Auxanography: pre-incub tenie series,
use Gal.
Probably 2 zones/plate.

Inoc T(B) plates heavily ~ 58-278. ~~58~~ 230 P21. Incubate to t~~x~~ at 38°. Then suppl. ~ drop of dl Gelan. 6:50 to 38° incub.

A22

1A 3:30
B. 6P >A.

2C 8P >>A. Best: 6 hr. preincubation
D 8P: K-12. Inhibition around streak; then zone of growth as supra.

3.

4.

auxanograms var. unidentified mutants. As above.

8P: ABCD.

58-5880 A, C, (B) C9., (6+7?) Methionine? Recheck.
175-3 — Inh. HCl
175-7 A.
175-8 D.
175-9 C?
175-11 Inh. A, C.
175-10 Inh. hydr. casein. Seems proto.
— 175-12 A✓ D1 inh D7 Proline inh tyrosine
175-14 —
— 172 28 A, D. D1 Inh. D7, 8 Proline " + cyst
— 172 30 A, D. D1 " " Proline. " + cyst

not a v.g. series.
Need better working.

Check densitometer calibration

4/22/46

Use culture of 278 which has given plate counts of ca. 3×10^9.

1:3 = ca. 10^9. Green filter 540.

	(73)	Absolute = 0.	Stand = 0.
A.		42[1]	
St		91[2]	

Red filter 660

	74.
A	53
St. 20 mg/ml	25
10 mg/ml	48[2]

Green filter Abs = 0.

71[1]

	Galv.	Dens.	Dens/dil
A.	43	367	367
A/2	65.	187.	374
A/3	75[1]	124	372
A/6.	87	060.5	363
A/9	91	041.0	369.

mean = 369.
m.d. = 5
= < 1%.

$367 = 3^2/_3 = {}^{11}/_3 \doteq 10^9$ cells.

1 d = ca. 3×10^6

ca. 10^6

$1/2 \cdot 3 \times 10^6$

$\frac{1}{50}$. $\frac{1}{50}$. $\frac{2}{50}$.

Ultra-violet mutations : tryptophane

4/22/46.

24 hour shaken tube culture 58-278. Irradiate at 11cm. for 2 mins. Incr 1 ml into another 10 ml and shake 24 hours. ( ).
9P22.
Incr 1 ml into colicar + ~~diffuse~~ da Growth quite clumpy, very slow. (Contamination?)

60
125
7500 dil 1:500 - ca. 7500

1:75,000 - 126 These figures checks.

50

∴ survival is ca. 500 × 7500 = 3750,000 out of 3×10^9
= 1%. = 2 mins.

N25. Dil $1:2.5 \times 10^6$ and plate into T(Φ,B). 38°. 5 plates.
1230P26 Larger colonies complete.

O
4P26. 9P26. 3P27. 10P28.

1
2
3
4
5

nothing

*

growing rather slowly.

73

* There are 12 prototroph colonies on this plate. In addition ca 50-60 new colonies have appeared, first noted at this time. The other plates are similar. Made protoplasts + continue incubation. In addition there is a single colony of intermediate type. 7P27 -

(see also 183)

Dec 194.

4/23/46. no good

Synteophum
EPA. 187.

I 58-278 (ϕ) & 58-161 (methionine). As. 181. Incub. 38°.
Inoc. 1230 A24. T(B) medium. .01 r. 10 r = 1 cc. Add 10 cc.

		ϕ	M.	24 hr. 38 hr. M24	2P25	11P25	G d. 11P25.	11P26
1	M	0	0		−	−	100	
2	"	0	1		−	±	98 .009	
3	"	0	3		−	+	98 .009	
4	"	~~ϕ~~ 5	5		−	++	95 .022	
5	"	0	10		−	+	98 .009	
6	"	10	10		−	++	95 .022	
7	ϕ	0	0		−	−		
8	"	1	0		−	−		
9	"	3	0		+	+	97 .013	+++
10	"	5	~~ϕ~~ 5		−	−		−
11	"	10.	0		−	−		−
12	ϕ	10	10		++	++	92^2 .034	+++
13	ϕ	250	0		−	−		−
21	ϕ + M.	0	0		−	−		−
22	"	~~ϕ~~ 5	~~ϕ~~ 5		++	+++	73 .137	+++
23	"	~~ϕ~~ 5	~~ϕ~~ 5		±	++	92 .036	+
24.	"	~~ϕ~~ 5	~~ϕ~~ 5		−	±	99 .004	+

II

	~~ϕ~~	ϕ	FPA	
31	ϕ.	100 r	0	
32		100 r	100 r	
33		200 r	100 r	
34.		200 r	200 r.	
		200 r	0.	See 13.
		0	0	See 7.
		0	+	See 181.

every thing still clear

−	−			
−	−			
++	+++	64. .114		
−				

77.

N. G. Old culture medium?
See 193.

58/r - Mutations + Killing.

4/26/46. Incr incomplete. U.V. radiation 11cm etc.

(KRAD)

26. 0. 7×10^8 1:12,500,000. 56.

A.	1	2 MIN.	250,000.		1	ca. 10^5. Killing. 10^{-3} surv.
	2		2,500,000.		0	$\rho S = 3/2\mu$.
B.	1	5 MIN.	1:1	.1	1	
	2		1:100	1:100	2	
	3		1:5000	1:5000	0	
	4		1:250,000	1:250,000		
	5		1:4,500000	1:2,500,000	0	

Incr flasks of 50 ml Complete Coli ± 1 ml of each dosage above.

D	1	6.	.1	
	2	7.	.1	
	3	8.	.1	
dil c	4	9	#1	
	5	10 ✓	#1	
	6	12 ✓	#1	
	7	15 ✓	#1	
	8	20	#1	
	9	30	#1	

2 presumably mut.

This strain is certainly less resistant than 58-278, and should hardly be designated 58/r.

191 A. 1:12,500,000 1030P27. into T(B, ∅) bottom + cover.

11						
12						
13	°	°	1 (31)	Y23-Arginine	0	51
14			1 32		1	48
15.			2 33,34	°		55
16 complete.					2	
					0	

250.

3P290 4A30X 10P30 ⊖ 1030P1 ▣ △

	Sm colon.	Total
	4	250

Pick ~~196~~. 197

Triple mutants.

4/26/46

24 hour culture shaken at 30° 58-278. ~~Incubate~~

4P26 Plate unir. culture 1:12,500,000. into T(B,φ). 3P26. .02 ml into CM 192 A.

	150A28	130P28	10P28	10A29	10P30 (35)	P1	
1	—	—	—	—	—		26
2	° —	° —	• —	• —	°	N. crassa contam.	37
3	—	—	—	—			29
4	—	—	—	—	2	36, 37.	38
5.	—	—	—	—	2	38, 39 → 5 total	31
							161

Incubate 2 mins.

Layer ē complete exc. 10P27 3+13.

10. 1:25,000. in coli complete. Centrifuge + resuspend. 750

ca. ½% survival

1:25,000 in T(Bφ,) etc.

11	° —	• —	° —	° —	4		172
12	—	—	—	' ③	6		253
13	—	—	—	—	0	0 (not layered).	1099 { 227
14	—	' ①	—	' —	1		242
15.	—	—	—	—	0		198

10P27. 1 ml into 50 ml coli. 192 B. 1:12,500,000. Bottom ~~Tower~~ T(φB)

	3P29 O	18h. 4A30X	36h. 10P30 Θ	1030P1. Δ	— 5P3
21	!	°	'		32
22		°	3		
23					
24		1	1		
25	2	°	°		
26	°	°	1		
27	°	°	3		
28	°	°	2		20.
29	°	°	°		
30.		°	2		35
31 complete.					31

x ~~25000~~
~~750~~

38 x 12,~~500,000~~
50 2

1/238 :

240 tested

N - contamination n.g. for pick.

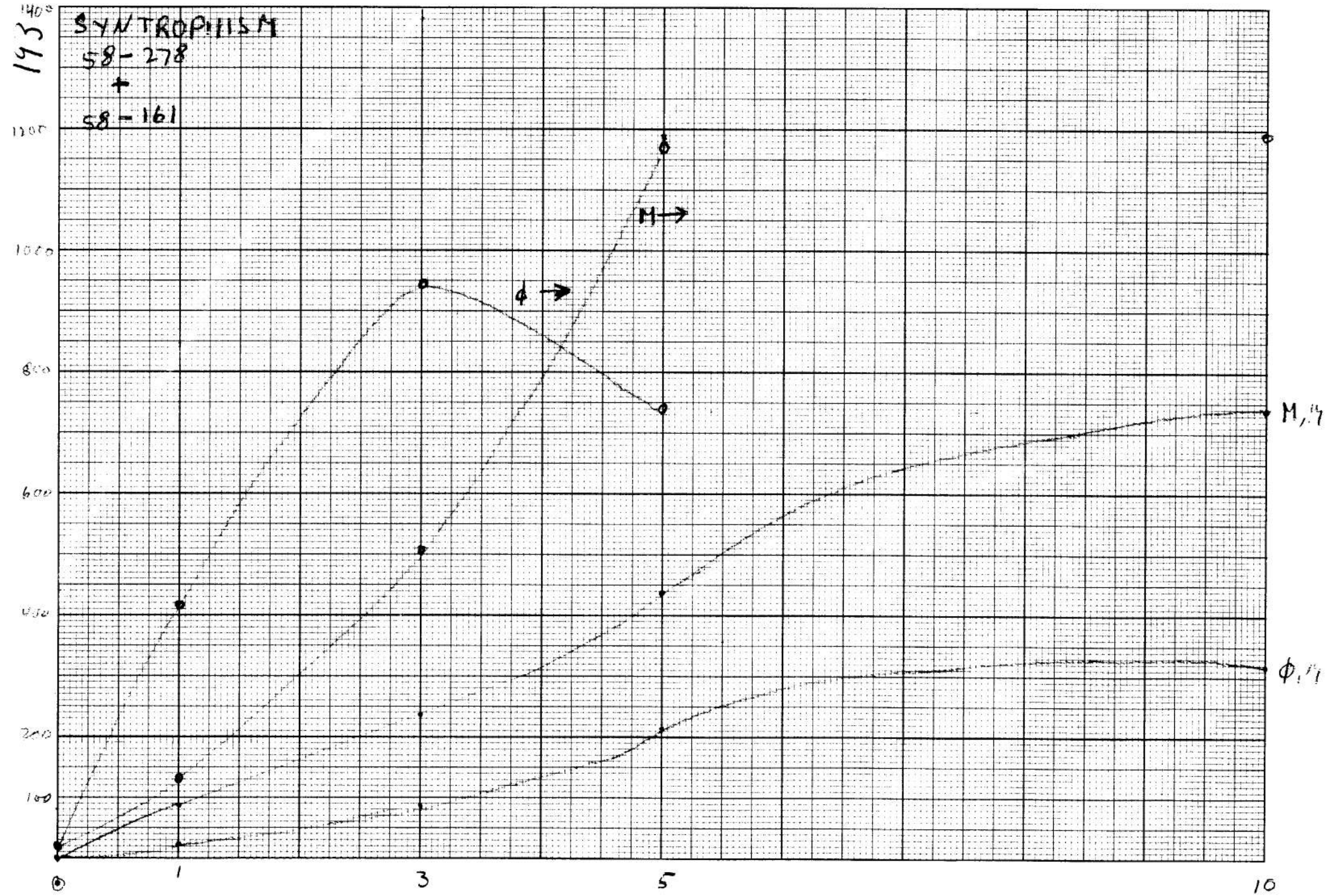
Tower ē saline - glucose - agar.
Layer ē complete 10A29.

Prototrophs unusually large here!

Incubate 1-15 at 31° after layering. do. 21-31.

Pick 1947

M5, Φ →



1933

Syntrophism

Meth. – ØAl.
Proline.
Use fresher cultures for inoculum.
4/27. Inoc 1230A28. 31°.

58-278 (Ø) and 58-161 (M.)

As before 10 ml T(B)+.

	Inoc.	Suppl Ø	M.	10P28 11A29 (77) G	D'	34h. 48h. 12M29. (77)	60h. 72h. 12N30. 11P30 72² 77	% M.
1	Ø M	5	0	— 100		100	100 100	
2	"	5	1	± 98	88	98³	98³ 98	
3	"	5	3	+ 94³	235	94²	94 94³	
4	"	5	5	+ 90²	434	90	91² 90	
5	1/10 "	5	10	+± 84²	731	84'	85 86²	
6	"	5	100	++ 63¹	1973	66	67² 66	
11	Ø	0	5	— 99²	22	100	100 100	
12		1	5	— 99²	22	98³	98³ 98³	
13		3	5	± 98	88	97²	96 92³	
14		5	5	± 95'	212	93³	88' 77² *	No protoh. on plating!
15		10	5	+ 93	315	90.	79 * 73 *	
16		200	5	+++ 61'	2129	60²	61' 61	
21	M+Ø	0	5	+ 90'	446 -M5 12	89'	81 72³	54
22		1	5	+ 82'	849 414	71	74³ 77³	44
23		3	5	+ 72³	1082 948	71	72' 72³	
24		5	5	+ 76'	1111 743	70	73² 73	
25		~~10~~	~~5~~					
31		5	0	+ 96	177 -D5	95'	62' 63	
32		5	1	+ 42'	321 139	74	67 69³	41
33		5	3	+ 84³	718 506	67	71 73	
34		5	5	++ 72³	1352 1170	67²	72' 72	50
plug wt 35		5	10.	++ 72'	1412 1200	71	73² 74	

679-183 (P) + 679-662 (G). in T (the. ½ mg). N2

		Proline.					
41	G	0	— 100	100	100 100	—
42		3	— 100	100	100 100	+++
43		10	— 100	100	99² 97'	+++
44		100	— 92²	84	83² 83	+++
51	P.	0	— 100	100	100 100	—
52		3	± 97'	97³	98² 96² 97²	+
53		10	± 96	95	96² 93² 93³ *	+
54		100	+± 69'	68'	68³ 68²	+++
61	P+G	0	— 100	100.	100 100	—
62		3	± 98'	96	81 79'	+++
63		10	± 96³	81³	74' 79 *	76³ +++
64		100	+± 66³	68	68 66'	+++

Note – change to EDST. 2A28.

* Adaptation.

1 A.M. 4/30/46.

Remove .1 ml of culture [illegible] from tubes 22 + 32 and plate into T(x) at a dil. of ca 1,250,000. Bottom + cover. After counting 1 mutant, layer c̄ other supplement + count the other. Dil. only [illegible] 500 for (.3) plates.

22a. x = biotin + methionine.
b. x = " + phenylalanine.
c. x = ".

etc

			3P2 L	S.	Total ✓	M %.	later:
22a.	52. (v. small colonies) (→ 117)	[illegible]	117	125	(251).	46.5	42.5
b.	114. 10P30. (→ 125)	[illegible]	w.T. 0 (1)	125	94? [illegible]	43.0	
32 a.	67. (later 114 →)	[illegible]	114.	149	d = 1308.	43.2	39.3
b.	141	[illegible]	w.T. 00.1%	145 [illegible]		do.	

✓ (1:500.)
32c 3 colonies seen 10P30. Divide by 2500 for rates c̄ mutants (Later, at 4P1, d = 1739.).

22c. 0. ✓

Notes from 1A30.

Layer c̄ letters: 4P1 (i.e., 22a c̄ phenylalanine).

At 3P2, examine + recount. Small colonies of the heterologous mutant have appeared.

Early counts are erroneous. Methionineless are much slower to develop initially on (?) layering. At 3P2 they are however quite distinct but too small to be counted readily.

930P2: ϕ smaller colonies intergrading with large but still distinguishable.
M/6 small colonies still minute, but enumerable. Count A3

N3 - M colonies still v. small. Not properly enumerable

Analyze syntrophic cultures.

4P1.

Colony counts on:

	Culture	M r	ø r	G d % M.	Deletion	T(B)	T(M)	T(ø)	F(M)+ø	T(ø)+M
F two major groups here.	193-21	5	0	54		10^3 >10^4-5$10^6$	194	F_10^6 170	369	380 ~~369~~
	-22	5	1	44		" 0	106	F 144	230	247
	31	0	5	—		>10^5	(N) —	(N) —		
	32	1	5	41		ca 3000	(N) 133	" 202		357.
	34	5	5	50		" 7	(N) 158	F 161		330.
but 58-278 adapted. What is sign. of adaptation here.	14	5	5,			(10^6) - 0 !	/////	F10^6 - 246		

62 10r — T(G)(T.) 10^6 (N) 392; T(T) 10^6 0 ✓; T(0 + ✓) (part of plate, ca 1/4 missing) 380.

∴ assume that another 1/4 ≐ prolineless.

1st counts 930P2

Layer ē complete 12 AHz. those marked ✓

Large differential is maintained indefinitely.

Thus, culture 31 was adapted (no detection kind of M or ø cells)

21 is also adapted, and contains, undoubtedly, 3 cell types.

% M.

		% M.	
21 M	52.5		
21 ø	55.3.		
R	53.2		
22 M	46.0	46.0	
22 ø	~~43.3~~ ~~[illegible]~~	43.7	
R	42.5	42.5	
31 M			
31 ø			
R	**		
32 M	—		
32 ø	43.5		
R	39.3		
	W.T. = .9		

% M.

34	M	
	ø	~~54.6~~ 49.7
	R,	49.5

Spontaneous mutants.
Small colony variants.

58-278 / [illegible]

A 3a Pick colonies on 183 plates to 1ml coli complete liq.

	No.	Complete	Test on T(B,Ø).	to CH plates P7.	Test on 1ml	BØ 5/11	BØ 5/18	Test – A20
	1	+	–		–	+	+	+
183-32	2	±	–		–	–	–	–
	3	–						
	4	+	–		–	–	–	✓
183-29	5		–		–			
	6	"	–		–	–	–	✓
	7		–					
183-31	8		–	v.sparse	– inoc. heavily	–	–	✓
	9	"	–	n.g.				
	10		–	do.	–	±	±	✓
183-30	11		–	ng P22				
58-278	12		–		–	±	±	✓
spont ↑	13	"	–	ng P22				
	14		–					
			P1 – 7.					
186-15.	21	+	–		–	–	–	✓
	22	"	–	ng	–	[illegible]		–
>70%	23		–	ng	–	–	–	✓
	24		–					
on 58-278	25		–		–	–	–	✓
+uv	26		–					
	27	"	–		–	–	–	✓
	28		–					
	29		–		–	–	–	✓
	30		– *			This had a gd control		

21a. to 3ml complete; 10 PØ; grow on shaker at 31°.
Came up slowly + to a low level.

* suspicious consistency.

medium later found n.g.

58-278 ++ ~~++~~

Every O was a photograph.
These are the 5 hour colonies.
One must examine plates daily
for 3–4 days.

Syntagheum

58-278 + 58-4899.

4/30/46. 530PM

T(B) 10 ml +

		10P1	N2
1. 5r del	58-278	++	++
2. "	58-4899	++	++
3.	58-278+58-4899.	++	+++

4.9. - too much del

7/29/46. Septicaemia - plate incor. 1945a

Pour plates of T(B) or T(T) & various organisms as indicated 230P29. Incubate at r.t. to 230A30. Incor. surface & homologous & heterologous E. coli & & Neurospora 5801. & 21863.

Inplate:	Incor:	1	2	4	3	
58-161		HC	-278	homologous	N. 21863	
		+	Sl. growth of streak	"		5P1.
			considerable "P3.			
58-278		HC	-161	"	N. 21863	
		+	—	—	—	
679-183		HC	662	"	N. 21863	
		++	l. growth str.	—	—.	Colonies plate & stim. zone
679-662. (turbid-campaw)		HC	183	"	N. 21863	
		+	—	—	—	
-662. no premicel.		+	N. 5801 growth; do coli stim.			
0					N. 21863	

679 – 662

Response of [redacted]
to glut + prol.

4/30/46. Is response to prol, which is delayed, an adaptation?
5:30PM.
Proc T(T) + suppl. ē 679-662

1. proline 200 r
2. glut. ac. 200 r

abandon temporarily.

Used 1–2 transfusions each solu.

* different colonial appearance. Stain c [illegible]: diplococci : ⁑

58-278

Cystineless

1) Spontaneous:	5/161			.031
2) U.V. - (t=0)	10/1099	.15 (1–2)	.014 (1–3) 197-61	.0099
3) u.v. (t=24h)	(24/240)	.001 (2–3)		.10

χ^2 tests:

1) - 2)

f_o	f	
2	5	161
13.	10	1099
	15	1260

$$\chi^2_{unc} = \frac{(2-5)^2}{2} + \frac{(10-13)^2}{13}$$

$= 5,2.$ $\quad p = .023$

$$corr = \frac{(3-5)^2}{3} + \frac{9}{13}$$

2.0.

$p = .15.$

1) - 3).

f_o	f	
12	5	161
17	24	240
	29	401

$$\chi^2 = \frac{(5-12)^2}{12} + \frac{(24-17)^2}{24}$$

$= 4.1 + 2.0$

$= 6.1.$

$p = .014$

2) - 3

f_o	f	
28	10	1099
6	24	240
34		1339

$$\frac{(10-28)^2}{28} + \frac{(24-6)^2}{6}$$

$= 11.6 + 54$

$= 66.$

$p = \ll .001$

See 194

Pick "mutant" colonies to coli complete

SP3

All colonies exc. O were of dest. - size at picking.

Plate	Design.	#	Complete test	Bφ (agar) CS		Bφ test 5/11 (From plate)	Bφ 5/18		A20	BφC [redacted]
91-12	X	31	+	A1		+ A12	−	−	✓	Arginine
58	X	32	+	2		+	±	±	✓	
91-13	X	33	+	3		+	+	−	✓	Leucine
91-14	X	34	++!	4 + ##		P11 + Seems to grow unusually rapidly. *				
92-1	Δ	35	+	5		−	−	−	✓	+
92-4	θ	36	"	6		−	±	−	✓	+
	θ	37	"	7		−	±	−	✓	+
92-5	θ	38	"	8	—					+
	θ	39		9		−	±	−	✓	+
		40.		10						
92-11	θ	41	"	11	x					+
	θ	42		12	x					+
	θ	43		13	?	−	−	−	✓	
	θ	44	"	14		−	−	−	✓	
92-12	O	45		15		−	++	✓	✓	
	θ	46	"	16	x					+
	"	47		17	x					+
	"	48		18		−	±	+	✓	+
	"	49	"	19		−	±	±	✓	
	"	51		20		−	+	+	✓	
	"	52		21		−	−	−	✓	
	"	~~53~~	"	22						
	Δ	54		23		−	−	−	✓	
	"	55		24		−	−	−	✓	
	"	56		25		−	−	−	✓	
	—	57		B1	✓	↓	+	+	✓	
~~92-14~~	~~θ~~	58	"	2			−	−	✓	
92-14	θ	59		3			+	+	✓	
	Δ	60		4			−	−	✓	
	θ	61		5			−	−	✓	−
92-15	Δ	62	"	6			−	−	✓	
	Δ	63		7			−	−	✓	
92-21	O	64	++	8		±	++	✓	++	
	θ	65	+	9			±	−	✓	
	Δ	66	+	10			+	+	✓	
	—	67	−	11			+	+	✓	
92-22	θ	68	[illegible]	12			+	+	✓	
	θ	69	−	13			−	−	✓	
	θ	70	+	14			+	+	✓	
	Δ	4c. ~~71~~	~~−~~	15						
92-23	—	71	~~+~~	16						
92-24	—	72		17			±	−	✓	+
	X	73		18	++	±	++	✓	✓	
	θ	74		19			,	−	✓	
	Δ	75		20			+	+	✓	
92-25	θ	76		21		±	++	++✓	✓	
	θ	77		22	++	±	++	++	✓	
92-27	θ	78		23			−	−	✓	
	θ	79		24			+	±	✓	
	θ	80		25			±	±	✓	

None grew, but were diff. 58-278 by P12.

1976.

								B ϕ C
192-29	—	81	+	C1	broken.	±	±	✓ +
-30	⊖	82	+	2				+
17-22	—	83	+	3		−	±	✓ +
				4		+++		
58-278				5				
58								

An exceptionally high proportion of mutants is indicated.
These have to be auxanographed now.

~~with~~ by series 197- 35 to 82, 58-278 treated ō u.v.

5 - grow on minimal

39 . grow on biotin + dal + cyst.

1 (#61) - ?

Auxanography: 19 ? nutrients. 197 C

Plates not sterile. Pour plates 10 PB. Inc 30°
1230A – A) ø10x B) etc. Inc 35°

5/18/46.

	5A19.9A		Sp			A 24 T(BCø) Final identity	Y–
Y 11	AD		D SA	1	Proline –		
Y 13							
Y 14							
Y 18	A C		C SA	Turbid			
Y 19	A B/C		B SA	– 1. (Thiamin) ✓			
Y 20	A; D, B C		D SA	Turbid			
2							
Y22 turbid 4		A					
6		A B?					
YY21	A D	turbid.					
25		A					
29	A	–					
Y12 D6 AD 5580 A 31	A C		C SA	– 8 turbid	Arginine.		(23)
32	A	–					
33	A	AD	D 11A.	1??			
34	A	D? A					
35	A	–				+	
36	A	–				+	
37	A	–				+	
39.	A	–				+	
43	A	C	C 11A			+	
44	A	C	C 11A	———		+	
48		–				+	
12 N– put palat C, B, D. 49	A	–				+	
51	A	–				+	
52	A	–				+	
54	A	–				+	
Proline del. 55	A	–				Growth on +	
56	A	–				T(cyst) +	
Error! 57		–				liquid. +	
58		AD	D 11A	6	BøC		24
59	AD	AD	D 11A	6	"		25
60		A			"	+	
61	turbid	A				–	
62		AD	D 11A	6	BøC		26
63		AD	D 11A	6	"		27
65	A	D	D 11A	6	"		28
66	AD		D SA	–6? (Cyst) ✓	"		29
where? 67							
68	A D	AD	D 11A	~~4~~ 6	BøC		30
69	–	A			"	+	
70		AD	D 11A	6	"		31
72	A	–			"	+	
74					"	+	
75	A D		D 11A	6	"		32
78		AD	D 11A	6	"		33
79	A	AD	D 11A	6 Cyst	"		34
80	AD		D SA	6?✓ Cyst	"		35
81	A, D?	D:	D SA	6	"		36
83	AD		D SA	6	"		37
21 ~~22~~	A	–					

Method A: Bact. hydrolysate C 198.

Inoc. 200 ml in 500 ml Erlenmeyer ~ K-12, as shaken 31°. SP 5/3

1. Coli complete

2. T(o).

① Harvest SP & (& wash superficially) into 20 ml 6N HCl. reflux 1A7 – 1P7 (calc 6 x 10" cells)
distill off HCl & water to volume of ca 5 ml.
Centrifuge humin down, dilute to 20 ml & store as hydrolysate 198.
(Light golden brown color.) for future titration & assay.

The T(o) has not been growing well.

B/r mutants.

5/8/48

Inoc 10ml exp ī B/r from constant. 1130A8.
1130P9 d = 1.4900

Irradiate as above for ~~30 min~~ t. (ps. calc. as .9/min.).
Construct killing curve points.

		Rad. t.	Dil.	Count.	ps.	ps/t
1.	1	0	$1:12\frac{1}{2}\cdot10^6$	86.	1.2×10^9	0
	1 "	"	"	83.		
2.		20 sec.	10^2	>>		
a	3		10^4	ca 10^4		
4.		.	10^6	~~508~~ ca 900	$.9\times10^9$	.12
5		60 sec.	10^2	>>		
6			10^4	ca 10^3		
6.	7.		10^6	558	$.56\times10^9$	.33
	8		.1	>>		

Inoc 1 ml of a and b into 10ml coli x.

Use. b. only. Apply mutant method. 1130P10.

$d_{opt} = 1.612$

dil. $2: 12.5\times10^6$

	10h.0	28h. Δ	60h.
	12M12	6P13	3A15
1	1,2,3,4		marked colonies all still dist. small
2			
3			
4			
5			
6	5	2	
7		1	
8		8	
9			
10. not layered.	6		
	6.	2	

nothing seen.

650.

1130P11 (24 hours) - colonies in T/O visible, but very small. Hold ∞ layer.
1145P12 38 hours. - good colony spread; sight sl. small but uniform.
layer ī ∞ 2U P12.

1130P12 colonies sl. larger than (10).

Pick to complete liq. 1ml:

Pick to complete. A21.

	G(∞)	G(0).
1.	O	+
2.	O	+
3.	O	+
4.	O	
5.	O	
6.	O	h.g.
7.	Δ	
8.	Δ.	
3/2		++

Kymograph 5/23.

3 - C8 - Arginine
4 - C1-2 - Leucine or isoleucine!

Lact. Hydrolysate D. 201.

5/9/46.

Broz K-12 into 200ml T(0) es198. Use fresh culture.

A. - unshaken 9P9.
B - unshaken

medium n.g. Repeat A11.

see 200, 202 ff.

Precursors + bacterial mass activity.

Remove alcohol from bacterial suspensions 2 and 7 by centrifuging + decanting, and hydrolysing by refluxing ~ 6N HCl 4 hours.

Calculate product in "density units"/ml by dividing original optical density by the ~~dd~~ ultimate concentration. e.g. #16 hydrolysate if final volume is 20 ml and 100% recovery is assumed would be:

$848 \times \frac{25}{20} = 1060$ d/ml. This may be useful in calculating recoveries of various substrates.

Precursors and filtrate activities

5/11/46.

1. Inoc c̄ K-12 the following. 10P11 31° (on shaker = ✓)

Coli Minimal +	Amt.	2P12	Growth 1130P	Final growth G 1:10.	d
1. + 0	500 ml ⟨250 unsh / 250 shake	+	2+	96	177
			3+	82'	848
✓ 2. glutamic ac. 5 mg.	50 ml	+++	++++	67^2	1707
✓ 3. anthranilic ac. 5 mg. Harvest 10A16	"	—	±	86	655
✓ 4. citrulline 5 mg.	"	++	4+	71^3	1442
5. pantoyl-lactone ~~ornithine~~ 1 mg.	"	++	4+	76^2 lost.	F163
✓ 6. phenylalanine 5 mg.	"	+++	4+	not done.	
7. 0	"	++	4+	71' (74)	1472

The most efficient growth is evidently in small shake flasks.
Harvest N14. Save filtrates & bacterial mass.

(Pool bacteria of # 1, [illegible], for hydrolysis. Preserve others in alcohol. collect in 25 ml 6NHCl + reflux 4P14. — 3A15 ca 11 hours) Lost

P14.

E. coli K-12 50 ml.
8. pantolactone 1 mg
β-alanine 1 mg

84
lost on centrifuging
(73)

N. crassa [illegible].5Y7 in Fries + 50 ml.
9. pantolactone 1 mg
β-alanine 1 mg.

Harvest. 11A17.
Sample 5 ml (st.) from 9 add to 5 ml F(0)
autoclave & inoculate c̄ 5531.

(79#)

3 A15. 30°

Inoc. 1 2 3 4 5 6

0 202-7 1ml 1ml 202-7 1ml 202-7

1+ +

1 58-3214. " " 1ml .1mg glut. 202-2 .1mg prol .1mg gl

– – ± ± – – ± ++++ – ±

100 100 100 99

2 R572-228 " " .1mg citr 202-4 .1mg arg. ~~.1mg~~ .1mg cit

– ± ± – ± ++++ ++++ ± ±

100 100

3 58-5030 " " .1mg del 202-6 .1mg tyr. ~~.1mg~~ .1mg del

– – ± ± – – ± ± ++++ ± ±

100 100

4 K-12 " "

+ +++ ++ ++

+++ +++

5 " "

6 " "

7 " "

all ± are ca 79, indicating traces of substrates. Larger quantities of filtrates should be tested, since these are dil. by 1:10.

1st reading " P15.
2d reading A16.
3d reading 9P16

Interaction of nutritional requirements

5/10/46.

679 – 183.

Inoc 1130P10. (10hour culture!)

	T. (r)	P. (r)	830P11. 21.4.	
1	500	0	100	
2	"	10	93	
3	"	30	(81')	proline limiting value.
4	"	100	62	
5	"	300	63	
6	1	500	100	
7	10	"	97	
8	30	"	93'	
9	100	"	–	
10	300	"	(83.5)	threonine limiting value.
11	1000	"	71'	
			59	
12	100	10	94	
13		30	84	← not depressed below T limit.
14		100	±	
15		300	±	
			±	
16	1	30	100	
17	10	30	97.3	} 11 to T response at higher proline.
18	30	30	95	
19	100	30	83 ←	
20	300	30	81'	
21.	1000	30.	82.	

(TB')

mis nion?

The requirements seem to be independent, with a sharp cut-off when the limited growth is reached. Set up another, cleaner expt. to establish this, using levels of

Proline = 40 r

Threonine = 100 r.

α = 679 · 185

β = 58 · 161.

5/13/46. Kroz BA vs. 30°

kroz		Biotin	Meth.	Thr.	P α r	11P15	10P16	8A17 (to M17)		
1	1	—	—	50	(α)	±	+	89	505	
2	α	—	—	(500)	10	±	+	98[3]	281	
3	3	3	(100)	—	—	+	++	183[1]	796	
4	3	(10)	3	—	—	±	+	94[1]	257	
									D+.	ΣD calc.
11	α + β	(10)	3	(500)	10	lost.	had grown to ++			
12	"	(10)	3	50	(100)	+	++	77[1]	1121	538
13	"	.3	(100)	500	100	+	++	76[1]	1177	1077
14	"	.3	(100)	50	100	+	++	73[2]	1337	1301
15	"	(10)	(100)	—		+	++	70[2]		
16	"	—	—	500	100	+	++	74[1]		
21	β	.3	3	—	—	+	+	+92[3]	339	
22	α	—	—	50	10	+	+	+ 91[1]	398	
23	α +	.3	3	50	10	++	++	++79[3]	982	>
									A18	A18
31	α + β	10	—	500	—	—	—	—		—
2		"	.01	"	—	—	—	—		+++
3		"	.03	"	—	—	—	100		—
4		"	.1	"	—	—	—	100		—
5		"	.3	"	—	±	±	+++59[1]	+++	+++
6		"	1	"	—	±	±	± 96[3]	+++	+++
7		"	3	"	—	+	++	+++65	+++	+++
41	β	"	—	"	—	—	—	100		—
2		"	.01	"	—	—	—	— 100		—
3		"	.03	"	—	—	—	— 100		—
4		"	.1	"	—	—	—	— 100		—
5		"	.3	"	—	±	±	± 99		±
6		"	1	"	—	±	±	± 97[2]		±
7		"	3	"	—	+	+	+ 93[2]		+
51	β	"	3	"		+		+		+++
52	"	"	3	"		+		+		+
53	"	"	3	"		+		+		+
54	"	"	3	"		+		+		+
61	58-278	"	β alanine 3			±		±	+	+++
62	"	"	3			±		±	+	+++
63	"	"	3			±		±	+	+++
64	"	"	3			±		±	+	+++
65.	α +	coli α								
66	β	—	100			—		++ 77[2]	? adapt?	

Left margin notes: 4; 6; 5 dual exchange; 7. Right margin: A20 (+++ beside rows 2–3).

77[3]

10P16 – mice 51 and 52 c 58-3214. (α)

See 207

Syntrophism - Sex. 20%.

679-183 x 58-161.

½ values - 81 ca. on galv.

This experiment is designed for:
a) critical conditions of syntrophism:
b) substrates in culture medium
c) recombinations.

679	200 r	T
183	30 r	P
58	.7 mr	B
161.	10 r.	M

1st. Need data on interaction of requirements. ~ Independent.

a. 4 1:1 interactions: use excess and .1 optimal

a. BT	+ PM
b. BP	TM
c. MT	BP
d. MP.	BT.

Analyse for recombination types.

2:2 .1 optimal for each.
e
f
g
h.

3:1. , 1 opt for
BM
TP

Critical conditions:
a. delayed inoculum
b. excess BT. provide M in range 0-5 r.
c. 278 adapt. series at 5 r.

Dysentophism.

10γ proline

All 58- cultures ± .01γ Biotin

679- ± .5 mg threonine

#1130P10. 30° 5/10/46.

	hor A	hor B.	Supp.	1130P11.	1130P12	1130A13	Ro (78)	
1.	58-3214.	—	BT.	+	+	+	85	
2.	—	679-183	BT.	+	+	+	89 3	
3.	—	679-662	BT.	−	±	±	98	
4.	58-3214	679-183	BT.	++	+	+	88	
5.	58-3214	679-662	BT.	+	+	++	74 2	Syn. !!
6.	58-3214	—	B	+	+	+	90'	
7.		Y1	"	+	+	+	93 3	
8.		Y1	"	+	+	+	93 3	
9		Y13	"	−	−	−		proline req?
10		Y13	"	+	+	+	89	
11		209-301R	"	++	+++	(autol?)	77	
12		209-301R	"	++	+++		79'	
13		58-2651		+	+	+	90'	
14.				+	+	+	90	
15		58-3232	"	+	+	+	90'	
16				+	+	+	89	
17		58-52.55	"	+	+	+	87 2	
18				+	+	+	84 1	
19		5450	"	−	+	+	91	
20				+	+	+	91'	
21		6049	"	−	+	+	91'	
22				+	+	+	92	
23		6177	"	+	+	+	89 2	
24				+	+	+	91	

All available prolineless are identical exc 679-662 which uses glutamine.

Note 1 cell contains $\frac{1}{2}$ 10^{-12} g H_2O.

at pH 7. the $[H^+] = 10^{-7} \times 6 \times 10^{23} \times 10^{-3}$ g.

$= 6 \times 10^{13}$ molecules/g.

This is $\frac{1}{2}$ 60 H^+/cell. at pH 7

.1% of the time, there will be only 30]

A daily requirement of 30 r/week is quite reasonable for protein 10-40% of the dry wt.

dry wt./cell $d = 3 \times 10^6$ cells. $\doteq$ ca 1 r

$\therefore$ 1 cell $\doteq$ .3 rr dry $= 3 \times 10^{-3}$ dry.

ca. 1rr wet.

This is less than previous estimates: $(8 \times 10^{-13}$

Bacteria production.

T(0) in 4 liter lots in 4 l. Pyrex bottles.

1.
2.
3. Without asparagine.

Aerate by aspirator suction; cotton - in glass air filter.

Hour 11-12 2A15. 5/15/46. Room temperature.

11A16 — 1, 2 ++ 3 ± growing slowly
12M11 — 1, 2 are nearly opaque; 3 ++. | A17. 3+++.
930P17 — all indistinguishable. Harvest - pool in large container + put in ice box.

Both are 90^2 at 10:1

l. 1.
2.
3.

Remove 250 ml samples of 2 + 3 for centrifugation.

Allow to settle in cold room at 0°C. for two weeks.

P2 - separate by siphon into (1) 4l., (2) 4l., (3) 3l., (4) 1l. fractions, ca.

and reject fraction 2. Allow others to settle further; centrifuge 500 ml samples from 3 + 4 and collect + accumulate the harvest.

P.S. Dry accumulated harvest after diluting in saline - note sticky nature (of cells) highly intact - and wash ≈ H_2O. Dry 1) over $ZnCl_2$ 2) at 100° / 2-3 hours. Extract ≈ 100cc Et_2O. overnight.

Dry weight was 3.406 g. Assume ca 75% yield of the bacteria, approx.
After extraction 3.318 ca 3%.

Thus were $12000 \times 10 \times 43 = 5,160,000$ 5.2×10^6 density units ≙ 3.406 gm. or 1 density unit ≙ 1γ dry wt. bacteria. and average culture as minimal 10 ml ≈ G = 60 has about 2γ dry bacteria in it.

Assays of Yhom hydrolysate Neurospora.

Mor 1A6. 30°.

A. Biotin. SY7A. 9A8.

1. ~~10~~ cc "Biotin"-free Fries. SY7. ±
2. do. + [redacted] 1ml 206A. +++
3. do. + 1ml 206A + .05 r biotin. +++

B. Inositol. 34701 a.

1. 10cc Fries. —
2. + 1ml 206A. —
3 + 5 ml 206A. —
4. + 5ml 206A + 5r inositol. +
5 5r inositol. +

C PAB. 1633A

1. 25cc Fries. —
2. " + 1ml 206A. +++
3. " + .1ml 206A +
4. " + 1r pab. +++
5. " + 1ml 206A + 1r pab. +++

While "appreciable" biotin and pab are available, there is no detectable inositol in this fraction of E. coli.

6/6/46.

Take ca 1 gm sample (1.014 g.) + reflux c 18% (6N) HCl at 100-110°.

at 4 hours digestion, 20.5 cc was present. Remove 5 ml sample + continue digestion. — make up to 20 cc. lost.

↓

Remove another 5 ml. $= \frac{1}{4} \times \frac{3}{4} \times 1.014$ g.

$\doteq$ 190 mg Concentrate + neutralize c NaOH. Store as 1% bacterial hydrolysate

assay 4 hr. hydrolysate 206a.

Continue hydrolysis of remainder to 24 hours. Concentrate, neutralize and dilute to a conc. of 20 mg/cc. Store in cold

See 234 for paper, arginine assay of hydrolysate

Use 204 - syntrophism cultures:
Mixtures of BM. + TP. P. 5/17/46.

#	Grown in excess:		Dilution	Medium		P19		Layer:	% BM.
1.	11. Lost								
2.	12 BT.	Plate	1:1000	in T(o).		0			
3.		"	1:1000	in T(BT)		3	1-3		
4		"	10^{-6}	BT		0			
5		"	10^{-6}	BM	✓			∞	
6		"	10^{-6}	BM.	✓			PT	
11	13 MT		10^{-3}	0.		0			
12			10^{-3}	BP		0			
13			10^{-6}	BP		0			
14			10^{-6}	BM	✓			∞	
15			10^{-6}	BM.	✓			PT	
21	14. MP		10^{-3}	0		0			
22			10^{-3}	MT		0			
23			10^{-6}	MP		0			
24			10^{-6}	BM	✓			∞	
25			10^{-6}	BM.	✓			PT	
	~~#5.~~ Wash:								
31	65.		~~10^{-7}~~	~~∞~~		~~[illegible]~~			
32			10^{-3}	0		0			
33			10^{-3}	BT		4	4-7		
34			10^{-3}	BP		0			
35			10^{-3}	MT		0 cont? ✓			
36			10^{-3}	MP		1	8		
37			10^{-7}	BM.	✓			∞	
38			10^{-7}	BM.	✓			PT.	

See 212.

Where possible recombination colonies are present, pick to a complete liquid ~~A~~. A21.
(BT).

Note: only B-T. Why?

P21. to slants + test on: (A, B, C)
Retest on various = #5.

	T(o) PP22	PP22	T(B)		T(T)		T(BT)	
1	—	✓	±	✓	±	✓	+++	✓
2	—	✓	±	✓	+	✓	—	✓
3	—	✓	±	✓	±	✓	—	✓
4	—	✓	±	✓	±	✓	—	✓
5	±	✓	±	+++	±	✓	+++.	
6								
7								

1: A BT strain? Call it 58-619x.

2–5: Why no growth? trace req.

Streak out 1 on a complete plate.

Sex

v. 204.   679-183 x 58-161
α   β

5/21/46.   30° 2A 23.

P2

	Inoc.	mγ Biotin	γ Methionine	γ Threon.	γ Proline	12h.
	1 α	10 ✓	3	500 ✓	10	+
	2 β	10 ✓	3	500 ✓	10	++
	3 α + β	10 ✓	3	500 ✓	10	++
	4 α + β	10 ✓	3	500 ✓	10	++
	5 α + β	10 ✓	3	500 ✓	10	++
BT.	6 α	10 ✓	3	500 ✓	100 ✓	+++
	7 α	10 ✓	3	500 ✓	100 ✓	+++
	8 ~~α~~ β	10 ✓	100 ✓	500 ✓	10	++
	9 β	10 ✓	100 ✓	500 ✓	10	++
	10 α	10 ✓	100 ✓	500 ✓	100 ✓	+++
	11. β	10 ✓	100 ✓	500 ✓	100 ✓	++
	12 α + β	10 ✓	100 ✓	500 ✓	100 ✓	++
	21. α	.3	100 ✓	500 ✓	10	+
MT.	β	.3	100 ✓	500 ✓	10	++
	α + β	.3	100 ✓	500 ✓	10	+
	31. α	10 ✓	3	30	100 ✓	+
BP.	β	10 ✓	3	30	100 ✓	+
	α + β	10 ✓	3	30	100 ✓	+
	41. α	~~10~~ .3	100 ✓	30	100 ✓	+
MP	β	~~10~~ .3	100 ✓	30	100 ✓	+
	α + β	.3	100 ✓	30M	100 ✓	+

— Do not use. ? autolysis or phage??

51. coli α, 50ml flask, shaken α +Y
52 " β +Y
53 " α + β +Y

Take off shaker 11A28