

Con-A Sepharose column binding 4/10/82.
of glycosylated DNA.

(A) Effect of Mannose +

(B) Effect of non-glycosylated DNA.

^3H TH DNA 2.5×10^5 epm per microgram

^{14}C E. coli DNA 1.1×10^4 epm per microgram.

d-Mannose 20 micrograms per ml.

Con-A Sepharose 18 μg Con A bound per
ml of settled gel.

Columns.

Con-A Sepharose columns 10 μg Con A.

0.6 ml settled gel

washed extensively with 1% Triton-X-100
~ 10 column volumes to remove stabilizer

Equilibrated with 20 column volumes

of PBS 0.01 M K-PO₄ pH 7.2

0.15 M NaCl

containing 10 μM MgCl₂

Aliquots of

4 micrograms ^3H T4 DNA

$\sim 1 \times 10^6$ ^3H cpm.

mixed \bar{e}

(A) PBS + Mg^{++} 0.2 ul

(B) d. Mannose 0.2 ul 4 micrograms.

(C) ^{14}C E. coli DNA 0.2 ul 4 micrograms

$\sim 4.4 \times 10^4$ ^{14}C cpm.

Each aliquot fractionated on cou-A sepharose columns as in earlier expts.

0.2 ul fractions collected

100 fractions.

Radioactivity checked.

plotted ~~as~~ column elution volume vs % of Input



³H T_H DNA on con A sepharose
 (c) Effect of ¹⁴C E. coli DNA

Total Volume
 (0.5 ul)

FR #	¹⁴ C epm			³ H epm		
	per Aliquot	Total	%	per aliquot	Total	%
Sample (5x)	400	40000	100	9560	956000	100
Sample (5x) E. coli	130	13000	32.5	10		> 0.01
PBST + MgPT						
200x	1	11600	11600	29	10	> 0.01
	2	7600	7600	19	20	
	3	4000	4000	10	10	
	4	2400	2400	6	10	
	5	1200	1200	3	10	
	6	400	400	1	10	
	7	186		0.145	10	
	8	60		0.15	15	
	9	30		0.075	10	
	10	40		0.1	8	
	1	60		0.15	10	
	2	30		0.075	6	
	3	20		0.05	10	
	4	10		0.025	10	
	5	15		0.03	6	
	6	20		0.05	5	
	7	15		0.03	8	
	8	28		0.04	10	
	9	10		0.025	8	
	20	8			10	
	1	18			10	
	2	10			10	
	3	20			20	
	4	30			10	
	5	60			10	
	6	30			10	
	7	30			8	
	8	60			6	
	9	16			8	
	30	5			2	
	1	28			10	
	2	28			10	
	3	80			10	
	4	16			10	
	5	30			10	
	6	20			10	
	7	10			10	

^3H DNA on con A sepharose (0.5 ml Total Volume)
 (B) Effect of d mannose.

FR #	^3H epm	FR #	^3H epm	Total % Input
	per aliquot			
Sample (5x)	9880	41	23	100
Sample wash (5x)	4610	45	20	> 0.001 %
PBS + Mg ⁺⁺ (5x)		50	10	
1	2470	55	5	
2	1285	60	8	
(100x) 3	34086	65	2	
(100x) 4	9880	70	3	
(100x) 5	24750	75	5	
(200x) 6	1620	80	8	
7	860	85	10	
8	220	90	3	
9	110	100	8	
10	36			
11	40			
12	33			
13	20			
14	24			
15	30			
16	56			
17	30			
18	11			
19	10			
20	16			
1	5			
2	10			
3	18			
4	13			
5	12			
6	13			
7	14			
8	13			
9	13			
30	13			
1	12			
2	10			
3	20			
4	25			
5	30			
6	36			
7	38			
8	30			
9	38			
40	40			

Tris pH 8.2

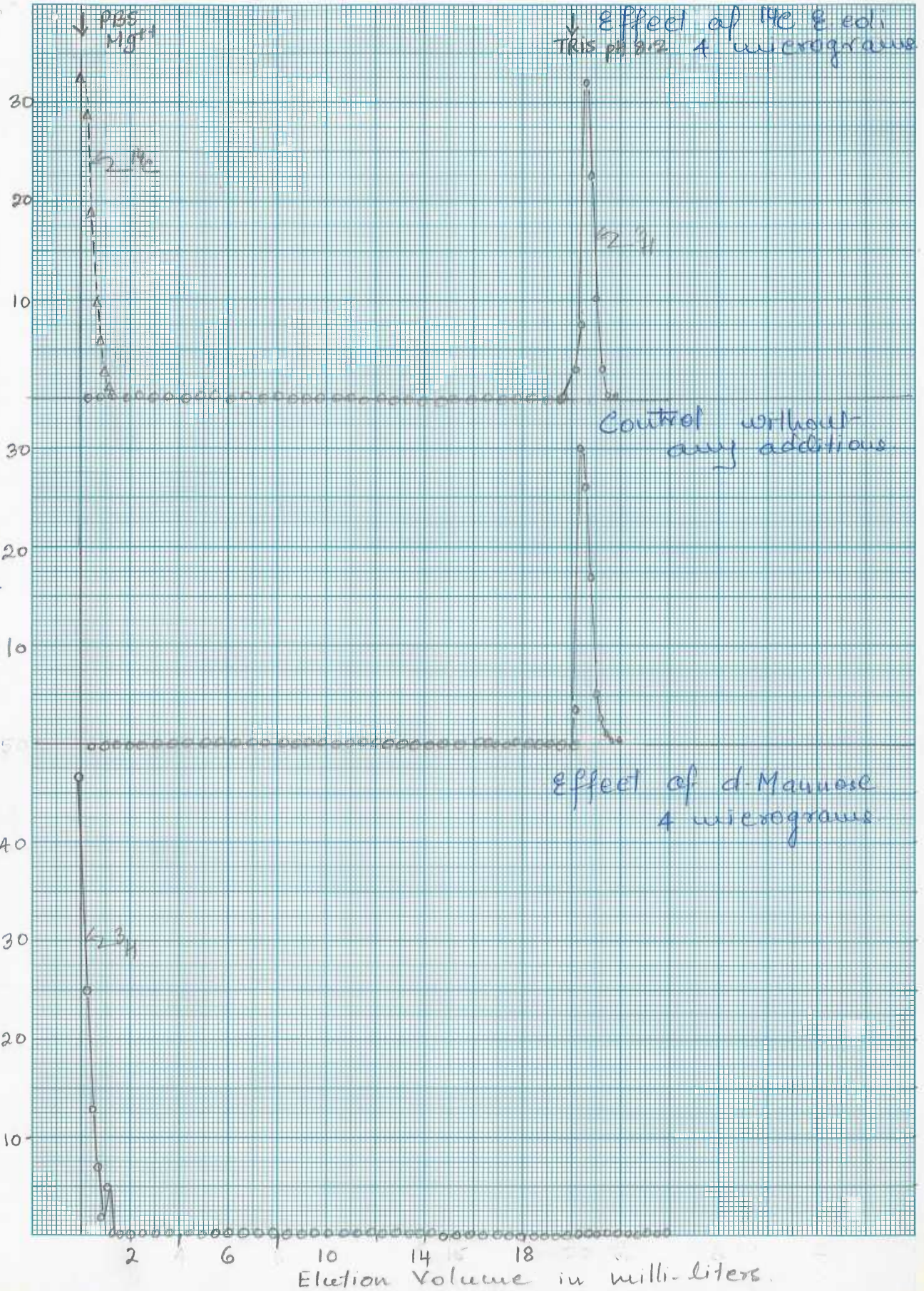
> 0.001

(C)

FR #	14 e epm			3 H epm		
	Aliquot	Total	% Input	Aliquot	Total	% Input
40	20		> 0.001 ↓	10		> 0.001 ↓
45	10			18		
50	10			16		
55	15			15		
60	30			19		
65	10			17		
70	30			16		
75	8			15		
80	6			12		
85	3			11		
90	2			10		
100	1					
T.Ris pH 8.2						
101	3		> 0.001 ↓	28680		3
2	2			71700		7.5
3	0			308788		32.3
4	3			215100		22.5
5	0			95600		10.0
6	6			27724		2.9
7	2			4780		0.5
8	0					
9	0					
110	2					
1	1					
2	0					
3	20					
4	0					
5	0					
6	30					
7	0					
8	3					
9	0					
120	0					

^3H T4 DNA on con A sepharose (0.6ml columns)

KE 10 X 10 TO THE CENTIMETER 18 X 25 CM.
 KEUFFEL & ESSER CO. MADE IN U.S.A.
 46,1521
 ^3H epm - percent of total suput



^3H epm - percent of Total suput Δ --- Δ --- Δ

5/25/82.

Denaturation of DNA

0.3 M NaOH 10' RT.

chill

T4 DNA 200 μ /ml in 10 mM NaCl
10 mM Mg⁺⁺

1. 25 λ DNA

112.5 λ NaCl Mg⁺⁺

18.75 λ 2.0 M NaOH

2. 125 λ DNA

18.75 λ 2.0 M NaOH.

3. 500 λ DNA 50 μ /ml

18.75 λ 2.0 M NaOH

λ p lac DNA 515 μ /ml.

4. 4 λ DNA

121 λ NaCl Mg⁺⁺

18.75 λ 2.0 M NaOH

⑥ 500 μ DNA 50 μ /ml

18.75 λ 2.0 M NaOH

5. 20 λ DNA

105 λ NaCl Mg⁺⁺

18.75 λ 2.0 M NaOH

10' at RT.

chill dilute to
equal vol. 10x sse.

Dot blot experiments.

5/25/82.

Nitrocellulose filters 8 $\frac{1}{2}$ S
47 mm size. 0.45 microns.

- ① Wash the filters in H₂O
- ② " " " in 5x SSC
- ③ Dry Air.
- ④ T₄ and λ plac DNA A260 1.0 ~ 50 micrograms per ml in 10 mM NaCl
10 mM Mg⁺⁺

denatured by incubating in boiling H₂O bath for 5'

chilled immediately to keep them denatured.

- ⑤ Na 20 x SSC added to DNA solutions to bring SSC concentrations to 5x SSC
- ⑥ Applied to washed filters.

T₄ NaCl Mg⁺⁺ λ plac

1x	1x	1x
1.5x	1.5x	1.5x
2.0x	2.0x	2.0x
2.5x	2.5x	2.5x

- ⑦ Filter was then air-dried

⑧ Filter washed with 5x SSC

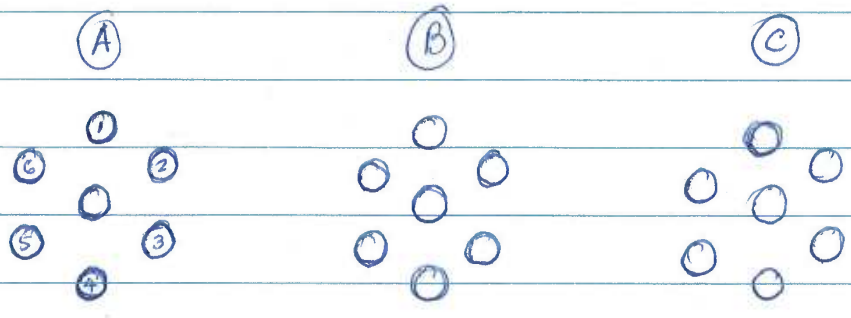
⑨ u baked at 80° for 2 hours.

⑩ u blocked \bar{c} 3% BSA in 2x SSC
at 37° for 2 hours in sealed tube-bag.

⑪ Washed \bar{c} 0.2 μ g cDNA in 5.0 ml 5x SSC
containing 20 mM Mg^{++}
incubated at RT. for 30-60 minutes.

⑫

Detection by Ochterlony techniques. 5/18/82



Center Well

Con A	1 mg / ul.	A
	2.5 mg / ul.	B
	7.5 mg / ul.	C

(2.30 pm.)

- 1 PBS
- 2 Mannose
- 3 Maltose
- 4 λ pla DNA
- 5 T₄ DNA
- 6 ATP

left at 4° in humid atmosphere for 42 hrs.

4.8.82.

DNA binding to cou-A sepharose column.

^{32}P DNA 2.15×10^5 cpm per microgram.
 ^{14}C E. coli DNA 1.1×10^4 cpm per microgram.

Cou-A sepharose 18 μg cou A bound per
ml of settled gel.

Column.

Cou-A sepharose column.

0.6 ml settled gel \approx 10 μg cou A
washed with 1% Triton-X-100
10 column volumes to remove
the stabilizer.

Equilibrated with 10-20 column volumes
of PBS 0.01 M KPO_4 pH 7.2
0.15 M NaCl
containing 10 mM MgCl_2

2-5 micrograms DNA in 100 microliters
of 10 mM TRIS NaCl pH 7.2
applied to the column.

Column washed extensively with equilibrating

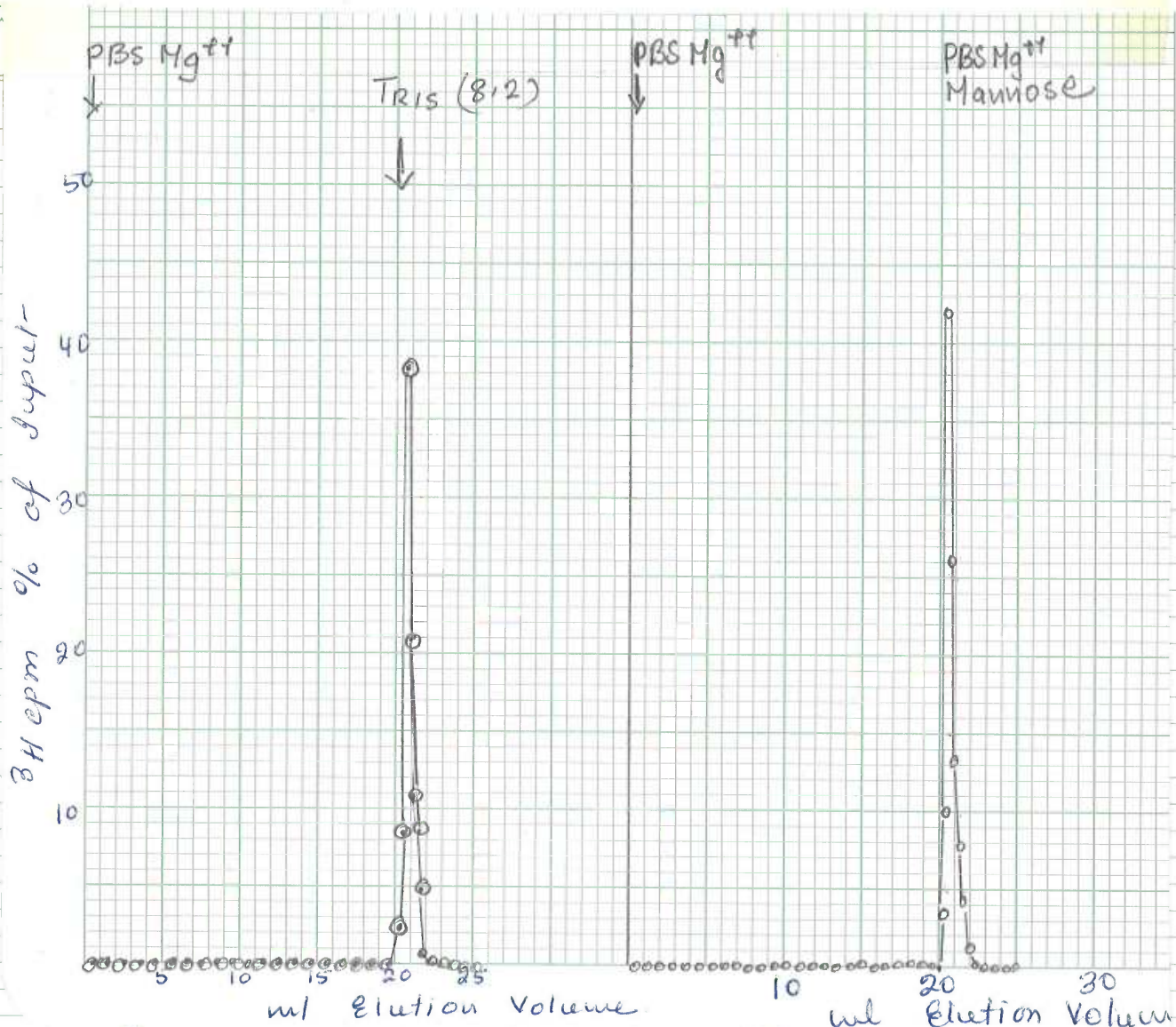
buffer \approx 30 column volumes
 \approx 20 ul.

0.2 ul fractions collected
aliquots checked for radioactivity.

Elution continued with ~~high pH~~ buffer

(a) high pH buffer Tris NaCl pH 8.2

(b) mannose containing PBS- Mg^{++} buffer



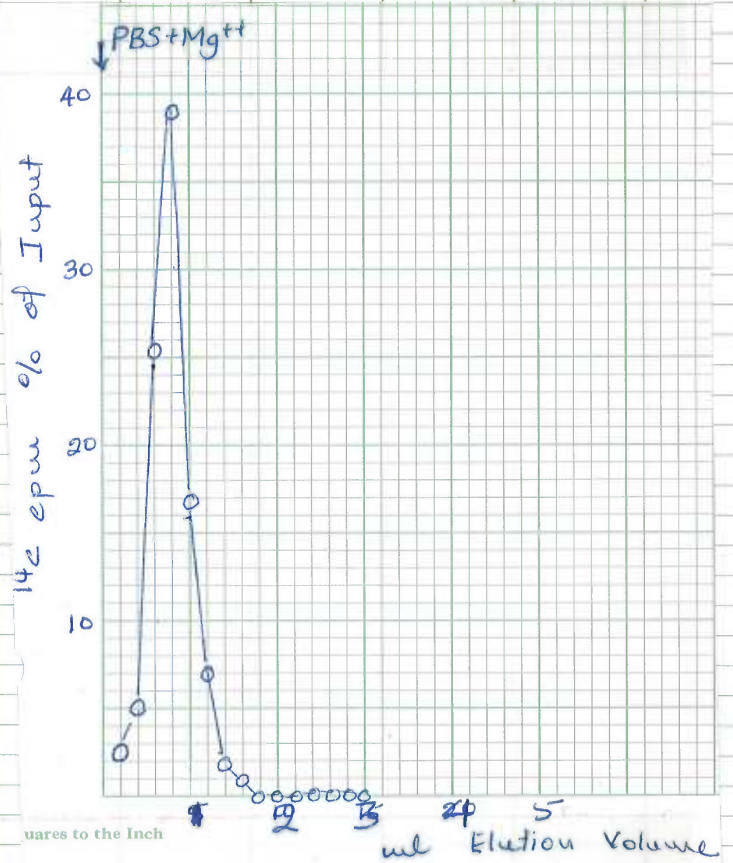
	ml Elution Volume	cpm	ml Elution Volume	cpm
3	11	5613	11	2260
4	12	2923	12	58460
5	13	1705	13	34100
6	14	983	14	19660
7	15	310	15	6200
8	16	39	16	780
9	17	23	17	460
10	18	4	18	220
11	19	3	19	26.28
12	20	3	20	13.68
13	21	3	21	7.98
14	22	10	22	4.60
			23	1.45
			24	0.18
			25	0.11
			26	0.052

FR #	⁸ Hepm 10x	Total	% Input
<u>Tris 8.2 wash</u>			
1	561	11220	2.63
2	1843	36860	8.63
3	8190	163800	38.34
4	4486	89720	21.00
5	2328	46560	10.9
6	1859	37170	8.7
7	1053	21060	4.93
8	200	4000	0.94
9	38	760	0.18
10	4	80	0.019

FR #	³ Hepm	Total	% Input
<u>Mannose 2 micrograms in PBS + Mg²⁺ Wash</u>			
1	765	15300	3.58
2	2238	44760	10.48
3	9056	181120	42.397
4	5613	112260	26.28
5	2923	58460	13.68
6	1705	34100	7.98
7	983	19660	4.60
8	310	6200	1.45
9	39	780	0.18
10	23	460	0.11
11	11	220	0.052
12	3		
13	3		
14	10		

¹⁴C E. coli DNA on Con A Sepharose

FR #	¹⁴ C per Aliquot	epm Total	% Input
Sample	(10x) 229	22900	100%
Sample Eluate	(200x) 448	448	1.96
PBS+Mg ⁺⁺ wash	(200x)		
1	613	613	2.68
2	1212	1212	5.29
3	5865	5865	25.61
4	8954	8954	39.10
5	3870	3870	16.90
6	1603	1603	7.00
7	458	458	2.00
8	241	241	1.05
9	36	36	
10	16	16	
11	10	10	
12	11	11	
13	36	36	
14	25	25	
15	13	13	
16	14	14	
17	30	30	
18	26	26	
19	15	15	
20	18	18	
21	10	10	
22	36	36	
23	30	30	
24	31	31	
25	21	21	
26	3	3	
27	5	5	
28	3	3	
29	2	2	
30	3	3	
31	3	3	
32	0	0	
33	2	2	
34	5	5	
35	10	10	



4.6.82.

Concanavallin A precipitation of radioactivity
from ^3H Thy labelled T4 DNA.

Comparison with ^{14}C labelled non-glycosylated
DNA

DNA (a) ^3H (Thy) T4 DNA from ^3H labelled
T4 phage T4 am 82 (44'62")
260 micrograms per ul in
10 mM Tris pH 7.2
10 mM NaCl

Sp act. 2.5×10^5 epm per microgram

(b) ^{14}C labelled E. coli DNA
hi mol wt 454 micrograms per
ul in 10 mM Tris pH 7.2
10 mM NaCl

Sp. act 1.1×10^4 epm per microgram.

Concanavallin A 20.0 mg per ul in 2.0 M
NaCl solution.

4.6.82

Incubation mixtures of 0.5 ml
consisted of

NaCl 1.0 M

PO₄ 0.01 M pH 7.2

³H or ¹⁴C DNA 1 - 10 micrograms.

con A 1 - 6 μg.

Reactions started by adding con A
to assay mixtures containing DNA
mixed well by vortexing

Incubated at 25° [RT.] for 10 minutes

Centrifuged and radioactivity determined
on aliquots of supernatant.

4.6.82

341 epm per aliquot Supernatant

DNA		con A	per aliquot 10 λ	Total	% non- pptd
3HT4 DNA	2	0	1650	82500	100
	4	0	3490	174500	100
	6	0	5000	250000	100
	8	0	7135	356750	100
	10	0	10,030	501500	100
	2	1 mg	48	2460	2.9
	4	↓	156	7800	4.46
	6		235	11750	4.7
	8		410	20500	5.7
	10		636	31800	6.3
	2		2 mg	23	1150
	4	↓	54	2700	1.5
	6		68	3400	1.36
	8		120	6000	1.68
	10		256	12800	2.55
2	4 mg		(100 λ aliquots) 22	110	0.13
4	↓	38	190	0.11	
6		49	245	0.098	
8		65	325	0.091	
10		75	375	0.074	
2		6 mg	36	180	0.218
4	↓	65	325	0.186	
6		83	415	0.17	
8		120	600	0.168	
10		195	975	0.194	

DNA		Con A	Radioactivity in Supernatant
			per aliquot Total
			(10x)
¹⁴ C E. coli DNA	2 Microgram.	0	250
	4	↓	386
	6	↓	800
	8	↓	966
	10	↓	1050
	2	1 mgm	248
	4	↓	350
	6	↓	900
	8	↓	958
	10	↓	1090
	2	2 mgm.	256
	4	↓	386
	6	↓	795
	8	↓	980
	10	↓	1100
	2	4 mgm	245
	4	↓	354
	6	↓	810
	8	↓	975
	10	↓	1076
2	6 mgm	256	
4	↓	400	
6	↓	754	
8	↓	1011	
10	↓	1210	

Con-A Sepharose Binding of glucosylated DNAs
3/2-9/82

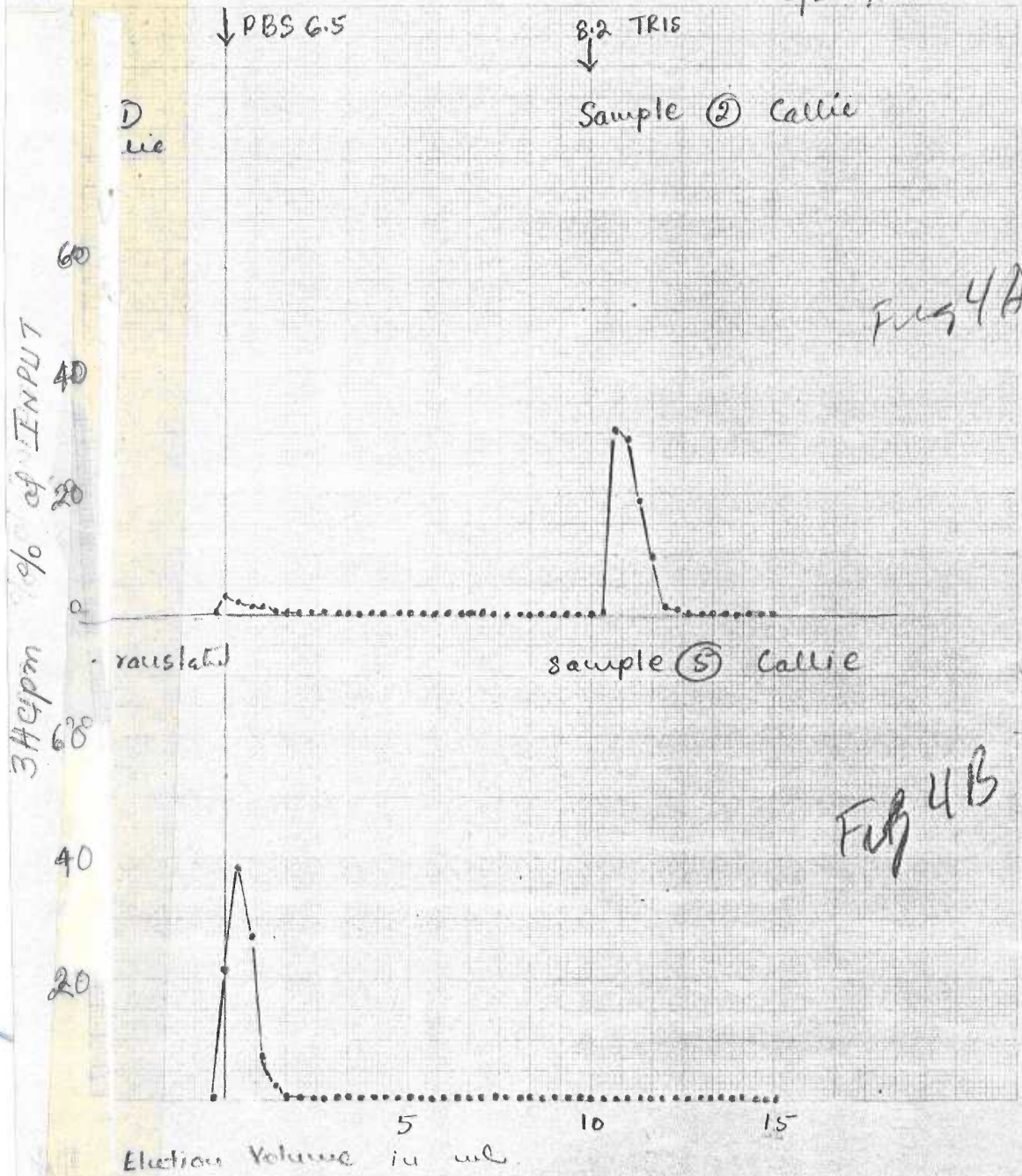


Fig 4A

Fig 4B

© Miller 1982

Con. A sepharose column

3/8/82.

Binding of glycosylated DNAs.

DNAs.

- ① 3HT λ DNA nick translated 2/26/82.
- ② Callie's sample I 10 λ
- ③
- ④
- | | | | |
|------|-----------|------|-----------|
| 1.41 | 001.00 | 1.65 | 001.00 |
| | 003.00 | | 003.00 |
| | 005594.00 | | 006848.00 |

DNA sample preparations

I. 100 λ DNA nick translated λ DNA. }
2.5 λ MgSO₄ · 7H₂O } 0.5 ml.
5 λ CaCl₂. }
392.5 λ PBS (6.5) } 10 λ for counting.

II. 100 λ DNA Callie sample I }
2.5 λ MgSO₄ · 7H₂O } 0.5 ml
5 λ CaCl₂. }
392.5 λ PBS (6.5) } 10 λ for counting.

III. 100 λ DNA Callie sample II }
2.5 λ MgSO₄ · 7H₂O } 0.5 ml
5 λ CaCl₂. }
392.5 λ PBS (6.5) } 10 λ for counting.

IV. 100 λ DNA Callie sample ⑤ }
2.5 λ MgSO₄ } 0.5 ml
5 λ CaCl₂. }
392.5 λ PBS (6.5) } 10 λ for counting.

3/8-9/82.

Con A- sepharose column.

1.0 ml column equilibrated \bar{c} PBS (6.5)
50-100 μ l
DNA sample applied Eluate collected
Aliquot of 0.5 μ l PBS 6.5 used to drive
the sample into the gel. Eluate collected.
Column washed with 10-15 μ l PBS (6.5)
0.33 μ l fractions collected - 30 fractions
Column was then eluted with 10 mM
Tris (8.2) 10 mM NaCl 5-7 μ l.
Fractions collected.

All fractions counted using 3.5 μ l
Reafluvor.

Results expressed as counts percent
of input vs elution volume.

1 01
001.00
005.00
002226.00
1 02
001.00
015.00
000346.00
1 03
001.00
001.00
050569.00
1 04
001.00
001.50
025009.00
1 05
001.00
005.00
002363.00
1 06
001.00
010.00
000512.00
1 07
001.00
015.00
000372.00
1 08
001.00
015.00
000252.00
1 09
001.00
020.00
000155.00
1 10
001.00
020.00
000134.00
1 11
001.00
020.00
000109.00
1 12
001.00
000.00
000098.00
1 13
001.00
000.00
000096.00
1 14
001.00
000.00
000065.00
1 15
001.00
000.00
000070.00
1 16
001.00
000.00
000062.00
1 17
001.00
000.00
000058.00
1 18
001.00
000.00
000066.00
1 19
001.00
000.00
000062.00
1 20
001.00
000.00
000052.00

1 65
001.00
003.00
004490.00
1 67
001.00
010.00
000727.00
1 68
001.00
015.00
000201.00
1 69
001.00
005.00
003753.00
1 70
001.00
007.00
001018.00
1 71
001.00
015.00
000320.00
1 72
001.00
015.00
000268.00
1 73
001.00
015.00
000224.00
1 74
001.00
020.00
000165.00
1 75
001.00
020.00
000157.00
1 76
001.00
020.00
000135.00
1 77
001.00
020.00
000135.00
1 78
001.00
020.00
000104.00
1 79
001.00
000.00
000082.00
1 80
001.00
020.00
000105.00
1 81
001.00
000.00
000088.00
1 82
001.00
000.00
000094.00
1 83
001.00
020.00
000105.00
1 84
001.00
000.00
000080.00



1 03
001.00
003.00
005125.00
1 05
001.00
007.00
000969.00
1 06
001.00
015.00
000354.00
1 07
001.00
003.00
007643.00
1 08
001.00
003.00
005257.00
1 09
001.00
005.00
002425.00
1 10
001.00
010.00
000784.00
1 11
001.00
015.00
000349.00
1 12
001.00
015.00
000235.00
1 13
001.00
020.00
000189.00
1 14
001.00
020.00
000134.00
1 15
001.00
020.00
000122.00
1 16
001.00
020.00
000116.00
1 17
001.00
020.00
000131.00
1 18
001.00
020.00
000109.00
1 19
001.00
000.00
000098.00
1 20
001.00
000.00
000084.00
1 21
001.00
000.00
000075.00
1 22
001.00
000.00
000075.00

1 03
001.00
003.00
006874.00
1 05
001.00
010.00
000632.00
1 06
001.00
020.00
000157.00
1 07
001.00
003.00
006804.00
1 09
001.00
005.00
002128.00
1 10
001.00
010.00
000587.00
1 11
001.00
015.00
000285.00
1 12
001.00
020.00
000179.00
1 13
001.00
020.00
000141.00
1 14
001.00
020.00
000132.00
1 15
001.00
020.00
000140.00
1 16
001.00
000.00
000098.00
1 17
001.00
000.00
000079.00
1 18
001.00
000.00
000078.00
1 19
001.00
000.00
000089.00
1 20
001.00
000.00
000081.00
1 21
001.00
000.00
000075.00
1 22
001.00
000.00
000080.00
1 23
001.00
000.00
000052.00

001.00	000.00	001.00	000080.00
000053.00	000075.00	000.00	1 23
1 22	1 86	000077.00	001.00
001.00	001.00	1 24	000.00
000.00	000.00	001.00	000052.00
000077.00	000075.00	000.00	1 24
1 23	1 87	000070.00	001.00
001.00	001.00	1 25	000.00
000.00	000.00	001.00	000066.00
000053.00	000090.00	000.00	1 25
1 24	1 88	000055.00	001.00
001.00	001.00	1 26	000.00
000.00	000.00	001.00	000049.00
000049.00	000069.00	000.00	1 26
1 25	1 89	000059.00	001.00
001.00	001.00	1 28	000.00
000.00	000.00	001.00	000061.00
000057.00	000077.00	000.00	1 27
1 26	1 90	000063.00	001.00
001.00	001.00	1 29	000.00
000.00	000.00	001.00	000052.00
000044.00	000069.00	000.00	1 28
1 27	1 91	000068.00	001.00
001.00	001.00	1 30	000.00
000.00	000.00	001.00	000050.00
000052.00	000074.00	000.00	1 29
1 28	1 92	000060.00	001.00
001.00	001.00	1 31	000.00
000.00	000.00	001.00	000041.00
000032.00	000063.00	000.00	1 30
1 29	1 93	000065.00	001.00
001.00	001.00	1 32	000.00
000.00	000.00	001.00	000059.00
000038.00	000070.00	000.00	1 31
1 31	1 94	000062.00	001.00
001.00	001.00	1 33	000.00
000.00	000.00	001.00	000054.00
000099.00	000056.00	000.00	1 32
1 32	1 95	000059.00	001.00
001.00	001.00	1 34	000.00
003.00	000.00	001.00	000047.00
004556.00	000063.00	000.00	1 33
1 33	1 96	000049.00	001.00
001.00	001.00	1 35	000.00
005.00	000.00	001.00	000058.00
002733.00	000054.00	000.00	1 34
1 34	1 97	000047.00	001.00
001.00	001.00	1 36	000.00
010.00	000.00	001.00	000057.00
000713.00	000067.00	000.00	1 35
1 35	1 98	000066.00	001.00
001.00	001.00	1 37	000.00
015.00	000.00	001.00	000053.00
000312.00	000049.00	000.00	1 36
1 36	1 99	000055.00	001.00
001.00	001.00		000.00
015.00	000.00		000035.00
000210.00	000056.00		1 37
1 37	1 00		001.00
001.00	001.00		000.00
020.00	000.00		000063.00
000110.00	000033.00		1 38
1 38			001.00
001.00			000.00
000.00			000048.00
000078.00			1 39
1 39			001.00
001.00			000.00
000.00			000037.00
000071.00			1 03
1 40			001.00
001.00			020.00
000.00			000130.00
000045.00			1 04
1 41			001.00
001.00			002.00
000.00			014847.00
000044.00			1 05
1 42			001.00
001.00			003.00
000.00			006423.00
000019.00			1 06
1 43			001.00
001.00			005.00

1 43	000715.00	001.00	1 07
001.00	1 06	015.00	001.00
000.00	001.00	000320.00	007.00
000016.00	015.00	1 45	000899.00
1 44	000373.00	001.00	1 08
001.00	1 07	020.00	001.00
000.00	001.00	000179.00	010.00
000028.00	015.00	1 46	000559.00
1 45	000244.00	001.00	1 09
001.00	1 08	020.00	001.00
000.00	001.00	000162.00	015.00
000016.00	020.00	1 47	000343.00
1 46	000181.00	001.00	1 10
001.00	1 09	020.00	001.00
000.00	001.00	000138.00	015.00
000024.00	020.00	1 48	000251.00
	000124.00	001.00	1 11
	1 10	000.00	001.00
	001.00	000090.00	020.00
	020.00	1 49	000192.00
	000104.00	001.00	1 12
	1 11	000.00	001.00
	001.00	000068.00	020.00
	000.00	1 50	000120.00
	000075.00	001.00	1 13
	1 12	000.00	001.00
	001.00	000050.00	000.00
	000.00	1 51	000089.00
	000055.00	001.00	1 14
		000.00	001.00
		000049.00	000.00
		1 52	000076.00
		001.00	1 15
		000.00	001.00
		000036.00	000.00
		1 53	000066.00
		001.00	1 16
		000.00	001.00
		000027.00	000.00
		1 54	000078.00
		001.00	1 17
		000.00	001.00
		000032.00	000.00
		1 55	000016.00
		001.00	
		000.00	
		000035.00	
		1 56	
		001.00	
		000.00	
		000031.00	

#<

1.4 ml
Total

1 03
001.00
003.00
004239.00

4023.5

Total = 563,290

1 04
001.00
005.00
003808.00

0.095, 822

2011 (24)
Date:

4239
3808

2 8047

4,023.5
X 140

1609400
40235
563,290.0

DNA sample I - Known negative control
 3HTTP - nick-translated λ DNA
 2/26/82.

	1 FR #	2 Hepun	3 % Input	4 -	5 FR #	6 Hepun	7 % Input	8	9
Original	107	2226			28	57	0.051		
1	X 50	111300	100 %		29	44	0.04		
2	S. Eluate	346	0.31		30	52	0.047		
3	W. Eluate	50569	45.44		TRIS(8.2)				
4	PBS #1	25009	22.47		31	32	0.029		
5	2	4556	4.09		32	38	0.034		
6	3	2733	2.46		33	78	0.07		
7	4	2363	2.12		34	71	0.064		
8	5	713	0.64		35	45	0.04		
9	6	512	0.46		36	44	0.04		
10	7	372	0.33		37	19	0.017		
11	8	312	0.28		38	16	0.014		
12	9	252	0.23		39	28	0.025		
13	10	210	0.19		40	16	0.014		
14	11	155	0.14		41	24	0.022		
15	12	134	0.12		42	16	0.014		
16	13	110	0.099						
17	14	109	0.098						
18	15	98	0.088						
19	16	96	0.086						
20	17	65	0.058						
21	18	70	0.063						
22	19	62	0.056						
23	20	58	0.052						
24	21	66	0.059						
25	22	62	0.056						
26	23	53	0.048						
27	24	53	0.048						
28	25	77	0.069						
29	26	53	0.048						
30	27	49	0.044						

DNA Sample II [# i of Callie]

	1 FR #	2 Hepu	3 % Input	5 FR #	6 Hepu	7 % Input	9
Original	10x	4490		# 26	63	0.17	
Column	Sany 10x	727		27	54	0.15	
3	x50	36350	100	28	67	0.18	
4				29	49	0.13	
5	S.E	201	0.55	30	56	0.15	
6	W.E	3753	10.32				
7	PBS.#1	4048	11.14	TRIS (8.2)			
8	2	1629	4.48	31	33	0.091	
9	3	1018	2.8	32	1820	5.01	
10	4	620	1.71	33	11058	30.42	
11	5	468	1.29	34	2364	6.5	
12	6	224	0.62	35	915	1.97	
13	7	165	0.45	36	393	1.03	
14	8	157	0.43	37	244	0.67	
15	9	135	0.37	38	181	0.50	
16	10	135	0.37	39	124	0.34	
17	11	104	0.29	40	104	0.29	
18	12	82	0.23	41	75	0.21	
19	13	105	0.29	42	55	0.15	
20	14	88	0.24				
21	15	94	0.26				
22	16	105	0.29				
23	17	85	0.23				
24	18	75	0.21				
25	19	75	0.21				
26	20	90	0.25				
27	21	69	0.19				
28	22	77	0.21				
29	23	69	0.19				
30	24	74	0.20				
31	25	63	0.17				

DNA Sample III [#2 of Callie]

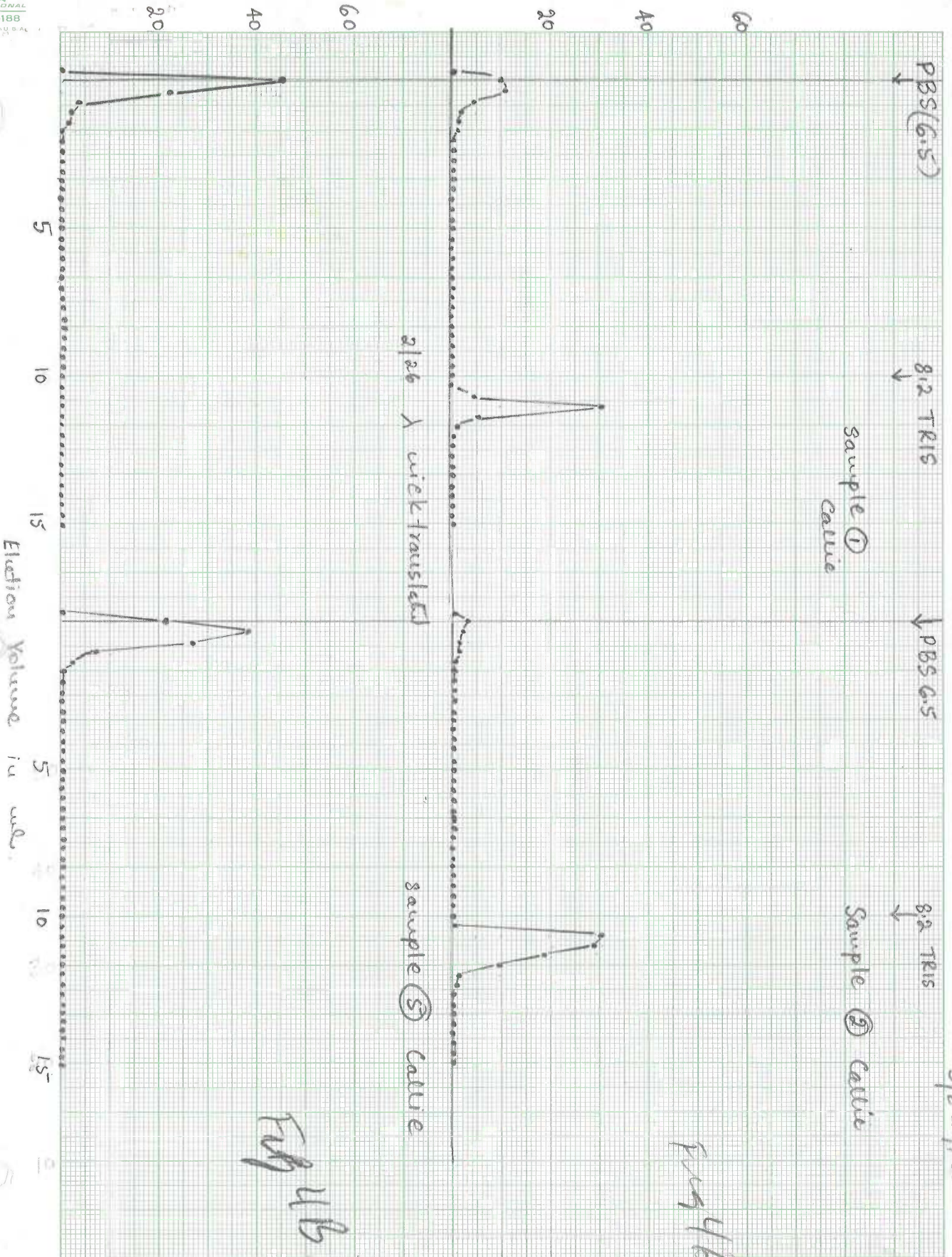
3/9/82

	1 FR #	2 Hepu	3 % Input	5 FR #	3 Hepu	% Input	9
	Original 10x	5125		27	47	0.098	
	Sample 10x	969		28	66	0.14	
3	XSD	48450	100	29	55	0.12	
4	SE	354	0.73	30	46	0.095	
5	W.E	1643	3.39				
6	PIBS 1	1257	2.60	Tris (8.2)			
7	2	845	1.75	31	282	0.58	
8	3	784	1.62	32	14913	30.78	
9	4	349	0.72	33	14021	28.94	
10	5	235	0.49	34	9168	18.93	
11	6	189	0.39	35	4588	9.47	
12	7	134	0.28	36	820	1.69	
13	8	122	0.25	37	479	0.99	
14	9	116	0.24	38	262	0.54	
15	10	131	0.27	39	138	0.29	
16	11	109	0.23	40	90	0.19	
17	12	98	0.20	41	68	0.14	
18	13	84	0.17	42	50	0.10	
19	14	75	0.15	43	49	0.10	
20	15	71	0.15	44	36	0.074	
21	16	77	0.16	45	27	0.056	
22	17	70	0.15	46	32	0.066	
23	18	55	0.11	47	35	0.072	
24	19	59	0.12	48	31	0.064	
25	20	63	0.13				
26	21	68	0.14				
27	22	60	0.12				
28	23	65	0.13				
29	24	62	0.13				
30	25	59	0.12				
31	26	49	0.10				

DNA sample #4 [sample (5) of callie]

	1 FR #	2 ³ Hcpm	3 % Input	5 FR #	6 ³ Hcpm	7 % Input	9
Original	10x	6874		# 27	58	0.18	
Callie Sample	10x	632		28	57	0.18	
3	X 50	31600	100	29	53	0.17	
4	S.E.	157	0.5	30	35	0.11	
5	W.E.	6804	21.53				
6	PBS # 1	12128	38.38	Tris (8.2)			
7	2	8587	27.17	31	63	0.20	
8	3	2285	7.23	32	48	0.15	
9	4	899	2.85	33	37	0.12	
10	5	285	0.90	34	30	0.095	
11	6	179	0.57	35	47	0.15	
12	7	141	0.45	36	28	0.09	
13	8	132	0.42	37	38	0.12	
14	9	140	0.44	38	19	0.06	
15	10	98	0.31	39	59	0.19	
16	11	79	0.25	40	43	0.14	
17	12	78	0.25	41	51	0.16	
18	13	89	0.28	42	42	0.14	
19	14	81	0.26	43	20	0.06	
20	15	75	0.24	44	19	0.06	
21	16	80	0.26	45	18	0.06	
22	17	52	0.17	46	16	0.05	
23	18	66	0.21	47	18	0.06	
24	19	49	0.16	48	16	0.05	
25	20	61	0.19				
26	21	52	0.17				
27	22	50	0.16				
28	23	41	0.13				
29	24	59	0.19				
30	25	54	0.17				
31	26	47	0.15				

3 Hepm % of INPUT



Cou-A Sepharose Binding of glucosylated DNAs
 3/8-9/82

① fractio B

35.8% 0.7×10^6

② fracti C

39.3% 0.8×10^6

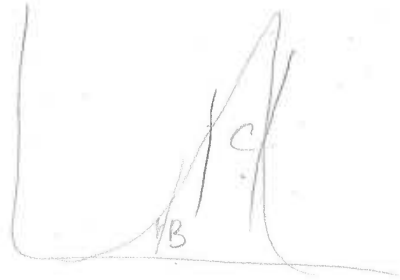
~~③ λ~~

0.6×10^6

% of

Approx.

6 K bases.



Cou-A sepharose binding

3/6/82

FR01

Samples

from

Stan

Jollie

Samples are numbered

(#1), ~~(#2)~~, (#3) and (#4).

They are in 4.0 ml test tubes in a beaker on top of the rack that we indicated in the freezer. In 50mm KPO₄ #2.0

1 02
001.00
003.00
005236.00
1 03
001.00
003.00
004671.00
1 04
001.00
007.00
001480.00
1 05
001.00
007.00
001448.00
1 06
001.00
007.00
001547.00
1 07
001.00
007.00
001402.00

Sample #	Vol.	cpm/10λ	cpm Total
# 1	2.2	5236 > 4953.5 4671	1089,770
# 3	1.9	1480 > 1464 1448	278,160
# 4	2.0	1543 > 1514.5	302,900

Samples for column.

0.5 ml

ie PBS 6.5

I

II

MgSO₄ 5 mM
CaCl₂ 1 mM

	I	II	III
① DNA sample ①	45λ	③ 180λ	④ 165λ
MgSO ₄ 7H ₂ O	2.5λ	2.5λ	2.5λ
CaCl ₂	5λ	5λ	5λ
PBS	447.5λ	312.5λ	329.5λ

3/6/82.

- ① One ml cou-A sepharose column equilibrated
50-100 ml 10 mM Na-K-PO₄ pH 6.5 } PBS
150 mM NaCl }

buffer.

- ② DNA samples under test were applied to
the column — 0.15 ml volume.

Eluate collected

- ③ Samples were driven into the gel & two
aliquots of 0.25 ml each PBS (6.5) buffer.

Eluate collected

- ④ Columns were eluted with 10 ml PBS (6.5)
buffer

0.33 ml — 10 drop — fractions collected.

- ⑤ 30 fractions total.

- ⑥ Column was then washed with

5.0 ml 10 mM TRIS } pH 8.2
10 mM NaCl }

10 drop ~ 0.33 ml fractions collected

To each fraction added 3.5 ml Reafluor
and all fractions counted.

- ⑦ Results are plotted as
3H cpm percent of total input
vs elution volume.

3/6/82

I

1 04	001.00
	005.00
	003628.00
1 05	001.00
	000.00
	000016.00
1 06	001.00
	010.00
	000556.00
1 07	001.00
	007.00
	000871.00
1 08	001.00
	015.00
	000259.00
1 09	001.00
	000.00
	000051.00
1 10	001.00
	000.00
	000023.00
1 11	001.00
	000.00
	000024.00
1 12	001.00
	000.00
	000019.00
1 13	001.00
	000.00
	000019.00
1 14	001.00
	000.00
	000026.00
1 15	001.00
	000.00
	000023.00
1 16	001.00
	000.00
	000028.00
1 17	001.00
	000.00
	000020.00

1 18
001.00
000.00
000012.00
1 19
001.00
000.00
000017.00
1 20
001.00
000.00
000026.00
1 21
001.00
000.00
000020.00
1 22
001.00
000.00
000019.00
1 23
001.00
000.00
000021.00
1 24
001.00
000.00
000024.00
1 25
001.00
000.00
000024.00
1 26
001.00
000.00
000016.00
1 27
001.00
000.00
000025.00
1 28
001.00
000.00
000010.00
1 29
001.00
000.00
000019.00
1 30
001.00
000.00
000021.00
1 31
001.00
000.00
000019.00
1 32
001.00
000.00
000021.00
1 33
001.00
000.00
000028.00
1 34
001.00

000.00
000031.00
1 35
001.00
000.00
000027.00
1 36
001.00
000.00
000019.00
1 37
001.00
000.00
000020.00
1 38
001.00
000.00
000012.00
1 39
001.00
000.00
000018.00

II

1 42
001.00
007.00
001500.00
1 43
001.00
000.00
000015.00
1 44
001.00
007.00
001209.00
1 46
001.00
007.00
001353.00
1 47
001.00
007.00
001105.00
1 48
001.00
010.00
000522.00
1 49
001.00
015.00
000327.00
1 50
001.00
020.00
000138.00
1 51
001.00
020.00
000140.00

1 52
001.00
000.00
000096.00

1 53
001.00
000.00
000061.00

1 54
001.00
000.00
000068.00

1 55
001.00
000.00
000067.00

1 56
001.00
000.00
000060.00

1 57
001.00
000.00
000049.00

1 58
001.00
000.00
000052.00

1 59
001.00
000.00
000057.00

1 60
001.00
000.00
000040.00

1 61
001.00
000.00
000046.00

1 62
001.00
000.00
000037.00

1 63
001.00
000.00
000028.00

1 64
001.00
000.00
000044.00

1 65
001.00
000.00
000039.00

1 66
001.00
000.00
000036.00

1 67
001.00
000.00
000029.00

1 68
001.00

000.00
000034.00
1 69
001.00
000.00
000027.00
1 70
001.00
000.00
000035.00
1 71
001.00
000.00
000031.00
1 72
001.00
000.00
000034.00
1 73
001.00
000.00
000034.00
1 74
001.00
000.00
000039.00
1 75
001.00
000.00
000032.00
1 76
001.00
000.00
000026.00
1 77
001.00
000.00
000023.00

III

1 76
001.00
007.00
001158.00
1 77
001.00
000.00
000069.00
1 78
001.00
003.00
004804.00
1 79
001.00
005.00
003590.00
1 80
001.00
007.00

000817.00
1 81
001.00
015.00
000234.00
1 82
001.00
020.00
000135.00
1 83
001.00
000.00
000099.00
1 84
001.00
000.00
000074.00
1 85
001.00
000.00
000076.00
1 86
001.00
000.00
000074.00
1 87
001.00
000.00
000042.00
1 88
001.00
000.00
000056.00
1 89
001.00
000.00
000040.00
1 90
001.00
000.00
000048.00
1 91
001.00
000.00
000040.00
1 92
001.00
000.00
000030.00
1 93
001.00
000.00
000023.00
1 94
001.00
000.00
000038.00
1 95
001.00
000.00
000045.00
1 96
001.00
000.00
000034.00
1 97

001.00
000.00
000039.00
1 98
001.00
000.00
000034.00
1 99
001.00
000.00
000039.00
1 00
001.00
000.00
000026.00
1 01
001.00
000.00
000019.00
1 02
001.00
000.00
000028.00
1 03
001.00
000.00
000030.00
1 04
001.00
000.00
000029.00
1 05
001.00
000.00
000026.00
1 06
001.00
000.00
000030.00
1 07
001.00
000.00
000033.00

T1
PH 8.2

1 08
001.00
000.00
000024.00
1 09
001.00
000.00
000021.00
1 11
001.00
000.00
000034.00
1 12
001.00
010.00
000485.00
1 13

001.00
005.00
003932.00
1 14
001.00
005.00
004625.00
1 15
001.00
010.00
000776.00
1 16
001.00
010.00
000468.00
1 17
001.00
015.00
000239.00
1 18
001.00
020.00
000161.00
1 19
001.00
020.00
000108.00
1 20
001.00
000.00
000089.00
1 21
001.00
000.00
000045.00
1 22
001.00
000.00
000056.00
1 23
001.00
000.00
000045.00
1 24
001.00
000.00
000040.00
1 25
001.00
000.00
000041.00
1 26
001.00
000.00
000055.00

I
elution 8.21

1 35
001.00

000051.00

1 36

001.00

003.00

005067.00

1 37

001.00

003.00

008356.00

1 38

001.00

007.00

001213.00

1 39

001.00

010.00

000511.00

1 40

001.00

020.00

000185.00

1 41

001.00

020.00

000110.00

1 42

001.00

020.00

000107.00

3/6/82

EFFICIENCY LINE® 22-206

	1	2	3	4	5	6	7	8	9	
1	Sample # 1. from Callie.									
2	DNA sample made up as follows.									
3										
4		DNA	45 λ			Total 0.5 μ l.				
5	1M	HgSO ₄	2.5 λ							
6	0.1M	CaCl ₂	5 λ							
7		PBS (6.5)	447.5 λ							
8										
9	FR #	³ Hepm	% Input	FR#	FR#	³ Hepm	% Input	FR#	³ Hepm % Input	
10										
11	10 λ DNA	} 3628			# 18	24	0.15	39	185	1.1
12	x 4.5		16326	100		19	24	0.15	40	110
13	S. Eluati	16	0.098		20	16	0.098	41	107	0.66
14	W Eluati	556	3.41		21	25	0.15			
15	PBS #1	671	4.11		22	10	0.06			
16	6.5	2	259	1.59		23	18	0.11		
17	(low)	3	51	0.31		24	21	0.13		
18		4	23	0.14		25	19	0.12		
19		5	24	0.14		26	21	0.13		
20		6	19	0.12		27	28	0.17		
21		7	19	0.12		28	31	0.19		
22		8	26	0.16		29	27	0.17		
23		9	23	0.14		30	19	0.12		
24		10	28	0.17		31	20	0.12		
25		11	20	0.12		32	12	0.074		
26		12	12	0.074		33	18	0.11		
27		13	17	0.10	TRIS	34	51	0.31		
28		14	26	0.16	(8.2)	35	5067	31.04		
29		15	20	0.12		36	8356	51.18		
30		16	19	0.12		37	1213	7.43		
31		17	21	0.12		38	511	3.13		

3/6/82

EFFICIENCY LINE® 22-206

	1	2	3	4	5	6	7	8	9
1		Sample	# 3	from	Callie				
2			DNA	180 λ		} Total 0.5 ul.			
3		1M	HgSO ₄	2.5 λ					
4		0.1M	CaCl ₂	5 λ					
5		0.5	PBS	312.5 λ					
6									
7	FR#	³ Hcpm	%Input	FR#	³ Hcpm	%Input	FR#	³ Hcpm	%Input
8									
9	Sample 10 λ	1520							
10	X 18	27000	100%	# 19	44	0.16	40	468	1.73
11	S. Eluak	15	0.06	20	39	0.15	41	239	0.89
12	W. Eluak	1209	4.48	21	36	0.13	42	161	0.6
13	<u>PBS 6.5</u>			22	29	0.11	43	108	0.4
14	1	1353	5.01	23	34	0.13	44	89	0.33
15	2	1105	4.09	24	29	0.1	45	45	0.17
16	3	522	1.9	25	35	0.13	46	56	0.21
17	4	327	1.2	26	31	0.11	47	45	0.17
18	5	138	0.51	27	34	0.13	48	40	0.15
19	6	140	0.51	28	34	0.13	49	41	0.15
20	7	96	0.36	29	39	0.15	50	55	0.21
21	8	61	0.23	30	32	0.11			
22	9	68	0.25	31	26	0.096			
23	10	67	0.25	32	23	0.085			
24	11	60	0.23	<u>TRIS 8.2</u>					
25	12	49	0.18	33	24	0.09			
26	13	52	0.19	34	21	0.08			
27	14	57	0.21	35	34	0.13			
28	15	40	0.15	36	485	1.8			
29	16	46	0.17	37	6932	25.64			
30	17	37	0.14	38	5625	20.83			
31	18	28	0.1	39	1796	6.6			

3/6/82

	1	2	3	4	5	6	7	8	9
1	Sample # 4 from Callie								
2									
3		DNA	volume	165 λ		}	0.5 ul Total		
4		1M MgSO ₄		2.5 λ					
5		0.1M CaCl ₂		5 λ					
6		PBS (6.5)		327.5 λ					
7									
8	FR #	3 Hepm	% Input	FR #	3 Hepm	% Input			
9	DNA 10 λ	1158							
10	x 16.5	19007	100%	19	39	0.21			
11	S.E	69	0.36	20	34	0.18			
12	W.E	4804	25.14	21	39	0.21			
13	PBS (6.5)	3590		22	26	0.14			
14	10 ul 1	3590	18.79	23	19	0.10			
15	2	2817	14.74	24	28	0.15			
16	3	1234	6.46	25	30	0.16			
17	4	735	3.85	26	29	0.16			
18	5	499	2.61	27	26	0.14			
19	6	174	0.91	28	30	0.16			
20	7	76	0.4	29	33	0.17			
21	8	74	0.39						
22	9	42	0.22						
23	10	56	0.29						
24	11	40	0.22						
25	12	48	0.25						
26	13	40	0.22						
27	14	30	0.16						
28	15	23	0.12						
29	16	38	0.2						
30	17	45	0.24						
31	18	34	0.18						

3/6/82

46 1521

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

34 cpm Percent of Input



3/1/82

Objective :

- A. To determine whether, glycosylated DNA, e.g. T₄ phage DNA, binds to concavallin-A-sepharose columns at neutral or slightly acidic pH
- B. To check ~~for~~ whether this binding is irreversible, i.e. whether the bound DNA can be replaced from the column, either by,
- (i) increasing the pH of the elution buffer,
 - or, by
 - (ii) monosaccharides such as mannose
- C. To compare binding of non-glycosylated DNA, e.g. phage lambda DNA to concavallin-A-sepharose columns under similar conditions.
- D. To determine whether presence of d-mannose will affect binding of glycosylated DNA to con-A sepharose columns.

Materials $\frac{1}{2}$ Methods:

DNAs

Nick-translated, $^3\text{H-T}$ labelled

(i) λ DNA 3.615 $\mu\text{g}/\mu\text{l}$
10780 $^3\text{Hcpm}$ per 10 μl .

(ii) T4 DNA 3.29 $\mu\text{g}/\mu\text{l}$
16372 $^3\text{Hcpm}$ per 10 μl .

in H_2O

Affinity Matrix

Concavallin-A sepharose

18 μg concavallin-A bound per
 μl of settled gel.

Buffers

Phosphate Buffer saline pH 6.5 (PBS)

10 μM TRIS-HCL } Buffers at
10 μM NaCl } pH 7.2

7.4

7.6

7.7

7.8

7.9

8.0

8.1

8.2.

Monosaccharide

d-Mannose at 0.056 M in d. H_2O

Experimental protocols:

1. One ml concavallin-A sepharose columns in sterile capillary pipettes were equilibrated with excessively with PBS [50-100 ul]
2. DNA sample [0.5 ul in PBS supplemented with 5 mM $MgSO_4 \cdot 7H_2O$ and 1 mM $CaCl_2 \cdot 2H_2O$] was applied to the column.
3. Sample was driven into the column with gel with 2 aliquots [~~of 1/2 volume~~ of [1/2 sample volume] PBS.
4. Column was successively eluted with
 - I (a) 10 ul portions of PBS
 - (b) 5 ul portions of Tris NaCl (7.2)
 - (c) " " " " Tris NaCl (7.4)
 - (d) " " " " " (7.6)
 - (e) " " " " " (7.7)
 - (f) " " " " " (7.8)
 - (g) " " " " " (7.9)
 - (h) " " " " " (8.0)
 - (i) " " " " " (8.1)
 - (k) " " " " " (8.2)
 - II (a) 10 ul portions of PBS
 - (b) 10 ul portion of d-Mannose (0.056 M)

5. Ten-drop fractions were collected, when the elution was started (three fractions per ml, of eluting buffer) directly into the scintillation vial.
6. 3.5 ml of scintillation cocktail Reafluor was added, mixed well and counted.
7. Results are plotted as
~~percent counts recovered~~
 counts recovered percent of the input
 vs elution volume.

Experimental data :

Nick-translated 3H T₄ DNA sample (0.5 ml.)
 10 λ of original 17622
 100 λ used in sample

Nick-translated 3H λ DNA sample 0.5 ml
 10 λ of original 10823 100 λ used/sample
 Total counts applied to the columns.

T ₄ DNA	column	①	176220
		②	176220
λ DNA	column	①	108230
		②	108230

I pH elution

T₄ DNA

		3# epm	Percent
Input epm		176220	100 %.
Sample eluate		30	0.017
Wash ₁ eluate		28	0.016
Wash ₂ eluate		36	0.02
PBS	FR # 1	31	0.018
(10 ml.)	2	38	0.022
	3	36	0.02
	4	35	0.02
	5	37	0.02
	6	35	0.02
	7	37	0.02
	8	40	0.023
	9	43	0.024
	10	45	0.026
	11	46	0.026
	12	47	0.026
	13	48	0.026
	14	45	0.026
	15	44	0.025
	16	43	0.025
	17	48	0.026
	18	49	0.026
	19	45	0.025

T4-DNA I pH elution

FR #	3H epm.	% of Input	FR #	3H epm.	% of Input
PBS 20	40	0.023	Tris NaCl (7.2) 43	38	0.021
10 ul. 21	41	0.023	(5 ul) 44	35	0.02
22	40	0.023	45	36	0.02
23	38	0.021	Tris NaCl (7.4) 46	32	0.018
24	36	0.02	(5 ul) 47	41	0.022
25	35	0.02	48	43	0.024
26	37	0.02	49	36	0.02
27	36	0.02	50	37	0.02
28	38	0.021	51	38	0.022
29	35	0.019	52	35	0.02
30	34	0.019	53	34	0.02
Tris NaCl (7.2) 5 ul. 31	32	0.018	54	20	0.011
32	33	0.018	55	21	0.011
33	38	0.021	56	35	0.02
34	32	0.018	57	36	0.02
35	36	0.02	58	37	0.02
36	31	0.018	59	30	0.011
37	32	0.018	60	31	0.011
38	38	0.021	Tris NaCl (7.6) 61	32	0.011
39	31	0.018	(5 ul) 62	33	0.011
40	32	0.018	63	35	0.02
41	33	0.018	64	32	0.011
42	33	0.018	65	38	0.022
			66	36	0.02

T₄ DNA I - pH elution.

FR #	3Hcpm	% Input	FR #	3Hcpm	% Input	FR #	3Hcpm	% Input
TRIS NaCl (7.6) 67	86	0.049	(7.8) 91	105	0.06	115	89	0.05
5ul 68	85	0.049	5ul 92	106	0.06	116	104	0.059
69	100	0.056	93	113	0.064	117	113	0.064
70	112	0.064	94	107	0.062	118	100	0.058
71	157	0.086	95	108	0.062	119	83	0.05
72	162	0.092	96	133	0.075	120	86	0.05
73	185	0.105	97	123	0.07	(8.0) 5ul 121	38	0.02
74	193	0.109	98	151	0.086	122	78	0.044
75	200	0.113	99	121	0.07	123	48	0.027
pH 7.7 (5ul) 76	212	0.12	100	130	0.071	124	35	0.02
77	215	0.122	101	121	0.07	125	69	0.039
78	1589	0.9	102	122	0.07	126	35	0.02
79	3875	2.198	103	186	0.106	127	90	0.05
80	6219	3.53	104	130	0.074	128	20	0.011
81	43212	24.52	105	115	0.065	129	100	0.058
82	80861	45.89	(7.9) 106	113	0.06	130	35	0.02
83	28183	15.99	5ul 107	119	0.068	131	86	0.05
84	8161	4.63	108	118	0.068	132	100	0.058
85	3809	2.16	109	129	0.073	133	80	0.05
86	1081	0.61	110	153	0.086	134	69	0.039
87	236	0.134	111	196	0.111	135	72	0.041
88	105	0.06	112	113	0.064	(8.1) 5ul 136	38	0.02
89	136	0.077	113	151	0.086	137	45	0.024
90	118	0.069	114	121	0.07	138	46	0.025

T₄ DNA

pH elution

FR #	3H epm	% Input	FR #	3H epm	% Input	FR #	3H epm	% Input
(8.1) 5wt 139	25	0.014	(8.1) 5wt 148	36	0.020	(8.2) 157	41	0.023
140	30	0.017	149	18	0.01	158	38	0.022
141	32	0.018	150	23	0.013	159	43	0.023
142	85	0.048	(8.2) 5wt 151	25	0.014	160	90	0.051
143	80	0.045	152	41	0.023	161	100	0.057
144	68	0.039	153	44	0.053	162	38	0.022
145	39	0.022	154	48	0.026	163	25	0.014
146	32	0.018	155	95	0.053	164	46	0.026
147	20	0.011	156	100	0.055	165	32	0.018

IV T₄ DNA

II

mannose elution

FR #	3H epm	% Input	FR #	3H epm	% Input	FR #	3H epm	% Input
S eluate	86	0.049	(PBS) 9	35	0.02	(PBS) 20	89	0.051
W ₁ eluate	45	0.026	10	48	0.028	21	35	0.02
W ₂ eluate	49	0.028	11	45	0.026	22	36	0.02
(PBS) 10 wt 1	41	0.023	12	61	0.035	23	90	0.051
2	46	0.026	13	41	0.023	24	45	0.026
3	38	0.022	14	48	0.028	25	38	0.022
4	35	0.02	15	56	0.032	26	60	0.034
5	38	0.022	16	59	0.033	27	30	0.017
6	39	0.022	17	51	0.029	28	50	0.028
7	41	0.023	18	50	0.029	29	36	0.02
8	46	0.026	19	53	0.03	30	90	0.051

T₄ DNA II Mannose Elution.

FR #	³ H epm	% Input	FR #	³ H epm	% Input	FR #	³ H epm	% Input
Mannose 0.056M 31	89	0.051	41	211	0.12	51	95	0.054
10 ul 32	90	0.051	42	220	0.13	52	196	0.11
33	84	0.048	43	280	0.12	53	201	0.11
34	7258	4.12	44	83	0.047	54	38	0.022
35	56295	31.95	45	200	0.12	55	49	0.028
36	80118	45.46	46	38	0.022	56	38	0.022
37	13913	7.895	47	69	0.039	57	101	0.057
38	1354	0.77	48	211	0.12	58	36	0.022
39	861	0.49	49	38	0.022	59	25	0.014
40	301	0.17	50	86	0.049	60	100	0.057

λ DNA Total Input 108230
 I pH Elution.

FR #	³ H epm	% Input	FR #	³ H epm	% Input	FR #	³ H epm	% Input
S. eluate	6227	5.76	21	19	0.011	TRIS (7.2) 44	40	0.02
W ₁ eluate	34230	31.63	22	14	0.011	45	35	
W ₂ eluate	34983	32.32	23	15	0.011	TRIS 7.4 46	85	
PBS 1	16831	15.55	24	23	0.02	47	30	
10x10 ⁴ 2	11000	10.16	25	23	0.02	48	92	
3	226	0.21	26	23	0.018	49	60	
4	112	0.10	27	29	0.02	50	35	
5	104	0.096	28	16	0.01	51	32	
6	40	0.02	29	25	0.02	52	38	
7	40	0.02	30	25	0.02	53	39	
8	43	0.02	TRIS (7.2) 31	61	0.022	54	30	
9	20	0.01	2	38	0.02	55	32	
10	29	0.015	3	33	0.02	56	28	
11	28	0.014	4	55	0.03	57	38	
12	35	0.022	5	32	0.02	58	88	
13	21	0.01	6	38	0.02	59	35	
14	32	0.018	7	89	0.05	60	69	
15	24	0.011	8	32	0.02	TRIS 7.6 61	30	
16	19	0.011	9	30	0.02	62	30	
17	18	0.01	40	49	0.024	63	31	
18	14	0.01	41	35	0.02	64	32	
19	21	0.02	42	38	0.02	65	33	
20	16	0.01	43	39	0.02	66	40	

λ DNA Input 108230
pH - elution

FR #	3Hcpm	% Input	FR #	3Hcpm	% Input	FR #	3Hcpm	% Input
<u>Tris 7.6</u> 67	22		<u>Tris 7.8</u> 90	41		<u>Tris 7.9</u> 112	35	
68	21		<u>Tris 7.8</u> 91	38		113	36	
69	22		92	40		114	30	
70	25		93	29		115	29	
71	38		94	51		116	38	
72	36		95	35		117	30	
73	32		96	43		118	81	
74	38		97	25		119	30	
<u>Tris 7.7</u> 75	41		98	86		120	41	
76	38		99	89		<u>Tris 8.0</u> 121	36	
77	44		100	38		122	38	
78	45		101	35		123	33	
79	96		102	32		124	25	
80	100		103	41		125	28	
81	38		104	40		126	31	
82	20		105	38		127	32	
83	151		<u>Tris 7.9</u> 106	39		128	30	
84	35		107	46		129	86	
85	36		108	52		130	56	
86	38		109	38		131	35	
87	49		110	39		132	45	
88	30		111	69		133	44	
89	31					134	29	
						135	10	

λ - DNA

Mannose ELUTION

INPUT 108230

3/1/82

FR #	³ Hcpm	% of Input	FR #	³ Hcpm	% of Input	FR #	³ Hcpm	% of Input
Sample Eluate	5804	5.36	PBS 21	82	0.05	Mannose 44	80	
Wash ₁ Eluate	82968	76.50	22	87	0.06	45	25	
Wash ₂ Eluate	13416	12.86	23	65	0.05	46	20	
PBS #1	2150	1.98	24	40	0.03	47	19	
10ul. 2	914	0.85	25	38	0.022	48	18	
3	820	0.76	26	36	0.022	49	16	
4	633	0.59	27	35	0.022	50	18	
5	375	0.35	28	38	0.025	51	35	
6	239	0.22	29	39	0.025	52	30	
7	200	0.18	30	40	0.02	53	31	
8	234	0.22	Mannose 31	23	0.02	54	32	
9	178	0.16	32	23	0.02	55	33	
10	148	0.14	33	23	0.02	56	34	
11	159	0.14	34	24	0.02	57	35	
12	136	0.13	35	25		58	36	
13	135	0.13	36	26		59	38	
14	173	0.15	37	29		60	30	
15	115	0.11	38	31				
16	131	0.12	39	30				
17	136	0.13	40	31				
18	136	0.13	41	34				
19	116	0.1	42	30				
20	98	0.09	43	30				

1 05
001.00
000.00
000078.00
1 06
001.00
000.00
000060.00
1 07
001.00
000.00
000087.00
1 08
001.00
000.00
000085.00
1 09
001.00
000.00
000072.00
1 10
001.00
000.00
000066.00
1 11
001.00
000.00
000076.00
1 12
001.00
000.00
000078.00
1 13
001.00
000.00
000068.00
1 14
001.00
000.00
000060.00
1 15
001.00
000.00
000066.00
1 16
001.00
000.00
000070.00
1 17
001.00
000.00
000067.00
1 18
001.00
000.00
000081.00
1 19

1 65
001.00
002.00
017622.00
1 66
001.00
000.00
000020.00
1 67
001.00
005.00
001836.00
1 68
001.00
001.00
005804.00
1 70
001.00
000.70
082768.00
1 71
001.00
002.00
013916.00
1 72
001.00
005.00
002150.00
1 73
001.00
007.00
000914.00
1 74
000.34
015.00
002082.35
1 75
001.00
010.00
000633.00
1 76
001.00
015.00
000375.00
1 77
001.00
015.00
000239.00
1 78
001.00
015.00
000200.00
1 79
001.00
015.00
000234.00
1 80

X 1 80
001.00
003.00
004596.00
1 81
001.00
003.00
006227.00
1 82
001.00
001.50
034230.00
1 83
001.00
001.50
034983.00
1 85
001.00
003.00
006831.00
1 86
001.00
007.00
001100.00
1 87
001.00
015.00
000226.00
1 88
001.00
020.00
000112.00
1 89
001.00
020.00
000104.00
1 90
001.00
000.00
000040.00
1 91
001.00
000.00
000040.00
1 92
001.00
000.00
000043.00
1 93
001.00
000.00
000020.00
1 94
001.00
000.00
000029.00
1 95

001.00
000.00
000091.00
1 20
001.00
000.00
000073.00
1 21
001.00
000.00
000059.00
1 22
001.00
000.00
000072.00
1 23
001.00
000.00
000049.00
1 24
001.00
000.00
000036.00

001.00
020.00
000148.00
1 82
001.00
020.00
000159.00
1 83
001.00
020.00
000136.00
1 84
001.00
020.00
000135.00
1 85
001.00
020.00
000173.00
1 86
001.00
020.00
000115.00
1 87
001.00
020.00
000131.00
1 88
001.00
020.00
000136.00
1 89
001.00
020.00
000136.00
1 90
001.00
020.00
000116.00
1 91
001.00
000.00
000098.00
1 92
001.00
000.00
000082.00
1 93
001.00
000.00
000087.00
1 94
001.00
000.00
000065.00

001.00
000.00
000028.00
1 96
001.00
000.00
000035.00
1 97
001.00
000.00
000021.00
1 98
001.00
000.00
000032.00
1 99
001.00
000.00
000024.00
1 00
001.00
000.00
000019.00
1 01
001.00
000.00
000018.00
1 02
001.00
000.00
000014.00
1 03
001.00
000.00
000021.00
1 04
001.00
000.00
000016.00
1 05
001.00
000.00
000019.00
1 06
001.00
000.00
000014.00
1 07
001.00
000.00
000015.00
1 08
001.00
000.00
000023.00
1 09
001.00
000.00
000023.00
1 10
001.00
000.00
000023.00
1 11
001.00
000.00

14

1 12
001.00
000.00
000016.00
1 13
001.00
000.00
000025.00
1 14
001.00
000.00
000025.00
1 15
001.00
000.00
000028.00
1 16
001.00
000.00
000014.00
1 17
001.00
000.00
000017.00
1 18
001.00
000.00
000014.00
1 19
001.00
000.00
000018.00
1 20
001.00
000.00
000021.00
1 21
001.00
000.00
000022.00
1 22
001.00
000.00
000019.00
1 23
001.00
000.00
000016.00
1 24
001.00
000.00
000024.00
1 25
001.00
000.00
000024.00
1 26
001.00
000.00
000020.00
1 27
001.00
000.00
000014.00
1 28
001.00

000018.00
1 29
001.00
000.00
000019.00
3

3/2/82

D Effect of mannose (in reaction mixtures) on DNA binding to cou-A sepharose columns.

Experimental protocols

1. One ml cou-A sepharose columns were equilibrated with PBS (6.5) 50-100 ml

2. DNA samples were made up in 0.5 ml volume containing at F.C.

10 mM Na-K-PO₄ 6.5

150 mM NaCl

5 mM MgSO₄ · 7H₂O

1 mM CaCl₂ · 2H₂O

40 ng DNA [T₄-DNA 21500 cpm(³H)

λ-DNA 12000 cpm(³H)

0.056 M Mannose,

Samples were applied to the columns.

Eluate was collected.

Columns were washed with 2 aliquots of 0.25 ml of PBS (6.5)

Both washes were collected separately.

3. Columns were eluted with 10.0 ml PBS (6.5).

4. Ten drop fractions [0.33 ul] were collected directly in scintillation cocktail vials.

Added 3.5 ul of cocktail and counted.

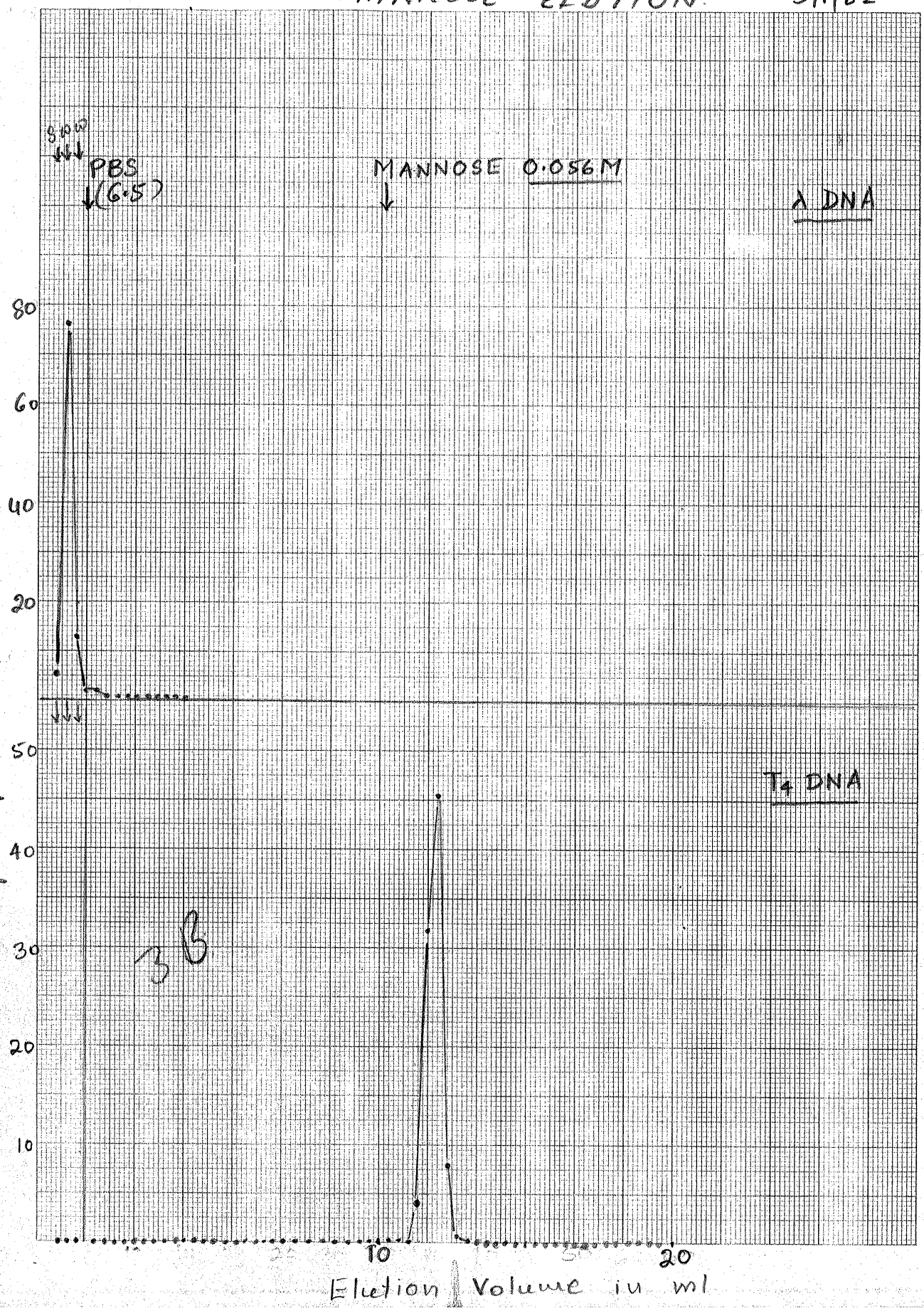
5. Results are plotted as

counts ~~per~~ recovered percent of input vs elution volume.

FR #	³ Hepm	% Input	FR #	³ Hepm	% Input	FR #	³ Hepm	% Input
<u>T4 DNA</u>	21500	100	13	14	0.065	30	16	*
E. Sample	1505	7	14	10	0.05	<u>λ DNA</u>	12000	100
Wash ₁	10110	47.02	15	19	0.09	E Sample	2800	23.3
Wash ₂	8243	38.34	16	16	0.075	E Wash ₁	4872	40.6
PBS 1	1005	4.67	17	30	0.14	E 2	3480	29.00
2	236	1.097	18	18	0.084	PBS 1	220	1.83
3	89	0.414	19	22	0.102	2	85	0.71
4	35	0.16	20	16	0.075	3	31	0.26
5	37	0.17	21	18	0.084	4	25	0.21
6	12	0.06	22	20	0.10	5	17	0.14
7	19	0.09	23	23	0.107	6	18	0.15
8	18	0.08	24	15	0.07	7	20	0.17
9	16	0.07	25	10	0.05	8	16	0.14
10	20	0.09	26	7		9	15	0.13
11	25	0.10	27	19	0.09	10	18	0.15
12	11	0.04	28	22	0.11	11	19	0.15
			29	17	0.075	12	20	0.17

DNA-BINDING TO CON-A SEPHAROSE
 MANNOSE ELUTION. 3/1/82

KEITHLEY 10 X 10 TO THE CENTIMETER 19 X 25 CM.
 KEUFFEL & ESSER CO. MADE IN U.S.A.
 3H cpm percent of total input 46 1521



2/23/82

Check of DNA binding to Cou-A Sepharose

Cou-A Sepharose 18 $\mu\text{g}/\text{ml}$ gel

DNA (A) 3H E. coli DNA

$\approx 140,000$ cpm/ λ

(B) T4 DNA 0.28 $\mu\text{g}/\text{ml}$

Eluting Buffer

PBS (6.5)

(A) 3H E. coli DNA

10 λ in 1.0 ml PBS containing

5 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$

1 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$

0.10 ml used. \approx cpm

1.0 ml cou A sepharose column equilibrated

with 50 ml PBS 6.5

DNA sample applied to the column.

Sample driven into the column with 2 aliquots
of ~~100~~ 50 λ each PBS.

Column eluted with PBS (6.5) ~~2.5 ml~~ 4.0 ml.

2 drop fractions collected
checked for radioactivity.

Column eluted successively with Tris.HCl pH 7.2,
pH 7.4, pH 7.6, pH 7.8, pH 8.0 buffers 4 ml
each.

1 05
001.00
002.00
014154.00
1 07
001.00
015.00
000303.00
1 08
001.00
000.00
000016.00
1 09
001.00
000.00
000020.00
1 10
001.00
000.00
000023.00
1 11
001.00
000.00
000023.00
1 12
001.00
000.00
000025.00
1 13
001.00
000.00
000024.00
1 14
001.00
020.00
000119.00
1 15
001.00
010.00
000542.00
1 16
001.00
005.00
001767.00
1 17
001.00
005.00
003814.00
1 18
001.00
005.00
002681.00
1 19
001.00
003.00
009008.00
1 20
001.00
002.00

1 21
001.00
002.00
011356.00
1 22
001.00
002.00
010341.00
1 23
001.00
003.00
008809.00
1 24
001.00
003.00
007328.00
1 25
001.00
003.00
004374.00
1 26
001.00
003.00
004069.00
1 27
001.00
005.00
003300.00
1 28
001.00
005.00
002436.00
1 29
001.00
007.00
001523.00
1 30
001.00
010.00
000464.00
1 31
001.00
007.00
001560.00
1 32
001.00
007.00
001459.00
1 33
001.00
010.00
000661.00
1 34
001.00
007.00
001136.00
1 35
001.00
010.00
000502.00
1 36
001.00
010.00
000792.00
1 37
001.00
015.00

1 38
001.00
010.00
000475.00
1 39
001.00
010.00
000417.00
1 40
001.00
015.00
000253.00
1 41
001.00
015.00
000389.00
1 42
001.00
020.00
000164.00
1 43
001.00
010.00
000413.00
1 44
001.00
015.00
000243.00
1 45
001.00
015.00
000313.00
1 46
001.00
015.00
000378.00
1 47
001.00
015.00
000364.00
1 48
001.00
015.00
000361.00
1 49
001.00
020.00
000115.00
1 50
001.00
020.00
000159.00
1 51
001.00
020.00
000144.00
1 52
001.00
010.00
000521.00
1 53
001.00
010.00
000497.00
1 54
001.00

010.00
000573.00
1 55
001.00
010.00
000536.00
1 56
001.00
015.00
000225.00
1 57
001.00
015.00
000202.00
1 58
001.00
015.00
000371.00
1 59
001.00
020.00
000191.00
1 60
001.00
015.00
000356.00
1 61
001.00
020.00
000186.00
1 62
001.00
020.00
000166.00
1 63
001.00
020.00
000130.00
1 64
001.00
015.00
000284.00
1 65
001.00
015.00
000224.00
1 66
001.00
020.00
000126.00
1 67
001.00
015.00
000274.00
1 68
001.00
020.00
000168.00
1 69
001.00
015.00
000273.00
1 70
001.00
015.00
000229.00
1 71

1 71
001.00
015.00
000246.00
1 72
001.00
015.00
000233.00
1 73
001.00
015.00
000255.00
1 74
001.00
020.00
000171.00
1 75
001.00
020.00
000154.00
1 76
001.00
020.00
000159.00
1 77
001.00
020.00
000119.00
1 78
001.00
020.00
000121.00
1 79
001.00
015.00
000224.00
1 80
001.00
020.00
000125.00
1 81
001.00
015.00
000284.00
1 82
001.00
020.00
000154.00
1 83
001.00
015.00
000268.00
1 84
001.00
015.00
000283.00
1 85
001.00
015.00
000274.00
1 86
001.00
015.00
000225.00
1 87
001.00

015.00
000225.00
1 87
001.00
015.00
000263.00
1 88
001.00
020.00
000197.00
1 89
001.00
020.00
000190.00
1 90
001.00
020.00
000173.00

FR #	³ Hcpm.	FR #	³ Hcpm.	FR #	³ Hcpm.
10x Sample	14154				
Sample Eluate	383	20	1523	43	521
Sample Wash	16	21	1464	44	497
2	20	22	1560	45	593
PBS FR # 1	23	23	1459	46	536
2	23	24	661	47	525
3	25	25	1136	48	202
4	24	26	582	49	371
5	119	27	792	50	191
6	542	28	302	51	358
7	1767	29	475	52	186
8	3814	30	417	53	166
9	5681	31	253	54	130
10	9008	32	383	55	284
11	10421	33	164	56	224
12	11356	34	413	57	126
13	10341	35	243	58	274
14	8809	36	313	59	168
15	7328	37	378	60	273
16	4374	38	364	61	229
17	4069	39	361	62	246
18	3380	40	115	63	233
19	2436	41	159	64	255
		42	144	65	171

TRIS
712
~~712~~

FR #	3H epw		
66	154		
67	159		
68	119		
69	121		
70	224		
71	120		
72	284		
73	154		
74	268		
75	283		
76	274		
77	225		
78	263		
79	197		
80	190		
81	173		
82			
83			
84			
85			
86			
87			
88			
89			
90			
91			

Last elution @ Tris NaCl pH 8.2.

All samples diluted @ 2.0 ul respective buffers and A₂₆₀ checked.

Buffer FR # A₂₆₀ Buffer FR # A₂₆₀

Sample 011 Tris 7.9 1 135

Wash I 006 2 091

II 003 3 041

PBS 1 004 4 011

2 005 Tris 8.2 1 006

3 006 2 004

4 008 3 003

Tris 7.2 1 004 4 002.

2 003 Sample } A₂₆₀ A₂₈₀

3 004 diluted to } 0.326 0.46

4 006 3.0 ul. }

Tris 7.4 1 001

2 002

3 003

4 004

Tris 7.6 1 006

2 010

3 023

4 043

Sample	A ₂₆₀	Alg DNA Corrected	Alg DNA
Diluted 3 fold	0.326	0.978	50
SE. x 3	0.011	0.033	1.65
W _I x 3	0.006	0.018	0.9
W _{II} x 3	0.003	0.009	0.45
PBS 1	0.004	0.012	0.6
2	0.005	0.015	0.75
3	0.006	0.018	0.9
4	0.008	0.024	1.2
Tris (7.2) 1	0.004	0.012	0.6
2	0.003	0.009	0.45
3	0.004	0.012	0.6
4	0.006	0.018	0.9
Tris (7.4) 1	0.001	0.003	0.15
2	0.002	0.006	0.3
3	0.003	0.009	0.45
4	0.004	0.012	0.6
Tris (7.6) 1	0.006	0.018	0.9
2	0.01	0.03	1.5
3	0.023	0.069	3.45
4	0.043	0.129	6.45
Tris (7.9) 1	0.135	0.405	20.25
2	0.071	0.213	10.65

Sample	A ₂₆₀	Corrected	lg DNA
--------	------------------	-----------	--------

Tris (7.9) 3	0.041	0.123	6.15
--------------	-------	-------	------

4	0.011	0.033	1.65
---	-------	-------	------

Tris (8.2) 1	0.006	0.018	0.9
--------------	-------	-------	-----

2	0.004	0.012	0.6
---	-------	-------	-----

3	0.003	0.009	0.45
---	-------	-------	------

4	0.002	0.006	0.3
---	-------	-------	-----

B) T₄ DNA 0.28 ug/ml in 10 mM TRIS (7.2)
10 mM NaCl.

0.180 ml sample in PBS (6.5) } 1 ml.
MgSO₄ 5 mM } sample.
CaCl₂ 1 mM }

1.0 ml con-A column equilibrated in
35.0 ml PBS.

DNA sample applied. eluate collected
Sample driven in 0.5 ml aliquots of PBS
two aliquots collected.

Column eluted in 4.0 ml PBS (6.5)
1.0 ml aliquot collected

Column eluted in 10 mM TRIS 7.2
10 mM NaCl

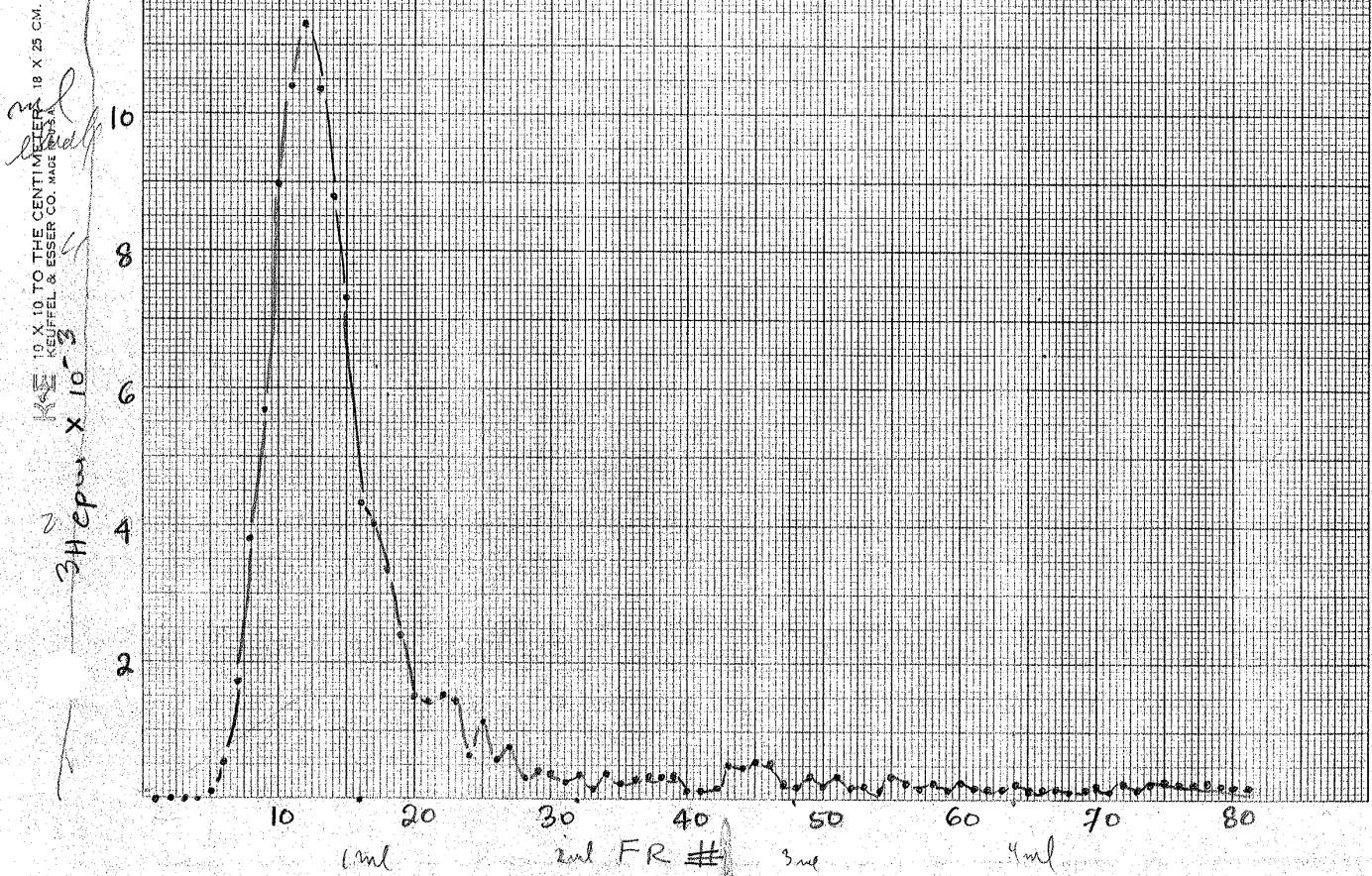
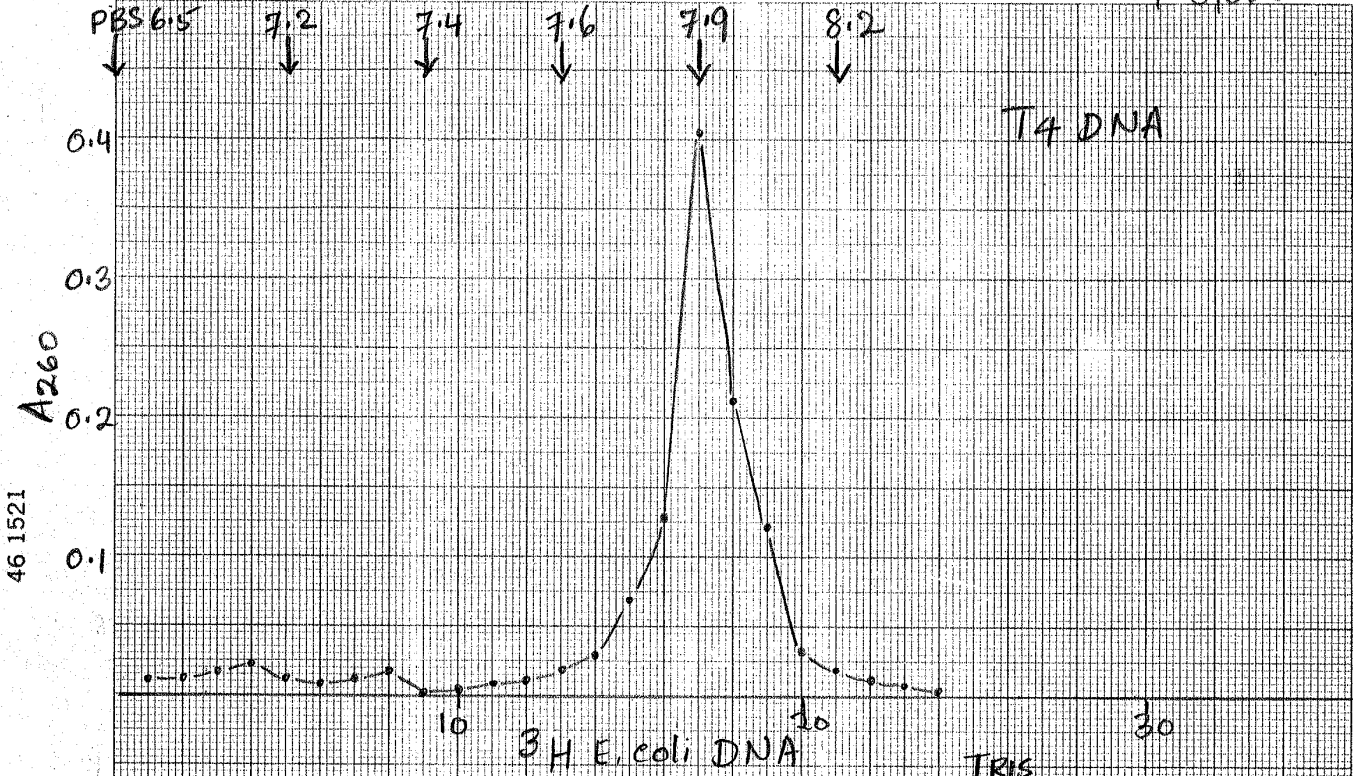
4 aliquots collected. of 1 ml each
Column eluted in 10 mM TRIS 7.4
10 mM NaCl

4 aliquots collected. 1 ml each.
Column eluted in 10 mM TRIS 7.6
10 mM NaCl

4 aliquots
Column eluted in 10 mM TRIS 7.9
10 mM NaCl

TRIS 10mM NaCl 10mM

2/23/82



10 X 10 TO THE CENTIMETER
 KUFFEL & ESSER CO. MADE IN U.S.A.
 3H E. coli DNA x 10⁻³

II Concavallin-A

Concavallin-A - Sepharose
affinity - matrix used.

One milliliter of settled
gel has 18 μg con-A bound
to it.

III Buffers

Phosphati. Buffered Saline pH 6.5

Tris.HCl 10 μM } pH 7.2
NaCl 10 μM }

Tris.HCl 10 μM } pH 7.4
NaCl 10 μM }

Tris.HCl 10 μM } pH 7.6
NaCl 10 μM }

Tris.HCl 10 μM } pH 7.9
NaCl 10 μM }

Tris.HCl 10 μM } pH 8.2
NaCl 10 μM }

Temp.

Room temp $25^{\circ} \sim 28^{\circ} \text{C}$

Experimental Protocol

1. 1.0 ml concavallin-A sepharose columns in capillary pipettes were equilibrated with PBS (6.5) excessively (50~100 ml).
2. DNA sample was applied to the column.
3. Sample was driven into the gel with 2 aliquots of ($\frac{1}{2}$ ~~volume~~ sample volume) PBS (6.5)
4. Column was successively eluted with ~~4~~ portions of
 - a. PBS (6.5) 4.0 ml
 - b. Tris-NaCl (7.2) 4.0 ml
 - c. Tris-NaCl (7.4) "
 - d. Tris-NaCl (7.6) "
 - e. Tris-NaCl (7.9) "
 - f. Tris-NaCl (8.2) "
5. Appropriate fractions were collected and checked for DNA either, by
 - a. counting radioactivity, or by,
 - b. checking UV absorbance at A_{260} against appropriate buffer.

~~Results~~ Experimental data

A. ^3H E. coli DNA

100 microliters DNA used. Aliquot of sample was checked for radioactivity to determine the radioactivity applied to the column.

DNA sample was driven into the column with 2 aliquots of 50 μl each of PBS (G15)

Sample eluate as well as sample wash-eluates were collected separately to determine the radioactivity when the

Column elution with different buffers was collected directly in scintillation vials 2 drop per fraction
16 fractions per ml.

Results were plotted as

percent radioactivity
percent radioactivity of the input
vs elution volume.

B. T₄ DNA

0.18 ml [50 µg] DNA in

1.0 ml PBS (G15)

MgSO₄·7H₂O 5 mM

CaCl₂·2H₂O 1 mM

applied to the column,

DNA sample driven into the column
with 2 aliquots of 0.5 ml each of
PBS (G15)

Sample eluate as well as wash-eluate
collected separately in sterile tubes
[nuclease-free].

Column elution with respective buffers
was collected as fractions of one
ml each.

4 fractions per buffer.

All the fractions were diluted with
200 µl of respective buffers and
A₂₆₀ vs that buffer was recorded.

Results were plotted as percent A₂₆₀
units of the total unit (input applied)
vs column elution volume.

$^3\text{H}(T)$ E. coli DNA Total Input 141540 ^3H cpm. 2/23/82.
 pH Elution.

FR #	^3H cpm	% of Input	FR #	^3H cpm	% of Input	FR #	^3H cpm	% of Input
Sample E.	383	0.27	(PBS) 21	1464	1.03	(PBS) 44	497	0.35
W ₁ E	16	0.01	22	1560	1.10	45	573	0.40
W ₂ E	20	0.01	23	1459	1.03	46	536	0.38
PBS # 1	23	0.01	24	661	0.47	3.681 47	225	0.16
(4 ul) 2	23	0.01	25	1136	0.80	48	202	0.14
3	25	0.01	26	582	0.41	49	371	0.26
4	24	0.01	27	792	0.56	14.75 50	191	0.13
5	119	0.084	28	302	0.21	51	358	0.25
6	542	0.38	29	475	0.34	52	186	0.13
7	1767	1.25	30	417	0.29	53	166	0.12
8	3814	2.69	31	253	0.18	54	130	0.09
9	5681	4.01	32	383	0.27	55	284	0.20
10	9008	6.36	51.99 33	164	0.12	56	224	0.16
11	10424	7.36	34	413	0.29	57	126	0.089
12	14356	8.023	35	243	0.17	58	274	0.19
13	18341	7.31	36	313	0.22	59	168	0.12
14	8809	6.22	37	378	0.27	60	273	0.19
15	7328	5.18	38	364	0.26	61	229	0.16
16	4374	3.09	39	361	0.26	62	246	0.17
17	4889	2.87	40	115	0.081	63	233	0.16
18	3980	2.39	41	159	0.11	64	255	0.18
19	2436	1.72	42	144	0.10	7.2 TRIS 65	171	0.12
20	1523	1.08	43	521	0.37	66	154	0.11

³H [T] E. coli DNA
PH

Total Input 141540 cpm. ^{2/23/82}
Elution.

FR #	³ H cpm	% of Input	FR #	³ H cpm	% of Input
67	159	0.11	(8.2) 89	89	0.06
68	119	0.085	90	113	0.07
69	121	0.085	91	158	0.11
70	224	0.16	92	133	0.093
(7.4) 71	125	0.088	93	211	0.15
72	284	0.2	94	230	0.16
73	154	0.11			
74	268	0.18			
75	283	0.2			
76 76	274	0.19			
(7.6) 77	225	0.16			
78	263	0.18			
79	197	0.14			
80	190	0.14			
81	173	0.12			
82	186	0.13			
(7.9) 83	153	0.11			
84	148	0.11			
85	113	0.08			
86	112	0.08			
87	118	0.08			
88	100	0.06			

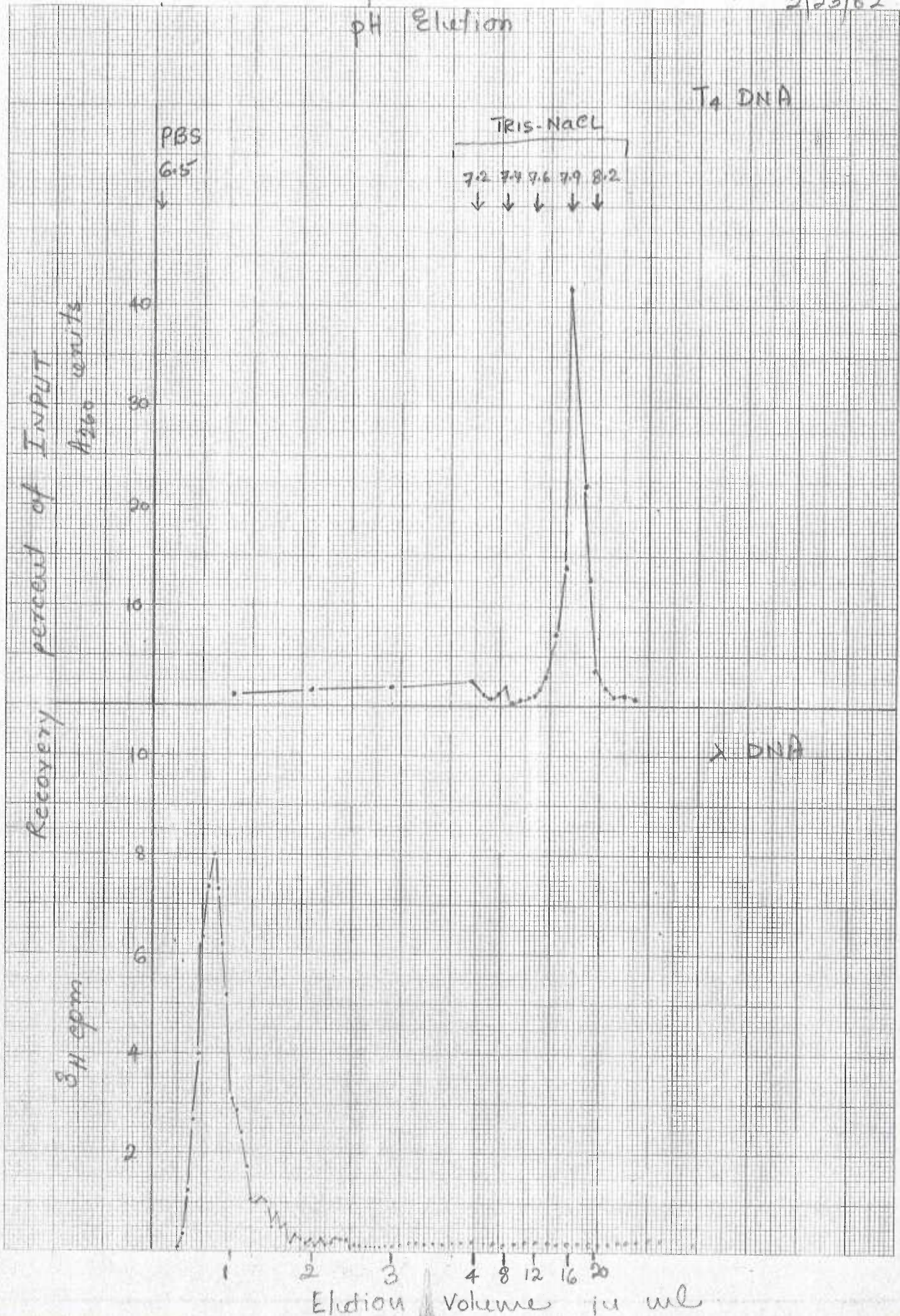
T₄ DNA Total A₂₆₀ units applied 0.978

FR #	A ₂₆₀	% of Input	FR #	A ₂₆₀	% of Input
Sample E	0.033	3.37	TRIS NaCl 7.9 17	0.405	41.41
Wash ₁ E	0.018	1.84	18	0.213	21.78
Wash ₂ E	0.009	0.92	19	0.123	12.58
			20	0.033	3.37
PBS 1	0.012	1.22	TRIS NaCl 21	0.018	1.84
G.S 2	0.015	1.54	8.2 22	0.012	1.22
3	0.018	1.84	23	0.009	0.92
4	0.024	2.45	24	0.006	0.61
TRIS NaCl 5	0.012	1.22			
7.1 6	0.009	0.92			
7	0.012	1.22			
8	0.018	1.84			
TRIS NaCl 9	0.003	0.31			
7.4 10	0.006	0.61			
11	0.009	0.92			
12	0.012	1.22			
TRIS NaCl 13	0.018	1.84			
7.6 14	0.030	3.07			
15	0.069	7.1			
16	0.129	13.19			

DNA - binding to Concanavallin A. sepharose

2/23/82

pH Elution



46 1521

K-E 10 X 10 TO THE CENTIMETER 19 X 25 CM. KUFFEL & ESSER CO. MADE IN U.S.A.

2/23/82

Objective To check whether,

- (a) DNA binds to concavallin A ;
- (b) glycosyl residues on DNA have anything to do with binding ;
- (c) the binding of DNA is irreversible ;
- (d) bound DNA can be eluted off by pH or any other compounds.

Materials & Methods:

~~DNA~~

T DNA - A ^3H E coli DNA - 140,000 cpm/ λ
in 0.1 M NaCl pH 7.0

10 λ diluted in

1.0 ml Phosphate buffer Saline

pH 6.5 containing

5 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$

1 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$

B T_4 DNA 0.28 mg/ml

in 10 mM TRIS (7.4)

10 mM NaCl

50 μg DNA diluted to 1.0

~~0.10~~ ml in PBS (6.5)

containing 5 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$

1 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$

2/22/82.

Objective:

To check whether,
glucosylated DNA, e.g. T₄ phage DNA
is precipitated by concanavalin A.

Materials & Methods.

T₄ DNA - T₄ am 82 [44'62'] DNA
0.28 mg/ml in
10 mM Tris } pH 7.2
10 mM NaCl }

Calf. thymus DNA - 0.5 mg/ml in
[worthington] 10 mM Tris } pH 7.2
10 mM NaCl }

Concanavalin A - 10 mg per ml in
(pharmacia) PBS pH 6.5

Experimental protocol

1. Fixed amount of DNA [either T₄ or calf-thymus] in a series of sterile 1.5 ml eppendorf tubes.
2. Added increasing amounts of eou-A [50 to 2500 µg]
3. Volume made up to 1.0 ml by addition of PBS, MgSO₄ and CaCl₂ so that final concentration will be
10 mM Na-K-PO₄ } pH 6.5
150 mM NaCl }
5 mM MgSO₄·7H₂O
1 mM CaCl₂·2H₂O
4. Mixed well and left at room-temp. for 60 minutes.
5. All tubes were spun at 12,000 x g for 15-20 minutes.
6. Supernates were carefully removed without disturbing the pellets and diluted 3 fold with PBS and A₂₆₀, A₂₈₀ determined.
7. Controls without DNA were run under similar conditions.

DNA Mg / Rx	Concanavalin A Mg Rx	Visible ppte	Supernatant	
			A ₂₆₀	A ₂₈₀
0	0	-	0	0
0	50	-	0.06	0.045
0	100	-	0.078	0.096
0	250	-	0.192	0.237
0	500	-	0.288	0.453
0	1000	-	0.522	0.864
0	2500	-	1.35	2.25
T ₄ 50 Mg	0	-	0.51	0.276
"	50	- 0.36	0.42	0.246
"	100	- 0.27	0.348	0.372
"	250	+ 0.06	0.252	0.513
"	500	+ 0.029	0.129	0.75
"	1000	+ 0.204	0.318	1.143
"	2500	+ 0.717	0.633	2.553
CT 50.4 Mg	0	-	0.51	0.279
"	50	- 0.51	0.57	0.324
"	100	- 0.522	0.6	0.36
"	250	- 0.511	0.702	0.519
"	500	- 0.522	0.81	0.756
"	1000	- 0.564	1.086	1.146
"	2500	- 0.525	1.875	2.55

2/22/82.

To check whether T₄ DNA is precipitated by con A.
compare with non-glycosylated DNA.

Rx mixtures of 1.0 ul.

PBS (6.5 pH)

MgSO₄·7H₂O 5 mM

CaCl₂·2H₂O 1 mM

T₄ ~~or~~ CT DNA 50.4 μg

(or CT DNA 50.00 μg)

con A 50 - 2500 μg

mixed well and incubated at RT for 60'

Tubes spun in microfuge for 15-20 minutes

Supernates diluted 3 fold with PBS (6.5)

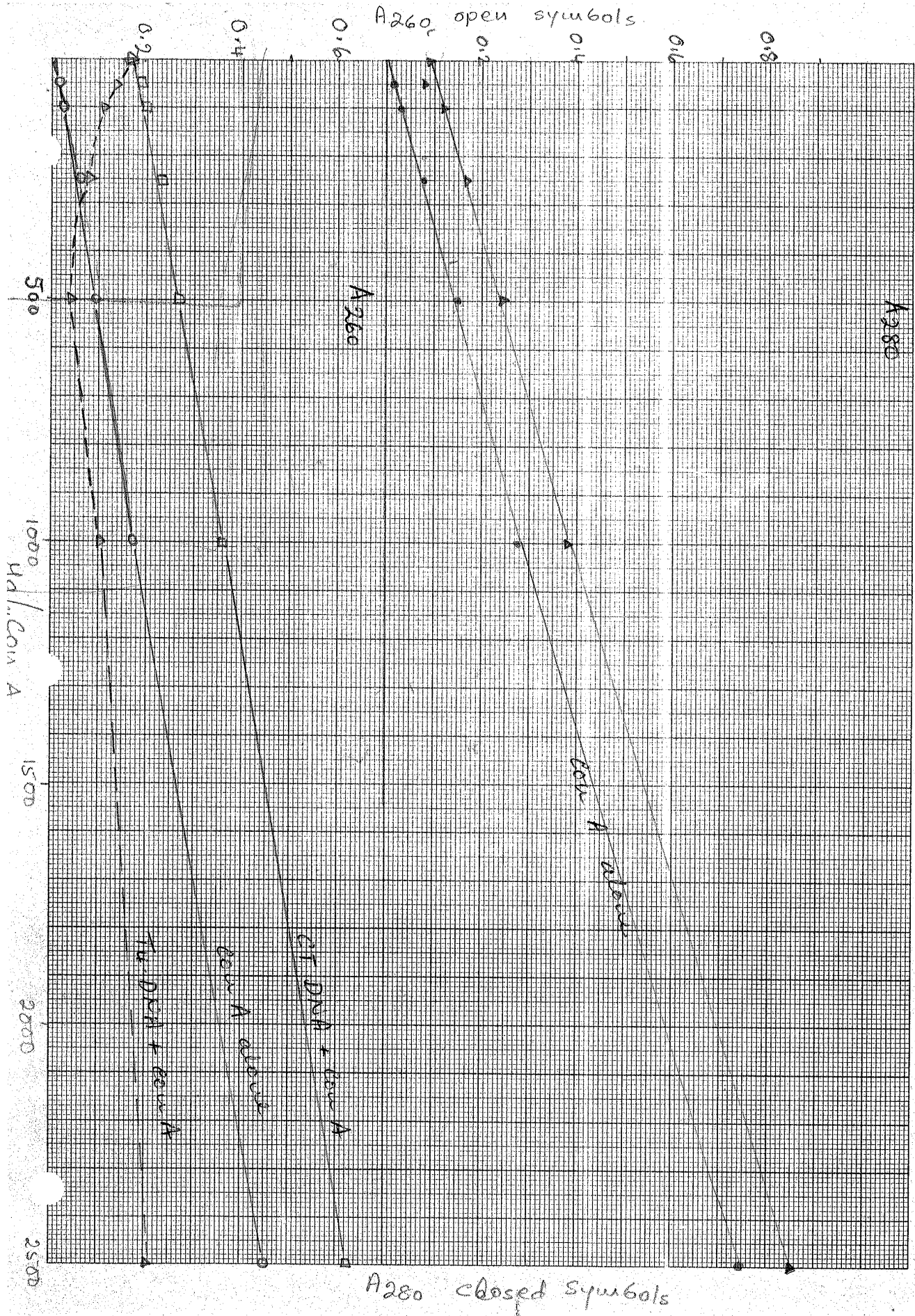
and A₂₆₀, A₂₈₀ checked.

Reviewed on 2/22/82 - 7:04 PM
 Norman Keller

2/22/82.

T₄ DNA 0.28 mg/ml Buffer PBS 6.5
 CT DNA 0.5 mg/ml RX at RT.
 Cou A 10 mg/ml.

	Cou A	T ₄ DNA	CTDNA	PBS	Hg	Ca	Visible ppt	X3	
								A ₂₆₀	A ₂₈₀
0	0	0	0	0.98	10λ	10λ	0	0	0
1	5λ	0	0	975	10		0	02	015
2	5λ	180λ	0	795			0	140	082
3	5λ	0	100λ	875			0	190	108
4	10λ	0	0	970			0	026	032
5	10λ	180λ	0	790			0	116	124
6	10λ	0	100λ	870			0	200	120
7	25λ	0	0	955			0	064	079
8	25λ	180λ	0	775			±	084	171
9	25λ	0	100λ	855			0	234	173
10	50λ	0	0	930			0	096	151
11	50λ	180λ	0	750			+	043	250
12	50λ	0	100λ	830			0	270	252
13	100λ	0	0	880			0	174	288
14	100λ	180λ	0	760			+	106	381
15	100λ	0	100λ	780			0	362	382
16	250λ	0	0	730			0	450	750
17	250λ	180λ	0	650			+	211	851
18	250λ	0	100λ	630			0	625	850
00	0	180λ	0	800			0	170	092
000	0	0	100λ	880			0	170	093



2/20/82.

^{32}P -DNA Cou A Mg^{++} Ca^{++} PBS. R.T. incubation.
 0.28 $\mu\text{g}/\mu\text{l}$ 5 $\mu\text{g}/\mu\text{l}$ 1 M 10 $\mu\text{g}/\mu\text{l}$ (6.5) 15' A260 of Sup.

①	0	0.5	10 λ	10 λ	0.48
②	0.36	0	10 λ	10 λ	0.62
③	↓	0.02	10 λ	10 λ	0.60
④		0.05	10 λ	10 λ	0.57
⑤		0.10	10 λ	10 λ	0.52
⑥		0.15	10 λ	10 λ	0.47
⑦		0.2	10 λ	10 λ	0.42
⑧		0.5	10 λ	10 λ	0
⑨		0	0	10 λ	10 λ

	Visible ppte			ofn at 4°
	15'	30'	60'	
①	+	-	-	
②	-	-	-	
③	-	-	+	
④	-	-	+	
⑤	-	+	+	
⑥	-	+	+	
⑦	-	+	+	
⑧	+	+	+	
⑨	-	-	-	

DNA binding to concavallin A

A priori assumptions.

1. Only glycosylated DNA will bind to conA.
2. Binding of glycosylated DNA is reversible
bound DNA can be released by increase in pH
3. Binding of DNA will be inhibited by carbohydrates [which have stronger affinity for conA].

Experimental set-ups.

check for binding of glycosylated DNA to conA

- a. precipitation.
- b. affinity chromatography.

a. Precipitation.

checked by observing

- ① A_{260} post Rx of conA and DNA
check
- ② radioactivity.

To check whether glycosylated DNA will bind to cou A.

T₄ DNA = T₄ am 82 [44⁻62⁻] DNA
in 10 mM Tris pH 7.4
10 mM NaCl
A₂₆₀ vs buffer

Cou A 5 µg/ml in PBS (pH 6.5)
[~99% pure protein
less than 0.1% CHO]
metal content at least
4 g ions [Mn⁺⁺ + Ca⁺⁺] per g mole
dimer.
In solution less dimeric form
at ~~this~~ pH range used.

Exptl. protocol

To Fixed amt of T₄ DNA is added
↑ amt of cou A in reaction mixtures
containing Ca⁺⁺ and Mg⁺⁺ and
PBS (6.5).

The samples are spun after ~~2 hrs~~
at after ~~of~~ at 4° 1 hour at room
temp. and supernates measured for A₂₆₀
checked also for visible precipitate.

Use

1. Lectins bind sugars

Lectin-binding to CHO \uparrow \bar{e} sugar residues
monosaccharides > disaccharides > oligo

2. DNA from phages T₄; T₆ is glycosylated

3. A priori assumptions.

(a) T₄ DNA will bind to ~~can A~~ ~~spharose~~ concanavalin A

(b) Binding not inhibited by non-glycosylated DNA

(c) Bound DNA quantitatively replaced by monosaccharides
such as mannose.

... ..
... ..
... ..
... ..

... ..
... ..
... ..
... ..

5 x 10⁵
329 | 163 | 20000
7
36157 | 1098000000
3