Hin Endo R preps – Summary

11-13-72 P-cell eluted c̄ 0.3M NaCl

1. Saved aliquots of 7x 3 to 14 (glycerol –15°)
2. Pooled rest + conc. on P-cell
   0.5 M eluate
   11-13 Prep A (#6-11) (Preps B + C = edges)
   Stored in small batches – 50% glycerol –15°

11-26-72 – Gradient elution of P-cell
0.15 to 0.35 M NaCl

1. Save aliquots of fx 25 to 47
   (all of 25, 26, 27 + 45, 46, 47)
2. Pools A (early clot + eluate) B, C, D, E
   Each ~~el~~ c̄ 0.5M NaCl to concentrate
   All in 50% glycerol at –15°.

11-13-72 Prep of Hin Endo R (1)

Strep super fr HOS (11-12-72)
from 300 g packed cells
in 25 mM Tris 8.3 1mM ME
~1% strep sulfate
~30-50 mg/ml protein
Vol = 1050 ml

AS fx

Add solid AS & stir for 30' at 0°
Cent in Sewall large rotor 9000 rpm
X 15'. Diss in min. vol 01 M NaP-1mM ME
pH 7.4

Initial Vol	AS fx	% (sat.)	g AS	Vol p̄ diss
1050	#1	0-30%	184	22 ml
1060	#2	30-40	66	130
1050	#3	40-50	66.5	83
1020	#4	50-60	67.5	41
1040	#5	60-70	72	30
1060	#6	70-80	76.5	17

pool

Assay by clot assay - see next page
In an attempt to ↓ vol of #4 + #5 + #6
combined fx (89 ml) ppt c̄ AS p̄ add'n
of 89 ml NaP-ME - 90 g
Stirred 40' + cent at 25000 x g 10'.
Diss in ~90 ml NaP-ME! (i.e
about same as before)

(2)

50 X 2.5 cm col.

G25 Med. seph. equil c̄ NaP-ME

3 - 30 ml batches - pool brownish run through. = ~160 ml (35 mg/ml protein by Lowry)

Clot

Pcellulose - P11

Washed c̄ 0.2 M NaOH X 2, water, + 0.2 M HCl X 2, water + .01 M Na_2HPO_4 til pH = ~4, then 0.01 M NaP pH 7.4 til effl = 7.4. ~~Equil overnight in cold~~

(HOS uses 1 ml bed vol/100 mg protein)

Pack 40 ml bed col. in 50 ml plastic syringe. Equil overnight in cold. c̄ .01 NaP.

Sample 95 ml G25 effl + 200 ml .01M NaP 7.4, apply overnight at flow of ~ 10-15 ml per hour.

Elution:
1) 0.01 M NaP - 2 vol (80 ml)
2) " + 0.1 M NaCl - 3 vol
3) " + 0.2 M " 5 vol
4) " + 0.3 M " 5 vol

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11-15 Endo R clot assay (p. 3)

10λ fx P-cell. ~10 ml fractions

35° x 35'

Hin Endo R
Label 11-15 fx#

add 0.5 ml glycerol

7x	2	0
	4	±
	6	4+
	8	4+
	10	4+
	12	2+
	14	0
	16	0
	18	0
	20	0
	22	0
	24	0
	26	0
	28	0
	30	0

Save 0.5 ml of each fx 3 to 14
Pool fx 5 to 11 + dilute to
0.1M NaCl c̄ NaP .01 - ME 1 mM
Conc. on 2 ml P-cell column ~
elute c̄ 0.5M NaCl - .01 NaP
Pool vol = 62 ml
Diluted vol = 190 ml

(Save 7x 3, 4; 12, 13)

Note: #5, 8 + 11 used to digest SV40 DNA by TL:
#5 1λ incompl
2λ compl
#8 1λ nearly compl
2λ " compl.
#11 1λ incompl
2λ → new pattern!

Exo assay

H. infl DNA (S)
4000 cpm / λ on 11-21
1215 n Moles/ml
(f Kent Wilcox)

.3
300
360 γ/ml

20X
0.10 ml
2 ml TMSH + Na .05 M
} .10

Eng fx 3 to 14 P-cell 10λ

35° x 30' Add 1 drop γ-RNA + 1 ml 5.5% TCA in cold. Count super

Cyo on P-cell fx

7x(10λ)

	TCA vol
3 –	5400 cpm
4 –	4300
5	3200
6	2300
7	5100
8	7400
9	7600
10	2180
11	6600
12	2000
13	3550
14	1040
0	1200
0	800
0.1 ml mix	44,700

11-13 Hin Endo R (con't)

Collect 3 or 4 fx per col. vol.

Enzyme gen. in 0.3 M eluate - 2nd + 3rd col. vol.

Concentration

Dilute enz. to 0.1 M salt c̄ 0.01 NaP-ME + load onto 2 ml P-cell col. overnight. (62 ml pooled fx + 125 ml NaP-ME)

Assay last wash through for enzyme. — neg. by clot test

Wash c̄ 0.01 M NaP 2-3 col vol

Elute c̄ 3 vol 0.5 M NaCl - 0.01 M NaP + collect 0.2 cc fx

Assay 1 λ to locate enzyme.

Add = vol 100% glycerol

HOS

May procedure → 2 col vol 0.1 M NaCl ; 3 col vol 0.2 M NaCl
5 col. vol 0.5 M NaCl (no SH in last)
~0.3 ml fx

Assay

35° x 20' 1) Clot 2 λ of each fx / 0.2 M | 0.5 M fx

fx 2 —	2 - —	3 —	
4 —	4 - —	5 —	
6 —	6 - 4+	7 4+	
8 —	8 - 4+	9 4+	
10 —	10 - ±	11 4+	
	12 - ±		
	14 - —		
	16 to 28 —		

2) Exo (over)

Exo - final conc. fx

Noeing = 440

.5M - fx 3 - 1990
4 785
5 2126
6 1770
7 960
8 1540
9 1410
10 1650
11 1480

Input = 129,400!

3 to 15 Add ~ = vol 100% glycerol + store at -15°C.

11-13

Pool fx 6 - 11 = prep A 11-13-72

leave in { ~~" 4+5 = prep B~~ "
~~" 12+13 = prep C~~ "

11-14-72

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Clot assay for EndoR

.3M MgCl2 — .1 ml

1M Tris 7.6 .03

5M NaCl .05

W → 5

SSDNA 1.2 mg/ml 0.5

TMSH + 50 mM NaCl x 2.5

} 0.1

Enzyme 5λ or 10λ of appr. dil.

35° x 30'
Chill
Add 1 drop 0.3% BSA in H_2O + 0.2 ml 10% TCA

Score clot vs fine ppt.

Assay for exonuclease

Buffer mix as above

0.1 { buf-salts 2.5 ml
P-P22DNA — 10λ (2 x 10^5 cpm)
SS DNA(s) — 50λ 200γ/ml

Enzyme 5 to 10λ

35° X 30' chill 1 drop YRNA + 0.2 10% TCA
Count super

11-16-72

Prot deter

50 ml Na_2CO_3 sol'n (2% in 0.1 N NaOH)
Mix $\bar{c}$ 1 ml of equal pts 2% KNa tartrate + 1% $CuSO_4$
~~10' RT~~

add 1 ml to 5 to 200γ prot in 200λ
10' RT
add 0.1 ml Folin rgt.
Read at A_{750} $\bar{p}$ 30'

AS fx $\bar{p}$ G25: (Vol = ~200 ml)

25λ	1→5	~~.630~~ 1.46	1.38	
5λ	1→5	.407	.329	= 34γ/1λ original or 34 mg/ml
5λ	1→25	.160	.082	
5λ	1→125	.098	.020	
50γ BSA		.560	.482	
100γ BSA		.630 -?	.552	
Blank		.078		

total protein
34 x 200 = 6.8 g

Use 68 ml P-cell bed volume or 35 ml for 1/2 of the AS fx

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11-13 Hin Endo R prep

					Endo R	TCA super Exo
	7x					
SM super	1 a	SM super (12)	1/10	5λ	fine ppt	1078
	b		1/10	10λ	f	2270
0-30 Norat	2 a	AS1 (22)	1/10	5λ	Coarse ppt	263
	b		1/100	5λ	c	20
30-40	3 a	AS2 (130)	1/10	5λ	c	1200
	b		1/100	5λ	c	77
	c		1/1000	5λ	c	16
40-50	4 a	AS3 (83)	1/10	5λ	f	1430
	b		1/100	5λ	c	155
	c		1/1000	5λ	c	69
50-60	5 a	AS4 (42)	1/10	5λ	f	3448
	b		1/100	5λ	f>>c	607
	c		1/1000	5λ	c	39
60-70	6 a	AS5 (30)	1/10	5λ	f	2910
	b		1/100	5λ	f<c	1110
	c		1/1000	5λ	c	423
70-80	7 a	AS6 (17)	1/10	5λ	f	2690
	b		1/100	5λ	f=c	1640
	c					
	no enz				c	28
					c	17
	Orig mix	0.1 ml				7260

Hold

Pool + re-ppt c̄ sat AS
Assay ppt (90 ml) + super (~200 ml) by clot assay 10' 35°

P		S	
5λ 1/10	f	5λ 1/10	c
5λ 1/50	c	5λ 1/50	c
5λ 1/250	c	5λ 1/250	c
no enz	c		

(over)

Clot assay of G25M $\bar{a}$ P-cell &

P-cell run through. $(= \bar{p})$

$\bar{a}$		$\bar{p}$	
5λ	— 3+(f)	5λ	— 0
5λ 1:5	— 0	5λ 1:5	— 0
5λ 1:25	— 0	5λ 1:25	— 0

SSDNA } Mix 0.1
THSH }

Eng .005

$35^\circ \times 20'$

11-21-72 2nd batch of Endo R
AS fx

65 ml of AS fx ~ G25 (35 mg gr./ml)
+ 130 ml of .01 NaP 7.4 - .001 ME

Apply to 50 ml col of P-cell overnight

Elute ~ 100 ml .01 M NaP - 1 mM ME
" " 250 ml " " + 0.15 M NaCl
~~" " ml " " + 0.15 M NaCl~~
" " linear gradient of 500 ml 0.15 M to 0.35 M
NaCl ~ NaP - ME - 52 fx of gradient
" " 0.35 M NaCl - 200 ml

11/23 Clot assay
Gradient fx G11, 13 ... 51
& 0.35 1, 3, etc

Repeat 4λ 7½' 35°

10λ each 30' 35°	G11	±	43	4+
	13	±	45	±
	15	±	47	0
	17	±	49	±
	19	±	51	0
	21	0		
	23	0	0.35-1	1+
	25	0	3	0
	27	±	5	0
29-30 Ia	29	4+	7	0
31-35 Ib	31	4+	9	0
	33	4+	11	0
34,35 Ic	35	4+	13	0
36-40 II	37	3+	15	0
	39	2+	17	0
42-44 III	41	3+	19	0
			21	0

G25		41
26		42
27		43
28		44
29		45
30		46
31		47
32	±	48
33	±	
34		
35		
36		
37		
38		
39		
40		

neg except 32

11-26 2nd batch of EndoR – Concentration
Gradient elution fx

Pool Gr–A 28–30 ✓ Try for EndoR II (IG)
B 31–33 ✓
C 34–37 ✓
D 38–40 ✓
E 41–44 ✓ — EndoR III (AEK) + f x 45,46,47

Each dil to ~0.1M NaCl (1:3) & conc on 1½ ml P-cel
Collect ~0.5 ml fx of 0.5 M eluate
(& 0.1M & 0.2M wash – 2 col vol each)

11-25 — 0.1 ml reac. vol 35° x 30'

A 2	5λ –	B2	2λ –	C2	2λ –	D2	5λ –	E2	5λ	4+
4	3+	4	–	4	2+	4	4+	4		–
6	±	6	–	6	–	6	–	6		–
8	–	8	–	8	–	8	–	8		–
10	–	10	–	10	–	10	–	10		–
12	–	12	–	12	–	12	–	12		–
14	–	14	–	14	–	14	–	14		–

Glycerol added → ~30–40%
Also to B – 0.2M eluate

11-26 Repeat – .05 ml reac. vol. 35° x 60'

A 3	5λ 1+	B1	5λ –	C3	10λ 4+	D3	5λ ±	E1	5λ –	–
5	" 1+	2	±	5	3+	5	3+	3		4+
3		3	2+					0.2M	10λ	–
~~0.2M~~	" –	4	3+							
		0.2M	10λ –							

Pools (in glycerol)
→ A 3,4,5; B 3,4,5 C 3,4,5; D 3,4,5; E 2,3

Endo R subfx on SV40 DNA

Ham's fx 2 → 5 fragments

R_{II} (GJ)

R_R

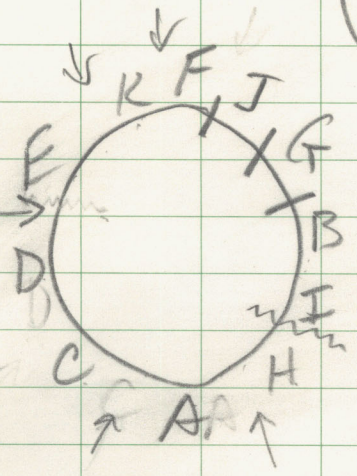
BIHACD 1) 32 mm (A=61)

HACD 2) 40 mm (A=61)

FKF 3) 66 mm (A=61 mm)

4) G = 183 mm

5) J = 246 mm



HACD = 48.
BI = 19.
EKF = 20
ED

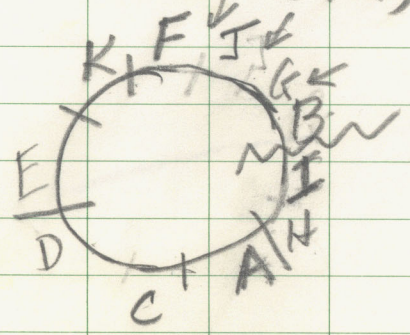
11-20 P-cell-1

R_{III} (AEK)

7x 5) → complete digest
7x 8) → " "
7x 11) → 5 fragments

1) 49 mm (G = 218 mm)
2) 76 mm (poss. this is A) - Broad
3) 166 mm (" " " CD) (")
4) 193 mm (prob " " E or F)
5) 297 mm (K)

This is the expected 2nd Dig.



JGB 26.5
HI 11.5
38.0

ED = 19