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GENETICS

Autoradiography.

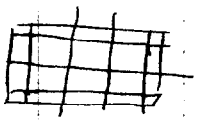
15/IV 1960

REF:

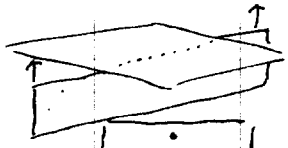
1. Treat slide-glass with gelatin.
   a. Clean slide-glass with soap, acid, rinse.
   b. Dip slide-glass in 0.5% gelatin solution for min.
   c. Let it dry for min.

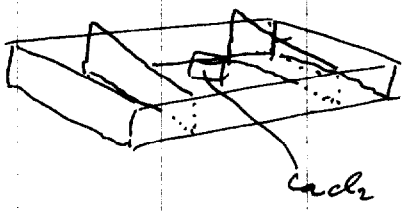
2. Put samples onto the slide-glass. Let it dry.

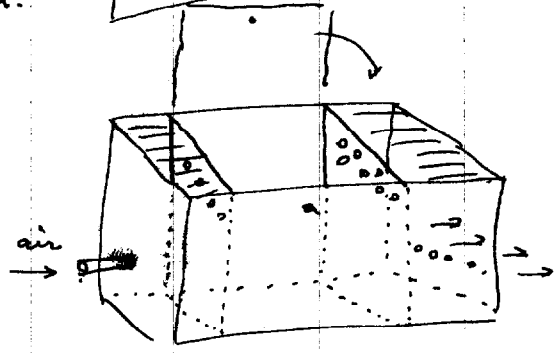
3. Cover the slide with gelatin film.
   a. cut gelatin-film into 6 pieces. →



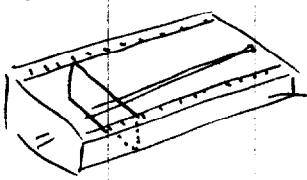
   b. ~~Take and~~ strip each pieces off from the plate.
   c. Let it place on surface of the water. (20 ~ 22°C)
      keep moisture ~~of the room~~ tight.
   d. Pick the film from water with slide. (film will attach very well on slide)
   e. Dry it with air for 30 min. in Dark box.
      Pinch with clamp & hang it from wire.
   f. Take the slide out. and keep it in refrigerator with $CaCl_2$ in small box.







4. ~~8~~. develop the slide.
   Kodak Developer D-19.



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4520 ⟶ × 3104
M F_8 Lp^+ Lp^s Gal_4^- F^-

18/11 ,1960 REF:

Purpose : ① DNA-Donor for transformation experiment.
② Test a posibility of location of Lp-locus on F8 Gal segment.

Experiment 1. Mix 4520 1ml & 3104 1ml, and incubate it overnight at 37°C.

2. Streak on M Gal. purify each isolates on B Gal.

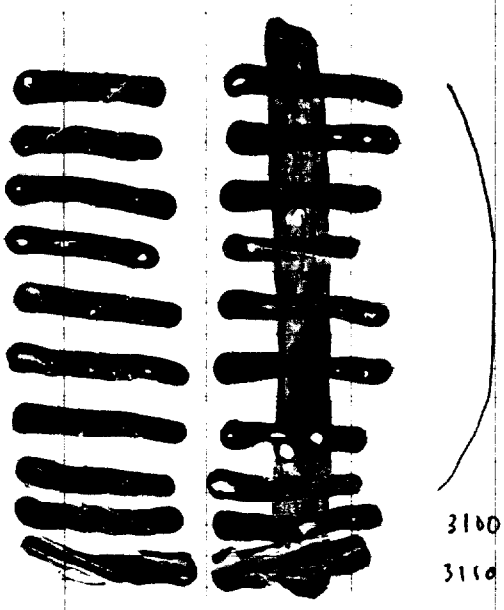
3. Test production of λ and sensitivity to λ. by cross-brushing on B-O.

Result :

all isolates (8) shows Lp^s character.

Save one.

λ ~~3100~~ 3110



3100
3110

on B-O

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So called Shigella, from Dr Yanofsky.

10/IV/1960 REF:

This strain was obtained on 3/2/60 + 4/9/60 : 5h/5.
as broth culture maintained in refrigerator.

1. purpose of this strain was for typing of Phage P_1.

2. Strange things happen when tested phage-sensitivity. The phenomenon is as follows:

This strain is sensitive to λ_2 434 hy (Same as λ except c loci).
But immune to λ.

Hypothesis given by Julian + Kaiser was that this shigella carries λ.

3. Cross-brushing exp. was done on B-O. vs. 3110 + 3100
φ^s φ^+
also UV + Un irradiated control.

Result shows that Shigella produce phage (plaque forming-center) on 3110 and lyse the 3110. But lysis was not observed on 3100. This lysis is enhanced after U.V. irradiation. (5 second/plate)

4. Sugar fermentations and nutritional marker were checked.

Result is as follows. When Shigella is streaked on Blac, all (the colonies shows Lac.

X	Lac	Mal	Mtl	Ara	Gal
−	+	+	+	+	+

This marker shows strong syntrophy on Mgglucose agar.

5. This strain can grow on SS-agar.

6. This strain is rod. Almost of the cells seems (were) Fla⁻

Conclusion: ① This strain produce phage which has same host range as λ.
② and it can ferment sugars.
③ This seems rod-unflagellated bacteria. (not quite sure yet.)

If this is shigella, it should be untypical strain from above data.

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Extraction of DNA from B120.

3110 4p+ (carries mutant marker)

12/11/ ; 1960

REF:

1. Grow up cells in 2.5 l ~~flask~~ per for several hrs. ~~in pan~~ at 37°c.
2. Collect cells and store it in refrigerator at 5°c for overnight.
3. Treat it with 15% duponol for 5 hrs at room temp.
4. p.p.t it with 95%, 100 ml of Et-OH, and suspend it in citrate-Na Soln. with the aid of glass-homogenizer. and wash it ~~two~~ twice.
5. ~~Extr~~ Extract DNA with Se 2M saline (40 ml) on Hag. mix. for 30'. twice.
6. ~~Dissolve it~~ p.p.t. with 100 ml of Et-OH, and redissolve it in 10 ml of 2M saline.

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Test of biological activity of DNA extracted from various strains.

20/IV /; 1960

REF:

1	2	3	4	5	6	7	8	9	10
	plate No.	DNA	DNase 30 µg/ml	#of Gal+ / #of Plaques	plate No.	DNA	DNase.	#of Gal+ / #of Plaques.	
	1	A	—	0 /526	27	I	0.1	2 / 2	
	2	A	—	0 /623	28	—	—	2 / 5	
	3	A	0.1	2 / 1	29	A	—	—	
	4	B	—	0 /347	30	B	—	many contaminants. ca. 1000	
	5	B	—	0 /325	31	C	—	—	
	6	B	0.1	0 / 3	32	D	—	—	
o	7	C	—	60 / 60	33	E	—	—	
o	8	C	—	108 /124	34	F	—	—	
	9	C	0.1	4 / 2	35	G	—	—	
	10	D	—	0 / 2	36	H	—	—	
	11	D	—	0 / 2	37	I	—	—	
	12	D	0.1	0 / 7					
✓	13	E	—	1 / 3					
✓	14	E	—	7 / 4					
	15	E	0.1	0 / 3					
	16	F	—	3 / 14					
	17	F	—	2 / 56					
	18	F	0.1	contaminants. / 4					
	19	G	—	3 / 377					
	20	G	—	1 / 323					
	21	G	0.1	1 / 5					
	22	H	—	0 / (993x2) 1986					
	23	H	—	0 / (1207x2) 2414					
	24	H	0.1	2 / 5					
50' 3000 o	25	I	—	contaminant / 196					
18 0008	26	I	—	98 / 168					

low
3 hrs. | 15
II II H1.

Recipient : B 7/1 infected with 434 kg.

DNA : 1. Duponol, 2M NaCl extracts of following strains.

A	W3100	: 3110 Lp+	+
B	W4520	: F_8 M- Gal+ (λ)	+
C	B 16	: Gal_4^- (λdg, λ) / λ_2	+
D	W3104 F_8	: Gal_4^- (F_8 Gal+)	o
E	B114	: Gal_4^- (λdg)	o
F	B120	: W3110 (λ ref)	o
G	W3094 ex 2	: Gal_4^- (λdg gal_2^-, λ)	+
H	W4687	: $Gal_{2,7}$, P_1 Gal_{27}^+ F_8 λ	+
I	III x dg dilute	.01/.25 .1/5 .1/5 TCM o	+

Summary : See back page.

Possibility : ① Only free phage DNA is active.

② Transduced DNA cannot make complementation : c.f. 7.8 vs. 19 20.

Strain number	genotype	plaque	gal-transduction.
3100	(Lp^+)	+	−
B120	Gal_4 (Lp ref)	+	−
B16	Gal_4 ($\lambda dg\ \lambda$)/λ_2	+	+
B114	Gal_4 (λdg)	−	− or ±
W4520	F_8 M^- Gal^+ (λ)	+	−
W3104 F_8	Gal_4 (F_8 Gal_4^+) Lp^+	−	−
W3094 ex 2	Gal_4 ($\lambda dg\ z^-\ \lambda$).	+	−
W4687	Lp^+ gal_{27} (P_{1++} F_8)	++	−

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DNA - Assay of DNA. samples. isolated from various strains. and RNA

21/IV 1960 REF:

Ball 5581 (remove filter) conc.

Sample of DNA		DNA Volume	DNA Reading	RNA Volume	RNA Reading
3100	0.1 ml / 0.3 ml	0.1	0.105		
B120	0.1 / 0.3	0.1	.036		
B16	0.1 / 0.3	0.1	.070		
B114	0.1 / 0.3	0.1	.056		
W4520	0.1 / 0.3	0.1	.034		
W3104 Fg	0.1 / 0.3	0.1	.025		
W3094 ex 2	0.1 / 0.3	0.1	.075		
W4687	0.1 / 0.3	0.1	.086		
50γ hole Standard	500γ / 50γ	0.1	.85 / .00		
	5γ				

4/28/60 Pentose determination

·1 & ·3 ml of unknowns

Standard curve. 5 → 50 γ

Stnd. 10 mg/ml. ·1 → 10 ml = 100 γ/ml (A, B, C, D) A. 10 γ B 20 γ

Tube #	Sample	H_2O	Acid reagent & orcinol	Klett reading	
1	·1	1·4		# 66	
2	·3	1·2			
3	·1 G 3094 ✓	1·4		138	
4	·3 4/cc 2 ✓	1·2		151	
5	·1 B 4520 ✓	1·4		404	
6	·3	1·2		> 900	F_8
7	·1 F B 120 ✓	1·4		79	
8	·3	1·2		124	
9	·1 H 4687 ✓	1·4		400	
10	·3	1·2		568	
11	·1 C B 16 ✓	1·4		165	
12	·3	1·2		600	
13	·1 A 3100 ✓	1·4		73	
14	·3	1·2		249	
15	·1 D 3104 F 8 ✓	1·4		520	
16	·3	1·2		> 900	F_8
17	·1 E B 114 ✓	1·4		300	
18	·3	1·2		540	
19	0	1·5			
20	0	1·5			
A	·1 ml std.	1·4		190	
B	·2 ml std.	1·3		271	

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Test hypothesis of compound structure of F.

Lac$_{91}$: ONPG (−)

24/11. 1960

4354 4520.
REF:

Purify it on BGal. before use.

Time

Procedure:

W4351 F⁻ Lac$_{91}$ Ara$_2$ V$_6^S$ Mal$_1$ Xyl$_2$ Mtl Gal$_2$

1:47 1. 4520 M F$_8$ → Gal$_2$ F⁻ S^S select on M gal.

Dilution 4$_{20}$ 10^{-2} to stop further infection of F$_8$)

1:37 2. Purify it on B Gal pick Gal⁺. Make sure their purity. Incubate them in Pen (37 tube) let it grow overnight.

ratio 1:5 1:5 ml pen. 3. Mix it with F⁻ S^R Gal$_2$⁻ 4573 and incubate it overnight at 37°C

×2 90%/plate Streak it on B Gal. Sm Count ratio of Gal⁺ & Gal⁻ on the EMB Sm agar.

~~4. Save [illegible] give free gal⁺ [illegible]~~

Gal⁺ 1 ml
F⁻ gal⁻ 1 ml
Pen 5 ml.

Ratio: F$_8$ 1 : F⁻ 100
In Pen.: Incubate the mix for 40' at 37°C on rotator.

Test Infectivity of Gal⁺ and sex-compatibility of Gal⁺ transductants.

4351 F$_8$ → 4573.

Expected character of defective F'

1. Not infective, a. (maybe sterile like as F⁻ in × F⁻ but segregate Lac⁻)
   b. (or may be fertile in cross after × F⁻ but cannot multiplies as F.)
   If it comes from revertants, it must be F⁻ and does not segregate Lac⁻.

2. It will become F' after infection of F to the hypothetical defective F$_8^+$.
   To detect this, just mix defective F' with F⁺ as exp. and F' def. with F⁻ as control, and see spreading of gal⁺ character in gal⁻ populations.

Result:

Isolation No	Infectivity	fertility	Isolation No	Infectivity	fertility
1	+	++	21	+	++
2	+	++	22	+	++
3	+	++	23	+	++
4	+	++	24	+	++
5	+	++	25	+	++
6	+	++	26	+	++
7	+	++	27	+	++
8	+	++	28	+	++
9	+	++	29	+	++
10	+	++	30	+	++
11	+	++	31	+	++
12	+	++	32	+	++
13	+	++	33	+	++
14	+	++	34	+	++
15	+	++	35	+	++
16	+	++	36	+	++
17	+	++	37	+	++
18	+	++			
19	+	++	Control 4520	+	++
20	+	++			

Infectivity & fertility.

+ ++ : gives more than 50% of gal⁺ colonies (by infection of F$_8$. ~~counted after infection of F$_8$~~)
from 4351 F$_8$ → 4573.

1 : 1 : 5 ml pen assay : 37°C overnight : Standing culture.

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Effect of RNase, omission of helper (434 hy) for DNA-transduction.

; 1960.

REF:

	1		2	3	4	5	6	7	8	9	10
			30 µg/ml + DNase	Boiled 20 µg/ml + RNase	+ helper	# of Plaque	# of Gal+		Strain No.	Genotype	
1	1	A	—	0.1	—	0	0	A	B16	gal4/ex dg+ λ/λ2	
2	2	B	—	0.1	—	0	0	B	B114	gal4/ex dg	
3	3	C	—	0.1	—	0	0	C	3100	gal+ (λ)	
4	4	D	—	0.1	—	0	0	D	3104F8	gal4/ex gal+F8	
5	5	A	[illegible] —	[illegible] —	—	0	0				
6			[illegible] —	—							
7	6	B	[illegible] —	—	—	0	0				
8	7	C	[illegible] —	—	—	0	0				
9	8	D	[illegible] —	—	—	0	0				
0	9	A	0.1	—	+	0	1				
	10	B	0.1	—	+	0	0				
1											
2	11	C	0.1	—	+	0	0				
3	12	D	0.1	—	+	1	0				
4	13	A	—	0.1	+	4	6 ←				
5	14	B	—	0.1	+	0	0				
6	15	C	—	0.1	+	28	0				
7											
8	16	D	—	0.1	+	0	0				
9	17	A	—	—	+	6	6 ←				
0	18	B	—	—	+	0	0				
[illegible]	19	C	—	—	+	42	0				
1	20	D	—	—	+	0	0				
2											
3											
4	B 7/1		c.f.g, resuspend in Pmed 0·125 + helper glucose.								
5											
6		+	OD	268	0.09	MgSO4 + cult →	4 ml + 0.04:434 hy	37°c 15', c.f.g.			
7		3	·26				~~Boiled~~				
8		127	1·32				~~[illegible]~~				
9		177	1·50								
0		203	1·66								
1											
2											
3											
4											
5											
6											
7											
8											
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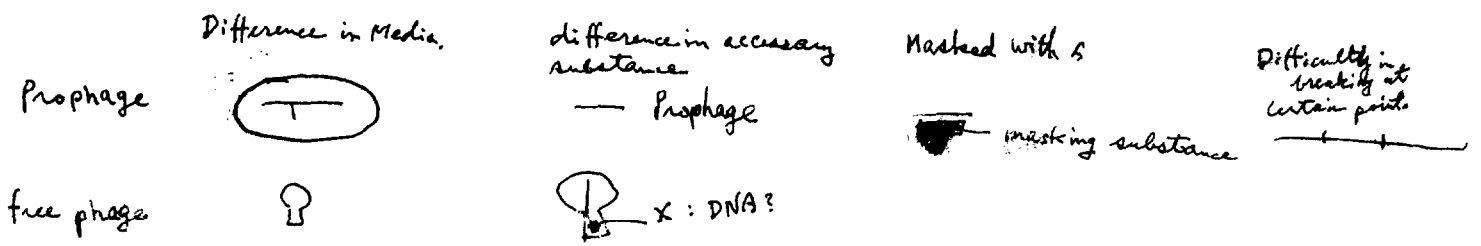
Difference in Media.
difference in accessory substance
Masked with S
Difficulty in breaking at certain points
Prophage
Prophage
masking substance
free phage
X : DNA ?

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Effect of UV-irradiation on transformability of Recipient cell.
W3350

5/14 1960 REF:

Idea. : U.V might block the immunity of recipient cell for transformation.
As in the case of recombination of prophage and exogenotic phage.
or in the case of transplantation of homograft-tissue.

Experimental design :

Recipient : 3350 1⁻ 2⁻ Lp^s F^- : from Weigle.

DNA Donor : DNA from 4520 M^- Gal^+ F_8
4687 Lp^+ Try Gal_2 Gal_7/ex+ P_1 F_8
3094D. Lp^+ ⁺/ex 2. (dg).

Method : Irradiate the recipient (3350) with various doses of U.V. (different time)
↓
Add DNA 0.1ml to 0.1ml of bacterial culture. + or − DNase
↓
Incubate it at 30°C for 1 ~ 2 hrs.
↓
Seed the mix on BGal. or on MGal.

Media : for transformation reaction.
a. penassay broth. b. Spiz + Y.E. c. D.O.

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Transformation of 3350 irradiated with UV.

1⁻2⁻

19 REF:

	Bacteria 3350 grown in Spiz.	Constant of DNA Donor	DNA Ext.	DNase	# of Gal⁺ B Gal (A)	B Gal (B)
1	0.1		0.1 from 4520'	0.1	0	0
2	0.1	Gal⁺ F8	0.1 4520	—	0	0
3 5'	0.1	2⁻7⁻/ex⁺⁺ + F8	0.1 4687	0.1	0	0
4	0.1	P, λ φ⁺	0.1 4687	—	0	0
5	0.1	4/ex 2.	0.1 3094	0.1	0	0
6	0.1		0.1 3094	—	0	0
7	0.1		—	0.1	0	0
8	0.1	+ F8	4520:0.1	—	0	0
9	0.1		4520:0.1	0.1	0	0
10	0.1	2⁻7⁻/ex⁺⁺	4687:0.1	—	0	0
11	0.1	P, λ φ⁺	4687:0.1	0.1	0	0
12	0.1	4/ex 2.	3094:0.1	—	0	0
13	0.1		3094:0.1	0.1	0	0
14	0.1		—	0.1	0	0

Cultural condition ① 3350 grown in Pen. for 5 hrs. → c.f.g resuspend in 5 ml. of Spiz + C.H. (5% 0.1 ml) & Pen.

② 5% [redacted] : 0.5 ml / 5 ml (2×10⁷) 2 tubes Spiz. glucose for 2 hr.

③ 2:45 —— 3:45. c.f.g. resusp. it in 1 ml of Spiz + C.H. [redacted]

④ UV 5', [redacted] : [redacted]. 1 ml of bacterial suspension in Spiz + C.H.

⑤ contact cell with DNA for 1 hr. at 30°C.
5:45 ~ ~~[illegible]~~ 8:00

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Isolation of DNA from 3100 (Lp^+ 3110)

7/[illegible] ; 1960 Lp^+ X^+ REF:

1. Grow up cells in 2.5 l ~~flask~~ of fln. for overnight. on shaker.
2. Collect cells & store in refrigerator at 5°C for overnight.
3. Add it into 15 % dupond soln. (pH. 7.0) and keep it at room temp. for 5 hr.
4. P.P.T. with 95 % Et-OH 10:30 ~ 3:30. & Wash it with 80 % Et-OH.
5. Wash it with citrate-NaCl soln. twice. Sediment saved.
6. Extract DNA with 2M NaCl. on Mag mix for 30'. twice.
7. P.P.T. DNA with 95% Et-OH. Dissolve it in 2M NaCl. (10 ml)
8. Keep it in refrigerator.
9. Deproteinize with $CHCl_3$-octanol mix for 5 times. (No interface).
10. ~~[illegible]~~ P.P.T with 95% Et OH. (100 ml.)
11. Dissolve it in 5 ml of 2M saline (steril).

Use this as DNA.

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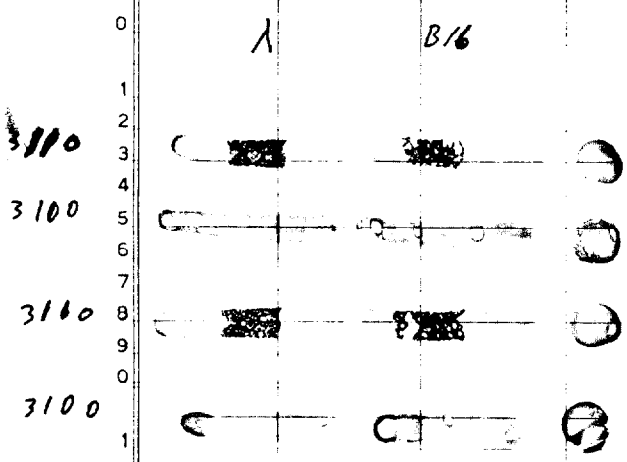
Extraction of DNA from B16

9 ~ 11/IV ; 1960 gal_4^-/ex λdg λ_2^R from Kaiser.

λ.

1. grow up cells in 2.5 l pen. & shake it overnight at 37°C.
2. Collect cells wash it with 0.15 M saline for 3 times. (cold room.)
3. Suspend cells in 10 ml saline. (0.15 M): Final vol. 16 ml.
   Add the 1 ml to 10 ml H_2O + $CHCl_3$, c.f.g. ~~final vol. 10^{-7} ... 10^{-5} ... / plate.~~ on 3110.
4. Add the 15 ml to 30 ml of 15% duponal soln. (pH. 7.0.)
   Keep it at room temp (20°C.) for 5 hrs
   1:30 ~ 6:30.
5. P.P.T. DNA with 95% cold Et-OH.: 100 ml in cold room.
6. Wash it with 80% Et-OH. twice.
7. Wash it with 100 ml of citrate-NaCl soln twice. Sediment saved.
8. Extract DNA from it with 40 ml of 2M saline twice. on Mag mix. (30')
9. P.P.T it with 95% Et OH. (100 ml).
10. Dissolve it in 10 ml 2M saline, Store in refrig. 2 days.
11. Deproteinize it with $CHCl_3$ - Octanol mix. 5 times.
12. P.P.T. it with 95% Et-OH (100 ml).
13. Dissolve it in 10 ml of atenil saline. Store in refrigerator at 5°C.



on B0.

# of Free phage-λ (on 3110) in 1 ml of original prep.

	# of plaques/plate.
10^{-1} : 0.1	194, 213.
10^{0} : 0.1	$\times 10^2$ (1 ml of original suspen.)
# of Free phage/~~plate~~ per ml. (×10)	2×10^4 / ml of original bacterial suspension.

total number of λ-particles.

15 ml × 2×10^4 ; 3×10^5 (# of λ in starting material.

plaques volume of DNA used
Ca. 100 : 0.1 ml ;
Total volume was dissolved in 5 ml of

$100 \times 10 \times 10 = 10^4$

% recovery $\frac{10^4}{3 \times 10^5} = 3\%$.

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Isolation of DNA from B114.

2/V. 1960 REF:

A : + 0.9 ml of λ ref.
B : No λ ref.

1. B114 was grown in 2.5 l ~~[illegible]~~ penassy on ~~[illegible]~~ shaker for overnight at 37°C.
2. Collect cells, ~~divide~~ wash twice, and divide it into 2 parts. (A, B).
   add λ ref to A, without + λ to B.
3. Add 14 ml of bacterial suspension to 30 ml of disposal (15%) soln.
   Keep it at room temp for 5 hrs. (1:15 ~ 6:15 PM).
4. P.P.T & wash it with 95% Et-OH. Homogenize it with potter-type glass-homogenizer.
5. ~~Extract [illegible]~~ Wash it with NaCl-citrate soln. 2 wice.
6. Extract DNA with 2M saline (40 ml.) twice. P.P.T it with 95% Et-OH.
   on Mag. mix for 30'. at 5°C.