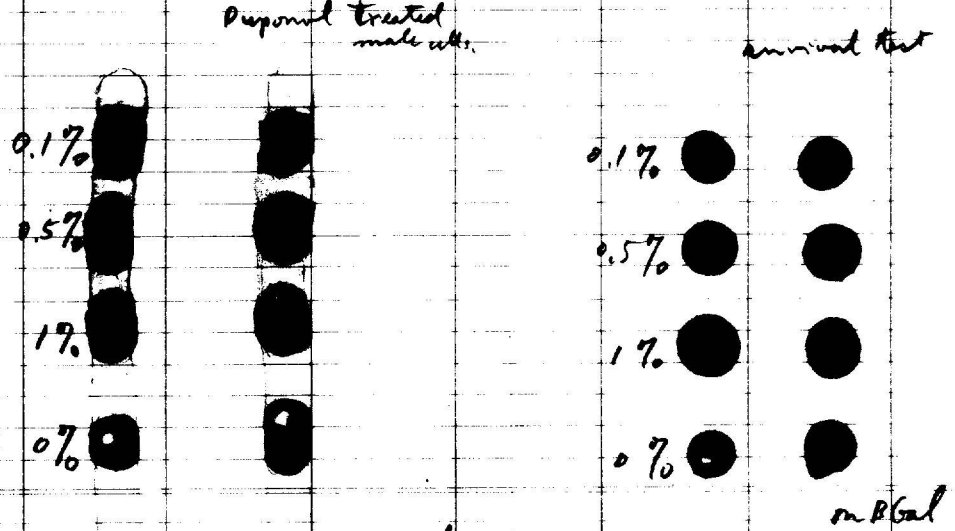
151

Trial on the killing of cells without loss of mating capacity.

17/xo 1957 REF:

5 ml overnight culture of W6 Fg. in Pen
↓
Centrifuge, discard the supernate, resuspend it's 1 ml of H_2O.
↓
distribute the 0.2 ml of W6 Fg into dupond soln. (sodium lauryl sulphate)
↓
Incubate it for 2 hrs. at 37°c. 1,000 rpm
↓
Wash it once and spot them on MGal + S73 & on BGal.

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



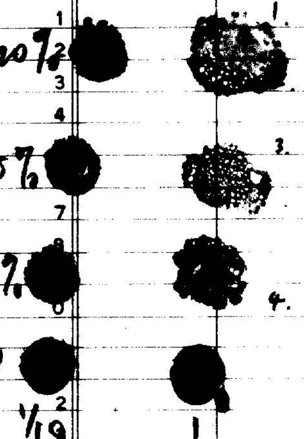
Conclusion: Use much conc. of dupond. (2 %)

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

17/IX ; 1959. REF:

1	2	3	4	5	6	7	8	9	10

20%
15%
10%
0
1/10



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

Centrifuge - Wash once - put 4 tubes togather - resuspend them into 1ml H_2O.

3. Add 0.2 ml of the Bacterial suspensions into duponol soln. 0% 10% 15% 20% and incubate them for 4 hrs. at 37°C.
3:00 PM ~ 7 PM.

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

Exp. I. Penassay grown. ← 2 days old.
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 ~~0.5~~ 1 ml
→ wash once → ~~spot~~ ~~them~~ the 0.1 ml on MGal. (7:30 PM ~ 8:00 PM.
(centrifuge for 5 min.)

Result.

	survival	Recombinant
10%	2×10^3	$>2\times10^3$
15%	"	"

Transfer of Lac.

Exp II. dilute the duponol-lysed material (10^{-1}) → spot on MLac on which W4573 was streaked.
non-diluted material (10^0) → "

untreated control.
0.

10%
15%
20%

~~Exp III. Centrifuge and resuspend into 1ml of H_2O. and spot on MLac x 4573.~~

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

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

x 4573

1/10 dil On MLac
1 undilute

Next step : Make exact experiment.

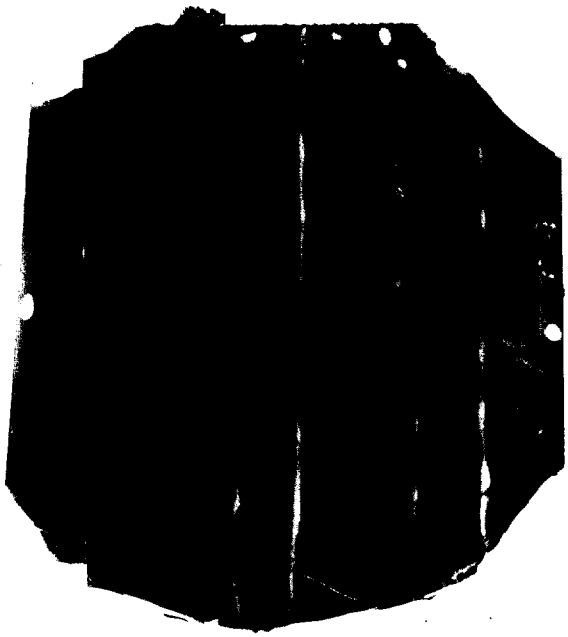
4:30

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.

F status of segregants from ♀ 3747 × 4574.

4574 : F^- P Ara_2 Mal_8 Mtl Ara_2 Xyl_2 Gal_2 SR.

primary exconjugants of (cf. P)

24/II ; 1960

REF:

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

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

(Lac$^-$ colonies (4574) are so crowded around the Lac$^+$, therefore, Lac$^-$ F^-(4574) can be contaminated with segregants)

#1 Lac$^+$.	# of Lac$^+$ tested	sex F_{13}	# 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$^+$ 876 : 1

See back page Ia, & Ib.

Exp. II Exp. does work beautifully.

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

Lac$^+$. tester : 4506 on DO.

① Mix 4hrs exponentially growing and culture of 3747 (10) & 4574 (1) Shake it on rotator for 25' at 37°C. Chill & Then blend the mix, dilute it into 10^{-3} c.c. plate on BLac Sm.

② pick well separated Lac$^+$.

③ purify them on BLac Sm.

Pick Lac$^+$ segregants & Lac$^-$ segregants. and test sex by cross-brushing method on DO. × 4506 (See back page II.

	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$^-$ segregant : 100.

• 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.

— : 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 marks) → 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 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 be 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.

Lac⁻

F⁻

F⁺

F₁₃

Lac⁻

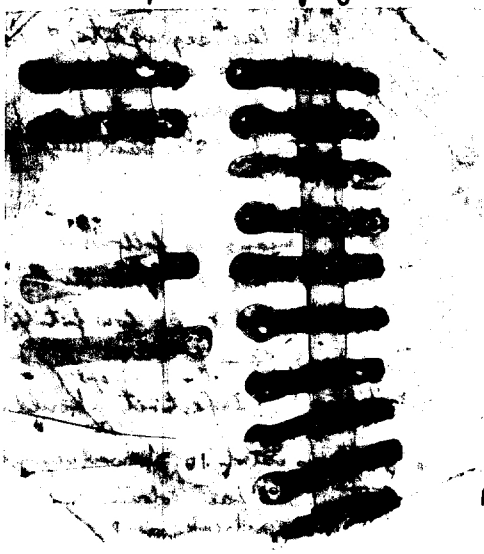
on DO ×4506

Lac⁺

~~Tested by~~

II. Sex of Lac⁻ segregants from primary zygote

Test of fertility of Lac⁻ segregants from Primary zygotes



#2

#3

control 4574

F⁺ 4574

#1 2 3 4 5 6 7 8 9 10

×4506 on DO. Pm F⁻

1 2 3 4 5 6

4574 (F⁻)

4574 (F⁺)

4574 F₁₃

on DO.

Blank 4297 4296 2985 4539 4627 4354 4295

F⁻ G₁ T A₁ H Try H L

Segregants

Segregants

Test for dual structure of F'-recs.

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

10/II ; 1960

1	2	3	4	5	6	7	8	9	10
		3.5 hrs							

Cultural age : 11:30 ~ 15:00 Inoculum size : 0.2 ml / 10 ml pen.
Shaker-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.

Experimental conditions to set it up. : tester { Lac$_{85}$ Pur^{-}. F^{-} 4^{+} W4642 / S^R W4647 } F_{13} V_6^R M

Use excess F^{0+} (10 times more). Blend the mix with mixer.
(Add 1/4 ml F' to 10 ml F^{-}) 20':10, 25':10, 30':10

Seed on Blac Sm. pick Lac$_v$. Purify each Lac$_v$ on Blac Sm.
Restreak the Lac$_v$ on Blac Sm. again.
Pick Lac^{-} and Lac^{+} from the segregants. Test sex selecting X^{+}
10 10
on M glucose seeded W6- on it.

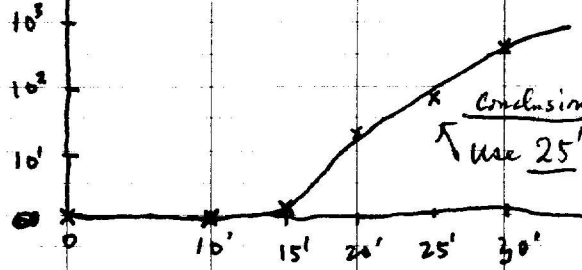
Time of Interruption : 0' 10' 15' 20' 25' 30'

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
Σ 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.

- cultural age : 13:00 ~ 16:00 at 37°C on rotator
- Inoculum size : 0.5 ml / 10 ml of pen. (overnight grown)
- Mix 10 : 3747 with [redacted]. 1 ml : 4574.
- Select on Blac Sm. 10^4 : 0.1, 0.5

4:20 ~ 4:45

Result :

Lac^{-} / Lac^{+}
428 : 4
967 : 4
419 : 3
429 : 4
454 : 3

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

continue to 153b

This data is in 153b.

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154

Experiments to show whether W4630 Lac_2 homogenote or heterogenote?

22/II 1960

REF: Lac_2 F^+ : 2242
S^R Lac_2 F^+ : 3112.

Experimental design:

1. Pick independent Lac^+_2 revertants and purify them on BLac. (Pick 20.)
2. Treat those Lac^+ with AO, and see are there any Lac^- segregate or not.

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

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

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^-_{ex}/Lac^+_2. (exogenotic revertants)
   b. Lac^-_2 will not be segregated after AO-treatment from Lac^+_2/Lac^-_{ex}. (endogenotic revertants)
   c. Suppressor mutation in outside of F13 will not segregate Lac^-.

Experiments:

1. Purify W4630 on EMB Lac Sm. and pick 20 Lac^- single colonies. Suspend these colonies in 1 ml distilled water and spread on BLac separately. Test all $F_{13}Lac_2$ by cross-streaking with 4573 (Lac_{95} F^-) on MLac. (See back page 1)
2. ~~Inoculate 0.2 ml of the culture on MLac and~~ Incubate them 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. / exponentially growing cell.

Results:

Medium : Nutrient broth : pH. 7.6 : AO : 20γ/ml : 37°C. overnight @100 rpm-Aero

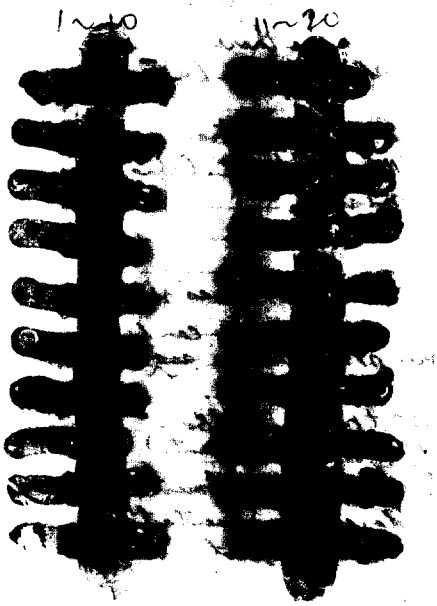
Inoculum size : one loop of 10^7 cells / 1 ml of medium. 20γ/ml of AO.
4630 Lac^+ Revertants

Isolation No. of 4630 Lac^+ Revertants.	Lac V or + before AO	after AO	Isolation No. of 4630 Lac^+-revertants	before AO. Lac V 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. 3112 Lac^+	No	No
7	No	No	3112 $Lac_2/ex\,Lac^+$	Seg	Seg
8	No	No			
9	No	No			
10	No	No			
11	No	No		Seg: 2	Seg: 6
12	No	No	Σ Lac V / total tested	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.

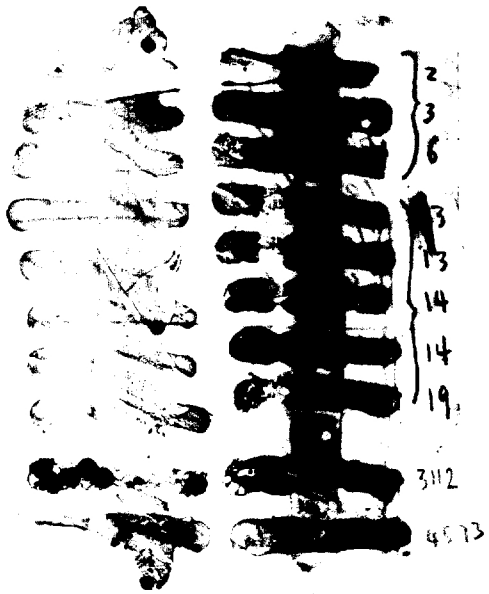
6. Restreak from 12, 13, 14, 15, 18, 19 (Single colonies and make sure.) 5. Pick Lac^- segregate and test the Lac marker. by cross × 3747 × Lac_2F_{13} on MLac

4630
Isolated from streaking
on Bhac Sm

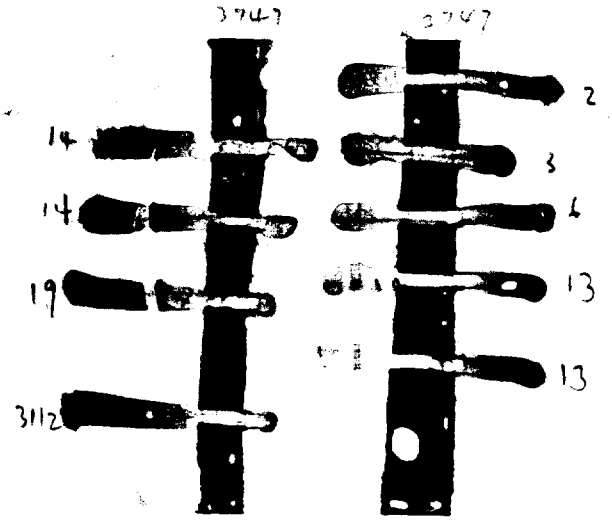


X 4573
m M Lac

segregants from Lac mutants of 4680



V4630 X3747
on M Lac



Compound structure of F' in 3747.
Testing for segregation of F' and F from primary exconjugate.

25/II ; 1960 REF:

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

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

↓

Keep them on rotator for 25'

↓

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

↓

Dilute it into 10^{-1} : 0.5 : 10^{-2} : $10^{-2} \times 5$: 0.1/plate

↓

Inoculate the 0.1ml on EMB Lac 3m.

Result :

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

Sex

	Lac⁺		Lac⁻	
	# of Lac⁺	Sex	# of Lac⁻	sex
# 1	3	F13	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 } M6low.
Spit.

[redacted] of colonies which segregate
: Lac⁻ F⁺ : 2
: Lac⁻ F⁻ : 7

Conclusion : ① F' segregate F to their progenies after mating. But it seems not always occurs. Some F' does but the others are not.
② Fertility of Lac⁺ varies, some shows high the others shows 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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Transformation of Gal with λ helper.

(DR. Kaiser's system)

22/III, 1960 REF:

Helper : 434hy C_{3}21 10^{12}/ml

dilute I, II in 2M NaCl.
ID, IID

Recipient :

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

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

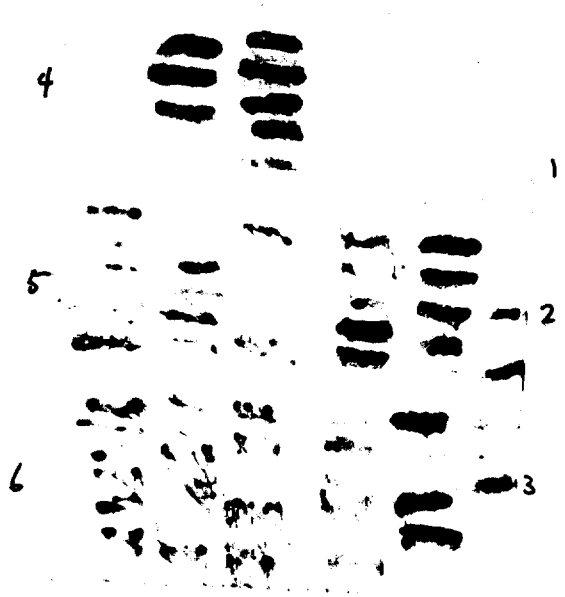
0.1 ml DNase (control).
0.1 ml DNA. (200µg, 20µg) W4687
0.1 ml Bacteria.

Keep the mix at 30°C for 1 hr.

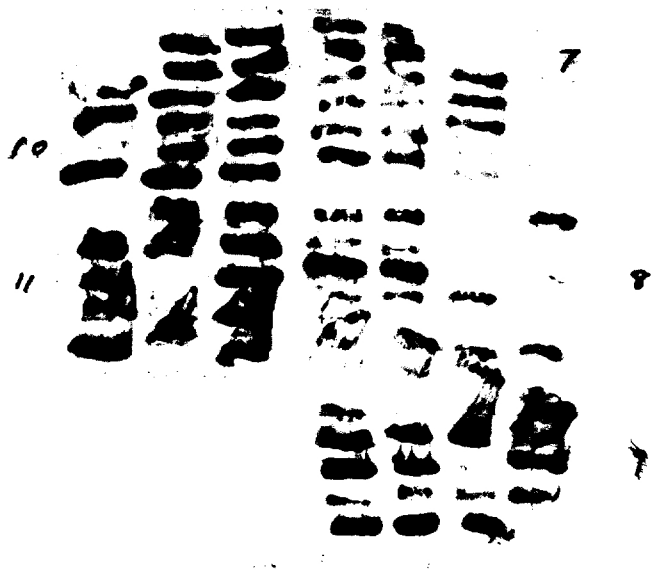
	on TTC Plaque	Gal	(Bact count)	on B Lac Sm	DNase 30µg	DNA	# of Gal+/- from replica (on BGal)
1			(1000)	0	—	I 0.1 ml	+48
2	341	198	(500) red	0	—	I 1/10 0.1 ml	+2/-6 ; ±4
3			(10) red	0	—	II 0.1 ml	+0, -6 ±2
4	73	41	(10) red	0	—	II 1/10 0.1 ml	+3, -5
5	(423×4) 1692		(500) blue	0	—	I D 0.1	+0, -10
6	(423×2)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

F8
2/4 on W3104
Gal+
λdg. ; λdg 0 — effective W3104
Gal+ lp+ / (3110 lp)

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



on B Gal.



on B Gal.

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Extraction of DNA from Gal-heterogenote W3094D. (4/ex2)

23/III 1960 REF:

1	2	3	4	5	6	7	8	9	10

21/III [redacted]-source : (from desired strain W3094 (4th Gal4 F⁻) 4/ex 2. (Made by HFT-Gal$_2$ transduction)

1. Shake it on shaker (☉ 2.5 l in 6 l flask.) at 37°C for overnight.
2. (22/III) c.f.g. collect, and wash the cells with 0.14M 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 : pH 7.5 15% : at room temp:) for 5 hrs. 7:10 ⟶ 12:10
5. p.p.t with 95% of Et-OH.
6. Wash the ppt 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 2M NaCl on Magi Mix. at 4°C for 30'.
11. C.f.g 20,000 xg for 20 min. at 4°C.
12. (23/III) Add it to 100 ml of 95% Et-OH.
13. Fibers suspended in 5 ml of 2M NaCl.
14. Deproteinize with sevag's method : Add equal volume of CHCl₃ & octanol mix. Shake it for 5 min. Repeat [illegible] times. No interface.
15. (24/III) P.P.T with Et-OH
16. Dissolve in steril 2M NaCl. soln.
17. Digest RNA with RNase. (50γ/ml. final. : 1 hr at 37°C.)

1 mg/ml . lysozyme 10 min ; 37°C

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Mating capacity of dupnonlated 3747.
(15% dupond).

11/III, 1960 REF:

Method:

Use 20 ml culture of 3747 (phenay 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 ~~0.5 ml of~~ the suspension to 5 ml of 15% dupond.

↓ keep it at 37°C.

Take ~~1.0~~ ml of the sample, add 2.0 ml of H_2O
↓ C.f.g. {12000 rpm 20' at each time 0 hr, 1 hr, 3 hr.
wash it once with 5 ml of H_2O.

↓ resuspend it in 1 ml of H_2O.
Use this as a dupnonlated cell.

↓ Use 0.2 ml.

Test mating capacity. for survival, for mating, for DNase digestion.
0.2 ml 3747 dupnonlated + 4573, 1 ml + Pen 8 ml.
Exp. grown.

Result:—

Volume of dupnonlated 3747 susp. used	3:00	4:00 +DNase		5:00	No DNase added.		
	1 hr	2 hr	3 hr		1 hr	2 hr	3 hr
# of survival colonies on EMB Lac (10⁻⁷ / 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. on Mlac Sm.	0, 0	0, 0	0, 0		0, 01 ↑	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. :		aerial ; 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/XII ; 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. :	small	; 12000 rpm. for 20 min.						
			3.5 ml H_2O + 1.5 ml duponolated 3747						
			Wash it with	↓	5 ml of water				
			Resuspend it in	↓	1.5 ml of H_2O.				
	• Result :								

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

14/III ; 1960 REF:

• Strains used:

DNA from 3747 : 1 month old. (Feb. 10.
Recipient ; 4573 : F- Lac^{-}S^{-} in 2M saline.
Helper : 4574 F+ : F+ Lac^{-}S^{-} kept in refrigerator.
4574 : F- Lac^{-}S^{-}
4049 ; Hfr, (mp ^{R} Lac^{-}S^{-} Hfr,)
m.

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

• cultural condition:
Inoculum size : 0.2 ml / 8 ml penassay. ; 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 Penassay broth.
(make whol volume into 2 ml.). 2:15 ~ ca. 10^{9} ~ 10^{9}.

→ Incubate it at 37°c for overnight on rotator. → Seed it on Hfac Sm.

• Result : After overnight shaking concentration of cells become Use 0.2 ml / plate EMLac Sm.

Mix :

Exp. #	Recipient	Helper	DNA	~~[illegible]~~	# of Lac^{+} X^{+} on Hfac Sm	~~[illegible]~~
1.	4573	+ 4574	+ 3747		0, 0	
2.	4573	+ 4574 + 4574F+	+ 3747		0, 0	
3.	4573	+ 4574F+	+3747		0, 0	
4.	4573	+ 4574F- 4049	+3747		0, 0	
5.	4573	+ 4049	+3747		0, 0	
6.	4573	+ 4574 4574F+ 4049	+3747		0, 0	
7.	4573	+ 4574F+ 4049	+3747		0, 0	
8.	4573	———	+3747		0, 0	

Test sterility of DNA. on Bfac. 0.1 ml / pan tube — OK ; Steril after overnight shaking on rotator which is same as 1 ~ 8.

order of additions of the DNA cultures. I II III.

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DNase-sensitivity of λ mating capacity of Sm-killed cell. ②

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 Penassay broth.
↓
0.2 ml bacterial suspension. + 0.1 ml enzyme soln.
+ DNase 200 γ/ml
+ RNase 100 γ/ml
+ lipase.

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

+ 4573 1 ml + Penassay 1 ml
↓
Incubate the mix for 1 hr. at 37°c.
5:00 ~ 6:00
↓
plate the mix onto MLac Sm. 10^{-1} : 0.1 ml / plate.

Result :

	● No enz.	+ DNase	+ RNase	+ lipase
# of Recombinants / plate EMLac Sm 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 be taking ⊕ at the time of infection of F^+ to F^- with
- Cultural age of strains used.
- 10:30 ~ 1:30
- 0.2ml / 10ml pen. at 37°C.
- Conc. of DNA. 20γ/ml : final concentration.
- Experimental condition for transformation of E. coli.

Mix these bacteria and ONA.

0.1 ml	4049	Hfr,	helper. (final Ca. 10^7 cells/ml for each strain)
0.1 ml	4574	F^-	
0.1 ml	4574	F^+	
0.1 ml.	4573		: Recipient
200γ/ml : 0.2 ml/2ml			: DNA. (kept in refrigeration for 1 month)

Make this mix into 2ml with pen. broth.

↓

- keep incubation for [3. ⊕ hrs / overnight] at 37°c without shaking. (just stand the mix.)

↓

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

↓

Inoculate 0.2 ml of these cultures on M Lac Sm agar.

- Result :

Vol. of Pen. (ml)	Exp. #	Constitution of the mix: Helper	DNA	Recipient	# of transformants/plate: 3.⊕ 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
10 Lac		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 Gesting)
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.

· Bactogen.

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Isolation of DNA from 4687.

P_1 → × 4520 → × 4621 F^- Ind 4^+ hcl_{27} 5^R

19/III ; 1960 REF: #74. (hcl+/- F? P_1^+? 4^+?

1. Strain W4687 grown in pm at 37°C with heavy shaking. (2
2. Collect cells with Sorvall centrifuge : 4000 rpm. for 10 min
   wash the cells twice with 0.14 M saline. Ca. 3~4 yrs ?
3. Let it suspend in H_2O by addition of 2 ml H_2O.
4. Keep it in refrigerator : 9:30 PM ~ 7:30 AM.
5. Add the bacterial suspension to 30 ml of detergent soln (1.5% pH 8.8)
6. Keep it at room temp for 5 hrs (7:30 ~ 12:30).
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 (7a. Brueck)
   (0.15 M) (0.005 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.
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.
16. Deproteinize with $CHCl_3$-octanol method (5) : (1) { $CHCl_3$ + octanol 1 = DNA soln 1
    Make 5 x Sevag.
17. p.p.t : DNA with 80% Et-OH.
18. Dissolve it in 5 ml 2M NaCl. → Sample I.
19. Take 2.5 ml of the (distilled twice) (sterilized) soln and add 50 γ/ml of RNase (Boiled) (free of DNase)
    Keep at 37°C for 60'.
20. p.p.t. DNA with 80% Et-OH and dissolve it in 2M salt. → Sample II.

11/18/59 EML 59.67

P1 + W4520 HFR

—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 is 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. read at [illegible] ~ 3/5 P.M.

Standard: 5γ, 500γ/ml of DNA.

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

Volume of Sample used.

conc. Standard		ml Sample I		ml. Sample II	
500γ	5γ	0.1	0.3	0.2	0.3
.85	.075	0.36	1.9	0.24	2.0

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.

Standard (deoxyadenylic acid. 5γ, 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 $FeCl_3$ solid 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-3-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 penassay (2.5ℓ) on rotary-shaker for overnight at 37°C.
2. C.f.g. & wash two times. with 0.14 M saline. Store in refrigerator at 5°C for overnight. 25/III
3. Add 1% (pH. 7.5 ajusted 0.5 ml of 0.01 N HCl) @, 30 ml of Onozuka sdn. 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 ppt with 80% Et-OH twice.
6. Suspend the ppt in 60 ml of saline-citrate sdn. with aid of glass-homogenizer.
7. C.f.g. (30,000 xg : 20 min) 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.
10. Add it to 100 ml of 95% Et-OH. 26/III
11. Fibers suspended in 10 ml of 2M NaCl.
12. Deproteinize with Sevag's procedure: $CHCl_3$ 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

80% 200 ml = 95% 168 ml + H_2O 32 ml

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Extraction of DNA from 4520. (H λ_L^2 F_8)

21/I ; 1960 REF:

1. Grow up cells for 6 hrs in 2.5 l per (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 was still 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.)
    o This sample seems more easily dissolved in 2 M Nacl 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/III ; 1960 REF:

	1	2 Lysogenic for	3	4	5	6	7 Condition:	8	9	10
	Recipient :	434 hy	: Gal+		Helper : 434 hy.		at 30°C. for 1 hr.			
1										
2										
3										
4	plate #.	Strain Extracted DNA	0.1 ml DNA 0.1 ml Bact.	0.1 ml DNA : 10 ml sol.	Media	# of plaques	# of gal+ on T.T.C & on MGal (T.T.C : soft agar)			
5	1	4687 "New"		0.1	TTC	0	37 ?			
6	2			0.1	EMGal	0	0			
7	3				TTC	Ca. 1000	0			
8	4				EMGal	527	0			
9	5	from 3094.		0.1	TTC	4	1			
10	6			0.1	EMGal	1	2			
1	7	$4^+ 4/4x^2$ (dg)			TTC	480	0			
2	8				EMgal	199	0			
3	9	4520		0.1	TTC	4	1			
4	10	4^+ Gal$^+$ / Gal$^+$ F_8		0.1	EMGal	10	0			
5	11	(Through this DNA seems no good must be repeated.			TTC	3	4			
6	12				MGal	0	0			
7	13	old 4687		0.1	TTC	3	6			
8	14			0.1	EMgal	5	1			
9	15				TTC.	395	2			
10	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 ~~or~~ in preparation during the process of preparation of DNA

2. There is no transforming activity for Gal.

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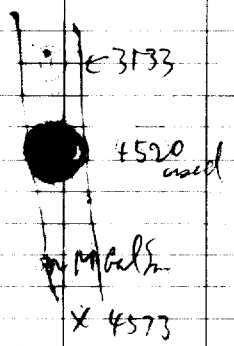
2nd Extraction of DNA from 4520.

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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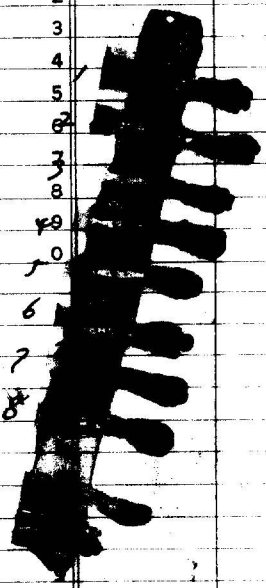
W 3104 F8

11/14. 1960 Made by 4520 F8 × 3104 REF:

Test of compatibility.

4520 × 3104



3104

4520

m MgSO4

Gal+ F8

1. W3104 F8 : 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 pestle 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 λdg) lyse by U.V. No λ release
Immune to λ infection.

15/10 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% detergent 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%~~ 75% Et-OH. ($CHCl_3$-treated. — c.f. 3 - Store in refrig.)
(100 ml.)
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.

3110

3110

B 114

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 m Mol.
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 (~120 ml)

glycerol	4g
NaCl	5 gr.
KCl	2 gr
NH4Cl	1
$MgCl_2$	1 m Mol
$CaCl_2$	0.1 m Mol
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
NH4Cl 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
KPO4 pH. 7.4 1 M 1.7 ml
} to (5 ml) 200 ml. pH. 7.3.

C). $NaSO_4$ - 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 steril 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/IV ; 1960 Exp. cell. Res. 12, 363-373 (1957) P^{32} : PO_4 in weak HCl conc. REF: ~~Specific activity~~ : 20 mc/mc.
Specific activity : 55350 mc/g.
date : 8 2-29-60.

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 10% of the culture to 3 med. made by method as follows.

a. To 0.8 ml H_2O cont. (1/40 conc ord.) add 0.01 ml (10%) stock.
2% CH : 0.1 ml / 10 ml H med. : final conc. : 0.2%.
5% CH : 0.1 ml / 20 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. 10^7
10% : .8 ml.

④ Wash cells 5 times. with ~~dil~~ water : 2 ml.

No. of Plate ①	# of Washing	before wash (total) counts/min. ×1000	
2	0 (Sup.)		50% 120,000
3	1		×10 1200,000
4	2	4553	
5	3	1493	10^{-3}
~~6~~ 8	6 (suspended in 2 ml)	12217 ×5 = 61085 count/min	

⑤ ~~Add~~ 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. 50,000 × 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 ×9,000×g

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