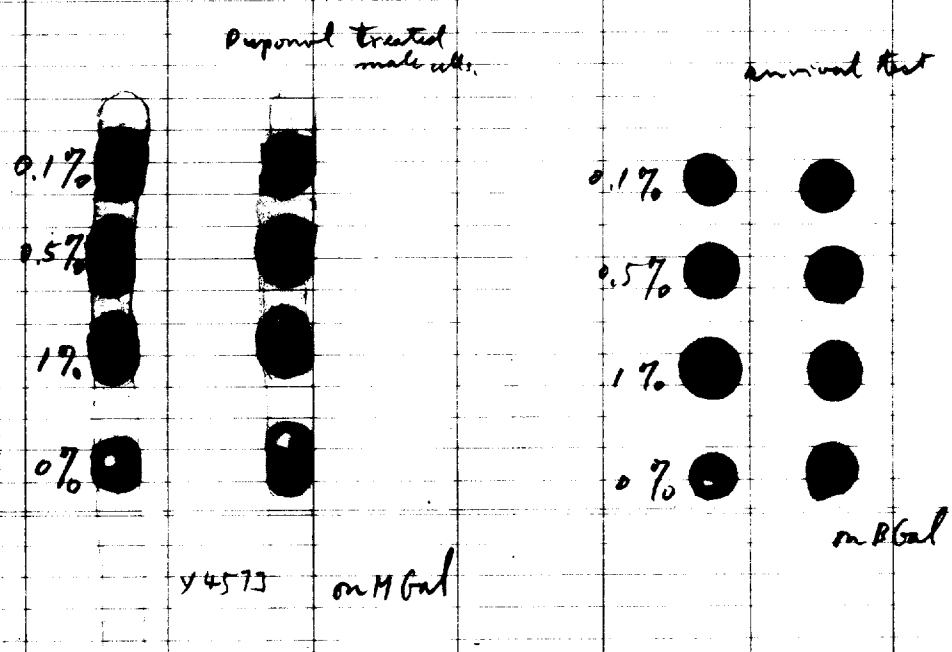
1st trial on the killing of cells without loss of mating capacity.

17/XII 1957 REF:

5 ml overnight culture of W6 F8 in Pen
↓
Centrifuge, discard the supernate, resuspend its 1 ml of H_2O.
↓
distribute the 0.2 ml of W6 F8 into duponol soln. {sodium lauryl sulphate
↓
Incubate it for 2 hrs. at 37°C. 1:100 PM
↓
Wash it once and spot them on MGal x 4573 & on BLac

	Conc. of duponol.	survival	fertility.
	1 %	+	+
	0.5 %	+	+
	0.1 %	+	+
control.	0 %	+	+



Conclusion: Use much conc. duponol. (20 %)

2nd trails on the killing cells without remove of mating capacity.
(duponol)

17/11 ; 1959. REF:

1. W6 F_8 overnight culture 8 ml / tube. 4 tubes.

20% Centrifuge - Wash once - put 4 tubes together - resuspend them into 1 ml of H_2O.

3. Add 0.2 ml of the Bacterial suspensions into duponol soln. 0% 10%

15% 15% 20%. and incubate them for 4 hrs. at 37°C.
3:00 PM ~ 7 PM.

10% 4. Test survivals & fertility of these treated and untreated W6 F_8.
on D Gal and M Gal x 4573.

0

1/10 1 Penassy grown. ← 2 days old.

Exp. I. 4573 + duponol-lysed F_8^+ (with Duponol) → incubate at 37°C for 30'

0.2 ml original 0.5 ml

0.2 ml 1/10 diluted 1 ml

→ wash once → ~~spot~~ ~~then~~ the 0.1 ml on M Gal. (7:30 PM ~ 8:00 PM,
(centrifuge for 5 min.)

Result.

	Survival	Recombinant
10%	2×10^3	$> 2 \times 10^3$
15%	"	"

Transfer of Lac.

Exp II. dilute the duponol-lysed material (10^{-1}) → spot on M Lac on which W4573 was streaked.

untreated control. 0 non-diluted material (10^0) → "

10%

15% ~~Exp III. Centrifuge and resuspend 5th into 1 ml of H_2O and spot on M Lac x 4573.~~

20%

Conclusion: ① Killed cells with duponol does not transfer Lac to F^-.

x 4573

1/10 dil on M Lac 1 undilute

② However, the treated material shows recombinating activity in transferring Gal as same numbers of survival cells.

Next step: Make exact experiment.

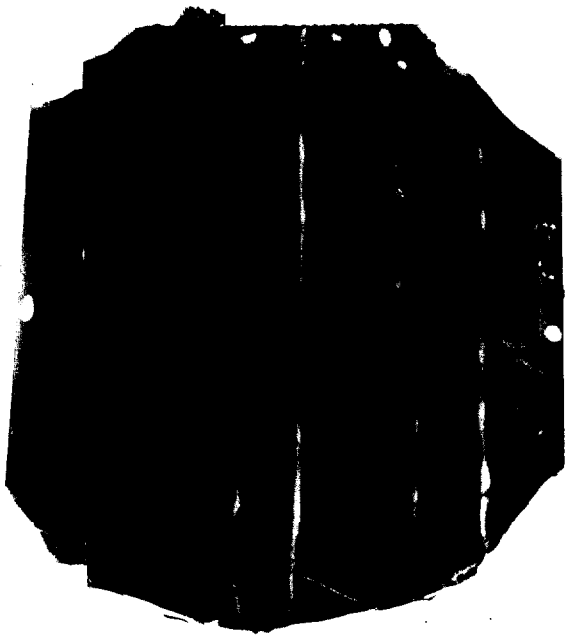
4:30

4297 4296 2985 4539 4627 4354 4295
G T A_1 H Trg M L

1
2
3
4
5

blank #295 #354 4627 4539 2985 4296 4297

on MGluc.



4297 4296 2985 4539 4627 4354 4295 blank

on MGluc.

Continued from 153a.

U.W. MICROBIAL GENETICS

153b

F status of segregants from 3747 × 4574.
primary exconjugants of (cf. P)

4574 : F^- P Ara_2 Mal_1 Mtl Ara_2 Xyl_2 Gal_2 SR.

24/II ; 1960 REF:

Exp. I This experiment does not work well. The fertility was so low in this time. only one Lac$_v$ was tested.

25' : at 37°C. in incubator. 1 recombinants (Lac$_v$) — purified on BLac Sm — Pick Lac$^+$ & Lac$^-$ onto BLac Sm — Replicate on DO + 4506 (F^- pur$^-$)

(Lac$^-$ colonies (4574) are as clouded around the Lac$_v$, therefore, Lac$^-$ F^- (4574) can be contaminated with Lac segregants)

#1 Lac$_v$.	# of Lac$^+$ tested	sex	# of Lac$^-$ tested	sex
	15	F_{13} : 15 F^- : 0	24	F^- : 21 F_{13} : 1 ← Save this F^+ : 2 (or F_{13}) recheck!

# of Lac$^-$ Lac$_v$ 876 : 1

See back page Ia, & Ib.

Exp. II Exp. does work beautifully.

25' : at 37°C : in incubator room : on rotator.

Lac$_v$. testes : 4506 on DO.

	Lac$^+$ tested	sex	Lac$^-$ tested	sex.
#1	8	F_{13} : 8	10	F^+ : 10
#2	10	F_{13} : 10	1	F^+ : 1
#3	4	F_{13} : 4	1	F^+ : 1
#4	8	F_{13} : 8	—	—
#5	6	F_{13} : 6	—	—
#6	7	F_{13} : 7	—	—
#7	8	F_{13} : 8	—	—
#8	6	F_{13} : 6	—	—
#9	5	F_{13} : 5	—	—
#10	6	F_{13} : 6		
Σ	58	58	12	12

% of F_{13} of Lac$^+$ segregants : 100. % of F^+ of Lac$^-$ segregants : 100.

- ① Mix 4hrs exponentially growing and culture of 3747 (10) & 4774 (1) shake it on rotator for 25' at 37°C. Chill & Then blend the mix, dilute it into 10^{-3} : 0.1 ml. plate on BLac Sm.
- ② pick well separated Lac$_v$.
- ③ purify them on BLac Sm. Pick Lac$^+$ segregants & Lac$^-$ segregants. and test sex by cross-brushing method on D.O. × 4506 (See back page II.

- F^+ means as follows :
  1. Not F^- from the cross × F^-
  2. Not F' by low fertility.
  3. Same fertile as F^+ in cross × F^-.

Fertility of F^+ & Infectivity of F^+ should be checked.

- 3 colonies out of 10 showed segregation of Lac$^-$ #1, 2, 3.

— : No Lac$^-$ colonies can be picked after streak on BLac Sm.

Conclusion : 1. Lac$^+$ becomes F_{13}. suggesting close linkage between Lac & F_{13}.
2. Lac$^-$ becomes F^+. (These F^+ (presumably?) should be checked. in its purity, and low fertility & infectivity for many markers) → These are really F^+! see back page.

Further experiment : Pick 3 colonies from #1, #2, #3. and test compatibility of each 3. 1, 11, 12. (see back page II)

Speculations : 1.) F'^+ cell carries wild type F, or at least F' which can distribute F into their progeny after mating in separate way from F'. This may be interpreted by 2 alternatives : 1. F'^+ carries wild type F ; 2. F' is easily separate from F and the newly formed F will spread over the progeny.
2) Hfr may be defective F^+. It carries defective F by recombination between F and bacterial chromosome.

Ia:

Lac⁻ ⟷ Lac⁺ on DO ×4506

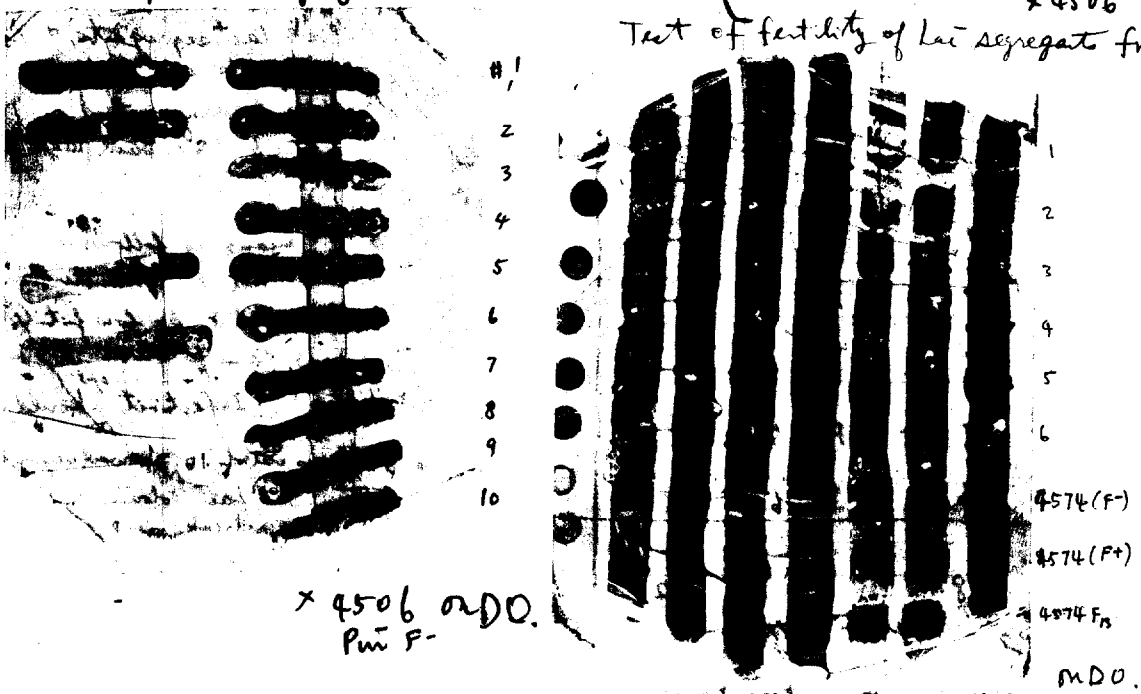
Ib. · Retest fertility of all Lac⁺ and 2 fertile Lac⁻ and sterile Lac⁻.

Make cross-streaking.

on DO ×4506 Lac⁻ / Lac⁺

II. ~~Tested by~~ Sex of Lac⁻ segregants from primary zygote

Test of fertility of Lac⁻ segregate from Primary zygote



× 4506 on DO. Pm F⁻

on DO.

Test for dual structure of F'-ness.

(Is F' defective? ~~Do~~ usual $F'^{+}_{REF.}$ contains standard F.)

10/II ; 1960

~~3 hrs~~ 3.5 hrs

Cultural age : 11:30 ~ 15:00 Inoculum size : 0.2 ml / 10 ml pen.
Shake it on rotator at 37°C. Overnight. Pen. grown culture

Principle : Does F' segregate out F after infection of F_{13} from F_{13}^{+} (3747) to F^{-} (4574)? F^{-} Lac_{85} P Ara_2 Gal_2 Xyl_2 Mtl, Mal, S^R.

F_{13} V_6^R M

tester { Lac_{85} Pur^{-}. F^{-} Cp^{+} W4642 / S^R W4647

Experimental conditions to set it up :

Use excess F'^{+} (10 times more). Blend the mix with mixer.
(Add 1.0 ml F' to 10 ml F^{-}.)

Seed it on BLac Sm. pick Lac_v. Purify each Lac_v on BLac Sm. 20' : 10, 25' : 10, 30' : 10
Restreak the Lac_v on BLac Sm again.

Pick Lac^{-} and Lac^{+} from the ~~BLac Sm~~ segregants. Test sex selecting X^{+}
10 10

on ~~Mal~~ M glucose. seeded W6- on it.

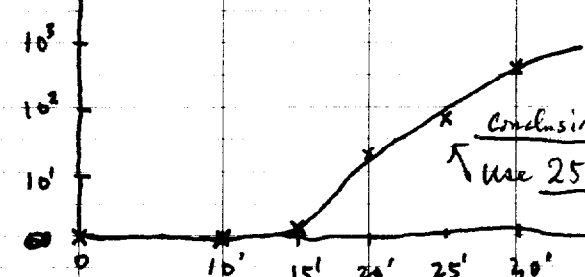
Time of Interruption : 0' 10' 15' 20' 25' 30'

Speed of blending 70 : 1 min. Dilute the mix into 5×10^{-4}

Result :

1). Timing experiment : at 34°C, standing in water bath.

Time of blending after mix	0'	10'	15'	20'	25'	30'
# of Lac^{+} S^R	0, 0, 0	0, 0, 0	0, 1, 0	11, 9, 12	38, 35, 23	155, 129, 121
$\Sigma_{Lac^{+}S^R}$	0	0	1	32	96	405



Lac^{+} transfer by F_{13}. (3747) in Pen. at 34°C.

Conclusion: Use 25' and try more diluted 1/10 sample. Use 10 plates for only 25'.

2). Repeat this experiment at 37°C. in incubator room. on rotator. in pen. 10 ml. at 37°C on rotator

- cultural age : 13:00 ~ 16:00
- Inoculum size : 0.5 ml / 10 ml of pen. (overnight grown)
- Mix 10 : 3747 with ~~[illegible]~~. 1 ml : 4574.
- Select on BLac Sm. 4:20 ~ 4:45 10^4 : 0.1, 0.5

Result :

Lac^{-} / Lac^{+}
428 : 4
467 : 4
419 : 3
429 : 4
454 : 3

Streak Lac_v on BLac Sm. and pick Lac^{+} & Lac^{-} colonies & test sex-compatibility.

continue to 1536

This data is in 1536.

Experiments to show whether W4630 Lac_2 homogenote or hemigenote?

22/II. 1960 REF: Lac_2 F^+ : 2242
S^R Lac_2 F^- : 3112.

Experimental design:

1. Pick independent Lac_2^+ reversions and purify them on BLac. (Pick 20.)
2. Treat those Lac^+ with AO, and see are there any Lac^- segregants or not.

As a control, use Lac^+ revertant from 3112. (This strain should not segregate Lac^- after AO-treatment.) also try 3112 Lac_2^-/ex Lac^+. (this segregate Lac^- by AO)

3. Check Lac^- segregant for Lac_2-ness.

Points expected for this expts.

1. If it is hemizygote for Lac_2, all the Lac^+ does not produce Lac_2^-.
2. If it is homozygote for Lac_2, three possibilities can be expected.
   a. Lac_2^- will be segregated after AO treatment from Lac_2^-/ex Lac_2^+. (exogenotic revertants)
   b. Lac_2^- will not be segregated after AO-treatment from Lac_2^+/ex Lac^-. (endogenotic revertants)
   c. Supressor mutation in outside of F13 will not segregate Lac^-.

Experiments:

1. Purify W4630 on EMB Lac Sm. and pick 20 Lac^- single colonies. Suspend those colonies in 1 ml distilled water and spread on BLac separately.
   Test all F_{13} Lac_2 by cross-brushing with 4573 (Lac_{85} F^-) on MLac.
   ~~Inoculate the colony in 8 ml pen. broth.~~ (See back page 1)
2. ~~Inoculate 0.2 ml of the culture on MLac and~~ Incubate the for 24 hrs. at 37°C. Pick Lac^+ from each Lac^- 4630 colonies.
3. purify the Lac^+ colonies on BLac. 20 colonies.
4. Treat these revertants with AO.

Results:

Medium : Nutrient broth : pH. 7.6 : AO : 20γ/ml : 37°C, overnight 4:00 PM–4:00 exponentially growing cell.

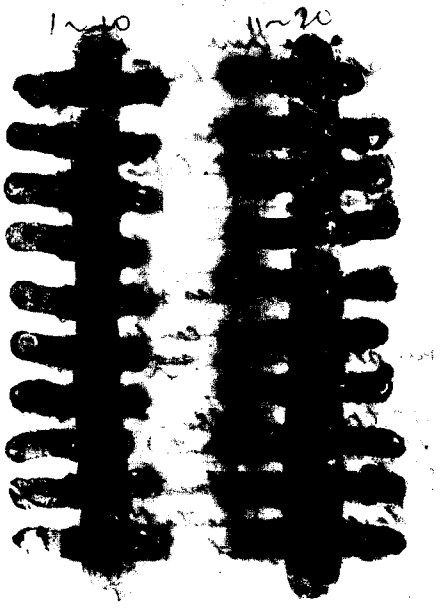
Inoculum size : one loop of 10^7 cells / 1 ml of medium. 20γ/ml of AO.
4630 Lac^+ Revertants

Isolation No. of 4630 Lac^+ Revertants.	Lac ✓ or + before AO	after AO	Isolation No. of 4630 Lac^+-revertants	before AO. Lac ✓ or +	after AO
1	No	No	16	No	No
2	No	Segregate	17	No	No
3	No	Segregate	18	No	No
4	No	No	19	No	segregate
5	No	No	20	No	No
6	No	Segregate	control.		
7	No	No	3112 Lac^+	No	No
8	No	No	3112 Lac_2/ex Lac^+	Seg	Seg
9	No	No			
10	No	No			
11	No	No	Σ Lac ✓ / total No. tested	Seg : 2	Seg : 6
12	No	No		No : 18	No : 14
13	Segregate	Segregate			
14	Segregate	Segregate			
15	No	No			

Streak the treated & untreated cells on EMB Lac. and see are there any Lac^- segregation or not.

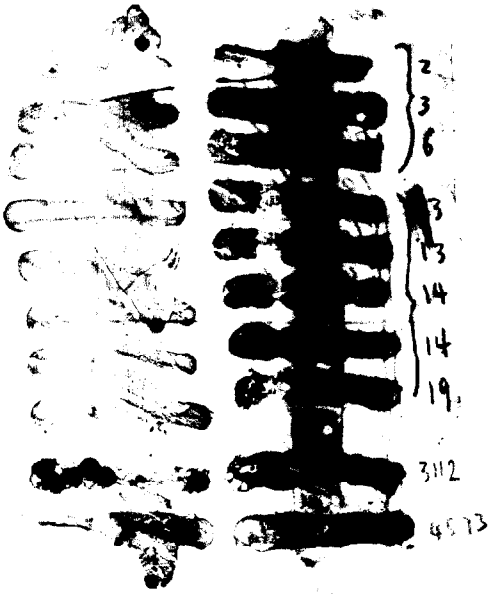
6. Restreak single colonies from 12, 13, 14, 15, 18, 19 and make sure. 5. Pick Lac^- segregants and test the Lac marker. by cross x 3747 x Lac_2 F_{13} on MLac

4630
Isolated from streaking
on Phac Sm



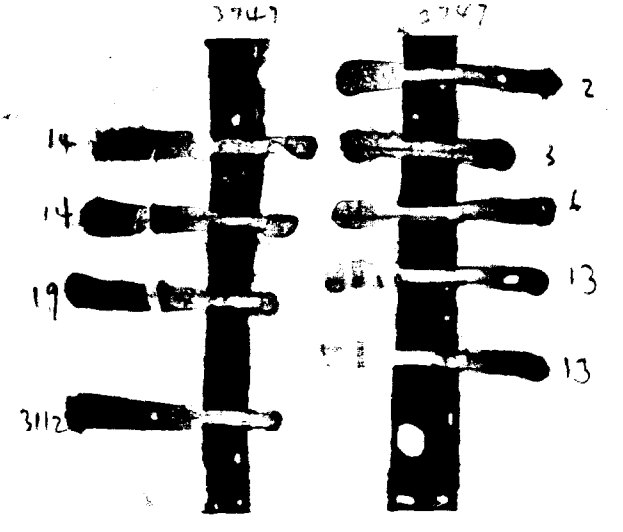
X 4573
m M Lac

segregants from Lac mutants of 4630



V4630

X3747
on M Lac



Compound structure of F' in 3747.

Testing on segregation of F' and F from primary exconjugals.

25/II ; 1960 REF:

Cultural age : exponentially growing culture in 10 ml pen. on rotator. (Inoculum size : 1 ml/10 ml pen)
4:30 ~ 6:30

Mix them : 3747 : 10 ml + 4574 1 ml.

↓

Keep them on rotator for 25'

↓

Dilute and chill them in distilled water, then blend the mix.

↓

Dilute it into ~~1/~~ 10^4 : 0.5 : 10^{-2} : 10^{-2} × 5 : 0.1/plate

↓

Inoculate the 0.1 ml ~~[illegible]~~ on EMB Lac Sm.

Result :

	# of Lac⁻	# of Lac⁺
	367	208
	319	196.

Sex

	Lac⁺		Lac⁻	
	# of Lac⁺	Sex	# of Lac⁻	sex
# 1	3	F 17	7	F⁻ 5 ; F⁺ 2.
# 2.	10	♂ 10	5	F⁺ 5
3.	8	" 8	9	F⁻ 9
4	3	" 3	9	F⁻ 9
5	5	" 5	9	F⁻ 9
6	4	" 4	9	F⁻ 9
7	2	" 2	8	F⁻ 8
8	2	" 2	10	F⁻ 10
9	6	" 6	9	F⁻ 9

Tester 4506
Media selected } MBlue. Spit.

~~[illegible]~~ colonies which segregate
: Lac⁻ F⁺ : 2
: Lac⁻ F⁻ : 7

Conclusion : ① F' segregate F to their progenies after mating. But it seems not always ~~[illegible]~~ occurs. Some F' does but the others are not.
② Fertility of Lac⁺ varies, some shows high the others show lo.
There seems no distinct difference.
Only a General principle is Lac⁺ is always male.

Test Infectivity of F and F'.

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GENETICS

Transformation of Gal with λ helper.
(Dr. Keien's system)

22/III. ; 1960 REF:

Helper : 434 hg Cs 21 10^{12}/ml

dilute 1, 2, in 2MNacl. 10, 10

Recipient :

W3104 (434 hg / T_1) infected with λ. :

grown in. Pmed { .02 M KPO_4 pH. 7.0 ; Conc. of Bact. 4×10^9/ml.
.001 $MgSO_4$
.001 $(NH_4)_2SO_4$
10^{-6}M Fe
0.5 mg/ml glucose.

OD: 1.78

DNA : Extract DNA from W4687 (c.f. 19/III/60). : Dialyzed at 5°C for 3 hr vs. 2l. vs. .15M NaCl .01 Tris pH. 7.4 ID IID.

Reaction Mix :-

0.1 ml DNase (control).
0.1 ml DNA. (200 μg . 2 μg) W4687
0.1 ml Bacteria.

Keep the mix at 30°C for 1 hr.

		on TTC Plaque	on TTC Gal	(Dilution)	on B Lac Sm	DNase 30 μg	DNA	# of Gal+/ from replicas (on BGal)
1				(1000)	0	—	I 0.1 ml	±48
2	2	341	198	(500) ml	0	—	I 1/10 0.1 ml	+2/+6 ; ±4
3	3			(10) ml	0	—	II 0.1 ml	+0, -6 ±2
4	4	73	41	(10) ml	0	—	II 1/10 0.1 ml	+3, -5
5	5	(423×4) 1692		(500) thin	0	—	I D 0.1	+0, -10
6	6	(423×2) 846	211	(500) thin	0	—	I D 1/10 0.1	+0, -17
7	7	58		(2000) thin	0	—	II D 0.1	+0, -17
8	8	44		(1000) thin	0	—	II D 1/10 0.1	+0, -18
9	9	0	660	(500) ml	0	0.1	I D 0.1	+1, -14
10	10	136		1/500 thin	0	—	λ dg DNA 1/6 10^4 0.1	+15, -0
11	11	0	73	100 ml	0	—	—	remeasure +0, -11 control.
12	12	0	0	0	0	—	I 0.1	
13	13	0	0	0	0	—	II 0.1	
14	14	0	0	0	0	—	I D 0.1	
15	15	0	0	0	0	—	II D 0.1	

F8

W3104 on 2/4.

Gal+ ↑ W3104

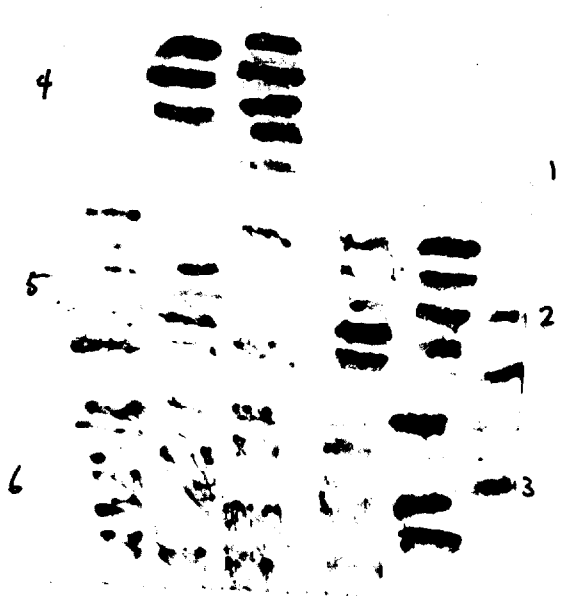
Gal+ ↑ W3104

λ dg. 0

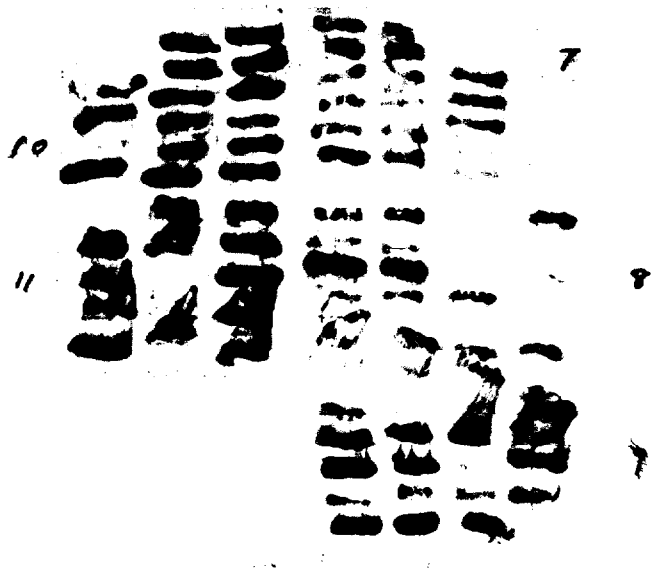
λ dg

Gal+ ↑ (T3110 Sp)

Conclusion : 1. DNA extracted from 4687 shows plaque forming activity on W3104. (434 hg/T_1)
2. Some colonies which form TTC+ shows Gal+ quality on BGal.
3. Such activity would be loose by treatment of DNase.



on B Gal.



on B Gal.

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Extraction of DNA from Gal-heterogenote W3094D. (4/ex 2)

23/III 1960 REF:

[redacted]-source : (from W3094 (derived strain) (Lpt Gal+ F⁻) 4/ex 2. (Made by HFT-Gal$_2$ transduction)

21/III

1. Shake it on shaker (2.5 l in 6 l flask.) at 37°C for overnight.

22/III

2. c.f.g. collect, and wash the cells with 0.14 M NaCl 3 times. 4000 g — 20 min.

3. Store it in refrigerator for overnight at ca. 5°C. (Ca. 20 hrs)

4. Treat with duponol (30 ml : 15 % : pH. 7.5 : at room temp :) for 5 hrs.
7:10 ⟶ 12:10

5. p.p.T with 95 % of Et-OH.

6. Wash the p.p.t with 80 % Et-OH twice.

7. Suspend the p.p.T in saline-citrate soln with aid of glass-homogenizer 60 ml.

8. C.f.g. 3,000 xg : 20 min and repeat this twice.

9. Sediment saved.

10. Extracted twice in 40 ml of 2 M NaCl on Magi Mix. at 4°C for 30'.

11. C.f.g 20,000 xg for 20 min. at 4°C.

23/III

12. Add it to 100 ml of 95 % Et-OH.

13. Fibers suspended in 5 ml of 2 M NaCl.

14. Deproteinize with sevag's method : Add equal volume of CHCl$_3$ [illegible] mix. Shake it for 5 min. Repeat [illegible] times. No interface.

24/III

15. p.p.T with Et-OH

16. Dissolve in steril 2 M NaCl. soln.

17. Digest RNA with RNase. (50 γ/ml. final. : 1 hr at 37°C.)

1 mg/ml lysozyme 10 min : 37°C

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Mating capacity of duponolated 3747.
(15% duponol).

11/III ; 1960 REF:

Method :

Use 20 ml culture of 3747 (pheasy grown for overnight at 37°C)
(2 tubes of 10 ml) Recipient 4573.

↓

C.f.g

↓

resuspend it in 1.5 ml of H_2O.

↓

Add 1.5 ml of the suspension to 5 ml of 15% duponol.

↓

keep it at 37°C.

Take 1.0 ml of the sample, add 4.0 ml of H_2O
↓ C.f.g. {12000 rpm 20' at each time 1 hr, 2 hr, 3 hr.
wash it once with 5 ml of H_2O.

↓

reasuspend it in 1 ml of H_2O.
Use this as a duponolated cell.
Use 0.2 ml.
for survival, for mating, for DNase digestion.

↓

Test mating capacity.
0.2 ml 3747 duponolated + 4573, 1 ml Exp. grown + Pen 8 ml.

Result :—

Volume of duponolated 3747 susp. used	3:00 +DNase 1 hr	4:00 +DNase 2 hr	5:00 +DNase 3 hr	No DNase added. 1 hr	No DNase added. 2 hr	No DNase added. 3 hr
# of survival colonies on EMBLac	—	—	—			
10^{-1} / 0.2 ml. of the treated suspension.	17, 20	4, 2	0, 1	14, 12	5, 2	4, 3
# of Recombinants / plate. 0.2 ml / plate. of mix on MLac Sm.	0, 0	0, 0	0, 0	0, 1	0, 0	0, 7

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Mating capacity of duponolated 3747. (10% duponol).

12/III ; 1960

REF:

	1	2	3	4	5	6	7	8	9	10
		• Cultural age :		overnight culture of 3747 in Penassay broth. (30 ml)						
		Recipient :		4573.						
		• Treatment :		10% duponol : final conc. at 37°C.						
		• Time :		1 hr. (2:30)	2 hr. (3:30)	3 hr. (4:30)				
		• c.f.g. :		aerval ; 12000 rpm. for 20 min.						
				3.5 ml H_2O + 1.5 ml. duponolated 3747						
				Wash it with ↓ 5 ml of water						
				Resuspend it into ↓ 1.5 ml of H_2O.						
		• Result :								

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Mating capacity of duponolated 3747.
(10 % duponol)

12/III ; 1960 REF:

1	2	3	4	5	6	7	8	9	10

- Cultural age : Overnight culture of 3747 in Penassy broth. (30 ml)
  Recipient : 4573. "
- Treatment : 10 % duponol : final conc. at 37°C.
- Time : 1 hr. (2:30) 2 hr. (3:30) 3 hr. (4:30)
- c.f.g. : serval ; 12000 rpm. for 20 min.
  3.5 ml H_2O + 1.5 ml duponolated 3747
  ↓
  Wash it with 5 ml of water
  ↓
  Resuspend it into 1.5 ml of H_2O.
- Result :

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Transformation in E. coli K-12.
using Helper (Hfr$_1$, F$^+$, F$^-$)

14/III ; 1960 REF:

• Strains used:

DNA from 3747 : 1 month old. (Feb. 10. in 2M saline. Kept in refrigerator.
Recipient : 4573 : F$^-$ Lac85
Helper : 4574 F$^+$: F$^+$ Lac85
4574 : F$^-$ Lac85
4049 : Hfr$_1$ (M P V_0^R Lac$_{89}$ Hfr$_1$) m.

(Those culture was taken from old broth culture). (several weeks)

• cultural condition:
Inoculum size : 0.2 ml / 8 ml penassy. ; Incubate at 37°C on rotator. 11:00 ~ 2:00
Use exponentially growing cells.

• Experimental condition:

Inoculate 0.1 ml of Recipient
+
0.1 ml of Helper
+
0.1 ml of DNA. (ca. 20γ / ml)
+
1.5 ~ 1.8 ml of Penassy broth.
(make whol volume into 2 ml.). 2:15 ~ ca. 10^9 ~ 10^{10}.
Use 0.2 ml / plate EMLac Sm.

→ Incubate it at 37°C for overnight on rotator. → Seed it on MLac Sm.

• Result : After overnight shaking concentration of cells become

Exp. #.	Mix : Recipient	Helper	DNA	# of Lac$^+$ X$^+$ on MLac Sm
1.	4573	+ 4574	+ 3747	0, 0
2.	4573	+ 4574 + 4574F$^+$	+ 3747	0, 0
3.	4573	+ 4574F$^+$	+ 3747	0, 0
4.	4573	+ 4574 F$^-$ 4049	+ 3747	0, 0
5.	4573	+ 4049	+ 3747	0, 0
6.	4573	+ 4574 4574 F$^+$ 4049	+ 3747	0, 0
7.	4573	+ 4574 F$^+$ 4049	+ 3747	0, 0
8.	4573	———	+ 3747	0, 0
order of additions of the DNA cultures.	I	II	II.	

Test sterility of DNA. on B Lac. 0.1 ml / pen tube

OK : Steril after overnight shaking on rotator which is same as 1 ~ 8.

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DNase - sensitivity of mating capacity of Sm-killed cell.

14/III ; 1960 REF:

Experiment : Strain. : 3747 overnight culture at 37°C.

↓

+ Sm 1 mg : final conc.

↓

keep incubation for overnight.

↓

C.f.g + resuspend it in 5 ml of Penassy broth.

↓

0.2 ml bacterial suspension. + 0.1 ml enzyme soln.
+ DNase 100 γ/ml
+ RNase 100 γ/ml
+ lipase.

↓ 4:00 ~ 5:00.
Incubate it at 37°C for 1 hr.

+ 4573 1 ml + Penassy 1 ml.

↓

Incubate the mix for 1 hr. at 37°C.

↓ 5:00 ~ 6:00

plate the mix onto MLacSm. 10^{-1} : 0.1 ml / plate.

Result :

	No enz.	+ DNase	+ RNase	+ lipase.
# of Recombinants/plate EMLacSm 10^{-1} : 0.1 ml				
# of survival cells/ml on EMB lac. 10^{-1} : 0.1.				

Conclusion : seems no difference.

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Transformation of E. coli K-12.
(Hfr, F^+, F^- Helper). without shaking.

15/III. 1960 REF:

$Lac_{85}^- \longrightarrow Lac^+$

- without shaking. Idea : DNA may will be taking at the time of infection of F from F^+ to F^-.
- cultural age of strains used.

0.2 ml / 10 ml pen. $\xrightarrow{10:30 \sim 1:30}$ at 37°C.

- Conc. of DNA. 20 γ/ml : final concentration.
- Experimental condition for transformation of E. coli.

Mix these bacteria and DNA.

0.1 ml 4049 Hfr, 0.1 ml 4574 F^-, 0.1 ml 4574 F^+ } helper.

0.1 ml. 4573 } : Recipient

20 γ/ml : 0.2 ml/2 ml : DNA.
(kept in refrigerator for 1 month)

Make this mix into 2 ml with pen. broth.

final. Ca. 10^7 cells/ml for each strain.

↓

- Keep incubation for 3.0 hrs overnight at 37°C without shaking. (just stand the mix.)

↓

- Score Lac^+ S^R X^+.

Inoculate 0.2 ml of these culture on MLacSm agar.

- Result :

Constitution of the mix

Vol. of Pen. (ml)	Exp. #.	Helper	DNA	Recipient	# of transformants/plate 3.0 hr	24 hr.
1.6	1	4574 F^+	3747	4573	0, 0	0, 0
1.5	2	4574 F^-	3747	4573	0, 0	0, 0
1.5	3	4049	3747	4573	0, 0	0, 0
1.4	4	4574 F^+ + 4574 F^-	3747	4573	0, 0	0, 0
1.4	5	4574 F^+ + 4049	3747	4573	0, 0	0, 0
1.4	6	4574 F^- + 4049	3747	4573	0, 0	0, 0
1.3	7	4574 F^+ + 4574 F^- + 4049.	3747	4573	0, 0	0, 0
1.6	8	——	3747	4573	0, 0	0, 0
1.4	9	4574 F^+ + 4574 F^- + 4049	—	4573	0, 0	0, 0
0.5 hr		3	1	2		

Conclusion : No transformants were observed under this condition.

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Extract DNA from W4687.

(Use this DNA as Gal-transfer.)

18/III. 1960 REF:

EML.

1. Grow up cells in penassay broth (Inoculated from broth given by Esthy)
10 ml. : overnight grown.

2. Inoculate the 10 ml into 2 l of Penassay in 6 l flask.
Shake it for overnight at 37°C. 2:00 ~

3. Collect cells by centrifugation.

· Continuous flow.
refrigerating centrifuge.

· Bactogar.

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Isolation of DNA from 4687.

$P_1 \rightarrow$ x 4520
$\rightarrow$ x 4621 F^- Ind Lp^+ Gal_{27}^{--} S^R
#74. (Gal +/-, F_8 P_1^+? 9 Lp^+?

19/III ; 1960 REF:

18/III 1. Strain W4687 grown in pen at 37°C with heavy shaking. for 6 hrs. on shaker. (2.5 l. in 6 l flask)

2. collect cells with Servall. centrifuge : 4000 rpm. for 10 min
wash the cells twice with 0.14 M saline. Ca. 3 ~ 4 grs ?

19/III 3. Let it suspend in H_2O by addition of 2 ml H_2O.

4. Keep it in refregerator : 9:30 PM ~ 7:30 AM.

5. Add the bacterial suspension to 30 ml of duponol soln (15% pH 6.8)

6. Keep it at room temp 7:30 for 5 hrs (7:30 ~ 12:30).

40°C 7. P.P.T. with 95% Et-OH.

8. Wash the p.p.T. with 80% Et-OH. twice.

9. Suspend the p.p.T in Saline-citrate soln. with the aid of glass-homogenizer (Ten Broeck)
(0.15 M) (0.05 M)

10. C.f.g. & save the pellet.

11. Extract DNA with 40 ml of 2M Nacl (twice) on Magi Mix. at 4°C for 30'

12. C.f.g at 20,000 xg for 20' at 4°C.

19/III 13. Add the supernate to 100 ml cold 95% EtOH.

14. Suspend p.p.T in 7 ml of 2M saline.

15. Keep the solution at 5°C for overnight.

20/III 16. Deproteinize with $CHCl_3$-octanol method (5) : (1) { $CHCl_3$ + octanol 1 = DNA soln 1

Make 5 x Sevag.

17. ~~Deproteinize~~ P.P.T : DNA with 80% Et-OH.

18. Dissolve it in 5 ml 2M Nacl. $\longrightarrow$ Sample I.

19. Take 2.5 ml of the soln (Sterilized) and add 50 γ/ml of RNase (Boiled) (final conc.)
Keep at 37°C for 60'.

20. P.P.T. DNA with 80% Et-OH and dissolve it in 2M salt. $\longrightarrow$ Sample II.

11/18/59 EML59.67

P-1 + W4520 MFg

——x W4621
F^- Ind^- Lp^+ Gal_2^- γ^- S^R

transductants. 73 & 74

Gal +/−
F'8
P_1^+ ?
Lp^+ ?
S^R Ind^-

reisol. 3/15/60 as W4687
#74
as Gal +/- heterogenote.

[. Segregation Pattern: details later)

♀ Gal_6 S^S 3096
S^R 3996

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DNA-analysis.

20/III : 1960 REF:

Ref: Burton, K. : Biochem. J., 62, 315~323 (1956)

Reagent:

1. Diphenylamine Reagent.

Dissolve 1.5 gr of Fisher certified diphenylamine in 100 ml of redistilled acetic acid and add 1.5 ml of conc. H_2SO_4. Store in brown bottle.

On the day it is to be used 0.10 ml of aqueous acetaldehyde (16 mg/ml) is added for each 20 ml of reagent ref'd.

Procedure:

The author states that extraction with 0.5 N $HClO_4$ at 70° for 15 min, (2x) will liberate 95% of the nucleic acid from E. coli. This is comparable to extracting at 90° for 15 min. with 5% TCA.

The final conc of $HClO_4$ must be 0.5 N before add'n of diphenylamine reagent.

1.0 ~ 2.0 (usually 1.5 ml) of sample is mixed with 2 volumes (3 ml) of diphenylamine reagent containing acetaldehyde.

Tubes containing known amounts of standard DNA. and a blank containing 0.5 N $HClO_4$ but no DNA are also prepared.

Color in developed by incubating at 25~30°C for 16~20 hr. Provided that all tubes are at the same temp, constancy of the temp. is not essential, and variations at least between 25°C and 35°C do not appreciably affect the readings

Read at 560 mμ.

Added. at 6:[illegible] P.M. ~ read at 315

Standard: 5γ, 50γ/ml of DNA.

Use 0.1 ml (5N) P.C.A to 1.5 ml of sample and 3 ml

Volume of Sample used. Exp. :- 0.1 ml + 1.4 ml H_2O + 0.15 ml PCA (5N)

0.3 ml + 1.2 ml H_2O + 0.15 ml P.C.A (5N)

↓

+ 3 ml diphenylamine reagent cont. CH_3CHO.

	conc. Standard		ml Sample I		ml. Sample II	
	50γ	5γ	0.1	0.3	0.1	0.3
600	.85	.075	0.36	1.9	0.24	2.0

Standard (deoxyadenylic acid. 50γ, 500γ/ml.)

0.1 ml + 1.4 ml H_2O + 0.15 ml P.C.A (5N)

blank

—— + 1.5 ml H_2O + 0.15 ml PCA (5N)

0.1 20 ml. EN 0.045
1.5 ml

0.015 3 ml.

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Pentose determination.

29/III ; 1960 REF:

Ref. Colowick & Kaplan "method in Enzymology" Vol III, p. 87.
(Dische modification: J. B. C., 204, 983 (1953))

Reagents:

Acid Reagent – 10% $FeCl_3 \cdot 6H_2O$
0.5 ml of $FeCl_3$ solution in to 100 ml of Conc. Hcl.

Orcinole Reagent – 6% soln of orcinol in 95% Et. OH.

Procedure:

To 1.5 ml of unknown is added 3 ml of the $FeCl_3 \cdot 6H_2O$ (acid reagent above).

Add 0.2 ml of freshly prepared Orcinol soln

Heat in Boiling H_2O bath for 20 min.

Read in Klett with #66 filter.

Comments:

Under these condition, both ribose & ribose-5-PO4 are completely developed in 20 min.

No other sugars besides pentoses can be present.

Standard soln of ribose used as control.

Standard: 5 to 50 γ/ml : final.
(1/10)
ribose. soln is used as standard

Vol. Sample (ml)
0.1 ml

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2nd Extraction of DNA From 4687.

25/III ; 1960 REF:

1. Grow up cells in penney (2.5 l) in 6 l flask on rotary-shaker for overnight at 37°C.

25/III

2. C.f.g. & wash two times with 0.14 M saline. Store in refrigerator at 5°C for overnight.

3. Add 15% (pH. 7.5 ajusted 0.5 ml of 0.01 N HCl) ~~@~~, 30 ml of Duponol soln. and keep it at room temp for 5 hrs. 7:30 ~ 12:30

4. p.p.T with 95% of Et-OH.

5. Wash the p.p.T with 80% Et-OH twice.

6. Suspend the p.p.T in 60 ml of saline-citrate soln. with aid of glass-homogenizer.

7. C.f.g. (30,000 xg : 20 min) ~~dis~~ Sediment saved. Repeat this three times.

8. Extract twice in 40 ml of 2M NaCl on Magi mix at 4°C for 30'.

9. C.f.g (20,000 xg : 20 min) at 4°C.

80% Et-OH 200 ml = 95% 118 ml + H_2O 32 ml

26/III

10 Add it to 100 ml of 95% Et-OH.

11. Fibers suspended in 10 ml of 2M NaCl.

12. Deproteinize with Sevag's procedures: $CHCl_3$ 5 : N-octyl alcohol 1
Mix 1 : 1. Repeat 5 times.
After 7 times Sevag, there is no interface.

13. p.p.T with 100 ml, 95% Et-OH.

14. Dissolve in 10 ml of 2M saline. —— 4687 I

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Extraction of DNA from 4520. ($H\ \lambda_L^2\ F_8$)

21/I ; 1960 REF:

1. Grow up cells for 6 hrs in 2.5 l pan (in 6 l flask) with shaking at 37°C.
2. Wash cells 3 times with 0.1 M saline. keep it in refrig. at 5°C for overnight
3. Treat with 15% duponol at room temp for 5 hr. 7:30 AM ~ 12:30
   cell suspension was not well homogenized : looks bad.
4. p.p.t. with 95% Et-OH.
5. Wash ppt with 80% Et-OH. twice
6. Suspend p.p.t in Saline-citrate soln. with the aids of glass-homogenizer
7. C.f.g. it (@ 30,000 xg). Saved p.p.t. Repeat 2 times.
8. Extract DNA with 40 ml of 2M saline. (2 times.) (20 min extraction at 5°C with Mag. Stir
9. p.p.t. DNA with 95% Et.OH. 100 ml.
10. Dissolve the p.p.t. in 2 M salt. Store in refrigerated at 5°C.
11. Deproteinize it 4 times by Sevag's procedure. there is very little interface insoluble remain.

    In the course of this procedure, the sample was kept at room temp. For 1 hr.
12. p.p.t with 100 ml of 95% Et.OH.
13. Dissolve it in 5 ml of 2M saline. (steril.)
    • this sample seems more easily dissolved in 2 M salt than the other samples. Also seems very thick.

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Biological activity of DNA extracted from several Strains. (Gal⁻ → Gal⁺ : plaque forming activity).

1/II ; 1960 REF:

1	2	3	4	5	6	7	8	9	10
Recipient :	Lysogenic for 434 hy	: Gal4		Helper : 434 hy.		Condition: at 30°C. for 1 hr.			
plate #.	Strain Extracted DNA	0.1 ml DNA 0.1 ml Bact.	0.1 ml DNA : 100 g/ml.	Media	# of plaques	# of gal⁺ on T.T.C & on MGal (T.T.C : soft agar)			
1	4687* New.		0.1	TTC	0	37 ?			
2			0.1	EMGal	0	0			
3				TTC	Ca. 1000	0			
4				EMGal	527	0			
5	from 3094.		0.1	TTC	4	1			
6			0.1	EMGal	1	2			
7	Lp⁺ 4/2x² (dg)			TTC	480	0			
8				EMgal	199	0			
9	4520		0.1	TTC	4	1			
10	Lp⁺ Gal⁺/Gal⁺ Fp		0.1	EMgal	10	0			
11	(Through this DNA seems no good must be repeated)			TTC	3	4			
12				MGal	0	0			
13	old 4687		0.1	TTC	3	6			
14			0.1	EMgal	5	1			
15				TTC.	395	2			
16				Mgal.	46	0			

Conclusions:

1. DNA from 4687, & 3094 have plaque-forming activity.
   a. It forms plaques.
   b. Such activity will disappeared by DNase-treatment.
   c. That activity is retained in preparation during the process of preparation of DNA

2. There is no transforming activity for Gal.

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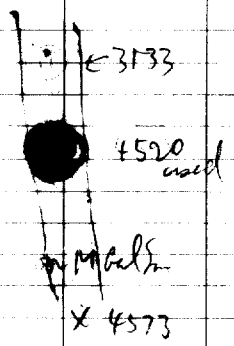
2nd Extraction of DNA from 4520.

2/14 1960 REF:

1. Let 4520 grow for overnight on rotator.
2. Collect cell with 0.14M saline. Wash 3 times with saline.
3. keep it at 5°C for overnight.
4. lyse cells with Duponol (15%) for 5 hrs. at room temp.
5. ppt. & rinse it with 95% Et. OH
6. Wash cells with Saline-citrate mix 60 ml. 3 times.
   0.14M 0.05M.
   Suspend the ppt in Saline-citrate mix. Mix with Mag-mix. for 20 min.
   c.f.g. x 50,000 g for 20'. Save sediment.
7. Extract DNA with 2M saline (40 ml : 2 times) with Mag.mix. twice
   30'. at 4°C. 2:15 ~ 2:45.
8. c.f.g. 20,000 xg for 20'. Sup. saved.
9. Add. it to 100 ml of 95% Et-OH.
10. Dissolve it in 10 ml of 2M NaCl.



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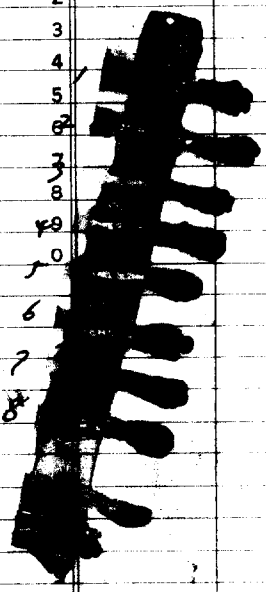
W 3104 F_8

11/14. 1960 Made by 4520 F_p × 3104 REF:

Test of compatibility.

4520 × 3104



3104

4520

m Mal+

Gal+ F_8

1. W3104 F_8 : grow up cells in 10 ml broth. 4. Add it in ~~25l~~ pm. Shake it for 5 hr. on rotator.
2. C.f.g & collect cells by small c.f.g. wash it twice with 0.14 M saline.
3. Treat with 15% dupanol at room temp for 5 hrs. 10:30 AM ~~—~~ 3:30 pm.
4. P.P.T. DNA with 95% Et-OH (100 ml) Rinse it twice
5. Wash p.p.t. with citrate - Nacl-Soln. twice, Save sediment. Homogenized by potter-type homogen.
6. Extract DNA with 40 ml of 2M Nacl twice.
7. P.P.T it with 100 ml of cold Et-OH. Dissolve it in 10 ml of 2M Nacl

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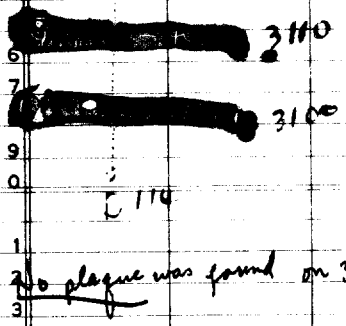
B 114 (W3104 1dg) lyse by U.V. No λ release
Immune to λ infection.

15/11 1960 REF:

1. Grow up cells in 2.5 l pen. for overnight at 37°C.
2. Collect cells keep it in refrigerator for overnight. (5°C).
3. Lyse the cells with 15% deoxycholate at room temp (11:00 ~ 4:00) for 5 hrs.
assay of plaque forming activity. 10^{-1} : 0.1 / plate on 3110.
4. Wash the cells with ~~95%~~ 70% Et-OH. (100 ml.) $CHCl_3$-treated. — c.f. g. - Store in refrig.
5. Wash the precipitate with citrate-saline soln. twice.
6. Extract DNA with 2M saline : 40 ml : twice. on Mag. mix. for 30'. at 5°C.
7. P.P.T the extract with 95% Et-OH. (100 ml), & Dissolve it in 2M saline (10 ml)
8. Deproteinize it with Octanol-$CHCl_3$ soln. (5 times) until no interface
9. P.P.T DNA with Et-OH and redissolve it in 5 ml of 2M saline.
10. Store in refrigerator at 5°C.

Test for λ production.



No plaque was found on 3110.

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Labeling DNA with P^{32}

1960. REF:

Medium low in phosphate for making P^{32} DNA in E. coli.

Ref. Lehman, Doesman et. al. : J. B. C., 233, 163 (1958)

The medium contains .34 μM of $PO_4^{\equiv}$ / ml.
Coli requires ca. .70 μM/ml for optimal growth.

Preparation of Medium.

Na-lactate 70 mMol.
to 100 ml of hot lactic acid (Mark 85%), add 40% NaOH (~100 ml) to pH. 7. Boil 3 min. Check pH.
Cool & filter if necessary. Soln is yellow viscus.
concentration ~ 6 M (~100 ml)

glycerol	4g
NaCl	5 gr.
KCl	2 gr
NH_4Cl	1
$MgCl_2$	1 mMol
$CaCl_2$	0.1 μMol
gelatin	0.01 gr
P (as ortho PO_4)	10 mg P
S (as $SO_4^{\equiv}$	10 mg S

To 1,000 ml should be pH. 7.
generation time : ~ 1 1/2 hr.
cells smaller than in most of the synthetic med.

Preparation of Stock Soln.

A.) Na-lactate ~6 M 58.0 ml
glycerol 95% 16.0 ml
NH_4Cl 5.0 g
→ 200 ml pH. 7.2

B)

NaCl	25g		
KCl	10		
$MgCl_2$	0.3 M	16 ml	
$CaCl_2$	1 M	0.5 ml	
gelatin	1%	5 ml	
KH_2PO_4	pH. 7.4	1 M	1.7 ml

to (5 ml) 200 ml. pH. 7.3.

C). Na_2SO_4 - 0.2% After autoclaving may be mixed with B (after B has been autoclaved)

Autoclave all 3 stock soln 20'

Preparation of complete medium.

92 ml sterile H_2O (+ P^{32})
4 ml A
4 ml B
4 ml C.

● continued to back page

prep. of P^{32} med. — Want 0.6 ~ 0.8 μM P/ml

Add hot P^{32} + adequate cold KPO_4 1 M.

pH. ~7. to ~~adjust~~ give difference from 0.34 μM/ml for E. coli

for P^{32} studies, the P^{32} should be added to the water, autoclaved, and then neutralized with sterile NaOH.

(Best autoclave ~~with~~ while P^{32} in dilute acid, since any polyphosphates will then be broken down.)

Inoculum — can be grown in Bunsen. Use a small inoculum, and grow with vigorous aeration.

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Labelling cells with P^{32}.

c.f. Ferro, F

13/III ; 1960 Exp. cell. Res. 12, 363~73 (1957)

P^{32} : PO_4 in weak HCl
conc. : 200 mc/ml.
Specific activity : 55350 mc/g.
of original P^{32}
date : 2-29-60.

REF.

purpose : 1. for autoradiography of bacteria.
2. Testing leakage of P^{32} from P^{32}-labelling bacteria after dilution.

Method ① grow up cells in H-medium for overnight at 37°C on rotator.

② ~~Wash the cell~~ Add the 10λ of the culture to ↑ med. made by method as follows.

a. To 0.8 ml H_2O cont. (1/40 cases ord.) add 0.01 ml (10λ) ↑ stock.
2% CH : 0.1 ml/10 ml H med. : final conc. : 0.2%.
5% CH : 0.1 ml/2 ml H : 0.25%

b Evaporate it and redissolve it in 0.8 ml H-medium.
adjust pH. 7.2. Add. 0.1 N KOH ca. 0.05 ml.

③ Grow cells for 3 hr on shaker at 37°C.
1:100 ~ 1:500 Inoculum size ca. 10^{6}
10λ : .8 ml.

④ Wash cells 5 times with water : 2 ml.

No of Planet ①

# of	Washing/ml	before wash (total) counts/min. ×1000
2	0 (Sup.)	
3	1	
4	2	4553
5	3	1493
~~6~~	~~4~~	
~~7~~	~~5~~	
~~8~~ 6	6 (suspended in 2 ml)	12217 ×5
		61085 count/min

50% 120,000
×10 1200,000

10^{-3}

⑤ ~~go~~ Add 0.25 ml of the suspension to 10 ml of protoplastic broth (c.f. back page) shake it on rotator.

⑥ Make high speed c.f.g. (5,000 ×g : 20') Save sediment. Add 1 ml of H_2O to both tube. Assay Radioactivity in supernate.
(Keep it 15 after + H_2O) c.f.g ×3,000 ×g

	7 + P	8 − P (×1000)
Count/.1 ml/min.	2284	2248

1200