

~~14/20~~ H. influenza Endo R

Final conc.

5X { 6.6 mM Tris pH 7.5
 6.6 mM MgCl₂
 6.6 mM ME } 2X (+45 mM NaCl)
 45 mM NaCl

Enzyme 20° 4 U/ml in 50% glycerol -
 0.2 M NaCl - .15 M KCl - .02 M Tris 7.5

2 conditions

A. E at 0.12 U/ml
 E : DNA 0.018 U / μ DNA
 37° 30' → complete

B. E at .04 U/ml
 E : DNA 0.006 U / μ DNA
 37° > 90'

Vol DNA < 5% total volume.

Electrophoresis : 5% gels 4 mAmp x 16 hr
 or 8 mAmp x 4 hrs \bar{p} appl sample
~~at 4 mAmp for 20'~~
~~then 8 mAmp tubes for 90' - see 32 only~~

11/24/70 HI Endo on ^{32}P + 3H DNA I
large scale

	A	B	C	D
DNA	^{32}P 11/18 .05	^{32}P 11/18 .05	3H 11/18 .05 568/ml	3H 11/18 .05
Mix	.20	✓	✓	✓
Endo R 40/ml	.01	✓	.015	✓

2^{30} 36° x 90' Then same amt enzyme as in 1st incub.
36° x 90' more

5x TMMSE 1.0
1M NaCl .15
BSA 10mg/ml .01
water 2.5

2×10^5
 10^5 .28
100,000 .02
30,000 4000
(1000) (6000)

at end of 3hr inc. remove 10 μ l of A + B
+ 20 μ l C + D to tubes E 1/5 vol .1M EDTA
+ 1/5 vol 2% SDS Freeze remainder at -70°.

Aliquots electro.

3 A
6 B
9 C
12 D

4m Amp/tube 6⁴⁵ PM - 9 AM
but temp got to 30°
(over)

11/25/70

5% acrylamide

10 cm gels

4 mA Amp/gel x 14 hrs

10x

got to 30°
Constant voltage

12/24

Cpm

400

200

400

200

600

400

200

200

400

300

100

200

100

A

10' ds

B

10' ds

C

10' - Has den

D

10' - Has den

450

140

80

670

-60

610

~ 60/40 = 1.5

10x
500x

280
120
80
480
-60
420

10x

4 x 10⁶/ml

x .05

2.0 x 10⁵

25

10⁴

8000

600
190
50
840

315
180
495

20x

2 x 10⁶/ml

280

102

146

106

634

200

434

190

88

116

394

-150

244

+30

274

137

411

20x

KE 20 X 20 TO THE INCH 46 1240
MADE IN U.S.A.
KEUFFEL & ESSER CO.

11/25

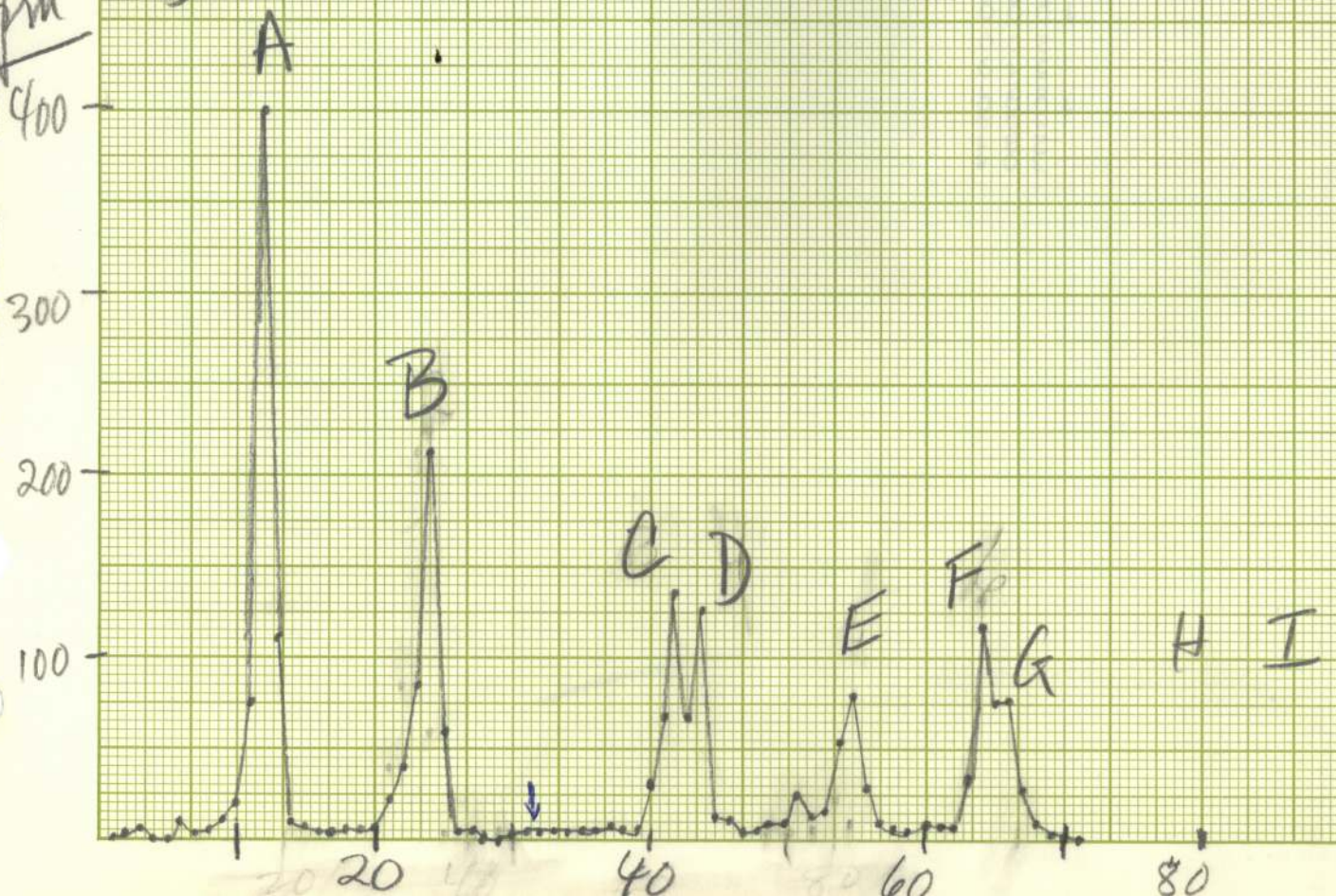
835

³²P HI Endonuclease Product
5% gel 14 hrs 4m Amp/gel

B

cpm
400

358-1
MADE IN U.S.A.
10 X 10 TO THE 1/2 INCH
KEUFFEL & ESSER CO.



SUBJECT

11/25

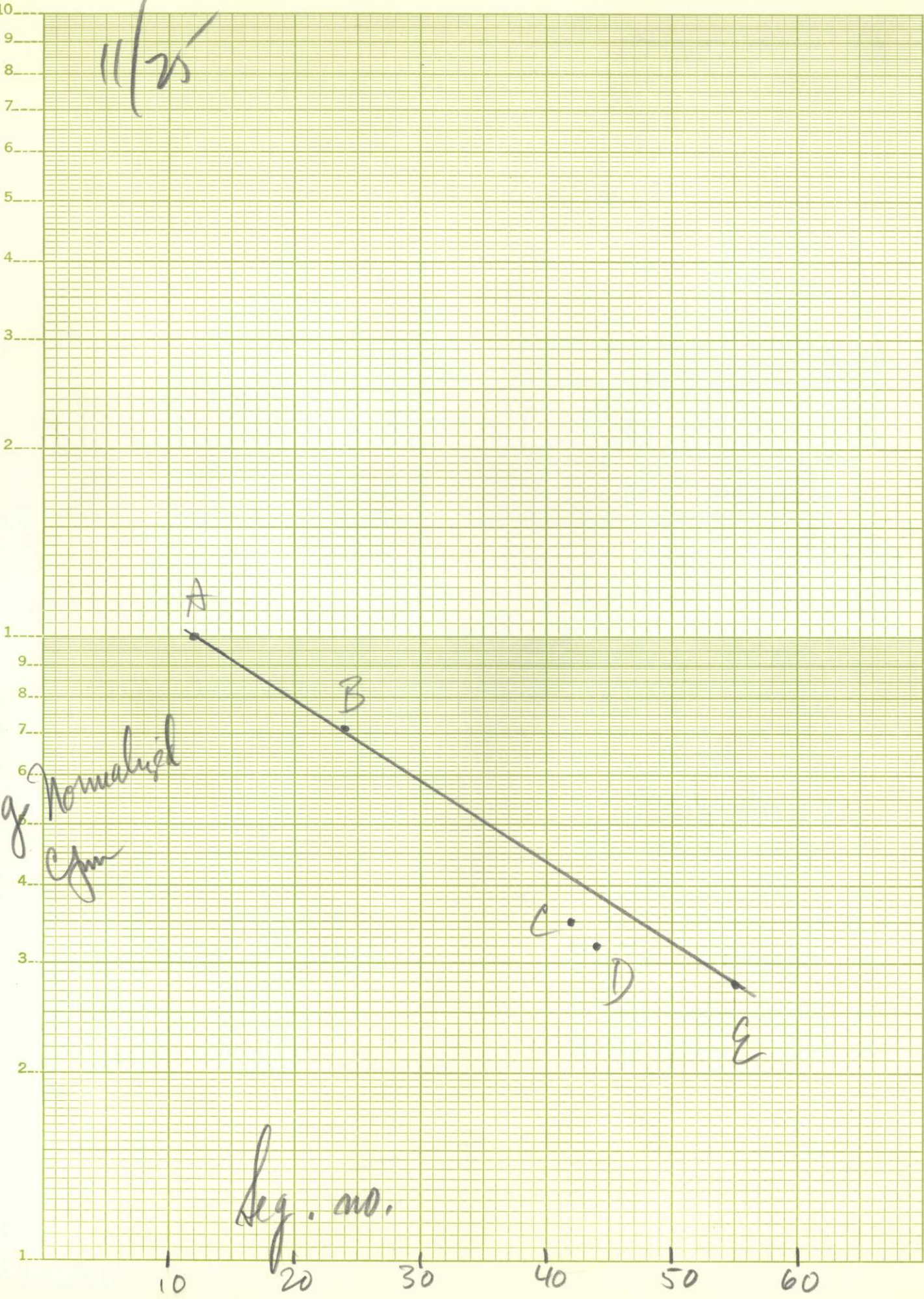
	<u>total</u> <u>cpm</u>	<u>Seq no.</u>	<u>Normalized</u> <u>size</u>		
Peak 1	585	12	1		
2	417	24	0.71	note 34 T label → .6	
all of 43 to 44	3	205	42		0.35
	4	189	44		0.32
	5	165	55		0.28

11/25

KE SEMI-LOGARITHMIC 358-61
KEUFFEL & ESSER CO. MADE IN U.S.A.
2 CYCLES X 10 DIVISIONS

Log Normalized
Gpm

Seq. no.



11/27/70

Electrophoresis of HI Endo Product

SUBJECT

SHEET NO.

BY

DATE

³P + ³H product from 11/24

25

Warm tubes of frozen samples. To each
add .01 0.5M EDTA + .01 ml 10% SDS
Incubate at 35° X 15' Add .05 ^{35%} sucrose-BPB

A+B: Apply .20 to each of 3 gels; 20 λ to
a 3rd gel + 25 λ \rightarrow .16 \bar{c} electrophoresis buffer
+ add .04 sucrose

C+D Apply .20 to each of 3 gels

5% gels - 10 cm

On 9 ³⁰ P.M.

3.5 mAmp / tube
const. voltage

gel #

2 A .20

4 A+B .20

6 B .20

8 B .03

10 B delete .20

12 C .20

1 C+D .20

3 D .20

(11 ~ .01 or .02 of D + .19 buffer suc. by error)

265
240
320
640
270
270
540
17

500
550

Gels sliced + distrib. into tubes \bar{c} 0.2 ml
electrophoresis buffer, as shown on
graph. Incubated in warm room 15-24 hrs,
then room temp.

40 500
 6

 3000

11/28/70

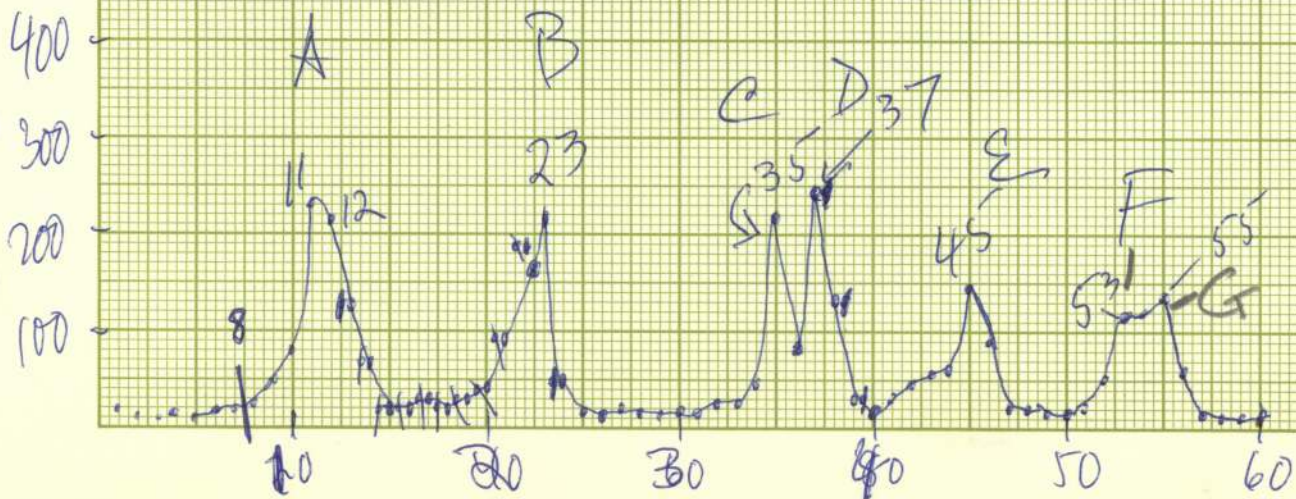
Note

#4 7-9 went into #2, 7-9

#4 10 " " #2 10-13

Eluted into electrophoresis buffer.
~ 0.2 ml / tube

Sample	Segments	Tube #	Tube #
#1 <u>Bally</u> <u>Sliced</u>	1. segments 7-9	41	61-2
	2. 10-13	42	63-4
	3. 14-16	43-47	65-6
	4. 17-19	48-49	67-8
	5. 21-24	50	69-70
	6. 25-27	51	71-2
A	8-16	32	73-4
B	19-26	33	75-6
C+D	32-41	34	77-8
E	43-48	35	79-80
F	50-58	36	
		37	
		38	
		39	
		40	
		52	
		53	
		54	
		55	
		56	
		57	
		58	
		59	



900

← all 10 ↓ →

	#2	4	6	36 ⁰⁰	3	HOS 31 cts/2'	12 HOS cts/2'	1
7-10								
A 10-13	114	37	77		40	108	248	
14-16	55	55	51		26	55	181	
17-19			14					
B 21-24	58	133	90		40	123	234	
25-27	58	66	38		36	119		
32								
34								
35	5	C			0	0		
36	9	26	23		0	0	67	24
37	9	47	35		0	0	70	42
38	34	85	63	D+C	5	14	83	69
39	56	26	32		13	26	48	51
40	45	6	6		21	61	20	38
41	37	D				90		
42								
E 43-47	135	138	105		70	188	52	
48-49		0	10		43	77	400	
50	5							
51	1							
52	5							
53	8	24			10	0	7	4
54	7	48			2	0	40	29
55	30	36			17	0	88	42
56	49	19			15	29	0	32
57	52	12			43	65	13	3
58	14				16	39	5	0
59	9						3	0

	✓	4	6		3		12		1
61-2	0 ^{10λ}								
63-4	3								
65-6	1			10λ		10λ			
67-8	6			2		0 ✓	(59) H	(53) H	15
69-70	10	10λ		2		25 ✓	(44) H	(60) H	16
71-2	(77) ✓	(78)		(24) H		(24) ✓ H	25	(24) I	17
73-4	(55) ✓	(60)		(25) H+I		(74) ✓ H	(35) I	(25) I	18
75-6	(74) ✓	(64)		(25) I		20 ✓	(10) I	(25) I	19
77-8	(52) ✓	(32)		5		(54) ✓ I	0	(48) I	20
79-80	18					(40) ✓ I			

all 10λ

3-41	12-67	12-36	1-36	1-65
3-67	↓	37	↓	↓
↓	79	38	40	75
3-79		39	↓	
		40	40	
		12-54	1-54	
		5	↓	
		6	8	
		7		
		8		