LABORATORY NOTEBOOK

DNA

GENENTECH, INC.

10840

NOTEBOOK NO. 10840

ISSUED TO John Ridgway

ON December 7 19 89

DEPARTMENT Cell Genetics

RETURNED _____ 19 ____

Witnessed by Rebecca Cazares
on March 1, 1991 pages #1-96.

—SCIENTIFIC NOTEBOOK CO.—
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Genentech Laboratory Notebook Procedures

Important steps for maintenance of your notebooks:

1) **Use dark permanent ink to record all of your work in your notebook on a daily basis. Sign and date each day's entries.**

2) **Permanently affix all attachments without covering any other entries. Please attach copies of any computer data entered into your computer files. These data are considered part of your experimental record.**

3) **Make any changes in data in dark permanent ink and, if appropriate, initial and date in the margin. Ensure that the original entry remains visible. Leave no open areas. When the book is completed line out any unused portion of a page at the end of each experiment.**

4) **Have a witness who understands and is aware of your work, but who is not directly involved in your project, sign and date your notebook entries (not later than one month after you do the work).**

5) **Don't hold back data for later entry and don't keep a "rough draft" notebook.**

6) **Report the quantitative or qualitative results only.** Avoid over broad and potentially inflammatory comments like "failed experiment", "doesn't work", or "toxic compound."

7) **The source and character of starting materials should be described.** Preferably, refer to the notebook pages describing the starting material and its method of preparation.

8) **Be sure your notebook record is understandable.** Omit abbreviations or slang that would not be understood by others working in your field. It is helpful to introduce each experiment with a statement of purpose, and make liberal use of cross-references to related experiments. While sufficient detail should be included to enable reproduction of experiments, it is acceptable to refer to conventional or published procedures. However, record any changes you may make to such procedures.

9) **Record your ideas too, not just experimental data.** This is important to demonstrate when an invention or thought occurred to you, and is an important part of establishing priority in inventorship contests. Don't hesitate to broaden the scope of your ideas; there is no reason to limit them to specific experiments planned for the next few days, although you should include as much detail as possible. Your notebook will not be published and there is no penalty for guessing wrong on notebook *idea* entries, so don't hesitate to let your imagination run.

10) **Try to keep a different notebook for each product and/or project. Please complete the table of contents indicating which product and/or project this work concerns.**

Genentech's success, and with it your own prosperity and research support, depends upon the quality and timing of your work. This may require that we prove what you did and when you did it in the face of a contrary challenge. Such challenges have and will continue to arise in judicial proceedings, in patent validity or infringement or challenge, inventorship priority contests and product liability actions. The key to winning in such cases is the ability to supply appropriate evidence.

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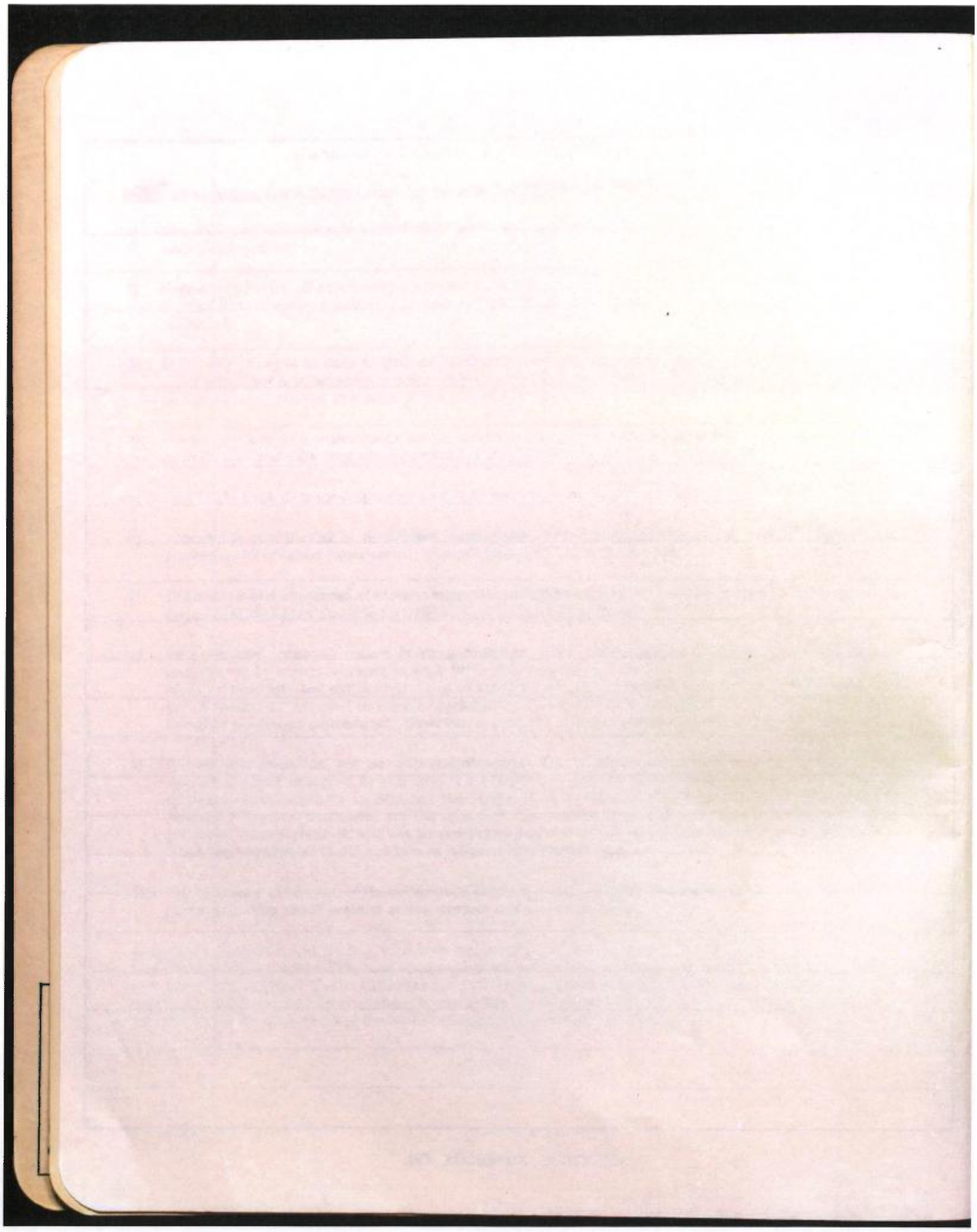

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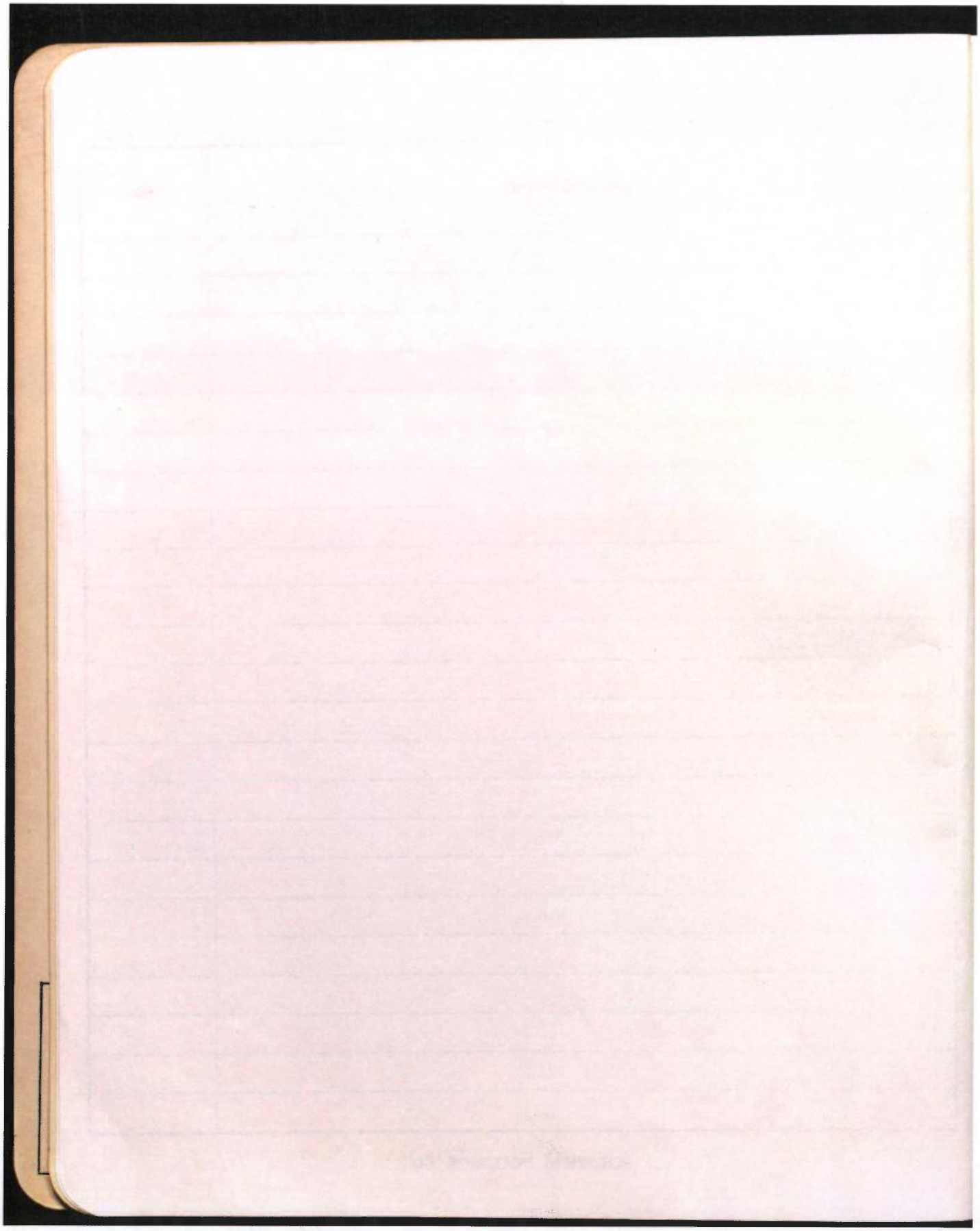

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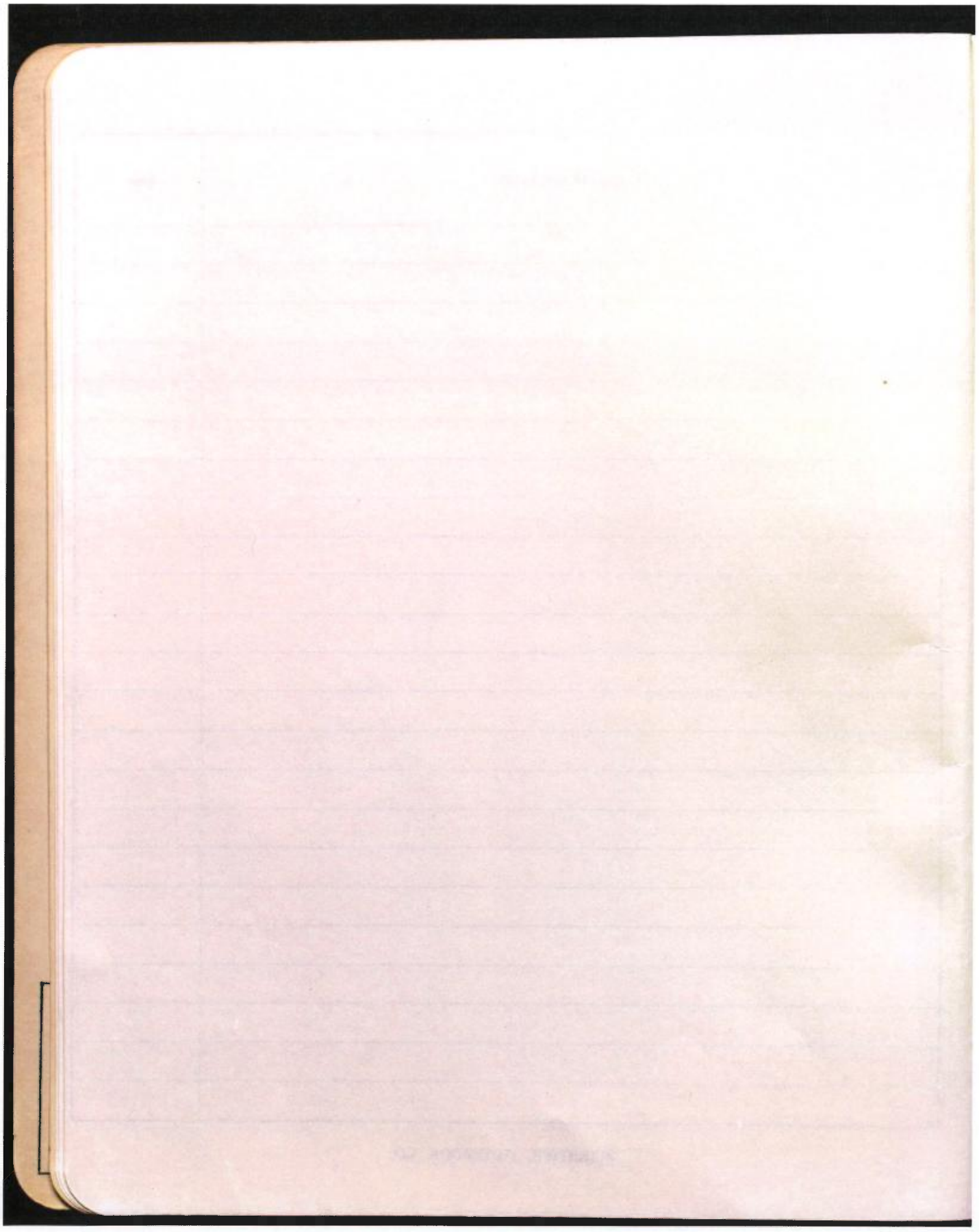

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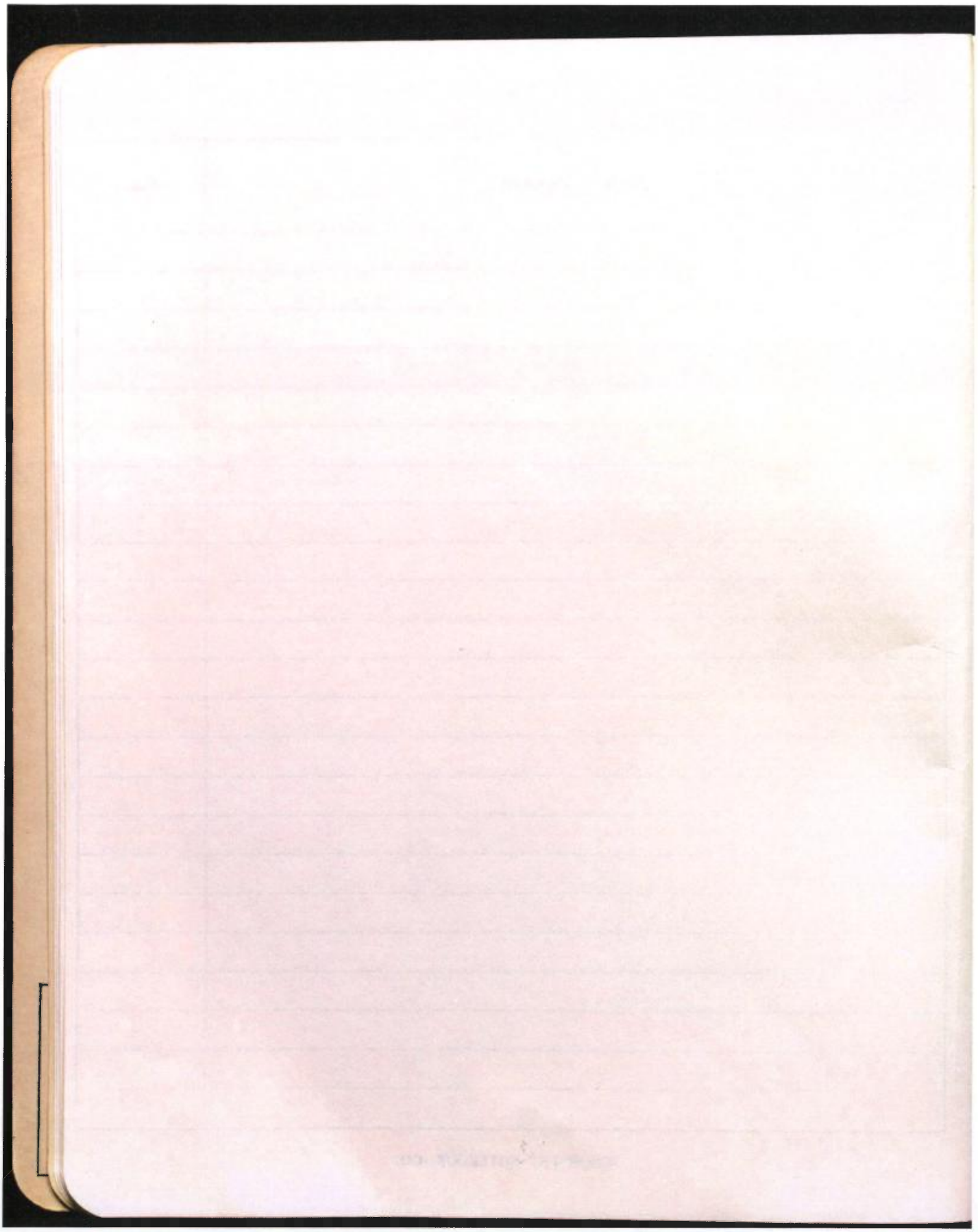

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Dmanifa	26.1.90	John Rdyn	7/12/90
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Dan Fraser	26.1.20	Recorded by	

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Dorfman	26.1.90	Recorded by	1/12/90

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[illegible]	26.1.90	[illegible]	1/12/90
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Dan Mann	26.1.88		1/11/90
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Dalmont	26.1.90	[redacted]	1/22/90
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Kaufmann	26.1.90		1/22/90
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Dan/mon AA	26.1.90	[signature]	1/22/90
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Dan Monnett	26.1.90	[signature]	1/24/90
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Daly Marnell	26.1.90	[redacted]	1/26/90
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Daufman	26.1.90	Recorded by	1/24/90

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[illegible]	13.4.20	[illegible]	3/9/20
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Dan Marriott	13.4.90	Recorded by	2/2/90

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Dafmann	13.4.90		2/4/90
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Dalmorich	13.4.90		3/5/90
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[illegible]	13.4.90	[illegible]	4/6/90
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Dalmarin	13.4.90		2/5/90
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Dan Smith	13.4.90		2/6/90
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Dayman	13.4.90		2/7/90
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Dafmanuff	13.4.90	Recorded by	2/8/90

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Dan Pearmain	13.4.90	Recorded by	3/9/90

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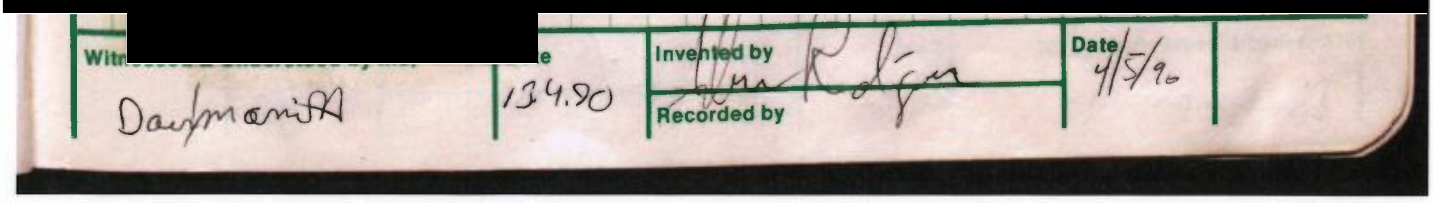

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Daufmann	13.4.90	[signature]	4/13/90
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Dorothy Marin	13.4.90	Recorded by	4/13/90

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Dashmanth	13.4.10	Recorded by	4/4/10

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	1.7.90	Recorded by	5/12/90

TITLE Variants of Humanized 4D5 Project No. Book No. 61

From Page No.

5/15/90 The first variant of humanized 4D5 called "wildtype" also called version "a" of VH, VL

Make Variants b and c of VH chain:

1) - Cut pA5 (wildtype VH (a) in pRK Vector) w/ PvuII and ApaI

this is the Vector.

- Cut clone 1/1 [VH(a)] w/ ApaI and PvuII → ~360bp fragment

Ligate frag into Vec

2) VH-c. Use Vector from #1 (pA5 PvuII-ApaI)

Insert: clone 2/3 PvuII-ApaI ~350bp frag

ligate Vec and fragment.

Variant VH-b should lose the PstI site in pA5.
" VH-C has no restriction site changes - need to seq.

3) Variants b and c of VL chain:

VL-b Cut pA3 (wildtype VL-a in pRK Vec) w/ EcoRII and AccI → Vector.
Cut 3/2 clone w/ RII and AccI → Insert VL-b frag ~550

4) VL-C Use same Vec pA3 as in #3.

Cut clone 4/3 RII-AccI Insert VL-c → frag ~550

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Project No._____
Book No._____ TITLE Variants of Humanized 4D5

From Page No. 61

5/17/90 1% Agar Sea Plaque Soft Agar in TAE buffer

Lanes 1) pA5 - PvuII + ApaI
2) clone 1/1 VH(b) - PvuII - ApaI
3) clone 2/3 VH(c) - PvuII - ApaI
4) pA3 - RII - AccI
5) clone 3/2 VL(b) - RII - AccI
6) clone 4/2 VL(c) - RII - AccI
7) PBR322 w/ BSTNI
8) ~~ØX174~~ HaeIII

Should be only 1 band at 365

Lane 1) Vector pA5 - has faint bands - poss. dly contamination of PvuII sites not deleted? (Recheck Vector)

Cut out bands next to dots for Constructions

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Dmainfa	1.7.90	[signature]	5/22/90
		Recorded by	

TITLE pA5 Vector check

Project No.

Book No.

63

From Page No.

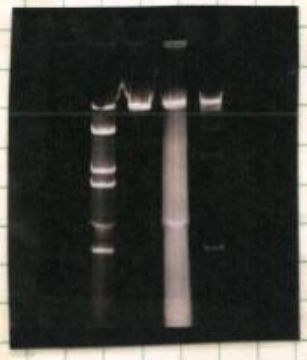
5/17/90

Lanes) 1) pA5 - PvuII + ApaI (left over from same digest run on lane 1 of Agarose gel #62.)

2) #316 clone 4/1 (pA5 mini screen) PvuII (forgot to run Rnase)

3) pA5 - PvuII

4) pBR BstNI

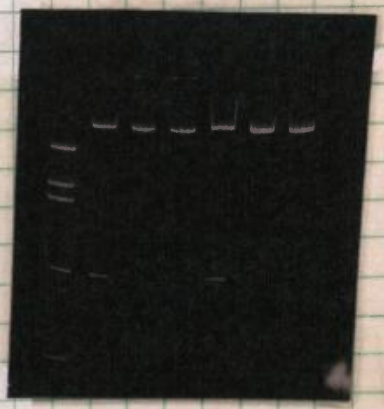


Results: 4) Marker lane has contamination from other lanes.

3) No apparent multi cuts from PvuII - Could it be the ApaI

5/18/90 Do more complete diagnostic

1) pA5 - PvuII
2) pA5 - ApaI
3) pA5 - PvuII + ApaI
4) #316 PvuII
5) #316 ApaI
6) #316 PvuII + ApaI
7) pBR 322 + BstNI



Results: pA5 Vect is O.K.
PvuII and ApaI are unique.

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Omar Ali	1.7.90	Recorded by	5/22/90

Project No._____
Book No._____ TITLE Ligation of HDS Hemoviral Variants

From Page No.___

5/18/90 Cut more pA5 w/ PvuII + AgeI for clean vector.

1) pA5 - PvuII + AgeI - Soft Agm remove Vector.
Results: Clean bands no problems.

Vector: Dilute 1:10 in H_2O. Remelt.
Insert 1:) clone 1/1 VH(b) Soft Agm
Insert 2:) clone 2/3 VH(c) Soft Agm

Lig Rx.
Vec 1 ml (1:10 dil)
Ins 3 ml (undiluted)
Lig Buffer 2 ml (Salts + ATP)
H_2O 14 ml

Ligase 1 ml Incubate 1 hr 37°C
1 hr 14°C

Remelt at 65°C
Add 50 ml Soft Agm dilution mix.
Cool.
Add 160 ml Competent 2% cells
25 mm on Ice.
5 mm 42°C Shock → plate.

Lig results: Lig >10X colonies over background Vec ctr.

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[signature]	1.7.90	[signature]	5/22/90
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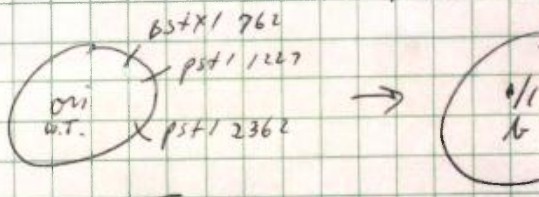
From Page No.

5/21/90 Variant of Heavy chain Variable region, clone 1/1 Called Version (b).

This Variant removes the pst1 site at 1227.

the original has: pst1 site at 1227 and 2362
BstX1 site at 762

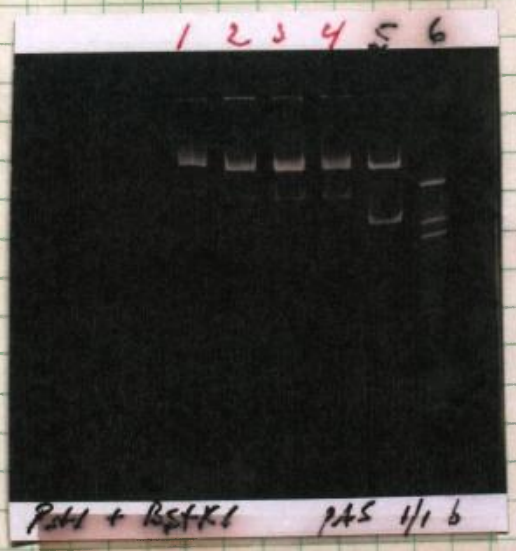


Vector clone w/o insert, then cut w/ pstI + BstX1 would be 3656p.

Frags
1135
465

frags
1680

1600 - 365 : 1235



Lane 1 pA5 VH(b) 1/1 - 1
2 " - 2
3 " - 3
4 " - 4
5 pA5 wild type (a)
6 pBR pstM I marker

Results: all cleaved.
Verification needed that frag in 1-4 is 1600 and 1235.

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	1.7.90	Recorded by	5/21/90

Project No._____
Book No._____ TITLE Verification of Human mid 405 PAS VH(b) 1/c

From Page No. 65

5/22/90 Main: Screen #4
Cut again w/ pst I + BstXI 1° pst I 37°C
2° BstXI 65°C

Also pvuII + ApaI - look for 365bp

Use marker ØX174 w/ HaeIII See p. 68

PAS VH(c) 2/3 done - has no changes in site
Cut w/ pvuII + ApaI to show insert went in.
then sequence to show correct bp. seq.
(Should be O.K. since background was low)

See gel p. 68, 70

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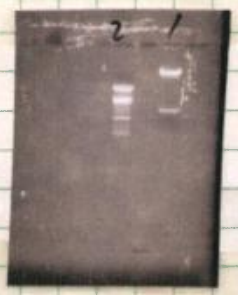
5/22/90 Vec cut from Sft Agar (RI - Acc I) pG2
Insert VL (b) clone 3/2
VL (c) clone 4/3 Soft Agar.

Lig Vec d.l 1/10 - Results: Vec control background too high
Vec CTR colonies Same # as lig.

Cut more Vec. 100 ml Rx Acc I - 6 hrs
RI + overnight.

B.I. Soft Acc I - A
RI - B



Cut out Vec Band.
Lig use Vec 1/100 d.l.

+ 3 ml undiluted Inserts B, C

Results: No Vec Background.
Insert Lig ~50 colonies.

VL(b) clone 3/2 Variant - loss of BglII site.
Cut w/ BglII and HindIII to check
Wild type pA3 will drop out ~ 448 bp frag

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Project No.
Book No.
TITLE Mini Screen Gel of 4D5 Humanized Variants

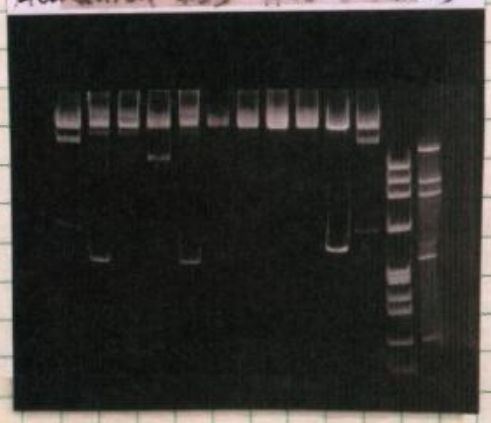
From Page No. 67

5/22/90 6% Acrylgel-

Lanes) 1) λ - HindIII markers
2) pA5 VH(c) clone 3/3 #1 - ApaI + PvuII
3) " #2 - "
DNA prep 4) pA5 VH(b) clone 1/1 #4 - pstI + BstXI (p65 #4)
5) " - PvuII + ApaI
6) pA3 VL(k) clone 3/2 #1 - BglII + HindIII Lig 5/10/90
7) " #4 - "
8) " #9 - "
9) " #11 - "
10) pA3 wildtype original Vκ - BglII + HindIII
11) λ - HindIII markers
12) ØX174 HaeIII
13) pBR BstNI

see p.67

Humanized 4D5 H+L Variants



Results:
Lane: 1) markers
2) VH(c) #1 has insert -.
Seq this one
3) VH(c) #2 - has not ligated
4) VH(k) #4 pstI-BstXI frg is over 1350 so it's 1600. (No pst Site)
5) VH(b) #4 has insert.
6, 7, 8, 9) VL(b) Suggests no BglII site
10) Control for 6-9 has BglII site.

∴ pA5 VH(b) #4 is correct

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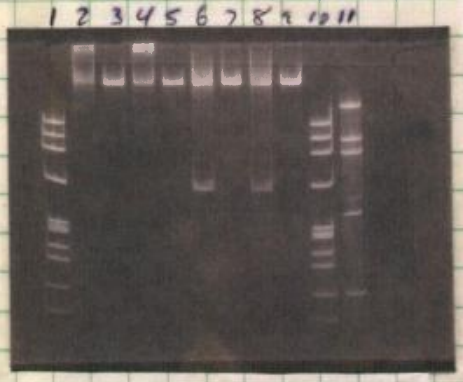
5/23/90 6% Acry Gel

Lanes)

1) ØX174 HaeIII
2) pA3(b) #9 RI+AccI - 570 bp whole Insert.
3) pA3(b) #9 BglII + HindIII - No bands.
4) pA3(b) #11 RI - AccI - 570bp
5) pA3 (b) #11 BglII + HIII - No bands.
6) pA3 (c) #7 RI - AccI - 570bp. * DNA prep.
7) pA3 (c) #7 Xho - H3 - No Bands.
8) pA3 (c) #10 Acc - RI - 570.
9) pA3 (c) #10 Xho - H3 - No bands.
10) ØX174 HaeIII
11) pBR BstNI



Results: pA3(b) #9, 10 have no inserts.

pA3(c) #7, 10 are correct!

To Page No.

Witnessed & Understood by me, [signature] Date 6.7.90

Invented by [signature] Date 5/23/90

Recorded by

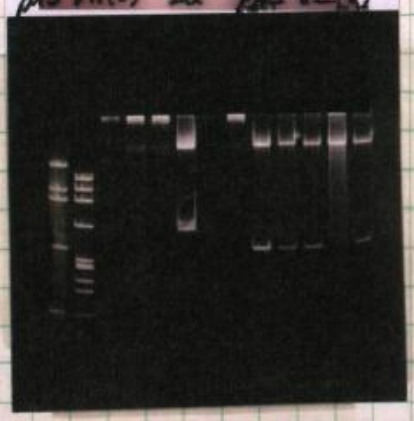
Project No._____
Book No._____ TITLE pA5 VH(c) and pA3 Vk(b)

From Page No.__

5/23/90 6% Ac#1

Lanes): 1) pA5 VH(c) #1 AgaI + PvuII (Repeat from p67)
2) " c #3 "
3) " c #4 " ☆ used this for Prep
4) " c #6 "
5) pA5 Vec Ctrl AgaI + PvuII
6) pA3 Vk(b) #6 AccI + [illegible] 570
7) " #7 "
8) " #8 "
9) " #10 "
10) " #4 "
11) " #1 "
12) Markers ØX174 HaeIII
13) Markers pBR 322 BglMI

pA5 VH(c) and pA3 VL(k)



Results: Lanes 1, 3, 4) pA5(c) Insert present 2 of these

8) pA3(b) Insert there cut to show absence of BglII site.

To Page No.__

Witnessed & Understood by me,	Date	Invented by	Date
Dmeinhardt	1.7.90	Recorded by	5/23/89

From Page No.

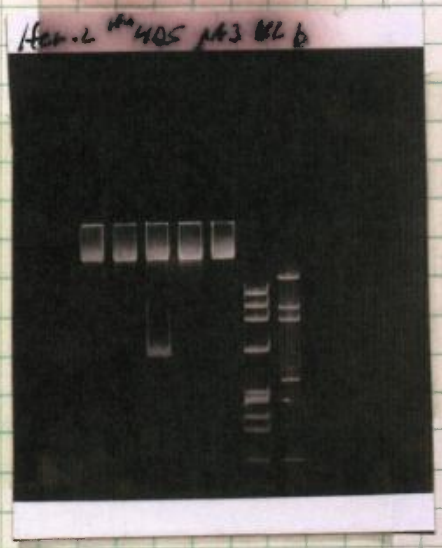
5/23/90 6% Acryl.

(Lanes) 1) pA3 VL(b) #13 From Ligation 5/22/90 p67 1/100 vec
ACCI + RII - 570 bp. frag
2) " BglII - H3 - N3 frag.

☆ 3) pA3 VL(b) #14 ACCI + RII 570 bp - ☆ - DNA prep
4) " BglII - H3

5) pA3 VL(b) #8 See p 70. BglII + H3
It has the AccI - RII frag.

6) Oxoy HaeIII
7) PM BsiNI



Results: ☆ pA3 VL(b) #14 is good
pA3 VL(b) #8 is also good.

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
	1.7.90	Recorded by	5/23/90

Project No. ______ ^{35}S labeling of 293 transfected Humanized 4D5 Antibody

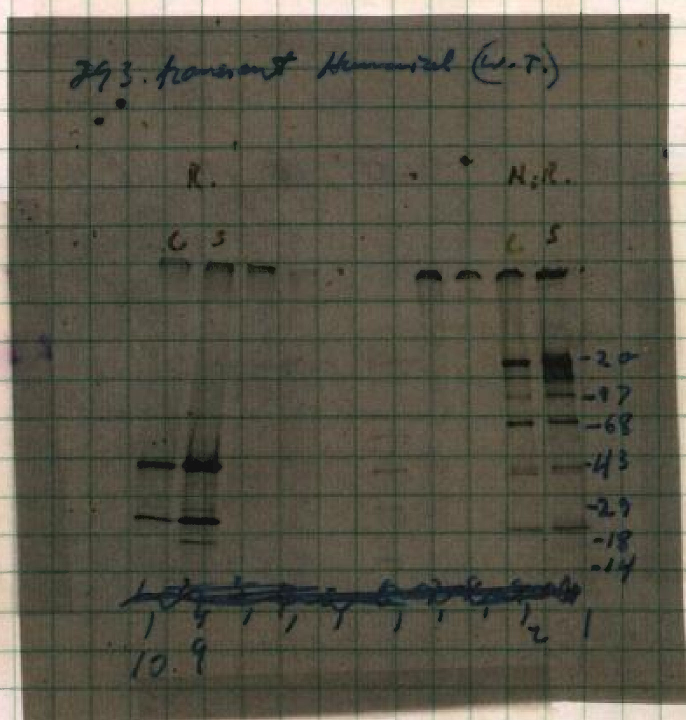
Book No. ______ TITLE ^{35}S 293 4D5 humanized pA3, pA5

From Page No. NB 1116 2 p 51 → I.P. as usual.

5/30/90

Lanes 4-20% precast mini gradient

1) 4D5 Sups - N.R.
2) Cells - N.R.
3) 293 Sups - N.R
4) Cells - N.R
5) ^{14}C MWM Highs
6) Prestained MWM Highs
7) 293 Sups - Reduced.
8) Cells - "
9) 4D5 Hu pA3,5 - Sups - Red.
10) " - Cells - Red.



To Page No. ______

Witnessed & Understood by me,	Date	Invented by / Recorded by	Date
[signature]	6-7-90	[signature]	6/4/90

From Page No.

6/4/90

Hu 4D5 DNA's

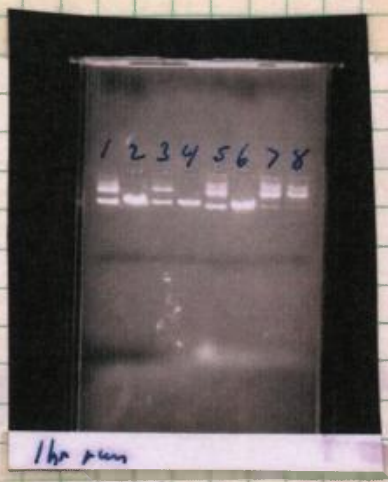
		S#	260.0	280.0	260/280
L	3-b-14	1	0.7967	0.4231	1.882
L	3-c-7	2	0.9493	0.5047	1.880
H	5-b-4	3	0.3877	0.2054	1.887
H	5-c-4	4	0.6207	0.3294	1.884

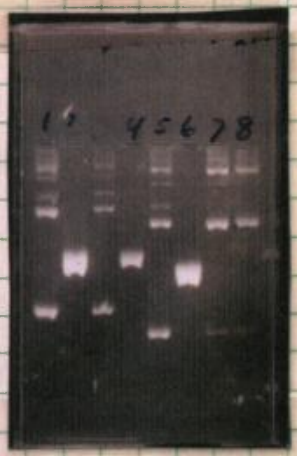
Note - 5-c-4 sequenced and confirmed correct for seq. change at residue 109

Gel — Cut + Uncut 1% Agar

Left to Right Cut w/ EcoRI ~ 1 ug/lane DNA

1) 5-c-4 uncut
2) " Cut
3) 5-b-4 uncut
4) " Cut
5) 3-c-7 uncut
6) " - Cut
7) 3-b-14 - uncut
8) " - Cut -





1kb ruler

To Page No. 75

Witnessed & Understood by me,	Date	Invented by	Date
	6.7.90	Recorded by	6/15/90

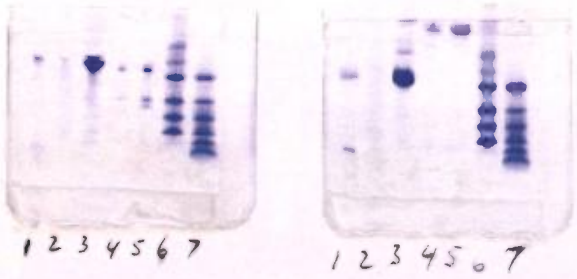
Project No._____
Book No._____ TITLE Protein phast gel of Ab

From Page No.___

5/30/90

4-20% Gradient Gel

Reduced Non-Reduced



Lanes 1) 293 4D5 chimera (3/9/90) - Inactive on BioAssay
50 ml of Sup + 10 ml protein buffer

2) 293 Humanized 4D5 tube #2 (5/14/90) - Inactive on BioAssay.
50 ml Sup + 10 ml buffer.

3) 2.16 Stable chimera 5/6/90 - BioActive. 50 ml Sup + 10 buffer

4) 293 4D5 chimera - purified on prot A column. 20 ml + 5 ml buffer

5) 4D5 hybridoma (monoclonal) purified

6) ~~Bio~~ BRL prestained markers. (Highs)

7) BRL " " (Lows)

To Page No.___

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	6.7.90	[signature]	6/15/90
		Recorded by	

TITLE Hu 4D5 L-Chain Variant 3-6-14 Project No. Book No. 75

From Page No. 73

6/5/90 the 1% Agar gel of 3-6-14 mini Cut and Uncut, didn't look good.

NoRI Cut and 1.#6 was supercoiled.

Do Diagnostics Cuts

6% Acry gel. 1ml in 50ml Rx.

Lanes

1) AccI + RI - -570 - Identity check

2) R1 + H3 - -710 to check R1 site

3) H3 + BglII - No band - BglII check.

4) XmnI - 1848, 1749, 397, 1400

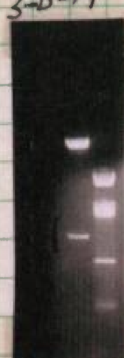
5) PBR w/ BstNI

6) ØX174 HaeIII

Results: #1 lane the 570bp is Very faint.

The other cuts are o.k.

3-6-14



Cut again w/ AccI + RI, using RE cut buffer 1 for AccI, add 50mM NaCl before RI.

worked much better

1.) 3-6-14 DNA AccI + RI

2) PBR w/ BstNI

To Page No.

Witnessed & Understood by me, [signature] Date 6.7.90

Invented by [signature] Recorded by

Date 6/15/90

Project No._____
Book No._____ TITLE Hu L-chain Variant 3-b-14

From Page No.__

6/13/90 Running the 3-b-14 DNA 1% Agar w/ relaxed DNA marker to see if the prep is ok

Previous gel w/ 3-b-14 run

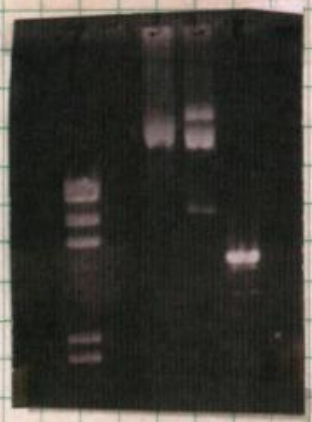
Lane 1) R1 cut once - linear
2) Uncut

Question - is this band enriched in singly dimers of the plasmid.

294 cells are RecA+ so the plasmid can be recombined to form dimers.

New Gel. 3-b-14

Lanes 1) R1
2) Uncut
3) Topo isomerase - Relaxes DNA
4) λ HindIII markers



Run at 100V 2hrs No EtBr

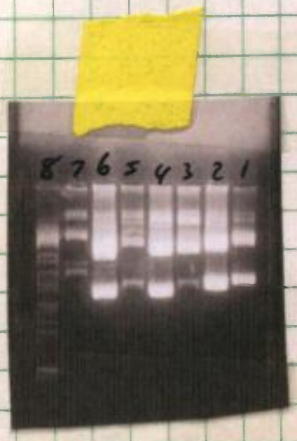
To Page No.__

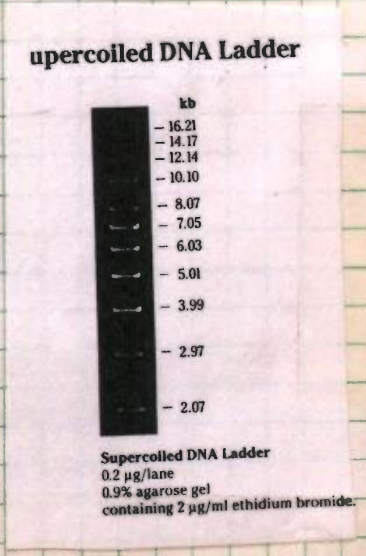
Witnessed & Understood by me,	Date	Invented by	Date
Amanda	6.7.90	Recorded by	6/15/90

From Page No.

6/14/90 1% Agar 1mg/ml EtBr TBE buffer

Lanes 1) Hu 4D5-H 5-b-4
2) Hu 4D5-H 5-c-4
3) Hu 4D5-L 3-b-14
4) Hu 4D5-L 3-c-7
5) Chimera 4D5-L dH158 (dennis's prep 4ug/ml 2.5.90)
6) dH158 " (JR prep 0.4ug/ml)
7) Chimera 4D5-H dH160 (dennis prep 1.3ug/ml
8) Supercoiled ladder marker.





Lane #3: Hu Lc 3-b-14 it Point.
#5: dH158 (dennis prep is Point)
#7 dH160 (")

To Page No.

Witnessed & Understood by me, Date 6.7.90 Invented by Date 6/15/90 Recorded by

Project No._____
Book No._____ TITLE Cm Cut DNA preps

From Page No.__

6/18/90 1% Agar Gel w/ 1 drop/ml EtBr in Gel

Lanes: 1) AdVA prep 0.88 ug/ml
2) AdVA prep 0.25 ug/ml
3) Hu γ1S-L chain pA3 (Pearl Carter Preps) Hu La
4) Hu γ1S-H chain pA5 (") Ha
5) Hu γ1S L chain Version b (3-b-14)
6/18/90 prep (Sample before banding)

6/19/90 1% Agar Compare old 3-b-14 prep to new one after banding

1) old prep
2) new prep
3) markers

Results: Lack of good % supercoiled is not due to prep problems.
Made up 3-b-8 (different clone of same construct)

To Page No.__

Witnessed & Understood by me,	Date	Invented by	Date
Amanda	6-7-90		7/6/90
		Recorded by	

TITLE Plasmid Preps. AdVA, 405 Hu 3-b-8 Project No._____ Book No._____ 79

From Page No.___

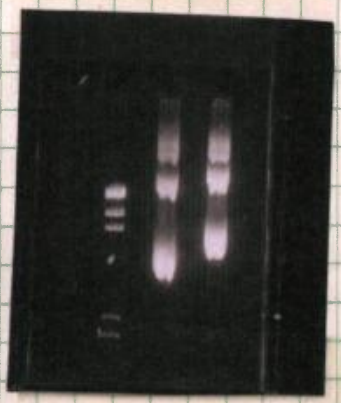
6/27/90 Prepared 1L preps of AdVA and 405 Hu 3-b-8

Banded in CsCl 1 time.

Run 7ml of each direct from Quick Seal Centrifuge tube. w/ CsCl and EtBr still in it.

1% Agar gel Uncut plasmid.

Lanes) 1) 3-b-8 - Light Chain
2) AdVA
3) λ H_{III} marker



Appears that there is good supercoiled DNA.

Delute some of each 1/100 take 260 OD for Conc

S# 260.0 S# 2

AdVA 1 0.4376 x5 = 2.2 mg/ml
3-b-8 2 0.3911 x5 = 2 ug/ml

To Page No.___

Witnessed & Understood by me,	Date	Invented by	Date
	6.2.90	Recorded by	7/6/90

Project No.____
Book No.____
TITLE Plasmid Preps pA9, pA10, pA13

From Page No.__

7/24/90.

4D5 Chimera

Plasmid	Expt #	Clone	Comment
→ pA9	340	2	4D5 light chain chimera differs from the original version (dh158) only in that the non-silent freebie mutations have been removed: V104L:T109A
→ pA10	339	1/4	4D5 heavy chain chimera differs from the original version (dh160) only in that the non-silent freebie mutation has been removed: Q1E

Hu4D5 Heavy Chain

Plasmid	Expt #	Clone	Hu4D5 Version	
→ pA13	342	1/6	e	
pA14			d	coming soon (hopefully)

1/100 pA9	1 0.6844	x 5	= 3.4 mg/ml
" pA10	2 0.6393	x 5	= 3.2 mg/ml
" pA13	3 0.5944	x 5	= 3.0 mg/ml

DNA prep uncut 5n Supercoiled

pA9 clone 2 → DNA not good for Xfn
pA10 clone 1/4

No good supercoiled!

pA13 - Heavy chain - e is Fine.

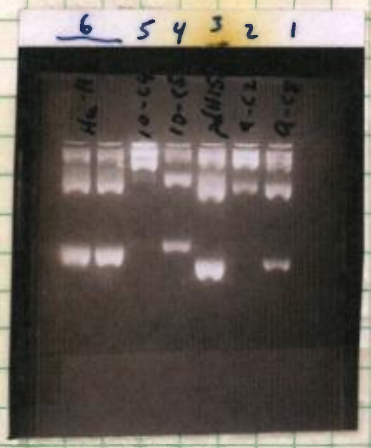
To Page No.____

Witnessed & Understood by me, [signature]
Date 10.8-90
Invented by [signature]
Recorded by
Date 8/10/90

From Page No.

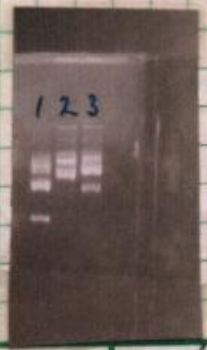
7/25/90 New preps of pA9 and pA10 from different clones were provided by Paul Carter. Also pA14 Now here.

	Expt #	Clone	[DNA] µ/λ	Vol mL
pA9	340	8	0.21	1.0
pA10	339	1/5	0.18	1.0
pA14	347	3/2	0.16	1.0



1% Agar uncut DNA

1) pA9 clone 8 - Good Supercoiled DNA
2) pA9 clone 2 - Bad Supercoiled
3) CHIMERIC L chain - Marker DNA for pA9
4) pA10 clone 1/5 - Good DNA
5) pA10 clone 1/4 - Bad DNA
6) Human Heavy Chain - Marker for pA10



1% Agar uncut.
1) pA14 - Good Supercoiled (clone 3/2)
2) pA10 clone 1/5 - Good DNA
3) pA9 clone 8 - Good DNA.

To Page No.

Witnessed & Understood by me, Date 10.8.90 Invented by Date 8/10/90 Recorded by

Project No._____
Book No._____ TITLE Construction of 4D5 Hu Var 5 and 6 dhFr

From Page No.__

For stable lines in CHO and Myelomas

8/20/90 4D5 Humanized Var 5 and 6 are chosen to make stable lines. The existing plasmids are pRK based and without selectable markers.

For best xfer efficiency and amplification possible. Construct Heavy + Light chain w/ dhFr on one vector.

Using: dhFr vector pSV16B5 del. d (one dhFr transcription unit).

	Light chain	Heavy chain
Var 5	c (3-C-7)	a (PA5)
Var 6	c (3-C-7)	c (5-C-4)

Strategy for Construction:

Light chain (3-C-7) used in both Var 5 and 6, is in pRK vector with a restriction map based on dh158 (Chimera Light)

Heavy chains PA5 and 5-C-4 are in pRK vectors based on dh160 map. (Heavy chimera).

1) Make pSV1 based 3-C-7 light chain:
Cut pRK 3-C-7 w/ R_1 + H_3 to Remove L-chain only.
Insert it into $R_1 - H_3$ of pSV16BΔd Named pSV1 Light chain 3C7

2) Make pSV1 based PA5, 5-C-4 heavy chains,
Cut out H chains from pRK versions → insert to R_1-H_3 of pSV16BΔd.
Named pSV1PA5 and pSV15-C-4.

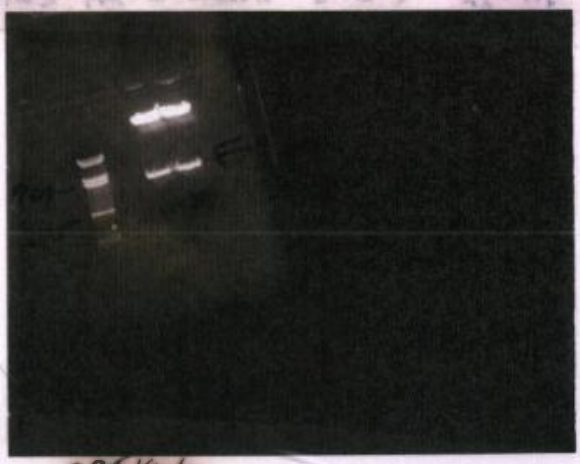
3) Remove pSV1 L-chain 3-C7 transcription unit w/ PvuII and insert into blunted XbaI site of pSV1PA5 and pSV15-C-4. Named pSV1Var5 and pSV1Var6 respectively.

To Page No.__

Witnessed & Understood by me,	Date	Invented by	Date
Dan Marini	12.10.90	Reporded by	8/20/90

From Page No.

8/21/90 Cut Light chain pRK 3C7 w/ R_1-H_3 in Soft Agar 1%.
Should be 710 bp
Marker is pRK 322 - BSFMI
Cut out at 4hrs



pRK Vector

Ligation: R_1-H_3 frag of pRK 3C7 w/
R_1-H_3 Vec of pSV1 6B ΔD.

8/23/90 Ligation Results. 0 Colonies on Vec plate
many colonies on Lig plate.

8/24/90 Digest mini Screens
w/ KpNI. one site in
L-chain - one in Vec.
564 bp - all Correct!

SV1 6B ΔD Light chain (3-C-7)

To Page No.

Witnessed & Understood by me, Date 12.10.90 Invented by Date 8/24/90
Recorded by

Project No._____
Book No._____ TITLE Const of pSV1 Var 5 and 6 w/ dhfr

From Page No. 83

8/27/90 Cut pRK PA5 and 5-C-4 Heavy chains w/ H_3+R_1 ~1450bp. each.

Marker of X174 HaeIII, λ HindIII

Run in 1% Agar, Cut out at dot

Do ligation w/ H-Chain H_3-R_1 w/ pSV16B Δ R_1-H_3

1) PA5 H_3+R_1

H chain only

SV1 5c4, SV1 PA5 XbaI cut

all Correct 8/30/90

8/30/90 MiniScreens of SV1 5C4 and SV1 PA5 Cut w/ XbaI ~1685bp

Lanes 1-3: SV1 5C4
4-6 SV1 PA5
7 ØX HaeIII

all Correct!

To Page No.____

Witnessed & Understood by me,	Date	Invented by	Date
Dan Mani	12.10.90	Recorded by	8/27/90

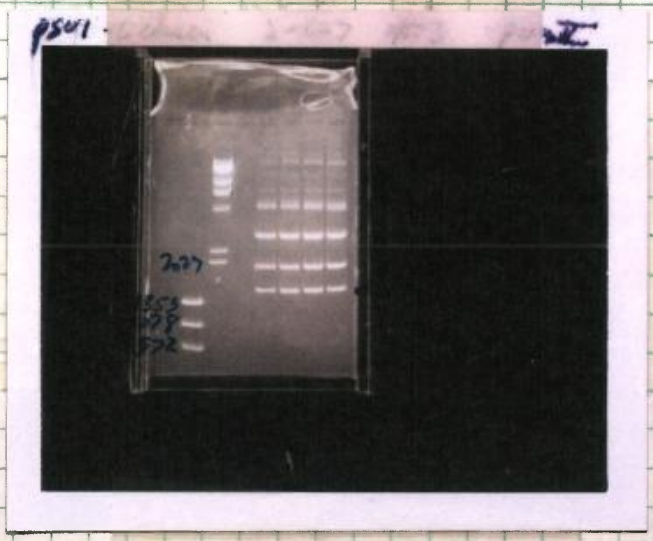
TITLE pSV1 Var 5 and pSV1 Var 6 w/dhfr

Project No.

Book No.

From Page No.

9/3/90 Cut pSV1 (3-C-7) light chain transcription unit out w/ PvuII



~1588bp frag

Use this insert in both pSV1 Var 5 and 6

9/5/90 Cut Vec SV1 PA5 (Heavy chain for SV1 Var 5) with KpnI.
Blunt by chew back w/ T4 DNA pol I → phosphatase
Gel Purify on in Soft Agar

Set up ligation: Vector - SV1 PA5 KpnI-blunted (above) H chain
Insert - pSV1 3C7 - PvuII Cut (blunt) L chain
Overnight at 14°C

9/6/90 Transform into 294 bacteria.

9/7/90 Results of ligation: Vec control — NO colonies.
Lig — 4 colonies.

SV1 Var 5 H+L

To Page No.

Witnessed & Understood by me, Dan Mansfield

Date 26.10.90

Invented by

Recorded by

Date 9/7/90

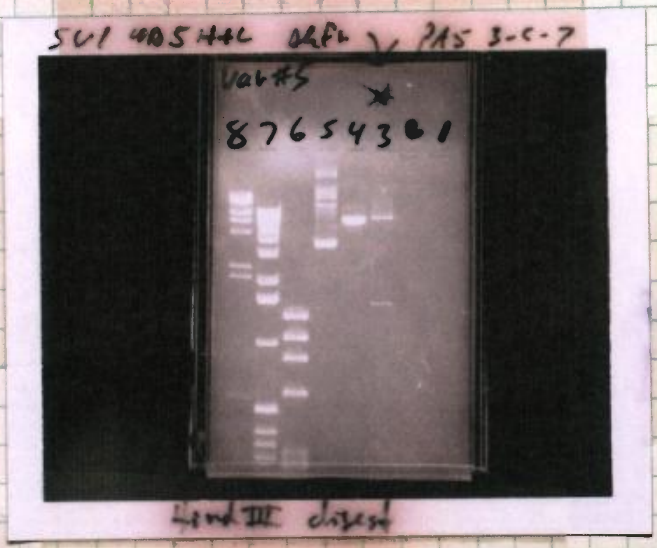
Project No.
Book No.
TITLE Construction pSV1 Var 5 + 6

From Page No. 85

9/10/90 pSV1 Var 5 4/min: Smears.

2 possible orientations. Digest w/ Hind III

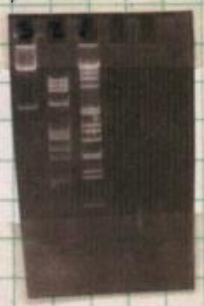
If light chain variant goes in Vec w/ same orientation a 1519 bp frag will come w/ Hind III.



#3 is Correct.

pSV1 Var 5
Test further digests
#4 - R_1 - ~ 2257 - yes
#5 - KpN - 562 - (NO!)
#6 - H_3 - 1519 - yes
#7 - ØX174 w/ HaeIII
#8 - 1Kb Ladder

pSV1 Var 5 KpN



Lane 2 - ØX174 HaeIII
3 - SV1 Va-5 - KpN
(yes!!)

re Cut.

To Page No.

Witnessed & Understood by me, Oatmanish
Date 26.10.90
Invented by
Recorded by
Date 9/10/90

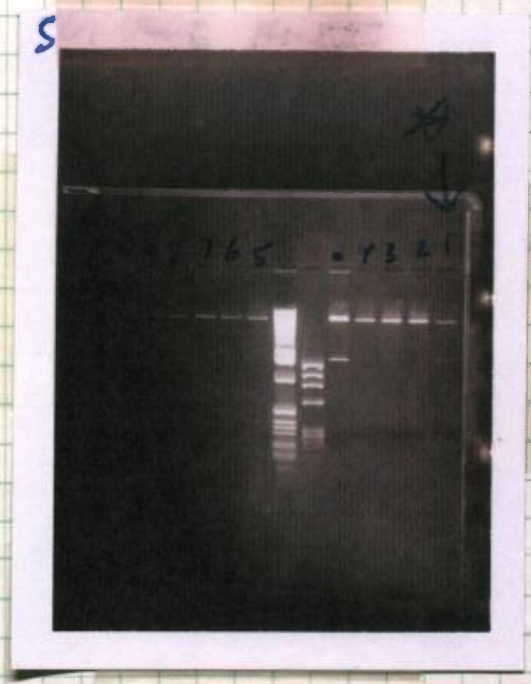
From Page No.___

9/11/90 Prepare vector heavy chain for Var 6 (SV1-5C4)
Cut w/ KpnI – blunt w/ T4 DNA pol I. → phosphatase
Gel purify.

Ligate Vec – KpN blunted (SV1 5C4)
Ins – 1581 bp PvuII frag of SV1 (3-C-7) (p. 85)

9/12/90 Transform 294 bact.
9/13/90 Vec-background 1/5 of ligation plate.

9/14/90 12 mini screens. Digest w/ HindIII ~1519 bp



#1 is cored.
• is SV1 Var 5 control number.

We have pSV1 Var 6 H+L - probably.

Better picture!?
Same gel.

To Page No.___

Witnessed & Understood by me,	Date	Invented by	Date
Dan Smith	26.10.90	Recorded by	9/14/90

Project No._____
Book No._____ TITLE Construction of pSV1 Var 6 4D5 Hu

From Page No.__

9/17/90 Check pSV1 4D5 Var 6 HHC w/ KpN I and.
Should be ~ 560

Hu 4D5 SV1 Var 6 KpN1

Corrected!

Test pSV1 Var 6 w/ R_1 ~2257

Lane 1) R_1 - Correct!

New plasmid Formal name:
pSV16B5 del.d. 4d5V6
1/8/92

To Page No.__

Witnessed & Understood by me,	Date	Invented by	Date
Daily manisha	26.10.90	Recorded by	9/17/90

TITLE Const. pRK Hu 4D5 Var 6 Project No. Book No. 89

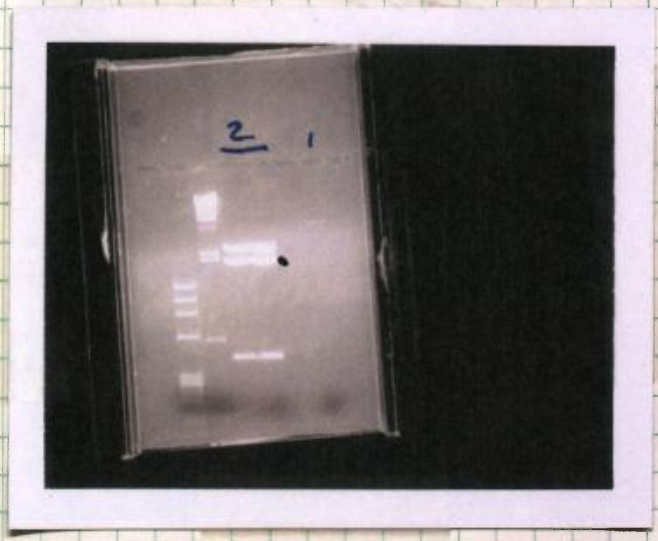
From Page No.

9/18/90 Combine the pRK vectors Hu 4D5 Hu Light chain Var C and pRK 4D5 Hu ~~Heavy~~ chain Var C on one plasmid. This is Hu 4D5 Var 6 in pRK Vectors.

Both H + L chains are in pRK Vectors now. (Supplied by Paul Carter).

Remove the L chain entire transcription unit w/ PvuII ~ 7056 bp.

Insert this into a blunted KpNI Vec of the H chain (pRK 5C4)



Lane 1) pRK 5C4 KpNI blunted w/ T4 DNA pol I phosphatased.

2) pRK 3-C-7 - Light PvuII 7056 bp.

Soft Agar Gel 1%

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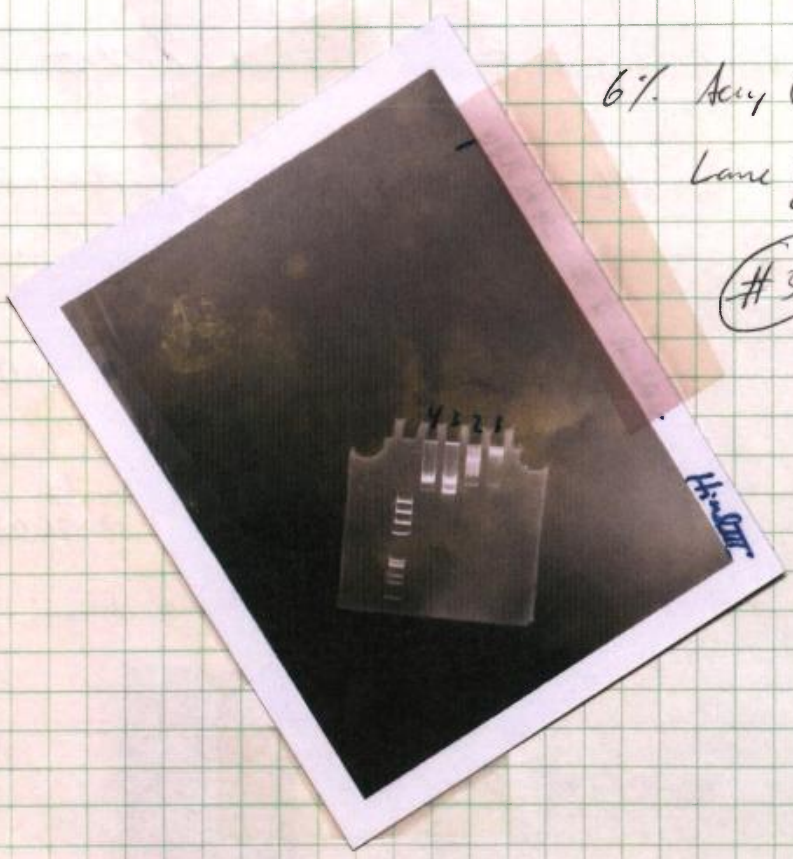
9/19/90 Ligation.
Vec - pRK 5C4 Xba N Blunted
Ins - pVHII 2056bp Frag of pRK 3-C-7.

9/24/90 Mini Screens Cut w/ HindIII ~ 2030



6% Acry Gel.

Lane 3? is Correct probably.
4?

#3

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9/21/90 Test mini Scrn #3 further!

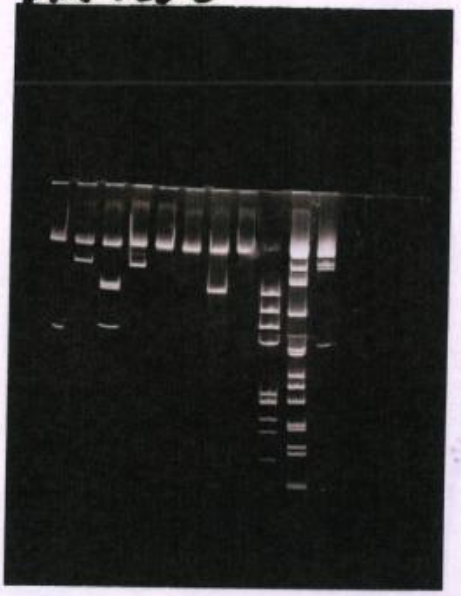
Lane 1) #3 KpnI ~562 ✓ O.K
2) #3 HindIII ~2030 ✓ O.K.
3) #3 AccI ~1962 ✓ O.K.
3) #3 R_1-H_3 ~1488 ✓
1340 ✓ Ok
710 ✓

5
6
7
8
) #6 No Good

9 Ø x174 HaeIII
10 1Kb ladder
11 λ HindIII

~~The~~ #3 is Good

pRK Var 6



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Invented by

Recorded by

Date 9/21/90

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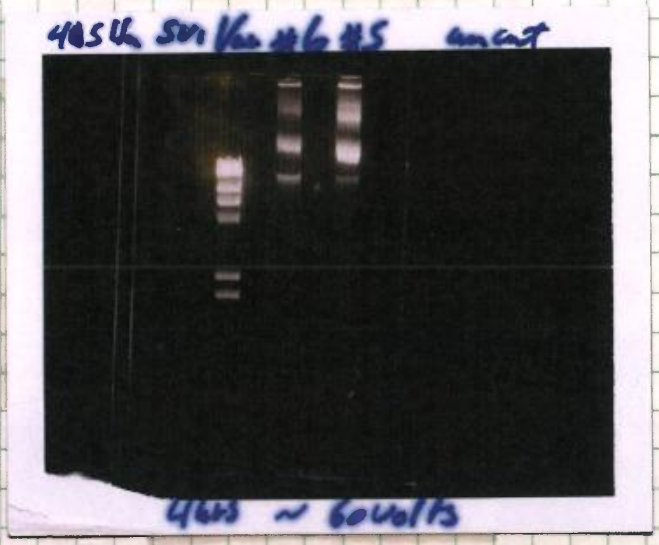
9/24/90 Qiagen prep SV1 Var 5 and Var 6

Not too good



9/25/90 the above DNA banded CsCl

Much better

uncut DNA

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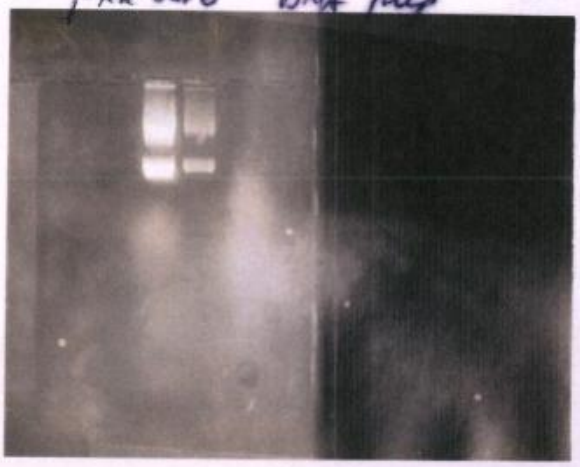
9/25/90 pRK Var 6 4/5 Hin H+L Qiagen prep

pRK Var 6 DNA prep



Hin cut DNA

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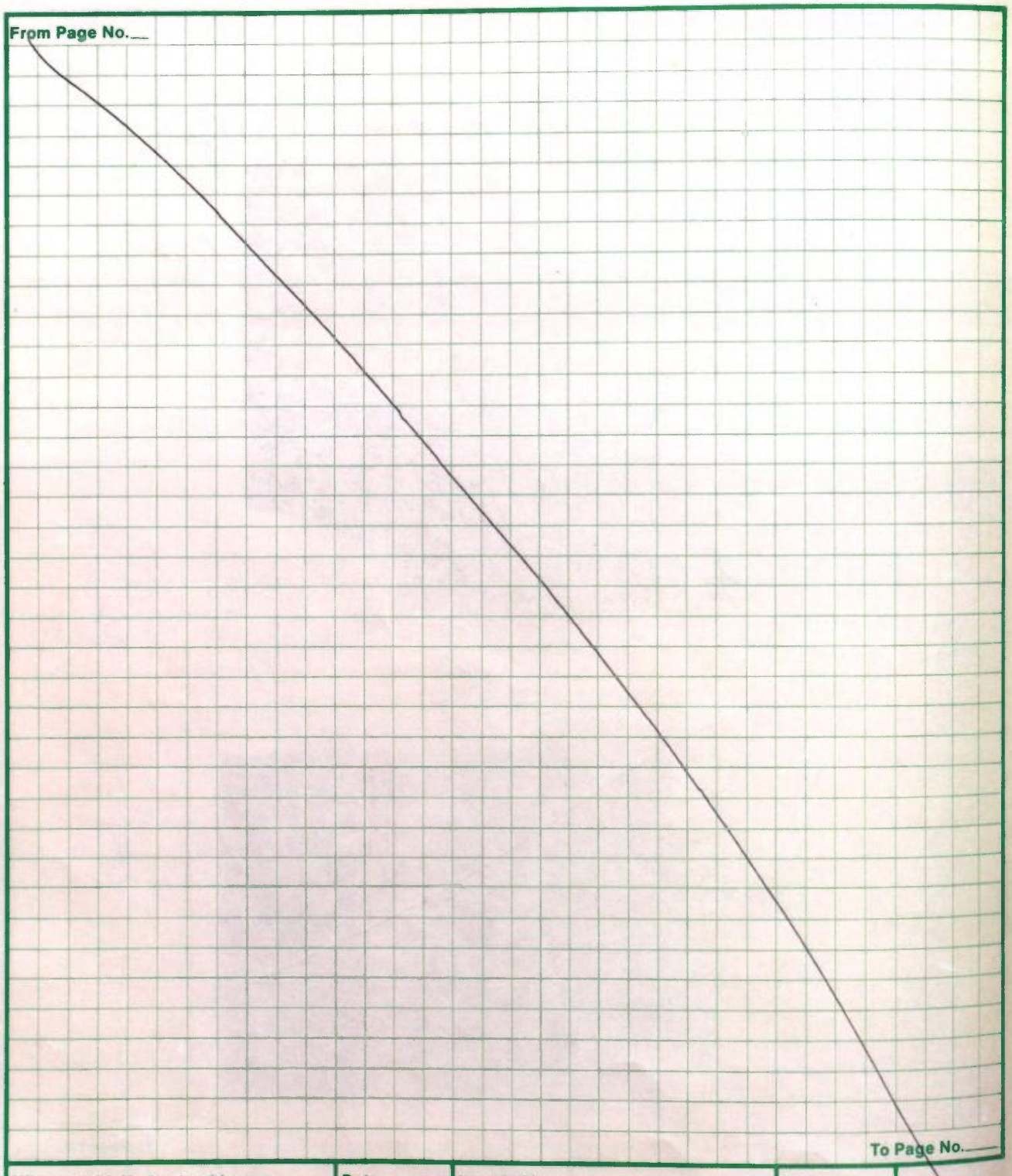

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		Recorded by		

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TITLE _____ Book No._____ 95

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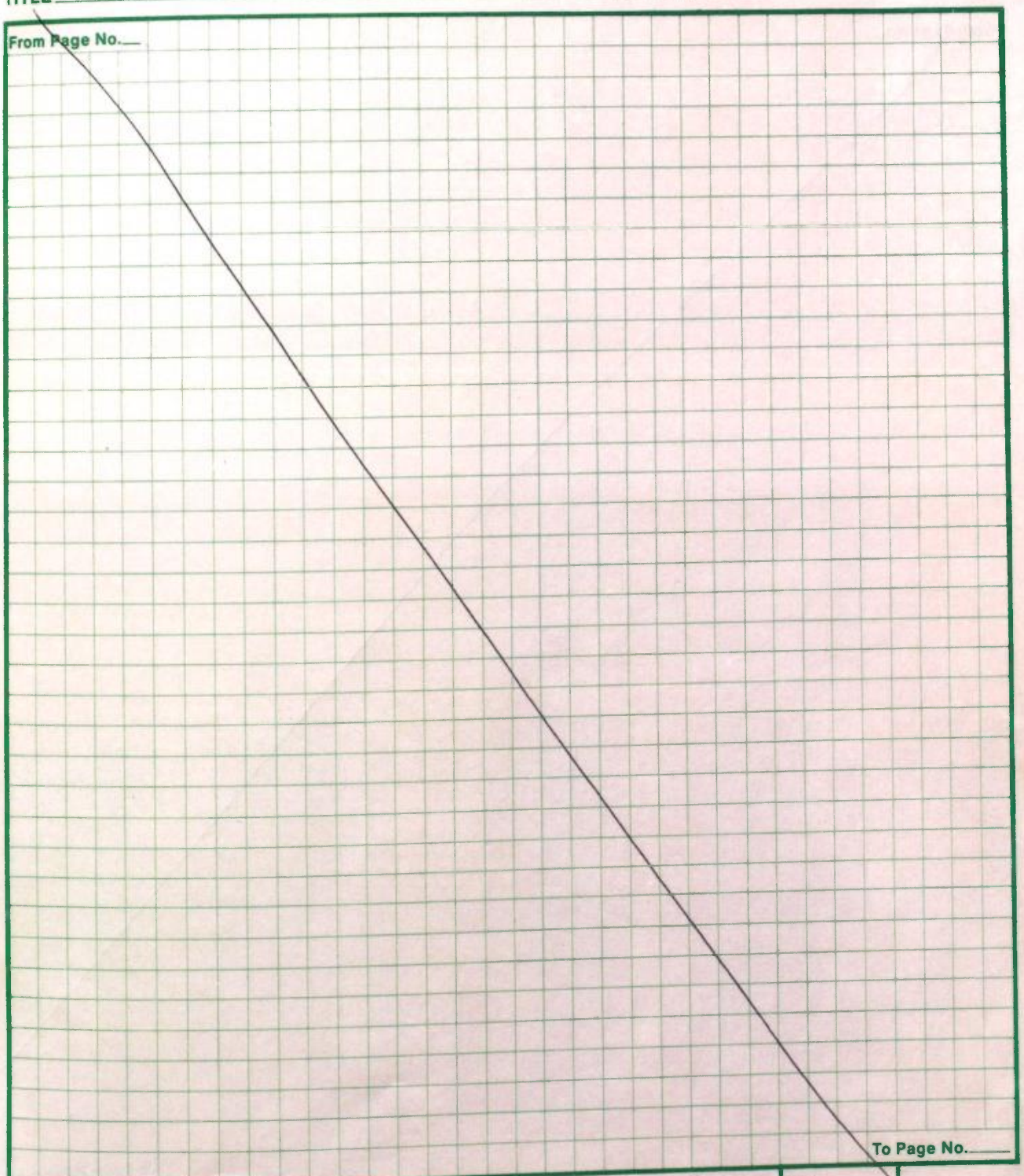

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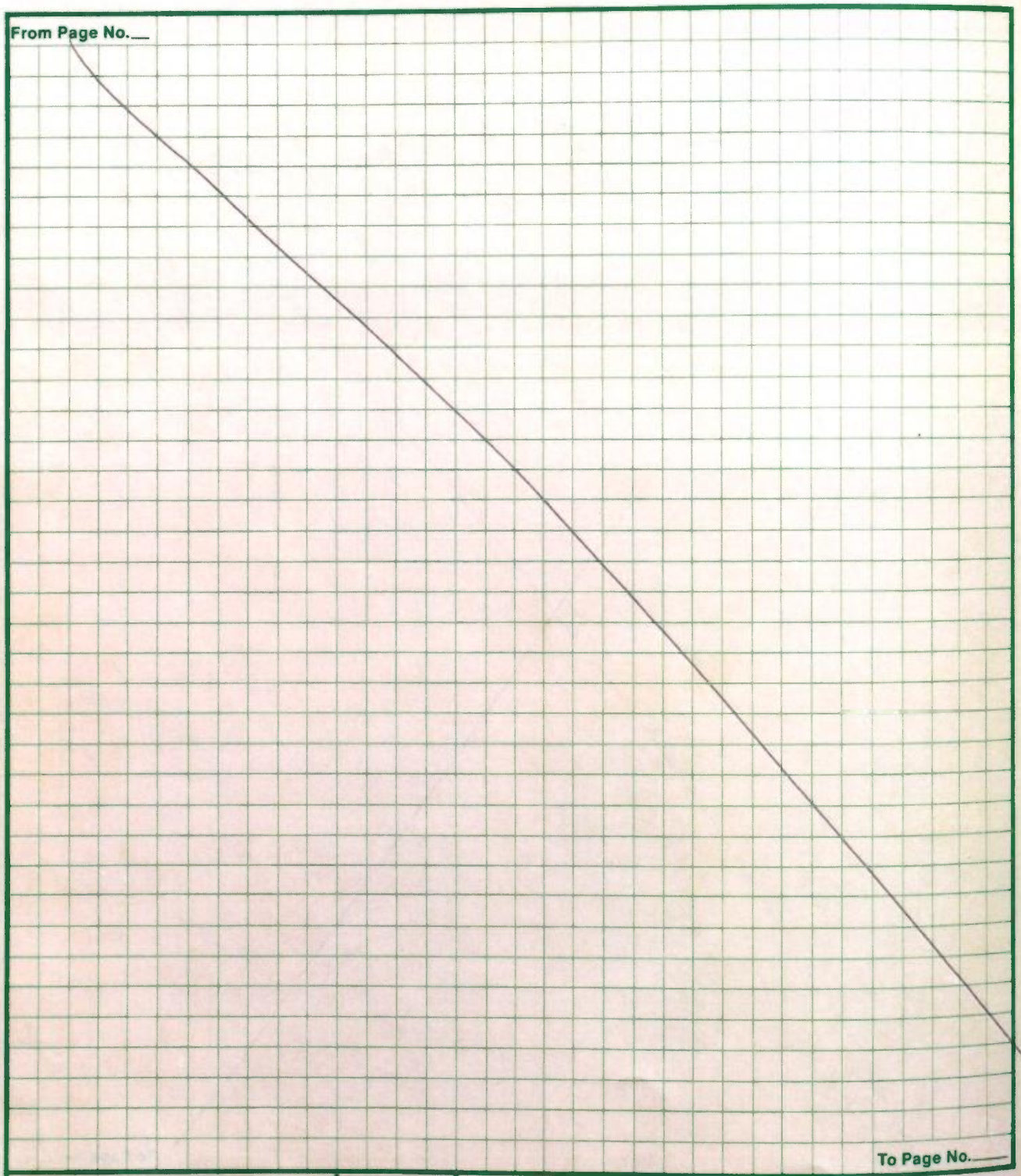

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		Recorded by	