

T4 Expts

ENZO-LNB-0001

LECTIN-BINDING OF T<sub>4</sub>  
DNA

Recombinant phage screening with glucosylated probe - Detection with cou A-enzyme system.

	1	2	3	4	5	6	7	8	9	
1										
2										
3		Recombinant phage					<del>λ Fel V</del>			
4							phage λ with Fel V insert.			
5										
6										
7										
8		Glucosylated probe					Fel V insert (isolated from λ Fel V DNA)			
9							nick translated with			
10							malto-triose d UTP			
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Recombinant phage screening

check of the isolated insert for nick-translation

	1	2	3	4	5	6	7	8	9
1				Control			Glycosylated		
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14° Incubation  
2 μl aliquots removed at 15 min. intervals  
in 1.5 ml epiendorf tubes containing  
2.5 μl of 0.2M EDTA  
2.5 μl carrier DNA  
Added 1.0 ml cold 10% TCA  
10' at 4°  
Insoluble material collected on EGF filters.  
washed 3 x and counted.



recombinant phage screening

AMAS EFFICIENCY LINE® 22-205

TTP  
 1 92 001.00 0'  
 010.00 0'  
 000477.00 0'  
 1 93 001.00 5'  
 010.00 5'  
 000587.00 5'  
 1 94 001.00 15'  
 010.00 15'  
 000637.00 15'  
 1 95 001.00 30'  
 010.00 30'  
 000502.00 30'  
 1 96 001.00 45'  
 010.00 45'  
 000558.00 45'  
 1 97 001.00 60'  
 010.00 60'  
 000581.00 60'  
 1 98 001.00 90'  
 015.00 90'  
 000216.00 90'  
 1 99 001.00 120'  
 015.00 120'  
 000249.00 120'

1 00 001.00 0'  
 015.00 0'  
 000307.00 0'  
 1 01 001.00 5'  
 015.00 5'  
 000394.00 5'  
 1 02 001.00 15'  
 007.00 15'  
 001229.00 15'  
 1 03 001.00 30'  
 007.00 30'  
 000902.00 30'  
 1 04 001.00 45'  
 010.00 45'  
 000618.00 45'  
 1 05 001.00 60'  
 015.00 60'  
 000374.00 60'  
 1 06 001.00 90'  
 010.00 90'  
 000483.00 90'  
 1 07 001.00 120'  
 007.00 120'  
 001580.00 120'

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 015.00 15'  
 000319.00 15'  
 1 31 001.00 35'  
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 000206.00 35'  
 1 32 001.00 45'  
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 000341.00 45'  
 1 33 001.00 60'  
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 1 34 001.00 90'  
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 1 35 001.00 120'  
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 1 36 001.00 150'  
 010.00 150'  
 000491.00 150'  
 1 37 001.00 180'  
 002.00 180'  
 010969.00 180'  
 1 38 001.00 210'  
 005.00 210'  
 001773.00 210'  
 1 39 001.00 240'  
 003.00 240'  
 0009174.00 240'

Repeat

1 40 001.00 15'  
 015.00 15'  
 000297.00 15'  
 1 41 001.00 30'  
 015.00 30'  
 000376.00 30'  
 1 42 001.00 45'  
 015.00 45'  
 000308.00 45'  
 1 43 001.00 60'  
 010.00 60'  
 000488.00 60'  
 1 44 001.00 90'  
 010.00 90'  
 000540.00 90'  
 1 45 001.00 120'  
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 1 46 001.00 150'  
 007.00 150'  
 001139.00 150'  
 1 47 001.00 180'  
 010.00 180'  
 000508.00 180'  
 1 48 001.00 210'  
 005.00 210'  
 002907.00 210'  
 1 49 001.00 240'  
 010.00 240'  
 000544.00 240'

M TTP

recombinant phage screening.

## Preparation of phage lysates

98.11.82

Wild type phage:

*E. coli* RR1 carrying  $\lambda$  CI857 phage strain used.

1. On L. broth culture grown at  $30^\circ$  10 ml inoculated in fresh 1000 luria broth in 2 L. Nalgene flask incubated at  $30^\circ$   $\bar{e}$  vigorous shaking.

$A_{600}$  vs medium checked periodically

Time	$A_{600}$
60'	0.048
120'	0.096
180'	0.263
210'	0.35
225'	0.399

2. Flask removed and incubated  $\bar{e}$  shaking in boiling water bath till the temp of culture reached  $42^\circ$

about 7 minutes

Held at that temp for 5'

3. Incubated  $\bar{e}$  shaking at  $37^\circ$  for  $2\frac{1}{2}$  hrs.

4. Cells spun at 10 K for 30' at  $5^\circ$  and resuspended in basal M-9 salts

100.0 ml

5. Added 0.5 ml  $\text{CHCl}_3$  shaken at RT for 30' and transferred to  $0^\circ$

# recombinant phage screening

8.12.82

6. Added DNase I (1 mg/ml in 0.1 M HgCl<sub>2</sub>)  
and RNase I [10 mg/ml boiled]  
at F.C. of 10 μg/ml and 50 μg/ml respectively.  
Incubated at 0° 30'

7. Spun at 11 K for 30'  
opalescent sup transferred to sterile container.

Determine phage titer on RW 262.

# plaques in 0.1 ml of dilution.

	10 <sup>-2</sup>	10 <sup>-4</sup>	10 <sup>-6</sup>	10 <sup>-7</sup>	10 <sup>-8</sup>	10 <sup>-9</sup>	PFU/ml
--	------------------	------------------	------------------	------------------	------------------	------------------	--------

λ WT	CL	>200	3	1	-	-	
------	----	------	---	---	---	---	--

λ FelV	-	-	-	-	-	-	Repeat
--------	---	---	---	---	---	---	--------

# plaques in 0.1 ml of dilution

	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	PFU/ml
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λ WT	TMTC	TMTC	TMTC	51	5.1 x 10 <sup>7</sup>
------	------	------	------	----	-----------------------

λ FelV	TMTC	TMTC	93	8	8.65 x 10 <sup>6</sup>
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# Recombinant phage screening

9/3/89

	1	2	3	4	5	6	7	8	9
1		Preparation of the glucosylated probe							
2									
3									
4									
5						125 $\lambda$			
6									
7					Control			Glucosylated	
8									
9									
10									
11		3Hd ATP			12.5 $\lambda$			12.5 $\lambda$	
12		NT buffer 10 $\lambda$			12.5 $\lambda$			12.5 $\lambda$	
13		nTP 0.3 $\mu$ M			12.5 $\lambda$			12.5 $\lambda$	
14		TTP 0.3 $\mu$ M			12.5 $\lambda$			—	
15		glu glu glu dDTP 0.3 $\mu$ M			—			11.72 $\lambda$	
16									
17									
18									
19		Fel V insect DNA 200 $\mu$ g/ml			50 $\lambda$			50 $\lambda$	
20									
21									
22		DNAse 100 $\mu$ g/ml			3 $\lambda$			3 $\lambda$	
23									
24		DNA Pol I 5 $\mu$ /ml			5 $\lambda$			5 $\lambda$	
25									
26		d. H <sub>2</sub> O			17 $\lambda$			17.78 $\lambda$	
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↓ hrs at 14°

Rx Terminated with 12.5  $\lambda$  of 0.2 M Na EDTA

mixture passed thru 4.2 ml G-50 column  
 [ equilibrated in 10  $\mu$ M Tris 7.6 ]  
 0.1  $\mu$ M EDTA ]  
 5 drop fractions collected.



Fed X insert  
 glycosylated G-50  
 9/3/82

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FR # 10 To 34  
 combined and evaporated  
 to small volume.

Colony Hybridization with glucosylated probe  
 Detection with CouA-enzyme system.

Glucosylated probe - PBR 322 nick-translated  
 with malto-triose dUTP

Preparation of the probe 7.29.82

Rx mixtures of 125 microliters

	Control	Glucosylated
$^3\text{HdATP}$	12 $\lambda$	12 $\lambda$
10 X NT buffer	12.5 $\lambda$	12.5 $\lambda$
dNTP 0.3 mM	12.5 $\lambda$	12.5 $\lambda$
TTP 0.3 mM	12.5 $\lambda$	-
MTdUTP 0.3 mM	-	12.5 $\lambda$
PBR 322 2 $\mu\text{g}/\mu\text{l}$	15 $\lambda$	15 $\lambda$
DNAse 10 $\mu\text{g}/\mu\text{l}$	3 $\lambda$	3 $\lambda$
DNA Pol. 5000 u/ $\mu\text{l}$	6 $\lambda$	6 $\lambda$
d. H <sub>2</sub> O	53.5 $\lambda$	53.5 $\lambda$

14<sup>o</sup> 2 hrs.

Rx terminated  $\bar{c}$  12.5  $\lambda$  of 0.2 M Na EDTA

Freed of unreacted nucleotides by G-50  
 column chromatography.

8/5/82

G-50 column chromatography

3.0 ml G-50 columns in 5.0 ml pipettes  
 equilibrated with 20 column volumes of  
 10 mM Tris-Cl pH 7.6  
 0.1 mM Na EDTA

Nick Translated DNAs passed thru the column  
 column eluted with equilibrating buffer  
 5 drop fractions collected.  
 10  $\lambda$  checked for radioactivity.

Control

Glycosylated

FR #	<sup>3</sup> Hepm		FR #	<sup>3</sup> Hepm
1	62	] pooled and concentrated	1	19
2	1796		2	20
3	7649		3	21
4	7293		4	20
5	5569		5	9
6	5939		6	17
7	7286		7	34
8	7130		8	4163
9	7104		9	14158
10	4555		10	9480
1	5147	] pooled and concentrated	1	6003
2	4812		2	5872
3	3762		3	6417
4	3807		4	
5	3253		5	
6	1454		6	
7	1257		7	
8	1122		8	
9	945		9	
20	514		20	



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#1

AMPAE EFFICIENCY LINE® 22-205

	1	2	3	4	5	6	7	8	9
1						1, 2,		3, 4	
2		Bacterial strains :							
3									
4									
5		A	E. coli C-600			1, 7, 15		10, 14, 24	
6									
7		B	JM 103			3, 11, 23		8, 20,	
8									
9									
10		C	RSG - OM 13			5, 9		6, 12,	
11									
12									
13		D	PBR 322			2, 10, 14		1, 15, 23	
14									
15		E	PBR 325			4, 12, 20		3, 13, 17	
16									
17									
18		F	PBR 328			6, 16, 22		5, 11, 21	
19									
20									
21		G	PHUB <sub>2</sub>			8, 18, 24		7, 9, 19	
22									
23									
24		H	Pst gent <sup>R</sup>			13, 19		2, 16, 22	
25									
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27									
28		I	Ps. aer. 140			17, 21		4, 18	
29									
30									

Single colonies on L. agar plates.

Tuebali 37° o/u.

8/6/82

Non-sterile filters used

Odd numbers for whatmann 3 um paper  
Even numbers for nitrocellulose membrane filters.

#1

Transfer method

- ① Filter papers layered carefully on surface of agar plates with colonies. When the paper is completely wet, let stand for 5 minutes at room temp. Then remove carefully.
- ② Soak on whatmann 3MM paper [in pyrex dish] saturated with 0.5 M NaOH 1.5 M NaCl for 5 minutes. Transfer to dry whatmann paper to blot dry.
- ③ Repeat step ②
- ④ Soak on whatmann 3MM paper (saturated with 0.5 M Tris-Cl pH 7.4, 1.5 M NaCl) for 5-10 minutes. Transfer to dry whatmann paper to blot dry.
- ⑤ Repeat step ④  
check pH  $\bar{c}$  pH paper
- ⑥ Only for whatmann 3MM paper.  
Dip filters in 95% EtOH  
Air Dry.
- ⑦a Bake at 80° for 2 hrs.
- ⑦b Irradiate for 10'  $\bar{c}$  UV lamp.
- ⑧ Wash at 65°  $\bar{c}$  in sealed bags in  
2 x SSC  
0.2% BSA  
0.2% PVP  
0.2% ~~Tris~~ x 100 Ficoll  
0.01% SDS

#1

8/9/82

⑨ Remove filters and blot dryish.  
 Soak in 2.5 ul Pre-hybridization Mix  
 50% Formamide  
 5x SSC  
 100 µg/ml Salmon Sperm DNA  
 1x Denhardt  
 incubate in sealed bags at 42° for one hour.

⑩ Denature probe glucosylated nick translated  
 pBR 322 at 80° for 3'  
 quickly cool in ice-bath.  
 Add microliters ~ 5 micrograms per filter  
 to each bag containing pre-hybridization mix  
 Seal and incubate at 42° for over the  
 week-end.

8/9/82

⑪ The filters are washed ~~three~~ times (60 min each)  
 in 20 ul [per filter] } once at Room temp  
 0.2% BSA }  
 0.2% PVP } thrice at 55-60°  
 0.1% Triton X-100 }  
 0.1% SDS }  
 2x SSC }  
 at ~~65~~° } Blotted dryish on  
 } Whatman 3 MM  
 } filters.



#1

Con A treatment

(12)

Filters transferred to a clean plate and solution of Con A 0.5 mg/ml in PBS-Mg<sup>++</sup> buffer added. ~~just to soak the plate~~ enough to saturate the filters.

Incubated at RT for 60 minutes

(13)

Filters washed 3x in PBS-Mg<sup>++</sup> buffer 20 ul per filter 10' each time at RT.

Blott  
Coating.

(14)

Filters washed 3x in  
0.2 M Tris-HCl pH 6.5  
0.15 M NaCl  
0.1 % BSA  
20 8 ul 10 minutes each.

(15)

Filters soaked in  
1 % BSA in 0.2 M Tris-HCl (6.5)  
0.15 M NaCl  
2 hrs at 37° or overnight at 4°

#1

1  
2 (16) Filters washed 3x in  
3  
4 PBS buffer containing  
5 0.1 % BSA  
6  
7 10' at RT. each time  
8  
9  
10 Blotted dry  
11

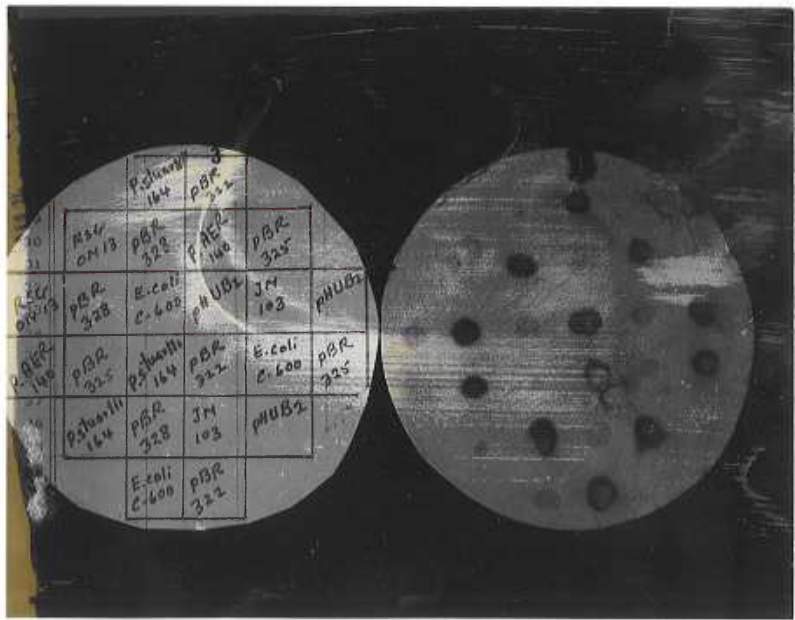
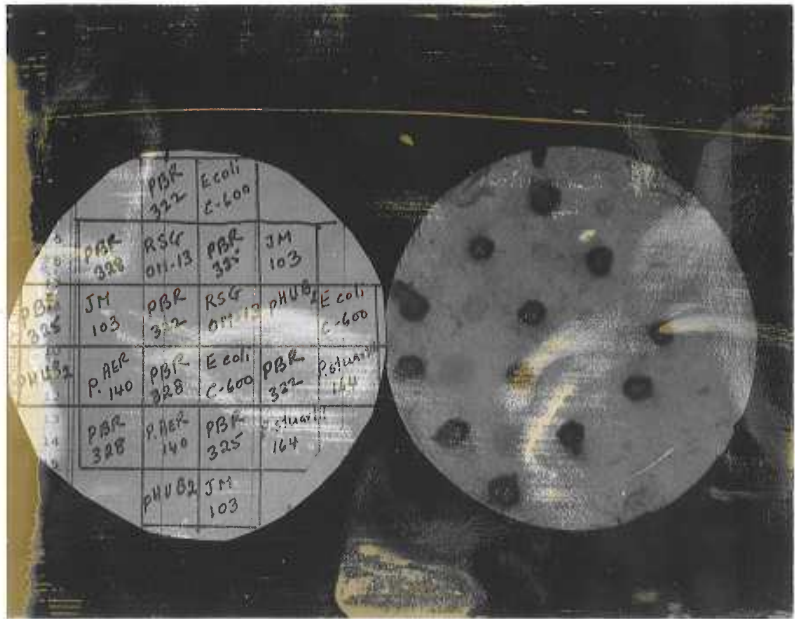
12  
13 (17) Treatment with HRP  
14

15  
16 To each colony applied HRP 1 microliter [1 mg/ml]  
17  
18 incubated in humid chamber 30' at RT.  
19

20  
21 (18) washed 3x in  
22  
23 PBS containing 0.1 % BSA  
24

25  
26 (19) Filters soaked in 6 ul of substrate solution  
27 prepared fresh  
28  
29 1 mg/ml DAB mixed with equal volume  
30  
31 of  $H_2O_2$  in  $H_2O$  [ 100  $\mu$  per 15 ul ] 0.2%  
32  
33 immediately added and plates stored in dark  
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#2

8/12/82

Bacterial Strains

E. coli C-600

RSG OM 13

PGP E1

PBR 322

P. stuartii 164

E. coli C

Single colonies resuspended in 0.5 ml

0.1 x SSC

① Streaked out on Whatmann 3MM Filter paper  
Make 6 Filters.

Allowed to DRY

② Filters soaked on Whatmann 3MM Filter paper saturated with 0.5 M NaOH  
1.5 M NaCl

For 5 minutes

Allowed to dry on another Whatmann paper

③ Repeat step ②

8/12/82

#2

- ④ Soak on Whatman 3 MM paper saturated with 0.5 M TRIS 7.4  
1.5 M NaCl  
5 minutes at RT.  
Allowed to DRY on fresh Whatman paper
- ⑤ Repeat step 4  
check with pH paper should be 7.4
- ~~⑥~~ ~~A~~ Soak the filter paper in  
Spot Proteinase K solution  
0.25 mg/ml in 1x SSC  
on bacterial streaks.  
Incubate at RT. for 15'
- ~~⑦~~ ~~B~~ Soak Filter in GTC cacodylate buffer  
15' at RT  
5 M GTC  
20 mM cacodylate pH 7.1  
0.1 % SDS
- ~~⑧~~ Soak
- ⑨ Soak Filters in 0.1 % SDS
- ⑩ Soak Filter in ~~GTC~~ ~~GG~~ in ~~EtOH~~ 0.1 % SDS
- ⑪ Same as A
- ⑫ Same as B

#2

8/12/82

EFFICIENCY LINE® 22-205

⑦ Remove solutions  
 and dip all the filters in  
 95% EtOH 2x 5' each  
 Air DRY

⑧<sub>a</sub> Bake at 75-80° for 2½ hrs

⑧<sub>b</sub> Irradiate 10' to UV lamp

⑨ Wash filters at 65° overnight in  
 sealed bags in

- 2x SSC
- 0.2% PVP
- 0.2% Ficoll
- 0.2% BSA
- 0.1% SDS

8/13/82

⑩ Soak filters in Prehybridization Mix 42° for  
 90-120 minutes

- 5x SSC
- 100 µg/ml S.S. DNA
- 0.02% BSA
- 0.02% PVP
- 0.02% Ficoll

⑪ Incubate filters in Hybridization Mix 42° o/n  
 Prehybridization Mix  
 +  
 pBR 322 glucosylated probe single stranded  
 at 80° 3'

12

Wash filters in

10x DK mix  
2x SSE

DK  
mix

0.02%	BSA
0.02%	PVP
0.01%	Triton X-100

65° for 4 hrs.  
buffer changed every hour.

13

Con A Treatment

Wash filters 2x 10' each  
with PBS-Mg<sup>++</sup> buffer

Soak the filters in conA solution  
0.5 µg/ml in PBS-Mg<sup>++</sup> buffer  
60' at Room Temp.

14

Wash with PBS-Mg<sup>++</sup> buffer containing  
0.1% BSA  
3x 10' each

15

Coat the filters with 1% BSA in  
PBS buffer  
37° 2 hrs.

16



Wash Repeat stop 14

17

Treatment with HRP enzyme.



AVANGARD EFFICIENCY LINE® 22-205

	1	2	3	4		8	9
1							
2		C-600					
3		RSG-OM13					
4							
5		PGPE1					
6							
7		pBR 322					
8							
9		PST 164					
10							
11		E. coli C					
12							
13							
14							
15							
16							
17							
18							
19							
20							
21		C-600					
22							
23		E. coli C-600					OM-13
24							pBR-322
25		OM-13					PGP-E1
26							
27							
28		OM-13					P. stuartii
29							
30							
31		PGPE1				P. stuartii	
32							
33							
34		E. coli C				pBR 322	
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E. coli C-600  
PBR-322  
RSG-OM-13  
E. coli C  
PGP-E  
PGP-E1  
PBR-322  
P. stuartii  
PGP-E1  
E. coli C  
PBR-322  
PGP-E1  
E. coli C-600  
PGP-E1  
PBR-322  
Mixture E1+322  
Mixture Coli C + C-600  
PGP-E1  
PBR-322



C-600  
PBR-322  
E. coli C  
PST  
E. coli C  
PGP-E1  
P. stuartii  
E. coli C-600  
PBR-322  
Coli C  
PGP-E1  
OM-13  
PBR-322  
P. stuartii  
E. coli C



E. coli C  
C-600  
PGP-E1  
PST  
PBR-322  
C-600  
E. coli C  
OM-13  
PST  
PBR-322  
Coli C  
C-600  
PBR 322  
PST  
C-600

# Colony hybridization - non-radioactive detection.

# 3

8/21/82.

Single colonies on L. agar plates prepared by Roberta. 8 plates. 1 plate used as master  
Strains used. ~~1 plate on~~

E. coli C-600 parent strain

E. coli RSG-014-13 xylose Isomerase negative

E. coli pGP E1 plasmid carrying Xyl. Isomerase.

E. coli pBR 322 vector plasmid.

## Preparation of filters.

Whatmann 3 min filter 9.2 cm diameter circles  
 Thickness

$$\text{Area } \pi r^2 = 70.11 \text{ cm}^2$$

① Filters pressed on colonies left for 5'

Filters lifted off carefully

① Placed on Whatmann filter paper soaked in

0.5 M NaOH  
 1.5 M NaCl

Left for 5'

DRIED ON Whatmann filter

② Step ① repeated

③ Placed on Whatmann filter paper soaked in

0.5 M TRIS pH 7.4  
 1.5 M NaCl

Left for 5 minutes



#

AMRAD EFFICIENCY LINE® 22-205

	1	2	3	4	5	6	7	8	9
1	④ Repeat step 3								
2	⑤ Soak filters in proteinase K solution 0.25 mg/ml in 1x sse								
3	15' at RT								
4	AIR-DRY Blot DRY								
5	⑥ Dip filters in 95% EtOH 2 times 5' each								
6	⑦ Irradiate 10' at 10 cm distance UV lamp.								
7	⑧ Bake at 80° for 2.5 hours in vacuo.								
8	⑨ Wash ofn at 65° in								
9	0.2% ficoll								
10	0.2% PVP								
11	0.2% BSA								
12	0.1% SDS								
13	2x sse								
14	⑩ Blot filters dryish								
15	⑪ Immerse filters in prehybridization mixture 2.5 ml per								
16	filter								
17	50% formamide								
18	5x sse								
19	100 µg/ml ss DNA								
20	1x Denhardt								
21	42° for one hour.								
22	⑫ Incubate filters 42° ofn in 1.29 µm.								
23	Prehybridization mix containing								
24	<del>glucosylated</del> DNA probes pBR 322								
25	3 Filters used. — 2 filters ± pBR 322								
26	DNA glucosylated								
27	① With no pBR 322 DNA								
28	non-glucosylated								

# colony hybridization  
glucosylated probe  
pBR-322

EFFICIENCY LINE® 22-205

- |    | 1    | 2  | 3 | 4 | 5 | 6 | 7 | 8 | 9 |  |
|----|------|--|---|---|---|---|---|---|---|--|
| 1  | (13) | Hybridization solution removed.                    |   |   |   |   |   |   |   |  |
| 2  |      | Filters washed in wash buffer <del>con</del>       |   |   |   |   |   |   |   |  |
| 3  |      | 30' twice at RT.                                   |   |   |   |   |   |   |   |  |
| 4  |      | 60' twice at 65°                                   |   |   |   |   |   |   |   |  |
| 5  |      | 30' once at RT. in 0.1% Triton X-100               |   |   |   |   |   |   |   |  |
| 6  |      | 0.1% BSA   |   |   |   |   |   |   |   |  |
| 7  |      | 2 x SSC.   |   |   |   |   |   |   |   |  |
| 8  |      | Blotted dry.                                       |   |   |   |   |   |   |   |  |
| 9  | (14) | Filters soaked in                                  |   |   |   |   |   |   |   |  |
| 10 |      | PBS-Mg <sup>++</sup> buffer containing con A       |   |   |   |   |   |   |   |  |
| 11 |      | 0.5 ug/ml.   |   |   |   |   |   |   |   |  |
| 12 |      | #1 and #6 filters only.                            |   |   |   |   |   |   |   |  |
| 13 |      | #5 soaked in PBS-Mg <sup>++</sup> buffer. - con A. |   |   |   |   |   |   |   |  |
| 14 |      | 60' at RT.   |   |   |   |   |   |   |   |  |
| 15 | (15) | Filters washed in PBS-Mg <sup>++</sup> buffer      |   |   |   |   |   |   |   |  |
| 16 |      | 3 x 20' each                                       |   |   |   |   |   |   |   |  |
| 17 |      | containing 0.1% BSA                                |   |   |   |   |   |   |   |  |
| 18 |      | 0.15 M NaCl  |   |   |   |   |   |   |   |  |
| 19 | (16) | Filters blocked 37° 2 hrs in                       |   |   |   |   |   |   |   |  |
| 20 |      | 1% BSA acidified                                   |   |   |   |   |   |   |   |  |
| 21 |      | 0.15 M NaCl  |   |   |   |   |   |   |   |  |
| 22 |      | PBS pH 7.0 } 2x SSC can be used.                   |   |   |   |   |   |   |   |  |
| 23 | (17) | Filters washed 2 x in                              |   |   |   |   |   |   |   |  |
| 24 |      | 0.1% BSA   |   |   |   |   |   |   |   |  |
| 25 |      | 2 x SSC  |   |   |   |   |   |   |   |  |

# Colony hybridization-glucosylated probes.

18

Horse Radish peroxidase 5 mg/ml  
diluted to 100 µg/ml in PBS pH 7.0  
0.1% BSA.

Filters soaked in 1.0 ml enzyme.

Incubated 30' at R.T.

19

Filters rinsed in  
PBS pH 7.0  
0.1% BSA  
0.15 M NaCl

3 x 20' each at R.T.

20

Filters soaked in 5.0 ml of substrate  
solution.

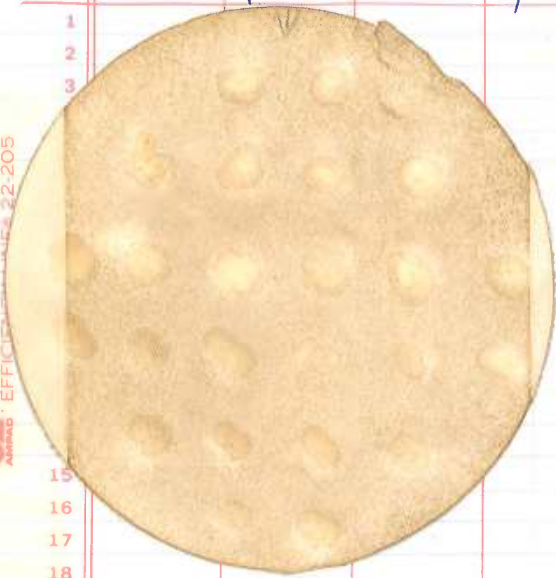
1 mg/ml DAB mixed with equal volume  
of 0.2% H<sub>2</sub>O<sub>2</sub>

Immediately added plates in the dark

Non glucosylated pBR 322  
Complete Detection System

glucosylated pBR 322  
Detection system without Cou A

AMRAD EFFICIENT LAMINATES 22-205



glucosylated pBR 322  
complete Detection system.



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# Colony Hybridization

glycosylated DNA  
probe.

9/3/82

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Colonies picked by Peter

Treatment of filter.

① 5' on filter soaked in 0.5M NaOH  
1.5M NaCl

② DRY on filter paper

③ Repeat step ①

④ 5' on filter soaked in 0.5M TRIS 7.4  
1.5M NaCl

DRY on filter paper

⑤ Repeat step ④

⑥ Soak filter in 0.25 mg/ml Proteinase K  
15' at RT.

Blot DRY

⑦ Soak filters in 95% EtOH 3x 5' each

AIR DRY

⑧ Bake 2-3 hrs at 70°

⑨ Store in a sealed bag

9/7/82

⑩ Incubate 65° 4 hrs in

2x SSC  
0.2% PVP  
0.2% Ficoll and 0.1% SDS.  
0.2% BSA

# Colony hybridization glucosylated DNA

AMFAS EFFICIENCY LINE® 22-205

	1	2	3	4	5	6	7	8	9
1									
2	(10)	Pre	Inc	Wash	filters	in			
3									
4			3 x SSC		37°	1 hr.			
5									
6					65°	1 hr			
7									
8					37°	1 hr.			
9									
10					RT	1 hr.			
11									
12									
13	(11)	Prehybridize	filter	at	42°	1 hr			
14									
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# Colony hybridization glucosylated $\mu$

11.

## Preparation of the probe.

	1	2	3	4	5	6	7	8	9
					Control	125 $\lambda$		glucosylated	
		3Hd ATP			12.5 $\lambda$			19.5 $\lambda$	
		10x NT buffer			12.5 $\lambda$			12.5 $\lambda$	
		0.3 mM nTP			12.5 $\lambda$			12.5 $\lambda$	
		0.3 mM TTP			12.5 $\lambda$			—	
		0.32 mM glucosyl dUTP			—			11.72 $\lambda$	
		pGPE-1 insert 1 $\mu$ g/ $\mu$ l.			10 $\lambda$			10 $\lambda$	
		DNase 100 $\mu$ g/ $\mu$ l			3 $\lambda$			3 $\lambda$	
		DNA pol. I 5 $\mu$ / $\mu$ l.			5 $\lambda$			5 $\lambda$	
		d. H <sub>2</sub> O.			57 $\lambda$			57.28	
					2 hrs at 14 <sup>o</sup>				
		Rx Terminated $\bar{e}$			12.5 $\lambda$		0.2 M	EDTA	
		Rx mixture passed thru			4.2 $\mu$ l G-50 column				
		equilibrated in			10 mM Tris 7.6				
					0.1 mM EDTA				
		First radioactivity peak collected							
		concentrated by vacuum evaporation.							



PGP E I  
 glucosylated  
 9/3/82  
 G-50

1 16	001.00	1
	000.00	
	000022.00	
1 17	001.00	2
	000.00	
	000016.00	
1 18	001.00	3
	000.00	
	000017.00	
1 19	001.00	4
	000.00	
	000020.00	
1 20	001.00	5
	000.00	
	000017.00	
1 21	001.00	6
	000.00	
	000009.00	
1 22	001.00	7
	000.00	
	000017.00	
1 23	001.00	8
	000.00	
	000022.00	
1 24	001.00	9
	000.00	
	000025.00	
1 25	001.00	10
	020.00	
	000111.00	
1 26	001.00	11
	015.00	
	000261.00	
1 27	001.00	12
	010.00	
	000444.00	

1 28	001.00	13
	010.00	
	000668.00	
1 29	001.00	14
	007.00	
	001022.00	
1 30	001.00	15
	007.00	
	001506.00	
1 31	001.00	16
	005.00	
	002330.00	
1 32	001.00	17
	005.00	
	003419.00	
1 33	001.00	18
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	005070.00	
1 34	001.00	19
	003.00	
	006994.00	
1 35	001.00	20
	003.00	
	008709.00	
1 36	001.00	21
	003.00	
	009473.00	
1 37	001.00	22
	003.00	
	009563.00	
1 38	001.00	23
	003.00	
	008620.00	
1 39	001.00	24
	003.00	
	005304.00	
1 40	001.00	25
	003.00	
	006012.00	

DNA binding to activated glass surface

8.2.82.

	1	2	3	4	5	6	7	8	9
1									
2									
3		T <sub>4</sub> DNA	4	μg/ml					
4									
5		diluted	to	contain					
6									
7									
8	A	1000	ng/ml						
9									
10	B	100	ng/ml						
11									
12	C	10	ng/ml						
13									
14	D	1	ng/ml						
15									
16	E	0.4	ng/ml						
17									
18									
19									
20		100	λ	of each solution	in	duplicate			
21									
22					in	activated	glass	tubes	
23									
24									
25					10'	at	RT.		
26									
27									
28					Remove	carefully.			
29									
30									
31		Rinse	tubes	3 x	̄	100	λ	of	2 x
32									
33									
34		100	λ	of	con A	added	0.1	mg/ml	in
35									PBS. Mg <sup>++</sup>
36									
37									
38	F	con A							
39									
40									
41	G	PBS. Mg <sup>++</sup>							
42									
43									
44									
45					left	at	RT	for	60'
46									11.47 am.
47									
48		Rinse	3 x	̄	100	λ	of	2 x	sse
49									
50	To	set	1	added	100	λ	PBS. Mg <sup>++</sup>		Incubate
		set	2	added	100	λ	1% Ficoll 400		RT 60'

	1	2	3	4	5	6	7	8	9
1									
2		Wash	3 x	$\bar{e}$	2 x	SSC			
3									
4		Add	100 $\lambda$		PBS-Mg <sup>++</sup>	to	set I		
5			100 $\lambda$		Con A	0.1 $\mu$ g/ml	in	PBS-Mg	to set II
6									
7									
8									
9									
10			60'		at	RT.			
11									
12									
13		Wash	3 x	$\bar{e}$	2 x	SSC			
14									
15									
16			3 x	$\bar{e}$	0.1 %	BSA	in	2 x	SSC
17									
18									
19		Add	100 $\lambda$		1 %	BSA	in	2 x	SSC
20							to		
21									
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8/3/82

	1	2	3	4	5	6	7	8	9
1									
2		Add	20 $\mu$	of	0.1 M	substrate			
3			80 $\mu$	of	0.2 M	imidazole buffer (6.5)			
4									
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Add 20  $\mu$  of 0.1 M substrate  
80  $\mu$  of 0.2 M Imidazole buffer (6.5)

Incuabali at 37° for one hour.

Diata

stop Rx @ 2.0 ml 5% NaHCO<sub>3</sub>.

check OD at A<sub>410</sub>.



	1	2 DNA	3 Cou A	4 Ficol	5 Cou A	6 Acid P.	7	A <sub>410</sub>	9
1									
2									
3									
4	A	50 ng	+	-	-	+		0.285 x 20	
5									
6									
7	A <sub>2</sub>	50 ng	+	+	+	+		0.35 x 20	
8									
9									
10	B <sub>1</sub>	5 ng	+	-	-	+		0.175 x 20	
11									
12									
13	B <sub>2</sub>	5 ng	+	+	+	+		0.23 x 20	
14									
15									
16	C <sub>1</sub>	500 peg	+	-	-	+		0.06 x 20	
17									
18									
19	C <sub>2</sub>	500 peg	+	+	+	+		0.12 x 20	
20									
21									
22	D <sub>1</sub>	50 peg	+	-	-	+		0.11 x 5	
23									
24									
25	D <sub>2</sub>	50 peg	+	+	+	+		0.22 x 5	
26									
27									
28									
29	E <sub>1</sub>	20 peg	+	-	-	+		0.144 x 2	
30									
31									
32	E <sub>2</sub>	20 peg	+	+	+	+		0.208 x 2	
33									
34									
35									
36	F <sub>1</sub>	-	-	-	-	+		0.006	
37									
38									
39	F <sub>2</sub>	-	+	+	+	+		0.007	
40									
41									
42	G <sub>1</sub>	-	-	-	-	+		0.006	
43									
44									
45									
46	G <sub>2</sub>	-	-	+	-	+		0.005	
47									
48									
49	Control	-	-	-	-	+		0.35 x 20	
50									

Con A - alkaline phosphatase binding.

7/28/82.

Elisa method in  
micro-titer plates.

Dilute con A (10  $\mu\text{g}/\text{ml}$  in 2.0 M NaCl) in  
PBS-Mg<sup>++</sup> buffer to give.

A 200  $\mu\text{g}/\text{ml}$

B 20  $\mu\text{g}/\text{ml}$

C 2  $\mu\text{g}/\text{ml}$

D 0.2  $\mu\text{g}/\text{ml}$ .

50  $\times$  from each dilution in triplicates

Vertical Rows.

Cover and leave at 4<sup>o</sup> o/n.

7/29/82.

Remove Con A

Wash wells 3x  $\bar{e}$

100  $\times$  of 0.1% BSA  
0.1 M TRIS-CL (pH 7.8)  
0.15 M NaCl  
0.02% Tween 20

Coated the wells with  
 100  $\lambda$  of 1% BSA  
 0.1 M Tris-Cl pH 7.6  
 0.15 M NaCl

at 37° for 2 hrs.

Washed 3 x  $\bar{e}$

100  $\lambda$  of 0.1% BSA  
 0.1 M Tris-Cl pH 7.6  
 0.15 M NaCl

"horizontal rows"  
 ←

Added ~~the~~ horizontal rows.

50  $\lambda$  of 200  $\mu$ g/ml } Alkaline  
 20  $\mu$ g/ml } phosphatase  
 2  $\mu$ g/ml } (1.8  $\mu$ g/ml  
 0.2  $\mu$ g/ml } stock solution

50  $\lambda$  of 200  $\mu$ g/ml } Horse  
 20  $\mu$ g/ml } Radish  
 2  $\mu$ g/ml } Peroxidase  
 10  $\mu$ g/ml } stock solution

Left at room temp for 60'

	1	2	3	4	5	6	7	8	9
1									
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47									
48									
49									
50									

Remove

Rinse wells 3 x  $\bar{e}$

100  $\lambda$  of 0.1% BSA

0.1 M Tris Cl (pH 7.8)

0.15 M NaCl

last rinse  $\bar{e}$  200  $\lambda$  of solution.

Add substrate 100  $\lambda$

Incubate 37° for one hour

left too long.

qualitative results.

repeat.



DNA Dot Blots.

8/2/82.

1  $\lambda$  DNA in 0.1 x SSC  
range 2 ng to 3  $\mu$ g.

Spotted on Nitro cellulose strips.

Strips placed on whatman 3mm paper soaked in  
0.5M NaOH  
1.5M NaCl

for 10 minutes

Strips washed 3 x in 10 ml of  
0.5M Tris.Cl pH 7.4  
1.5M NaCl

for 10 minutes each time.

Strips baked at 70° in vacuo for 3 hrs.

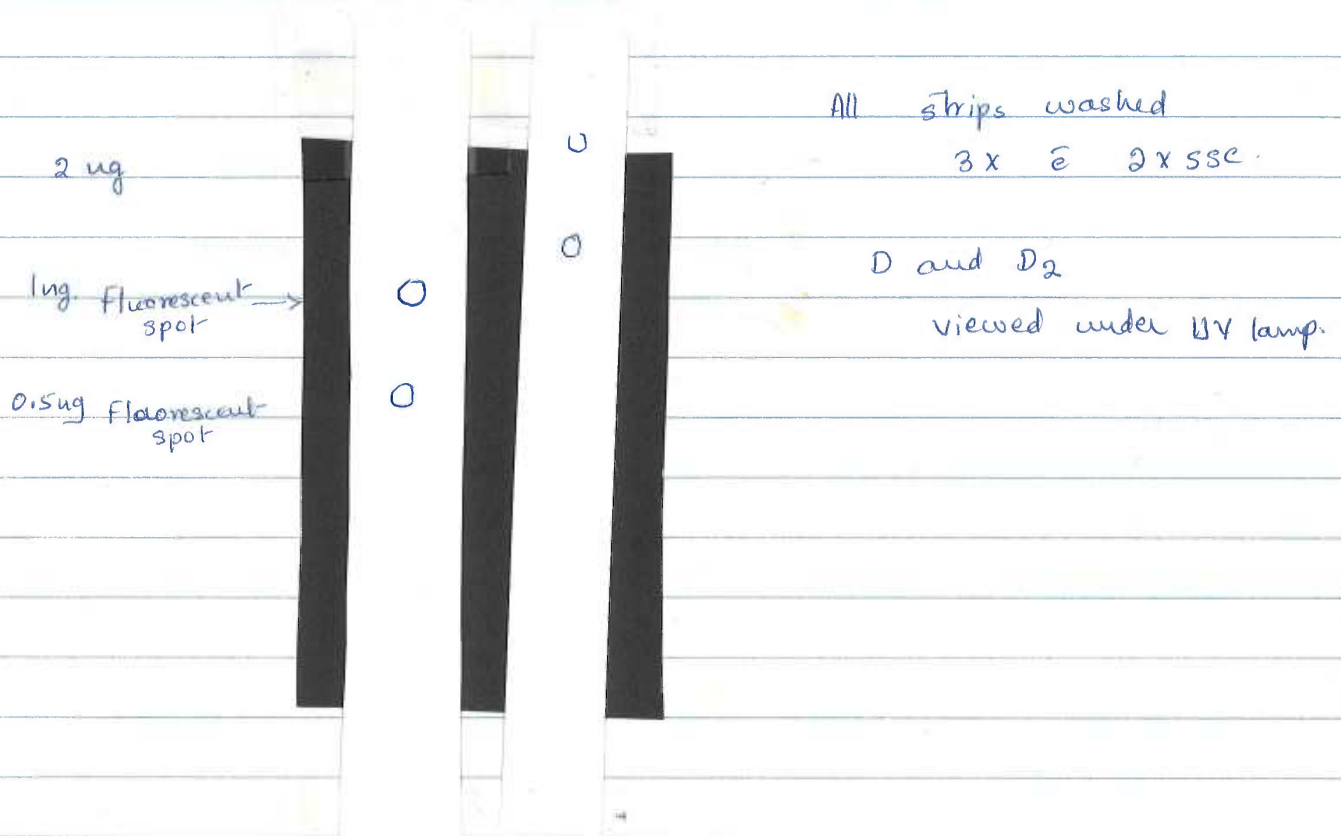
Strips blocked in  
0.2 % BSA  
0.2 % PVP  
0.1 % Triton X-100  
2 x SSC  
0.1 % SDS.

at 60° for 2-4 hrs. 10 am.

Con A binding.

Strips D<sub>1</sub> and D<sub>2</sub> soaked in FT-Con A 0.1  $\mu$ g/ml  
in PBS-Mg<sup>++</sup> RT 60' 2.0 ml total in the dark

Strips E<sub>1</sub>, E<sub>2</sub>, F<sub>1</sub>, F<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> soaked in  
10 ml cou A 0.1 mg/ml in PBS-Mg<sup>++</sup>  
RT 60'



Strips Strips E<sub>1</sub>, E<sub>2</sub>, F<sub>1</sub>, F<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>

washed 3x in 0.1% BSA in 2x SSC.

soaked in 10 ml 1% BSA in 2x SSC  
37° for 2 hours.

washed 3x in 0.1% BSA in 2x SSC.

E <sub>1</sub> and E <sub>2</sub>	10 ul	0.1 % BSA	2 x sse
		0.1 ug/ml	HRP
F <sub>1</sub> and F <sub>2</sub>	10 ul	0.1 % BSA	2 x sse
		0.1 ug/ml	Alk. Phosph.
G <sub>1</sub> and G <sub>2</sub>	10 ul	0.1 % BSA	2 x sse
			units Acid Phosp.

Incubated at 37° one hour



Con A - alkaline phosphatase

7/26/82

1      2      3      4      5      6      7      8      9

Dilute con A (10 µg/ml stock solution) in PBS Mg<sup>2+</sup>  
to give

A      200 µg/ml

B      20      "

C      2      "

D      0.2      "

Deliver 50 µl of each solution in plastic wells  
in triplicates vertical rows:

Incubate 4° overnight

7-27-82

Remove con A

Wash 3 x  $\bar{c}$       2 x SSC

Wash 3 x  $\bar{c}$       100 µl of 0.1% BSA in  
0.1 M TRIS-CL (7.8)  
0.15M NaCl  
0.02% Tween 20

~~Deliver~~ to  
Coat wells  $\bar{c}$

100 µl of 1% BSA in  
0.1 M TRIS-CL (7.8)  
0.15M NaCl  
Incubate at 37° ~~2 hrs~~



left at 4° o/n.

7/28/82

Remove coating solution.

Wash 3 x  $\bar{e}$  100  $\lambda$  of 0.1% BSA in  
0.1M TRIS.Cl (7-8)  
0.15M NaCl

Enzyme Alkaline phosphatase.

0.5 mg/ml.

diluted in 0.1 M TRIS pH 7.8 to give

200  $\mu$ g/ml

20  $\mu$ g/ml

2  $\mu$ g/ml

0.2  $\mu$ g/ml

0.02  $\mu$ g/ml

50  $\lambda$  of each dilution applied to wells.

horizontal rows.

Expt. did not work

Should have used calf intestine  
alkaline phosphatase

Repeat

Dot Blots

7.28.82.

glucosylated DNA

detection with con A - alkaline phosphatase

pH curve.

T<sub>4</sub> DNA 200 µg/ml in 10 mM TRIS pH 7.4  
10 mM NaCl

5λ DNA diluted to 225 λ 0.1xSSC  
Final con. DNA 4 µg/ml.

### Denaturation

18 λ DNA (4 µg/ml) treated at RT  
for 10' in 2 λ of 3.0 M NaOH

F.C. NaOH 0.3 M

diluted in 20 λ (equal vol.) of 2.0 M  
Am Acetate

F.C. DNA 2 µg/ml

10 λ diluted serially in two fold  
dilutions in 1.0 M Am Acetate

① 2 µg/ml

③ 0.5 µg/ml

② 1 µg/ml

④ 0.25 µg/ml

Spotted 1  $\lambda$  on 8 strips Nitrocellulose.

~~Baked at 80° in vac~~

Washed 2 x @ 1.0 M Am Acetali

Baked at 80° in vacuo for 2 1/2 hrs.

Coated ofu at 65° in

0.2 % Acidified BSA

0.2 % PVP

0.1 % Triton X-100

2x SSC

0.1 % SDS.

7.29.82

Strips soaked in 5.0 ul solution  
0.1  $\mu\text{g}/\mu\text{l}$  CouA in PBS-Mg<sup>++</sup>  
at RT. for 60'

Washed @ 2x SSC three times.

Washed in 1% BSA in SSC (2x)  
3 times.

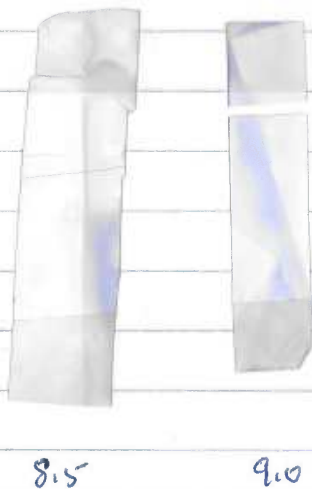
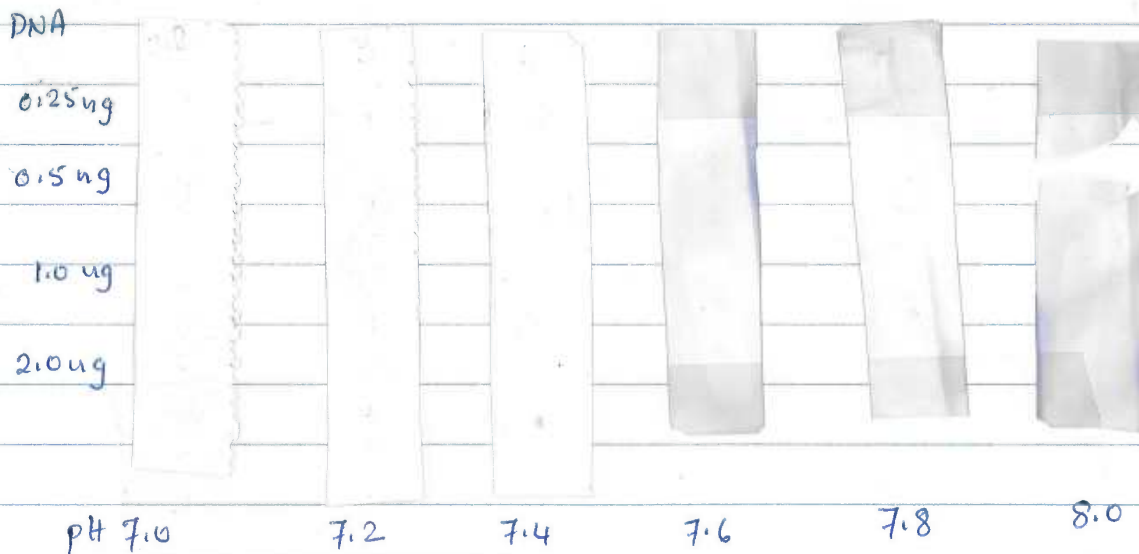
Soaked in Alkaline phosphatase  
200  $\mu\text{g}/\mu\text{l}$  at RT. for 30'

Washed repeatedly in 0.1% BSA in  
2x SSC.

Substrate Naphthol AS-BI phosphate  
0.1 mg/ml in TRIS 0.1 M  
Dye Fast Blue BB 0.5 mg/ml in  
0.1 M TRIS.

range pH of Buffer 7 to 9.

Kept at 37° for one hour.



detection system couA-Alk Phosp

best at pH 7.4



N. Kellm  
7/26/82

Con A binding of glycosylated DNA

7.23.82.

Eliza method Sandwich Technique

Con. Curve.

T<sub>4</sub> DNA 0.2 µg/ml in 10 mM Tris 7.2  
10 mM NaCl

<del>20</del> A	0.2 µg/ml
B	0.1 µg/ml
C	0.05 µg/ml
D	0.025 µg/ml
E	0.0125 µg/ml
F	0.00625 µg/ml.

Spotted on Nitrocellulose strips at 1.5 cm distance

0.6 µg	3 λ	} Air dried, placed on 3 whatman sheets. soaked in 0.5 M NaOH 1.5 M NaCl for 2' washed 2 x in 0.5 M Tris 7.4 1.5 M NaCl 10' each.
	2 λ	
	1 λ	
	0.5 λ	
	0.25 λ	
	0.1 λ	

Air dried

Baked at 80° under vacuum for 2.5 hours.

Coated at 65° o/v in 1.25 µl per strip

0.2% BSA  
0.2% PVP  
0.2% Ficoll  
0.1% SDS  
2 x SSC

N. Kellm  
7/26/82

7.24.82

Remove strips from coating solution.

Blot dry on filter paper.

### Cou. A treatment

Soak strips in Cou A solution. 10 ul Total  
0.1 ug/ul in PBS. Mg<sup>++</sup> buffer. for 6 strips  
at RT for ~~2~~ - 60 minutes.

Wash  $\bar{c}$  0.1 M TRIS pH 7.2 3 X

Air Dry.

### Enzyme Treatment

~~Apply alkaline phosphatase 4 X of 0.5 ug/ul solu.~~  
~~air to the spots.~~

Soak the strips in alkaline phosphatase solu.

10 ug in 0.25 ml Tris 0.1 M pH 7.2.

per strip.

leave at R.T. for 30 minutes.

Wash  $\bar{c}$  0.3 M NaCl 10 ul each time.

3 - 5 X

Soak the strips in 10 ul of substrate-dye solution.

0.5 ug/ul Fast Blue BB

0.1 ug/ul Naphthol AS-BI

} in 0.1 M Tris

pH 7.5

Incubate at  $\bar{c}$  37° for one hour

N. K. Keller  
7/26/82

con A+ couA-

EFFICIENCY LINE® 22-205

	1	2 A	3 B	4 C	5 D	6 E	7 F	8 G	9 H
1									
2									
3	DNA	600 ng	300 ng	150 ng	75 ng	37.5 ng	18.75 ng	-	-
4									
5									
6		400 ng	200 ng	100 ng	50 ng	25 ng	12.5 ng	-	-
7									
8									
9		200 ng	100 ng	50 ng	25 ng	12.5 ng	6.25 ng	-	-
10									
11									
12		100 ng	50 ng	25 ng	12.5 ng	6.25 ng	3.125 ng	-	-
13									
14									
15		50 ng	25 ng	12.5 ng	6.25 ng	3.125 ng	1.5625 ng	-	-
16									
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18		20 ng	10 ng	5 ng	2.5 ng	1.25 ng	0.625 ng	-	-
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N. Keller  
7/23/82

Binding of glycosylated DNA to lectin  
- detection by sandwich technique

7.22.82

T<sub>4</sub> DNA 0.2 µg/µl.

λ plaes DNA 0.516 µg/µl.

4 Nitro cellulose strips 1.5 x 7 cm [S.S.]  
marked 1, 2, 3, 4

On 1 and 2

T<sub>4</sub> DNA at 1.5 cm distance.

3 λ = 0.6 µg

2 λ

1 λ

0.5 λ

On 3 and 4

λ plaes

3 λ

2 λ

1 λ

0.5 λ

Strips placed on 3 mm paper soaked in

0.5 M NaOH

1.5 M NaCl

2' at RT.

Washed 5' in 0.5 M TRIS HCL pH 7.4

1.5 M NaCl

2 x

Baked at 80° 2.5 hrs. in vacuo.



N Keller  
7/23/82<sup>2</sup>

Strips coated at 65° c/fu in  
(1 ml solution per strip)

0.2 % BSA  
0.2 % PVP  
0.2 % Ficoll  
0.1 % SDS  
2 x SSC

7.23.82

~~Washing solutions~~

Coating solution removed.

Strips are air-dried

placed in clean petri-dishes.

1 and 3 in one

2 and 4 in another.

Con. A treatment

2 and 4 soaked in PBS. Mg<sup>++</sup> buffer  
0.25 ml per strip

1 and 3 soaked in PBS. Mg<sup>++</sup> buffer  
containing 0.1 mg con A per strip  
0.25 ml solution per strip  
at R.T. for 30 minutes

Washed 4 x in 5.0 ml 0.1 M TRIS pH 7.4  
10' each time.

## Enzyme Treatment

Strips soaked in (0.25 ul per strip)

0.1 mg/ul Alkaline phosphatase  
30' at RT.

Washed in 0.15 M NaCl 5 ul 10' thrice

Soaked in substrate-dye solution.

~~5.0 ul~~ 2.5 ul for 4 strips

0.5 mg/ul Fast Blue BB

diacetylated 4'-amino

2',5' Diethoxybenzoyl chloride

Zinc chloride

0.1 mg/ul Naphthol AS-BI phosphate

in 0.1 M TRIS pH 7.5

( Dye 10 mg/ul in 0.1 M TRIS 7.2 )  
( substrate 10 mg/ul in 0.1 M TRIS 7.2 )

Incubated at 37°

Blue spots immediately within 5'

on # 1 strip only

N. K. Kelly  
7/23/82

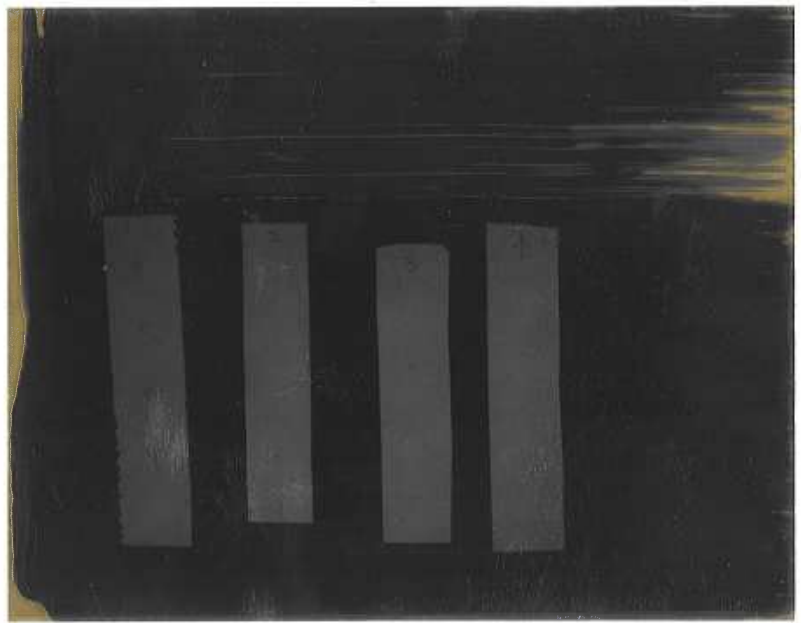
7/22 - 7/23/82



5.1 in. [unclear] [unclear]  
2.111 [unclear] [unclear]  
[unclear] [unclear]  
[unclear] [unclear] [unclear]  
[unclear] [unclear] [unclear]  
[unclear] [unclear] [unclear]  
[unclear] [unclear] [unclear]  
[unclear] [unclear] [unclear]

analysis of [unclear]

7/22 - 7/23/82



[unclear]  
[unclear]  
[unclear]  
[unclear]  
[unclear]  
[unclear]  
[unclear]  
[unclear]

Detection of glucosylated DNA with 7.20.82  
lectin enzyme system  
effect of glutaraldehyde

2 x 8 cm nitrocellulose strips

1  $\lambda$  DNAs spotted on all strips.

T<sub>4</sub>

$\lambda$

glucosylated  $\lambda$

PST

glucosylated PST

DNA denatured 1' in 0.5 M NaOH  
1.5 M NaCl.

washed 2 x 5' each in

0.5 M Tris pH 7.4

1.5 M NaCl

Blotted Dry.

Baked at 80° under vacuum for  
2 hrs.

Incubated at 65° o/n. in  
coating solution.



0.2 % Ficoll  
0.2 % PVP  
0.2 % BSA  
0.10 % SDS  
2 X SSC

7/21/82.

Coating solution removed.  
Strips are air-dried.

A, C, E and G strips are soaked in  
10 ml 50 mM K-PO<sub>4</sub> pH 7.2  
150 mM NaCl

containing 0.1 % glutaraldehyde  
for 3 hrs at RT.

Strips:

B, D, F, and H are soaked in  
10 ml 50 mM K-PO<sub>4</sub> pH 7.2  
150 mM NaCl

for the same length of time.

Solutions removed.

Strips washed 3x in PBS-Mg<sup>++</sup> Gutter.

A, C, E } Soaked in 8.0 ml PBS-Mg<sup>++</sup>  
B, D, F } containing 0.5 mg/ml

cou A  
G and H in PBS-Mg<sup>++</sup> Gutter at RT for 30'

7-21-82

Treatment with acid phosphatase

Strips A and B washed 3 x  $\bar{e}$  5.0 ml  
each time of 0.2 M Imidazole buffer  
pH 6.5

acid phosphatase 0.005 units in 0.2 M  
Imidazole pH 6.5 containing 1 mg/ml  
phosphatase free BSA [1.0 ml].

Filters soaked in enzyme solution 30' at  
RT.

washed repeatedly with 0.15 M NaCl  
5-10 x

Filters soaked in 5.0 ml of  
0.1 mg/ml Naphthol Bs. 1  
in 0.2 M Imidazole pH 6.5  
containing 1.0 mg/ml Fast Red TR dye

Incubated at 37° for 30-60'

Some indication that glutaraldehyde  
enhances reaction.

qualitative data.

Should be worked out more.

7.19.82

Filter hybridization of glucosylated probe  
detection with *eu A*  
effect of glutaraldehyde

### Preparation of filters.

Nitrocellulose membrane filters. washed 5'  
in  $H_2O$

Soaked 15' in 1.0 M Ammonium Acetate  
air-dried.

### Denaturation of DNA.

DNA is denatured in 0.3 M NaOH 10' at RT.

Quickly chilled in ice

Diluted with equal volume of 2.0 M Am Ace.  
cold.

DNA concentration not more than 20  $\mu\text{g}/\text{ml}$ .

### Application of DNA to NC filters.

DNA applied manually using siliconized  
micropipette

air Dry the filters.

Rinse filters in 1.0 M Am Acetate 5'

air Dry

Bake filters under vacuum at  $80^\circ$  for 2 hrs

1. T<sub>4</sub> DNA 0.28 mg/ml 5λ
2. λ Fel V 1.0 mg/ml 5λ
3. PST 1.4 mg/ml 5λ
4. PBR 322 2.0 mg/ml 2.5λ

	1	2	3	4
DNA	5λ	5λ	5λ	2.5λ
Tris Buffer	3.5λ	3.5λ	3.5λ	5.5λ
2.0M NaOH	1.5λ	1.5λ	1.5λ	1.5λ

10' at RT.

add ice-cold 2.0 M Am Acetate 10λ to each vial.

Apply 5λ to nitrocellulose strips

A	B
1	1
2	2
3	3
4	4

Air dry

Rinse 5' in 1.0 M Am Acetate

Air dry

Bake 80° under vacuum 2 hrs



Filter Pre hybridization.

Prehybridize filters at  $42^{\circ}$  for 90' in solution containing

50 % Formamide

5 x SSC

100  $\mu$ g/ml sonicated salmon sperm DNA

10 x Denhardt 0.2 % Ficoll

0.2 % PVP

0.2 % BSA

Hybridization.

Buffer 0.025 M NaP pH 6.5

50 % Formamide

5 x SSC

10 x Denhardt

10 % Dextran Sulphate

Hybridize filters in 1.0 ml above buffer containing  $10 \lambda$  of heat-denatured probe [95° for 5' quickly cooled] at  $42^{\circ}$  for 16 hours.

Wash the filters at  $37^{\circ}$  thrice in 2 x SSC 10' wash each time.  
dry the filters.

Con A Binding

Treatment with glutaraldehyde

Wash the filters in 0.05 M K-PO<sub>4</sub> pH 7.2  
0.15 M NaCl

Soak set of A filters

A-1 to A-4

2 Blanks.

in 10 ml 50 mM K-PO<sub>4</sub> 7.2  
150 mM NaCl

0.1 % ~~of~~ glutaraldehyde

at RT. for 3 hours.

Set of B filters.

in 10 ml 50 mM K-PO<sub>4</sub> pH 7.2  
150 mM NaCl.

Buffers removed.

Washed 3 x in 50 mM K-PO<sub>4</sub> 7.2  
150 mM NaCl.

Treatment with Con A.

Filters soaked in 5.0 ml

0.5 mg/ml Con A in  
PBS 6.5 containing  
MgCl<sub>2</sub> 10 mM

30 minutes at RT.

Con A removed.

Filters washed 3 x  $\bar{e}$  0.1 M TRIS pH 7.4.  
at RT. 10 min. each

Alkaline Phosphatase treatment.

Filters soaked in 1.0 ml solution containing  
0.5 mg alkaline phosphatase in 0.1 M TRIS  
pH 7.4.  
30' at RT.

Washed 3 x  $\bar{e}$  0.1 M TRIS pH 7.4 containing  
0.15 M NaCl.

Added 1.0 ml substrate in 0.1 M Tris pH 7.4  
37° Incubation.

No spot seen.

Enzyme is there because good  
color development.

NEGATIVE

preparation of *Eco* V insert

7.19.82

$\lambda$ <i>Eco</i> V DNA	1.0 $\mu$ g/ $\mu$ l
<i>Eco</i> RI	5000 u/ $\mu$ l

500  $\lambda$  Assay Mix

10x Buffer	50 $\lambda$
DNA	100 $\lambda$
<i>Eco</i> RI	10 $\lambda$
d. H <sub>2</sub> O	340 $\lambda$

37° 90 minutes

Added 10  $\lambda$  SDS, EDTA  
200  $\lambda$  dye solution.

200  $\lambda$  mixture electroforesed  
0.7% agarose gel.

No digestion.

phenolize to recover the DNA.



Eco R I digestion of

7.16.82.

$\lambda$  DNA and  $\lambda$  Fel V DNA

$\lambda$ DNA	FROM	Chris Brackel	1.6 $\mu$ g/ $\mu$ l.
$\lambda$ Fel V	FROM	Henry Cudney	1.0 $\mu$ g/ $\mu$ l.
EcoRI	FROM	NBL	5000 u/ $\mu$ l.

	1	2	3	4
10x Buffer	1 $\lambda$	1 $\lambda$	1 $\lambda$	1 $\lambda$
$\lambda$ DNA	1 $\lambda$	1 $\lambda$	-	-
$\lambda$ Fel V DNA	-	-	1 $\lambda$	1 $\lambda$
EcoRI	-	1 $\lambda$	-	1 $\lambda$
dist. H <sub>2</sub> O	8 $\lambda$	7 $\lambda$	8 $\lambda$	7 $\lambda$

37° 60'

Rx Terminated @ 2  $\lambda$  SDS EDTA

70° for 5'

Added 12  $\lambda$  dye solution

electrophoresed 1% agarose



KB.  
 25.6  
 7.31  
 6.16  
 5.55  
 4.84

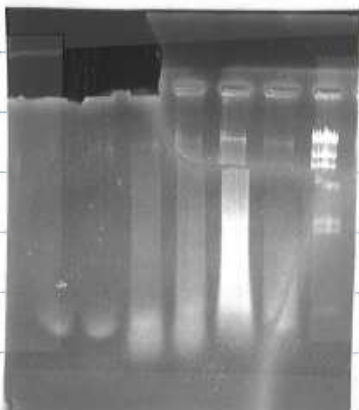


Run parallel @  
 Hind III digested  
 $\lambda$   
 8kb. piece  
 of interest

Gel electrophoresis of DNA probes

7.14.82.

1. TTP PBR 322 2.5  $\lambda$
2. glucosyl. PBR 322 "
3. TTP Pst "
4. glucosyl Pst "
5. TTP  $\lambda$  Fel V "
6. glucosyl  $\lambda$  Fel V "



23.5

preparation of glucosylated probe  
PST DNA

7.8.82.

PST DNA 1.4 mg/ml  
mT dUTP 0.3 mM  
DNA POL I 5 u/microliter

	Control	Test
	Rx 250 $\lambda$	
$^3\text{HdATP}$	60 $\lambda$	60 $\lambda$
10 x NT buffer	25	25
dNTP 0.3 mM	25	25
TTP 0.3 mM	25	-
mT dUTP 0.3 mM	-	25
PST DNA 1.4 mg/ml	0.142 ml	0.142 ml
DNase I 10 $\mu\text{g/ml}$	20 $\lambda$	20 $\lambda$
DNA Pol 5 u/ml	12 $\lambda$	12 $\lambda$
dist. H <sub>2</sub> O	0	0

14° for 2 hrs

Rx Terminated with 25  $\lambda$  of 6.2 M EDTA

2 microliter to check for incorporation TCA insoluble

G-50 column chromatography of  
Nick. translated DNAs.

7.9.82

	1	2	3	4	5	6	7	8	9
	FR #	3Hepm							
1									
2	1	277				PST	glucosylated DNA		
3	2	77							
4	3	17							
5	4	65							
6	5	14611							
7	6	58098							
8	7	63499							
9	8	38848							
10	9	35985							
11	10	35838							
12	1	34654							
13	2	32986							
14	3	27563							
15	4	2114							
16	5	27496							
17	6	25260							
18	7	20777							
19	8	536							
20	9	12031							
21	20	9006							
22									
23									
24									
25	FR #	3Hepm							
26									
27									
28	1	44				PST	non-glucosylated		
29	2	7							
30	3	22							
31	4	33							
32	5	9							
33	6	12							
34	7	14							
35	8	4280							
36	9	63318							
37	10	66291							
38	1	53561							
39	2	49679							
40	3	48543							
41	4	38140							
42	5	38457							
43	6	34734							
44	7	36834							
45	8	40663							
46	9	23944							
47	20	24963							
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Dollie #19182

Dollie #19182

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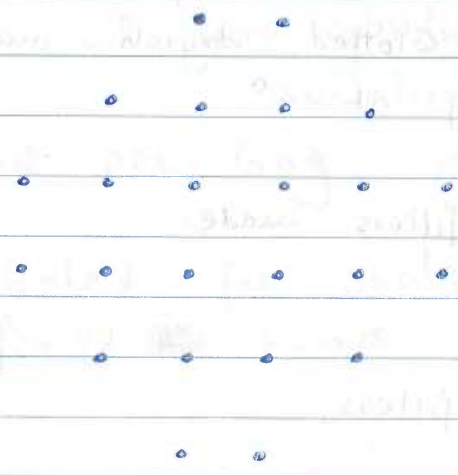
Colony Hybridization  
PBR 322  
glucosylated-  
lectin HRP



7/7/82

Colony hybridization with glucosylated probe  
and detection with lectin.

pBR 322 single colonies were grown on  
L-agar plates at 37° overnight



F7

The colonies were transferred to nitrocellulose  
membrane filters. Filters were dipped into  
0.5 M NaOH solution for 30 seconds  
1.5 M NaCl

and then into twice into  
0.5 M Tris-Cl (pH 7.4)  
1.5 M NaCl

for 2 minutes each time.

Filters were blotted dry and baked at 80°  
for 3 hours in vacuo.

Filters were soaked in wash buffer

0.2 % Ficoll  
0.2 % PVP  
0.2 % BSA  
2 x SSC  
0.1 % SDS

} in sealed Gags  
10 ul per Gag  
4 filters per Gag.  
at 65° overnight.

7.8.82.

Filters were Glotted dryish and stored  
in sealed Gag at 4°

7/11/82. - 7/12/82.

Some more filters made.

7/13/82.

Total 16 filters.

Filters soaked in pre-hybridization mixture  
8 ul per Gag & 4 filters per Gag.

Pre-hybridization Mixture Total 40 ul.

20.0 ul Formamide.  
10.0 ul 2 x SSC.  
0.4 ul 100 x Denhardt mix  
0.44 ul S.S. DNA.  
H<sub>2</sub>O to 40.0 ul.

At 42° for 2 hours.



7.13.82.

Pre-hybridization mixture carefully removed  
and filters soaked in

Hybridization mixture

125  $\lambda$  probe (heated at 80° for 3'  
20  $\mu$ l pre-hybridization and quickly cooled  
mixture

10  $\mu$ l per bag 4 filters per bag.

Incubated in sealed bag at 42°  
for 72 ~~h~~ hours.

N 30 $\mu$ g	$2.75024 \times 10^6$	cpm	glucosylated probe
N 25 $\mu$ g	$3.172955 \times 10^6$	cpm	TTP probe.

7.16.82.

Filters removed and washed 3 x in wash buffer  
Cl<sub>2</sub> - dried.

7.21.82.

Filters treated with con A 0.5  $\mu$ g/ $\mu$ l in  
PBS. Mg<sup>++</sup> buffer  
1.0  $\mu$ l per filter.

Soaked at RT. for one hour.

Controls without conA run at the same  
time.

7.21.82

~~Four filters~~ w-

4 Filters were washed free of con A  
using 0.1 M TRIS pH 7.2.

Treatment with Horse Radish peroxidase.

HRP 5.0  $\mu\text{g}/\text{ml}$  in dist.  $\text{H}_2\text{O}$ .

diluted to 0.1  $\mu\text{g}/\text{ml}$  in 0.1 M TRIS pH 7.2

Filters soaked in enzyme solution 30' at RT.

enzyme solution removed and filters were  
washed free of enzyme using 0.1 M TRIS  
pH 7.2 containing 0.1  $\mu\text{g}/\text{ml}$  BSA.

Blotted dry and placed in another  
petri dish.

Substrate solution made fresh.

1  $\mu\text{g}/\text{ml}$  DAB mixed with  
equal volume of  $\text{H}_2\text{O}_2$  in  $\text{H}_2\text{O}$  [100  $\lambda$  per 15 ml]  
25  $\mu\text{l}$  solution made and quickly  
added to filters. 5.0  $\mu\text{l}$  per filter.

~~brown spots~~

Colonies hybridized with glucosylated probes  
gave brown spots.

Controls were negative.

Still background problem.

Use of glucosylated-lectin system for  
colony hybridization.

7.13.82

pBR 322                      2.0  $\mu$ g/ $\mu$ l  
 $^3$ H dATP  
 malto-triose }                      0.3  $\mu$ M  
 dUTP }

	Control	Glucosylated
$^3$ H dATP	25 $\lambda$	25 $\lambda$
10X NT buffer	12.5 $\lambda$	12.5 $\lambda$
MT dUTP	—	12.5 $\lambda$
dNTP 0.3 $\mu$ M	12.5 $\lambda$	12.5 $\lambda$
TTP 0.3 $\mu$ M	12.5 $\lambda$	—
pBR 322 2 $\mu$ g/ $\mu$ l	25 $\lambda$	30 $\lambda$
DNase 10 $\mu$ g/ $\mu$ l	6 $\lambda$	6 $\lambda$
DNA Pol 5 $\mu$ l/ $\lambda$	6 $\lambda$	6 $\lambda$
dist H <sub>2</sub> O	45.5 $\lambda$	45.5 $\lambda$

2 hrs 14<sup>o</sup>

1.40 pm.

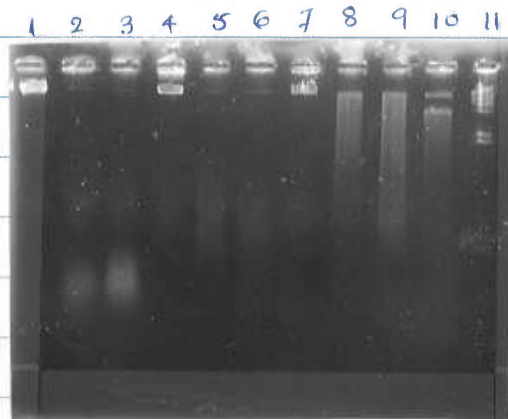
Rx Terminated  $\bar{c}$  12.5  $\lambda$  of 0.2 M EDTA  
 Rx mixtures passed thru G-50 1.0 ml in  
 Syringes.

First peak radioactivity collected  
 15  $\lambda$  checked for counts.



Gel electrophoresis of nick translated DNAs 6.29.82

- |     |                           |                 |              |             |
|-----|---------------------------|-----------------|--------------|-------------|
| 1.  | Ad-2 DNA                  |                 |              | ~ 1 $\mu$ g |
| 2.  | Ad-2 DNA                  | wick translated | control      | "           |
| 3.  | "                         | "               | glucosylated | "           |
| 4.  | $\lambda$ Fel V DNA       |                 |              | "           |
| 5.  | "                         | wick translated | control      | "           |
| 6.  | "                         | "               | glucosylated | "           |
| 7.  | Pst DNA                   |                 |              | "           |
| 8.  | "                         | wick translated | control      | "           |
| 9.  | "                         | "               | glucosylated | "           |
| 10. | PBR 322                   |                 |              | "           |
| 11. | $\lambda$ Hind III marker |                 |              |             |





G-50 column chromatography.  
nick translated DNA  $\lambda$  Fel V.

7/19/82

	1	2	3	4	5	6	7	8	9
1									
2	1	18							
3	2	12							
4	3	14							
5	4	11							
6	5	21							
7	6	15							
8	7	1871							
9	8	8728							
10	9	21008							
11	10	18183							
12	1	22063							
13	2	24978							
14	3	28155							
15	4	23958							
16	5	21710							
17	6	16482							
18	7	10920							
19	8	7082							
20	9	185							
21	20	4152							
22	1	1987							
23	2	1270							
24	3	38							
25	4	799							
26									
27									
28	1	13							
29	2	20							
30	3	13							
31	4	89							
32	5	15							
33	6	11							
34	7	1355							
35	8	13075							
36	9	23475							
37	10	20874							
38	1	23071							
39	2	28437							
40	3	21240							
41	4	37682							
42	5	27418							
43	6	19345							
44	7	13977							
45	8	10390							
46	9	5918							
47	20	7117							
48	1	4872							
49									
50									

$\lambda$  Fel V nick translated  $\bar{e}$  TTP

6 drop fraction.

3.5 ml column.

lyophilized

pH 7.5 TRIS 10 mM } buffer  
EDTA 0.1 mM }

$\lambda$  Fel V nick translated  $\bar{e}$   
~~for~~ malto-Triose  
& UTP.

3.5 ml column

lyophilized

TRIS 10 mM } pH 7.5  
EDTA 0.1 mM }

6 drop fractions.

Dollie  
7/9/82

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Preparation of glucosylated probe.

6.28.82.

$\lambda$  Fel V DNA.

	control	glucosyl-DNA
	0.5 ml Rx	
$^3\text{HdATP}$	40 $\lambda$	40 $\lambda$
10X NT buffer	50 $\lambda$	50 $\lambda$
dNTP	50 $\lambda$	50 $\lambda$
TTP	50 $\lambda$	-
MT dUTP	-	50 $\lambda$
$\lambda$ Fel V	100 $\lambda$	100 $\lambda$
DNAse 0.1 $\mu\text{g}/\text{ml}$	40 $\lambda$	40 $\lambda$
DNA Pol. 20u/ $\lambda$	5 $\lambda$	5 $\lambda$
d. H <sub>2</sub> O	165 $\lambda$	165 $\lambda$

2 hrs at 14°

Rx Terminated  $\bar{c}$  50  $\mu\text{l}$  of 0.2M EDTA

2  $\mu\text{l}$  aliquot mixed  $\bar{c}$  5  $\mu\text{l}$  carrier DNA

TCA insoluble epm determined.



Large scale preparation of  
glucose-substituted PST DNA

6.25.82.

Pst DNA 1.4 mg/μl.  
maltotriose dUTP 0.3 mM  
DNA pol. 20 u/microliters.

	A.	B.
<sup>3</sup> H dATP	12 λ	12 λ
10 x NT Buffer	12.5	12.5
dNTP	12.5	12.5
TTP	12.5	-
MT dUTP	-	12.5
PST DNA	50 λ	50 λ
d. H <sub>2</sub> O	26 λ	26 λ
DNAase 0.1 Mg/μl.	10 λ	10 λ
DNA pol.	1.5 λ	1.5 λ

*Douglas  
Chapman*

1 58	010.00	
	020.00	
	00018.60	
1 59	010.00	Pst A
	000.50	
	031499.40	
1 60	010.00	Pst B
	000.50	
	018294.60	

14° 2 hours.

2 μl aliquot for checking incorp.

R<sub>x</sub> terminated ē 10 λ of STOP buffer.



6.25.82.

Nick translation of PBR 322 plasmid DNA  
with malto-triose dUTP

PBR 322	mg/ml.
malto-triose dUTP	0.3 mM
DNA polymerase	20 u per microliter

	C	D
$^3\text{H}$ dATP	6 $\lambda$	6 $\lambda$
10x NT Buffer	2.5 $\lambda$	2.5 $\lambda$
dNTP mix	2.5 $\lambda$	2.5 $\lambda$
TTP	2.5 $\lambda$	-
MT dUTP	-	2.5 $\lambda$
PBR 322	10 $\lambda$	10 $\lambda$
DNase 0.1 mg/ml	2 $\lambda$	2 $\lambda$
Pol I <del>30 u/l</del> 3 u/l	2 $\lambda$	2 $\lambda$
d. H <sub>2</sub> O	-	-

14° incubation.

2 ul aliquots at 15' intervals in  
vials containing

2 ul stop buffer

5 ul carrier DNA.

Added 1.0 ul cold 10% TCA - 10' at 4°  
Insoluble material collected on EGF  
as washed and counted.

6.25.82

AMRAS EFFICIENCY LINE® 22-205

1	2	3	4	5	6	7	8	9	10
	Incubation		TEA	insoluble		SH ep	per	2 ul	Rx.
1									
2	14°	in Min.		C			D.		
3									
4									
5	3'			1251			1202		
6									
7									
8	15			2255			2268		
9									
10									
11	30			3222			3311		
12									
13									
14	45			4013			3732		
15									
16									
17	60			4006			4123		
18									
19									
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21	90			5544			4426		
22									
23									
24	120			5050			5174		
25									
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27									
28	150			5377			5663		
29									
30									
31	180			5754			5876		
32									
33									
34	210			3676			6138		
35									
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37	240			912			6008		
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6.25.82

46 1521

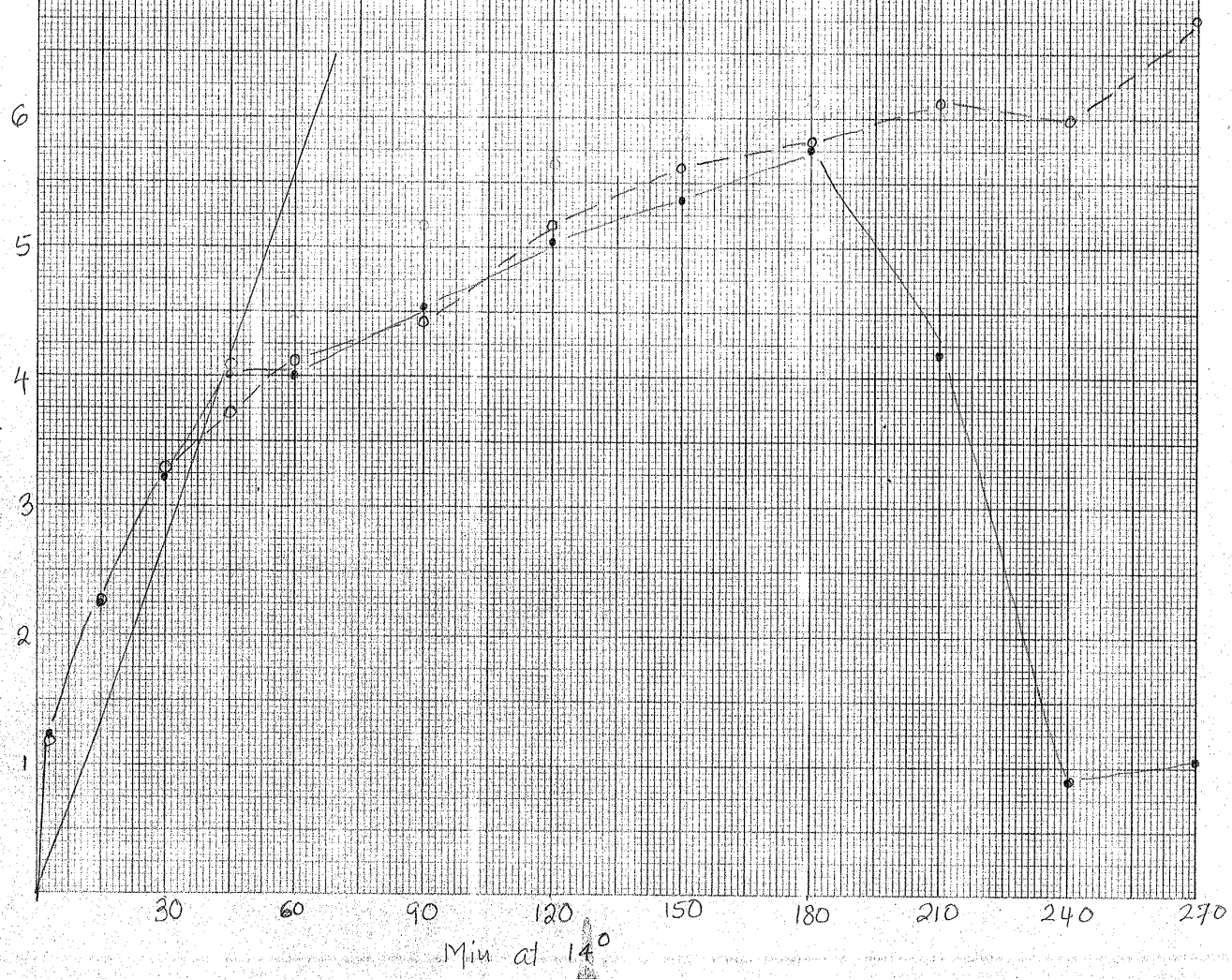
12 X 10 TO THE CENTIMETER 18 X 25 CM.  
KEUFEL & ESSER CO. MADE IN U.S.A.

TCA insoluble  $344 \mu\text{m} \times 10^{-3}$

Nick-Translation PBR 322

with  $\text{O}$  malto-kinase dUTP 0-0-0

$\text{O}$  TTP



6.25.82.

## Preparation of phage plaques.

\* Lambda. Fel V.

Recombinant DNA lab used.

L-agar plates freshly prepared  
dried at  $37^{\circ}$  for 24 hours.

Permissive host RW 262 from Henry.  
Phage  $\lambda$  Fel V

$10^{-2}$   $10^{-4}$  and  $10^{-5}$  dilutions in  
L Broth containing Mg<sup>++</sup> and Ca<sup>++</sup>  
made.

0.1 ml of each phage dilution mixed with  
0.3 ml of 6 hour L Broth culture of  
RW 262

mixed. after 5' added 3.0 ml  
of 0.7% soft L agar.  
mixed well without bubbles and  
entire contents poured on dried plate

Incubated at  $37^{\circ}$  cfe.

2 plates  $10^{-2}$  dilution.

3 plates  $10^{-4}$  dilutions

4 plates  $10^{-5}$  dilution.

6.26.82.

# plaques / 0.1 ul.

$10^{-2}$	~ 200 , 150
$10^{-4}$	48 , 80 , 50
$10^{-5}$	10 , 5 , 6 , 0

Phage filters made according to std. procedure  
from  $10^{-2}$  and  $10^{-4}$  dilution.

7 80 cm. filters.

6 47 cm filters.

Washed  $s'$  in 0.5 M NaOH

1.5 M NaCl

$s'$  2 twice in 0.5 M Tris (pH 7.4)

1.5 M NaCl.

Blotted ~~dryd.~~ dryish.

Baked  $80^{\circ}$  in vacuo. for 3 hours.

Incubated in wash buffer (5 ul per  
filter in sealed Gags.) at  $65^{\circ}$   
for 16 hours.

Blotted dryish.

Stored stacked in sealed Gags.

ofu.

$\lambda$  Fel V DNA Hybridization to wick-translated probe 6-28-82.

phage Filters 7 large  
6 small.

Pre-Hybridization:

Filters soaked in 8.0 ml of pre-treatment soln.  
pre-treatment soln. F.e.

20 ml Formamide 50%

10 ml 20x SSC 5x SSC

0.4 ml 100x Dehardt Mix. (2% Ficoll)  
1.0 ml S.S. DNA (2% PVP)  
(2% BSA)

H<sub>2</sub>O to 40 ml.

4 filters per bag - 8 ml pre-treatment soln. per bag.

Bags are sealed and incubated in water bath at 42° for 1.5 hrs.

Pre-treatment soln removed and added hybridization mix.

20 ml Pre-treatment soln.

250 microliters wick-translated  $\lambda$  Fel V DNA.

denatured at 80° for 3' and quickly cooled.

[ probe is either TTP  $\lambda$  Fel V DNA or HTdUTP  $\lambda$  Fel V DNA.

Bags sealed and incubated at 42° water bath for 24 - 48 hours.



6.30.82

Hybridization mixture sucked off and  
the filters washed quickly into  
four changes of wash buffer at room Temp.  
6 hours at 65° buffer changed every  
hour.

Blotted dry.

A Filters soaked in 10 ml of CoNA solution  
in PBS Mg<sup>++</sup> [1.0 ug/ml CoNA].  
for 2 hours at R.T.

washed 3 times in PBS Mg<sup>++</sup> buffer.  
washed 3 times in 0.2 M Tris glycine  
pH 6.5 buffer

Blotted dry.

~~7/1/82~~

~~Two small and two large from  
control and experimental filters.  
-treated with acid phosphate  
1 ml solution~~

(A)  $\lambda$  Fel V glucosylated probe. 7.1.82.

Detection @ Acid phosphatase  
Sandwich

~~Acid ph~~

The filters are soaked in phosphatase-free BSA 10' at RT.

Blotted dryish.

Soaked in enzyme solution at RT for 30'

0.5 ul	Acid phosphatase	units/ml
0.8 ul	for large filter	diluted in
0.4 ul	for small filter	phosph-free BSA

Filters washed with 0.15 M NaCl  
5 times @ 5.0 ul per filter

~~Filters soaked in substrate solution.  
5 Bromo-4-chloro-3 Indoyl phosphatase  
in Veronal Acetate Buffer containing  
NBT Dye.~~

~~1 ul for large filter  
0.5 ul for small filter.~~

~~Incubated at 37° for 30 minutes~~

wrong substrate Discard.

see over.

Filters soaked in substrate-dye solution.

Substrate Naphthol AS-BI Phosphate

Sodium salt 0.1 mg/ml.

Dye - Fast Red TR salt

5-chloro-2-toluenediazonium chloride

benzidine chloride ~~0.5 mg~~ 5 mg/ml.

at 37° for 30 minutes.

where the DNA (glucosylated probe) has hybridized - con A binds.

and since acid-phosphatase will

bind to con A, the enzyme

sticks to those plaques. and

pink-red spots are seen.

Control plate (non-glucosylated probe)

is treated similarly, does not show any reaction.

(B)  $\lambda$  Fel V glucosylated probe  
sandwich technique with  
alkaline phosphatase

7.1.82.

Filters are soaked in phosphate-free BSA  
10' at RT.

Blotted dryish.

Soaked in alkaline phosphatase 0.5 mg/ml  
at RT. for 30'

1.0 ml for large filter

0.5 ml for small filter.

Filters washed free of enzyme

0.15 M NaCl 5-6 times 5 ml per  
filter.

Filters soaked in substrate solution  
containing Dye.

Substrate: 5-Bromo-4-chloro-3-Indoyl  
phosphate in Veronal acetate buffer  
~~NBT Dye~~ Nitro B.T. tetrazolium salt -

1.0 ml for large filter

0.5 ml for small filters.

Incubated at R.T. for 30 minutes

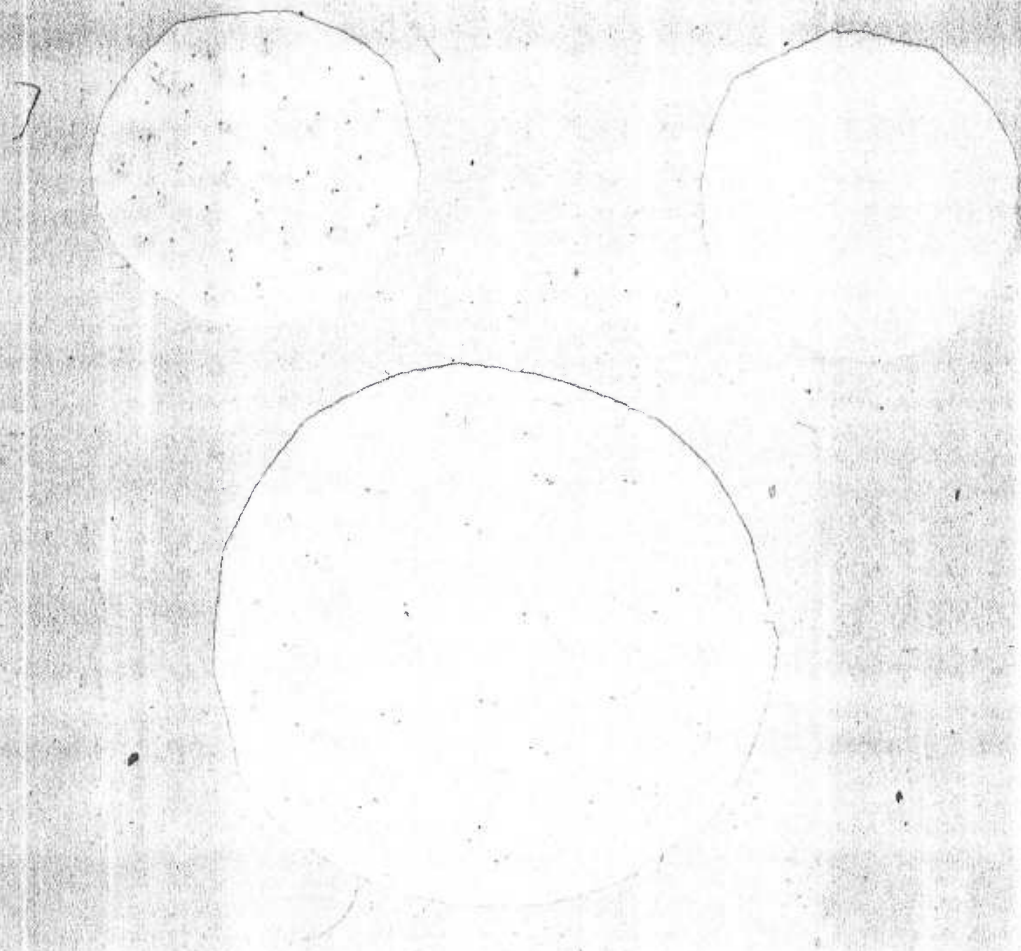
blue spots started appearing on plates  
where glucosylated  $\lambda$  Fel V DNA was hybridized  
to  $\lambda$  Fel V phage plaques.



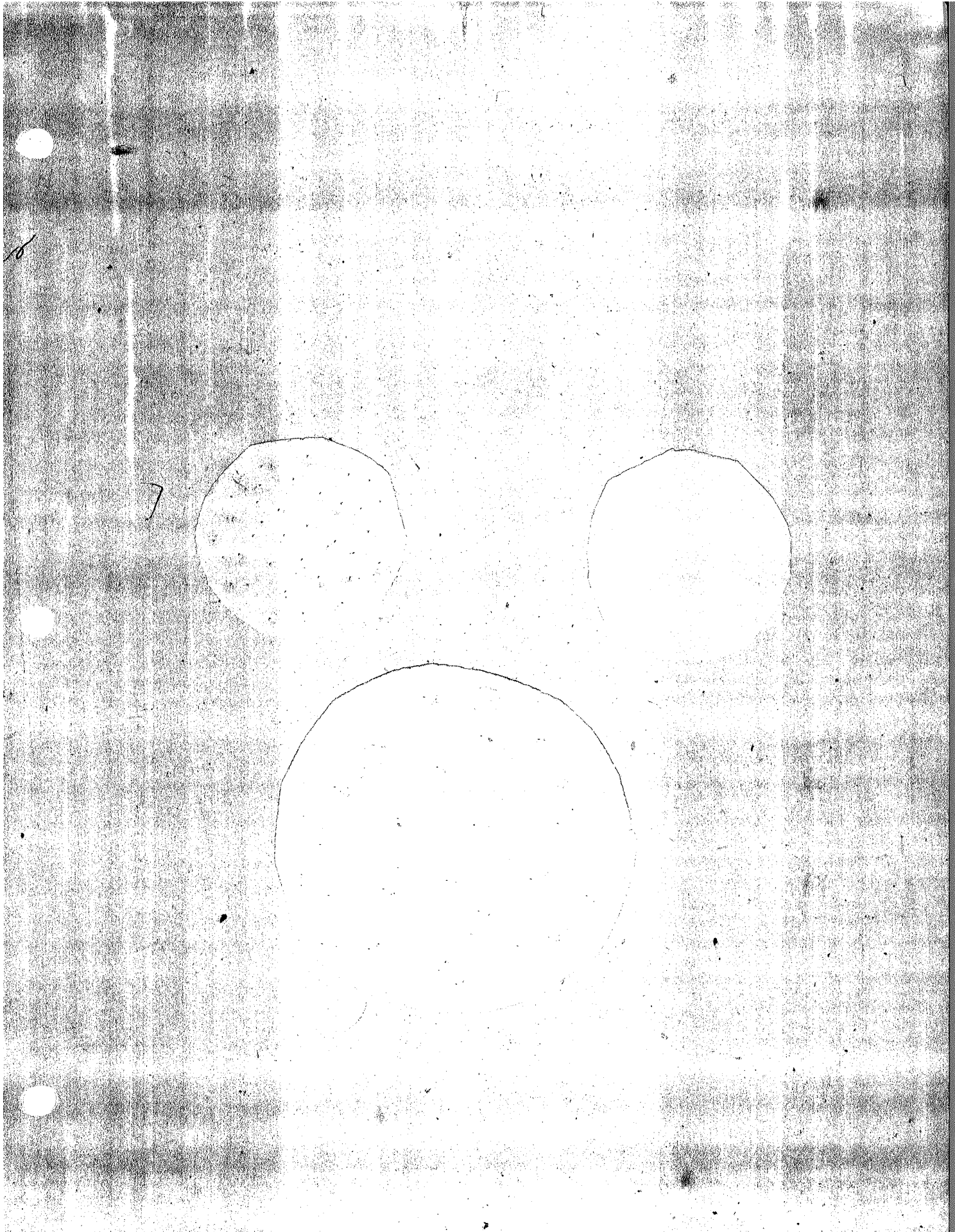
7/1/82

glucosylated probe hybridized to phage  
con A binding plaques  
Sandwich Technique

Acid phosphatase - ~~Tetrazolium method~~  
diago  
Fast Red TR. Salt  
~~s-ecto~~







6.25.82.

Hybridization of malto-triose d UTP substituted  
Ad-2 DNA to Ad-2 virus in fixed cells.

Virus Fixation Fixed slides from Pat O'Hearn.

Adherent virus infected cells [Ad-2 virus]  
grown on coverslips

Non-adherent cells cytocentrifuged into  
microscope slides.

Both are fixed in 0.4% paraformaldehyde  
in pH 7.4 PBS for 20-30 min.

The slides are washed once in 3x PBS  
twice in 1x PBS both for 5' each.

Slides are dehydrated in 30, 60, 80, 95, 100  
percent ethanol [2 min each except for  
5 min in 80%; 95% and 100% two  
containers 1' each used]

and air-dried

slides are stored in humid chamber  
at 4° for 2-4 wks.

Hybridization.

Eight slides used. Each has Ad-2 infected  
cells and uninfected cells.

Checked microscopically to ensure of the  
purity and quality of the preps.

- ① Slides are rehydrated in 100 ul PBS for 10'
- ② slides treated with nuclease-free pronase (0.1 mg/ml in PBS prepared just prior to use 100 ul) for 5'
- ③ Slides immersed in wash buffer once for 5' (2 mg glycine / ml PBS) to stop pronase reaction
- \* Checked microscopically to see extent of pronase digestion.
- ④ Slides washed in PBS twice for 5' each.
- ⑤ Slides dehydrated in 30, 60, 80, 95, 100% ethanol [1 min each except 5' for 80% 1 min twice for 95 and 100%]

⑥ Air dry.

⑦ Hybridization Mixture prepared.

Deionized formamide (pH 6.8-7.2) 50 ul

50% dextran 80<sub>4</sub> 20 ul

20 X SSC 20 ul

Salmon-sperm DNA (acid<sup>†</sup> phenolized) 8 ul  
12 ug/ml

MT-dUTP Ad-2 DNA or 20 ul

TTP Ad-2 DNA

(54 ug/ml)

Mixed well.

20 ul applied to the slide

A - 4 slides TTP Ad-2

B - 4 slides MT-dUTP Ad-2

Covered with coverslip and sealed with white 1 inch tape.

8) Steam heat to  $80^{\circ}$  and held there for 5 minutes  
cooled slowly to  $37^{\circ}$   
Incubated in humid chamber  $37^{\circ}$   
over the week-end.

6/28/82.

①

Detection never let slides dry out.

① Coverslips removed. Slides soaked in 2x ssc ~~10'~~  
at  $32^{\circ}$  (water bath) twice for 10 min each.  
at R.T. once for 10'

② Acetylate 10 min in 0.1 M TEA pH 8.0 (100 ml)  
containing 0.25% acetic anhydride  
prepared just prior to use - (10 seconds.)

③ Slides washed once in 2x ssc for 10'

④ " " twice in PBS 5 min each

⑤ FITC con A (10 mg/ml in 2.0 M NaCl)  
or unlabelled con A (10 mg/ml in 2.0 M NaCl)  
diluted in PBS containing 10 mM Mg<sup>++</sup> to  
give 0.25 mg per ml

0.1 ml applied per slide covered  $\bar{e}$  coverslip.  
Incubated at RT for one hour

⑥ washed thrice for 5 min each in PBS

6.

nothing definite

either probe was no good

A. larger size

B. not enough glycosyl residues.