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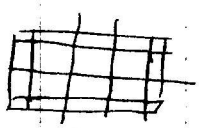
Autoradiography.

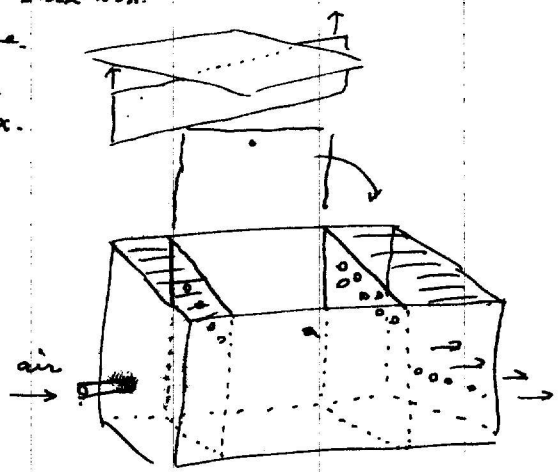
15/IV 1960

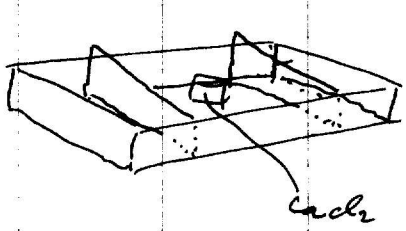
REF:

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.

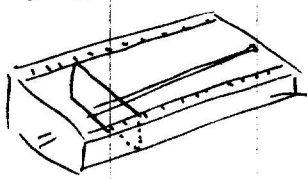
2. Put samples onto the slide-glass. Let it dry.

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~~ (right).
   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.
H F8 Lp+ Lp^s. Gal- 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.

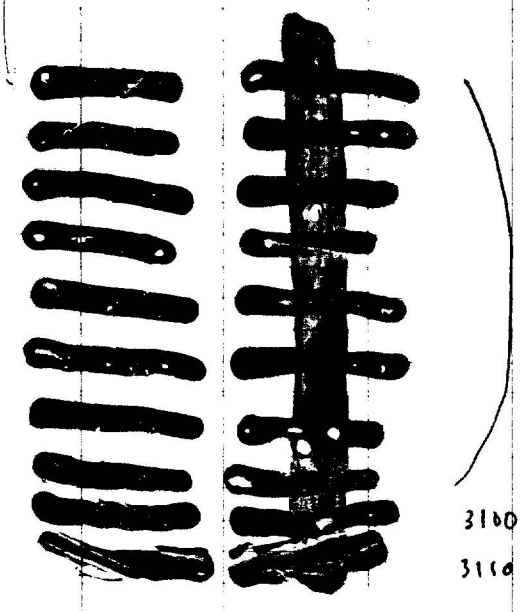
2. Streak on M Gal. purify each isolates on BGal.

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

Result :
all isolates (8) shows Lp^s character.

Save ml.

λ 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/s.
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 1_2 434 hy (Same as 1 except C loci).
But immune to 1.

Hypothesis given by Julian & Kaiser was that this shigella carries 1.

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

Result shows 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 Highlose agar.

5. This strain can grow on SS-agar.

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

Conclusion: ① This strain produce phage which has same host range as 1.
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)

12/14/ ; 1960

REF:

1. Grow up cells in 2.5 l ~~flask~~ per. for several hrs. ~~in per.~~ 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 Sol'n. with the acid of glass-homogenizer. and wash it ~~twice~~ Ex twice.
5. ~~Extract~~ Extract DNA with 2M saline (40 ml) on Mag. mix. for 30'. twice.
6. ~~Dialyze it~~ P.P.T. with 100 ml of Et-OH, and redissolve it in 10 ml of 2M saline.

Test of biological activity of DNA extracted from various strains.

20/IV / ; 1960

REF:

1	2 plate No.	3 DNA	4 DNase 30μg/ml	5 # of Gal+ / # of Plaques
	1	A	—	0 / 526
	2	A	—	0 / 623
	3	A	0.1	2 / 1
	4	B	—	0 / 347
	5	B	—	0 / 325
	6	B	0.1	0 / 3
o	7	C	—	60 / 60
o	8	C	—	108 / 124
	9	C	0.1	4 / 2
	10	D	—	0 / 2
	11	D	—	0 / 2
	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 / (993×2) 1986
	23	H	—	0 / (1207×2) 2414
	24	H	0.1	2 / 5
150' 3000 o	25	I	—	contaminant / 196
18 000 g	26	I	—	98 / 168

15 / 3 hrs. / 1.5 8 / HI. / 正 示

6 plate No.	7 DNA	8 DNase	9 # of Gal+ / # of Plaques
27	I	0.1	2 / 2
28	—	—	2 / 5
29	A	—	—
30	B	—	many contaminants. ca. 1000
31	C	—	—
32	D	—	—
33	E	—	—
34	F	—	—
35	G	—	—
36	H	—	—
37	I	—	—

Recipient : B 7/1 infected with 434hy.

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

A	W3100	: 3110 Lp+	+
B	W4520	: F_8 M− Gal+ (λ)	+
C	B16	: Gal4− (λdg, λ) / $λ_2$	+
D	W~~3104~~ F_8	: Gal4− (F_8 Gal+)	0
E	B114	: Gal4− (λdg)	0
F	B120	: W3110 (λref)	0
G	W3094 ex 2	: Gal4− (λdgal2−, λ)	+
H	W4687	: Gal$_{2,7}$, P1 Gal$_{27}^{+}$ F_8 λ	+
I	III × 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. 78 15,19 20.

Strain number	genotype	plaque	gal-transduction.
3100	(φ^+)	+	−
B120	Gal_4 (φ ref)	+	−
B16	Gal_4 ($1dg\ 1$)/1_2	+	+
B114	Gal_4 ($1dg$)	−	− or ±
W4520	$F_8\ M^-\ Gal^+$ (1)	+	−
W3104 F_8	Gal_4 ($F_8\ Gal_4^+$) φ	−	−
W3094ex2	gal_4 ($1dg\ 2^-\ 1$).	+	−
W4687	$\varphi^+ gal_{27}$ (P_1^{++} F_8)	++	−

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

21/IV 1960 REF:

	1	2	3	4	5	6	7	8	9	10
	Ball- 5581 (remove filter) core.		DNA				RNA.			
		Sample of DNA		Volume	Reading.		Volume	Reading		
1										
2										
3										
4										
5		3100	(0.1 ml 0.3 ml	0.1	0.105					
6										
7		B120	(0.1 0.3	0.1	.036					
8										
9		B16	{0.1 0.3	0.1	.070					
0										
		B114	(0.1 0.3	0.1	.056					
1										
2		W4520	(0.1 0.3	0.1	.034					
3										
4		W3104 Rg	(0.1 0.3	0.1	.025					
5										
6		W3096 ex 2	(0.1 0.3	0.1	.075					
7										
8		W4687	(0.1 0.3	0.1	.086					
9										
0			500γ		.85					
		50γ/ml Standard	50γ	0.1	.00.					
1										
2			5γ							
3										
4										
5										
6										
7										
8										
9										
0										
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										

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. 100 γ B 20 γ

Tube #	Sample	H_2O	Acid reagent	Klett readings
1	·1	1·4	4 ml	# 66
2	·3	1·2		
3	·1 (G) 3094	1·4		135
4	·3 4 Kec 2	1·2		151
5	·1 (B) 4520 ✓	1·4		404
6	·3	1·2		> 900 F8
7	·1 (F) B 120 ✓	1·4		79
8	·3	1·2		124
9	·1 (H) 4657 ✓	1·4		400
10	·3	1·2		565
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 F9
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

Test hypothesis of compound structure of F.

Lac₉₁ : ONPG (−)

24/II. 1960

REF: 4354 4520.
Purify it on BGal. ~~before use.~~

Time

Procedure : W4351 F⁻ Lac₉₁ Ara₂ V₆ˢ Mal₁ Xyl₂ Mtl Gal₂

1:07 1. 4520 M F₈ ⟶ × Gal₂ F⁻ Sˢ select on Mgal.
Dilutition H₂O 10^{-2} to stop further infection of F₈)

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

ratio 1:5 : 5 ml pen. 3. Mix it with F⁻ Sᴿ Gal₂⁻ 4573 and incubate it overnight at 37°C
×2 90⁴/plate Streak it on BGal. Sm Count ratio of Gal⁺ & Gal⁻ on the EMB Sm agar.

~~4. Save ... which ... give free gal⁺ infections.~~ (~~get gal⁺ segregant ... infection~~)

Ratio : F₈ 1 : F⁻ 100
In Pen. : Incubate the mix for 40' at 37°C on rotator.
Gal⁺ 1 ml
F⁻ gal⁻ 1 ml
Pen : 5 ml.

4. Test Infectivity of Gal⁺ and sex-compatibility of Gal⁺-transductants.
4351 F₈ ⟶ × 4573.

Expected charactor of defective F'

1. Not infective, a (maybe steril like as F⁻ in × F⁻ but segregate Lac⁻)
b. (or may be fertile in case of × F⁻ but cannot multiplise 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 F₈⁺.
To detect this, just mix defective F' with F⁺ as exp. and F def with F⁻ as control, and see spreading of gal⁺ charactor in gal⁻ population.

Result :

Isolation No	Infectivity	fertility
→ 1	+	++
2	+	++
3	+	++
4	+	++
5	+	++
6	+	++
7	+	++
8	+	++
9	+	++
10	+	++
11	+	++
12	+	++
13	+	++
14	+	++
15	+	++
16	+	++
17	+	++
18	+	++
19	+	++
20	+	++

Isolation No	Infectivity	fertility
21	+	++
22	+	++
23	+	++
24	+	++
25	+	++
26	+	++
27	+	++
28	+	++
29	+	++
30	+	++
31	+	++
32	+	++
33	+	++
34	+	++
35	+	++
36	+	++
37	+	++
Control 4520	+	++

Infectivity & fertility.
+ ++ : gives more than 50% of gal⁺ colonies (by infection of F₈.) ~~counted after infection of F₈~~
from 4351 F₈ ⟶ × 4573.

1 : 1 : 5 ml penassay : 37°C overnight : Standing culture.

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

; 1960. REF:

		+ DNase (30 μg/ml)	+ RNase (Boiled 20 μg/ml)	+ helper	# of Plaque	# of gal+
1	A	—	0.1	—	0	0
2	B	—	0.1	—	0	0
3	C	—	0.1	—	0	0
4	D	—	0.1	—	0	0
5	A	[redacted] –	[redacted] –	—	0	0
		[redacted] –	—			
6	B	[redacted] –	—	—	0	0
7	C	[redacted] –	—	—	0	0
8	D	[redacted] –	—	—	0	0
9	A	0.1	—	+	0	1
10	B	0.1	—	+	0	0
11	C	0.1	—	+	0	0
12	D	0.1	—	+	1	0
13	A	—	0.1	+	4	6 ←
14	B	—	0.1	+	0	0
15	C	—	0.1	+	28	0
16	D	—	0.1	+	0	0
17	A	—	—	+	6	6 ←
18	B	—	—	+	0	0
19	C	—	—	+	42	0
20	D	—	—	+	0	0

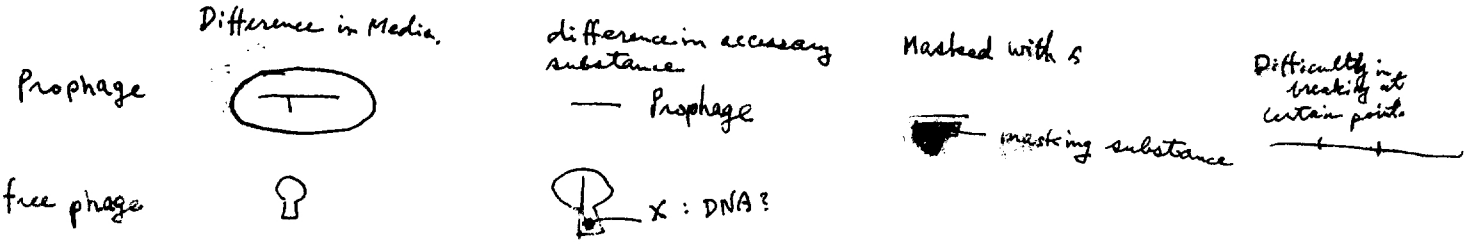
	Strain No.	Genotype
A	B16	gal4/ex dg+ λ/λ2
B	B114	gal4/ex dg
C	3100	gal+ (λ)
D	3104F8	gal4/ex gal+F8

B 7/1 c.f.g, resuspend in Pmed 0.125 + helper glucose.

+	OD
3	.26
127	1.32
177	1.50
203	1.66

268 0.09 MgSO4 + cult → 4 ml + 0.04 434hy 37°c 15' c.f.g.

~~Boiled~~ ~~[illegible]~~


Difference in Media.
difference in accessary 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 knock 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 ⟸ i^- 2^- Lp^s F^- : from Weigle.

DNA Donor : DNA from 4520 ur^- Gal^+ F_8
4687 Lp^+ Try Gal_2 Gal_1 $/_{ex+}$ 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 + γE. c. DO.

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Transformation of 3350 irradiated with UV.

1⁻ 2⁻

19 REF:

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

Cultural condition ① [redacted] 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] (ca. 2⁺ 7⁻) : 0.5 ml / 5 ml 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 [redacted] : [redacted]. 1 ml of bacterial suspension in Spiz + C.H.

⑤ Contact cell with DNA for 1 hr. at 30°C.
5:45 ~ ~~9:45~~ 8:00

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Isolation of DNA from 3100 (4p+ 3110)

7/ ; 1960 — 4p+ x+ REF:

1. Grow up cells in 2.5 l of flm. for overnight on shaker.
2. Collect cells & store in refrigerator at 5°C for overnight.
3. Add it into 15 % duponol 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). 7. 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.
10. ~~Dissolve~~ p.p.t with 95 % Et-OH. (100 ml.) (No interface).
11. Dissolve it in 5 ml of 2M saline (steril).

Use this as DNA.

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Extraction of DNA from B16

9~11/14 ; 1960

gal⁻/ex ~~bdg~~ λ_2^R from Kaiser.

1. grow up cells in 2.5 l pen.A 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.
   ~~from 2nd vol. 10^{-1} ml, 10^{-2} ml, 10^{-3} ml / plate.~~
4. Add the 15 ml to 30 ml of 15% dry-mal solu. (pH. 7.0.) on 3110.
   Keep it at room temp (20°C.) for 5 hr.s
   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 solu 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 1 ml of atmil saline. Store in refrigerator at 5°C.

λ B16

3110

3100

3160

3100

on B0.

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

	# of plaques/plate.
10^{-1} : 0.1	194, 213.
10⁰ : 0.1	×10² (1 ml of original suspen.)
# of Free phages per ml. (×10)	2 × 10⁴ /ml of original bacterial suspension.

* total number of λ-particles.

15 ml × 2 × 10⁴ ; 3 × 10⁵

(# of λ in starting material.

phages volumized. # of DNA

ca. 100 : 0.1 ml ↑ ;

Total volume was dissolved in 5 ml of

100 × 10 × 10 = 10⁴

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

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Isolation of DNA from B114.

2/17 : 1960 REF:

A : + 0.9 ml of 1 ref.
B : No 1 ref.

1. B114 was grown in 2.5 l ~~[illegible]~~ synthesis on ~~[illegible]~~ shaker for overnight at 37°c.
2. Collect cells, ~~[illegible]~~ wash twice, and divide it into 2 parts. (A, B). Add 1 ref to A, without + 1 to B.
3. Add 14 ml of bacterial suspension. to 30 ml of deoxycholate (5%) Soln. Keep it at room temp for 5 hrs. (1:15 ~ 6:15 pH).
4. P.P.T & wash it with 95% Et-OH. Homogenize with potter-type glass-homogenizer.
5. ~~Extract~~ Wash it with Nacl-citrate Soln. 2 twice.
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.