafter, before separation, second division in one line. C.410, after separation other one also divided, less regularly.

Nearest fission was D3 — C1 Add fluid

A16. Examined for motility. Thus most descendants are immotile, but D3 would have formed a stream.



Other isolates

- C5. Initially M+, then M−. Initial growth poor ⟶ NM. Lp^3
- D5 Removed motile for some time unless stuck ⟶ NM. (~~Fl~~ Lp$^+$)

E: 1 deep had many motile: E 2, 3, 4, 5 ⟶ all M E1 = mess ("NM").

(late). 1 clone? [E1, E4 Lp$^+$, other Lp^3.]

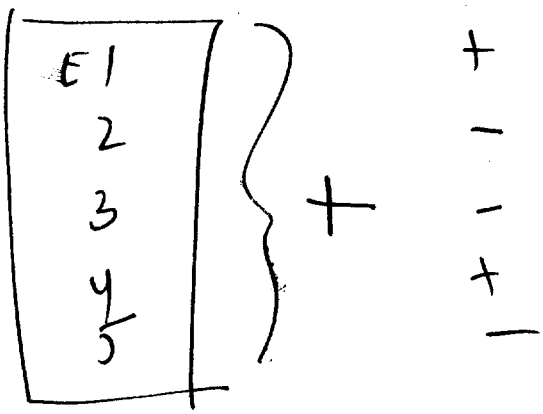
A: Controls: Lp$^+$ 2, Lp$^+$ 3, 4 ⟶ NM (~~Fl~~) (4 n.g.) 5 = deposit ⟶ NM. (Lp$^+$)

Blank control OK.

Pick for homogeneity test and serotyping.

Fla (armotagan) 666

A2	−	+
A3	−	−
A5	−	+
B5	−	+
C1	−	−
C2	−	−
C3	−	−
C4	−	−
C5	−	−
D2	−	−
(D3)		−
D4	−	−
D5	−	−



~~A~~. Test $H_1^?$ of Fla_1^- conjugants of clone 1131CD. Broc motility tubes ± D5, etc. and FA12. [Should have used 9!]. Look for $Fla^- H_1^i$ crossovers ~~D. + FA9 (or should be used!)~~ of course FA 9 → Fla+ gave no Fla+

B. Test lysogenicity character on SW 666.

	Fla	H_1	Lp_{22}
C1	−		−
C2	−		−
C3	−		−
C4	−		−
D2	−		−
D3	+	i	−
D4	−		−
D5	−		−

C. Test single colonies from D3 for motility (incr. droplets under oil)

12 Fla^- : 8 Fla^+ Save 1, 2, 3, 4

(1, 2, 5, 6, 8, 10, 12, 13, 16, 17, 20) (3, 4, 7, 11, 14, 15, 18, 19)

∴ Conclude that final segregation occurred at (or since) the 4th fission. Phage apparently not penetrant (as noted frequently in this system).

B5, C5 were evidently stock formers.

1 ✓ = H^- ✓

3 + i^{+1} b^{+2}

4 + i^{+1} b^{+2}

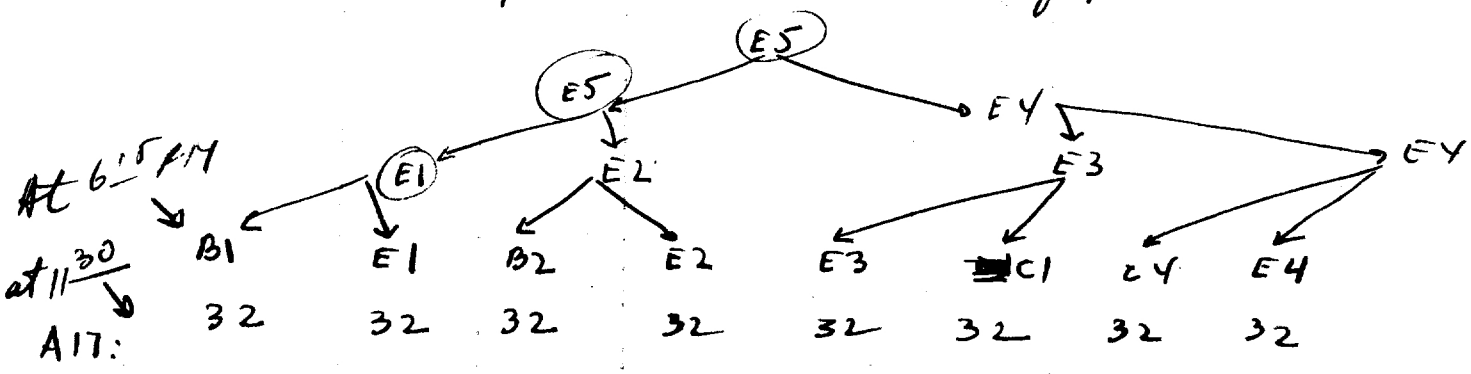
✓ Alec finds same result + will check further. All are 1, 2 ...

2/19 D FA 10 → 1, 2. generally b.

A

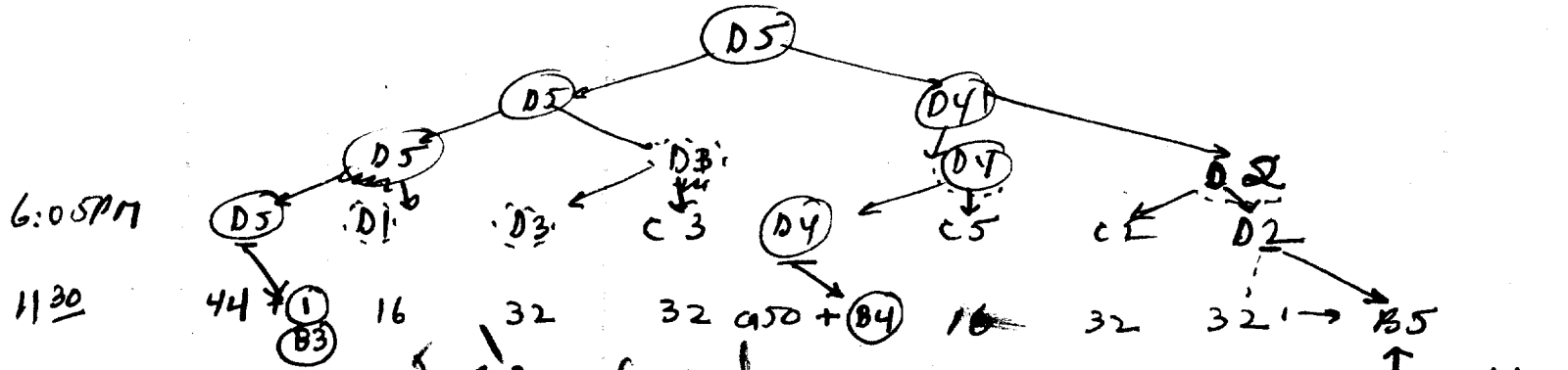
2/16.

See 1131. 12^{25} to 3:50 PM before spotting drops. ○ = phen. mutle
2 cells initially motile and at 1st transfer



∴ at room temperature about 1 fission per hour. Held rested decks among to warm persistently.

+ 8 generations of track in each branch.



A17: slight plaque - found worked.

A - n.g.
B 1 - 2 - 3 - 4 - 5 -
1 - 2 - 3 - 4 - 5 -
D1 - 2 - 3 - 4 - 5 -
E1 - 2 - 3 - 4 - 4 -

all to flab

∴ above all tracks equivalents.
Definite "branching": phenotypic delay?

added fluid ca 11^{45} PM. Pick all drops (no visible Fla^{+}) A17 to nutrient agar (i media).

[In future might be better to follow such tracks more closely by chilling overnight + sorting to find even late residual + by plating in soft agar!].

Febr. 17, '54

FA12 →x SW666. At room temperature, but 11:30 AM – 3:30(+) PM. probably allows too long. Rather large drops provide quite easy selection of Fla+ from large populations, Fla− falling to interface. See dictated record for details of manipulation technique.

Separate originals (E5), (D5), (E3), (C5), (B5), (E2), (E1), (B2)   → H1 b

No pedigree on —.

O motile when last seen or until fission + separation — all.

E5 < C1 (0) ; [3] < E4 + H1 i ; [2] < D3 + H1 i ; E5 + H1 [illegible] i

E5 → (E5) (D3) (E4) (C1)
x i i 0
[they split off early, ?]

D5 → D5 < (D4) — ; (D5) —

(C5) → C3 [illegible] —

(C5) → C4 ±[2] ; (C5) → C3 — ; C5 —

(B5) → B4± — ; (B5) —

A18: E3 — ; C5 — ; B5 — ; E2 —

E1 + (? fed) ; B2 —

[Plan somewhat disorganized. Probably best to collect a number of motiles early and not separate clones, or else isolate a few for full clonal analysis.]

B	2, 3, 4, 5
C	2, 3, 4
D	3, 4
E	2, 3

LP22 ... not all are + necessarily pure. 8.0...

Test sub cols — Streak before? Fla+ for purity

E1 10: all −, 12: 1+, 11−. {Ab...says b for mess.
E4 7 all + i
E5 6 all − (b) i on [illegible] tube test
D3 5 all + i A.B. is [illegible]

E5 pedigree ~~suggests mixed clones!~~
But finds 1133E1 Fla+ = Lp+ ; Fla− = Lp−

E4 / E5 | .... | 000 E5
0000 | 000000 D3

Feb. 18, 1954. 2/21/54.

FA12 – ∠ SW6G6. 1 hour 37°; Refrigerate.

(a). 2/18. FIRST ISOLATIONS 4:45 – 5:25. In this interval also, A5, B5, had divided. All cells actively motile. But note that the two early divisions both gave 1 lethal!

Day 1. These isolations included:

A5
A4
A3
B5
C5
C3 } from 1 drop.
C4 }
D4
E5.

(b) At 10PM, ~10-45 cells had given clones of 2-25 individuals, 2/19. usually with 1 motile. This was separated as indicated. Leave at R.T.

2/19 (c) 9:30 – 12N. Reexamine drops, transfer to ~~the~~ second group as indicated. R.T.

2/19 (d) Brief exam. 5PM. Refrigerate.

(e) Reexamine A20. Where 1 motile cell already obvious, transfer to another drop in same site. Incubate at 37° from ca 12N – 4PM, then R.T. (already too long!) Refrigerate ca 6PM – 10PM. (or at 9PM?)

But this gave very large clones, perhaps also impaired motility. Some dried out. It is therefore uncertain whether this is natural termination or whether 37° played some part.

(f) Reexamine P20. Refrigerate for reexamination of those clones that are not too large, and for subsequent plating.

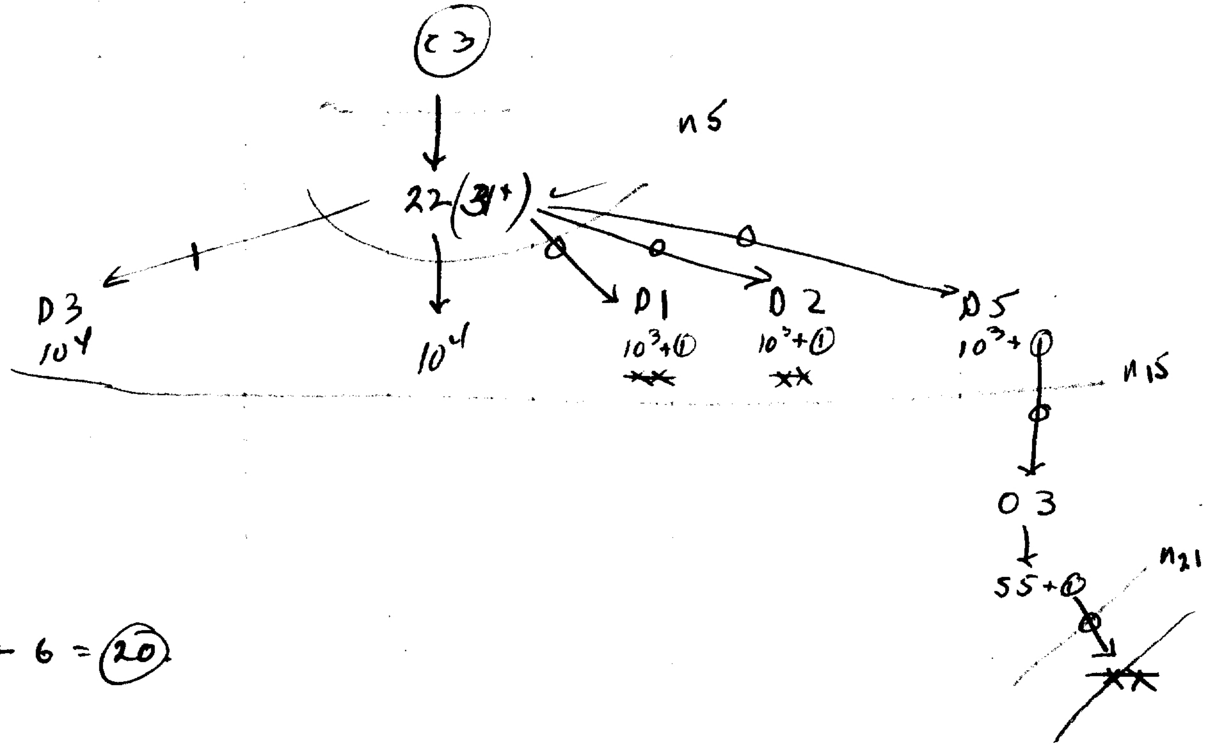
Note: wh[illegible] (1) due [illegible] of [illegible]

C3

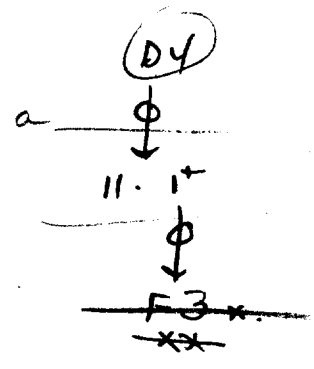
check notes on time of isol. of D1-5.

∴ limited early replication only.

O 3: $N = 4 + 10 + 6 = 20$

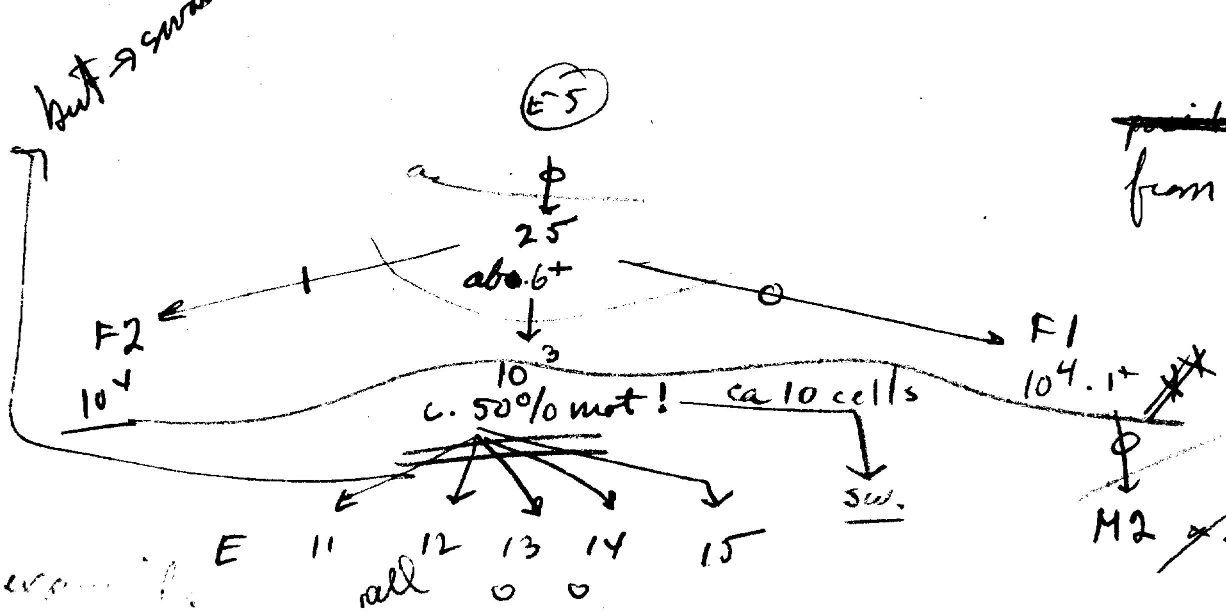


D4.



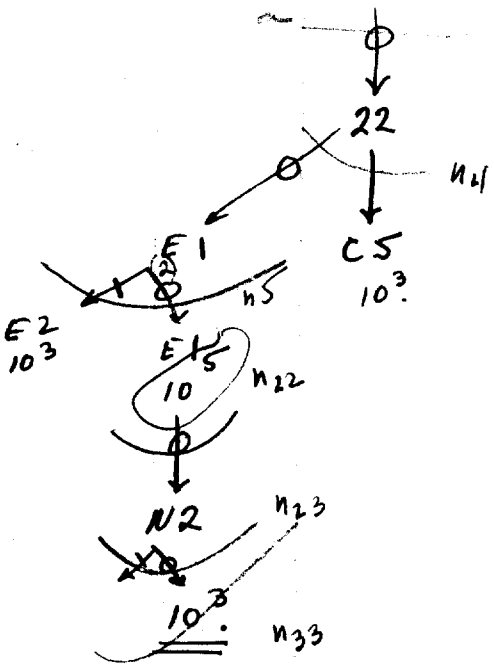
E5.

T+S. from 1 cell!



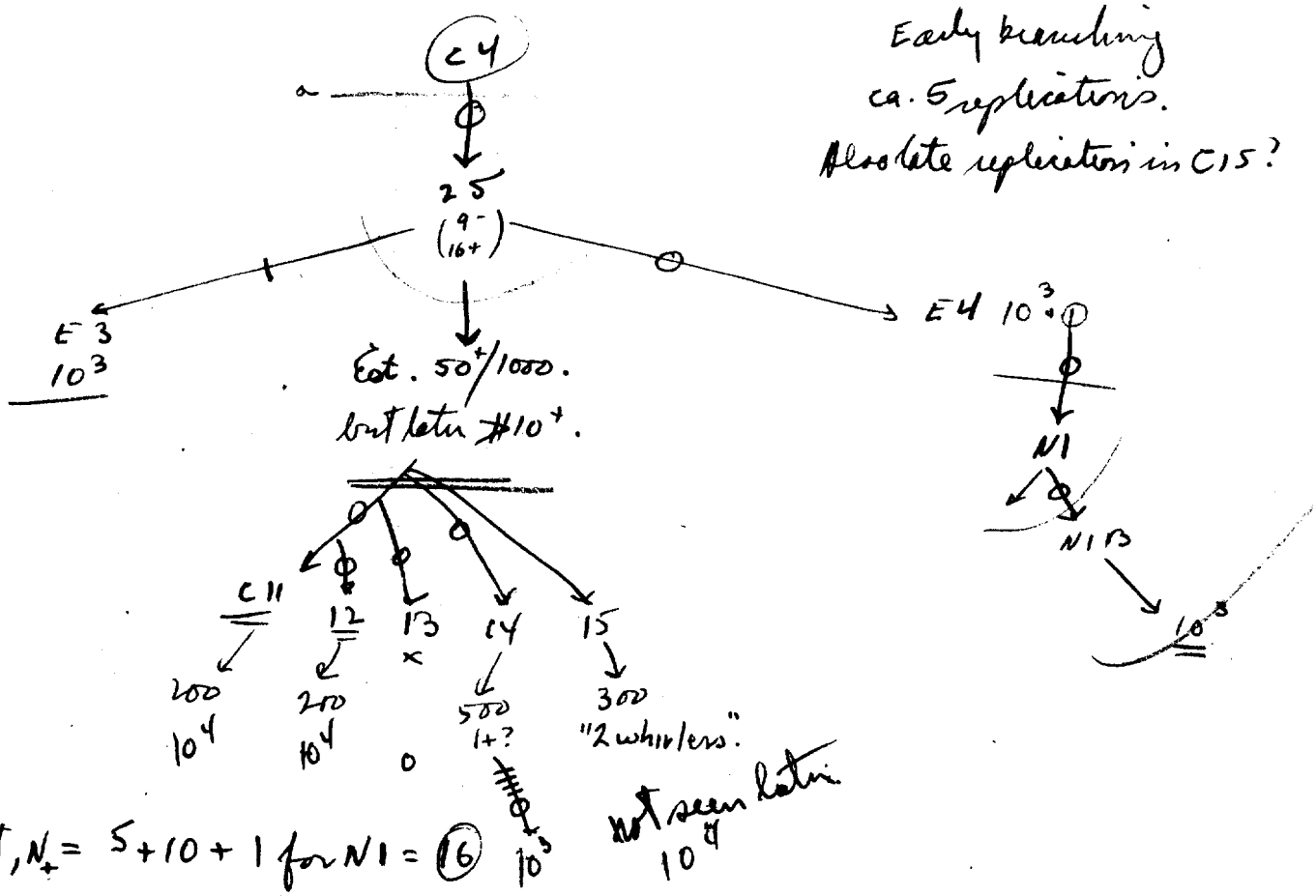
not yet exam[illegible]

C5



No branching.
Linear track =
4 + 1 + 17 + 1 = (23).

C4



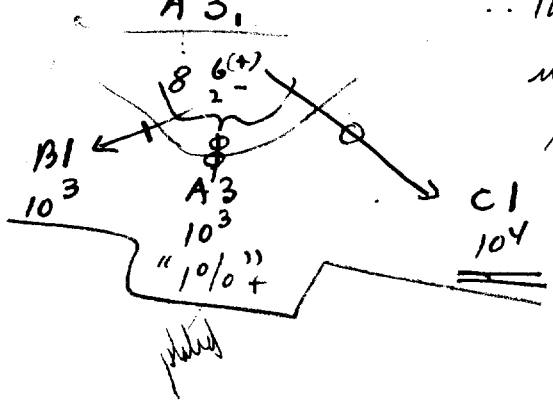
Early branching
ca. 5 replications.
Absolute replications in C15?

If correct, N_+ = 5 + 10 + 1 for N1 = (16)

C15: 5 + 10 + 9 = (24)

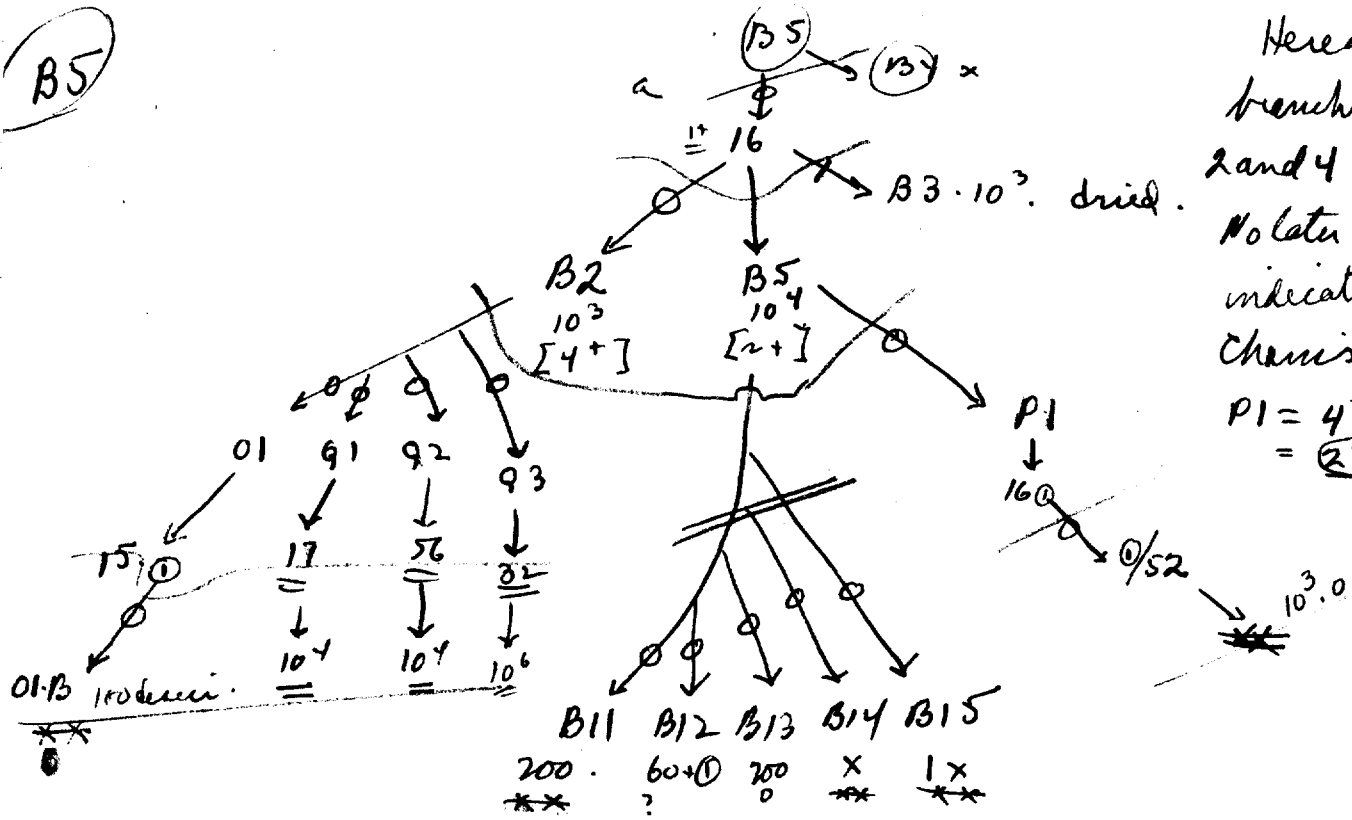
A3

A3 should have been included under 1135 but was plotted instead.



∴ This also probably an initial branching of semiclone, probably not quite completed at N = 8.

B5



Here again, covert branching between 2 and 4 divisions. No later branching indicated.

Chemis: not!

P1 = 4 + 13 + 4 + 6 = 27

Leaves fooled by such clones as B5 thinking that numerous + bitobranched stabilizations of a late clone.

Total N for All ... →

is $4 \times 10^4 \times 70 \times 100 \times 4 \times 2 \times 6144$

$= 32 \times 70 \times 6144 \times 10^6$

$= 10^3 \times 10^4 \times 10^6 = 10^{13} = 2^{40}$.

Total transfers actually: 16.

Semimatch sibs: 8 actually followed;

estimate ca 20 total.

Latest replication: $\times n = 12$.

Should follow earlier divisions more closely.

Resume as of 2/21/54 A.M.

1134 —+→ 1 non motile
—⊖→ 1 motile cell
× n.g.

Platings on motility mixed.

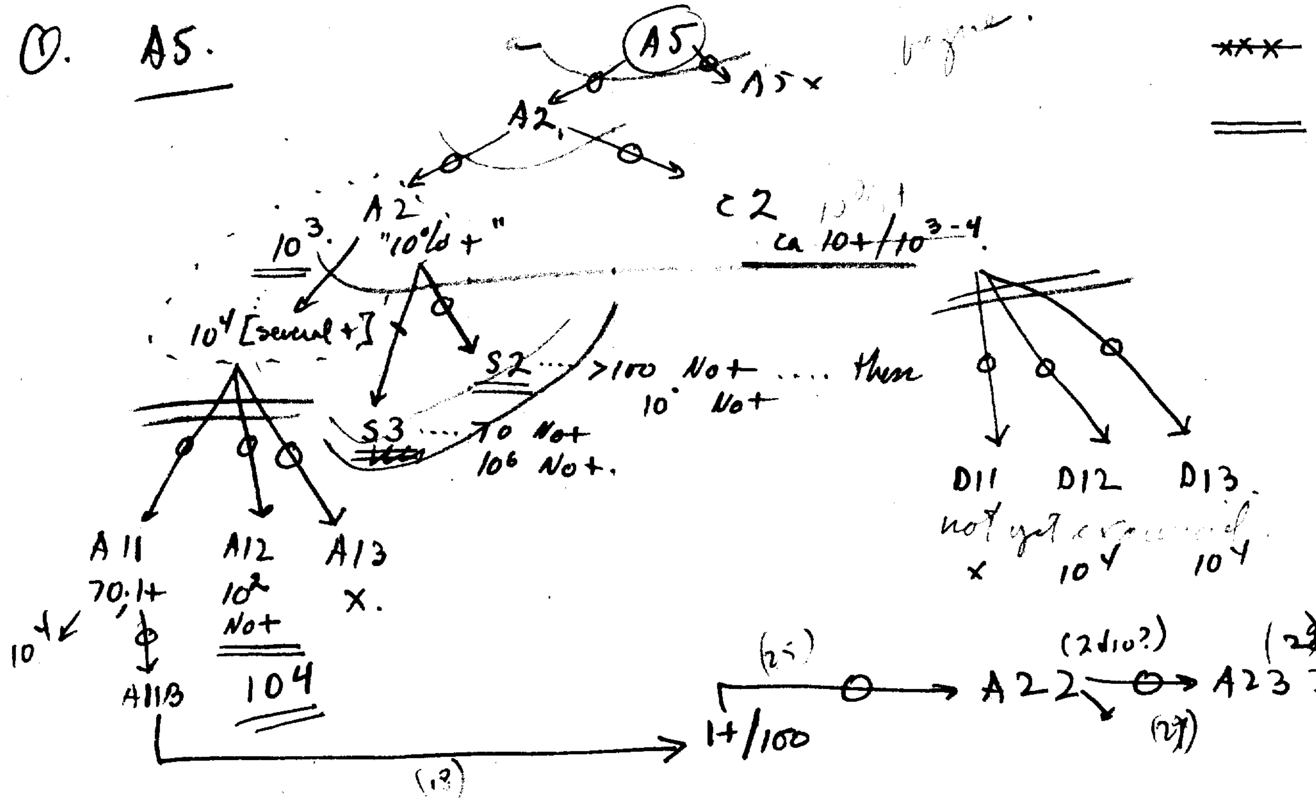
××× on technical acc., end of semiclone.

═══ apparent sp. termination (may include some misses in v. large pop.)

(n=40)

log₂ 614 gen. 8 transfers

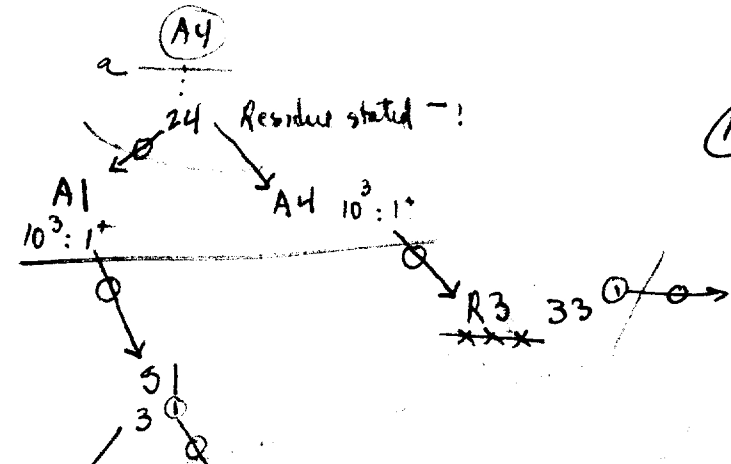
① A5.



∴ Semiclone branched at least at A2, – C2 and in these clones also gave ca. 10 each / 10^{3-4} descendants. Further branching not indicated and clonal propagation for ca. 5 generations would acct. for results.

Max. chain here is A11B : Generations = 1 + 1 + 13 + 6 = (21)

A4.



Max. chain (*) = S1
(16) = 4 + 10 + 2 generations
(19) = 4 + 10 + 5 R3 gen.

1 branching at (A4) and this originally covert!

1134n
plating

Reinserted p23.

		Lp
S1		−
	2	+
	3	.
R3		
Q3		+
	2	+
	1	+
P1		+
O3		
	1	
N2		+
	1	+
M2		
A11	→ A22	+
	12	+
	13 x	.
B 11		+
	12	+
	13	+
	14 x	
	15 x	
C 11		+
	12	+
	13 x	
	14	+
	15	+
D 11 x		
	12	+
	13	+
E 11	0	
	12 0	
	13 0	
	14 0	
	15 0	

Sent to Dot (DCG) for Lp tests.

2/20/54.

Recorded as 1135

at 6P20, inoculate motility tubes with residues of A–F: in toto.

E5 plated directly, and washings in 1ml, not 0.1ml, 0.01ml, and on agar p

	Growth	Tracks	Swarm	Lp22 (DCG) 2/24
A1	✓			+
A2	✓			+
B1	✓			+
2	✓			±
3	✓			−
4				-
5	✓			+
C 1	✓			+
2	✓			+
3	✓			+
4	✓			+
5	✓			+
D 1	✓			+
2	✓			+
3	✓			+
4	✓			+
5	✓			+
E 1	✓			+
2	✓			−
3	✓			+
4	✓			+
5	✓	✓ .1 ✓ .01		+ .1ml: − & phage .01ml
F 1	✓			− colonies
2	✓			− 10

no tracks seen (6 hours)
Uncertainty of eff. of transfer
or whether motile cells
remained, as well as
efficiency of detection.
Try single motile cells
to agar.

Note numerous contamin
colonies in E5! (Use
anti-22 serum?).

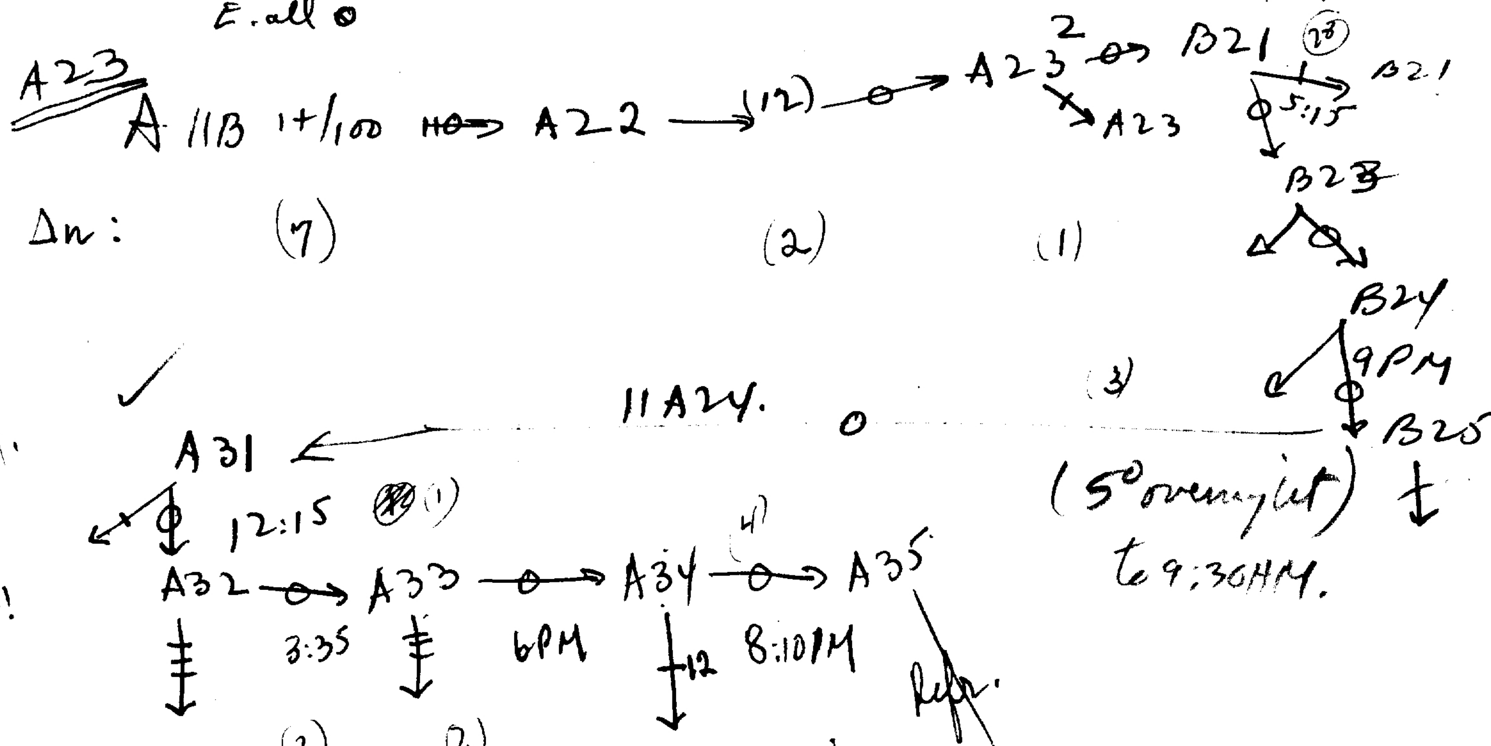
February 20, 1954. see 1134

Data of 1134 received P19, and evidence of multiple semi clones noted. ~~Reso~~ Reisolate motile cells from indicated multiple semiclones about 3:15P20. ~~At 9P20~~, Incubate at 37°! At 9P20, examine.

Although taken at = stage 1134c, these will be treated separately under heading 1135.

Some examined at 9P20 c discouraging results. Refrigerate overnight for later study. (No certain + semi clones. See note 1134e

D, E examined 11A23. D1 0 D2,3 10^4 no + seen.
E. all 0



9:05 AM–12N R.T.

warmed by lamp!

diaphasic fiber content, and 1 swarm only (nonmotile). In course of day at RT gave 36 progeny after slow start, but none motile.

Plate semi clones, Fla+ × SW666

1136

DATE: 2/23/56

REF:

	1	2	3	4	5	6	7	8	9	10
A	FA12 × SW666									
	5 planted.			1 swarm						
				4 surface growth only (one or 2 loci).						
				1 Do not save						
10	Presumably few cells give traces from surface plating. Try pour plate.									
B.	2.									
20										
30										
40										
50										

Feb. 26, 1954.

FA9 (SW 666) × SW 967 11-12^{30} 37° Refrs. Collect Flg^{+} 3:30-4:20

4:25 PM : A1-5, B1-5, C1, C5. (A1-2, B2-C5 prob. sibs).

By 5:45 PM most above had divided once, some gave 2 motile, some 1. Refr. 5:45 PM - 9:20 A 27. Follow clones at room temperature throughout the day., separating further. (Refrigerate overnight). Most clones no longer had descernable motility. Followed in separated subclones during 2/28. Overnight P28-A21, set out at room temperature, but the larger clones likewise were pure —. (Compare 1134-35)

This suggests that SW 967 engenders many v. short generations or else conditions here used are unfavorable.

DATE: March 1, 1954. REF:

1	2	3	4	5	6	7	8	9	10

0: 60A (SW967) → × SW666 12^{15} – 1^{35}.
Studied to 7:45 no Fla⁺. / >10⁴ [illegible]

FA12 → × SW666 3^{15} – 4^{15} 37°. No Fla⁺ seen at this time / >>10⁶
5:30 – 6 PM, several easily isolated.
× Transfer to single drops at 6 PM refrigerate overnight (A1-2-3-4-5 B1-2-3). A1-2 are prob. sibs; B1-2 ?Fla⁺ after transfer.

A2. Remic. under lamp 9:40 – 10:55. Reexamine.
overheated! T.O.

3/2/54. 5-6 PM. From earlier mixture, refrigerated (ca 2 hours intermittent incubation previous). Isolate 12 cells. (some divided before being put away).

	a.	12:30 PG.	
A1	× ①	×d.	
A2	①	×d.	
3	. . ① [illegible]	×d.	
4	①① . .	×d.	(1 group only)
5	① . . .	×d.	
B1	lysed		
2	. .	3.	
3	→ 5. to D1 →	3+, 7− lysed	
4	large clone. By 5+ PM had formed 15 later pairs (total A11 – F15)		all pairs ±.
5	. . . .	ca 16. each almost d.	
C1	①	1.	
2	1.	d.	
3	1. + ~ . . .	.	ca 8. each, some lysed
4	1.	1	
5	1.	ca 20. some lysed.	

T.O.

B3 → D1
a = 2:10 PM 3/3. (Refr. overnight, at R.T. to 2:10)
. = unmot.

b. 3/3 5 PM transfer the B4 isolates (A11 – F15) to new coverglass, and incubate these overnight at R.T. Original in Frig. to 10A6; to 30° 10A6 —

P3.

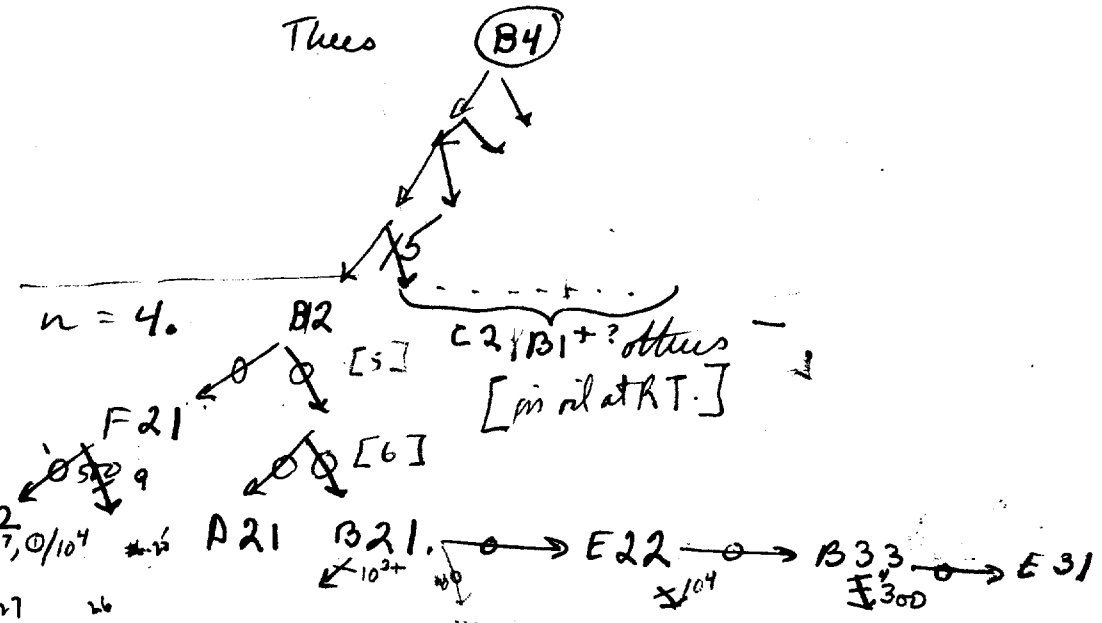
By GPH, those subclones had undergone 1-3 further divisions and were separated within the replicates.

(c) A4. Examine clones of A11-F15

But by this time, most were already mixtures.

(e). Most have dried out. But of the 15 sites, only following were recorded as motile at this time:

C2, B2, B1. C2 probably unviable. C2 and B1 dried out, but B2 clones OK.



d. 2 PM. B21. ca 10^3 total, 1 Fla+ = A21-25 B22-25 C21-22 E21, E22

D21 ca 400 4 Fla+ = D22-24 E24 ? E21?

F21 ca 500 1 Fla+ = F22.

15 + 3

e. 4:20 PM. Separate some subclones.
(Visit by Francis Bello from fortune).

f. ~~9:05 AM~~ 2:30-5:30 PM 3/5. Ca 10^4 clones in each.
From 10 clones identify + separate Fla+.

g. 8:30 PM — Research remaining clones for +. Record already transformed for next cycle

over.

4-10A6 Lewannie clones

only B33, C32 still semiclonal.

These ranged to i = P6, j = A7.

Total n = 44, n = 42 resp.

involving 11, 7 actual transfers

Replication occurred beginning n = 3, 4? to
n = 10 (no greater than 19 poss.)

Detailed examination of early subclones seems now necessary. Also, repeat (n=36) transfer of cells to motility agar to verify multiple trail origins. (Assume that most semiclones never get into ~~agar~~ soft agar)

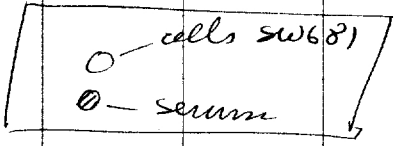
Serum eff; phenol ~~tactism~~ taxis

DATE: March 6, 1954. REF:

A). Test efficacy of immobilization of SW680, 681 by homologous antiserum. Nearly complete agglutination in slide droplets under oil. Individual immobilized bacteria are also seen. Some seem stuck at one end. Other single bacteria still swimming at first. Suggest agglut. in tubes, then transfer under oil.

[10 Also try more dilute susp. for immobilization to determine whether single cells can be diagnosed by dilute serum! J.

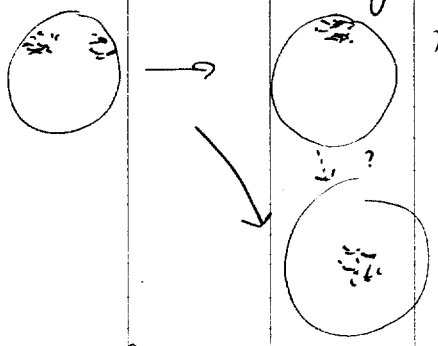
B) While setting up expt., separate droplets were set up as



& a polarization in the cell drop was noted.

20 As the serum droplet was entirely isolated, a oil-soluble component was suspected, namely the phenol preservative. This was promptly confirmed by the arrangement resulting: The polarity was transient, and cells randomized again after 1-2 hours.

3/7/54. Experiment ℓ 1/2% phenol repeated ℓ clearcut results. No serious effect on non-motile bacteria (SW967). Also noted moving bands of cell concentration becoming either apical or (later?) central. Chemotaxis very marked. These might suggest either an optimum conc. zone or faster diffusion through oil vs. water.

40 

SW681 diluted 1:50 in non-motile SW967 showed same tactic effect but only for the motile cells (ca 5+ fold concentration distally.

Possible mechanisms? ℓ phototaxis (Clayton)

3/8/54. In agglutination tubes, definite ring formed over phenol (resorcin + lactose) 10% ← ring in 15-20 minutes. ← clearing Results are more clearcut in oil

March 8, 1954

Plate single motile cells in motility agar to learn possible multiple branching. Mixtures at R.T. 12⁰⁰ – 3⁰⁰ PM

FA92-X5W866 } no + seen! Refr to 4:30 PM, Incubate 4:30 – 6 PM.
FA92-X8W967 }

Most efficient isolation: let Fla⁻ settle, scan only top focal plane. Catches perhaps ½ all motile cells, but with a fraction of effort required for complete search.

By 6 PM, a dozen Fla⁺ picked out, some separated.

Refrigerate to 8:30 PM, then continue. Separate 13 cells total.

at 8:40 PM:

2P9.

A	1	①	
	2	①	swam
	3	①	
	4	①	
	5	①	
B	1	②	3.
	2	①	
	3	① 1.	
	4	1.	swam!
	5	①	
C	1	①	
	2	1	
	3	①	

no tracks.

Expt. rather sloppy

Ca 9–9:20 PM, Fleshout dilns, plate to motility plates. Add first agar to embed. Then impregnate dilnly in additional layers.

Incubate 37°. (0.5% agar formula)

7/13/55 Analysis → ϕ diff

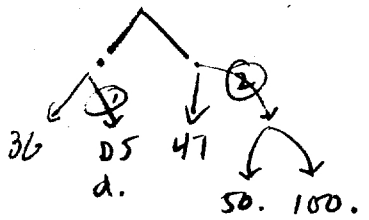
A1 ϕ

A2 ×

A3 ϕ

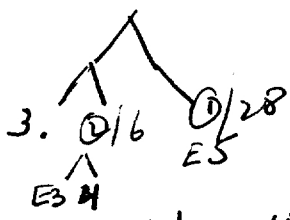
A4 see polygin

A5.

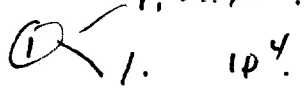


B1 — V.sh D (8.14.)

B2



B3



B4 v.i

B5 n.g., stayed motile

C1 diff ϕ

C2 —

FA12 — × SW666
single cells added.

1141

March 9, 1954.

Further survey of pattern of establishment of semicloses
Terminology: coverglasses now ruled in advance, (A-H)(1-5)
and marked with serial numbers 101...

12 → × 666 kept overnight in frig to 9:45 AM.

Examined 10:20-10:55: no Fla+ seen. [Sample refrigerated and examined again later also negative at 4:10 PM].

Preps. at room temperature 1:45— 3 PM Isolations (ca. 3-4 hours).

Numerous Fla+ but isolated at uppermost focal plane.

101 at ca 3:40 PM. collected:

	a	b. 4:50 PM	c 11 A 10	d 4 P 10	e 10 A 11
A 1	+	1 1	✓	1.	A: 1/10⁴
2	× ... Reservoir.			0.	BCD: 10⁴.
3	1	+	0	0	
4	1	1 +	1.A 1.C 1.D T.D	A. 15. → D2, D1	
5	1	1	1 →	B. 2/9.	
B 1	1	1	+ . 1 . 1 .	1/36. → D5	d, 10⁴
2	1	1	1 . 3,	2/47 → E1, E2	10⁴.
3	1	1, 1,	1. 1.	8.14.	10³. 10³. 10⁴
4	~	T 1. 1.	2. 1. 1.	2/6.1 → E3 E4 E5	10⁴. 10⁴.
5	1	1	1.	1/8	(14)/10³⁺ (6) → H2
C 1	1	1	1	1.	1
2	1	1 1	1	0	0
3			T_A T_C 16+- 8 1 2 T_B T_D 22	1. A2 → .	100. 10⁴. 10⁴. 10⁴.

probably abandoned inadvertently

Record lost

b. Refrigerate 5 PM to 9:30 A 10.

d-e R.T. overnight

e 10 A 11.

FA92 — × 666 37° 9-12 N, Refr to 4 PM. No Fla+ seen.

FROM	10-12N11	N12	
D1 } A2D	②/$10^{3\,000}$ → G1G2	10^{4+}	
D2 } ②/11.	⑪/10^3 ③ → H3	10^{4+}	
D3 ①/32 A2D	10^4.	10^{4+}	
D4 A2C ①	①	①	This all long note but in g.
D5 A5 ①/36	d.	0	
E1 } A5	50.	10^2.	
E2 } ①/47	100.	10^4.	
~~E3~~			
F1 B4A.	10^3.	10^3.	
G1 D1 } ①/10^3		10^4.	
G2 D1 } ①/10^3		$+18/10^{3+}$ → 5P12 → 105 (G, 1+) (1-5)	
H1 } B4A ①/10^3		25 (d).	by 3P13, each gave $20-10^3$.
H2 } B4A ①/10^3			
E3 } ②/6. B2		→ 10d.	
E4 } ①/28 B2			no record on E4-5
E5 }			
H3 D2 ⑧/10^3		10^5. Not (incl top)	

Confusion re G1-G2 H1-H2

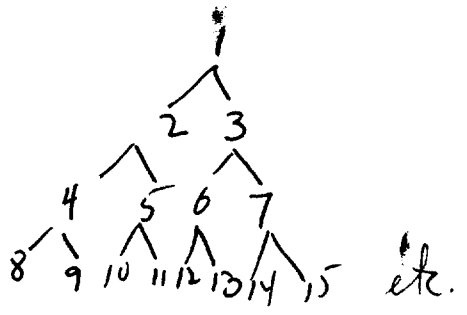
1 group is from B4, ⑥/10^3

1 group is from D1-D2, ①/10^3

Note: no H1 is listed.

∴ H2 is probably from B4. Where F1? Maybe C3 - But unimportant.

Many of these pedigrees are there shortlived! (Cf. 1142A-B!)



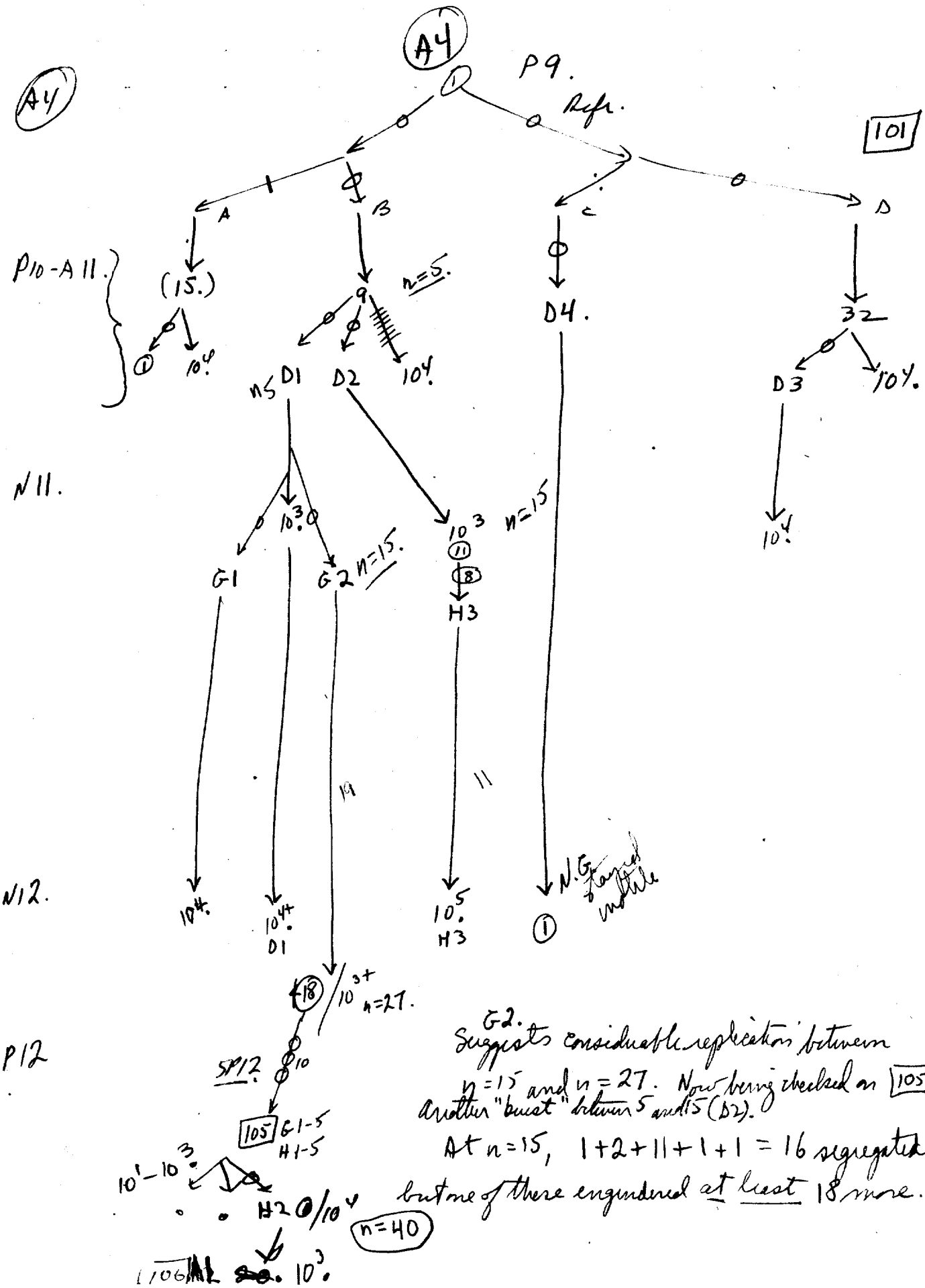
1141 A 4. line 62 showed replication #19 #27. Then only semiclonal for up to $n = 40$.

Total semiclonal yield:

Cells	4	5	6	7
#	1	20+11	1	1
		31		
Min fate:	K6.	40,	15	7.

Time of division: 19-27; 9-15.

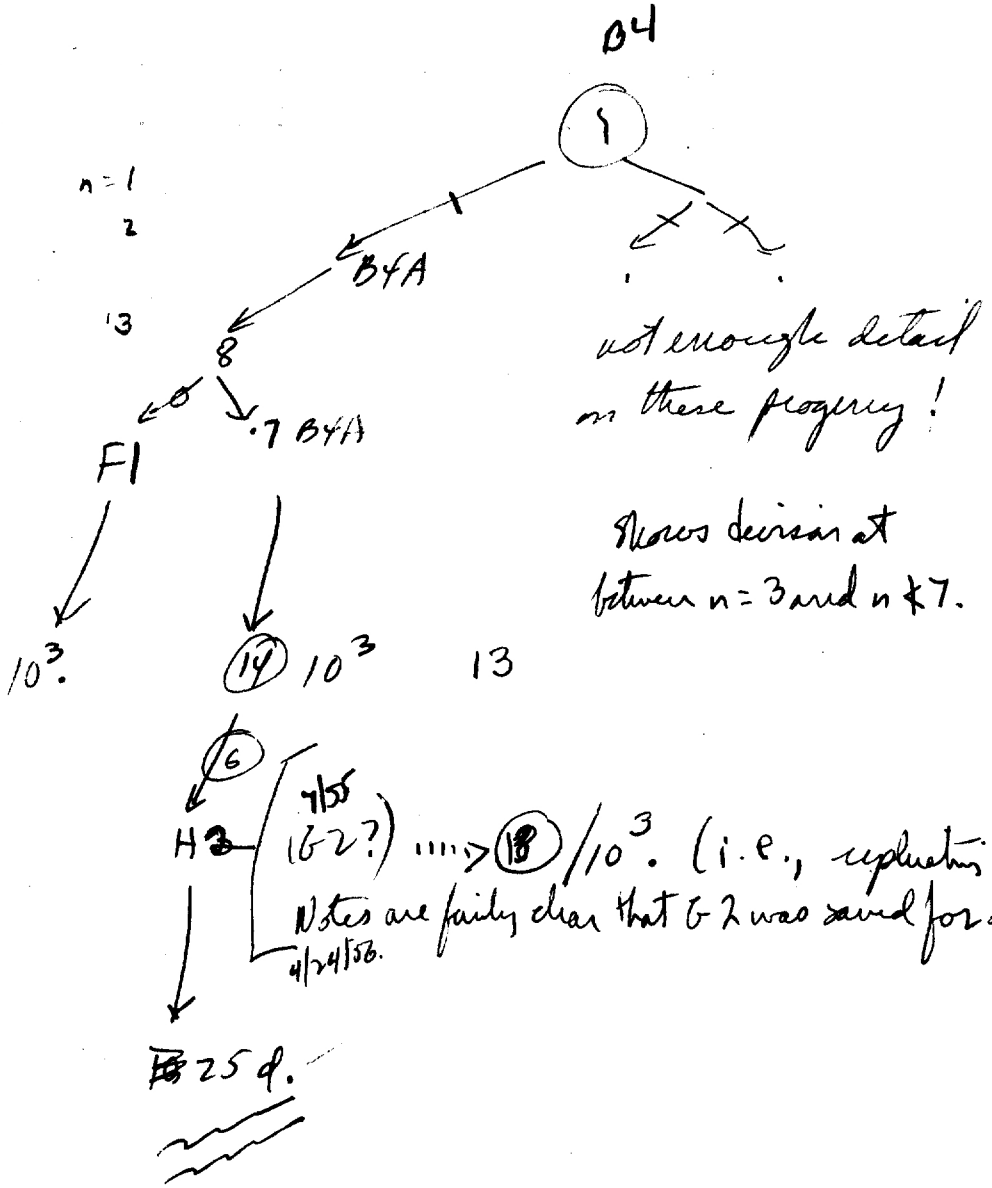
11/4/
pedigree



G2.
Suggests considerable replication between $n=15$ and $n=27$. Now being checked on 105 →.
another "burst" between 5 and 15 (D2).

At $n=15$, $1+2+11+1+1=16$ segregated semidarks but one of these engendered at least 18 more.

B4



COMMENT: I do not yet have a really adequate pedigree or genuine origins. Partly, it has been necessary to sketch out the background. Previous observations had suggested that division was restricted to $n < 10$.

Hyp: ① Irregular replication

② Segregation of particle

A: polytenic loci, aborted haploid sectors

B: gene product, inherently heterocatalytic but particles.

Best basis of distinction would be total extent of replication and irregular later initiation of swarms to support A. Both might be possible!

From this plating, motile + non motile recovered.
F4 (mislabelled 1142E3).

PA 60 → Fla^- gave +
9 → did not.
22 → Fla^- → H_1^b.
∴ Fla_1^- H_1^b ; sub is Fla^+ H_1^b.

No + found from > 40 isolates from "E3"

60 → x SW666.
and plating

[104]
[105]
[106]

March 11, 1954.

PA60B-x SW666 12·30 – 3:15 PM. Turn Refr. ca 4:40 – 6:40 isolate + plate.
Use entrapment.
Plate EFGH on .4% motility agar. 6:40 PM. 2 P12 See over.

(a) Survey yields from (1)

ca 6P11. A 1 — a 1.
2 — 1.
3 — (1)
4 } — (1)
5 } — (1)

B 1
2 — (1)
3 — (1)
4 — (1)

Essentially N.G!!

C 2 — (1) — (1)/500
3 — (0) — (50)/10^3! ——o——→ [105] C1-5 5P12
4 — (1) — (10)/10^3
5 — (1) — (1)/10^4

D 1 — (1) — 10^4: ~~ca~~ ~~x100~~ motile! —o→ [105] (D,E)(1-5). 5P12
2 — (1) — (3)/10^3
3 — (1) — 0/10^3 plating also → no motile swarms.
4 — (1) — 0/10^3
5 — (1) — (1)/10^3

E→H plated. ←

Plate (hastily, no flush) (on surface 0.4% agar) Res. on [104] P12

		A12	A13	
E	3	0		swarms → DCG from plating E/H 60
	4	1.	:	0
	5	1.	:	0
F	1	1.	.	F/a –
	2	0	:	⊕/–
	3	1.	:	0
	4	swarm – to DCG to isol +/–.		0
	~~4~~ A4	0		0
G	1	0		0
	2	0		netwole.
	3	smeared..	.	0
	4	1.	3T?	0
	5	1.	2T?	0
H	1 [(+)]	1.	trail?	F/a.
	3 ~~4~~	0		⊕/–

But no appreciable trails were seen later. Thus v. few trails are manifested upon isol count.

Good correlation!! 0/0 picked = Trails not very striking.

Fluid trap method (had been tried before on possibly negative samples) now found to be most effective.
Use FA60-x as including another factor possibly linked to H_1. Swarm isols wanted for crossover test.

5P12 — 3^{15}P13 16° 4P13 4P15

[105] not to [106] 4P14

no add'l +.

42C3 C1	10^4						
2	10^4	①	C1	50.			
3	10^4						
4	10^4						
5	10^4	①	C2	①/100	0/2	50. 10^3	∴ ended semiclones.
42D1 D 1	10^1	②	< C3	50.			
2	10^4	①	C4	①/200	0/2	20. 10^3	
			C5	0			
3	10^4						
4	10^4						
5	10^4	①	B5	4.			
E 1	10^{3+}					3	
2	10^4	→ F1	B1	28.		10	
3	10^4	①	B4	50.			
4	10^4	F3	B3	50.			
5	10^4	F2	B2	8.			

∴ each was 1141H2① A1 100. 10^3

semiclonal not clone!

Pedigree results

By n = 10-15, many clones showed only a few +. Not followed further. However, C3 had ca. 50+. 5 sampled ~~etc~~ terminated by n = 23 except for 2 which ~~terminated~~ terminated n ≥ 23 < 31 and 5 which was considered a semiclone ~~+++~~. +41.

a)

n): Thumps replication +6 +10. Semiclones ~~+~~ +41.

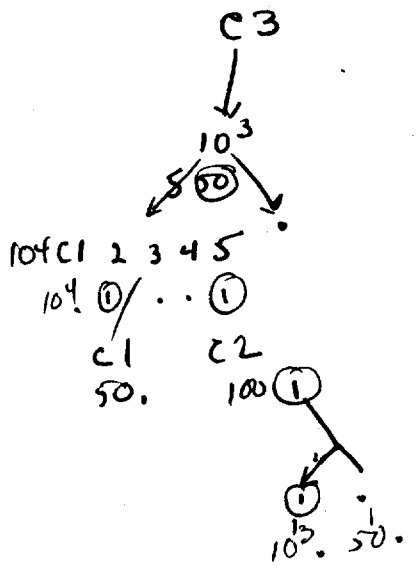
max semiclones

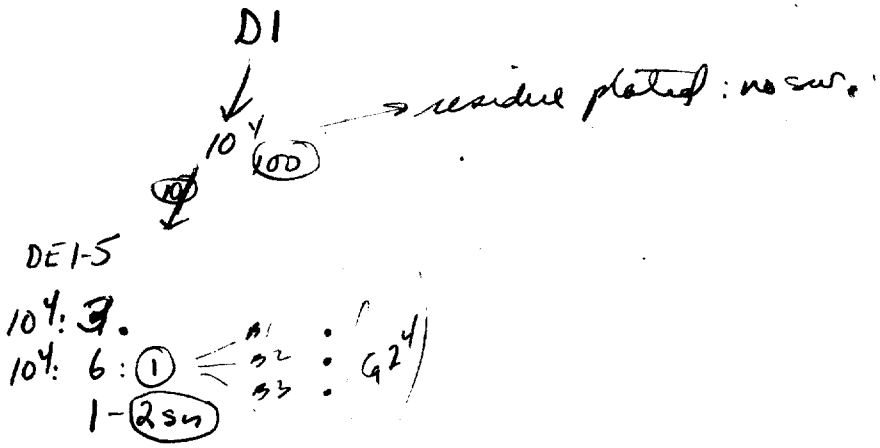
b) D1. at n = 13, ca 2^7 motile. 10 tested and showed repl. at 14.

∴ replication to +14. Semiclone then to +35. Others terminated earlier.

What was remarkable here was simply the very large crop of semiclones.

Need more detail on distributions of motile in earlier versions. It is now quite clear that replication does not extend beyond n = 15, usually ends sooner.





1143

110
107
112

DATE: 3/16/54. REF:

F460B × 40666 (old susp) 11:25 – 1:45 at 37°. Rpfr. as 1143 stock
Isolate trapped motile cells to [107] A1–D5, E1 and plate.
[Should have labelled + followed residues but did not.]
D1 = 4 app. NM cells E1 = 8 motile cells pooled. For most platings, embed in layered .4% motility agar, chilling to immobilize. Layers seal perfectly.

E1: swarm D1 2 dup colonies.
not chilled 2/3 i colonies. (contam?)
chilled: 14/16 had dup "colonies": no swarm, no distinct feathers
at 10A17. Reincubate. Medium is quite fluid. Φ 1/plate ∴ contam. swarms
A19: no tracks. See over for results on swarms.

Also, using old, rpfr. susp. (1141-42) Isolated A1–D3 on [110] F1–5
but decide later not to use. However D4, 5, E1–5 isolated ca 2:30 PM from ~~xxx xx~~ 1143 stock above.
Refrigerate at 5:55 PM. at 3:40 PM, all there were just ① except E2 (②) and F1 (②).

at 8P16, Check some of these, on [110] and transfer [110] E1-5 to [107]. 110 – F1 showed ○, ⊕; others not checked. Incubate [107] at 29°; Rpfr [110] Residue 8PM – 9A17. Then RT tel index.

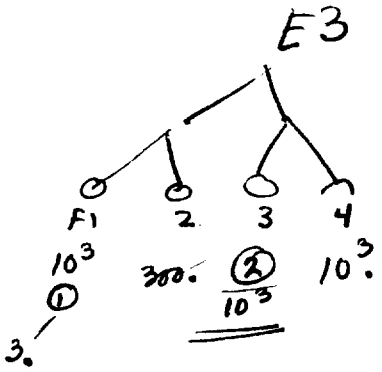
[107]: GH eso. n.g. (see protocol). 10:30A17

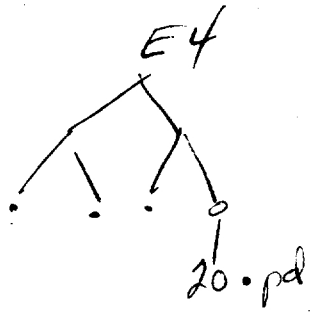
E	2	+2+/300	to [112] B1, B2
	3	50.	
	4	+7+/500	– 112 : B3-4-5-C5.
	5	post d 2.	
F	1	⊕/10^3	→ A1.
	2	200.	
	3	①/10^3	
	4	10^3.	

seeb

Intact further replication.
Inc 30° 12:30P17 to ...

107





suff for E2

3/18 [107] 8P16 — 10^{30}A17 → [112]

	[112]	3P17 [112]	6A17 [112]	8^{15}P17 [112]	
B1	①/19	①/8	①/8	→ D1	
B2	①/24	①/18	①/6 n=2	D2 v.sl.	
B3	①/86	①/8	①/5 n=3	D3	
B4	①/8	①/8	①/5 n=2	D4	
B5	①/10	7.	40.		
C5	16.	50.	10^2.		
A1	3.		50.		

to 16° overnight.

110 E2 { E2 + 4②/300 B1, B2
3 +
4 + 47 50/10^{2+} B3 4 5 C5.
5 + part d. 2

110 E3 { F1 + ①/10^3 A1 → immobile
2 + 2-300.
3 + ②/10^3 . ..
4 ± 10^3.

110 E4 { G1 − p.d. 100
2 − 200.
3 − 60.
4 + 20 p.d.

110 E5 { H1 + 50d
2 + "
3 + "

110F1 0

RT to 10^{30}A17

to 38
at 11^{30}P17 − 3PM
Thresh. T. various
times

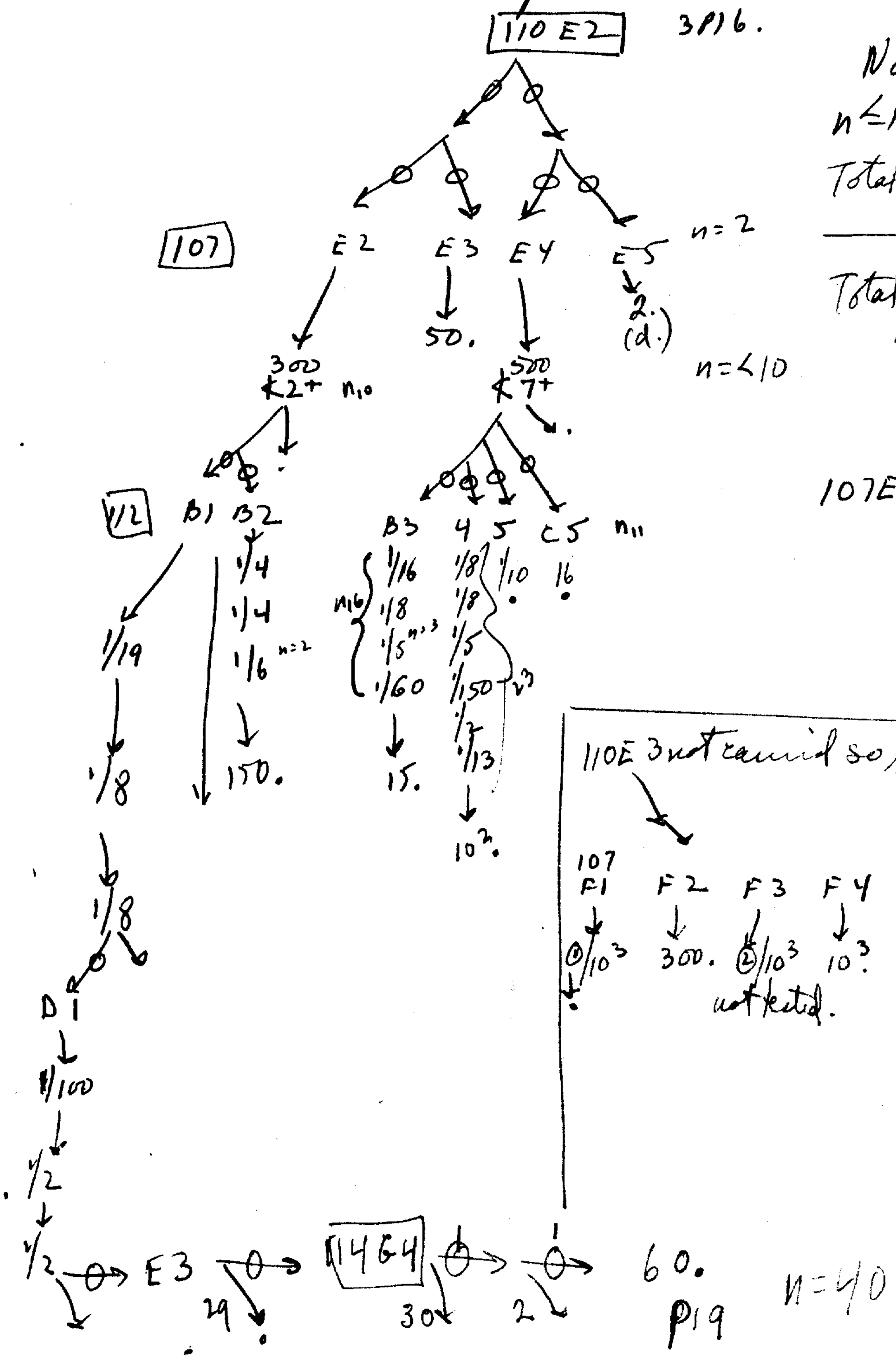
~~1^{30}P18~~ [112] — 11A18 to 3P18 — RT to 6P18. Refr. to 9^{30}P18 — 16° — A19 — P19

D1	①/100	①/2 ①/2 → E3.	①/29 → [114] G4	①/30	①/2	60.	
D2	150.						
D3	①/10		ca 15.				
D4	150.	①/2 → E1. v.sl.	13. → [114] G3	10^2.	10^4.		

Summary.

a) Platings: cells grew out in dep agar; no tracers

b) 8 clones: fun + at 10^{2-3} / 4 [illegible] tested for later replication. 2 clones essentially n.g. (died) 2 clones followed:



No replications after $n \leq 10$, and mostly prior
Total crop. 2, 1, 7(1).

Total fate: 107E2 = 2 + 8 + 4,3,3,7,1,1, 5,5,1

40 = 10+30 in [illegible] and 10 + 6 in another

107E5. 10+16 = 26

10+20 = 30

10 + 3 = 13

10 + 0. = 10.

∴ have no ~~later~~ decisions seen after n = 3 but not properly tested.. F! shattered.

109

111
H4

60B → × SW666

3/17/54. Refrigerated 1143 susp. 9 40 AM. — 10 20 AM.

Then 30° — to 16°

8 30 P17

all residues unless indicated

109

245 P18 114

A2 ✓ 52. ① → 111·A1 dried out. Res. 10⁴.

3 ✓ n=6 ② → .. R 10⁴; ①/300 ①/200 → D1, D2 see G1,2

5 ✓ 26 ① → . 10⁴. no further +.

B1 ✓ 1? (+10)/60 (16)/150

2 ✓ 25 (10) 4 → ....

SWARM 3 ✓ (50) swarm 1? → +also. Pure + Pure gns (A.B.) save

4 50 (11) isolated insertion A–K see below. R 10⁵.

5 57. ③ → ... (...) 3 not seen.

C 3 not exam.

5 " .

30° — 3 40 clones already large. Refrig. to 8 30 PM.

Note: manipulator broke down 9P17.
Ed Tatum visited A18.

3P18: ① from B4 to each of 11 droplets (A–K). No exchange
Ca. 250 each. Motile as indicated.

109 B4 / 114

A	0
B	+15
C	0
D	0
E	0
F	3
G	2
H	1
I	1
J	4
K	1

D4 ✓ F3 E4 D3 ✓ C3 - F3

A1-2-3
A4-5 (recorded at first division (but not necessarily this product).
B5
C1
B1-4 B4?
C2

16 P18 · A19 P19 (RT)

A 1	1.		1.
2		①/8	13.
3	1/25	1/2	10³.
4	0		0
5	0		0
B 1	①/16		200.
2	7 ①/26		60.
3	82.		61.
4	31.	①/10³ 1/2 → H2	
5	①/26	1/150 1/8 → H	
C 1	①/9		40.
2	①/45 1/2	1/60 1/2 → H3	
3	①/28	1/60 1/2	
4	52.		10³.
5	①/20		44.
D 1	71.		10³
2	36.		10³
3	73.		10³

D4	21.	300
D5	17.	10³.
E1	①/28	①/118 : 2? → F5
2	28.	32.
3	16.	10².
4	22.	10².
5	31.	400
F 2	33.	200
3	①/34	70
G 1	7150.	10⁴
2	60.	10³
3	10²?	10⁴
4	①/30	60

H 114

A20

H 1.
2.
3
F 4-5.

N19: Sherman visited

8 30 p 21

A 22

∴ One line reached n = 59

others n = 33, n = 52 before

primogeniture ended.

Isolate residual non-motile sibs to check

their propensity to give long descendances.

1144 F 2

G 3

1144 Pedigrees

10 clones pedigreed cumulatively.

Had planned to isolate clones at $n = 3-5$, but let them go too long. ∴ picked the notable from B4.

A2 1/52 – 1.

A3 2/26 no further replication. Fate: $n = 13\pm$.

A5 1/26 → 10%. 1 Limited semiclone (or others appeared and passed!) $n < 13$

BA ~~10/60~~ ca 16/100 not pursued. Grew late. Early division apparent

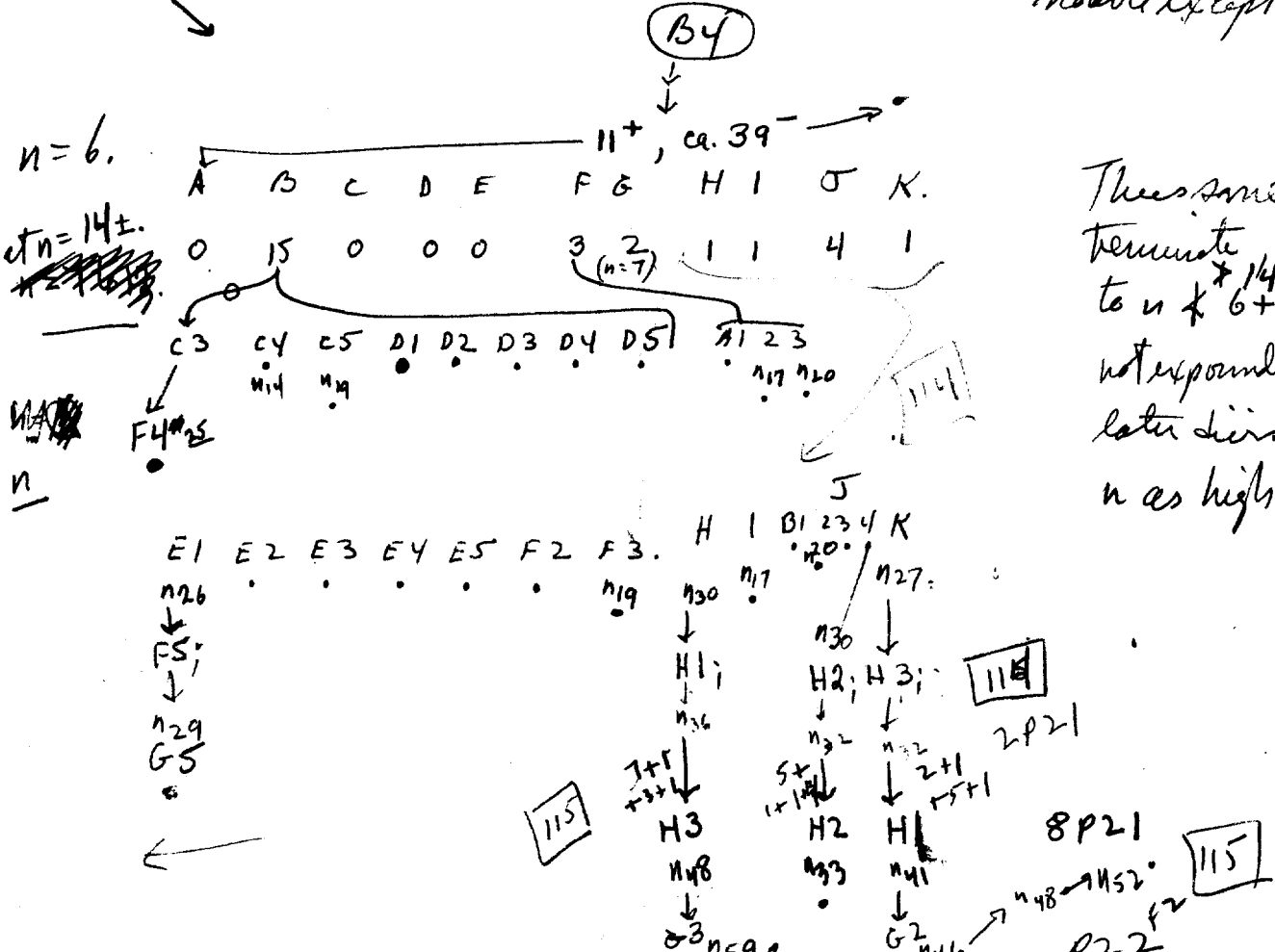
B2. ca 10/25 4 isolated, A 0/10^3 B ~~8~~ C ~~6~~ D ~~1~~.

∴ substantial replication $n > 5$ ~~8~~ but not pursued.

B3. Pure savarin. Check H, statues.

B4. N.i. B5. ③/57 lost.

Thus all clones not notable except B4.



These same semiclones terminate, others divide to $n \sim 6+4 = 10$, but not expanded fully. No later division seen to n as high as 59!

3/19/54.

5-6 PM. Isolates from pepsin refrigerated from 3/18. Plant at R.T. to see whether gross semi clone pattern applies here. A few previous isolates were discouraging, but agar this combination gives the largest tracks + should be the most suitable for transplant tests.

[116] 7 colds isolated 5P19, kept at R T overnight.

A 20. 6/7 were ca 10^3. C1 had 2+ to [115] H4, H5.

N 21: H4: lysed H5... 1. lysed.

Time Pla+ x — in re growth

[1/3]

1148

3/17/54.

A; 2:05 PM Mix 60B, SW66 (old) in c.g. R.T.
(Isolate cells singly & small groups) at 2^{20}–2^{40}.
Motile cells not seen in suspension at 2:40, (3:00), 3:35
Then R.T. By 5:40, ++ motile.
No substantial increase of isolated cells, but probably did in main drops after all. Will have to be done by plate count.

swarms. SW666x —
1144 H3x —.

1147

DATE: March 30, 1954.

REF: [118].

For past few days have had poor luck c SW666x — to find motile cells. possibly suspensions too old, used too small drops?

3/28. Ca 3 hours then refrigerated to   SW666x — FA60B.

(1) 3/29. 2 Fla+ → [118] E1, E2.   P30: E1: 0 E2 ca (5)/20. leave as is.

(2). 3/30. Suspension as above. 1144 H3 x — 60B. (= trail end sib of SW666x — 60B)
Isolations from 3:55 PM — ca 6 PM. Ca 1 division during this interval for some cells. Single motile cells to drops, a few sibs separated etc. 26° overnight.   [118]

3/31   log₁₀ cells   mot.   to fate [119].

A1 —
2
3   2   1?   .
4   2   0?
5   2   2

B1   (4.0)   (1) → B2
2   2
3   2   .   1 → B1 [illegible]
4   2 C   A(20)   ca 50, 20+?
5   0 A B   B, C 0

(mito & 3 [119] ←)

C1   4   1 →   C1
2   (2) 2   1
3   2   1+   C3
4   (2.)   1+, 1−
5   (2 smears)

D1   3. 3   4 4   (1) D1
2   0
3   (0)
4   4   1.   C. 4
5   4

E1   0
2   4+   K1   D2
3   3+   K1   D4
4   3   .
5   3   1?

F1   4   1?
2   4, 4   K1   K2 E4 E5
3   1.3   1+
4   3, 2   1, 0   E1.
5   0

G1   3   ca 50%+
2   2, 3   1   F3
3   2.3   3 4B   F4 (all 8)
4   3   2   F5 (7)
5   2   1?

H1   1, 3   .
2   4−, 4−   >7100+. K2 (5± each)
3   3, 3   all+.   to H1, 2
4   4   1   G5
5 —

did not search carefully for individ motile cells. . = 0 or 1 or 2...   This expt. designed for info on swarms.

Resumm. 4/5/54. 6PM

3/30/54.
35 motile cells to [118]. 8 clones had few or no +
3 failed
19 had a few
? 1 48/200 (118G3) →8 119F4 → 121F3 42/104... (5?).

4 definite resumes

1. pure S variable uppr. 118 B4 → 119G3 (20)/50 → 121G3. (pure motile. c contaminant came in later)
2. pure S G1 → 119G1 → 121G1-G4. any non-motile? prob not.
3. T+S! A2 119H3, [H5, F1, F2.] — form nonmotile branch
4. Pure S. H3 → 121 H1, H3. (2 initial sites. Both pure +.

Other lines were not followed up. [120] lost P3 interrupted analysis of possible segregating lines.

picked.

1. ~~not yet analyzed.~~ pure +. 119G3 Erratic expression of + gene.
pure S 2: ~~119G3, G4.~~ 121 G3, G4 same motile line.
3: 119 : H5, F1, F2 non motile branch
S.T. + ^{119}H3 } motile bands ~~D1 single cell +.~~
− ^{121}D3

4. 2 branches: 121H1, 121H3 both motile
pure S

DATE: 3/31/54. REF:

See 1147.

SW666, overnight culture. X-60B R.T., 10^{30} – 1. (2½ hours).

~~Tube~~ Set up 4:10 – 4:45 [1120] No+ seen. R.T.

5:20. No+ . heat c̄ lamp (R.T. too low now?

(ca 21-22° today)

5:45 Rare +

becoming > numerous ca 6+ PM.

A 1
2
3 5:52
4
5

B 1
2
3
4 6:03
5 6:10.

10-20+/300 → d. (few+)/10^3.

×30+/10^3

Pl. Trees of 10 cells, 2 failed, 2 not+, 4 few+, 2: B1, B4: no further multiplication on further transfer.

[120] lost P2. ∴ no swarms. 2 clones moderate exp. semi-clones

∴ 10 cells 8 viable no swarms.

Cultigrity of 124F5 and 124H2-3.

F5. (122E4)

Initially, ① 6.

10⁴. → >40/10⁴

∴ n = 13

Ca 20+ / 20- → 40 → 123C2

10⁴ K7⁺ → 7 → C5, 10⁴.

∴ $\Delta n = \log \frac{10^4}{40} = 8$

n = 21

R2D5.

①①① ⑤

n=11   10³. K35. Ca 40 [⑳] → C1

n = 19   10⁴ K8⁺ → 10[⑦] → D5 123

n = 29   10⁴ K4⁺ → 0 → 124F5 ②/10⁴

Φ' lost

became contam: i coli C. accident recalled (stopped e.g. too fast, drops came off).

① ① 0

n = 42   125E1   125D1.

4.   40.

P6:   ↓

124H2   10⁴.   10⁴.

occ mutate.   124H3

probably contam: check OC etc.

(might be contam coli)

To be checked

see next page

/5/54
PM

April 2, 1954
60B × 666 10:00 – 12:20 at 30°. Refr.
~~? suspension (same as 1148?)~~ Extra 20–30 mins at R.T.?
[122] ? Start to separate some at 2–4 cell stage, but too far along!

3:40 – 4:25 isolations [122]: A1 – H4. F1, G5, H5 X.
37 single motile cells + 11 pooled at Z1.

all grew. SWARMS: 122 B5, F5, Z1. ∴ 3/48 swarms.

A few large semiclones also transferred:

[122] →	[123]	✓ = uninteresting
C2	A1 – 4	✓
D1	A5	✓
D3	B1 – 5	✓
D5	ABC(D) 435 → C1 (8)/10^4 → D5 44	
E4	> 40. C2 (7)/10^4 → C5	
F4	40+/10^4 . C3. → (3?)/10^4 → C4 440	

D5 44 .
C5 7. (1) → lost; (1) → [124] F5
C4 440: 4?2 → lost; (1) → 124 – H1,2,3.

ca 10^4/clone. plated ... no TS! N4. P4 (ca 10^3 each). P4 10^4 each.

B5 ∴ pure +

F5 ∴ + , [rough! –] pure ↑

D1. Almost pure +.
F1. Try to separate stationary cells.

0/22 non motile.

3/24 stationary cells gave non-motile clones (E1, 2, 3).
E3 (1)→ E4⁺ Rough?
[123]

~~G5 ... ~~

F3 pure s.c+

see 1149E.

Also set up reconstruction mixture in E5, F5 (– from C1). Seem to give Fla > Fla⁺.

In this exp., I had intended to separate subclones, but these were too numerous and was finally abandoned. B5 and F5 are unseparated clones.

122FY

① ③

②/10⁴

440/10⁴ → 123C3 440/10⁴ →(34) C4

n = 13.

must have been considerable late replication

no further replication

449/10⁴ →(42) lost

→(7..) 124H2, 3. (A-F) → 40.3,

F 10³ ①

P5, some of these ①/1000 (n = ~~30~~ 31)

n = ~~13~~ 21

125H1

n = 31

P6. n = 33 125 H2. → 10⁴.

Crossovers in "back progeny."

1149B.
C

4/5/54. 35 non-motile clones from 1149 picked to broth P4. (Ignore rare semidomal cells). Test control × 60C P5. (DCG. Of early expts. on macro-nucleus.

C: also test 1147-3 branches: [119]-H5, #F1, F2 [121]-D3.
(1 2 3 Y)
control OK. 60C × F1, H5, D3 all +. 60B × F2 +.

Note FA60C (22/967) transmutated each culture but also the current strains of SW666 and SW967. ∴ not valid test.

In a repetition, 60B × 967: o (most expts.)
60C × 967 ++, Not tried to identify this by
60C. o type of swarm.
967 •

Tests continued by DCG — see her summary.

Not tried 90C × 122 series. Each gave only b; each responded

JAN 24 1955

See note above on ambiguity of "60C", supposed to be 22/967

4/5/54. [E3] E1-2-3 are clones from a few stationary cells isolated in transduction clones [122] B5 and F5. They appear rather rough
E4 is a motile clone from a cell in E3 at ca 200 cell stage. (Reversion?)

P5: test E1-3 for motility reversion and × FA60C. (cf 1149B.

Howeve! E1-3 prove to be galactose positive! E4 is Gal⁻.

Unless a serious error has been made, at least E3 was segregating Fla⁺ Gal⁻ / Fla⁻ Gal⁺. Could latter be SW967? whence? (crossing?) Test on EMB lactose.

There is a remote possibility that there are W2049 = Lac⁺ S^R. Droplets of 2049 + W2438 were on an adjacent coverglass that broke in course of ' expt. and conceivably might have contaminated some drops! Morphology of E1 is rough rods, ∴ different from 2438

Great care must also be exercised in removing coverglasses for exchange.

✓ Lac⁺ S^R ! Φ.

N6. FA60C = 22/967. But OCG finds control × 967 gave T+S. Possibly contaminated c̄ FA22? She is rechecking.

60C × E1-3 gave no swarms

P6 Try SW967 × SW666. Mixed culture plated gave numerous swarms! New transductive phage? (cf FA26!) — ask Abele to check. — Yes. supernates × 967, not 666 to motility. Source of the phage? All 8 were Gal+ jm.

Transduction of lac_3 - W435. [1150]

W1409

April 11, 1954.

Two coincidences of lp^s & lp-linked mutations have been recorded: W-518 and W-1650.

A. A third coincidental mutation, W435 lac_3^- has never been tested for transduction or linkage to lp.

B. Recurrent lp^s should be checked for other mutations, viz. auxotrophy. Cf. EML.

A: (1) Reisolate W435 from lyophil. First tube proved to be substantially Glu$^-$ – but ca 1% Glu$^+$. Test against λ (Hfr Gal_2^-) and λ2 $^{ex\,EMP}_{Glu^-}$. Proved sensitive to both, no indication of transduction.

(2) Do. W1409 stock now repurified (lac_1^- lac_3^-) lp_2^s. λ2^s; No transduction.

A (1) should be repeated on repurified cultures; lp-Gal-lac_3 linkage should be tested. [Cf. W1741-1744]. (W618 = Gal_2^-).

Conclude mutation W435, W1409 not subject to transduction. EML will check lac_3 for linkage to lp-Gal, & DCG also will look for other mutations coupled c lp^s.

B) DCG isolated 3 suppressors (lac^+ Glu^-) from W1409. Tested by Boris – none of these are constitutive-lactase.

All EML program on isolated lp^s-auxotrophs

April 12, 1954.

Written up 5/12/54.

No motile seen at 2 hours. First seen at 2½ hours! Begin isolations 2½ hours - 3:30. Some divided at least once by this interval! 40 isolated to clones. As far as possible, separate at 2-cell stage. Most clone pairs showed very few, occ. none of motiles. Record ~~numerous~~ clones with "numerous" motile at 10^3-10^4 cells.

	134 →		126 F4 (43) → Save! F3, F5	
A1 (1) (1)				
Sco A2 (1) →	Swarm studied stationary also → F3, F5			pure swarm
E3 (1) (1) →	(4)	(8)		
E4 "	(7)	(4)		(13)
E5 "	(1)	(4)	G1	(20)
F3 "	(30)	(6)	G4	(2+)
H4 (1)	(50)		H3	(24)
G1			H4	(26)
F1			G3	(27)
B2			F2	(12)

No - formed from stationary cells of A2 (126F3 - F5). Save as pure swarm.

Note (since Stocker's question) most numerous swarm clones separate quite unequally. E3, E4 most exceptionally. But we should get more explicit data on congeners of swarms.

Need better method to isolate early pedigree lines.

→ × paralytics

1,57.

spont. trailers?

April 14, 1954.

22 × 578. Pool A2 - F4. [127]

3h. 30° 9^30 - 12^30. Then isolate motile cells. Most inviable.

9 inviable + 5 — 14 dead

6 - nonmotile + 4 — 10 nonmotile at 10^3.

1 5+/20 - later 10^35.

3 (2-4).

1 ca 30 wght + (1?) → 10^3.

3 c semi-clones. B1, C1, E2

1 (how?) many +. F1.

On transfer of single cells, motility very sluggish & petered out so that direct study of phenotypic delay was not successful.

Roughness?

Platings:

PA 22 × 578 trailers and swarms.

60 × 578 1S 0T; 1S 1T.

60 × 536 0

534 0

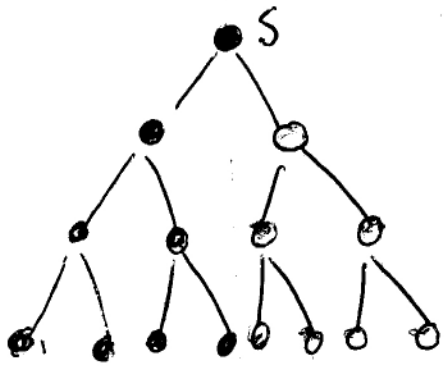
Controls:

578 0, 0, 1Sw no T.

580 0, 0

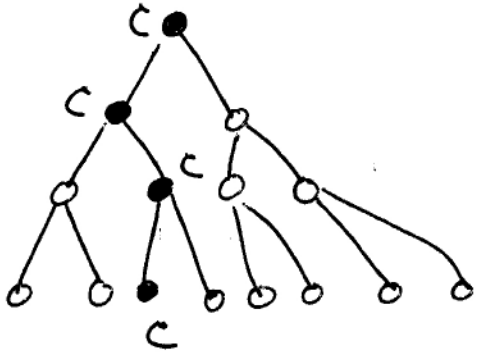
534 1 ? trailer 5± colonies of BHI5 note

536 1Sw, 2T? relating sprinkling of subsurface colonies.



Single clonal

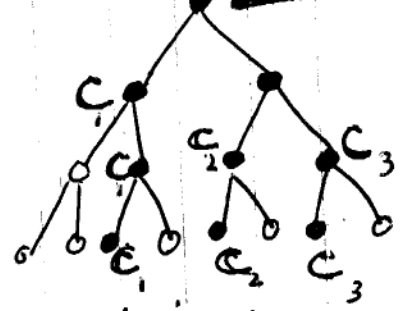
segregational.



.etc.

unicatenate

(single, unbranched chain)



. pluri-etc.

catenate

(n = 3 in this case).

pluricatenate or [illegible]

Strain C.

~~1149~~
" 1151

Note: away to ORNL week of 4/19/54.

4/4/54.

[123]. Isolate motile cells to G2. —growth + P5.→ [124] G1, G2.

picks for selection on mot. agar.

Showed no motion.

W-2438 = C received from Murray for cytological comparison. Strain C W2049 as rec'd from Weigle is overtly different (SR, rough). I had set up W-2438 for possibility of selecting a more bacillary form, but after several hours noticed occasional motile bacteria. Picked these as stated. Ones appear erratically motile + stodgy. Possibility of selecting more motile variants? In mot. agar, C is stationary.

N6 123 G2 in broth (37°): no motion at all. Inoc. from this to Penassay, incubate 11:30 AM 37°, 30°, 19°. for temperature effect.

8:30 P6: at 37°: homogeneous suspension; very rare motile ±
30° rough mod. +
20° smooth fair. ++

Best chance for selection is at lower temperatures in agar c̄ gelatine. Use 20° culture as inoculum.
= 1151B.

N11: All cultures swarmed appreciably, 20° < others. Retransfer 30° and 37° swarms to the same, 3P11. P12: 30° showed faster spreading but still sluggish. P13 Both about ½ through tubes, 30 > 37.

4/30 4 transfers in gelatin-motility tubes 37°.

DATE: 4/25/54. REF:

D. Leave plating of W2441 & Dot Boating.
She has separated
1. Lac⁻ — (v. slow + ?)
2. Stable lac⁺ pure + ?
3. " " prob +, −
4. Reversion " strong +
5. " " +, −.

Restreak these + prepare cultures for constitutive test. Hand over to Bou

0, 1, 2, 4.

DATE: April 12, 54. REF:

W2441 = "Lac$_1^-$ Lac$_c^c$" as received from Monod.

P13 Streaked out on EMB Lac., Gal. All Gal$^+$. Lac: colony types noted: separate #1 = Lac$^-$ (v. faint pink at 24 hrs) #2, 3 not pure. Re-streak

P15: both these lines showed sectored (slow Lac$^+$), Lac$^-$ colonies. No pure + seen!

Crude NPGase test: intact cells Enzyme

(Pneumococcus)	W1301 −	++	±
	#1	−	±
both r, −	#2,	−	±
	3	±	± scè.

1151A. Restreak Lac$^-$ from #1 of W1427? Mal$^-$.

B. Lac$^-$, Lac$^\pm$ from #2.

C Lac$^-$ " "

D Original W2441.

DCG purified Lac$^-$ from plate D = W2455$^-$ and Lac$^+$ reversion from this (strong +) = 2456.