NATIONAL
42-383
Made in U.S.A.

14/20 H. influenza EndoR

Final conc.

5X { 6.6 mM Tris pH 7.5
6.6 mM $MgCl_2$
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 Tris 7.5

2 conditions

A. E at 0.12 U/ml
E:DNA 0.018 U / γ DNA

37° 30' → complete

B. E at .04 U/ml
E:DNA 0.006 U / γ DNA

37° > 90'

Vol DNA < 5% total volume.

Electrophoresis: 5% gels 4 mAmp x 16 hr
or 8 mAmp x 4 hrs p̄ appl sample
~~at~~ 4 mAmp for 20'
~~then 8 mAmp/tube for 90' = slice 32 only~~

11/24/70 HI Endo on ^{32}P & ^{3}H DNA I
2×10^5
large scale

	A	B	C	D
DNA	^{32}P 11/18 .05	^{32}P 11/18 .05	^{3}H 11/18 .05 56 8/ml	^{3}H 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

5 x TMME	1.0	2×10^5	
1 M NaCl	.15	10^5	.28
BSA 10 mg/ml	.01	100,000	.02
water	2.5	30,000	1000
		1000	6000

at end of 3 hr inc. remove 10 λ of A & B
& 20 C & D to tubes c 1/5 vol .1 M EDTA
+ 1/5 vol 2% SDS Freeze remainder at −70°.

Aliquots electro. 4 mAmp/tube 6 45 PM – 9 AM
but temp got to 30°
3 A
6 B
9 C
12 D
(over)

B - rest of gel sliced 2 days later
(kept at -15°) 31-70 (last 4 segments discarded)

11/25/70 5% acrylamide

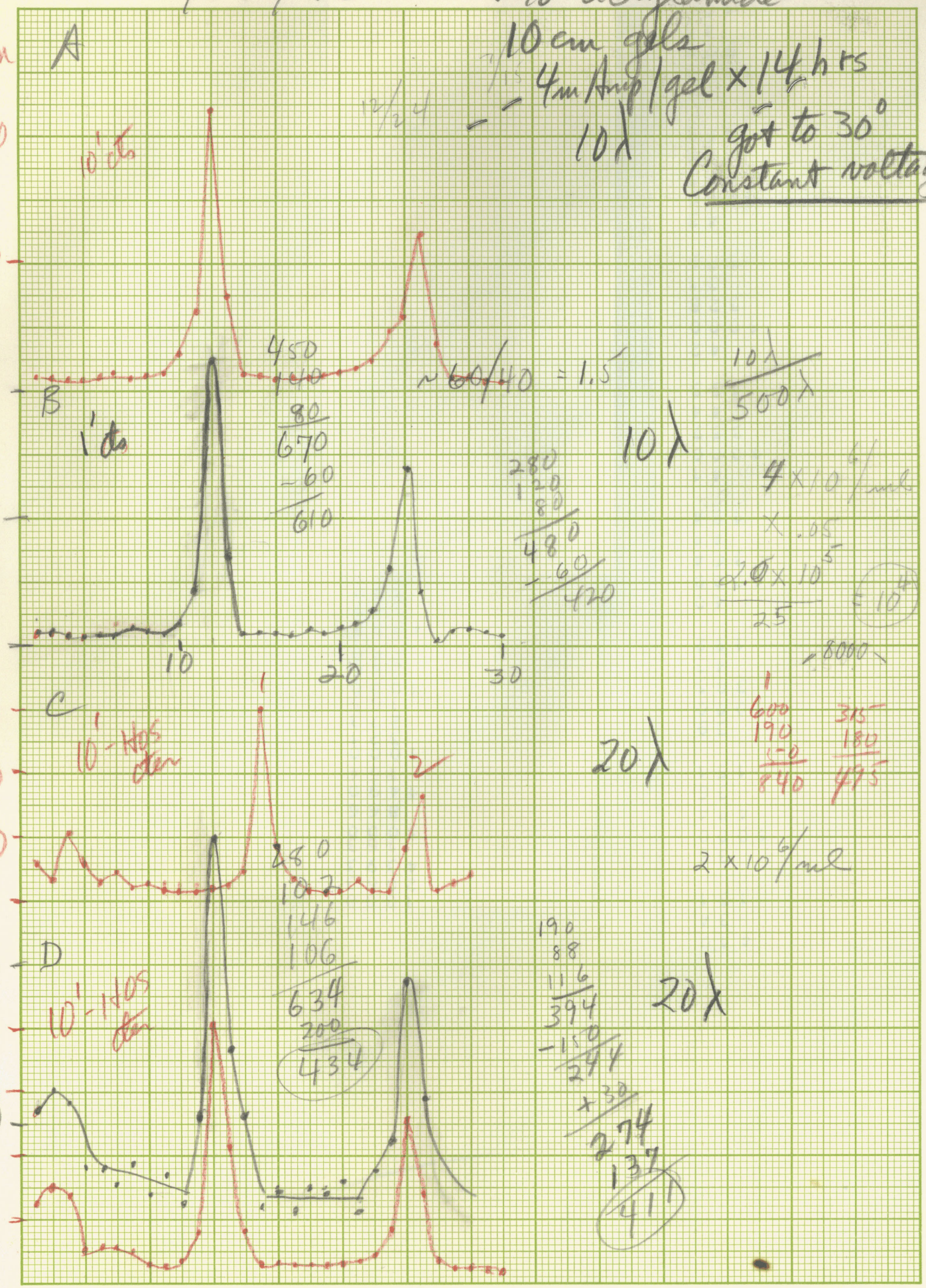
10 cm gels

4m Amp/gel x 14 hrs

10λ

got to 30°

Constant voltage

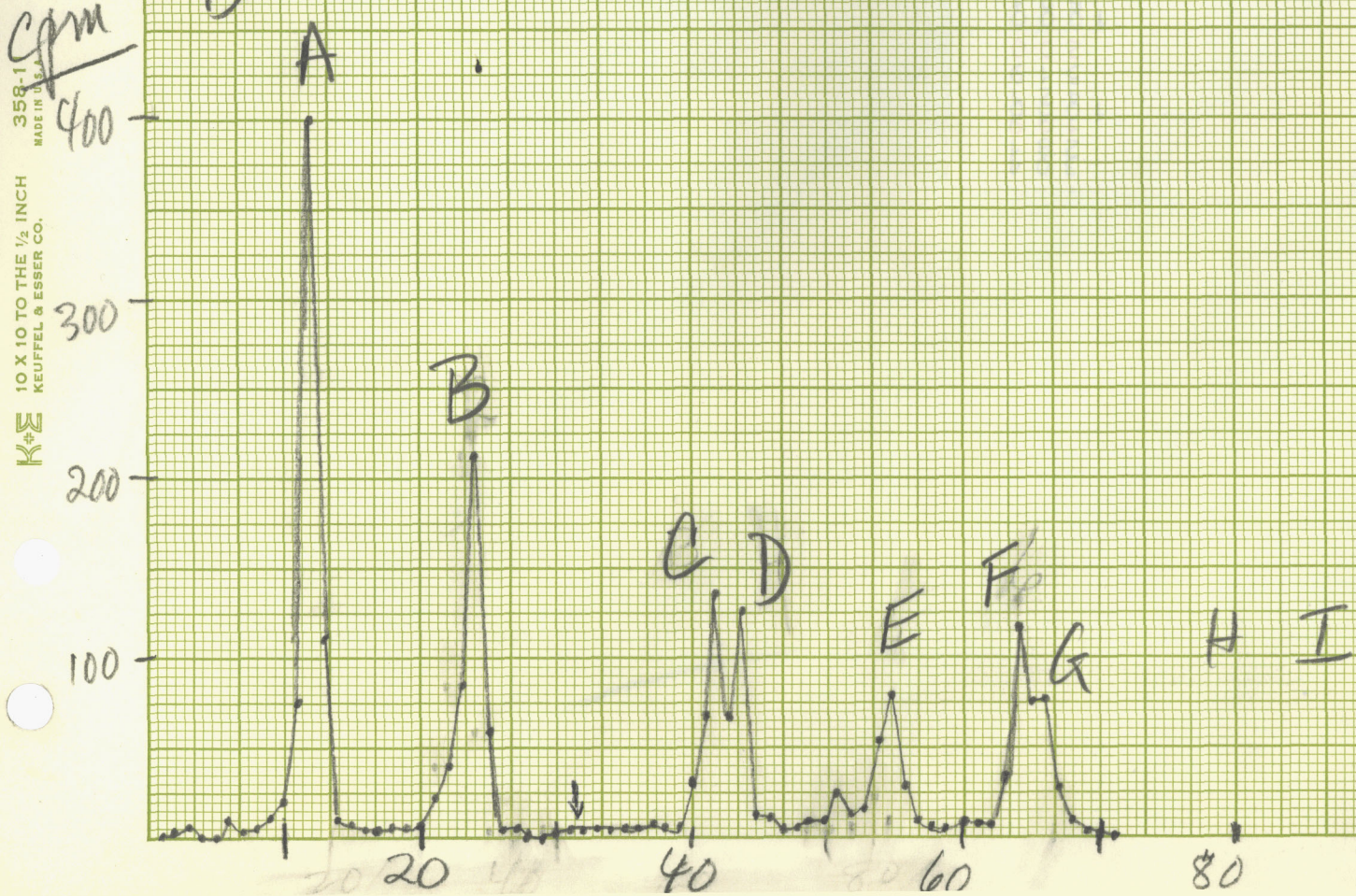


K&E 20 X 20 TO THE INCH 46 1240
7 X 10 INCHES MADE IN U.S.A.
KEUFFEL & ESSER CO.

11/25

^{32}P HI Endonuclease Product

5% gel 14 hrs 4mAmp/gel



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

C-190

11/25

SUBJECT

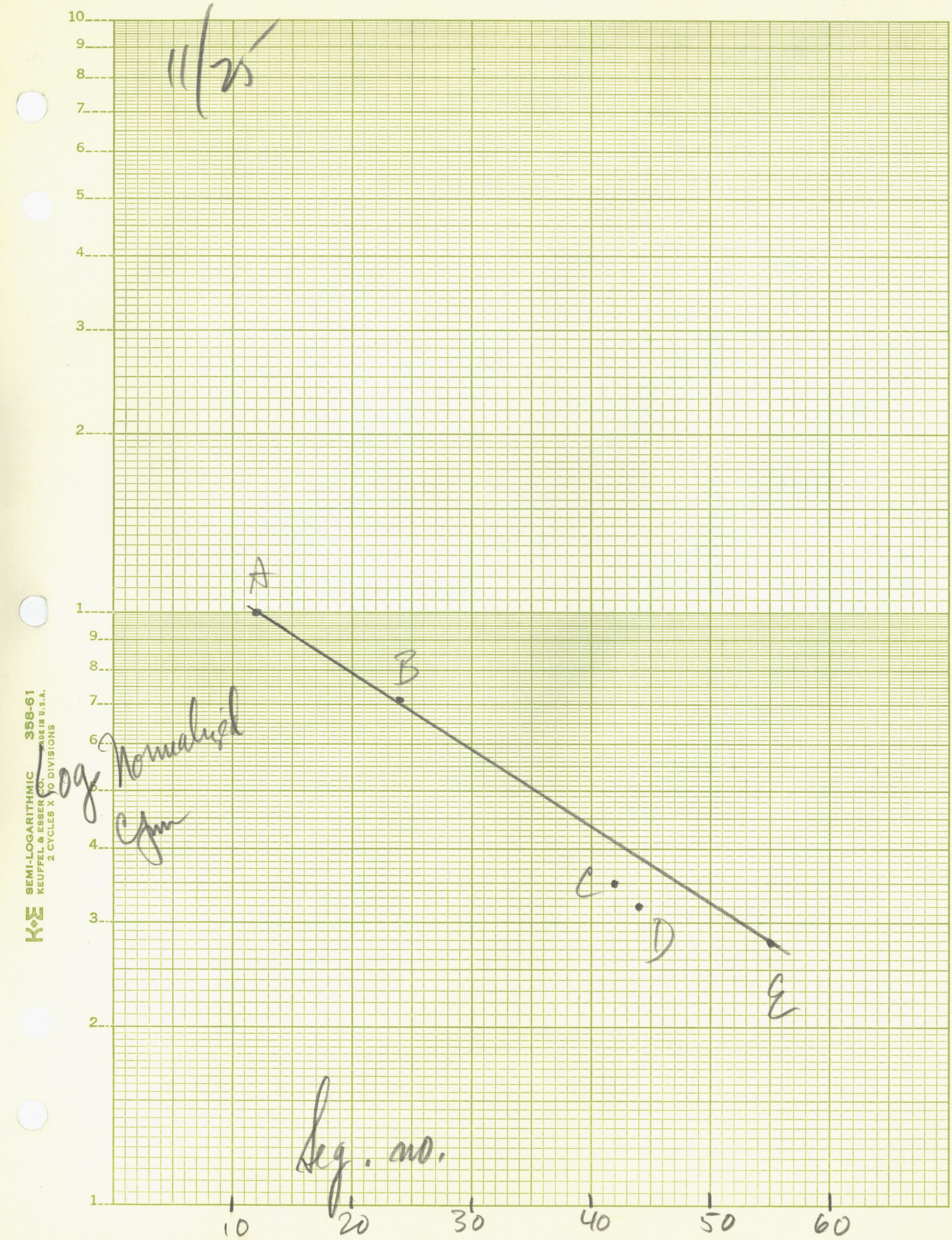
SHEET NO.

BY

DATE

	total cpm	Seg no.	Normalize size
Peak 1	585	12	1
2	417	24	0.71
all of 43 to 44 { 3	205	42	0.35
4	189	44	0.32
5	165	55	0.28

note 3/4 T label → .6


11/25
A
B
C
D
E
Log Normalized Gm
Seg. no.
10
20
30
40
50
60
SEMI-LOGARITHMIC 358-61
KEUFFEL & ESSER CO. MADE IN U.S.A.
2 CYCLES X 70 DIVISIONS

C-190

SUBJECT 11/27/70 Electrophoresis of HI Endo Product

SHEET NO.
BY
DATE

^{32}P & ^{3}H product from 11/24

Warm tubes of frozen samples. To each add .01 0.5M EDTA & .01 ml 10% SDS
Incubate at 35° X 15' Add .05 50% sucrose-BPB

A+B: Apply .20 to each of 3 gels; 20λ to a 3rd gel + 25λ → .16 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 dilute .20
12 C 0.20
1 C+D 0.20
3 D 0.20
(11 ~ .01 or .02 of D + .19 buffer - succ. by error)

Gels sliced + distrib. into tubes c̄ 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

#1 badly sliced

Eluted into electrophoresis buffer ~0.2 ml / tube

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
⋮	21-24	50	69-70
	25-27	51	71-2
	32	52	73-4
	33	53	75-6
	34	54	77-8
	35	55	79-80
	36	56	
	37	57	
	38	58	
	39	59	
	40		

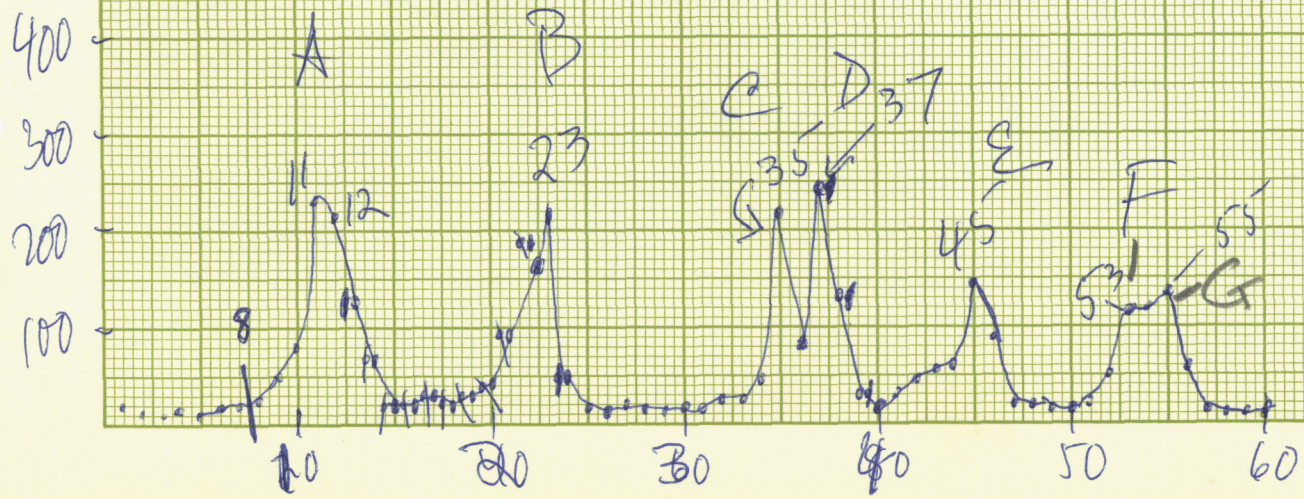
A 8-16

B 19-26

C+D 32-41

E 43-48

F 50-58



K+E 10 X 10 TO THE ½ INCH 358-11 KEUFFEL & ESSER CO. MADE IN U.S.A.

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900
3600

← all 10 λ →

	#2	4	6	3	HOS 3' 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		
33							
34							
35	5	C		0	0		
36	9	26	23	0	0	67 C	24 C
37	7	47	35	0	0	70	42
38	34	85	63 D+C	5	14 C	83	69
39	56	26	32	13	26	48 D	51 D
40	45	6	6	21	61	20	38
41	37	D			90 10λ D		
42	✓					52 ✓	✓
E 43-47	135	138	105	70	188	400	✓
48-49	✓	0	10	43	77		
50	5					7 ✓	4 F
51	1					40 F	29
52	5					88 F+G	42
53	8	24	F	10	0	0 ✓	32 G
54	7	48		2	0	13	3
55	30	36	F+G	17	0 F	5	0
56	49	19	G	15	29	3	2
57	52	12		43	65	0	0
58	14			16	39 G	0	0
59	9						

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	✓	4	6		3		12		1	
61-2	0 10λ									
63-4	3									
65-6	1									
67-8	6		10λ 2		10 λ				53	15
69-70	10	10λ 17	24	H	0	✓	69	H	60	H 16
71-2	77 ✓	78	2	H + I	25	✓	44		9	17
73-4	55 ✓	60	10		24	✓ H	25		240	I 18
75-6	74 ✓	64	25	I	74	✓	35	I	25	19
77-8	52 ✓	32	5		20	✓	10		48	20
79-80	18				54	✓	0			
					40	✓ I				

all 10λ

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