11/5/53.

A. Mix ca 4 hours in [illegible]assay. Plate out on EMB Lac.
P6. (C.M.) Pick and streak Lac v colonies (ca 1% of total) onto EMB Lac

Morphology (1 or 28)

	Lac	Mal		S	Mtl	Xyl		D(0) (dl⁻ / lac)	Mal	S	Mtl	Xyl
1	+	– ↓	–	S	R	–	+	+ ↓	– ↓	+		
	+		–	S	R	–	+					
	+			R	R	+	+					
	+			R	R	+	+					
2	+			R	R	+				+		
	+			R	R							
	+			R	R							
	+			R	R							
3	+			R	R	+				+		
	+			R	R							
	+			R	R							
	+			R	R							
4	–	no Xyl.		R	R	+ (Xyl.)	+			+	no Mal	no Mal
	+			R	R	+	+					
	+			S		–						
	+			S		–						
5	+			S	R	–	+			•+	+ 28	28 no Mal
	+					–	+			+	+	
	+					–	+			•–	+ 1?	
	+					–	+			–	+	
6	+			R	R	+	+	↓	↓	+		
	+					+						
	+					+						
	+					+						
7	+	omitted		S		–						
	+			S		–						
	+			R		+						
	+			R		+						

(Morphology)

W2057 = wg 1 Hfr TLB, - Lac+ S^S Mal- Xyl- Mtl-
W2333 = wg 28 F- Lac- S^R + + +

B. Repeat:

W2333 x W2057.

IF– : Mtl–Mal–Xyl–S^{S} / +++^{R} completely linked.

13 Lac+/– tested.

8: Lac+ Mal+ / Lac– Mal+ — R1/P1

4: Lac+ Mal– / Lac+ Mal+ / Lac– Mal+. — R1/P1/P2

1: Lac+ Mal– / Lac– Mal+ — P1/P2

P2 type was morphologically distinguishable: lighter color.

Note (as before) absence of Mal– recombinants. Recovered P2 should be tested for Hfr. (incl. λ status). Also [illegible] morphology.

November 5, 1953.
W2057 + W1[illegible]

24h. Penassay cultures 1+1 + 5 ml Penassay 4.40 PM. — 9.40.
Dilute [illegible] on EMB lac ± sm.

Test SR, Xyl, Lac + Mal — for analysis

EMB lac sm. No SR+ at first. Ca 36 hours, weak lac+ appear (presumably Gal-).

A. EMB lac: from ca 2 plates, only 4 possible lac+ at first.
Two gave lac+/-; two pure lac+. Test components as A1-2.
[illegible] Gal+/-. Later, more formed and streaked out. Little likelihood of colony admixture, especially with lac+. lac- → lac+.

B. after ca 36 hours, weak lac+ appear, almost certainly diagnostic of lac+ Gal-. Test s.c. from A, B.
↓
all pure Gal- in spot tests → 17: all S^R Mal+ Xyl+ Lac± Gal− #2 MtlMtl −+ ($\frac{15}{16}$?)

A1.

	Lac	Gal	S, Mtl, Xyl, Mal	
1	+✓	+ +	+ +, R	−
	−, +˙	+ −, +	+ + R	−
		+	+	−
		+	+	−
2	−	+ −	+ +	−
	−	+ −	+ +	−
	−	+ −	+ +	−?(R)
	−	+ −	+ +	−
3	−, +˙	+ −, +	+ −, +R	−
		+	+	−
		+	+	−
		±	−	+R
		±	−	+R

some v. tiny lac− colonies.

A2.

	Lac	Gal	Mtl, Xyl	Mal, S
10	−	+ −	+ +	− +R	−+(R)
		±✓	−	+	+R
		±✓	−	+	+R
		+	+	−	− S
		+	+	−	− S
11	−+	±✓ −+	− +	+ +R	+R
		±✓	−	+	+R
		±✓	−	+	+R
		+	+	−	− S

Added. Lac +, − from 2, 4, 6, 7, 8, 9, 11.
In 11, lac± verified. Others either ++ or − but check on replica to EMB Gal.

Aim of expt. is to determine the incidence of parent genotype and of Mal/Gal recombinants. These seemed more frequent than Lac/S.

A1 cont.

	Lac	Gal			Mal, Xyl, MH, SM	
4	–	+	–	+	+ R	– S
	–		–		+ R	
	–+		–		+ R	
	–		–		+ R	
5		+		+		–
		+		+		–
		+		–	+ R	
		+		–	+ R	
6	–	+	–	+	+ R	–
	–	+	–	+	+ R	–
	–	+	–	+	+ R	–
	–	+	–	+	+ R	–
7	–	+	–	+	+ R	–
	–	+	–	+	+ R	–
	–	+	–	+	+ R	–
		+	–	+	+ R	–

	Gal	Lac		Mal, Xyl, MH, S	
8	– +	–	+	+ R	–
	– +	–	+	+ R	–
	+		+		–
	+		+		–
9	– +	–			
	– +	–	+	+ R	–
	+		+	+ R	– + MH, Mal
	+		+		–
	+		+		–

Inoculated 4-8 colonies of A 2 4 6 7 8 9 11, except in #11 are Lac+, were Gal+ S^s; Lac–, Gal– S^R

Notes: 5: No Lac⁻ obvious nonsegm. but restreak poss.: 3 classes seen on restreak ⟶ Repl to EMB Lac + sm

4: Lac+ are Gal+ S^s (i.e. are Lac+ Gal– S^R prob. seen)

✓1: P1 + P2

5: P1 + P2 seen + R1

Better procedure in colonies for recomb. might be to replicate as in 78A.

DATE: 11/14/53. REF: 1077 SUM.

A. Test for synkaryons. W2057 × W1321

Hfr TLB, Lac+ S^S Mal·Xyl·Mtl—   Lac− Gal− S^R M−F−.

call. P2.   P1   and R1 = P1 Lac+.

11 Lac v colonies:

✓further

1. P2+P1 ✓   Purity of A1−1, 2 +P1 + ?   Probably P+P2 ✓ + trace P1/P2 from lac slows?
2. P1. P2 ✓
3. P1, P2, R1
4. P1, P2 ✓
5. P2, R1   −+P1 ✓ not P1?   Test: list:
6. P1, P2 ✓
7. P1, P2 ✓
8. P1, P2 ✓
9. P1, P2 ✓
10. P1, P2, R1
11. P1, P2, R1

bipar	7
bipar+R1	4
orthopar+R1	0. (not easily sought)

no further record of presence of Lac±: may have been present in some of these.

#5 Lac− auxtal− S^R = P1

B. Lac±. No effort to identify Lac− components. 17 isolated were all Mal+ S^R Xyl− Gal− (orthotypic). 16/17 also Mtl−. Class B might have been favored by less restricted conditions for picking. [Gal and Mal-S generally concordant, but exceptions not yet looked for.]

Notes: defer more detailed analyses for single cell; Gal− Lac+ × Gal+ Lac−.

These results are now interpreted as synkaryons from which recombinants may or may not issue.

11/10/53.

Pinesassay overnight (or 48 hours). 1:1:10 pinesassay 2-5PM

A. W1895 x ~~W1177~~. 1956. Plate on EMBlac ± sm E 11/17 Check W1956 on EMBMal OK.

B. W2057 x W1321 " "

C. W2057 x W2333

11/11 (D) W2058 x W1578. P12: no lac s noted. (Hfr??)

C:) Ø ram. 10^{30} All. 12 plates EMBlac + ? sm. No SR+ noted.
These plates have Lac- > Lac+, well separated colonies. Only well-isolated Lac s/ picked for further study.

1- irregular margins. dark center, spreading edges. No definite sectoring. No likelihood of contamination unless noted.
4,5 merely fuzzy. 6 near Lac- but not touching. 7 ⊙ 11 ~~[struck out]~~
9: def. sectoring ⊙ 10 ⊙ touching +. Disseminate.

P11. 12: small, fuzzy (cont. c +) 13. ● These colonies generally smaller than others. C.M. streak out from my pick

B) - hold E). Lac+ >> Lac-. SR+ ca 5% of Lac-. Lac+ too numerous for present purposes. SR+ to EMBXyl. All Xyl-. By replica test, 9/19 were Lac+ V_1^S, 10/19 Lac+ V_1^R

P11 → B): Pick only Lac s, whether weak or strong +/-, that are not likely contam. c Lac+. Separate some possible adjacent +/-.
Pick only Lac s that are not merely simple sector.
14 picked, all of type ● 15-20 are simple conjunctions.

A. EMBlac Lac s of two types: ⊙ (1) and ● (2). Streak on EMBMal
(v W1956 Mal!) 9/14 type 1 (1-9) and 1/3 type 2 (1) had Mal + (presumably the paratype parent. Restreak to analyse: spot s.c.i. Lac+ and replica to EMBlac sm. (1 plate inadvertently [C.M.] streaked on EMBGal: note several phenotypes!). (over)

Mal (misorder)

A: + 3
± 2 − 4 − 14
+ 5 + 15
+ 6 + 13 − 16
+ 7
+ 8
+ 9
− 10
+ 11
− 12 ✓

✓ i.e. Lac ⁿ sequence used in streaks.

On EMB Lac, #2, 6 papillae

3, 6, 8 Lac+

13 – 16 on EMB Gal.
13 Gal++ > Gal− > Gal±
14. Gal± > Gal−
15. Gal± >> Gal−
16 Gal± > Gal±

	P2	R1	P1
	Lac+ S^s	Lac+ S^R	Lac− S^R
1	No	Rare ✓	−
2	No	✓	✓
3	✓✓	✓ occ.	✓ occ.
4	No	✓✓	✓✓
5	✓	✓✓	✓✓
6	✓ [Rare (secondary ?) →]	No!	✓✓
7	✓✓	✓	✓✓
8	✓✓	Rare sec.? No	✓✓
9	No	✓✓	✓✓
10	No	✓	✓✓
11	✓	✓	✓✓
12	No	✓	✓✓
13	✓	✓✓	✓✓✓
14	No	✓✓	✓✓
15	✓✓	No	✓
16	No	✓	✓✓

Notes:

(Lac+ only papillae in orig.)

∴ assume these were misorder by C.M.

Note v. many + to test ±. Recheck for presence of Mal+!

If we accept 9 as P2 ✓ we have:

5 P1+P2+R1 : 3, 5, 7, (9), 11, 13,
3 P1+P2 (+R1??) : 6, 8, 15
7 P1+R1 : 1, 2, 4, 14, 16, 10, 12

A9 = Lac + Mal + S^R + Lac − Mal + S^R and Lac − Mal − S^R

(P1+R1 not detectable), S^R mutant?

Restreak

1, 2 | Lac+
9 | Mal+ → mostly Mal+ on restreak

→ ! check types on other machines!

11/16/53 Clean up A, B, C.

A9 Mal+ >> Mal− (1, or 2 − noted as streaks). Replica streak plate to EMB Lac ± sm. Mal− are Lac− S^R Mal+ are Lac+ S^R / Lac− S^R
No S^S noted!

A1 — Mal− only Lac+ = S^R. — P1 + R1

A2 Lac− only. (+ papillae) — Mal− only. — P1 + R1

E: 19 all Xyl−. Replica to EMB Lac T1.

B. Streak out possible Lac+ Gal− (11: 1−4; 8:4)
both → Lac ±.

C. Plates heavily inoculated and overincubated hinder scoring Lac+/−. 4,5 pure Lac+ S^S 6: Lac+ S^R
Inconsistencies in scores! Repeat replicas to Lac, Mal, [illegible], Xyl, Mtl.

Concordance of Mal− Xyl− Mtl− S for: Lac F

Mal	Xyl	Mtl	S	Lac	F
+	+	+	R	−	−
−	−	−	S.	+	+

Mal →

	Lac−	Lac+	
1	3− 1+	4−	R1 P1 P2
2	1− 3+	4−	
3	4+	4−	
4		4−	
5		4−	
6	4+		
7	2+	3+ (weak lac+) 3−	
8	2+	3− 3 weak lac+ Mal+	
9	4+	4+	
10	4+	2+	
11		4−	
12	3−	3+	

	Lac− (orthotype pos).	Lac+	
1	4+	3− 1+	P1 P2 R1
2	4+	3+ 1−	P1 P2 R1
3	4+	4−	P1 P2
4		4−	P2
5		4−	P2
6	4+		P1
7	4+	3− 3 Mal+ weak lac+	P1 P2 R1
8	2+	3− 3 Mal+ weak lac+	P1 P2 R1
9	4+	4+	P1 R1
10	2+	4+	P1 R1
11		4−	P2
12	3+	3−	P1 P2

∴ 4 tritype + 4 < 3 P2 / 1 P1.
2 bipar / cf. R1 1,2 vs 7,8.
2 bi ortho

(over)

7078A9. (c) Lac+ Mal+ : Gal + Xyl − MH − S^R
(b) Lac − Mal+ : Gal ± " " "
(a) Lac − Mal − (1) : Gal ± " " "

(a) = W1177 = P1

(c) = R1 Mal+ } Mutation ?
(b) = P1 Mal+ }

Synkaryon tests.

DATE: 11/14/53. REF: 1078 S4M.

1	2	3	4	5	6	7	8	9	10

A. W1895 x W1956. Find Lac± 10/17 had a Mal+ component.

Test isolates for fraction also showing an SR+ component:

≡ Tentative conclusion: of 16 lac+/-, are biparental; are biparental + orthotype recomb. and are orthotype parent + recombinant.

Nutritional markers not tested.

B. W2057 x W1321. 14 Lac± isolated and types picked. [Adj. mixtures showed no Lac± instabilities of this set!].

Lac± seen is following: (not recorded for 2 days!) 4 Lac+, 4 Lac- from each eye: #3 has Lac- only.

	+	±	-	
1	·		·	P1 P2
2		·	·	P1 P2
3		·	·	P1
4	·	·	·	P1 P2
5		·	·	P1 P2
6	·		·	? P1 P2
7	·	·	·	P1 P2
8	·	·	·	P1 P2
9	·	R		P1
10	·			"
11	·		·	P1 R1
12		·	·	P1 P2
13	·		·	"
14	·		·	"

Parentals only except poss: # Lac+ Gal−
#8/4 " Restreak for check ✓

R1 P — Test: biparental + ortho recomb. / bipar only. / par + orthorec.

see 77A → 4:7

Total 77, 78: 6/19

In view of ambiguities, test replicas but do not pursue in place of forthcoming Gal- Lac x Gal+ Lac-

What tests? No record except for Lac, Gal, S.

C. W2057 x W2333 Lac+ and - except in 4, 5, 6, 11 (of spots +)
(4, 5, 11 had not been Lac±?). 12 n.g. 14 n.g.

P1.P2 colonies suggest that, judging from colony morphology units probably single cells are synkaryotic. Occurrence of recombinants strengthens the argument. (Look for V1/S recombinants?)

11/16/53.

ca. 1:1 in Snl broth 1^{15} PM – 4^{45} PM.

A. W1895 }
B. W2341 } x W2033 on EMB Lac ± Sm.

Lac+ Gal– S^S x Lac– Gal+ S^R

AA. Lac+/– central AB: sectorial and peripheral
A.C. portions of parents. Replicate streakes to Lac Sm.

No easy way to detect protype parents or Gal/S recombinants.

SR+:
AA. 46 colonies. 44 SR+ ✓✓ 1 Lac+ rare 1 Lac+ inf. mostly S^S. Not certain whether secondary SR+ are completely controlled by AC. Note low proportion of P1+P2 in this entire experiment!

AB
10 probable P1+P2 (no or secondary SR+) / 36 total.

AC. 16 Spotty +/– only.

B. 9 EMB Lac (accurately plated .1ml from 10^{-6} dil.) and 9 EMB Lac Sm.

On EMB Lac, score all Lac++ : almost all variegated with Lac– or Lac±. Scores may include some Lac±/Lac–. On EMB Lac Sm score Lac+ and Lac+/–. May include some S^R Lac± but probably not.

BB. (Lac)

	Lac+...	Lac–	Total
1	23		551
2	24		534
3	18		592
4	18	255	569 {255 are Gal+}
5	22		563
6	34		545
7	19		576
8	19		516
9	22		560
Mean:	22.1		
Σ	199		

BA

	Lac–	Lac+	Lac+/–	Total S^R
11		1	23	343
12		1	14	331
13		0	2? 22	308
14		0	33	302
15		2	22	295
16		1	33	320
17		1	18	335
18		0	23	328
19		0	24	345
Mean:			24.2	323.0
		6 (only 2 as Lac others →)	212 / 218	

Averages not appreciably different. Unlikely that any genotypes are confused. I.E. SR+ are almost certainly Lac+ Gal+ S^R. To identify Gal– would require separation of Lac+ components and get total counts. All appear ++. Check pure + = 79BB. (over)

EMBGal: Low count of fuzzy Gal+ or +/- colonies. 1-4/plate
= BC. scored, but not very distinctive

BB. On rechecking, 2 were found to be pure Lac+, others had rare Lac-

Parent (means).

Lac- = 323.0
- 14.2
298.8

Lac+ = (545)
323
-
222.

BC: 15 Gal +/-? or Gal ±: Streak on EMB Lac

	P1	P2	R1
1	✓	✓	✓
2.		✓	✓
3	–	–	
4	✓		
5	✓	✓	✓+
6	✓	✓+	✓
7	✓	✓	✓
8	✓	✓	(✓+)
9	✓	✓	✓+
10	✓	✓	✓+
11	✓	✓	✓+
12	✓	✓	✓
13	✓	✓+	✓+
14	✓	✓	
15	✓	✓	✓+

sic = F3

1 R1+P2
3 P1+P2
10 P1+P2+R1
(1 P1)

EMBlac sm. Prog. yields of SR+ similar to 79B. 17/17 are in lac+/- colonies
Lac+ sector usually small. Save for picture.

EMB Lac. Numerous ⊕ colonies. All that can be learned here is the incidence of SR+ among these. Distinguish type 1 (A1-2) —, central + c radiations but surrounded by Lac - and type 2 (A-3) — multisectored peripheral lac+/- . On EMB lac sm, type 1: type 2 = 7:5
12:9:1+
(Type 2 are less characteristic on EMB Lac). Do not use this series (in preference to 79B) to test persistence of paraparental component.
Save some plates for photography. Note that Lac+ recomb. are distinguishable here from W1895 lac+ also.

11/18. In replicas from streaks, A3: virtually all had numerous SR+.
A1-2 not yet tested; also conjunct P1/P2 colony tests to be done.
Ca 9/36 had a lower incidence of SR+ than others. But this test is essentially too crude.

BAEMB lac. Mark clear +/− — and fuzzy +/±/− —

Pick only clearly isolated colonies. Among scoreable, isolable colonies:

BA		—
2	11	3
2	2	8
3	7	2
4	5	2
5	3	2
6	7	1
7	5	3
8	7	3
9	6	3

— are far less conspicuous as (and are lac ±/− with possible lac+ component) isolable colonies.

+ an addnl. unnumbered plates:

—	—
11	2
1	1
6	3
7	7
4	2
1	0
2	0
7	1
3	0
5	2
1	2
3	0
5	4
9	4
5	3
2	1
4	2
9	2
7	2
92	38
D	E

Gal/Lac "Interaction" (EML Thesis) a prominent feature.

Parents: W2431 - pure Gal− Lac± W1895 Lac+ Gal+ W2033 Lac− Gal+

E = BA yellow p. 1

D = BA red. ~~6A 1-60.~~ 1-136 (G.O.)

DATE: 4/18/53 REF: 1079

	5	6 (Σ scored.)	P_1+P_2	R_1+P_1	$R_1+P_1+P_2$	R_1+P_2
D (uncor.)				12~~6~~	8	
E.		57	7	5	43 (20?)	2

D:E had been estimated at the same · actually found.

D+E			7	131	51	2

but there is a probable bias favoring the detection of R_1+P_1. (R_1+P_1) is evidently distinguishable from ~~R+P~~ $(R_1+P_1+P_2)$ in original plates

P_1 = ~~Hfr~~ F- parent
P_2 = Hfr parent
R_1 = Recomb

Should check incidence of R1 from conjoint $P_1 \cdot P_2$ colonies.

1179 G: P_1 dil. zones (and streak) at junction of P_1 and P_2 in distinct colonies (4). On EMB lac, no R1 in distinct colonies. EMB lac con – SR+ nlyco are papillae in their streak. Those distinct from types referred to above as x in R1 column.

	P1	P2	R1	4
D. 101				
110				
120				
30				
40				
50				

12 to are R1+P1
8 R1+P1+P2, though the low incidence of R1 suggests some of these are secondary.
1 R1 only.
1 P1

Scores of streaks of 1079D on EMBLac

DATE: 11/18/53 REF: 1079.

	P1	P2	R1	EMBLac sm 3R+ = R1	5	A1	A2	R1	9	10

D 1 … 10 … 20 … 30 … 40 … 50

51 … 60 … 70 … 80 … 90 … 100

P1 = F- Lac- wg 28

P2 = Hfr Gal- Lac+ wg 1

R1 = Lac+ Gal+

1079E
scores.

DATE: 11/18/53 REF: 1079D.

	P1	P2	R1	S1+	5	P1	P2	R1	9	10

least is marked at n1.

sic. = F1

sic. = F2

58.

∴ This series, selected for the identifiability of P2 in the colony, shows

6+1		P1 + P2
43	(23 certain + 20?)	P1 + P2 + R1
5		P1 + R1
2		P2 + R1 (= F)
1		P1

F: (1, 2, 3) (See also BC). Save after verification of absence of P1. F1-2 also checked — on EMB lac sm. No Lac− or Lac± here

(too affective in detecting recombinants)

✓ if Lac+ is considerable
all have at least papillate +
very few colonies in SR1 tests 1-33

	Notes.	P1+P2	P1+R1	P1+P2+R1	Lac.
		1	8	4	Lac+/-
1076A W2057 x W2333	sample colonies tested MalMtl Xyl-S concordant.				
Possibilities of synkaryon analysis conceived from this					
1077 A W2057 x W1321	EMBLac. MalXylMtlS concord.	7	0	4	Lac +/-
B	EMBLac ser. 17 all $S^R Mal^+ Xyl^- Gal^- Lac\pm$ {16 Mtl- 1 Mtl+}.				
1078 A W1895 x W1956	EMBLac. Test as EMBMal for Lac+/- 9/16 [illegible] had Mal+	3	(+A9) 7	5	Lac+/-
#9 had	P1 Lac+Mal+ S^R & Lac-Mal+ S^R				
Lac, Mal, S tested only.	(addnl. Recomb type: Mal X S or Mal+ mutant?)				
1A: SR+: 9/19 V_1^S					
B. W2057 x W1321		11	1	1	Lac+/-
Limited sample of colonies tested Lac, Gal, S.					
C. W2057 x W2333		2	2	4	Lac+/-
Mal XylMtl S concordant limited sample of colonies from each.					
1079 A. W1895 x W2333.	Test Lac+/- for SR+: cf. [illegible]				
B W2(4)31 x W2333.	Also 2(R1+P2)	7	131	51*	Lac++ associate[illegible]
? *Maybe biased. Some R1 might be secondary, or many such colonies miscored as not uni-cell origins.					
BC.	(P1+R1 of course not picked!). (+1 P2R1)	3	0	10	Gal+/-

11/19/53

The general conclusion is that the Hfr parent is frequently associated with the F- in recombinant containing colonies. Critical question still whether these are unicellular in origin - considered likely from the colony appearance of and from EKL findings.

It is difficult to calculate exactly what proportion of Lac+/- colonies have SR+ recombinants.

Single Cell isolations of synkaryons.

11/16/53.

ca. 11/10 ff. Preliminary trials.

A. Prep'n mercury droplets – overlooked Topley description. Had attempted to use needle directly; then used fine bore needle c syringe. Later found simple technique, and could pick droplet off glass plate by sliding needle + drop across the edge. Droplets ca 1/2 – 2/3 diameter of 43X field found adequate and could still permit study under phase contrast.

B. Quartz o.K. – Plastic coverglass shutting opaque. #1 coverglass: no killing even for 5 mins! Kodak film base allows partial transmission – need 3-5X usual dose, but poor optical quality. Visking absorbs water too readily.

C. Troubles: with several blocks: growth along and up sides. Peril of moving completed work by accidentally touching edge of coverglass

BB. Plastocel 10 ~~[illegible]~~ mils showed partial transmission (killing at 1 min dose). May be too scratched?

D. 11/22/53. After various casual efforts, try the method using 5 cm squares of agar blocks c plastocel squares about same size. 6 tests: 2 controls, 4 droplets. On controls, found 2,1 colonies. On others, total of 26 but only 1 even close to a droplet. Complete failure!! UV dose may have been inadequate.

wg 28Lac Screen for best crossing markers.

DATE: 11/18/73. REF:

W2341 × Mix 1:1:5 12-N18.

A W2334
B W2335
C W2336
D W2337 - Stock already had lac+. (revertible?)
E W2338.

Rev each of these on EMB gal!

	Lac v	SR+	EMB Gal	EMB lac.
A	✓✓	—		—
B	no	no		flat, v. small colonies
C	✓✓	✓✓	no v	—
D				already some +
E	✓?	no		rather small

Use either A or C for future work. W2333 has the disadvantage of showing slow +.

Must be confused!. W2336 is Gal −, + mixed (of D)

Rev	Gal	Lac	
W2333	+	− → ±	
4	+	− → ±	
5	+	—	
6	+, −	+, −	Mal +, −
7	—	− thin	
8	+	—	

Use 3, 5 or 8. Try 8.

(over).

Motility.

W-2333-8, W1208A, W 2059 (wg 51) are non-motile under microscope; 2333 also by motility tube.

W1208 (lyophil) is motile. W1258 (old vial) is motile + (non-motile?)?

8A. W1258 o.v. small cols. (11/19-11/20)
1B " " large.

"wg 28"

11/22/53

W1258 = wg 28 = NCTC 123 as received from Cavalli.
Is now microscopically motile; grows poorly on EMB; Lac +.
[also should be S^s; λ^s; ...]. Reisolate from lyophil 11/20/53.

W1258A = wg 28A. Recovered by EML from an old vial # 12/11/...?

Recorded as prototrophic S^R. Mutants have morphology similar to that of wg 51 and are likewise also non-motile. Present stock wg 28A also non-motile. 2 types on EMB lac A1 = gummy. A2 - not

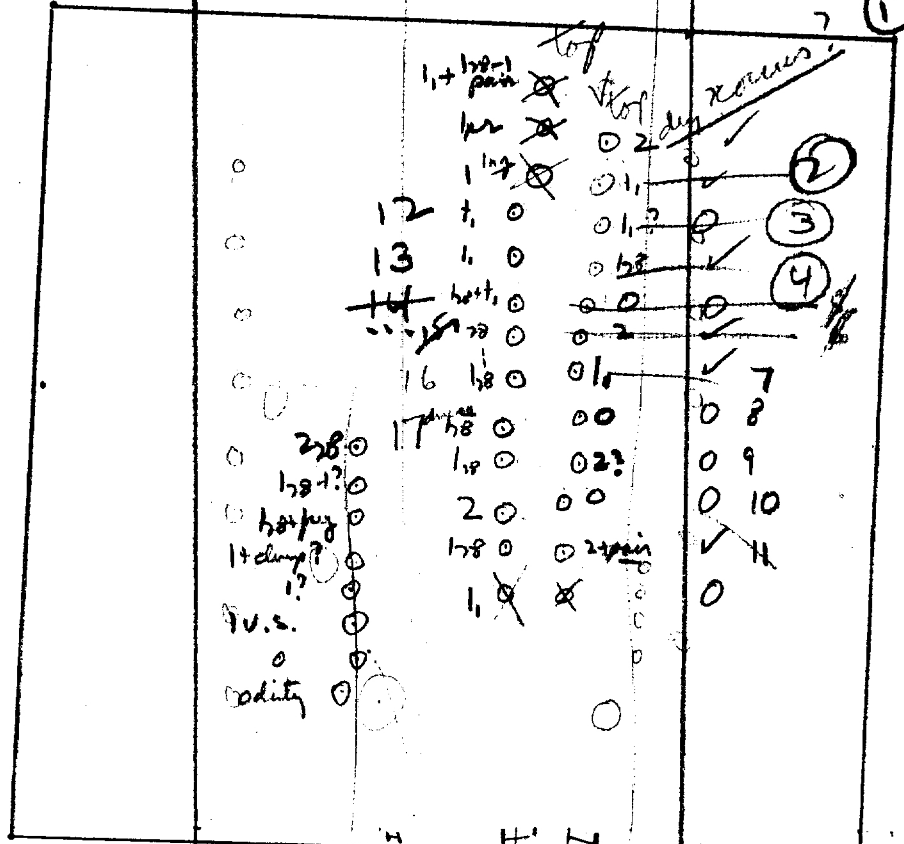
---

Old vial. Streaked out directly gave only lac - colonies (81A, B. both are distinctly motile. (Picked for presence of lac+ = 81D).

Add broth, streak out after the mostly lac - S^R, as above. Occ. some Lac+ S^S = 81C.

	Mot	Lac	SM		TI
1258	+	+ slow (s)			
A	+	−	R	R	R
B	+	−	R	R	R
C	rare +	+	S	R	R
wg 28A 1	−	+	(R)	R	R gummy.
2	−	+	(R)	R	S
2338...					S

1081



1081

DATE: REF:

	1	2	3	4	5	6	7	8	9	10
1–10 Expo-ction.	2 ✓	1, ✓	1, 0	1+8 ✓	=	=	1, ✓	0	2? 0	0 0
Growth (table)	✓ (mucus?)	? mod	✓	—			—	—	—	✓
type fac loc	P2 ✓	n.g. ✓	P1-R1 ✓							R1-P2
11–20	2 + pa ✓	+, ✓	1,	—	1+1	1+8	1+8	1+8	2	1+8
Gr (table)	—	✓	✓	=	✓	—	—	✓	✓	—
type fac loc		P1-P2 ✓			P1 only			P2 ✓	P1 ✓	

uppermost disappeared

[illegible] have died.

Segregation again is arguable

Some single cell possible

This expt. only explanatory method results n.g.

nodye

11/24/53. 2338?

A. W3~~4~~44 × W2341 12:30 – 6PM 1:1:10 Pen
(also use for oil chamber isolations (1:1000))
measured carefully.

1. EMBlac

2 EMBlac Sm (2X)

3 EMBlac Sm + T1 (2X) (20X)

4 EMBGal + T1. (2X)

B. ~~Single cells. Droplets 10^{-3} dil. in oil chamber. Study and consider 0, 1, >1 cell types. Allow to grow in chamber overnight. Pick~~

2: Brush lac+ S^R colonies (mostly also lac- S^R) / T1 on EMBlac
50 tested, 26 R lac+, 24 S lac+. All but one, if lac-present this was lac- V_1^S (unless masked by lac+ V_1^R).

1: lac+ V_1^S / lac- V_1^R = #18.

→ 1, 2, 3, 4, 5, 12, 13, 16, 17, (18), 19, 23, 24, 27, 28, 29, 31, 38, 40, 41, 43, 47, 48, 50

~~[illegible]~~:

∴ ca 50% of SR+ are V_1^R. (independence of Lac, V_1 here?) [Neither of the factors is known to be allelic with lac_1, V_1 of line 1].

Re 1117 B

DATE: 11/25-26/53 REF:

1	2	3	4	5	6	7	8	9	10

A2. SR+/plate = 22, 19, 23,

A3 20X. 233+, 130−

2X [9+ 14−; 10+, 3−; 5+ 11−;] → includes 3 sectored colonies.

Therefore there are an appreciable number of Lac−V_1^R S^R, but no (Gal−Lac+) $S^R V_1^R$ were noted in this experiment.

A4 (no T1) fuzzy colonies (total 18). Streak EMB Lac for components

	Lac−	Lac ±	Lac ++
1	✓	✓	
2	✓	✓	✓
3	✓	✓	✓
4	✓	✓	✓
5	✓	✓	✓
6	✓	✓	✓×
7	✓	✓	✓
8	✓	✓	✓
9	✓	✓	✓
10		✓	✓
11	✓	✓	✓×
12	✓	✓	✓
13	✓	✓	✓
14	✓	✓	✓
15	✓	✓	✓

∴ 12 P1+P2+K1
2 P1+K1
1 P2+K1

+T1. Gal+ counts/plate 23, 21, 14, 12 − (386 Gal−)

Phage probably inadequate for total immediate lysis. Cf A2/A3.

A2: Lac+S^R = 21/plate., expect 11 to be V_1^R.

A3: ca 8 V_1^R found, not in disagreement so no evidence of lag. But phage amount needs to be checked, also character of the Lac−$V_1^R S^R$. See also Gal+V_1^R, comparable (perhaps low or heterogeneous) c̄ S^R+.

See note 1/12/54

1/12/54

To do. a) Haploid crosses on independence of Lac, T1.

b) Het diploids for ind. segr. of Lac, T1 (unless linked to auxomarkers. Use SR+ if necessary)

Try S × M_7

c) Transfer Hfr to this stock by Gal linkage. I.E.

find a Gal⁻ V_1^R recombinant in appropriate setup.

d) Study the Gal^+/Gal^- ratio among Lac+ V_1^R recombinants (direct scoring!)

e) Look in Fig.

There may well be many Lac⁻ V_1^S recombinants (there are 1/2 as many as Lac⁺ S^R) (inducted along) and with P1.

Some "P1+P2" might have such recombinants. (Either test at random or replicate cross plates.) For present work this means testing Lac isolates on SM_7 T1. Later study "P1+P2" in plates, and try to find P1+ (R2). [R2 = Lac⁻ V_1^S recombinant]

Also do (d) above for the record.

1/12/54 see 1082

No data on V_1 distr. among lac$^+$/lac$^-$

$\underline{\underline{S}}^R$ lac$^+$ V_1^S usually accompanied by $\underline{\underline{s}}^R$ lac$^-$ V_1^S $\left(\frac{20+}{24}\right)$ = P1

rather than lac$^-$ V_1^R = recipr. recombinant.

Should now test Gal$^+$ lac$^+$ ctg. colonies fr comps. of. 1076-1079 Experiments

probably nothing saved.

More likely to be with Lac⁻ parent. I.E. $\frac{S^R Gal^+ Lac^- V_1^S}{S^R Gal^+ Lac^- V_1^R}$.

3 = recomb. classes 2 are Lac+, now detected.

1 is Lac−V_1^R.

If associated with P1. not now detected as sig. colonies. nor readily detectable in segregates.

∴ all 1-cell isolates must also be scored on T1.

1) 2338 $V_1^R S^R$ (2344).

×

ϕH- Het lac⁺ Mal⁻ (Gal⁻)

---

alleles in 2338 lac.

1/12/54

082. ? How many zygotes are missed

a). Are there recombinants not detectable as $Lac^+ S^R$
b) Are there segregates other than Lac^+/Lac^-.

a.] 2344 x 2341 = $Lac^- S^R V_1^S$ (F^-) x $Lac^+ Gal^- S^S V_1^R$

$Lac^+ S^R$ essentially all Gal+. 26 V_1^R 24 V_1^S.

S^R/V_1 recombinants = (A 3.) 20X showed { 33 Lac+, 130 Lac− }

2X showed 8 V_1^R Lac^+, 8 V_1^R Lac^-

(A2) $S^R Lac^+$ (2X) numbered ca 21 per plate.

∴ One should have predicted that 1/2 these would be V_1^R = ca 11.

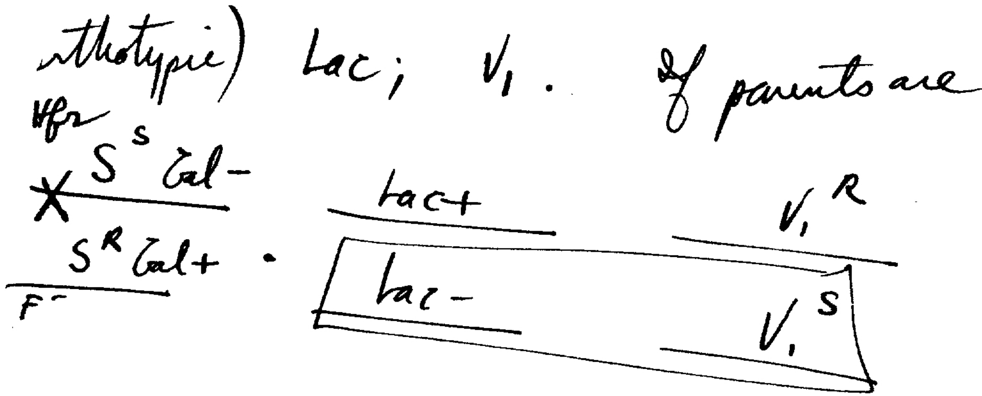
(A3) Found $S^R V_1^R$ Lac^+ = 8 per plate.

1/12/54

Per 2X plate: $S^R \cdot Lac^+ \begin{cases} V_1^R & 11 \\ V_1^S & 10 \end{cases}$

$S^R \cdot V_1^R \begin{cases} 8\ Lac^+ \\ 8\ Lac^- \end{cases}$.

These experiments suggest that here Lac and V_1 are unlinked to each other and segregate independently one of the other. ∴ 3 groups indicated S–Gal (almost always orthotypic) Lac; V_1. If parents are



Then recombinants are generally S^R Gal+ · $\frac{Lac^+}{Lac^-}$ · $\frac{V_1^R}{V_1^S}$

but one should test the Gal S character of V_1/Lac recombinants for final verification.

The missed recombinants are therefore probably $Lac^-\ V_1^R\ S^R$. Do these occur in association with either parent? Would be detected now with the Lac^+ parent. P1/P2 combinations should be renewed for other [illegible]

(Preliminaries on F transfer)

EML: #655

A) W2338 F- line 28A.

(Lac- S[R])

~~W6 F+ line 1.~~
~~W1607~~
W218
W1655

Mix 10^8 each (young cells) in 10ml broth for 1 hour. Plate out on EMB Lac. Test 20 Lac- colonies for F status (x W1607 W-6 ?).
1802

B) The same in large droplets under oil.

C) Then I will touch single cells together.

1083

Use 1:100 for minute drops.

L = large drop.

	A	B	C	D	E	F
1	+L	+L	4-6+ ?	0		
2	0	+L	(1) 116.8 / 27.6	L		
3	+L	+L	(3)	5 + (3) + 3?		
4	+ drops L	+L	(2)	1		
5		1 div	L 3 + pair	v. sm. 1 + (4)		
6		~	5 + pair + drops	1 v. small		
7			3	— 0		
8		L		9 (1 pair) + pair		
9						

D-8 two cells
incipient pairing
1×78
definitely ~~came~~ together
(not nec. mobile)

Single cell method.

DATE: 11/25-26/53. REF: 1083

11/26. W2338 x 2341.

(same pattern?) — whence 7 cells?

	1	2	3	4	5	6	7	8	9	10
C exp.	3 + frag.	1? + frag	0	1?	··	··	··			
found	2- 1+ (3 frag?)	1	1	1	3-2+	4-5+	0			
D exp.	0 + debris	2+ large	++	? v.l.	3	··	··			
found	0	4L- 1L+	no cool.	1	0	5L-4L+	2L-			
C	4+ ?	1	3	2	3 + pair	2+ pr + clump	3			
~~found~~	2- + 1+, 1-?	1-	1-	1-	3- 2+	3-4+ 1pr.	0			
D.	0	··	5 + 3 + 3	1	1 + 4	1 snake	0	9 + pair		
found	0		1+ 4-	1	0	4+5-	2-	?	sequence?	

CM bloc

sequence probably disturbed but argues for moderate eop.

Need to use fewer, better distributed droplets and a marker dye!

No cells in both side tubes. This can be omitted

Single cell Hfr x F −
W 2338 x 2341

1084

DATE: 11/20/53.   REF:

12[15] — 4:00

5 picked from 10 droplets, direct.

+ = daughter pair attached

	cells seen.	EMB lac.	
1	1 + +	2+ , 2−	may have grown? or mislabeled?
2	1	0	
3	1 pair + doubtful debris	0	
4	7 (clump)	3+, 4−	
5	1 pair.	2 +	

all parentals re lac.

(Use eosin, small drops 1:200. Culture too old!)

Cells observed in oil over chamber.

Try ≤ chamber directly!

ERLively 4/53.

$Lac^+ S^S Hfr$ X $Lac^- S^R F^-$

Summary of microscopic manipulation experiments with W-1895 X W-1956

I method: Single cells separated from mixture; microcolonies plated on EMB lac.

purpose: To detect recombinants as plates having both lac+ & lac- cols.

proportion of parental cultures:

exp.	W-1956/W-1895	lac-/lac+	# single cells isolated	plated on:	cols. lac-	# plates lac+	mixed
5/13/52			6	EMB lac	2	4	0
5/14		(by assay) 3/4	14	"	5	9	0

S character not tested

II method: Small numbers of cells (1-50) were deposited on complete medium agar in holes cut from filter paper. Early growth was observed; then the piece of paper was laid on an EMB lac Sm plate.

purpose: To detect recombinants as lac + or lac v S^R colonies developing from a known number of cells at a given spot. (I have no record of separating or testing the components of v. cols.)

exp.	mixture W-1956/W-1895	lac-/lac+	approx. number # fields of cells	Total viable cells	plated on	colonies lac- S^R	lac+	lac v S^R	approx. no. S^S cells (by subtraction)
5/16 & 5/19	omitted (growth failure?)								
5/?/52			7	9	EMB lac Sm	3			6
5/21			9	52	"	5			47
5/26			18	140	"	1		1	138
5/28			16	67	"	4			63
5/30			16	79	"	2			77
6/4	1cc culture/.5cc		15	80	"	3		1	76
6/7	"		7	45	"	2			43
6/7	"		8	31	EMB lac	1?	8		30
Red Tz added to W-1956									
6/24	1cc/.5cc		13	not observed	EMB lac Sm	2		2	
6/26	1cc/.25cc		13	73	"	3			70
6/30	1/.5		26	95	"	3			92
7/2	5cc/.5		15	48	"	9		5	34

Summary W-1956 x W-1895

II continued	mix't by assay	lac−/lac+	no. fields	no. viable cells	plated on:	Lac− S^R	Lac+ S^R	S^S not recovered
exp.	(T2 labeled W-1956)							
7/3/52	4.5 cc/.5cc		14	34	EMB lac Sm	2	1	31
7/4	4.5/.5cc	4/5	14	39	"	1		38
7/7	4.5/.5cc	3/4	8	36	"	0	2	34
7/8	4.5/.3cc	4/1	8	24	"	1		23
totals			207	852		42	12	802

III method — single cell isolation of red marked W-1956 from mixture

		lac−/lac+	viable cells	plated on:	# plates lac−	lac+	mixed
7/10	4.5cc/.4cc	3/1	4	filter paper transf. to EMB lac Sm	2		2 S^R v cols
7/15	4.5/.4	3/1	8	spread plate EMB lac	5	1	2
7/17	4.5/.4	7/1	11	EMB lac	11	0	0
7/22	4.5 red/1cc + blue T2	3/5	2	"	2	0	0
7/24	4.5 red/1cc blue T2	1/1	9	"	8		1 * (1 col lac− S? + 2 col lac+ S^R)
7/29	4.5 red/.4 unmarked	7/1	12	"	12		
Totals			46		40	1	5

#3 only grew.
Found them 11/22/53 and tested.

* colonies saved
No trace of 7/15 cultures, more critical.

UNIVERSITY OF ILLINOIS
DEPARTMENT OF BACTERIOLOGY
362 NOYES LABORATORY OF CHEMISTRY
URBANA

Nov. 24, 1953

My dear Dr. Lederberg,

Did you really think I would remember? I'm afraid I can't tell any more about the experiments than what is recorded, which isn't much; is it? This is the best I can do by way of summary.

I do remember that in the filter paper transfer experiments, before I started using Fz and selecting marked (Lac-) cells, I was plagued by a persistent excess of Lac + and/or S^s cells, those which started to grow under direct observation but failed to produce colonies on sm agar. Several times I assayed the parental mixture to affirm that this excess was greater than might be expected from a higher titer of W-1895. I also tried inoculating fresh broth from the mixture at the time the cells were deposited in the micro-chamber and reassaying at the time the microcolonies were plated, but something always happened to make these assays unreliable, and I don't know what happens to the proportion of the mixture in broth. Do you?

The single cell isolations, 7/15 -7/29/52, seem to have been plated on Lac without sm. I recorded that the Lac +colonies from the mixed plate, 7/24 were tested and found to be S^r. Probably those from the two mixed plates, 7/15, were also tested and found to be S^s. I don't know about the Lac-. It could have been the result you suggest, but I wouldn't base any conclusions on it. I think I saved the cultures, but if you can't find them I don't suppose I could. The Lac +plate in that (7/15) experiment probably arose from an unmarked cell that stuck to the needle and got pulled out by mistake (see drawing of isolation).

Good Luck & amd Happy Thanksgiving to you and Esther and Seymour.

Ethelyn

Conclusions (11/28/53) apparent morphology of zygotes (normal)

Ethelyn's experiments were directed at a different objective, A few cases of cells giving SR+ recombinants are recorded. Unfortunately most of the experiments involved plating directly to EMB Lac sm. Part of series III was plated on EMB Lac. There were two occasions of Lac+/- from 1-cells. But the two from 7/15 (presumably sisters) were not saved and there is no explicit record of tests for S. ERL thinks they were both Lac+S^s/Lac-S^r (bi-parentals). 7/24 were saved, presumably P1 + R1, are being checked now. Her work is therefore not too useful. [My recollection agrees with ERL on the 7/15 expt.]. Is there any reference in any correspondence?

1-cell exol crosses.

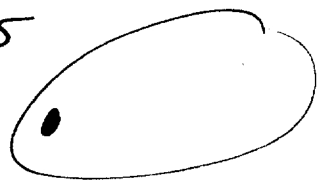
11/27/53.

A. W2344 × W2338.

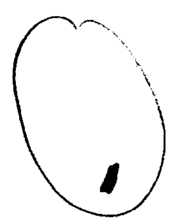
1:1:5 and ditto 10^{-1}.
B.

5:25 D1

D5



E2



12 others, 2 not formed 1 dirty others 0. hold for clones in chamb

8PM. D1 dried up. D5 dried up E2 dried

∴ n.g. Same with G-H series. no chambers. Larger drops wreck.

ABC - 4 1-cell drops [ca 15 laid down) But none grew on transfer. [agar was rather dry].

Comments: These experiments c̄ v. flat drops on slides or coverglass. Observation is quite as satisfactory, and keeps the outer surface better (optically). ∴ Dispense c̄ chambers in short term.

These cells are being used in small cell phase, not so good for observation. But increase in size is noticed in droplets.

Recommend: ① Use younger cells ② Use flat droplets but add fluid before incubating. ③. Note that in this series oil and slide had been heated and probably desiccated. Water may

11/20/53.

Reinoculate 85B 1:20, 1:100 in Genessay AM —
Technique: Mark 3x1" slides with india ink. Cover other surface c mineral oil. Add droplets, (culture + agar) moderately flat. (After mass warm add addnl fluid?) Pick up cells or clones by pumping addnl. fluid back and forth in pipette and then expelling this onto agar.

DATE: 11/2 REF: 1086 D.

	1	2	3	4	5	6	7	8	9	10
A.	dirty	dirty	drop 4+?	0 + debris	0	* 1 + dirt ✓	* 1+ dirt ✓ ? all	* 1 v. dense mobile ✓	message deviated forms 10 cells ✓	* 1 + broken? ✓

* add fluid and incubate in jar 2:20 ✓ after.

	1	2	3	4	5	6	7	8	9	10
B.	0	3 v. flat 1 infr. + 1 ✓ motile +	1 ~ ?	0	0	2. + ! sic	0	1 small	0	0

no dash.

inc 2:50.

oil added small drops in medium & dried out 7:15

	1	2	3	4	5	6	7	8	9	10
B 7 15 PM		1 ghost only n.g.				1 ghost?		ng		

something prob. inhibitory

even large control drops showed little growth.

(erosion mated oil..?)

slide was planted in mineral.

	1	2	3	4	5	6	7	8	9	10
A.						2??	ng	0	0	0

over

add droplets with considerable
cells each to test inhibition

Inc. overnight

5 c, 5 s eosin

20 - 30/drops.

These chicks grew very well (overnight)

but exptl. droplets still empty. Cells invisible?

DATE: 11/29/53. REF:

Reinoculate Abr cross 1:1000 10AM - 5PM. Pick eosinated droplets, moderate size, immediately.

	1	2	3	4	5	6	7	8	9	10
A.	8 + bubble	6	9, dirt	2	1, +	8 singles	③, ②, 6	4, +	3	1, †
Picked.				✓						
Recovered on EMB lac				0	1 –	2 –		2 +	0	0
B	++	++	++	1, 1 dry?	4	10±	4	3, ③	1	2
				0			1	0	0	0

all parentals

Why such poor recovery? Flat droplets deleterious?

~~Transfer these notes on single-cell isolation to cytology notebook and renumber.~~

Main problem: Get large young cells. Use ~~oil~~ large oil droplet as source?

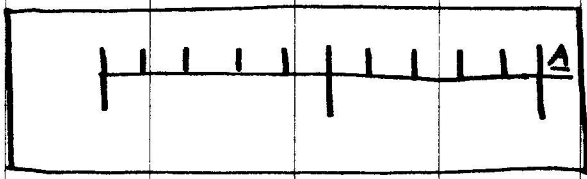
DATE: 11/28/53. REF:

1	2	3	4	5	6	7	8	9	10

MIX W2344, 2388 .05 + .05 + 10 10 AM.

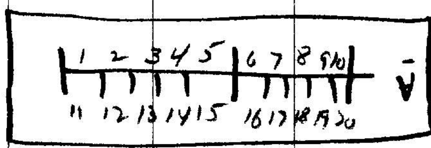
Make large droplets 11:50 AM as source of large bacteria. These droplets noted to have large cells at this time! also remove broth tubes.

Mark 1×3" slides with india ink:



no obverse:

number:



Flame to sterilize. India ink is resistant to oil but not water.

1/1000 dil. at 2:45 ca 10 cells/drop. dil. 1/10 in H_2O; in buffer. No dye really needed.

B

	1	2	3	4	5	6	7	8	9	10
1-10	5 + 6!	>1 (3) dead	1	1	1	3	0	2	[illegible]	d
11-20	✓	0	0	0	2	0	0	0	1	0

use 20 × 10 optics

add broth to B 4, 5, 14, 19 for clones

Pick B 1, 3, 6, 8, 11, 15.

Incubate 4 PM

14. growth limited probably contam

DATE: 12/1/53. REF: SUA1.

12/1 Single cells allowed to form dropcolonies:

Plate as EMBloc from 1ml Pen assay after 4h. further incubation. P1.

B4	— P1
B5	—
B14	NG
B19	+ P2
E2	—
E9	+
E13	+
G5	+
G10	+
G14	+
G20	+

all pure parentals.

B14?, E15, E16, G12 did not develop.

From direct picks:

— = cells seen

B1	0	5 (Res 5+)	E1	3-4+ =1	3 !
B3	0	1 small	6	2-1+ =3	3
B6	4+	3	10	1+ 1- =2	2
B8	4+	2	11	2+ 1- =3	4
B11	1+	2	14	0 =0	3
B15	0	2			

all parental het n

Score = 24/31 Note excess of colonies found in some instances. Might be due to subsequent divisions before plating! Repeated washing might be more effective than pumping back and forth!

DATE: REF: 88

	1	2	3	4	5	6	7	8	9	10
D water did'n 4:10	4 (incl +)	x deliv.		—						
E 1-10 water	3 (incl +)	1	+ + incl	4+	6	3	10	++	1	2
11-	4	0	1	2+	2	3	0	0	0	x

20 add fluid to E 2, 9, 13. 15, 16. 0 Try to [illegible] 1, 6, 10, 11, 14,

Over 4:40

30 capillary too large

F 505	1	too large x	x	++						
11										
G	1	x	+	6	0	1?	4	5	4	1?
	11	++	1? v. short +	+	1	0 or v.s.	0	0	0	1

5:25 add fluid to 5, 10, ~~1~~, 14, 20

50

DATE: REF: 1089

	1	2	3	4	5	6	7	8	9	10
L 945	0	+	+	0	0	0	0	+	0	X
	+	0	0	0	0	++	++	0	0	X

n.g. period!

N. acc. discarded.

① Need better control of numbering directions.

O. Set up sterile drops. overnight.

12/2/53 plating results: EMB Lac.

H. (direct picks)

1	7+ 5-		9	1+ 2-	
2	5+ 1-	1 ++/-	10	2+ 2-	
3	4+ 1-	1 ++/-	11	3+ 2-	
4	7+		12	5+ 1-	
5	8+ 3-		13	1+ 1-	
6	2+		16	2+	1 ++/-?
7	4+		17	4+	
8	2+ 2-		18	2+	

These drops initially were too large for careful observation. Not excluded that rather long cells or pairs were zygotes. However, syngamy may have been after picking: note high yield.

streaks + plates:

I	18	pure Lac+	ca 10^2		5: N.G.
	17	"	"	"	
	3	"	"	"	
	12	"	"	"	
H	14	"	"	"	

(serially grown cultures show overgrowth of the M-Hfr parent)

88 B. (streaks) 4- 5- 19+

DATE: REF:

	1	2	3	4	5	6	7	8	9	10
4:55– L 5:05 1	1	0	0	0 + dirt	0	0	0	1	3	0 ?
–	0	2	0	2+ ?	0	1	0	0	2	0?
5:10– 10 M	1	2+	1+	2	7	8	3	0 dirty	2	4 4

~~M 1 2+ 1+ 2 7 8 3 0 dirty 2 4 4~~

[illegible] too dense

	1	2	3	4	5	6	7	8	9	10
N 5:30 1	4	3	1 0	1 +	1	1	0	1	1	1
11				≥ debris	1	1	+	0	0	0
	0	0	0 0?	~~[illegible]~~ 0						

Inadvertently thrown out

12/1 — 12/2.

O 20 sterile drops. 24 hours: 18 sterile

1. had a few plumps of highly pigmented matter

2 had a denser, non coli. thrown out.

~~Screen on~~ V 1 N?

DATE: 12/1/53. REF:

	1	2	3	4	5	6	7	8	9	10

examine drops begin ca 8PM

H. 3- 8:20 — 14 ca 100 drops v. large. (circled) — 15 1022 bacteria! ? hold overn. — 19 × — 20 0

9P20: H14 +++ plate
H15 − ca. 30 cells seen as residue!
H20 now ++ with a coccus. May have been noted in first examination! 1-cell condensers appear justified.

I. 845

	1	2	3	4	5	6	7	8	9	10
1–20 EMB	* 1 / 0	None obv. / 0	ca 40 1 clumped / ✓	0 / 0	* 10 / 0 NG (circled)	0 / 0	0 / 0	0 / 0	0 / 0	0
11	0 / 0	>200 / ✓1 ✓	0 / 0	0 / 0	0 / 0	0 / 0	✓ 10² clumps. ✓1	✓ <10² clumps. 12	0 / 0	0.

drop space too large for observation.

Plate 3, 5, 12, 17, 18. Replenish fluid.

Reincubate 24 hours.
12/2: I5: a few "bacteria" but no further growth. Others (3, 12, 17, 18 are +++). I8 — indefinite shape; others empty by low power.

J 9:30

	1	2	3	4	5	6	7	8	9	10
1	ca 100 partly connected 1 group	0	0	0	0	0	>100 drop + clumps	>100	0	0
46	0	++	0	>100 at one edge						

~~slide was dirty n.g. any how~~

K.

	1	2	3	4	5	6	7	8	9	10
1	×	++	0	>100	0	++	0	0	0	0
9:35 11	0	++	++	0	0	++ at edge	0	0	0	++

order must have been inverted !! hold overnight over

K, order [illegible], 24 hours:

	1	2	3	4	5	6	7	8	9	10	
I	+++ 2	0 0	0 0	0 0	+++ 1	0 0	0 0	+++ 1?	+++ 2	1	cocci
II.	+++ 0	+++ ?	0	+++ –	+++	+++	+1	0	+++	X	
	(coccoid?)	?	0	1	2	?1?	4	0	1		

5 may have coli +?

Plate K5, 8, 10, 14, 16, 19.

Culture # 10, 11, 12,

lac

5 ±

8 ±

10 ng

X 11 ± fewer than others ∴ probably mixed

X 12 ng

16 ± 1 colony " "

19 ±

why no lac–?

all fits well.

why 14 not plated

DATE: REF:

	1	2	3	4	5	6	7	8	9	10

Freshcross 10-12N. Remove 1:100 12N - 2:20 = 89-1.
Also remove 2:20 1:100, 89-2

	1	2	3	4	5	6	7	8	9	10
H	++ / ' 0 ' +	' / '	/ ' + '	5 all rather small	'' + '	' '	+	/ ' ''	'	' + + ' ' '
Tot.	7 incl 1 pr.	3	4							

drops too deep

	1	2	3	4	5	6	7	8	9	10
II	+ λ '	+ ' '	' -	-	0	/ 0	' /	' '	0 '	0

[illegible] both [illegible] ' +

3PM Plate all these ab init. but add fluid to and hold. 14, 15, 19, 20.

	1	2	3	4	5	6	7	8	9	10
I 4PM.	0	0	1	0	0	0	0	0	1 + debris	
same as above shallower drops.	0	1	0	0 or ?	0	0	1	1	0	

add fluid each tube. 4:15

	1	2	3	4	5	6	7	8	9	10
~~J~~	~~1 x~~	~~x~~	~~x~~	~~2~~	~~x~~					
K 4:30	2	0	0	0	1	0	0 1?	2	1	1 ?
	0 deep	minute + and deep	0	1	2	1? at edge [illegible]	(4)	0	1	~~4~~

add fluid one. 4:50

Try filtered medium?

12/3/53.

A 1 +2 X 2 0 . 0 – 0 1 . 0 0 + 0 + X
11 . 1 0 0 0 0 0

~~dried up~~

X

B 1 + 0 0 0 0 0 0 0 X 0
1 0 0 0 0 1 x 0 0 0

~~dried up~~ 30 Rev. 5:40

~~C ++ 8 5 4 x 6 x~~

D 1

E 1 X 2 ~~0~~ (1) 3 ~~#~~ 3 2 0 0 2 (1)
11 4+ 0 0 ~~0~~2 0 0 ++1 0 0 ~~++~~

add fluid 5:30

Fluid must be added!

Growth by 1 X ✓ ✓ ✓ ✓ ✓ 0 0 ✓ ✓
Py 11 + – + – (+) + – – +
2 –, ±
6 ±
9 ±
10
14 all –
16 ±
17
rod! ±, –

EMB lac 12/5 Plate MG 2, 6, 9, 10, 14, 16, 17

why 2 not plated?

DATE: 12/6/53. REF:

		1	2	3	4	5	6	7	8	9	10
4PM		Add cross 2338 × 2344.				1:1000 [illegible] at 12			[illegible] 1:10 [illegible]		
D.	1	1 · 0? 8		0	×						
	11	0									
A	10	0	0	0	0	0	1++	1++	0	0	8 0
	11	×	×	7	>10	0	2++	0	1 [illegible]	×	0

add broth to 6, 7, 10, 16. (4:30) Plate A6, A7, A16

4:55 [illegible] 2-3 [illegible] along ink

		1	2	3	4	5	6	7	8	9	10
C	20	2++	1 0 0	1 1 0	0 0	0 0	0 0	0 0	1 ++	1 0 dirt	dirt
	11	×	×	×	0	×	0 dirt	2 -	0	0	0

add to 1-10 Plate C1, C8

[illegible]

		1	2	3	4	5	6	7	8	9	10
5:15 B	30 1	1 ++	0	3	0	0	0	1 [illegible] +	3+	0	[illegible]
		1 0 0	0 0	0 0	0 0	0 0	0 0+dirt	2 ++	0 0	×++ dirt	2: 1 ++ [illegible]

or oil drop?

add fluid to 11-20, 1, 7. 5:30

40 (P): Plate B1, B17, B20

2 cells.

[illegible] out after growth in broth, EMB lac

11/9/53. A. 6, 7, 16 : all 2344 type = P2
B. 1, 17 P2 only. (B20 P1+P2 [illegible])
C. 1, 8 P2 only D18 P2 only.

(over)

1092 –

DATE: 11/7 REF: 1093

A. S. fragilis 13, 15 both 1-cell. latter c bud. both → ca 10[3] cells overnight.

D ~~B.~~

	1	2	3	4	5	6	7	8	9	10
1	x dist	~~x~~	0?	0	1?	1	0	1?	0 dist	0
11	x	0?	1	0	0?	1	0	1 +d?	0	1?+d?

drop starts; cells small

all crosses 1:1000 shown!

E ~~C~~

	1	2	3	4	5	6	7	8	9	10
1	+	1 +dist	0	0	0	0	2?	1	2?	+
11						1+d.	0	x	pair? 1	+

11/8. E all but 12, 14 0 by low power.

D only 18 ++ See 1092 results.

plate A13, 15 for single cell S fragilis

#	cells ctd.	types
A 6	1	p2
A 7	1	p2
A 16	2	p2
B 1	1	p2
17	1	p2
20	2	p1 + p2
C 1	2	p2
8	1	p2
D 18	1	p2

DATE: 11/8/53. REF: 1094

W2338 × 2344. ⊗. 8:45 - 10:45. Dilute ca 1:20, incubate large droplet under oil.

2:10 Cells large. Some clumps, chains, etc. Dilute large droplet and proceed per routine. Use pattern: Trapezoid.

10

0 0 0 0 0 0 0 0 0 0 0	A.
• • • • • • • • • • •	

2:35 F 1 / 9:20 — 6 ++ clumps; 0; 1.0 + d. 30 × cf; 1. + large clump; 8 + 0 (3) ++; 0 8 2 +; 0 1 + v.s.; dirt 0 + vs; 3 + large clumped; 8 1 +

whole slide v dirty — d. X; 00 0 3+; 0 1; dirty +; 0 ? +; 0 1 + VS; 0 1 ? V. dirty; 0 2.+; 0 ? 3+ ++; ++ clumps

2:50 add fluid.

3:30 A — ✱; 0 1.; ++; ++; ++; ✱++; 3; 0; 1 pale short; 1 V. dirty X

30

0; 0; 5 (1 snake); 3; 0 ++; 0 2 0; 5 incl snake; ; 6; 0

9 PM

3:40 B. 1 — × ++; 0 0; ? 0 dir; 0 1 ?; 0 0; 0 0; 0 1.; 0 0; 0 0; 0 0 0

40 / 11 — X; 0 0 + vs; 0 v dirty!; 0 0; 0 1 2.5 VS; 0 20 v.s.; 1. 0 20; 0 dirt; 8 ?; 1 at edge pale lost

add fluid A, B 3:50

Room Temp

840 PM. A 2 empty except: (13.) ca 10² misc (14.) 60 15 ++
(16) ca 30, some rather small (17) ca 10² 18 50 (19) ++ 20 0

50

7 (30) 8: 0 9: 0 10 1?

DATE 1/4/53 REF: 1094/results

#	Cells detd originally :		EMB lac P1	P2	R1	Discordances
A						
13	5 1 smaller		✓ P1 sic			
14	3 incl pair					
16	2 v.s.		✓	✓		
17	5 incl smaller		✓	✓	few (2: 1 intact +1−)	
19	6 ...	1		24	1 intact	
7	3		✓	✓		
B						
12	1 vs ?	0				
15	1 vs	0				
16	0	0				
17	1	0	—			
F						
~~3~~	~~1~~			✓		
~~4~~	~~1~~		✓			
5	3 (1 pr)		✓	✓	no	
~~6~~	~~1~~		✓			
7	1	0				
10	1	0				
12	3			✓		
14	?		✓			
15	?	0	4A			
~~17~~	~~1 dirty~~			✓		
18	2		✓			
~~20~~	~~1 pedaghtic.~~		✓			

clones had messed to ca 10^2 before plating

4/9/53.

Old cross. Remove c & s aeration. Remove s aeration.
10-3 3-5+

New technique. Score coverglass, examine, break glass and plate fragments (add considerable fluid)

A — mostly dry. G+ × ~~[struck out]~~ 0. or 1 tiny × 0, dirt ~~smear~~ P+ ? 1. — × × × 0 .

B ~~× +++ × +++ ×~~ .

C ~~×~~ .

D ~~0 8' 0 ×~~ .

notes: ~~A optics v poor~~ ?

plate directly:

A ~~A1 × +++ × +++ +++ +++ +++~~

A 4. A4

B. × 1. — 0 ? 0 × 0 ? 0 0 0 0

? ? 1 large — 1 — 0 end.

Not too clearly done

plate 2, 12, 13 separately and remainder collect.

11/10 → A1 0 B — 0

EMBlue A4, P1, P2, R/P1 B2 1 P2

B12 — P2 (1) [one of 4 colonies dense]

B13 P1

Technique is therefore OK

A4: possibilities: Long cell divided when plated → segregants or A1 ⟺ A4 confused. ∴ This one is doubtful. (over)

DATE: 12/11/53.

REF: 1096

	1	2	3	4	5	6	7	8	9	10
	old cows	.	cG together							
A/ 235 1	x	3	8	4+(4)	6	1				
4	6	4+(2)(2)	3	0	4					

plate 2, 3, 4, 5, 6 (c) extra fluid, 11, 12, 13, 15 (s) ?

	1	2	3	4	5	6	7	8	9	10
B 3[10]-3[50] 1	~~4~~	~~1+(1)+(2)~~	~~2+(1)~~	~~0~~	~~0~~	2+(1)	•?	•	0	3 sh (1)
11	+	0 dup ?	0	8	0	3 sep.	2+	0	8	0, dif

(added fluid).

3[10] / [illegible] 3[30]

(10[30]) 12 mixed dil 1/100 ti [illegible]

Best to separate squares first.

Set up ... or

C 1 + x x (2) 1 ? 8

keeps mostly, n.g. method o.k. Cutting squares takes too long - have them premade! plate c/ [illegible]

DATE: 12/10/53. REF: 1095–1096

	1	2	3	4	5	6	7	8	9	10

Od cross, 12 30 – 3 PM c/s aviator
1:100

coverglass method. Strips of 2–5.

A.	1	3?	1+d.	1. –+ d.	0+d.	2 thick drops.	x	o	x	o	x
H	O	o	O	⊕	O	O	o	O	O	O

Plate 11–15, 16–20, 1, 2, 3, 5
N negus.

1096 platings. (If unrecorded, not successfully transferred!) Some difficulties
12/12 in handling the coverglass strips – at first tried to break previously scored segments, but this was too messy. Here used pre-broken fragments of glass. Plastic, if it could be properly cleaned, would be better as it could be cut in strips.

A. Cells counted ↓ types: — each cell accted for
Use extra fluid henceforth.

2	3	3 P2
4	4+④	0
~~5~~	6	
3	1 ghosty?	0
6	1 large	0
11	6	2 P2
12	4+2③	4 P2 + 1 P1
13	3	0
15	4	2 P2

B

6	2+1	1 P1
7	1 v.s.	0
~~8~~		
~~9~~		
10	3 short + 1	2 P2 + 1 P1
11	1	1 P2 — under oil.
13	1	1 P2 } all under coverglass.
14	1	1 P1
17	2 +.	1 P1
18	0	0
19	1	1 P1

C 4 ② 2 P1

12/12

Old cross. (24h+). Remix 1:20, ~~~~ 10:30, 1PM, 3:15PM.

3:30 A. (1+?) (11) m (0) (3) (1 eroded) 0 very small × 0 v. flat +

0 0 0 0 0

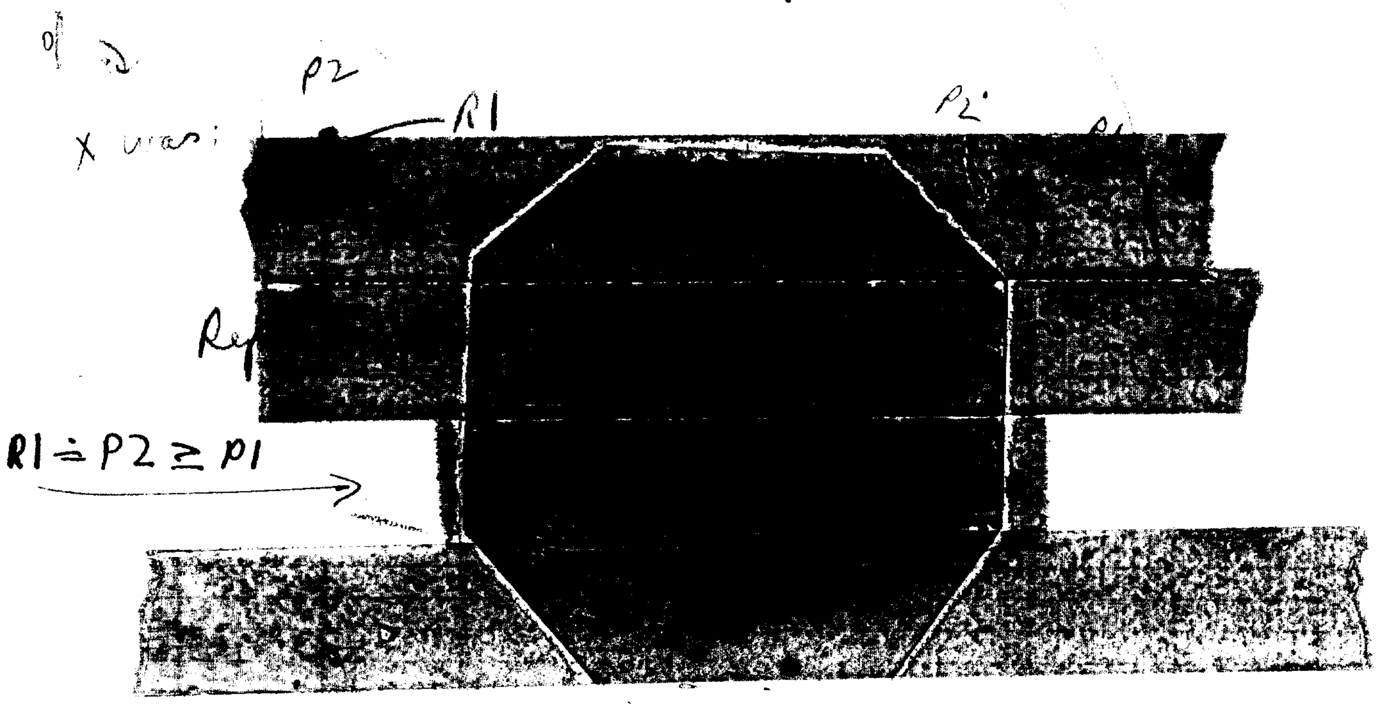
plate, add fl., 3:50

P13: [illegible] colonies

1	1	2P2
2	2(?)	2P1 + P2
3	1 + (2)	3P2 + P1 + (zygote)
4	1	P1
5	3	2P1 + P2
6	1?	0

[illegible] A3, all parts [illegible] 10% for parts

[illegible]



12/13
(11am)

del 4PM

A 12+1 1? 0 + 1? 1+ ? x 0 0 0

12/14. EMB lac.

	cells	cols.
1	3	1 P1
2	1?	0
3	1	1 P1
4	0	0
5	1?	0
6	1	0

12/14/53.

a). New cross .5 + .5 + 10 from old parent susp. 9:05 AM. — 10 30.
all with H_2O.

20
A. 1 x 0 0 0 0 0 0 oo X 1
(legged) thick

B 1. 3+ pair 0 0 0 x 0 0 0 oo P2 ?+
(v. small. rather large. possibly not a cell.) (P1, P2) (?)

C 0 0 0 0 3+ 11 2+ 11 0 2 1 0 0
4+. 4 mixed. 5+

2:05

D 0 1 1 + dirt? clump looser. 0 1. 0 1. 0 1. 1 0 0
2:13 — (drops to plating) 12:27

E 5+ 2+ 1.0+ ? 0 0 1 v.s.? 0 . 0 ? X 0 —
1:51 —

0+. 0 X 0 — 4+ (11 dirt)

5:21

DATE: 12/15. REF: 1099

Platings.

A.

	1	3
7	1	1 P1
8	+	2 P1

B.

	1	3
1	4	1P1 + 3P2
19	2 +	2P1
10	1	1P2

C

	1	3
1	4+	1P1
22	4	1P1
3	+	1P2
4	5	1P1 + 2P2
5	4	3P2
7	2	1P2
8	1	1P1

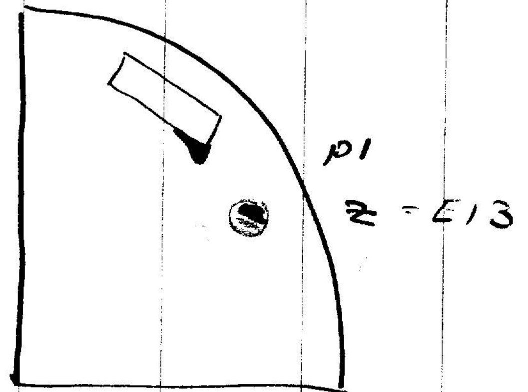
why yields so much lower?

D.

	1	3
32	1	1P2
3	1	0
5	2	1P1
7	1	0
4	3-clump	0
8	1	1P2

E

	1	3
1	7 + [illegible]	3P1 + 3P2
42	1	P2
5	1?	0
8	1?	0
10	1	P2
11	1	P2
13		P1 + Z!
1?	1	0
16	1	0
17	4	4P2



large cells recorded.
save components.

50

E13 → P1, R1 No P2 seen. Save mixture and [illegible] [illegible] colonies.

12/15/53.

12/14. Received cultures from Pomper — lyophil tubes. Inoculate in YEX medium.

WY-				
1	62	+++		Round cells (occ. oval!)
2	63	++		"
3	62-10-194	— did not grow out.		(tryptophane, uracil)
4	67-1	++		" (meth, adenine)

12/17. WY-1 grew well and promptly on yeast-sucrose agar
WY 2, 4 grew very poorly initially, but some large colonies suggest ~~incip~~ incipient adaptation.
Hand over to Rubbo for this
(probably temper (These do better at 30 than 37°
After 3 days, WY3 finally grew. Handle as above. [Reversion?)
Transfer from these initial broths to slants for "cultures as received"

WY6 = dipl S. cerevisiae WY ~~7~~8, ~~9~~ acriflavine-induced petites (Rubbo).
SOR reports that petites are defective in utilization of various sugars (cellobiose, rhamnose, ~~m~~altose, galactose) suggesting adaptive loss generally.

A. Check β-glucosidase in 6 vs 8 grown c̄ glucose, cellobiose.

B. Most sugars, WY 8, 9... showed mostly very poor growth, occasional larger colonies. On EMB Gal, WY8 showed two types of large colonies (fermenters vs slow fermenters). 1... and single colonies from EMB Gal of WY8. Also replate SOR's strains on EMB...

Further tests, nutrition

RL medium base. add niacin 1mg/liter to media

WY5 (S. fragilis) +++ (~~perf~~ previous failure presumably nic requirement)

± metals No effect on WY1, WY5 in liquid c̄ moderate ~~medium~~.

WY1 +++ s̄/c̄ metals

WY2 +++ confluent flor!

---

48 hours:

WY3 TR + YNA faint growth. Uracil + TR — TR, YNA only —

WY4 Meth ±

Meth Ad +

Yx +++

Hyd-Cas ++ (enzym.)

HC + pur ++

Meth, pur ±

Meth YNA +++.
hyd.

∴ something in YNA besides adenine for WY4. another amino acid?

WY3 unsatisfactory re morphology as well as growth requirements.

Try adenosine
guanosine ....

1100 4 A

Saccharomyces β glucosidase

DATE: REF:

A. Grow WY6,8 in Nutrient glucose, cellobiose broth.
Growth in cellobiose v. poor for either culture + harvest v. poor.
12/27: test c ONPG. No immediate rx but during several hours, all cultures "adapted" and split off o-n-φOH.

12/29/. Retest. Harvest WY6, 8 from YE agar and resuspend with glucose, cellobiose, 10:50 — 3 in pH 5 buffer + NB. maltose. ca M/10

Set up 3²⁵

v. poor
β glucosidase induced

Y30 faint color only, in (8, benzyl, cellobiose!)

Try S. fragilis / β-galactosidase
5³⁰: 6C 6GC 8G ~~benzene-treated~~ ... +
8C —
others all —

reaction too feeble to be useful

12/26-29 in Fries sucrose + vits 29°
Random Pompei mutants.
C WY1-2 grow well in F(S), + glucose, + 4 supplements ↓
(WY2 extremely flocculent: large clumps). (Also well at 37°).
WY3-4 failed to grow in F(S) ± glucose ± 4 suppl.
(meth, uracil, trypt, adenine each ca .05 mg/10 ml)
after 2 days delay, WY3 + 4 grew in F(glucose) and more slowly still in F(S). (presumably diploid hybrid) see over
WY4 also grew (poorly) in M, M + Ad but not Ad
Further work needed

D (over)

Burkhard Cross Brush on F(s) agar:

W Y

1	+++
2	+++
3	±
4	±

3 x 4 ± and scattered prototrophs at intersection. Yields ca like E coli cross, came up very slowly.

Replate these prototrophs as 1100 D1

12/30. 3 x 4, both F(s) and F(s) + glucose fully grown in tubes plate these as 1100 D2, D3.

Comparisons i/s MB, anaerobic supplement in liquid, aeration, covered with oil showed maltose generally better growth than glucose, W46, W48.

Cf. SDR comparisons. This cannot be ascribed to anaerobic or aerobic differences in maltose use.

DATE: REF:

	1	2	3	4	5	6	7	8	9	10

ca 12/26. Replate WY 6,8 etc.

12/29: WY6 gives faster growing scalloped colonies
WY8 (1-4 cols): slowergrowing, smooth. on YEagar

EMB. Glu — WY6 — WY8 — WY9. all give ++ ferm. reaction
WY8, 9 and 8(1-4) all considerably slower growth.

Gal. all grew more slowly and gave virtually ≡ ferm. rx
on replating, 1-4 were indistinguishable on EMB Gal!
(total reaction more nearly − than on S.K.'s plate

	Ferm	Gr	Ferm	Gr		
Sucrose	±	++	−	+	~~±~~	− +
Maltose	−	+±	−	−		− −
Cellobiose		±		−		−

v. sharp difference in growth!

S.K.'s plates did finally show moderate growth on cellobiose ~~+ maltose~~
c sec. papillae possibly better fermenters. Do ~~~~ WY 8 on maltose

Concl: Maltose shows sharpest differential.

10 20 30 40 50