T4 Expts

ENZO-LNB-0001

LECTIN - BINDING OF T4
DNA

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

Recombinant phage	~~λFel V ias~~ phage λ with Fel V insert.
Glucosylated probe	Fel V insert (isolated from λFel V DNA) nick-translated with malto-triose dUTP

Recombinant phage screening

Check of the isolated insert for nick-translation

	Control	Glucosylated
^{3}H dATP	6 λ	6 λ
10 X NT buffer	2.5 λ	2.5 λ
0.3 mM dNTP	2.5 λ	2.5 λ
0.3 mM TTP	2.5 λ	~~2~~
0.3 mM MTdUTP	—	2.5 λ
DNA	5 λ	5 λ
DNAse 100 µg/ml	2 λ	2 λ
DNA Pol 5u/λ	2 λ	2 λ
dist. H2O	2.5 λ	2.5 λ

14° Incubation

2 µl aliquots removed at 15 min. intervals in 1.5 ml epindorf 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

TTP

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

MTdTTP

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

TTP

Tape	Values	Time
1 30	001.00 / 015.00 / 000319.00	15'
1 31	001.00 / 015.00 / 000206.00	35'
1 32	001.00 / 015.00 / 000341.00	45'
1 33	001.00 / 015.00 / 000202.00	60'
1 34	001.00 / 015.00 / 000244.00	90'
1 35	001.00 / 015.00 / 000276.00	120'
1 36	001.00 / 010.00 / 000491.00	150'
1 37	001.00 / 002.00 / 010969.00	180'
1 38	001.00 / 005.00 / 001773.00	210'
1 39	001.00 / 003.00 / 009174.00	240'

Repeat

MTdTTP

Tape	Values	Time
1 40	001.00 / 015.00 / 000297.00	15'
1 41	001.00 / 015.00 / 000376.00	30'
1 42	001.00 / 015.00 / 000308.00	45'
1 43	001.00 / 010.00 / 000488.00	60'
1 44	001.00 / 010.00 / 000540.00	90'
1 45	001.00 / 007.00 / 000994.00	120'
1 46	001.00 / 007.00 / 001139.00	150'
1 47	001.00 / 010.00 / 000583.00	180'
1 48	001.00 / 005.00 / 002907.00	210'
1 49	001.00 / 010.00 / 000544.00	240'

recombinant phage screening.

Preparation of phage lysates

8.11.82

Wild Type phage:

E. coli RR I carrying λ CI857 phage strain used.

1. On L. broth culture grown at 30° 10 ml inoculated in fresh 1000 luria broth in 2 L. Nalgene flask incubated at 30° c̄ 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 c̄ shaking in boiling water bath till the temp of culture reached 42°
   about 7 minutes
   Held at that temp for 5′

3. Incubated c̄ shaking at 37° for 2½ hrs.

4. Cells spun at 10 K for 30′ at 5°
   and resuspended in basal M-9 salts
   100.0 ml

5. Added 0.5 ml $CHCl_3$ shaken at RT for 30′ and transferred to 0°

recombinant phage screening

8.12.82

6. Added DNase I (1 mg/ml in 0.1 M $MgCl_2$)
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'
opalascent sup transferred to sterile container.

Determine phage titre on RW 262.

	# plaques in 0.1 ml of dilution						
	10^{-2}	10^{-4}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	PFU/ml
λ WT	CL	>200	3	1	–	–	
λ Fel V	–	–	–	–	–	–	Repeat

	# plaques in 0.1 ml of dilution				
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	PFU/ml
λ WT	TMTC	TMTC	TMTC	51	5.1×10^7
λ Fel V	TMTC	TMTC	93	8	8.65×10^6

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Recombinant phage screening

9/3/89

Preparation of the glucosylated probe

125 λ

	Control	Glucosylated
3Hd ATP	12.5 λ	12.5 λ
NT buffer 10x	12.5 λ	12.5 λ
nTP 0.3 mM	12.5 λ	12.5 λ
TTP 0.3 mM	12.5 λ	—
glugluglud UTP 0.3 mM	—	11.72 λ
Fel V insert DNA 200 μg/ml	50 λ	50 λ
DNAse 100 μg/ml	3 λ	3 λ
DNA Pol I 5 u/μl	5 λ	5 λ
d. H_2O	17 λ	17.78 λ

2 hrs at 14°

Rx Terminated with 12.5 λ of 0.2 M NaEDTA

mixture passed thru 4.2 ml G-50 column [equilibrated in 10 mM Tris 7.6 0.1 mM EDTA]

.5 drop fractions collected.

Fel V inser glucosylated G-50 9/3/82

1 16
001.00
000.00
000012.00 1

1 17
001.00
000.00
000022.00 2

1 18
001.00
000.00
000017.00 3

1 19
001.00
000.00
000019.00 4

1 20
001.00
000.00
000022.00 5

1 21
001.00
000.00
000025.00 6

1 22
001.00
000.00
000024.00 7

1 23
001.00
000.00
000022.00 8

1 24
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000.00
000019.00 9

1 25
001.00
010.00
000555.00 10

1 26
001.00
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001419.00 11

1 27
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001484.00 12

1 28
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001785.00 13

1 29
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005.00
001801.00 14

1 30
001.00
005.00
001980.00 15

1 31
001.00
005.00
002188.00 16

1 32
001.00
005.00
002876.00 17

1 33
001.00
003.00
004059.00 18

1 34
001.00
003.00
006113.00 19

1 35
001.00
003.00
007047.00 20

1 36
001.00
003.00
009299.00 21

1 37
001.00
003.00
009843.00 22

1 38
001.00
002.00
011107.00 23

1 39
001.00
002.00
010640.00 24

1 40
001.00
002.00
010541.00 25

1 71
001.00
002.00
010002.00 26

1 72
001.00
003.00
006699.00 27

1 73
001.00
003.00
006344.00 28

1 74
001.00
003.00
005673.00 29

1 75
001.00
003.00
004447.00 30

1 76
001.00
005.00
003062.00 31

1 77
001.00
005.00
002733.00 32

1 78
001.00
005.00
002138.00 33

1 79
001.00
007.00
001163.00 34

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1 80
001.00
007.00
001358.00 35

1 81
001.00
007.00
001068.00 36

1 82
001.00
010.00
000683.00 37

1 83
001.00
010.00
000782.00 38

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

Colony Hybridization with glucosylated probe
Detection with Con A - 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}H dATP	12 λ	12 λ
10 X NT buffer	12.5 λ	12.5 λ
dNTP 0.3 mM	12.5 λ	12.5 λ
TTP 0.3 mM	12.5 λ	—
MTdUTP 0.3 mM	—	12.5 λ
pBR 322 2 mg/ml	15 λ	15 λ
DNAse 10 μg/ml	3 λ	3 λ
DNA Pol. 5000 u/ml	6 λ	6 λ
d. H_2O	53.5 λ	53.5 λ

14^{0} 2 hrs.

Rx terminated c̄ 12.5 λ 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 λ checked for radioactivity.

Control FR #	^3H cpm		Glucosylated FR #	^3H cpm	
1	62		1	19	
2	1796	pooled and concentrated	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	pooled and concentrated
9	7104		9	14158	
10	4555		10	9480	
1	5147		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		

1 13
002.00
020.00
000062.50

1 14
002.00
005.00
001796.00
1 15
001.00
003.00
007649.00
1 16
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003.00
007293.00
1 17
001.00
003.00
005569.00

1 18
001.00
003.00
005939.00
1 19
001.00
003.00
007286.00
1 20
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007130.00
1 21
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007104.00
1 22
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1 26
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005.00
003807.00
1 27
001.00
005.00
003253.00
1 28
001.00
007.00
001454.00
1 29
001.00
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001257.00
1 30
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1 31
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1 32
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1 33
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000020.00
1 37
001.00
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000009.00
1 38
001.00
000.00
000017.00
1 39
001.00
000.00
000034.00
1 40
001.00
003.00
004163.00
1 41
001.00
002.00
014158.00
1 42
001.00
003.00
009480.00
1 43
001.00
003.00
006003.00
1 44
001.00
003.00
005872.00
1 45
001.00
003.00
006417.00

#1

Bacterial Strains :		1, 2,	3, 4
A	E. coli C-600	1, 7, 15	10, 14, 24
B	JM 103	3, 11, 23	8, 20,
C	RSG - OM 13	5, 9	6, 12,
D	PBR 322	2, 10, 14	1, 15, 23
E	pBR 325	4, 12, 20	3, 13, 17
F	pBR 328	6, 16, 22	5, 11, 21
G	pHUB2	8, 18, 24	7, 9, 19
H	Pst gent^R	13, 19	2, 16, 22
I	Ps. aer. 140	17, 21	4, 18

Single colonies on L. agar plates.

Incubate 37° o/n.

8/6/82

Non-sterile filters used
Odd numbers for whatmann 3 mm 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 3 MM 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° o/n in sealed bags in
   2 x SSC
   0.2 % BSA
   0.2 % PVP
   0.2 % ~~Triton X-100~~ Ficoll
   0.01 % SDS

(#1)

8/7/82

(9) Remove filters and blot dryish.
Soak in 2.5 ml Pre.hybridization Mix
50% Formamide
5 X 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 ml [per filter]
0.2 % BSA
0.2 % PVP
0.1 % Triton X-100
0.1 % SDS
2 x SSC
~~at 65°~~

once at Room temp
thrice at 55-60°

Blotted dryish on whatmann 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^{++} buffer added ~~just to soak the solu~~ enough to saturate the filters.

Incubated at RT for 60 minutes

(13) Filters washed 3x in PBS-Mg^{++} buffer

20 ml per filter 10' each time at RT.

~~Blott~~
Coating.

(14) Filters washed 3x in

0.2 M Imidazole pH 6.5
0.15 M NaCl
0.1 % BSA

~~20~~ 8 ml 10 minutes each.

(15) Filters soaked in

1 % BSA in 0.2 M Imidazole (6.5)
0.15 M NaCl

2 hrs at 37° or overnight at 4°

#1

(16) Filters washed 3x in
PBS buffer containing
0.1 % BSA
10' at RT. each time
Blotted dry

(17) Treatment with HRP

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

(18) washed 3x in
PBS containing 0.1 % BSA

(19) Filters soaked in 6 ml of substrate solution
prepared fresh
1 mg/ml DAB mixed with equal volume
of H_2O_2 in H_2O [100 λ per 15 ml] 0.2 %
immediately added and plates stored in dark

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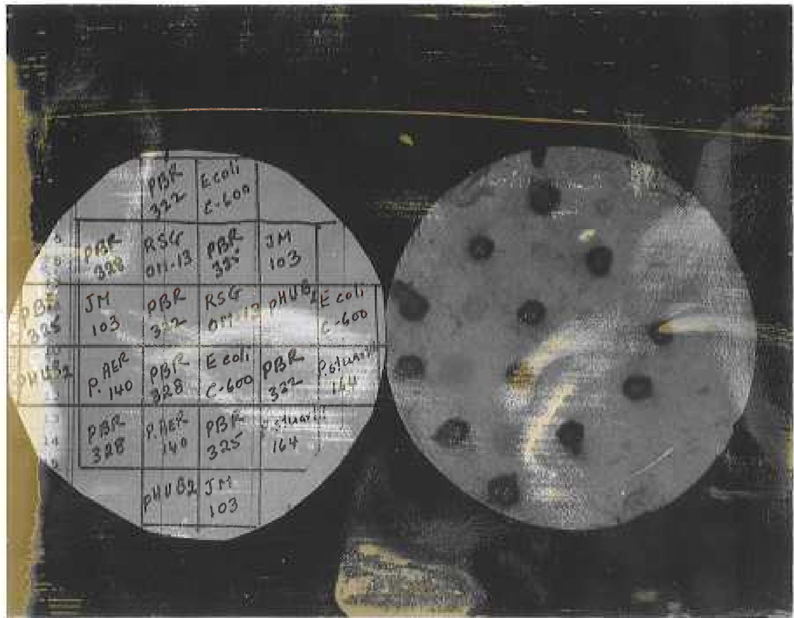

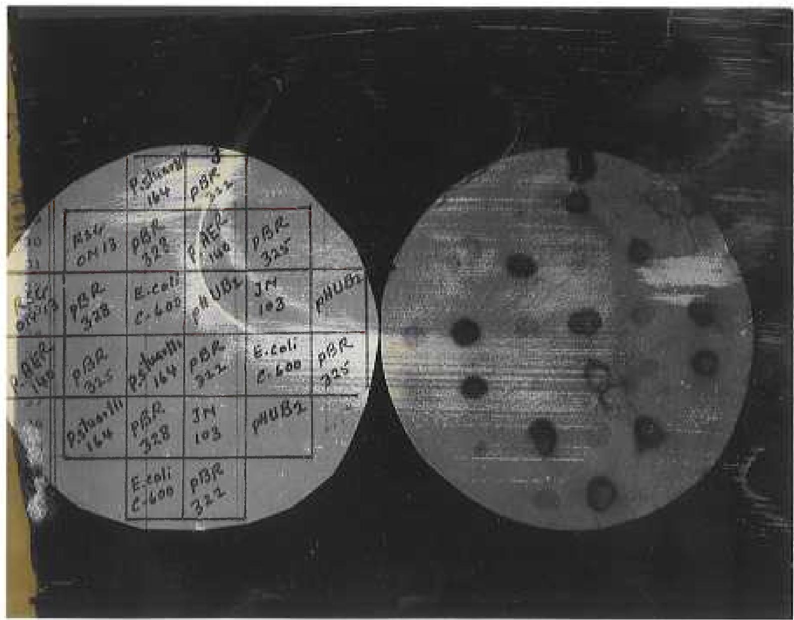

#2

8/12/82

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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 3 MM Filter paper   Make 6 Filters.

Allowed to DRY

(2) Filters soaked on whatmann 3 MM 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)

#2

8/12/82

(4) Soak on whatmann 3 MM paper saturated
with 0.5 M TRIS 7.4
1.5 M NaCL

5 minutes at R.T.

Allowed to DRY on fresh whatmann paper

(5) Repeat step 4

Check with pH paper should be 7.4

(6) ~~A Soak the filter paper in~~
(A) Spot Proteinase K solution
0.25 mg/ml in 1X SSC

on bacterial streaks.

Incubate at R.T. 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

~~(C) Soak~~

(C) Soak Filters in 0.1 % SDS

(D) Soak Filter ~~in~~ ~~GTC CG~~ ~~in EtOH~~ 0.1% SDS

(E) Same as A

(F) Same as B

#2

8/12/82

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

Air DRY

(8)a Bake at 75-80° for 2½ hrs
(8)b Irradiate 10' c̄ UV lamp
(9) Wash filters at 65° overnight in
sealed bags in

2 x 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

5 x 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 SSC

65° for 4 hrs.
buffer changed every hour.

DK mix:
- 0.02% BSA
- 0.02% PVP
- 0.01% Triton X-100

(13) **Con A Treatment**

Wash filters ē 2x 10' each
with PBS-Mg^{++} buffer

Soak the filters in con A solution
0.5 mg/ml in PBS. Mg^{++} buffer
60' at Room Temp.

(14) Wash with PBS. Mg^{++} buffer containing
0.1 % BSA
3x 10' each

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

(16) ~~Wash~~ Repeat step (14)

(17) Treatment with HRP enzyme.

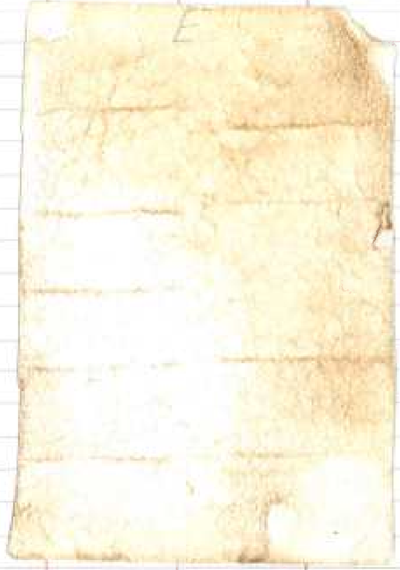
C-600
RSG-OM13
pGP E1
pBR 322
PST 164
E. coli C

C-600
Coli C-600
OM-13
OM-13
pGP E1
E. coli C

OM-13
pBR-322
pGP-E1
P. stuartii
P. stuartii
pBR 322

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 as~~

E. coli C-600 parent strain

E. coli RSG-OM-13 Xylose Isomerase negative

E. coli pGPE1 plasmid carrying Xyl. Isomerase.

E. coli pBR 322 vector plasmid.

Preparation of filters.

Whatmann 3 mm filter 9.2 cm diameter Circles

Thickness

Area $\pi r^2 = 70.11\ cm^2$

(a) 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

#

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(4) Repeat step 3

(5) Soak filters in proteinase K solution 0.25 mg/ml in 1x SSC

15' at RT

~~AIR DRY~~ Blot DRY

(6) Dip filters in 95% EtOH 2 times 5' each

(7) Irradiate 10' at 10 cm distance UV lamp.

(8) Bake at 80° for 2.5 hours in vacuo.

(9) Wash o/n at 65° in

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

(10) Blot filter dryish

(11) Immerse filters in prehybridization mixture 2.5 ml per filter

50 % formamide
5 x SSC
100 µg/ml SS DNA
1 x Denhardt

42° for one hour.

(12) Incubate filters 42° o/n in 1.29 pm.

Prehybridization mix containing ~~glucosylated~~ DNA probes PBR 322

3 Filters used. — 2 (5 & 6) filters c̄ PBR 322 DNA glucosylated

1 with ~~no~~ PBR 322 DNA non-glucosylated

(1)

colony hybridization glucosylated probe pBR-322

(13) Hybridization solution removed
Filters washed in wash buffer ~~con~~
30' twice at R.T.
60' twice at 65°
30' once at R.T. in 0.1 % Triton X-100
0.1 % BSA
2 x SSC.
Blotted dry.

(14) Filters soaked in
PBS. Mg++ buffer containing con A
0.5 mg/ml.
#1 and #6 filter only.
#5 soaked in PBS. Mg++ buffer. - con A.
60' at RT.

(15) Filters washed in PBS - Mg++ buffer
3 x 20' each
containing 0.1 % BSA
0.15 M NaCl

(16) Filters blocked 37° 2 hrs in
1 % BSA acidified
0.15 M NaCl } 2xSSC can be used.
PBS pH 7.0 }

(17) Filters washed 2x in
0.1 % BSA
2 x SSC

Colony hybridization
glucosylated probes.

(18) Horse Radish peroxidase 5 mg/ml
diluted to 100 ug/ml in PBS pH 7.0
0.1 % BSA.

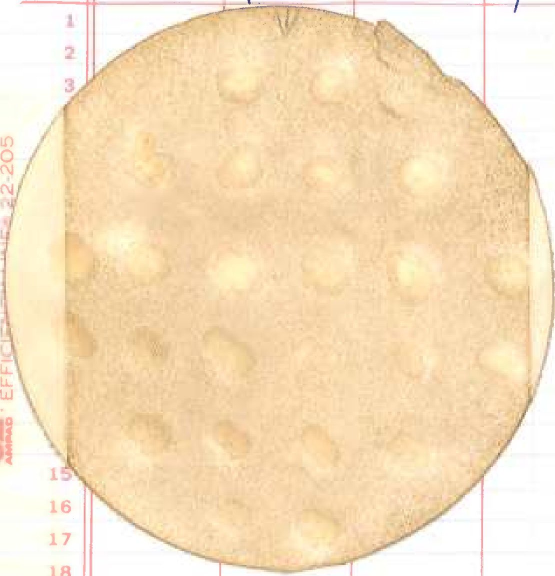
Filters soaked in 1.0 ml enzyme.
Incubated 30' at R.T.

(19) Filters rinsed in
PBS pH 7.0
0.1 % BSA
0.15 M NaCL

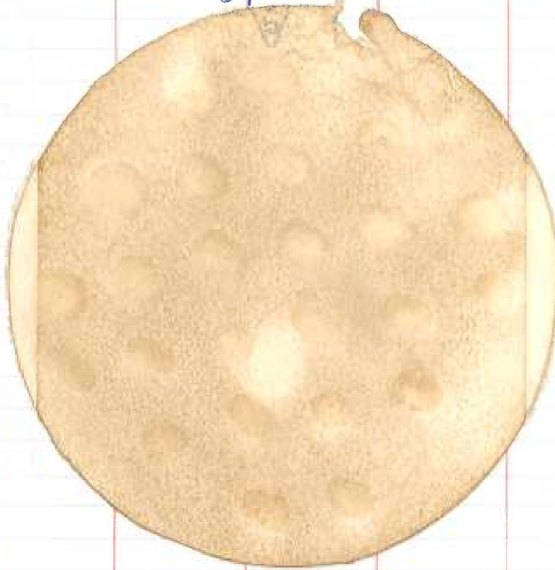
3 x 20' each at R.T.

(20) Filters soaked in 5.0 ml of substrate
solution.
1 mg/ml DAB mixed with equal volume
of 0.2 % H_2O_2
Immediately added plates in the dark

Nonglucosylated pBR 322
Complete Detection System



glucosylated pBR 322
Detection system. without Con A



glucosylated pBR 322
Complete Detection system.

Colony Hybridization glucosylated DNA probe 9/3/82

Colonies picked by Peter

Treatment of filter.

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

② DRY on Filter paper

② Repeat step ①

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

DRY on Filter paper

④ Repeat step ③

⑤ Soak filter in 0.25 mg/ml Proteinase K
15' at RT.

Blot DRY

⑥ Soak filters in 95% EtOH 3x 5' each

AIR DRY

⑦ Bake 2-3 hrs at 70°

⑧ Store in a sealed bag

9/7/82

⑨ Incubate 65° 4 hrs in

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

Colony hybridization glucosylated DNA

(10) ~~Pre-fue~~ Wash filters in

2 X SSC 37° 1 hr.

65° 1 hr

37° 1 hr.

RT 1 hr.

(11) Prehybridize filter at 42° 1 hr

in 50 % Formamide

EFFICIENCY LINE® 22-205

Colony hybridization glucosylated p

71.

Preparation of the probe.

	Control	125λ	glucosylated
^{3}Hd ATP	12.5λ		12.5λ
10x NT buffer	12.5λ		12.5λ
0.3 mM nTP	12.5λ		12.5λ
0.3 mM TTP	12.5λ		—
0.32 mM glucosyl dUTP	—		11.72λ
p GPE-1 insert 1 μg/μl	10 λ		10 λ
DNase 100 μg/ml	3λ		3λ
DNA pol. I 5u/μl.	5λ		5λ
d. H_2O.	57λ		57.28

2 hrs at 14°

Rx Terminated c̄ 12.5 λ 0.2 M EDTA

Rx mixture passed thru 4.2 ml G-50 column equilibrated in 10 mM Tris 7.6
0.1 mM EDTA

First radioactivity peak collected concentrated by vacuum evaporation.

PGP E1 glucosylation 9/3/82 G-50

Tube				Fraction
1 16	001.00	000.00	000022.00	1
1 17	001.00	000.00	000016.00	2
1 18	001.00	000.00	000017.00	3
1 19	001.00	000.00	000020.00	4
1 20	001.00	000.00	000017.00	5
1 21	001.00	000.00	000009.00	6
1 22	001.00	000.00	000017.00	7
1 23	001.00	000.00	000022.00	8
1 24	001.00	000.00	000025.00	9
1 25	001.00	020.00	000111.00	10
1 26	001.00	015.00	000261.00	11
1 27	001.00	010.00	000444.00	12
1 28	001.00	010.00	000668.00	13
1 29	001.00	007.00	001022.00	14
1 30	001.00	007.00	001506.00	15
1 31	001.00	005.00	002330.00	16
1 32	001.00	005.00	003419.00	17
1 33	001.00	003.00	005070.00	18
1 34	001.00	003.00	006994.00	19
1 35	001.00	003.00	008709.00	20
1 36	001.00	003.00	009473.00	21
1 37	001.00	003.00	009563.00	22
1 38	001.00	003.00	008620.00	23
1 39	001.00	003.00	005304.00	24
1 40	001.00	003.00	006012.00	25

DNA binding to activated glass surface 8.2.82.

T_4 DNA 4 mg/ml

diluted to contain

A 1000 ng/ml

B 100 ng/ml

C 10 ng/ml

D 1 ng/ml

E 0.4 ng/ml

100 λ of each solution in duplicates in activated glass tubes

10′ at RT.

Remove carefully.

Rinse tubes 3 x c̄ 100 λ of 2 x SSC

100 λ of con A added 0.1 mg/ml in PBS. Mg^{++}

F con A

G PBS. Mg^{++}

left at RT for 60′ 11.47 am.

Rinse 3 x c̄ 100 λ of 2 x SSC Incubate RT 60′

To set 1 added 100 λ PBS. Mg^{++}

set 2 added 100 λ 1% Ficoll 400

Wash 3 x $\bar{c}$ 2 x SSC

Add 100 λ PBS·Mg^{++} to set I

100 λ Con A 0.1 mg/ml in PBS·Mg to set II

60' at RT.

Wash 3 x $\bar{c}$ 2 x SSC

3 x $\bar{c}$ 0.1 % BSA in 2 x SSC

Add 100 λ 1 % BSA in 2 x SSC to

all tubes.

Cover $\hat{c}$ parafilm

Keep at 4° o/n.

8/3/82

Rinse 3 x $\bar{c}$ 2 x SSC containing 0.1 % BSA

Add 10 λ of 1/5 diluted acid phosphatase

40 λ of diluent containing 0.1 % BSA

Incubate at RT for 30'

Remove

Rinse 5 x $\bar{c}$ 0.3 M NaCl

Add 20 λ of 0.1 M substrate
80 λ of 0.2 M Imidazole buffer (6.5)

Incubate at 37° for one hour.

~~Dilute~~
Stop Rx c̄ 2.0 ml 5% $NaHCO_3$.

Check OD at A_{410}.

1	2 DNA	3 Cou A	4 Ficol	5 Cou A	6 Acid P.	7	A410	9
A	50 ng	+	−	−	+		0.285 x 20	
A2	50 ng	+	+	+	+		0.35 x 20	
B1	5 ng	+	−	−	+		0.175 x 20	
B2	5 ng	+	+	+	+		0.23 x 20	
C1	500 pcg	+	−	−	+		0.06 x 20	
C2	500 pcg	+	+	+	+		0.12 x 20	
D1	50 pcg	+	−	−	+		0.11 x 5	
D2	50 pcg	+	+	+	+		0.22 x 5	
E1	20 pcg	+	−	−	+		0.144 x 2	
E2	20 pcg	+	+	+	+		0.208 x 2	
F1	−	−	−	−	+		0.006	
F2	−	+	+	+	+		0.007	
G1	−	−	−	−	+		0.006	
G2	−	−	+	−	+		0.005	
Control	−	−	−	−	+		0.35 x 20	

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Con A - alkaline phosphatase binding. 7/28/82.

Eliza method in micro-titer plates.

Dilute con A (10 mg/ml in 2.0 M NaCl) in PBS·Mg++ buffer to give.

A 200 μg/ml

B 20 μg/ml

C 2 μg/ml

D 0.2 μg/ml.

50 λ from each dilution in triplicates vertical rows.

Cover and leave at 4° o/n.

7/29/82.

Remove Con A

Wash wells 3x c̄

100 λ 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 λ of 1 % BSA
0.1 M Tris.CL pH 7.6
0.15 M NaCL

at 37° for 2 hrs.

Washed 3 x ē
100 λ of 0.1 % BSA
0.1 M Tris-CL pH 7.6
0.15 M NaCL

"horizontal rows" ←

Added ~~Ver~~ horizontal rows.

50 λ of 200 ug/ml
20 ug/ml
2 ug/ml
0.2 ug/ml
} Alkaline phosphatase (1.8 ug/ml stock solution)

50 λ of 200 ug/ml
20 ug/ml
2 ug/ml
} Horse Raddish Peroxidase 10 ug/ml stock solution

Left at room temp for 60'

Remove

Rinse wells 3 x $\bar{c}$

100 λ of 0.1 % BSA

0.1 M Tris Cl (pH 7.8)

0.15 M NaCL

last rinse $\bar{c}$ 200 λ of solution.

Add substrate 100 λ

Incubate 37° for one hour

left too long.

qualitative results.

repeat.

DNA Dot Blots. 8/2/82.

1 λ DNA in 0.1 x SSC
range 2 ng to 3 pcg.

Spotted on Nitro cellulose strips.
Strips placed on whatman 3mm paper soaked in
0.5 M NaOH
1.5 M NaCL
for 10 minutes
Strips washed 3 x in 10 ml of
0.5 M Tris.CL pH 7.4
1.5 M NaCL
for 10 minutes each time.

Strips baked at 70° in vacuo for 3 hrs.

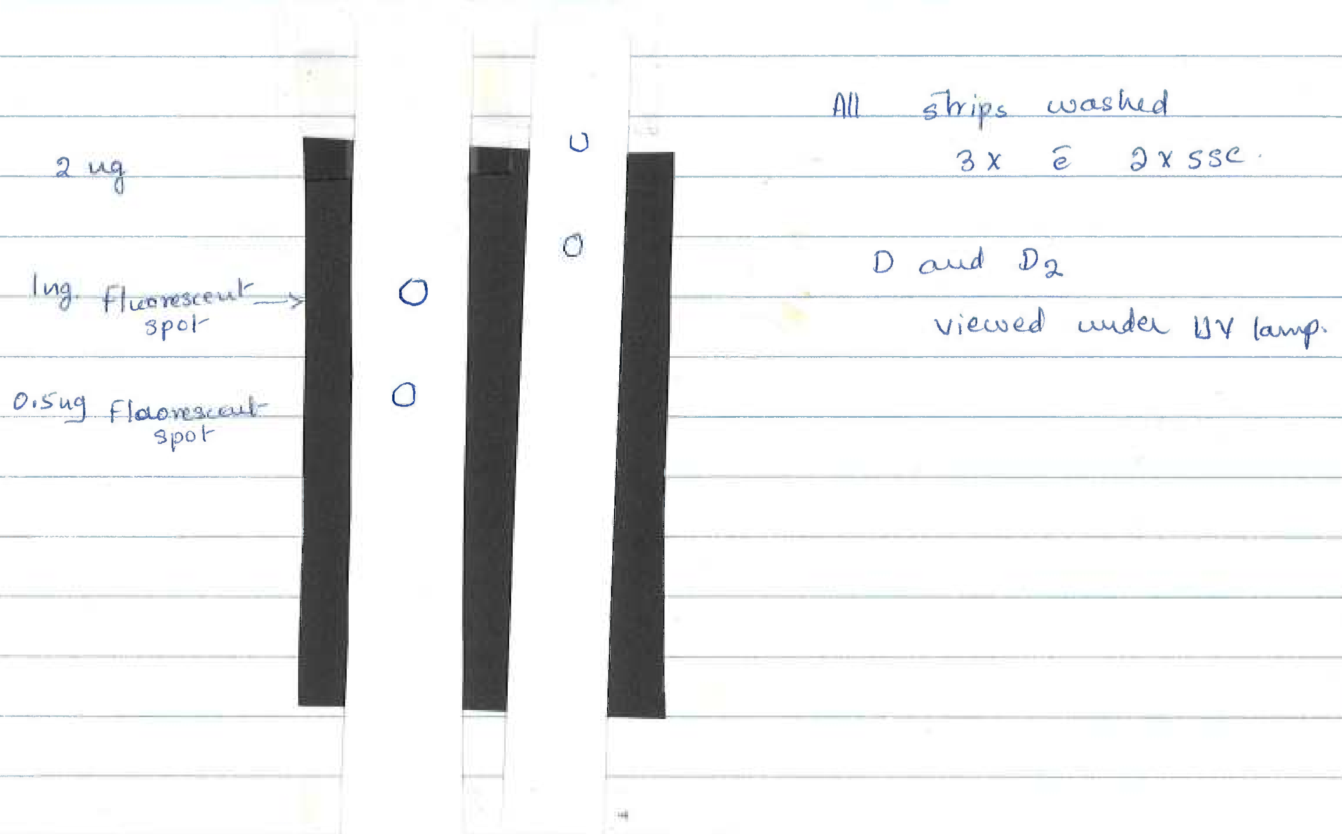
Strips blocked in
0.2 % BSA
0.2 % PVP
0.1 % Triton X-100
2 X SSC
0.1 % SDS.

at 60° for 2 - 4 hrs. 10 am.

Con A binding.

Strips D_1 and D_2 soaked in FT-Con A 0.1 ug/ml
in PBS.Mg^{++} RT 60′ 2.0 ml total. in the dark

Strips E_1, E_2, F_1, F_2, G_1 and G_2 soaked in
10 ul con A 0.1 ug/ul in PBS·MgCl
RT 60'



All strips washed
3x c̄ 2x SSC.

D and D_2
viewed under UV lamp.

~~Strips~~ Strips E_1, E_2, F_1, F_2, G_1 and G_2

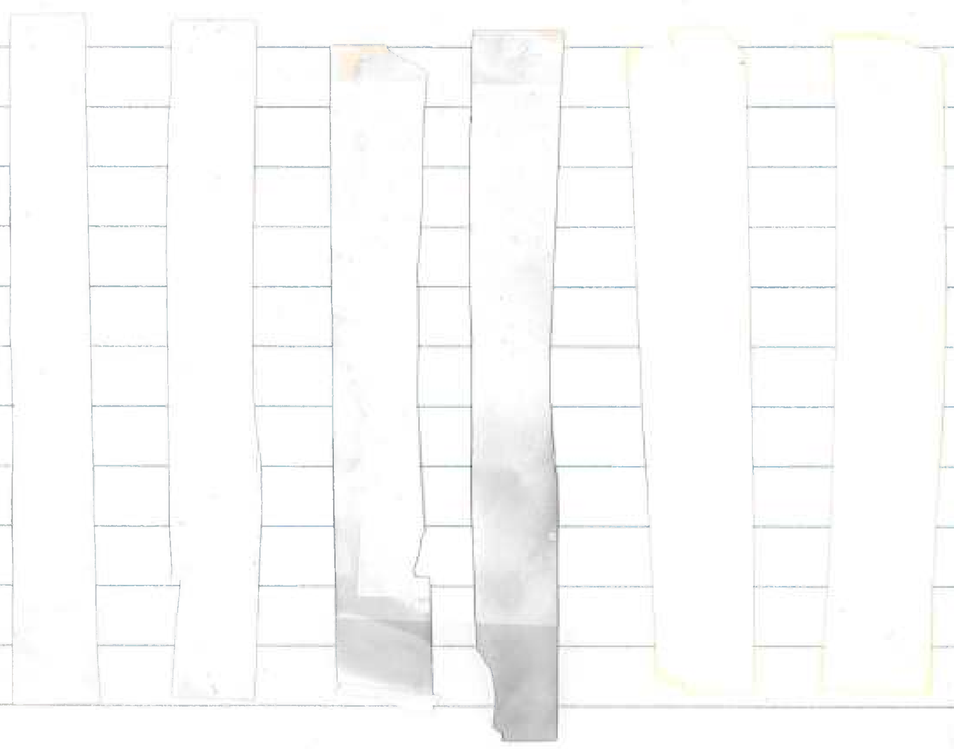
washed 3x c̄ 0.1 % BSA in 2x SSC.

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

washed 3x c̄ 0.1 % BSA in 2x SSC.

E_1 and E_2	10 ml	0.1 % BSA 2 x ssc	
		0.1 mg/ml HRP	
F_1 and F_2	10 ml	0.1 % BSA 2 x ssc	
		0.1 mg/ml Alk. Phosph.	
G_1 and G_2	10 ml	0.1 % BSA 2 x ssc	
		units Acid Phosp.	

Incubated at 37° one hour



Con A - alkaline phosphatase 7/26/82

Con. Curve.

Dilute con A (10 mg/ml Stock solution) in PBS Mg++

to give

A	200	μg/ml
B	20	"
C	2	"
D	0.2	"

Deliver 50 λ 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 λ of 0.1 % BSA in
0.1 M TRIS-CL (7.8)
0.15M NaCL
0.02 % Tween 20

~~Deliver 10~~
Coat wells $\bar{c}$ 100 λ of 1 % BSA in
0.1 M TRIS CL (7.8)
0.15M NaCL

Incubate at 37° 2 hrs ~~2 hrs~~.

left at 4° o/n.

7/28/82.

Remove coating solution.

Wash 3 x $\bar{c}$ 100 λ of 0.1% BSA in
0.1 M TRIS.Cl (7.8)
0.15 M NaCl

Enzyme Alkaline phosphatase.
0.5 mg/ml.

diluted in 0.1 M TRIS pH 7.8 to give

200 ug/ml
20 ug/ml
2 ug/ml
0.2 ug/ml
0.02 ug/ml

50 λ ~~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.

T4 DNA 200 μg/ml in 10 mM TRIS 7.4
10 mM NaCl

5λ DNA diluted c̄ 225 λ 0.1x SSC
Final con. DNA 4 μg/ml.

Denaturation

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

F.C. NaOH 0.3 M

diluted c̄ 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 λ on 8 strips Nitrocellulose.

~~Baked at 80° in vacc~~

Washed 2 x ē 1.0 M Am Acetate

Baked at 80° in vacuo for 2½ hrs.

Coated o/n at 65° in

0.2 % Acidified BSA
0.2 % PVP
0.1 % Triton X-100
2 x SSC
0.1 % SDS.

7.29.82

Strips soaked in 5.0 ml solution
0.1 mg/ml Con A in PBS.Mg^{++}
at RT. for 60'

Washed ē 2 x SSC three times.

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

Soaked in Alkaline phosphatase
200 μg/ml at RT. for 30'

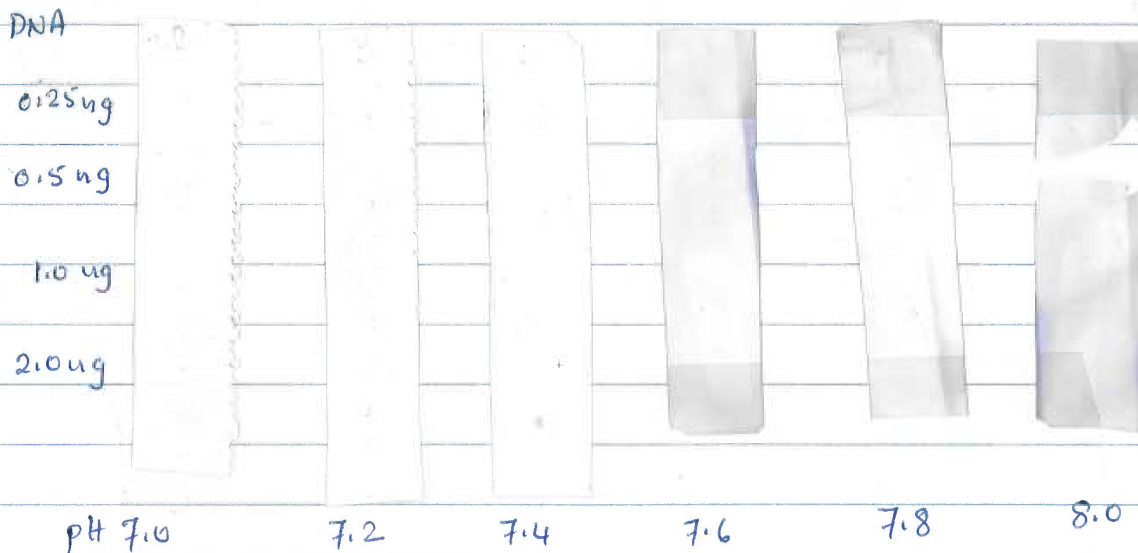
Washed repeatedly in 0.1 % BSA in 2 x SSC.

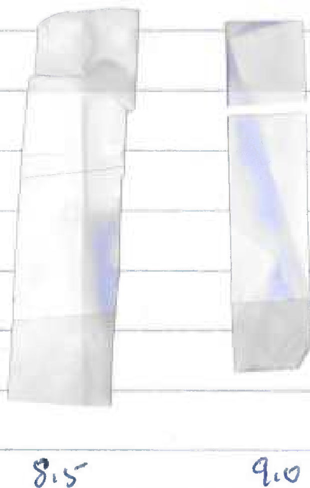
Substrate Naphthol AS-BI phosphate 0.1 mg/ml in TRis 0.1 M

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 con A - Alk Phosp.

best at pH 7.4

N. Keller
7/26/82

Con A binding of glucosylated DNA 7.23.82.

Eliza method Sandwitch Technique

Con. Curve.

T4 DNA 0.2 mg/ml in 10 mM Tris 7.2
10 mM NaCl

~~20~~ A 0.2 mg/ml
B 0.1 mg/ml
C 0.05 mg/ml
D 0.025 mg/ml
E 0.0125 mg/ml
F 0.00625 mg/ml.

Spotted on Nitro cellulose strips at 1.5 cm distance

~~0.6 H2O~~ 3 λ
2 λ
1 λ
0.5 λ
0.25 λ
0.1 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.

Air dried
Baked at 80° under vacuum for 2.5 hours.
Coated at 65° o/n in 1.25 ml 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.

Con. A treatment

Soak strips in Con A solution. 10 ml Total

0.1 mg/ml in PBS. Mg^{++} buffer. for 6 strips

at RT for ~~30~~ - 60 minutes.

Wash c̄ 0.1 M TRIS pH 7.2 3 X

Air Dry.

Enzyme Treatment

~~Apply alkaline phosphatase 4 λ of 0.5 mg/ml solu.~~
~~Air to the spots.~~

Soak the strips in alkaline phosphatase solu.

10 μg in 0.25 ml Tris 0.1 M pH 7.2.

per strip.

leave at R.T. for 30 minutes.

Wash c̄ 0.3 M NaCL 10 ml each time.

3 — 5 X

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

0.5 mg/ml Fast Blue BB } in 0.1 M Tris

0.1 mg/ml Naphthol AS-BI } pH 7.5

Incubate at ~~R~~ 37° for one hour

N. Kelley 7/26/82

con A + con A −

1	A	B	C	D	E	F	G	H
DNA	600 ng	300 ng	150 ng	75 ng	37.5 ng	18.75 ng	−	−
	400 ng	200 ng	100 ng	50 ng	25 ng	12.5 ng	−	−
	200 ng	100 ng	50 ng	25 ng	12.5 ng	6.25 ng	−	−
	100 ng	50 ng	25 ng	12.5 ng	6.25 ng	3.125 ng	−	−
	50 ng	25 ng	12.5 ng	6.25 ng	3.125 ng	1.5625 ng	−	−
	20 ng	10 ng	5 ng	2.5 ng	1.25 ng	0.625 ng	−	−

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N. Keller 7/23/82

1

Binding of glucosylated DNA to lectin — detection by sandwitch Technique

7.22.82

T_4 DNA 0.2 mg/ml.

λ plac 5 DNA 0.516 mg/ml.

4 Nitro cellulose strips 1.5 x 7 cm [S.S.]

marked 1, 2, 3, 4

On 1 and 2

T_4 DNA at 1.5 cm distance.

3λ = 0.6 ug

2λ

1λ

0.5λ

On 3 and 4

λ plac 5

3λ

2λ

1λ

0.5λ

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

2x

Baked at 80° 2.5 hrs. in vacuo.

N Keller
7/23/82

2

Strips coated at 65° o/n 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^{++} buffer

0.25 ml per strip

1 and 3 soaked in PBS. Mg^{++} 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 TRS pH 7.4

10' each time.

Enzyme Treatment

Strips soaked in (0.25 ml per strip)

0.1 mg/ml Alkaline phosphatase

30' at RT.

Washed c̄ 0.15 M NaCL 5 ml 10' thrice

Soaked in substrate-dye solution

~~5.0 ml~~ 2.5 ml for 4 strips

0.5 mg/ml Fast Blue BB

diazotized 4'-amino

2', 5' Diethoxybenzanilide

Zinc chloride

0.1 mg/ml Naphthol AS-BI phosphate

in 0.1 M TRis pH 7.5

(Dye 10 mg/ml in 0.1 M TRis 7.2

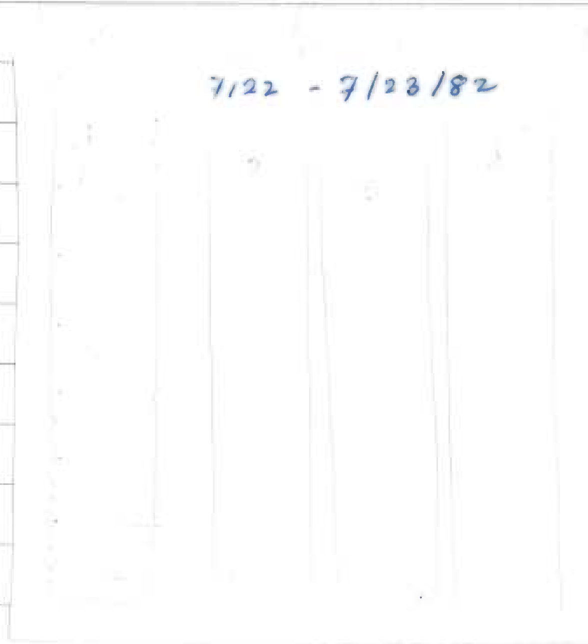
Substrate 10 mg/ml in 0.1 M TRIS 7.2.)

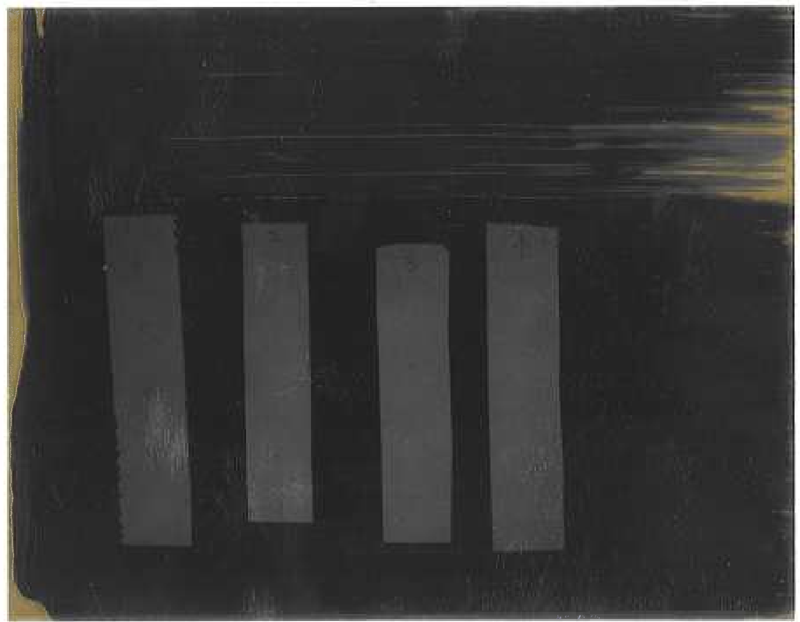
Incubated at 37°

Blue spots immediately within 5'

on # 1 strip only

N. Kelln 7/23/82





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

2 X 8 cm nitrocellulose strips

1 λ DNAs spotted on all strips

T_4
λ
glucosylated λ
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 vaccum 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_4 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_4 pH 7.2
150 mM NaCl
for the same length of time.

Solution removed.
Strips washed 3x c̄ PBS.Mg^{++} buffer.

A, C, E } Soaked in 8.0 ml PBS.Mg^{++}
B, D, F } containing 0.5 mg/ml
Con A
G and H in PBS.Mg^{++} buffer at RT for 30'

7.21.82

Treatment with acid phosphatase

Strips A and B washed 3 x ē 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 con 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 μg/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 vaccum at 80° for 2 hrs

1. T_4 DNA 0.28 mg/ml ~~10λ~~ 5λ

2. λ Fel V 1.0 mg/ml 5λ

3. PST 1.4 mg/ml 5λ

4 PBR 322 2.0 mg/ml. 2.5 λ

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

10' at RT.

Chill in ice bath.

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

Apply 5λ To nitro cellulose 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 vaccum 2 hrs

Filter Prehybridization.

Prehybridize filters at 42° for 90' in
solution containing
50 % Formamide
5 x SSC
100 μg/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 Dehardt
10 % Dextran Sulphate

Hybridize filters in 1.0 ml above buffer
containing 10 λ of heat-denatured probe
[95° for 5' quickly cooled]
at 42° for 16 hours.

Wash the filters at 37° 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_4 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_4 7.2
150 mM NaCL
0.1 % ~~glut~~ glutaraldehyde
at R.T. for 3 hours.

Set of B filters
in 10 ml 50 mM K-PO_4 pH 7.2
150 mM NaCL.

Buffers removed.
Washed 3 x $\bar{c}$ 50 mM K-PO_4 7.2
150 mM NaCL.

Treatment with Con A.

Filters soaked in 5.0 ml
0.5 mg/ml conA in
PBS 6.5 containing
$MgCl_2$ 10 mM

30 minutes at R.T.

Con A removed.
Filters washed 3 x c̄ 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 c̄ 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 developement.

NEGATIVE

preparation of FeL V insert 7.19.82

λ FeL V DNA 1.0 ug/ul
EcoRI 5000 u/ul

500 λ Assay Mix

10 x Buffer	50 λ
DNA	100 λ
EcoRI	10 λ
d. H_2O	340 λ

37° 90 minutes

Added 10 λ SDS. EDTA
200 λ dye solution.

200 λ mixture electro forezed
0.7% agarose gel.

No digestion.

phenolize to recover the DNA.

Eco R I digestion of 7.16.82.
λ DNA and λ Fel V DNA

λ DNA	FROM	Chris Brackel	1.6 ug/ul.
λ Fel V	From	Henry Cudney	1.0 ug/ul.
EcoRI	FROM	NBL	5000 u/ul.

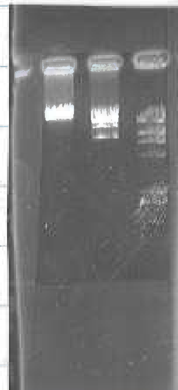
	1	2	3	4
10 x Buffer	1λ	1λ	1λ	1λ
λ DNA	1λ	1λ	—	—
λ Fel V DNA	—	—	1λ	1λ
EcoRI	—	1λ	—	1λ
dist. H_2O	8λ	7λ	8λ	7λ

37° 60'

Rx Terminated c̄ 2 λ SDS EDTA
70° for 5'

Added 12 λ dye solution electroforessed 1% agarose

KB.
25.6
7.31
6.16
5.55
4.84

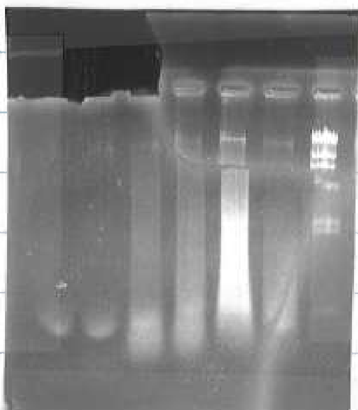


Run parallel c̄
Hind III digested
λ

8kb. piece
of interest

Gel electroforesis of DNA probes 7.14.82.

1. TTP pBR 322 2.5 λ
2. glucosyl. pBR 322 "
3. TTP Pst "
4. glucosyl Pst "
5. TTP λ Fel V "
6. glucosyl λ Fel V "



23.5

preparation of glucosylated probe 7.8.82.

PST DNA

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

	Control	Test
	Rx 250 λ	
^3HdATP	60 λ	60λ
10 x NT buffer	25	25
dNTP 0.3mM	25	25
TTP 0.3 mM	25	—
mTdUTP 0.3 mM	—	25
PST DNA 1.4 mg/ml	0.142 ml	0.142 ml
DNase I 10 μg/ml	20λ	20λ
DNA Pol 5 u/μl	12λ	12λ
dist. H_2O	0	0

14° for 2 hrs

Rx Terminated with 25 λ of 0.2 M EDTA

2 microliter to check for incorporation TCA insoluble

G-50 column chromatography of Nick-translated DNAs.

7.9.82

FR #	^{3}H cpm
1	277
2	77
3	17
4	65
5	14611
6	58098
7	63499
8	38848
9	35985
10	35838
1	34654
2	32986
3	27563
4	2114
5	27496
6	25260
7	20777
8	536
9	12031
20	9006

PST glucosylated DNA

4.8 ml column.

6 drop fractions.

10 mM TRIS pH 7.5 buffer
0.1 mM EDTA eluent.

FR #	^{3}H cpm
1	44
2	7
3	22
4	33
5	9
6	12
7	14
8	4280
9	63318
10	66291
1	53561
2	49679
3	[illegible]543
4	38140
5	38457
6	34734
7	36834
8	40663
9	23944
20	24963

PST non-glucosylated

Dollie 7/9/82

Dollie 7/9/82

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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 times.

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 bags, 10 ml per bag, 4 filters per bag. at 65° overnight.

7.8.82.

Filters were blotted dryish and stored in sealed bag 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 ml per bag 4 filters per bag.

Pre-hybridization Mixture Total 40 ml.

20.0 ml Formamide.
10.0 ml 20 X SSC.
0.4 ml 100 x Denhardt mix
0.44 ml S.S. DNA.
H_2O to 40.0 ml.

At 42° for 2 hours

7.13.82.

Pre-hybridization mixture carefully removed and filters soaked in

Hybridization mixture

125 λ probe | heated at 80° for 3'
20 ml pre-hybridization | and quickly cooled
mixture

10 ml per bag 4 filters per bag.

Incubated in sealed bag at 42°
for 72 ~~24~~ hours.

~ 30 μg 2.75024 $\times 10^6$ cpm glucosylated probe
~ 25 μg 3.172955 $\times 10^6$ cpm TTP probe.

7.16.82.

Filters removed and washed 3 x in wash buffer
Air-dried.

7.21.82.

Filters treated with con A 0.5 mg/ml in
PBS. Mg^{++} buffer
1.0 ml per filter.
Soaked at R.T. 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 mg/ml in dist. H_2O.
diluted to 0.1 mg/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 mg/ml BSA.
Blotted dry and placed in another petri dish.

Substrate solution made fresh.
1 mg/ml DAB mixed with
equal volume of H_2O_2 in H_2O [100λ per 15 ml]
25 ml solution made and quickly added to filters. 5.0 ml per filter.

~~brown spots~~
Colonies hybridized with glucosylated probe
gave brown spots.
Controls were negative.
Still background problem.

Use of glucosylation-lectin system for colony hybridization.

7.13.82

pBR 322 2.0 mg/ml
3H d ATP
malto-triose } 0.3 mM
d UTP }

	Control	Glucosylated
3H d ATP	25 λ	25 λ
10X NT buffer	12.5 λ	12.5 λ
MT d UTP	—	12.5 λ
dNTP 0.3 mM	12.5 λ	12.5 λ
TTP 0.3 mM	12.5 λ	—
pBR 322 2 mg/ml	25 λ	30 λ
DNase 10 μg/ml	6 λ	6 λ
DNA Pol 5 u/λ	6 λ	6 λ
dist. H2O	45.5 λ	45.5 λ

2 hrs 14° 1.40 pm.

Rx Terminated c̄ 12.5 λ of 0.2 M EDTA
Rx mixtures passed thru G-50 1.0 ml in Syringes.

First peak radioactivity collected
15 λ checked for counts.

3 #2
001.00
000.00
000011.00

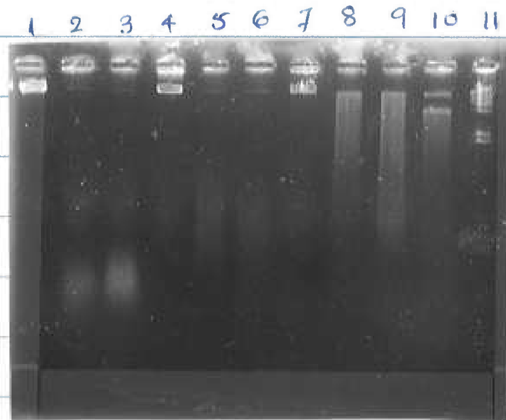
3 #3
001.00
000.30 TTP
684591.00 PBR 322

3 #4
001.00 gluessy
000.30 PBR
550043.00 322

Gel electroforesis of nick translated DNAs   6.29.82.

1. Ad-2 DNA   ~ 1 μg
2. Ad-2 DNA nick translated control   "
3. " " " " glucosylated   "
4. λ Fel V DNA   "
5. " nick translated control   "
6. " " " glucosylated   "
7. Pst DNA   "
8. " nick translated control   "
9. " " " glucosylated   "
10. PBR 322   "
11. λ Hind III marker

1 2 3 4 5 6 7 8 9 10 11



G-50 column chromatography.
nick translated DNA λ Fel V.

7/19/82

λ Fel V nick translated c̄ TTP

6 drop fraction.

3.5 ml column.

pH 7.5 TRIS 10 mM, EDTA 0.1 mM } buffer

Fraction	Count	
1	18	
2	12	
3	14	
4	11	
5	21	
6	15	
7	1871	lyophilized
8	8728	lyophilized
9	21008	lyophilized
10	18183	lyophilized
1	22063	lyophilized
2	24978	lyophilized
3	28155	lyophilized
4	23958	lyophilized
5	21710	lyophilized
6	16482	lyophilized
7	10920	lyophilized
8	7082	lyophilized
9	185	
20	4152	
1	1987	
2	1270	
3	38	
4	799	

λ Fel V nick translated c̄ ~~Bio~~ malto-Triose dUTP.

3.5 ml column

TRIS 10 mM, EDTA 0.1 mM } pH 7.5

6 drop fractions.

Fraction	Count	
1	13	
2	20	
3	13	
4	89	
5	15	
6	11	
7	1355	lyophilized
8	13075	lyophilized
9	23475	lyophilized
10	20874	lyophilized
1	23071	lyophilized
2	28437	lyophilized
3	21240	lyophilized
4	37682	lyophilized
5	27418	lyophilized
6	19345	lyophilized
7	13977	lyophilized
8	10390	lyophilized
9	5918	
20	7117	
1	4872	

Dollie
7/9/82

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Preparation of glucosylated probe. 6.28.82.

λ Fel V DNA.

	control	glucosyl. DNA
	0.5 ml Rx	
^{3}HdATP	40 λ	40 λ
10 X NT buffer	50 λ	50λ
d NTP	50λ	50λ
TTP	50 λ	–
MT d UTP	–	50λ
λ Fel V	100λ	100 λ
DNAse 0.1 µg/ml	40λ	40λ
DNA Pol. 20u/λ	5λ	5λ
d. H_2O	165λ	165λ

2 hrs at 14°

Rx Terminated c̄ 50 µl of 0.2 M EDTA

2 µl aliquot mixed c̄ 5 µl carrier DNA

TCA insoluble cpm determined.

Large scale preparation of glucose-substituted PST DNA — 6.25.82.

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

	A.	B.
^{3}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. H2O	26 λ	26 λ
DNAase 0.1 μg/ml.	10 λ	10 λ
DNA pol.	1.5 λ	1.5 λ

Dollie 6/[illegible]

1 58
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020.00
000018.60
1 59
010.00 Pst A
000.50
031499.40
1 60
010.00 Pst B
000.50
018294.60

14° 2 hours.

2 ml aliquot for checking incorp.

Rx terminated c̄ 10 λ of STOP buffer.

6.25.82.

Nick translation of PBR 322 plasmid DNA with malto-triose d UTP

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

	C	B
^{3}H dATP	6 λ	6 λ
10 x NT Buffer	2.5 λ	2.5 λ
dNTP mix	2.5 λ	2.5 λ
TTP	2.5 λ	—
MT dUTP	—	2.5 λ
PBR 322	10 λ	10 λ
DNase 0.1 mg/ml	2 λ	2 λ
Pol I ~~30u/λ~~ 3u/λ	2 λ	2 λ
d. H_2O	-	-

14° incubation.
2 ml aliquots at 15' intervals in vials containing
2 ml stop buffer
5 ml carrier DNA.
Added 1.0 ml cold 10% TCA - 10' at 4°
Insoluble material collected on EGF
& washed and counted.

6.25.82

Incubation 14° in Min. — TCA insoluble ^{3}H cpm per 2 µl Rx.

Incubation 14° in Min.	C	D.
3'	1251	1202
15	2255	2268
30	3222	3311
45	4013	3732
60	4006	4123
90	4544	4426
120	5050	5174
150	5377	5663
180	5754	5876
210	3676	6138
240	912	6008
270	1055	6774

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PBR 322 A

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PBR 322 B

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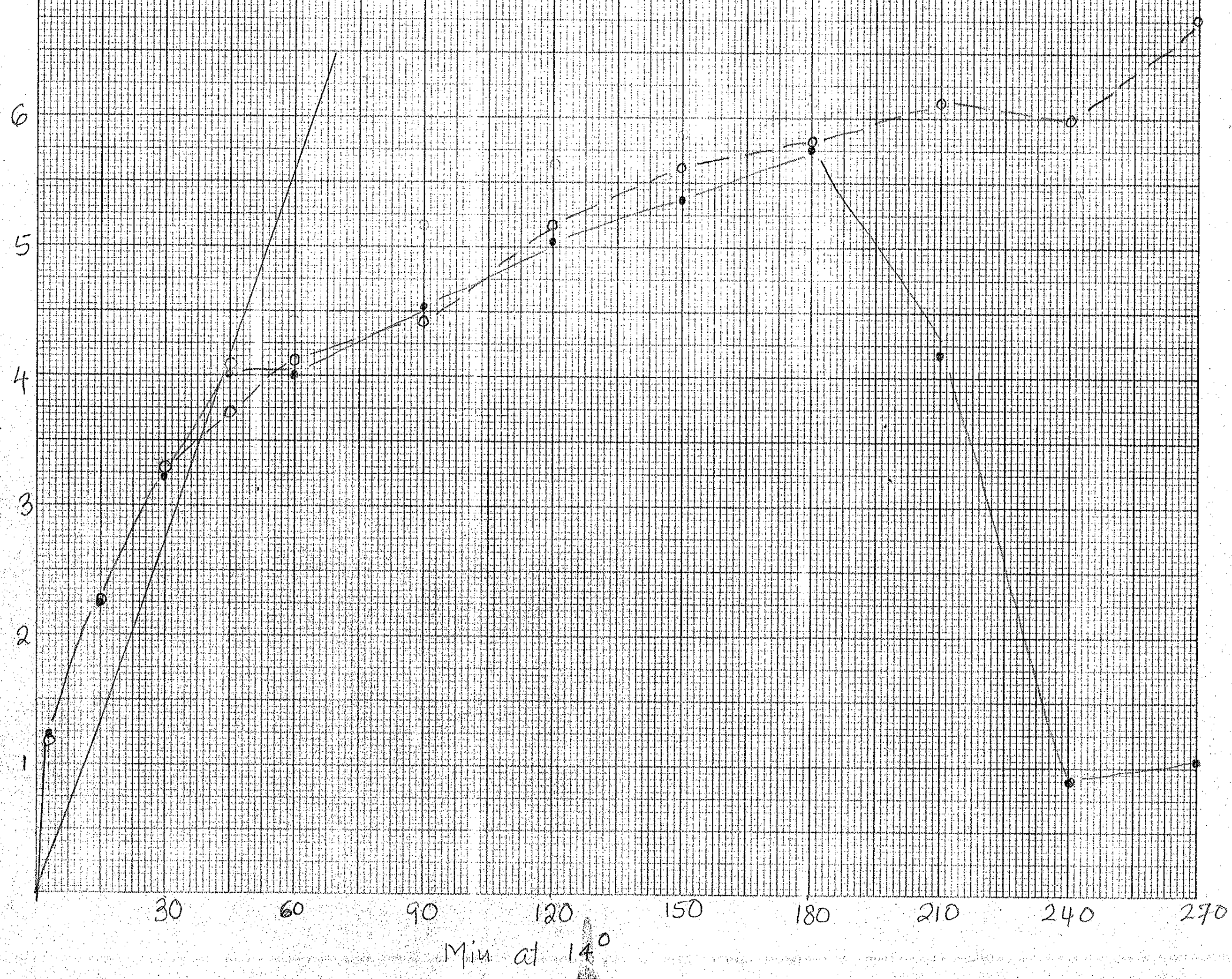
6.25.82



K+E 10 X 10 TO THE CENTIMETER 18 X 25 CM. KEUFFEL & ESSER CO. MADE IN U.S.A. 46 1521

6.25.82.

Preparation of phage plaques.

* Lambda. Fel V.

Recombinant DNA lab used.

L-agar plates freshly prepared

dried at 37° for 24 hours.

Permissive host RW 262 from Henry.

Phage λ Fel V " "

10^{-2} 10^{-4} and 10^{-5} dilutions in

L Broth containing Mg^{++} and Ca^{++}

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° o/n.

2 plates 10^{-2} dilution.

3 plates 10^{-4} dilutions

4 plates 10^{-5} dilution.

6.26.82.

plaques /0.1 ml.

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 5' in 0.5 M NaOH
1.5 M NaCL
5' 2 twice in 0.5 M Tris (7.4)
1.5 M NaCL.

Blotted ~~dryd~~ dryish.

Baked 80° in vacuo for 3 hours.

Incubated in wash buffer (5 ml per filter in sealed bags.) at 65° for 16 hours.

Blotted dryish.
Stored stacked in sealed bags.
[initials]

λ Fel V. DNA. Hybridization to nick-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.C.

20 ml Formamide 50%
10 ml 20 x SSC 5 x SSC
0.4 ml 100 x Denhardt {2% Ficoll, 2% PVP, 2% BSA}
Mix.
1.0 ml S.S. DNA

H_2O to 40 mls.

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 nick-translated λ Fel V DNA. denatured at 80° for 3' and quickly cooled.

[probe is either TTP λ Fel V DNA or HTdUTP λ 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 Con A solution in PBS Mg^{++} [0.5 mg/ml CoN A].
for 2 hours at R.T.
washed 3 times in PBS Mg^{++} buffer.
washed 3 times in 0.2 M Imidazole pH 6.5 buffer

blotted dry.

~~7/1/82~~

~~Two ~~One~~ small and two ~~one~~ large from control and experimental filters.~~
~~treated with acid phosphatase~~
~~1 ml solution~~

(A) λ Fel V glucosylated probe. 7.1.82.

Detection @ Acid phosphatase

(Sandwitch)

~~Acid ph~~

The filters are soaked in phosphate-free BSA 10′ at RT.

Blotted dryish.

Soaked in enzyme solution at RT for 30′

~~0.5 ml~~ Acid phosphatase units/ml

0.8 ml for large filter ⎤ diluted in

0.4 ml for small filter ⎦ phosph-free BSA

Filters washed with 0.15 M NaCl

5 times c̄ 5.0 ml. per filter

~~Filters soaked in substrate solution.~~

~~5 Bromo-4-chloro-3 Indoyl phosphate in Veronal Acetate buffer containing NBT Dye.~~

~~1 ml for large filter~~

~~0.5 ml 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-toluene diazonium chloride
hemizinc 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)
~~at~~ treated similarly, does not
show any reaction.

(B) λ Fel V glucosylated probe 7.1.82.
sandwitch technique with
alkaline phosphatase

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-Indoyle
phosphate in Veronal acetate buffer
~~NBT Dye~~ Nitro B.T. tetrazolium salt —

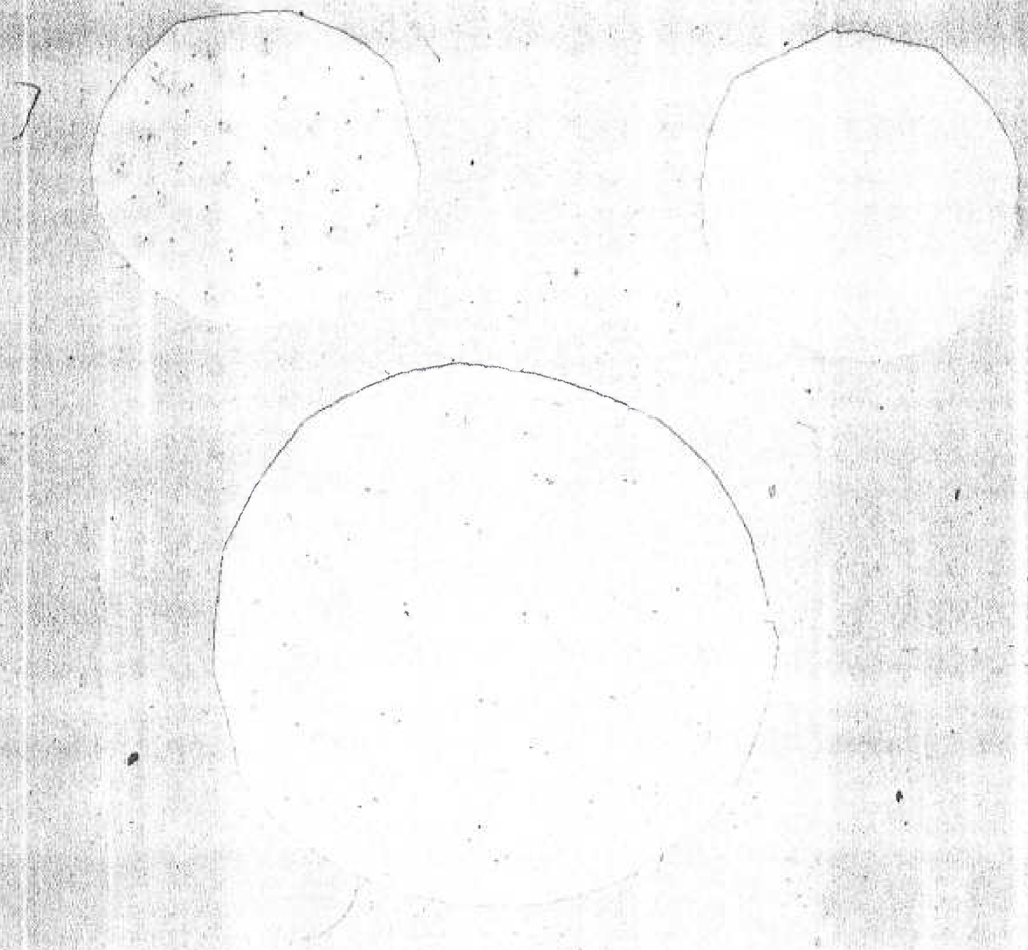
1.0 ml for large filter
0.5 ml for small filters.

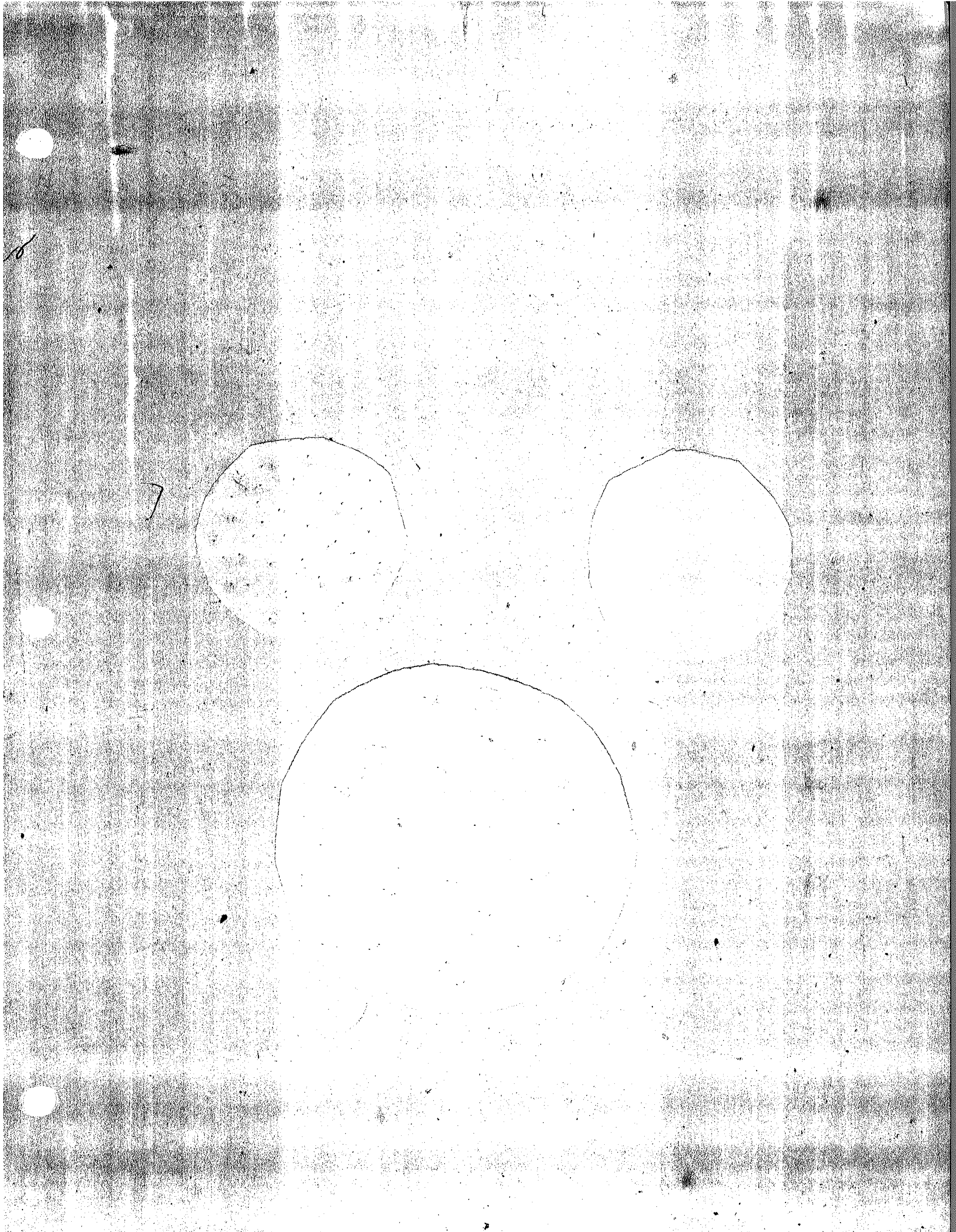
Incubated at R.T. for 30 minutes
blue spots started appearing on plates
where glucosylate λ Fel V DNA was hybridized
to λ Fel V phage plaques.

7/1/82

glucosylated probe hybridized to phage plaques
Con A binding
Sandwitch Technique

Acid phosphatase - ~~tetrazolium method~~
~~diazo~~
Fast Red TR. Salt
~~5-chlo~~





6.25.82.

Hybridization of malto-triose dUTP 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 cyclocentrifuged 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 3 x PBS twice in 1 x 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/ul in PBS prepared just prior to use 100 ul) for 5'

(3) Slides immersed in wash buffer once for 5' (2 mg glycine /ul 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 SO_4	20 ul
20 X SSC	20 ul
Salmon-sperm DNA (acid treated phenolized) 12 mg/ul	8 ul
MT-dUTP Ad-2 DNA or TTP Ad-2 DNA (54 ug/ul)	20 ul

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° and held there for
5 minutes
Cooled slowly to 37°
Incubated in humid chamber 37°
over the week-end.

6/28/82.

(9)

Detection never let slides dry out.

(1) Coverslips removed. Slides soaked in 2 x SSC ~~15'~~
at 32° (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 2.0M NaCl)
diluted in PBS containing 10 mM Mg^{++} to
give 0.25 mg per ml
0.1 ml applied per slide covered ē coverslip.
Incubated at RT for one hour

(6) washed thrice for 5 min each in PBS

E.

nothing definite.
either probe was no good
A. larger size
B. not enough glycosyl residues.