Enzo Exhibit 2037
Hologic, Inc. v. Enzo Life Sciences, Inc.
Case IPR2016-00820

LECTIN - BINDING OF T_4 DNA

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

Recombinant phage	~~λ Fel V ins~~ phage λ with Fel V insert.
Glucosylated probe	Fel V insert (isolated from λ Fel V DNA) nick translated with maltose-biose dUTP

Recombinant phage screening

Check of the isolated insert for wiek-translation

	Control	Glucosylated
^{3}HdATP	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	—
0.3 mM MTdUTP	—	2.5 λ
DNA	5 λ	5 λ
DNAse 100 µg/µl	2 λ	2 λ
DNA Pol 5 u/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 0.2 M 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

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

MuTdOTP

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

TTP

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

Repeat

15' 30' 45' 60' 90' 120' 150' 180' 210' 240'

1 40
001.00
015.00
000297.00
1 41
001.00
015.00
000376.00
1 42
001.00
015.00
000308.00
1 43
001.00
010.00
000488.00
1 44
001.00
010.00
000540.00
1 45
001.00
007.00
000994.00
1 46
001.00
007.00
001139.00
1 47
001.00
010.00
000508.00
1 48
001.00
005.00
002907.00
1 49
001.00
010.00
000544.00

MuTdUTP

recombinant phage screening.

Preparation of phage lysates

8.11.82

Wild Type phage:

E. coli RR1 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° ē 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 ē shaking in boiling water bath till the temp of culture reached 42°
   about 7 minutes
   Held at that temp for 5′

3. Incubated ē 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.

Determining 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

Recombinant phage screening.

9/3/89

Preparation of the glucosylated probe

125 λ

	Control	Glucosylated
^{3}Hd ATP	12.5 λ	12.5 λ
NT buffer 10 λ	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 Mg/ml	50 λ	50 λ
DNAse 100 Mg/ml	3 λ	3 λ
DNA Pol I 5 u/ml	5 λ	5 λ
dl. H2O	17 λ	17.78 λ

1 hrs at 14°

Rx Terminated with 12.5 λ of 0.2 M Na EDTA

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 iuser glucosylated G-50 9/3/82

1 16
001.00
000.00 1
000012.00
1 17
001.00
000.00 2
000022.00
1 18
001.00
000.00 3
000017.00
1 19
001.00
000.00 4
000019.00
1 20
001.00
000.00 5
000022.00
1 21
001.00
000.00 6
000025.00
1 22
001.00
000.00 7
000024.00
1 23
001.00
000.00 8
000022.00
1 24
001.00
000.00 9
000019.00
1 25
001.00
010.00 10
000555.00
1 26
001.00
007.00 11
001419.00
1 27
001.00
007.00 12
001484.00
1 28
001.00
005.00 13
001785.00

1 29
001.00
005.00 14
001801.00
1 30
001.00
005.00 15
001980.00
1 31
001.00
005.00 16
002188.00
1 32
001.00
005.00 17
002876.00
1 33
001.00
003.00 18
004059.00
1 34
001.00
003.00 19
006113.00
1 35
001.00
003.00 20
007047.00
1 36
001.00
003.00 21
009299.00
1 37
001.00
003.00 22
009843.00
1 38
001.00
002.00 23
011107.00
1 39
001.00
002.00 24
010640.00
1 40
001.00
002.00 25
010541.00

1 71
001.00
002.00 26
010002.00
1 72
001.00
003.00 27
006699.00
1 73
001.00
003.00 28
006344.00
1 74
001.00
003.00 29
005673.00
1 75
001.00
003.00 30
004447.00
1 76
001.00
005.00 31
003062.00
1 77
001.00
005.00 32
002733.00
1 78
001.00
005.00 33
002138.00
1 79
001.00
007.00 34
001163.00
1 80
001.00
007.00 35
001358.00
1 81
001.00
007.00 36
001068.00
1 82
001.00
010.00 37
000688.00
1 83
001.00
010.00 38
000782.00

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
d NTP 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 ng/μl	15 λ	15 λ
DNAse 10 μg/μl	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 ē 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 #	^{3}H cpm	
1	62	
2	1796	pooled and concentrated
3	7649	
4	7293	
5	5569	
6	5939	
7	7286	
8	7130	
9	7104	
10	4555	
1	5147	
2	4812	
3	3762	
4	3807	
5	3253	
6	1454	
7	1257	
8	1122	
9	945	
20	514	

Glucosylated

FR #	^{3}H cpm	
1	19	
2	20	
3	21	
4	20	
5	9	
6	17	
7	34	
8	4163	pooled and concentrated
9	14158	
10	9480	
1	6003	
2	5872	
3	6417	
4		
5		
6		
7		
8		
9		
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
001.00
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
001.00
003.00
007130.00

1 21
001.00
003.00
007104.00

1 22
001.00
003.00
004555.00

1 23
001.00
003.00
005147.00

1 24
001.00
003.00
004812.00

1 25
001.00
005.00
003762.00

1 26
001.00
005.00
003807.00

1 27
001.00
005.00
003253.00

1 28
001.00
007.00
001454.00

1 29
001.00
007.00
001257.00

1 30
001.00
007.00
001122.00

1 31
001.00
007.00
000945.00

1 32
001.00
010.00
000514.00

1 33
001.00
000.00
000019.00

1 34
001.00
000.00
000020.00

1 35
001.00
000.00
000021.00

1 36
001.00
000.00
000020.00

1 37
001.00
000.00
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

		1, 2,	3, 4
Bacterial Strains :			
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 3 MM 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 3 MM 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 & 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' & 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/µl Salmon Sperm DNA
1 x 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 nicrodilers ~ 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 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^{++} buffer added. ~~just to soak the paper~~ 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

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

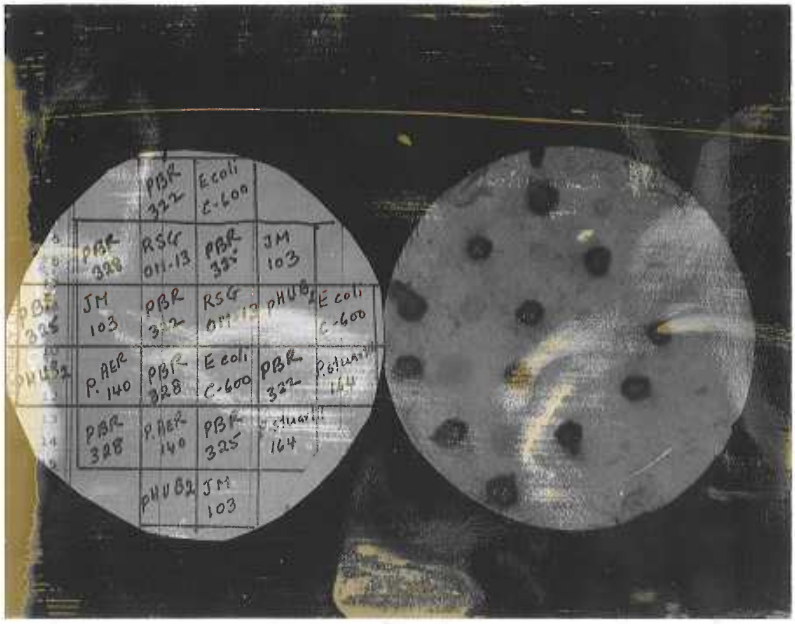
(16) Filters washed 3x in
PBS buffer containing
0.1 % BSA
10' at RT. each time
Blotted dry

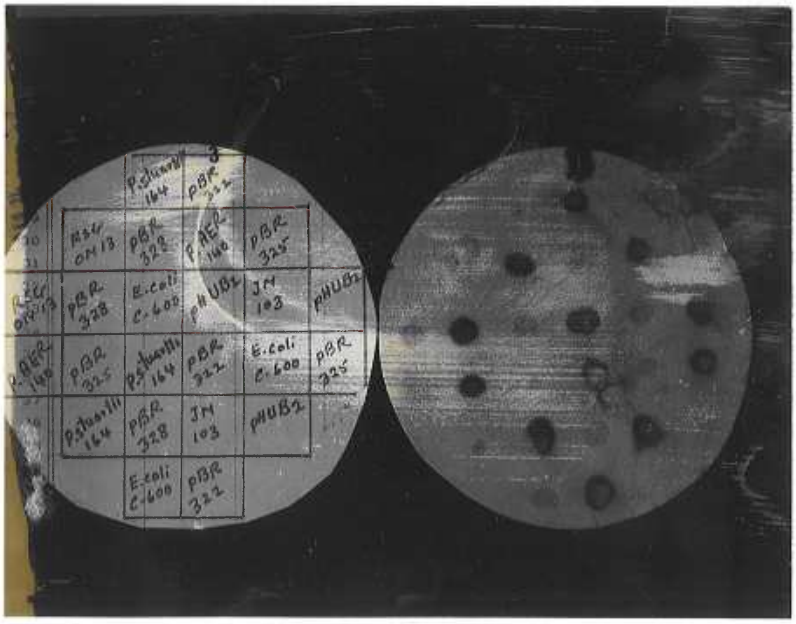
(17) Treatment with HRP

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


PBR 322
E coli C-600
PBR 328
RSG OM-13
PBR 325
JM 103
JM 103
PBR 322
RSG OM-13
PHUB2
E coli C-600
P. AER 140
PBR 328
E coli C-600
PBR 322
P. stuartii 164
PBR 328
P. AER 140
PBR 325
P. stuartii 164
PHUB2
JM 103


P. stuartii 164
PBR 322
RSG OM-13
PBR 328
P. AER 140
PBR 325
PBR 328
E. coli C-600
PHUB2
JM 103
PHUB2
PBR 325
P. stuartii 164
PBR 322
E. coli C-600
PBR 325
P. stuartii 164
PBR 328
JM 103
PHUB2
E. coli C-600
PBR 322

#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 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 RT.

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 1 X SSC

on bacterial streaks.

Incubate at RT. for 15'

(~~3~~ B) Soak Filter in GTC cacodylate buffer
5 M GTC
15' at RT 20 mM cacodylate pH 7.1
0.1 % SDS

~~C Soak~~

(C) Soak Filters in 0.1 % SDS

(D) Soak Filter ~~in GTC GG 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' ± 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 fillers 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 fillers 2 x 10' each
with PBS. Mg^{++} buffer

Soak the fillers 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 fillers with 1% BSA in
PBS buffer
37° 2 hrs.

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

(17) Treatment with HRP enzyme.

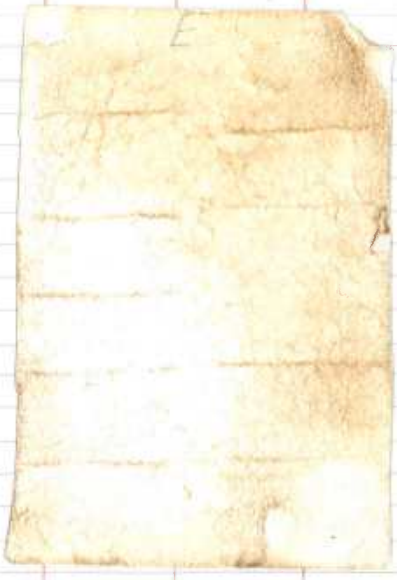
C-600
RSG-OM13
pGP E1
pBR 322
PST 164
E. coli C

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
PGR-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

~~1 plate on~~

Shains used.

E. coli C-600 parent shain

E. coli RSG-014-13 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 πr^2 = 70.11 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

#

(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 vaccuo.

(9) Wash o/n at 65° in
0.2% Ficoll
0.2% PVP
0.2% BSA
0.1% SDS
2x SSC

(10) Blot filter dryish

(11) Immerse filters in prehybridization mixture 2.5 ml per filter
50% formamide
5 x SSC
100 mg/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. — (5&6) 2 filters c 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 RT.

60' twice at 65°

30' once at RT. 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 filters 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
PBS pH 7.0 } 2x SSC can be used.

(17) Filters washed 2 x in

0.1 % BSA
2 x SSC

Colony hybridization
glucosylated probes.

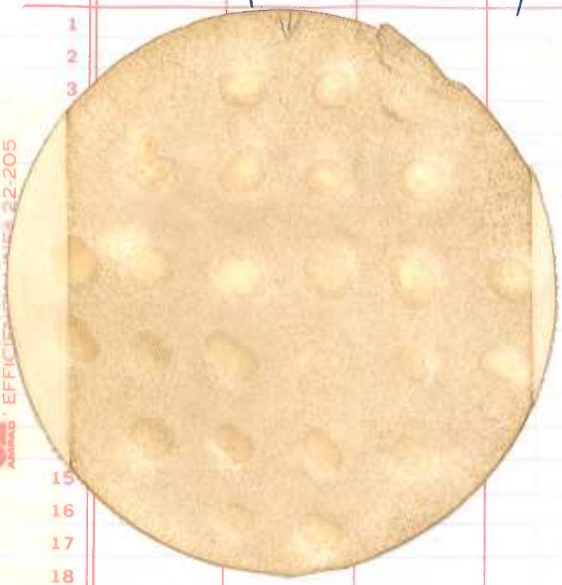
(18) Horse Radish peroxidase 5 ng/ml
diluted to 100 ng/ml in PBS pH 7.0
0.1 % BSA.

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

③ Repeat step ①

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

DRY on Filter paper

⑤ Repeat step ③

⑥ Soak filter in 0.25 ug/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

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~~ ~~Inc~~ 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

Colony hybridization glucosylated p

11.

Preparation of the probe.

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

2 hrs at 14°

Rx Terminated ē 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 vaccum evaporation.

PGP E1 glucosylated 9/3/82 G-50

```
1 16
     001.00
     000.00
  000022.00
1 17
     001.00
     000.00
  000016.00
1 18
     001.00
     000.00
  000017.00
1 19
     001.00
     000.00
  000020.00
1 20
     001.00
     000.00
  000017.00
1 21
     001.00
     000.00
  000009.00
1 22
     001.00
     000.00
  000017.00
1 23
     001.00
     000.00
  000022.00
1 24
     001.00
     000.00
  000025.00
1 25
     001.00
     020.00
  000111.00
1 26
     001.00
     015.00
  000261.00
1 27
     001.00
     010.00
  000444.00
1 28
     001.00
     010.00
  000668.00
1 29
     001.00
     007.00
  001022.00
1 30
     001.00
     007.00
  001506.00
1 31
     001.00
     005.00
  002330.00
1 32
     001.00
     005.00
  003419.00
1 33
     001.00
     003.00
  005070.00
1 34
     001.00
     003.00
  006994.00
1 35
     001.00
     003.00
  008709.00
1 36
     001.00
     003.00
  009473.00
1 37
     001.00
     003.00
  009563.00
1 38
     001.00
     003.00
  008620.00
1 39
     001.00
     003.00
  005304.00
1 40
     001.00
     003.00
  006012.00
```

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 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.

Riuse tubes 3 x ē 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 a.m.

Riuse 3 x ē 100 λ of 2 x SSC Incubati

To set 1 added 100 λ PBS. Mg^{++} RT 60'

set 2 added 100λ 1% Ficoll 400

Wash 3 x ē 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 ē 2 x SSC

3 x ē 0.1 % BSA in 2 x SSC

Add 100 λ 1% BSA in 2 x SSC to all tubes.

Cover ē parafilm

Keep at 4° o/n. 8/3/82

Rinse 3 x ē 2 x SSC containing 0.1 % BSA

Add 10 λ of ½ diluted acid phosphatase

40 λ of diluent containing 0.1 % BSA

Incubate at RT for 30'

Remove

Rinse 5 x ē 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 ∑ 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	A_{410}	9
1									
2									
3									
4	A	50 ng	+	−	−	+		0.285 x 20	
5									
6									
7	A_2	50 ng	+	+	+	+		0.35 x 20	
8									
9									
10	B_1	5 ng	+	−	−	+		0.175 x 20	
11									
12									
13	B_2	5 ng	+	+	+	+		0.23 x 20	
14									
15									
16	C_1	500 pcg	+	−	−	+		0.06 x 20	
17									
18									
19	C_2	500 pcg	+	+	+	+		0.12 x 20	
20									
21									
22	D_1	50 pcg	+	−	−	+		0.11 x 5	
23									
24									
25	D_2	50 pcg	+	+	+	+		0.22 x 5	
26									
27									
28									
29	E_1	20pcg	+	−	−	+		0.144 x 2	
30									
31									
32	E_2	20pcg	+	+	+	+		0.208 x 2	
33									
34									
35									
36	F_1	−	−	−	−	+		0.006	
37									
38									
39	F_2	−	+	+	+	+		0.007	
40									
41									
42	G_1	−	−	−	−	+		0.006	
43									
44									
45									
46	G_2	−	−	+	−	+		0.005	
47									
48									
49	Control	−	−	−	−	+		0.35 x 20	
50									

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

Elisa method in
microtiter plates.

Dilute Cou 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 Cou A

Wash wells 3x ē

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 ng/ml
20 ng/ml
2 ng/ml
0.2 ng/ml
} Alkaline phosphatase (1.8 ng/ml stock solution

50 λ of 200 ng/ml
20 ng/ml
2 ng/ml
} Horse Raddish Peroxidase 10 ng/ml stock soluti

Left at room temp for 60'

Remove

Rinse wells 3 x ē

100 λ of 0.1 % BSA
0.1 M Tris Cl (pH 7.8)
0.15 M NaCl

last rinse ē 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 peg.

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 vaccuo for 3 hrs.

Strips blocked in

0.2 % BSA

0.2 % PVP

0.1 % Triton X-100

2 x SSC

0.1 % SDS.

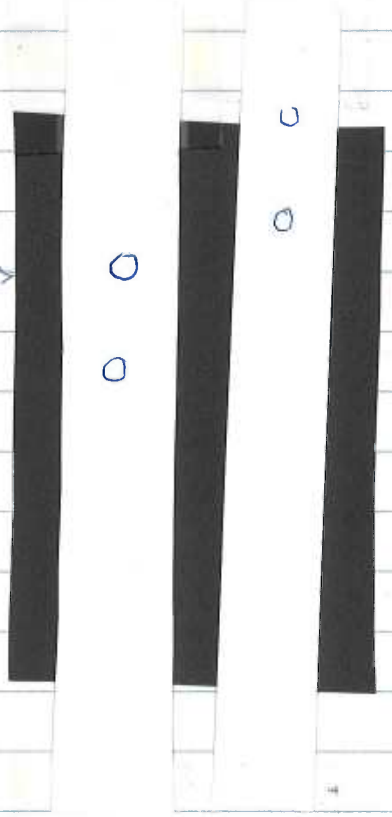
at 60° for 2 - 4 hrs. 10 a.m.

Con A binding.

Strips D_1 and D_2 soaked in FT-Con A 0.1 mg/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 ml each A 0.1 ng/ml in PBS-Mg^{++} RT 60'



All strips washed 3x ē 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 ē 0.1% BSA in 2x SSC.

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

Washed 3x ē 0.1% BSA in 2x SSC.

E_1 and E_2	10 ul	0.1 % BSA 2 x ssc 0.1 ug/ul HRP
F_1 and F_2	10 ul	0.1 % BSA 2 x ssc 0.1 ug/ul Alk. Phosph.
G_1 and G_2	10 ul	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 Mg/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 ē 2 x SSC

Wash 3 x ē 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 ē 100 λ of 1 % BSA in
0.1 M TRIS CL (7.8)
0.15M NaCL

Incubate at 37° ~~2hrs~~.

left at 4° o/n.

7/28/82

Remove coating solution.

Wash 3 X ē 100 λ 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 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.

T_4 DNA 200 Mg/ml in 10 mM TRIS 7.4
10 mM NaCl

5 λ DNA diluted ē 225 λ 0.1xSSC
Final con. DNA 4 Mg/ml.

Denaturation

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

F.C. NaOH 0.3 M

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

F.C. DNA 2 Mg/ml

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

① 2 Mg/ml ③ 0.5 Mg/ml
② 1 Mg/ml ④ 0.25 Mg/ml.

Spotted 1 λ on 8 strips Nitrocellulose.

~~Baked at 80° in vacc~~

Washed 2 x & 1.0 M Am Acetate

Baked at 80° in vaccuo 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 ConA in PBS. Mg^{++}
at RT for 60'

Washed & 2 x SSC three times.

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

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

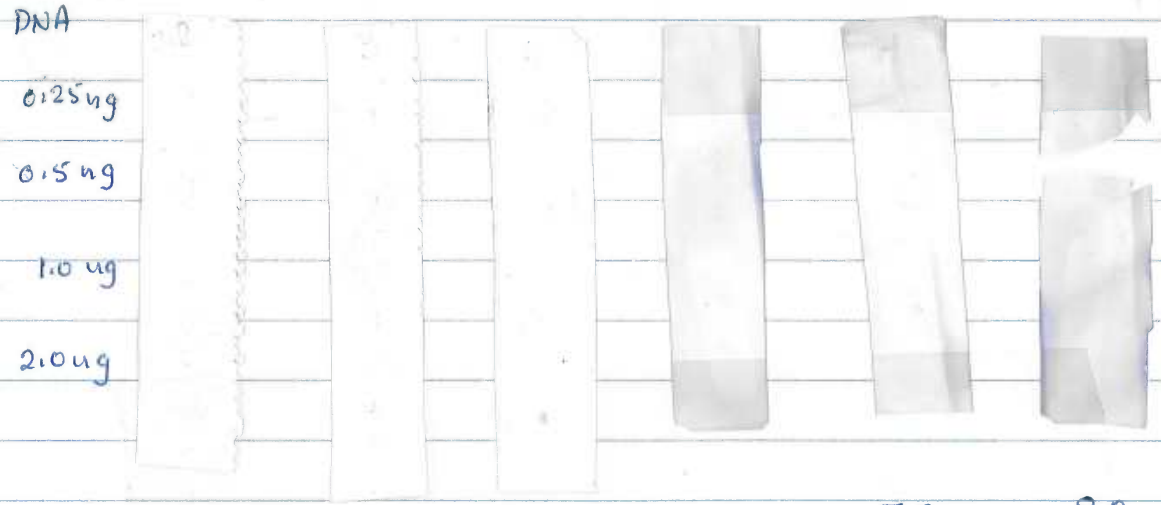
Washed repeatedly in 0.1 % BSA in 2 x SSC.

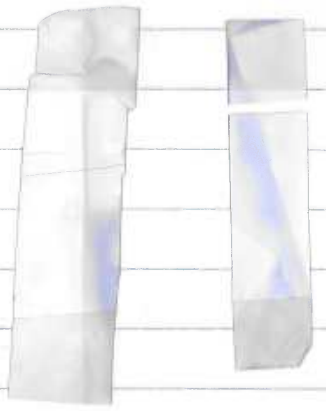
Substrate Naphthol AS-BI phosphate 0.1 mg/ml in TRis 0.1 M

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 conA - Alk Phosp.

best at pH 7.4

N. Kellen 7/26/82

7.23.82.

Con A binding of glucosylated DNA
Eliza method. Sandwitch Technique

Con Curve.

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

~~20~~ ~~2~~	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 ul~~ 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. Kelly 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 ~ 0.1 M TRIS pH 7.2 3 X

Air Dry.

Enzyme Treatment

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

Soak the strips in alkaline phosphatase solu.
10 Mg in 0.25 ml Tris 0.1 M pH 7.2.
per strip.
leave at R.T. for 30 minutes.

Wash ~ 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
0.1 mg/ml Naphthol AS-BI } in 0.1 M Tris pH 7.5

Incubate at ~ 37° for one hour

N. Kelly 7/26/82

con A + conA -

	1	2 A	3 B	4 C	5 D	6 E	7 F	8 G	9 H
1									
2									
3	DNA	600 ng	300ng	150ng	75 ng	37.5 ng	18.75ng	-	-
4									
5									
6		400 ng	200ng	100ng	50 ng	25 ng	12.5ng	-	-
7									
8									
9		200 ng	100 ng	50ng	25 ng	12.5 ng	6.25 ng	-	-
10									
11									
12		100 ng	50 ng	25 ng	12.5ng	6.25ng	3.125ng	-	-
13									
14									
15		50 ng	25 ng	12.5 ng	6.25ng	3.125ng	1.5625ng	-	-
16									
17									
18		20 ng	10 ng	5 ng	2.5 ng	1.25ng	0.625ng	-	-
19									
20									
21									
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26									
27									
28									
29									
30									
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49									
50									

N. Kelly 7/23/82

Binding of glucosylated DNA to lectin 7.22.82
— detection by sandwitch Technique

T_4 DNA 0.2 mg/ml.
λ plac 5 DNA 0.516 mg/ml.

4 Nitrocellulose 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.
3 λ = 0.6 ug
2 λ
1 λ
0.5 λ

On 3 and 4
λ plac 5
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.

NKelly 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 ē 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°

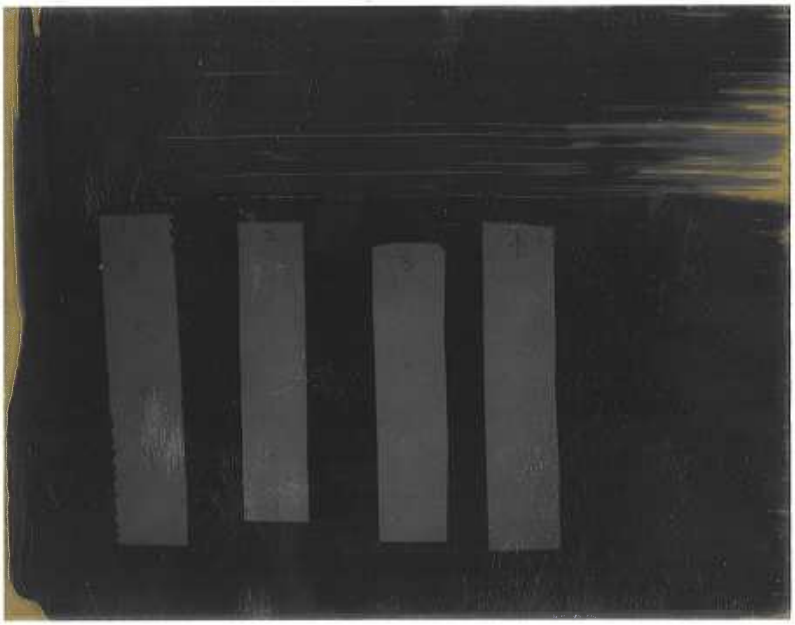
Blue spots immediately within 5'

on # 1 strip only

N. Kelly
7/23/82

7/22 - 7/23/82

7/22 - 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

T4
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-PO4 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-PO4 pH 7.2
150 mM NaCl
for the same length of time.

Solution removed.
Strips washed 3x ē 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 @ 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 Mg/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 2hrs

1.	T_4 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.	2.5 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 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 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 Denhardt
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 $K\text{-}PO_4$ pH 7.2
0.15 M NaCL

Soak set of A filters
A-1 to A-4
2 Blanks.
in 10 ml 50 mM $K\text{-}PO_4$ 7.2
150 mM NaCL
0.1 % ~~glut~~ glutaraldehyde
at R.T. for 3 hours.

Set of B filters.
in 10 ml 50 mM $K\text{-}PO_4$ pH 7.2
150 mM NaCL.

Buffers removed.
Washed 3 x ē 50 mM $K\text{-}PO_4$ 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 @ 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 ug alkaline phosphatase in 0.1 M TRIS
pH 7.4.
30' at RT.

Washed 3 x @ 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
dl. H20	340 λ

37° 90 minutes

Added 10 λ SDS. EDTA
200 λ dye solution.

200 λ mixture electroforesed
0.7% agarose gel.

No digestion.

phenolize to recover the DNA.

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

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	1 λ	1 λ	1 λ	1 λ
λ DNA	1 λ	1 λ	—	—
λ Fel V DNA	—	—	1 λ	1 λ
EcoRI	—	1 λ	—	1 λ
dist. H2O	8 λ	7 λ	8 λ	7 λ

37° 60'

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

Added 12 λ dye soletion electroforessed 1% agarose

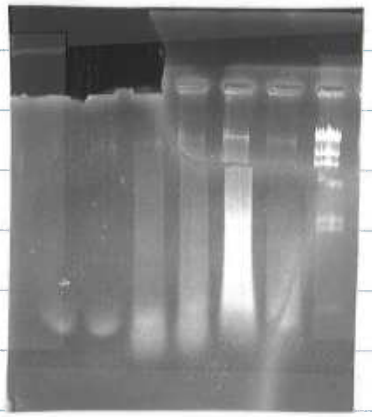
KB
25.6
7.31
6.16
5.55
4.84

Run paralled ē Hind III digested λ

8kb piece of interest

Gel electrophoresis of DNA probes 7.14.82.

1.	TTP	PBR 322	2.5 λ
2.	glucosyl.	PBR 322	"
3.	TTP	PstI	"
4.	glucosyl	PstI	"
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	
3H dATP	60 λ	60 λ
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 Mg/ml	20 λ	20 λ
DNA Pol 5 u/ml	12 λ	12 λ
dist. H_2O	0	0

14° for 2 hrs

Rx Terminated with 25 λ of 6.2 M EDTA

2 microliter to check for incorporation TCA insoluble

G-50 column chromatography of Nick. Translated DNAs.

7.9.82

FR #	^{3}Hepm
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

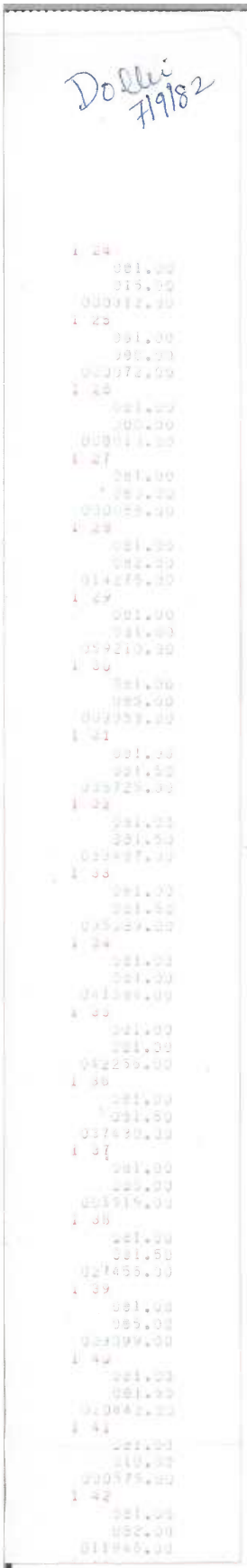
4.8 ml column.

6 drop fractions.

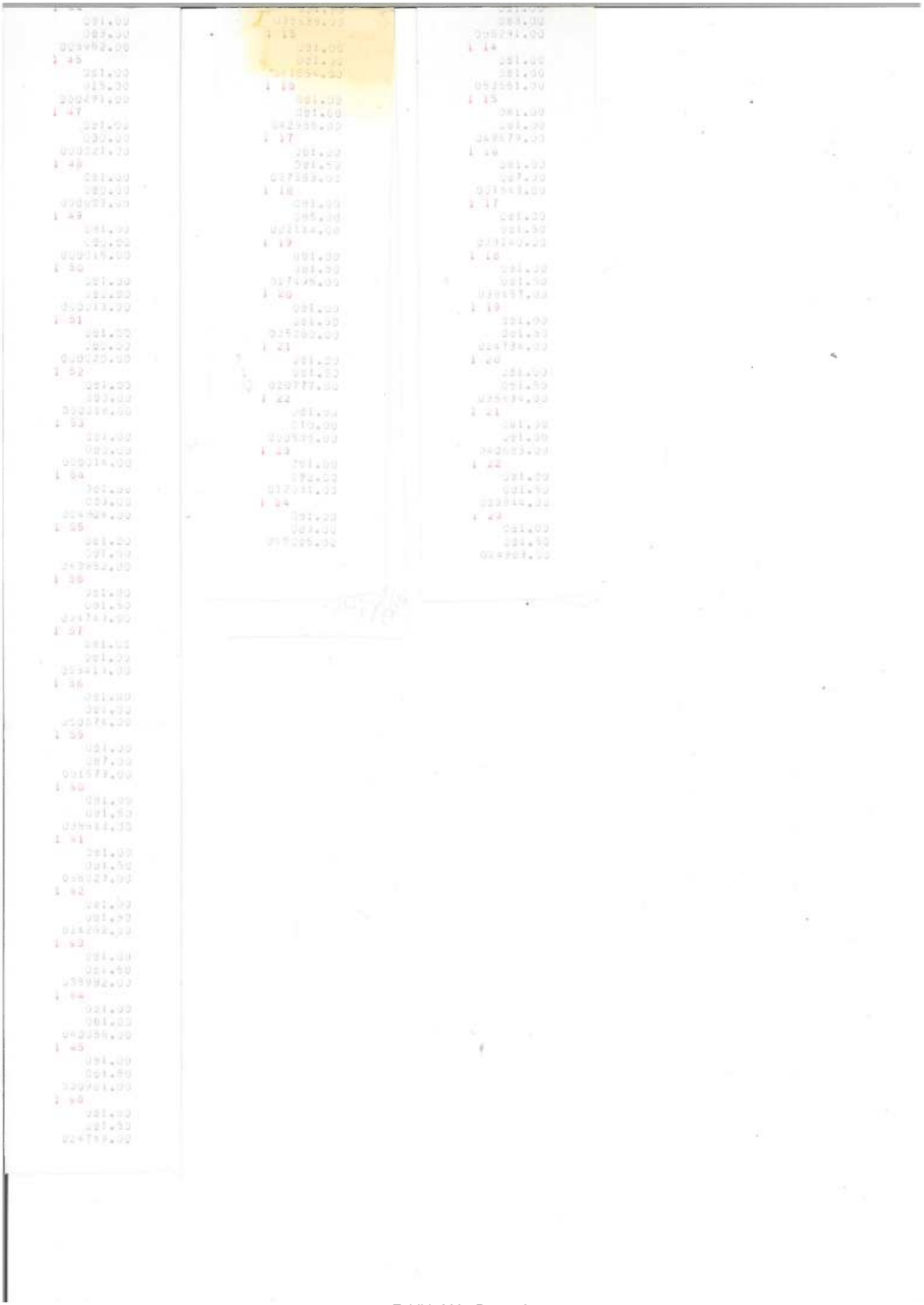
10 mM TRIS pH 7.5 buffer
0.1 mM EDTA eluent.

FR #	^{3}Hepm
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	41543
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

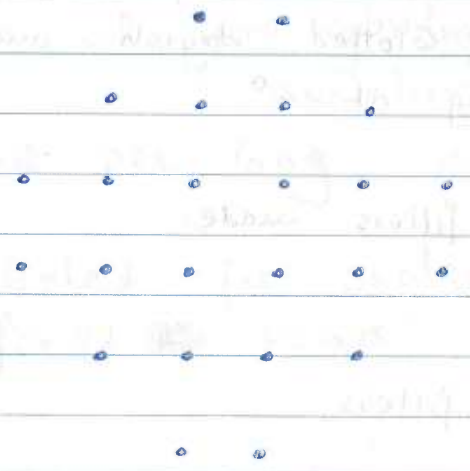


Colony Hybridization
pBR 322
glucosylated-
lectin HRP

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



~~7-7~~

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 vaccuo.
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' and quickly cooled)
20 ul pre-hybridization mixture

10 ul per bag 4 filters per bag.

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

~ 30 Mg. 2.75024×10^6 cpm glucosylated probe
~ 25 Mg 3.172955×10^6 cpm TTP probe.

7.16.82.

Filters removed and washed 3 x in wash buffer
Clr - 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~~ 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 [100 λ 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 back ground problem.

Use of glucosylated-lectin system for Colony hybridization.

7.13.82

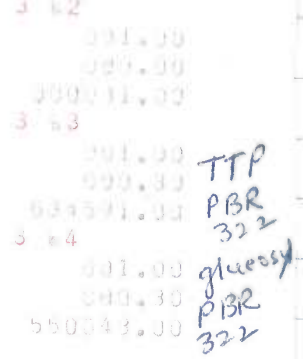
pBR 322	2.0 ug/ul
3H d ATP	
malto-Triose }	0.3 uM
d UTP }	

	Control	Glucosylated
3H d ATP	25 λ	25 λ
10X NT buffer	12.5 λ	12.5 λ
HT d UTP	—	12.5 λ
d NTP 0.3 uM	12.5 λ	12.5 λ
TTP 0.3 uM	12.5 λ	—
pBR 322 2 ug/ul	25 λ	30 λ
DNase 10 ug/ml	6 λ	6 λ
DNA Pol 5 u/λ	6 λ	6 λ
dist H_2O	45.5 λ	45.5 λ

2 hrs 14° 1.40 pm.

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

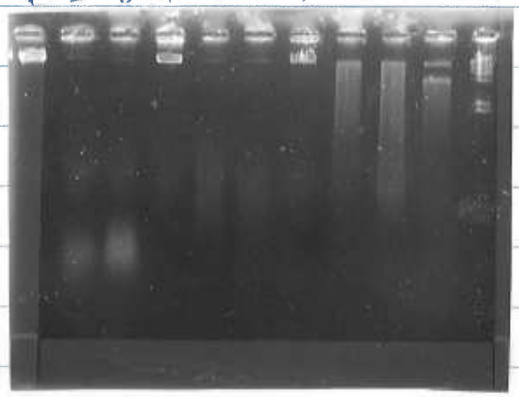
First peak radioactivity collected
15 λ checked for counts.


TTP
PBR
322
glucose
PBR
322

Gel electrophoresis 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

1	18	
2	12	
3	14	
4	11	
5	21	
6	15	
7	1871	
8	8728	
9	21008	
10	18183	
1	22063	
2	24978	
3	28155	
4	23958	
5	21710	
6	16482	
7	10925	
8	7082	
9	185	
20	4152	
1	1987	
2	1270	
3	38	
4	799	

lyophilized

λ Fel V nick translated ē TTP

6 drop fraction.

3.5 ml column.

pH 7.5 TRIS 10 mM } buffer
EDTA 0.1 mM }

1	13	
2	20	
3	13	
4	89	
5	15	
6	11	
7	1385	
8	13075	
9	23475	
10	20874	
1	23071	
2	28437	
3	21240	
4	37682	
5	27418	
6	19345	
7	13977	
8	10390	
9	5918	
20	7117	
1	4872	

lyophilized

λ Fel V nick translated ē
~~Bio~~ malto-Triose
d TTP.

3.5 ml column

TRIS 10 mM } pH 7.5
EDTA 0.1 mM }

6 drop fractions.

Dollie
7/9/82

```
1 35
     001.00
     000.00
  000018.00
1 36
     001.00
     000.00
  000012.00
1 37
     001.00
     000.00
  000014.00
1 38
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     000.00
  000011.00
1 39
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     000.00
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1 40
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     000.00
  000015.00
1 41
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     005.00
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     003.00
  008728.00
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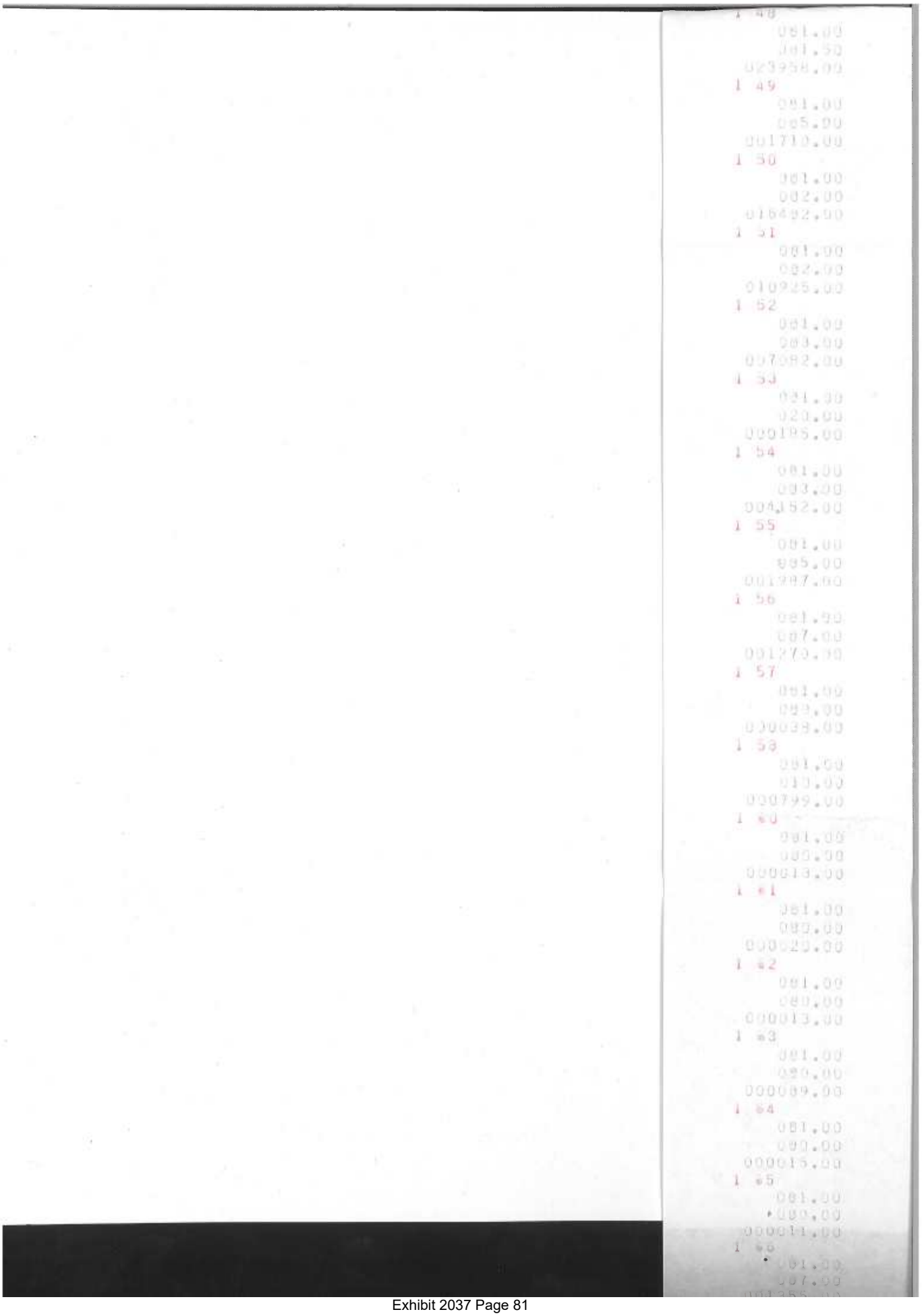
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1 66
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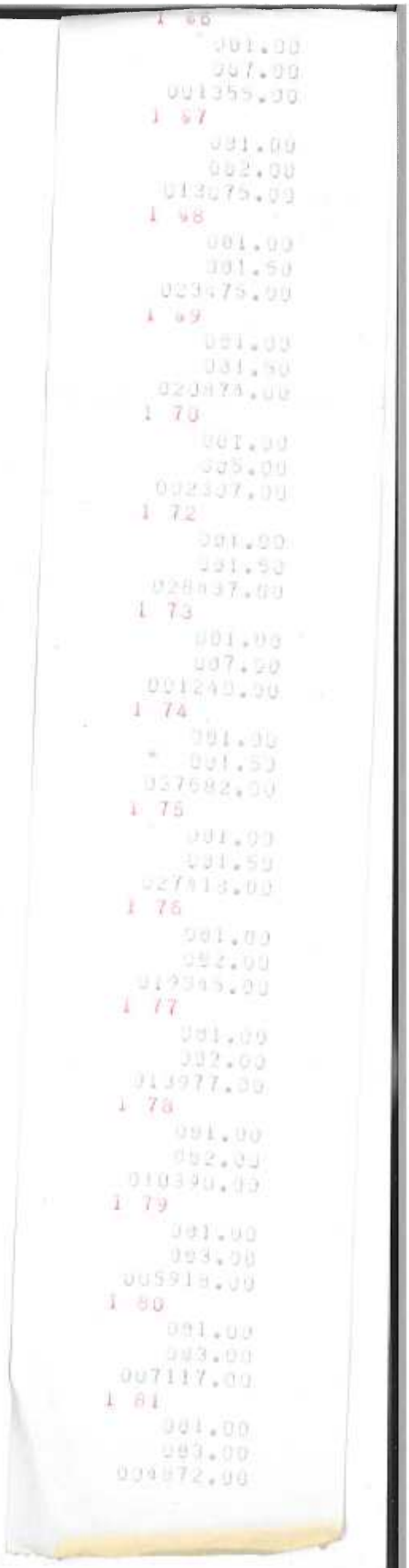
007.00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1 80
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003.00
007117.00
1 81
001.00
003.00
009072.00

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	50 λ
d NTP	50 λ	50 λ
TTP	50 λ	–
MT dUTP	–	50 λ
λ Fel V	100 λ	100 λ
DNAse 0.1 µg/µl	40 λ	40 λ
DNA Pol. 20u/λ	5 λ	5 λ
d. H_2O	165 λ	165 λ

2 hrs at 14°

Rx Terminated ē 50 µl of 0.2 M EDTA

2 µl aliquot mixed ē 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.
malto Niose 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 Mg/ml.	10 λ	10 λ
DNA pol.	1.5 λ	1.5 λ

Dallas Peterson

1 58
010.00
020.00
000018.60
1 59
010.00 Pst A
000.50
031499.40
1 60
010.00 Pst B
000.50
018294.50

14° 2 hours.

2 ml aliquot for checking incorp.

Rx 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}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 ~~30 u/λ~~ 3 u/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 a washed and counted.

6.25.82

Incubation 14° in Min.	TCA insoluble C	3H cpm per 2 ml Rx. D.
3′	1251	1202
15	2255	2268
30	3222	3311
45	4013	3732
60	4006	4123
90	~~4~~5544	~~4~~4426
120	5050	5174
150	~~5~~7777	5663
180	5754	5876
210	3676	6138
240	912	6008
270	1055	6774

```
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     005.00
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1 02
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     015.00
  000030.10
1 03
     010.00
     007.00
  000098.30
1 04
     010.00
     003.00
  000787.40
1 05
     010.00
     003.00
  000837.00
1 06
     010.00
     005.00
  000274.40
1 07
     010.00
     003.00
  000830.80
1 08
     010.00
     003.00
  000721.50
1 09
     010.00
     003.00
  000814.30
1 70
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     003.00
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     000.00
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PBR 322 A

PBR 322 B

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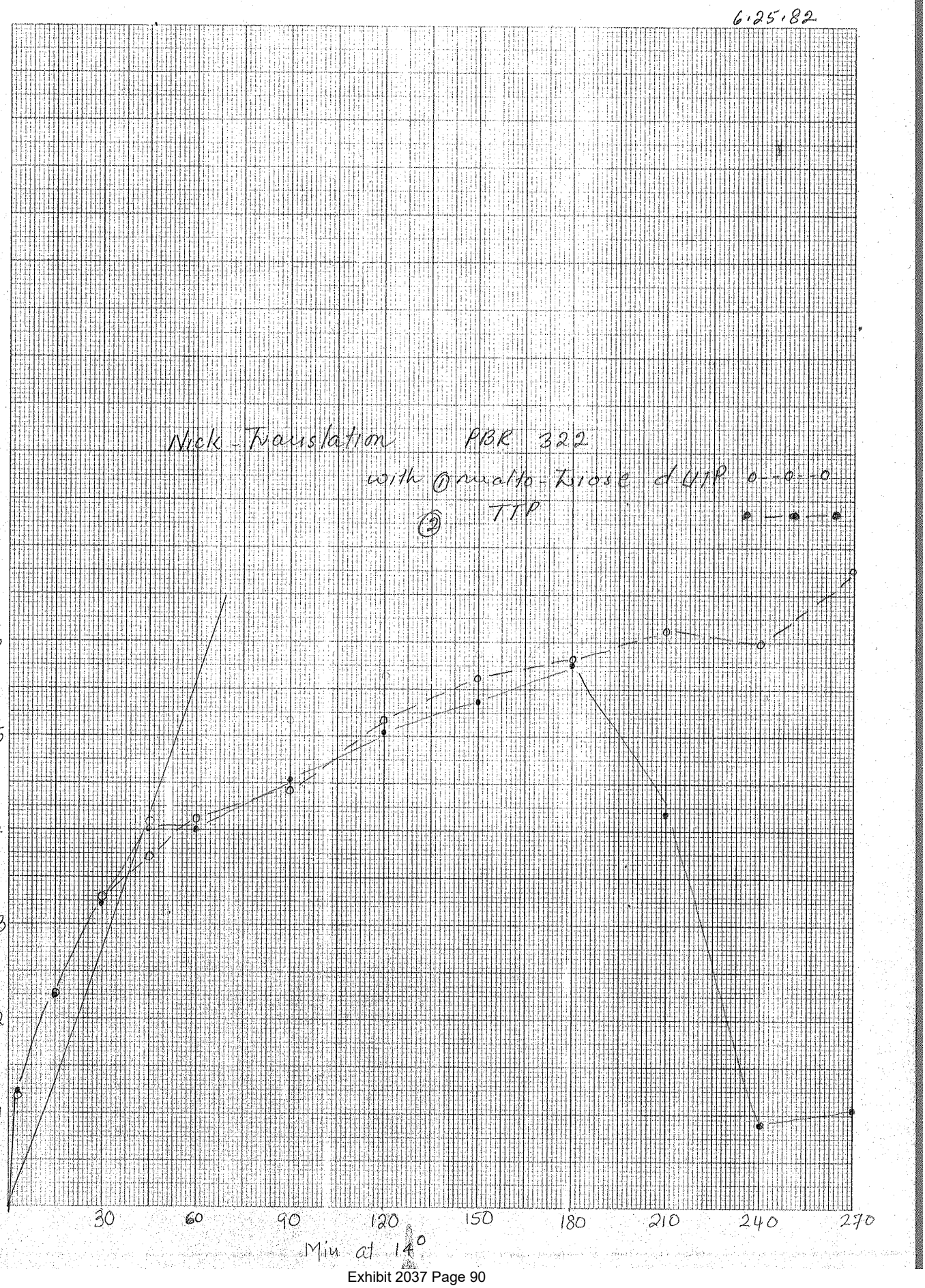


6.25.82.

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 S^{-1} 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 mm. filters.
6 47 mm 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 ~~dried~~ 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.
0/n.

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 solu.

pre-Treatment solu.			F.C.
20	ml	Formamide	50 %
10	ml	20 x SSC	5 x SSC
0.4	ml	100 x Denhardt Mix.	2 % Ficoll, 2 % PVP, 2 % BSA
1.0	ml	S.S. DNA	
H_2O to 40 mls.			

4 filters per bag – 8 ml pre-treatment solu. per bag.

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

Pre-treatment solu removed and added hybridization mix.

20 ml Pre-treatment solu.
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 HT dTP 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.

Filters soaked in 10 ml of Con A solution in PBS. Mg^{++} [1.0 mg/ml ConN 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 diluted in phosph-free BSA

0.8 ml for large filter

0.4 ml for small filter

Filters washed with 0.15 M NaCl 5 times ē 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)
st treated similarly, does not
show any reaction.

(B) λ Fel V glucosylated probe sandwitch 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 ug/ul at RT. for 30'

1.0 ul for large filter

0.5 ul 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 ul for large filter

0.5 ul for small filters.

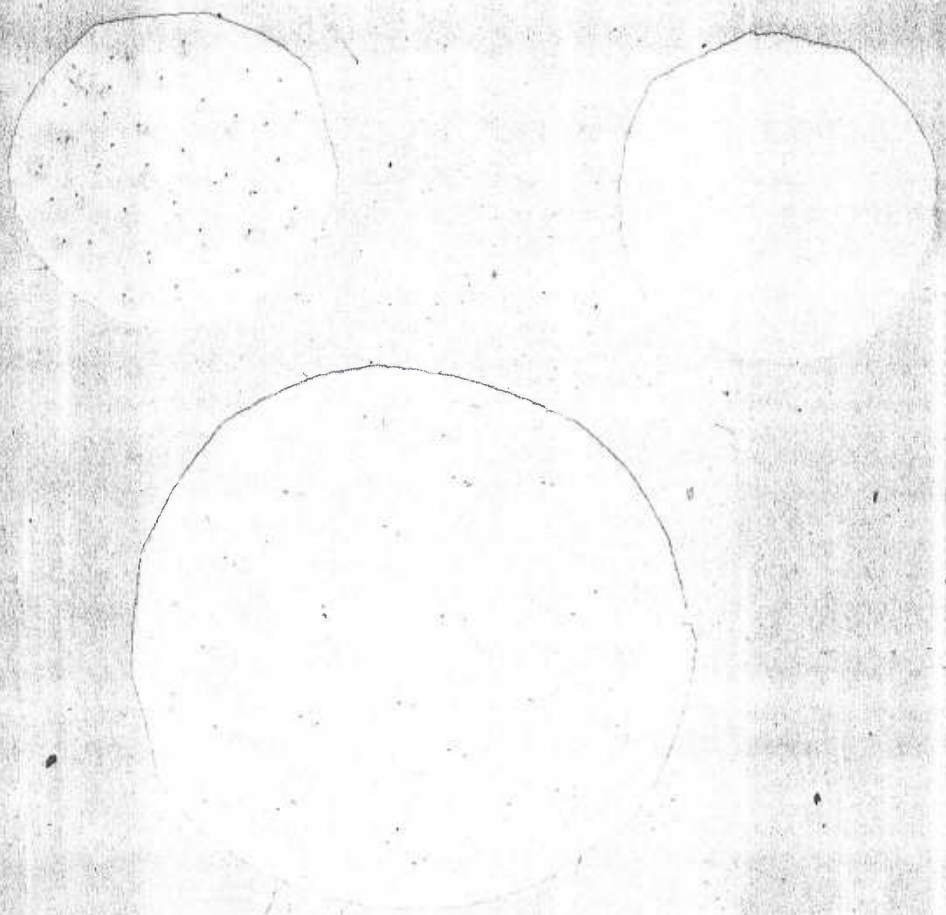
Incubated at R.T. for 30 minutes

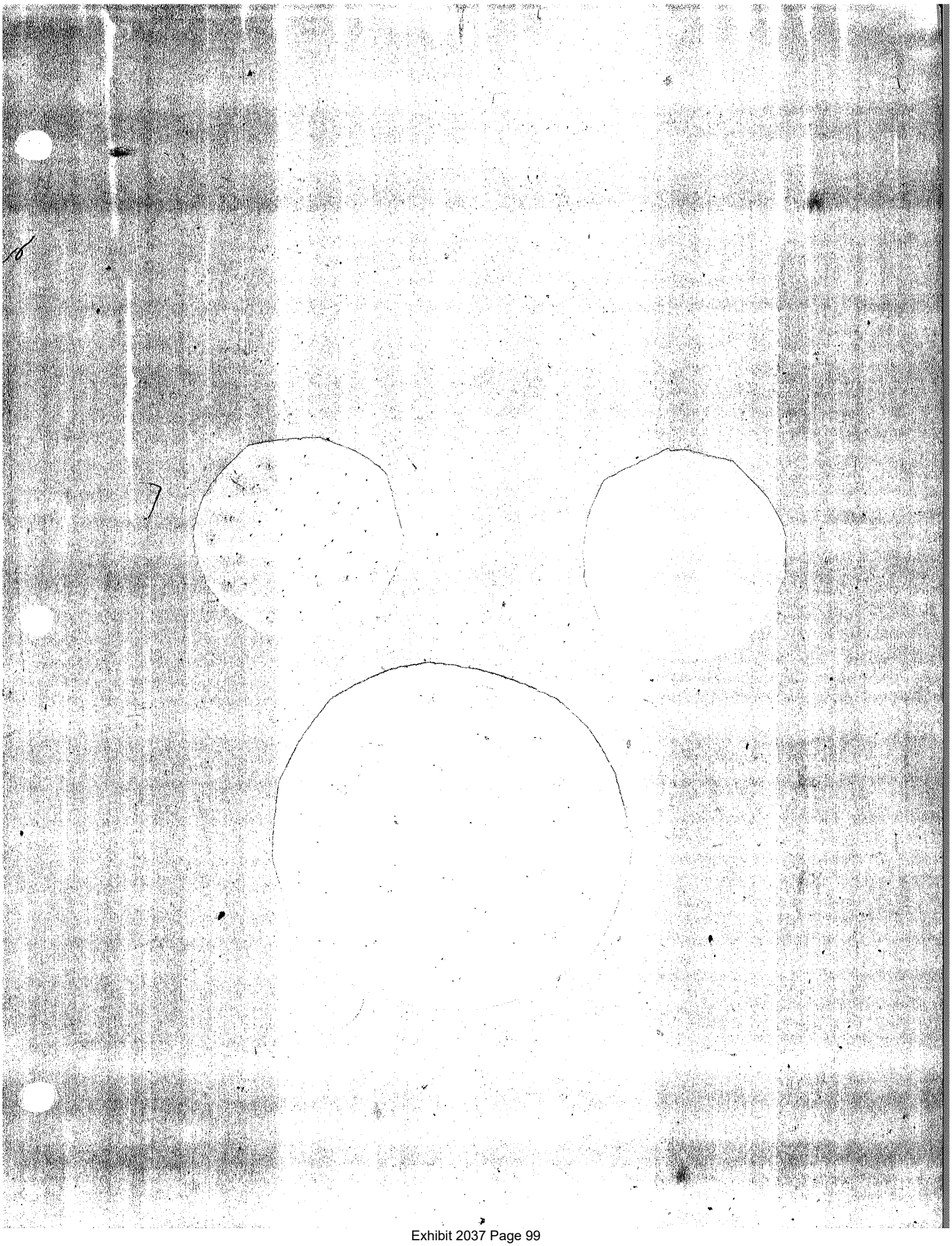
blue spots started appearing on plates where glucosylate λ Fel V DNA was hybridized to λ Fel V phage plaques.

7/1/82

glucosylated probe hybridized to phage plaques
Con A binding
Sandwitch Technique

Acid phosphatase - ~~Tetrazolium method~~
diazo
Fast Red TR salt
~~5-chl~~





6.25.82.

Hybridization of malto-Wiose 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 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/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

4 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 [2 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/ml	8 ul
MT-dUTP Ad-2 DNA or TTP Ad-2 DNA (54 ng/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.

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 2.0 M 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

&.

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