

# LABORATORY NOTEBOOK

DNA

GENENTECH, INC.

Celltrion v. Genentech  
IPR2017-01374  
Genentech Exhibit 2005

10840



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**NOTEBOOK NO.** 10840  
**ISSUED TO** John Ridgway  
**ON** December 7 **19** 89  
**DEPARTMENT** Cell Genetics  
**RETURNED** 19

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

—SCIENTIFIC NOTEBOOK CO.—  
2831 LAWRENCE AVE.  
P.O. BOX 238  
STEVENSVILLE, MI 49127  
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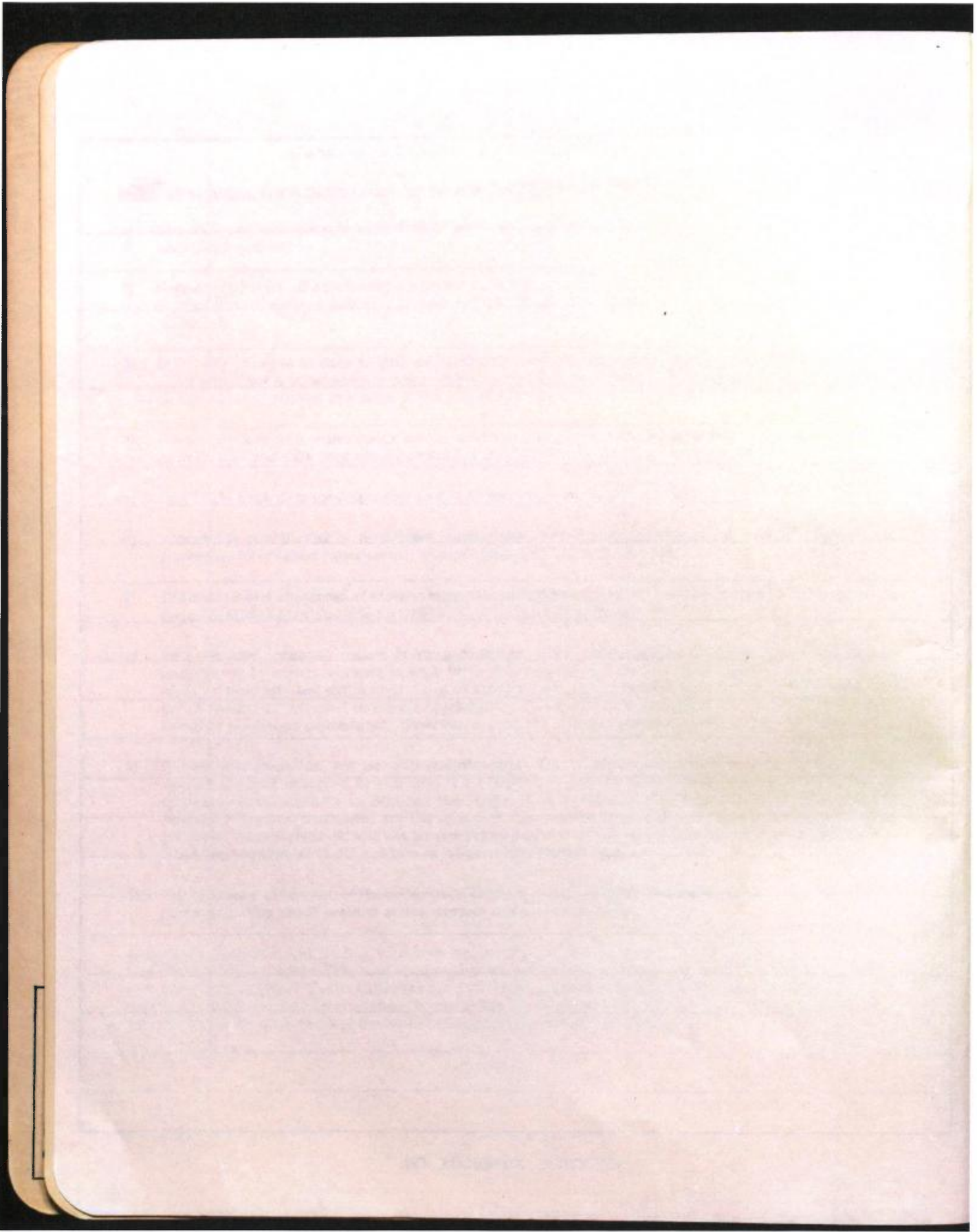
## Genentech Laboratory Notebook Procedures

### Important steps for maintenance of your notebooks:

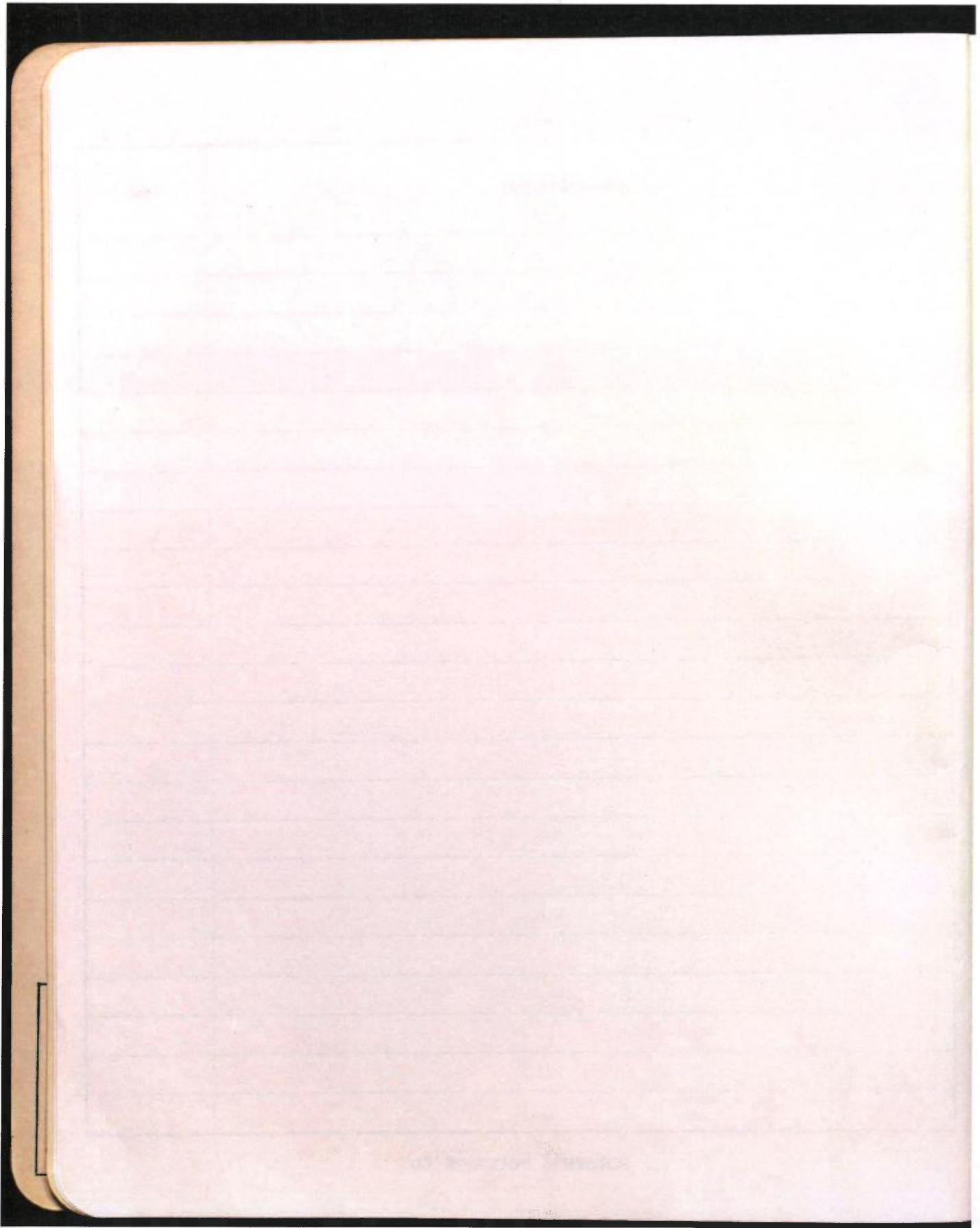
- 1) Use dark permanent ink to record all of your work in your notebook on a daily basis. Sign and date each day's entries.
- 2) Permanently affix all attachments without covering any other entries. Please attach copies of any computer data entered into your computer files. These data are considered 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.



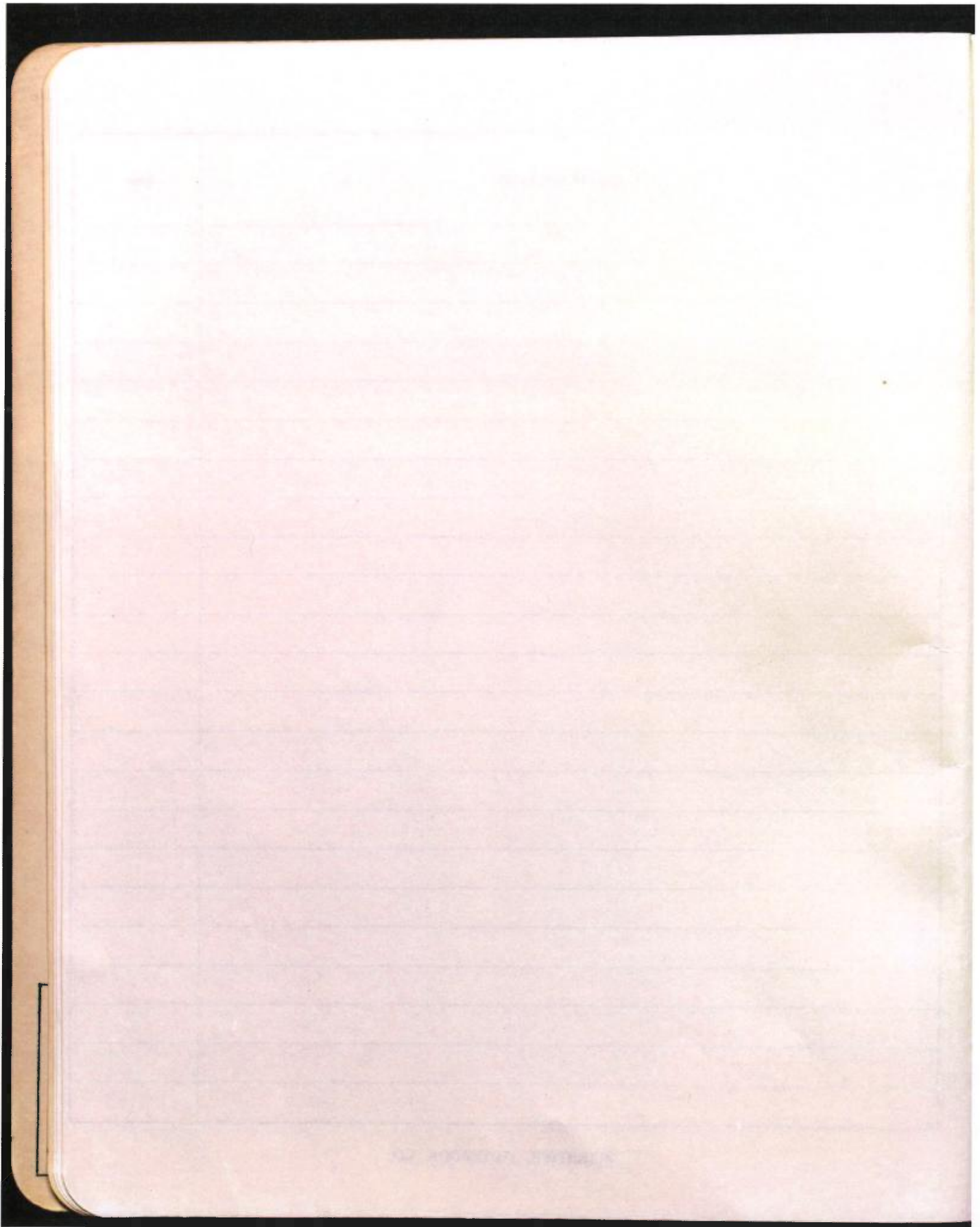




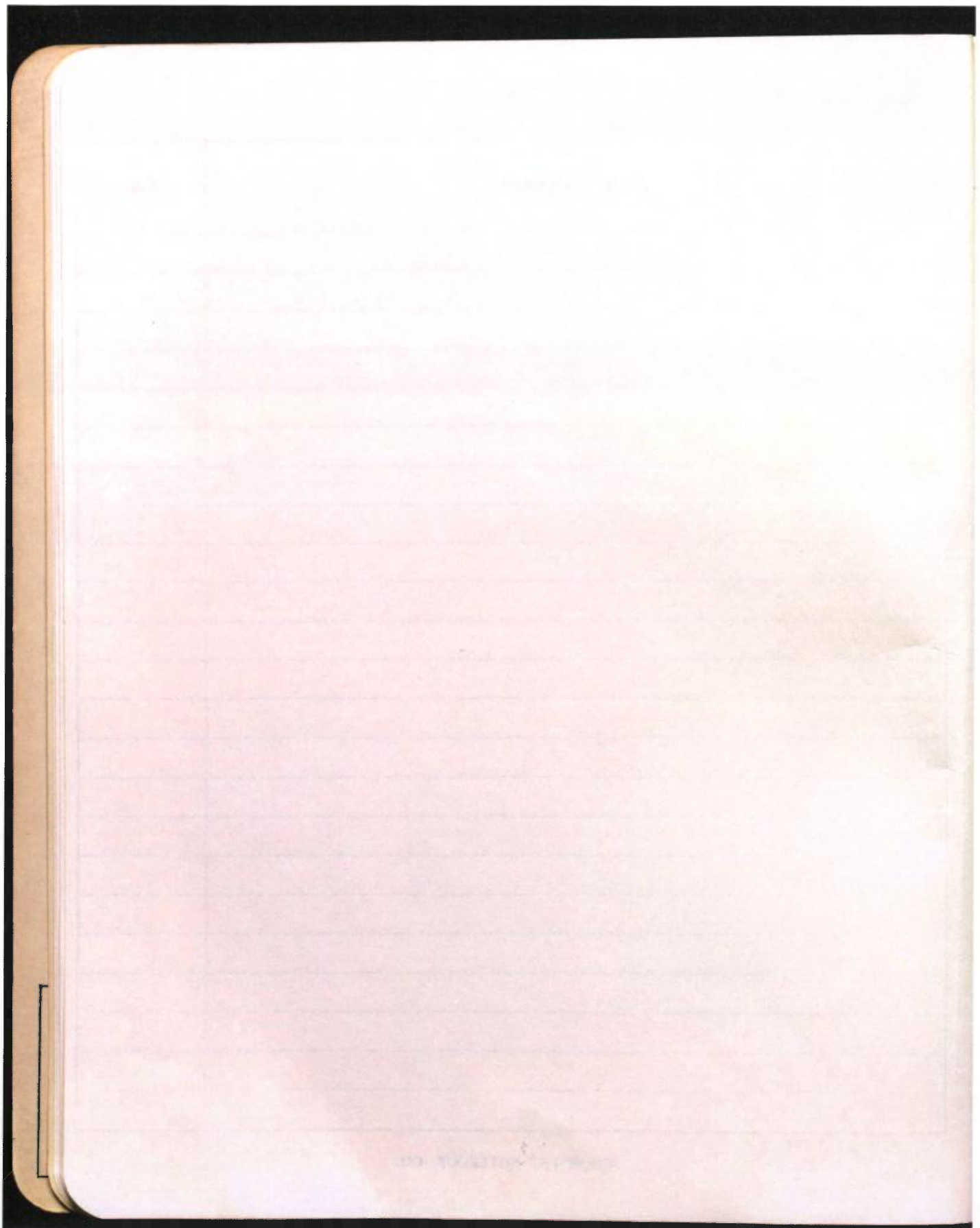


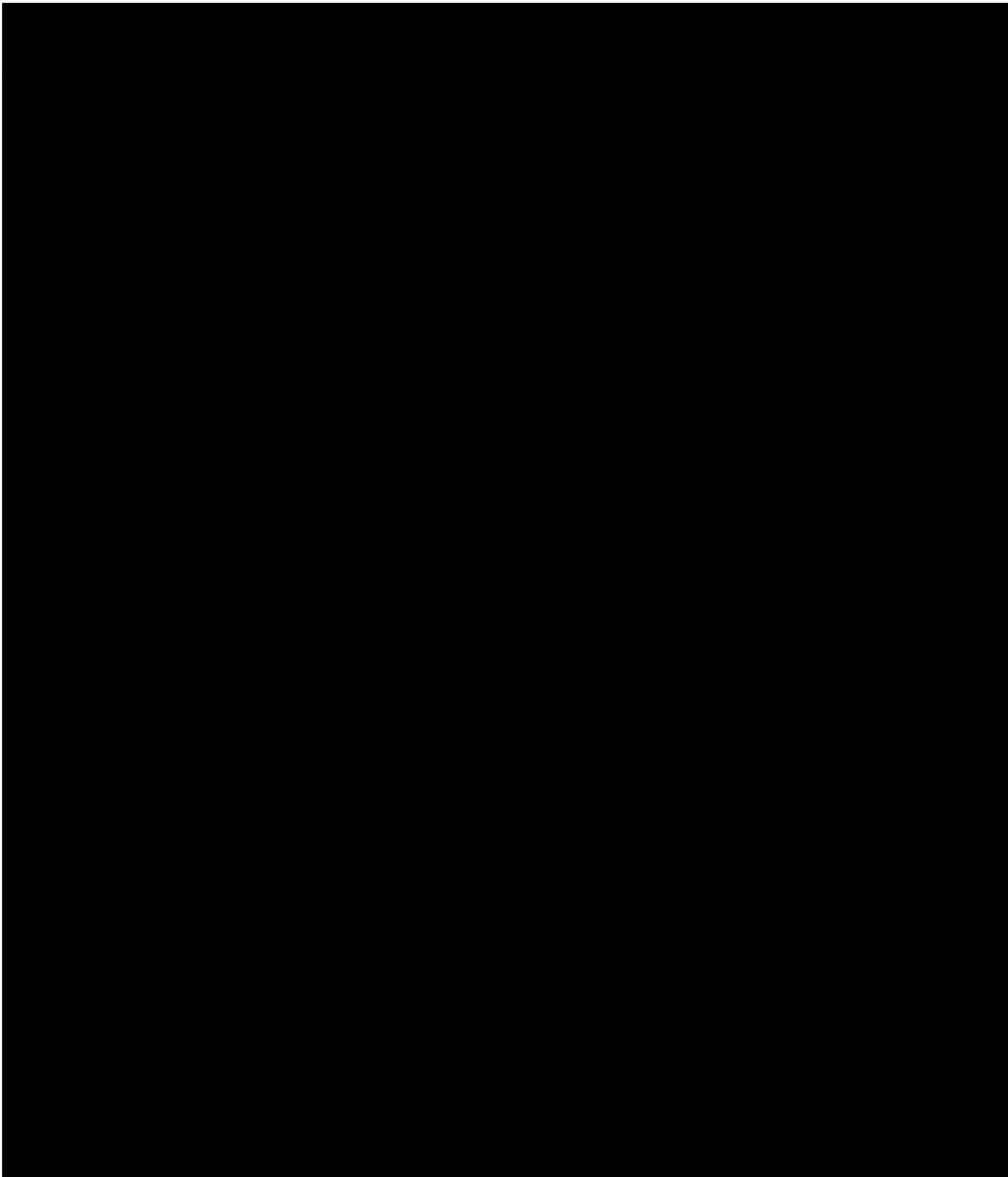












Witnessed & Understood by me,

*Dalman A*

Date

*26.1.90*

Invented by

Recorded by

*John Ruffin*

Date

*1/12/90*

Witnessed & Understood by me,

*D. Mantel*

Date

26.1.90

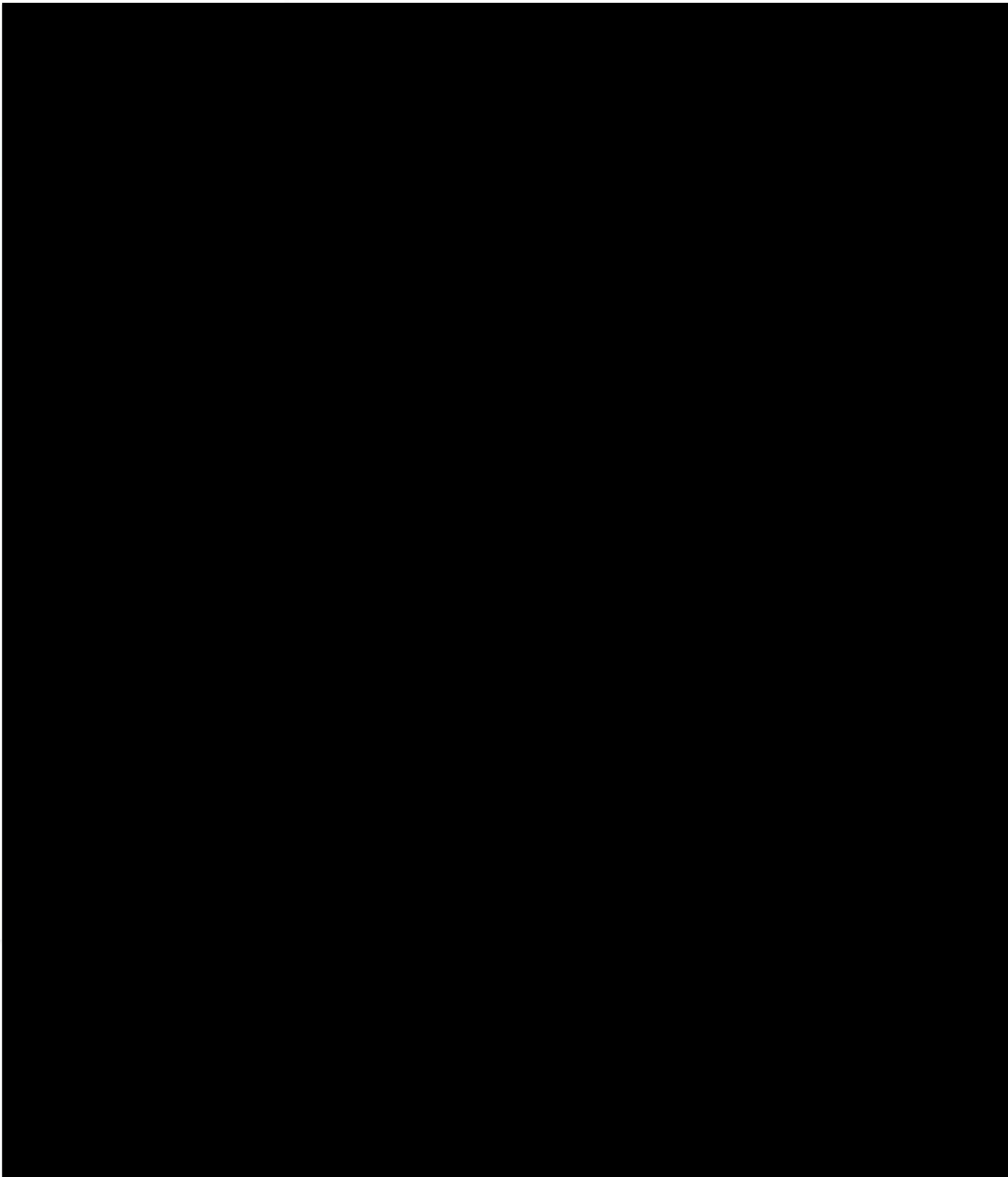
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*John Ryle*

Date

1/12/90



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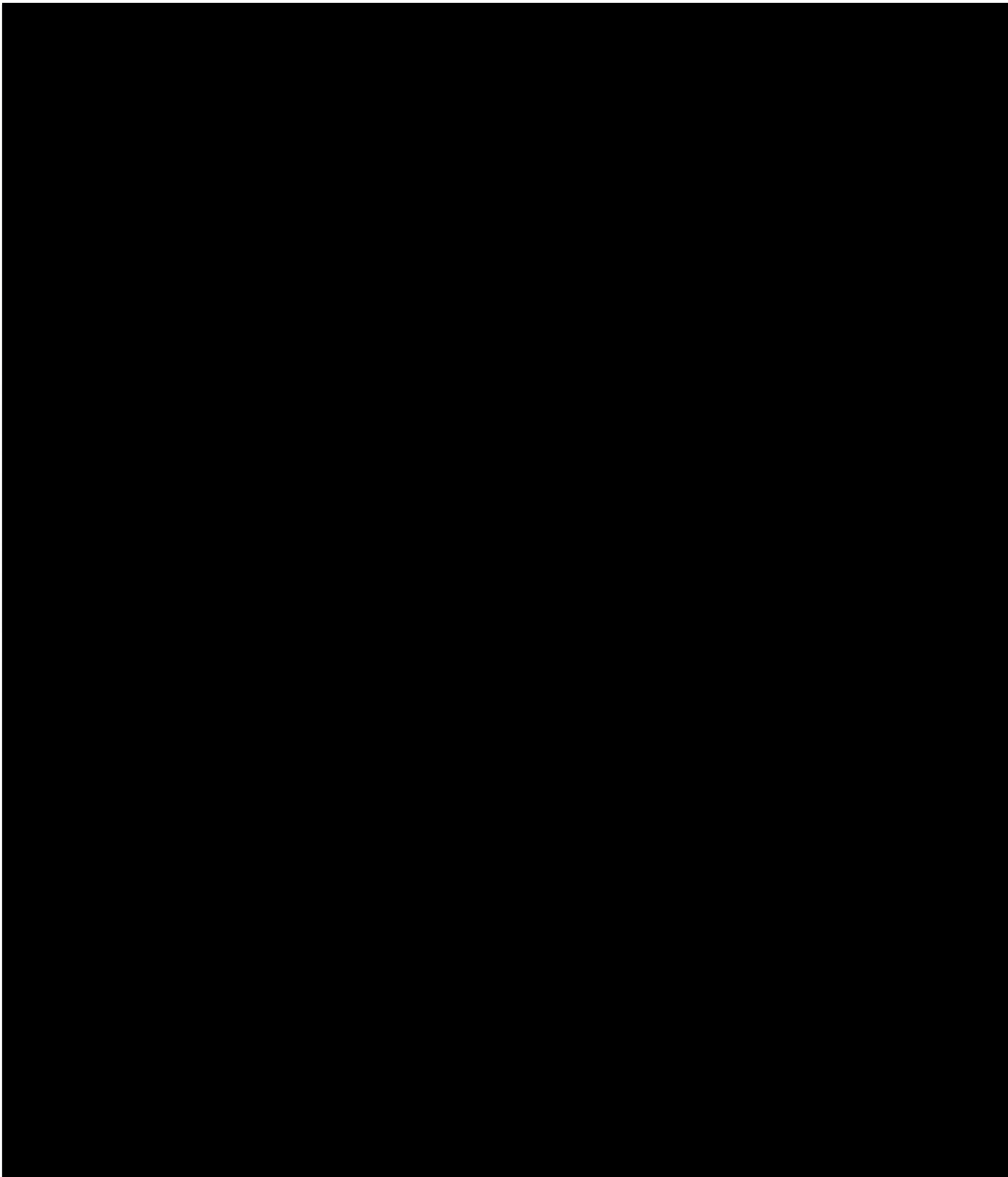
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*D. Mani*

Date

*26.1.90*

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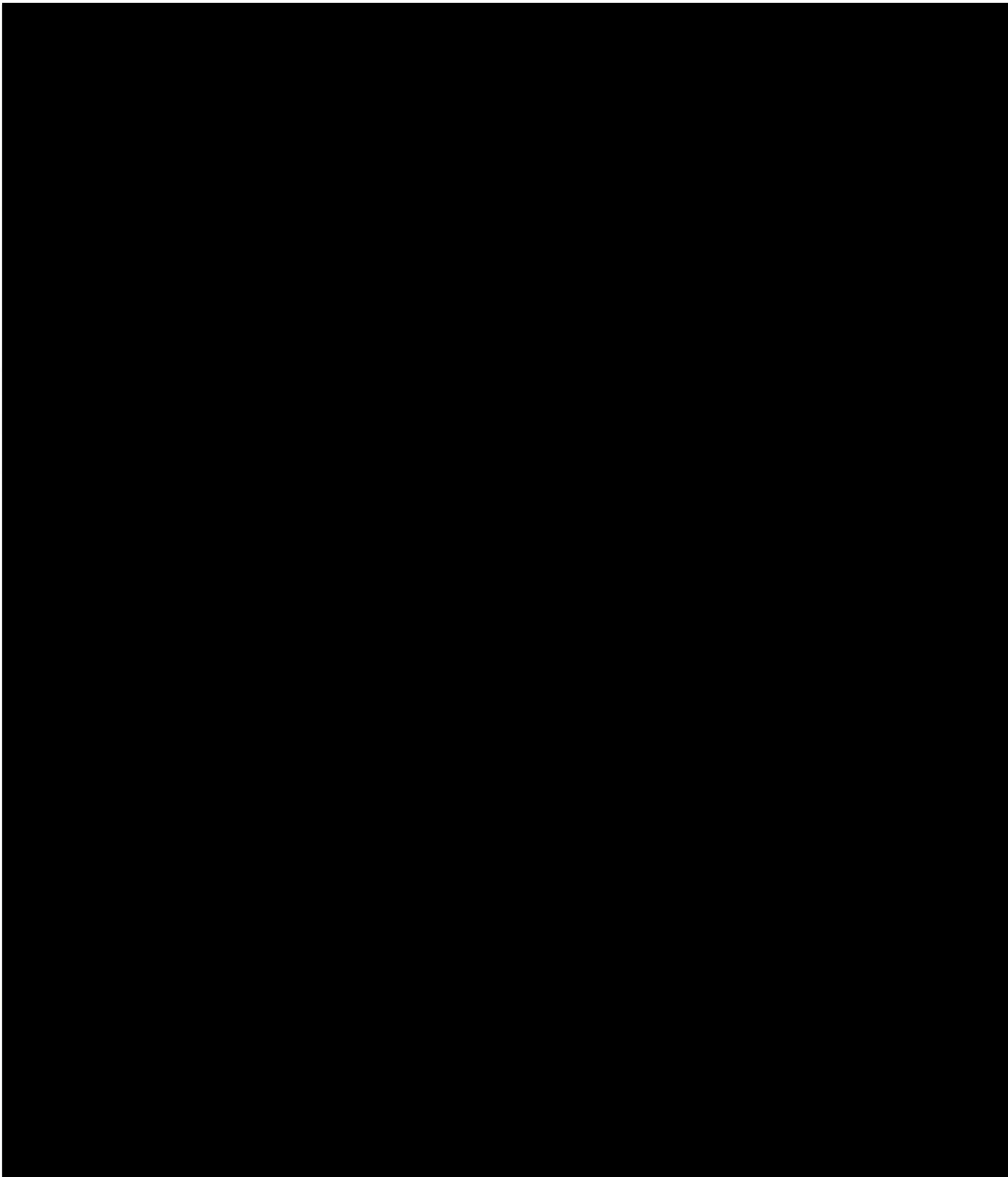
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*John Redman*

Date

*1/2/90*





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*Amanda*

Date

*26.1.90*

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Recorded by

*J. K. Redman*

Date

*1/12/90*

Witnessed & Understood by me,

*Dayman A*

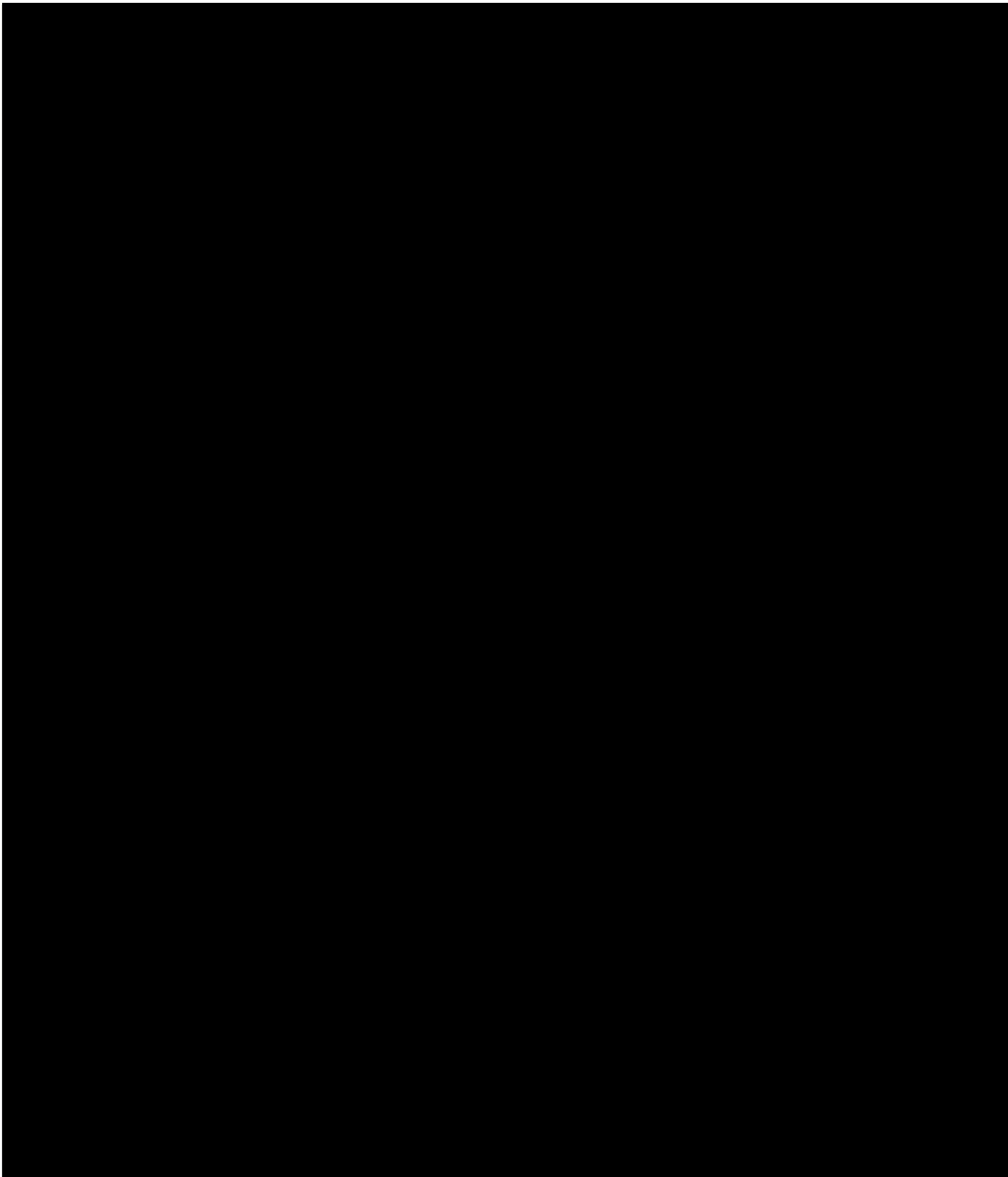
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*D. Mann*

Date

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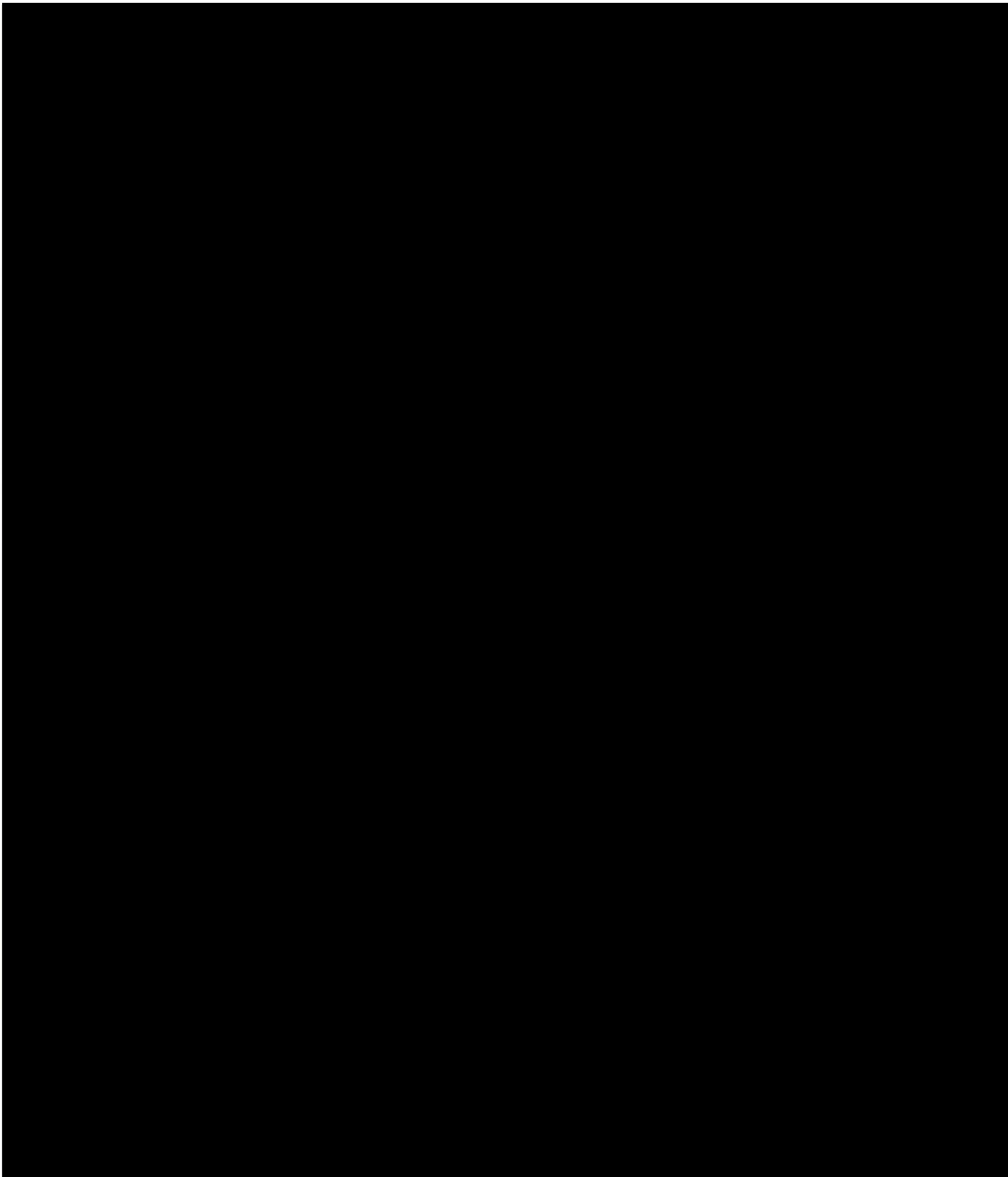
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*John Redman*

Date

*1/12/90*



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*Barbara M. [Signature]*

Date

*26.1.90*

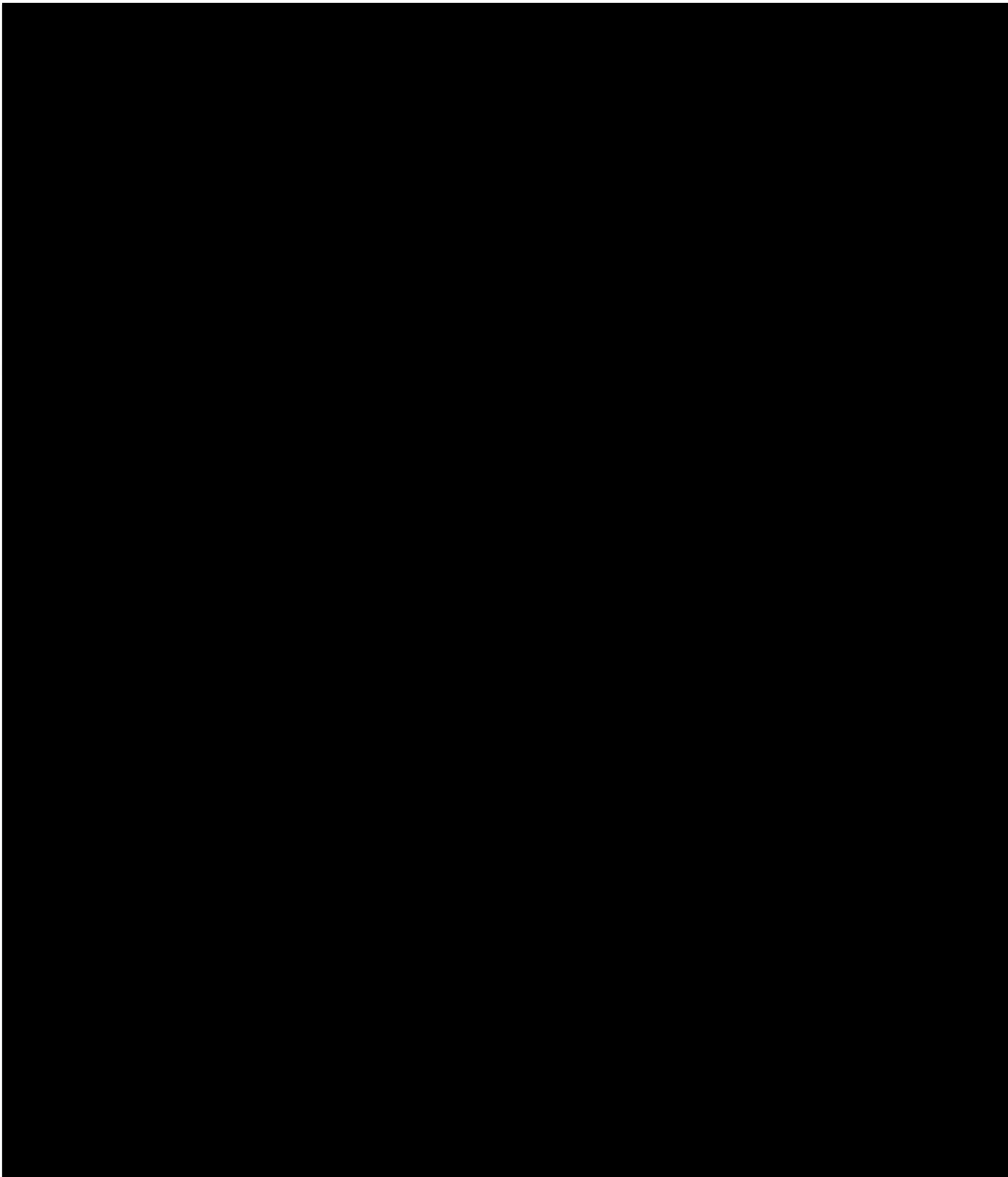
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Witnessed & Understood by me,

*Raymond A.*

Date

*26.1.90*

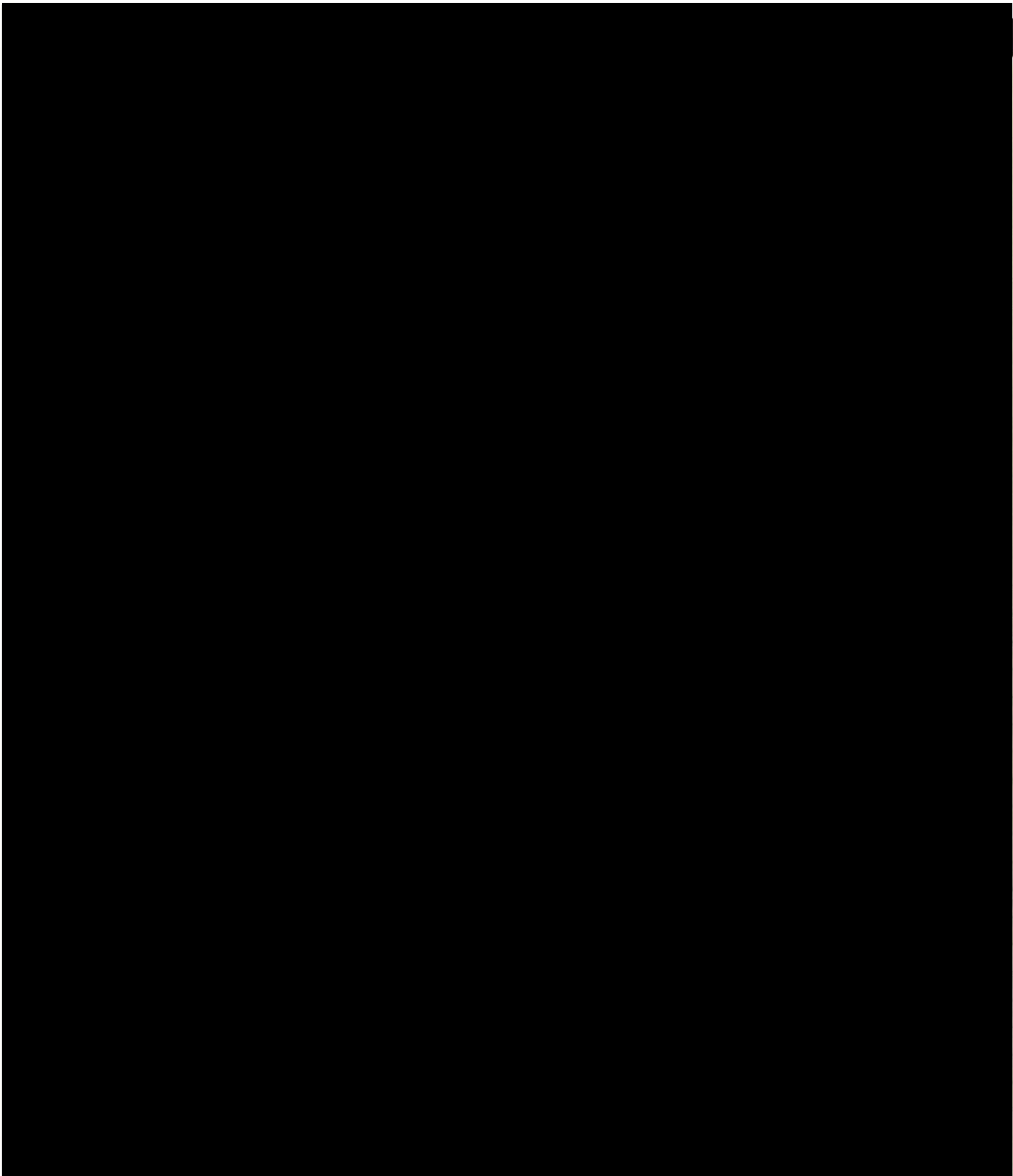
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Date

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Witnessed & Understood by me,

*Ormanth*

Date

*26.1.90*

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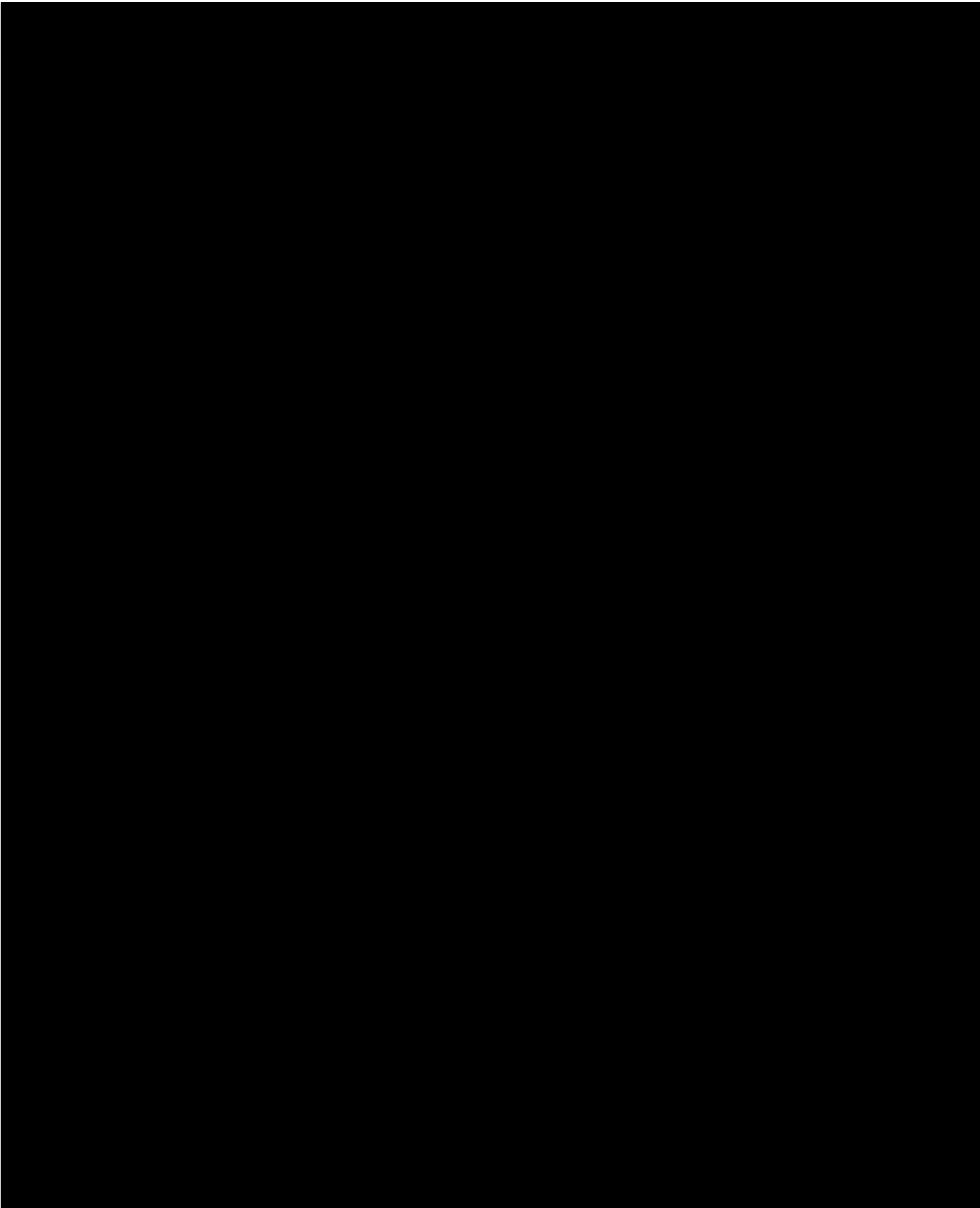
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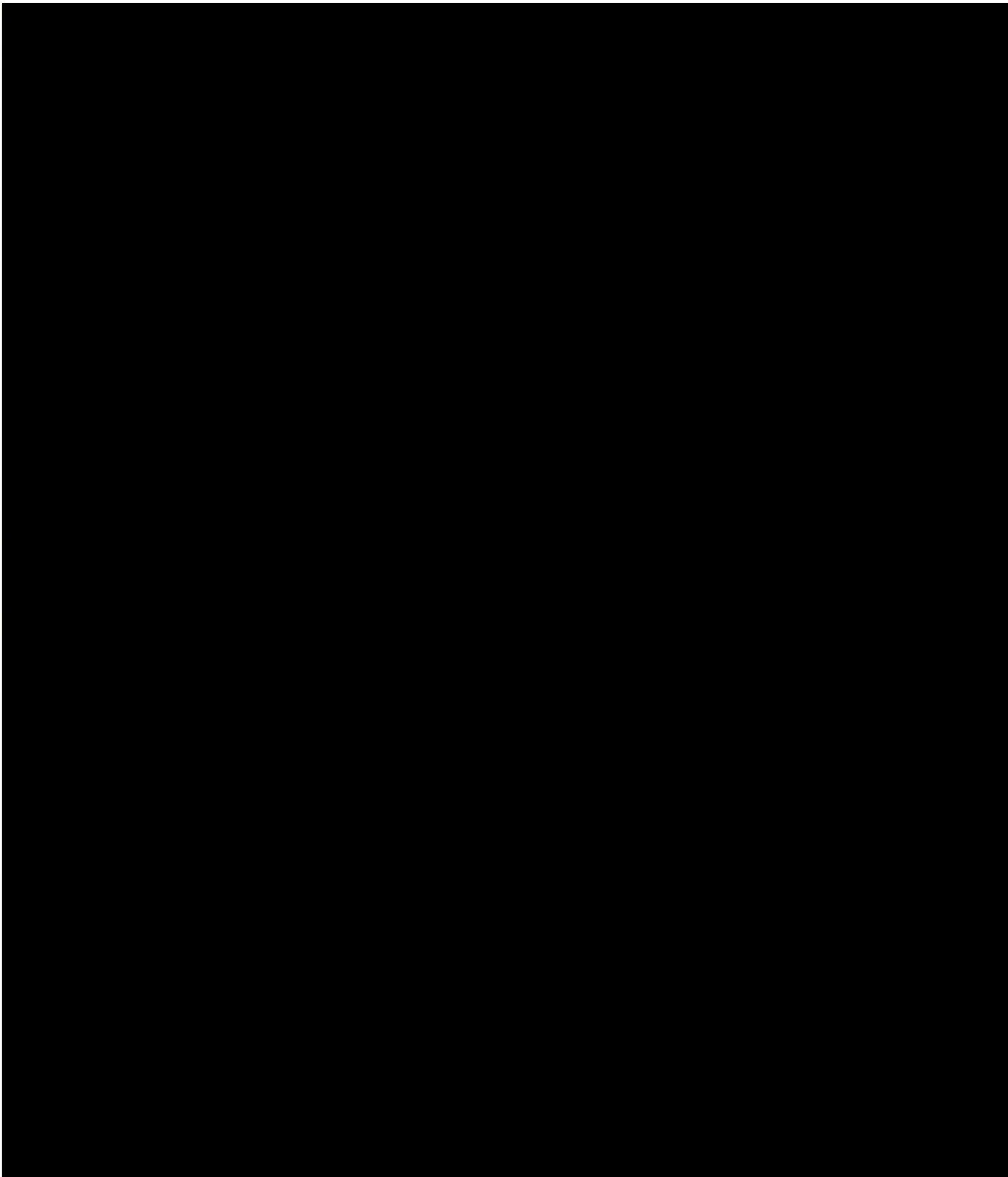
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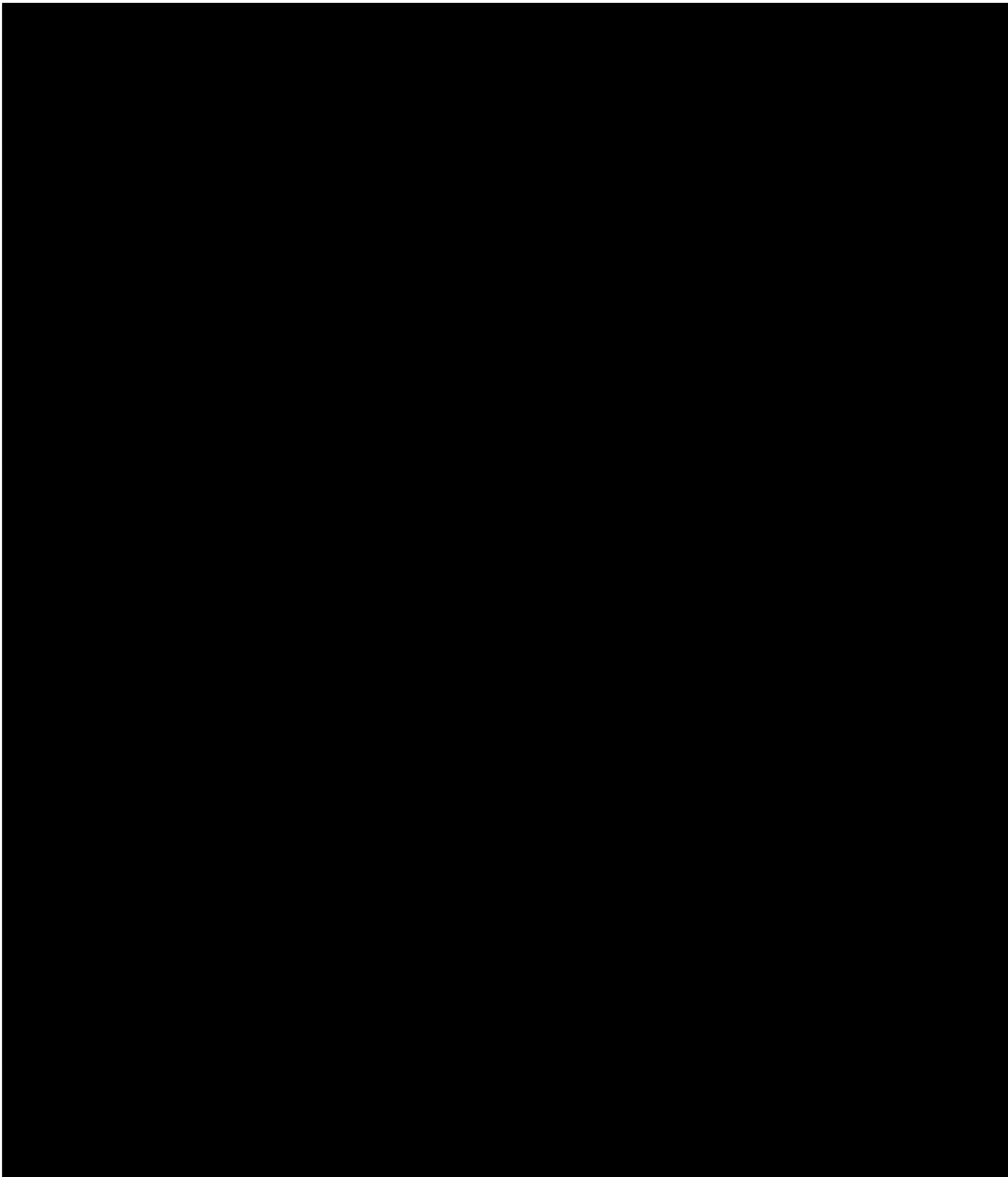
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*Dajmamth*

Date

*26.1.20*

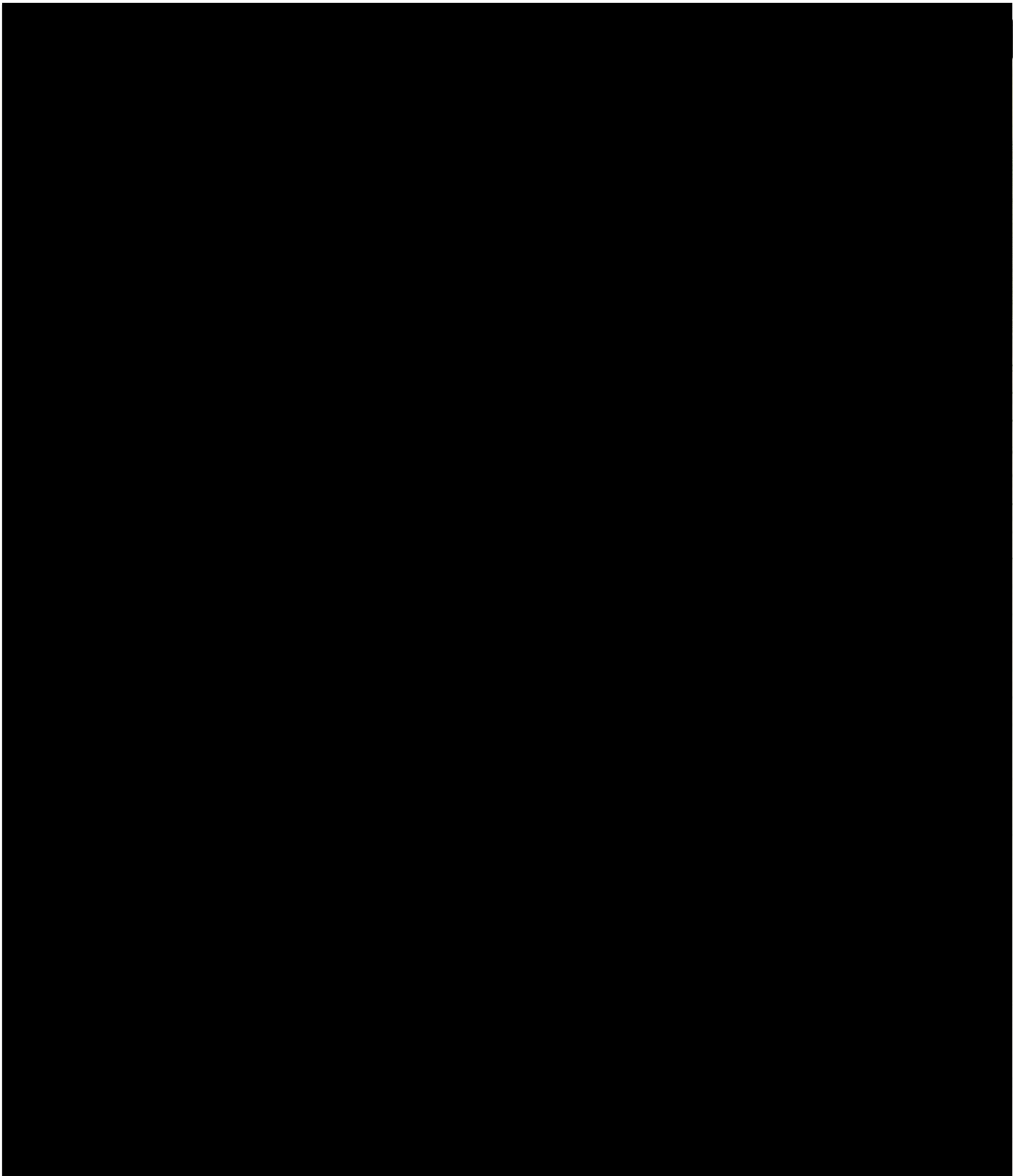
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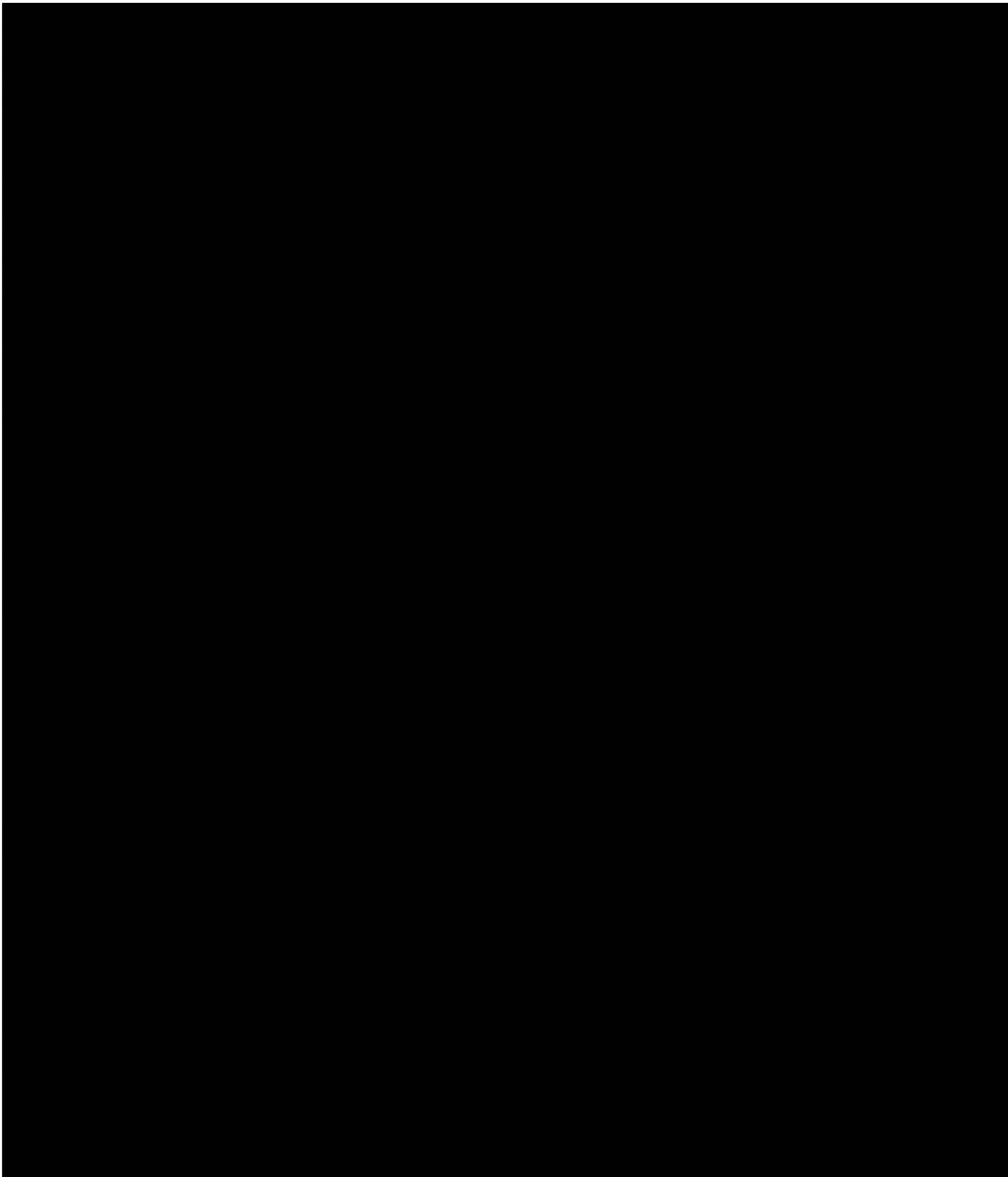
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Witnessed & Understood by me,

*Danman*

Date

*26.1.80*

Invented by

*Alan Turing*

Recorded by

Date

*1/17/90*

Witnessed & Understood by me.

Dalman

Date

26.1.90

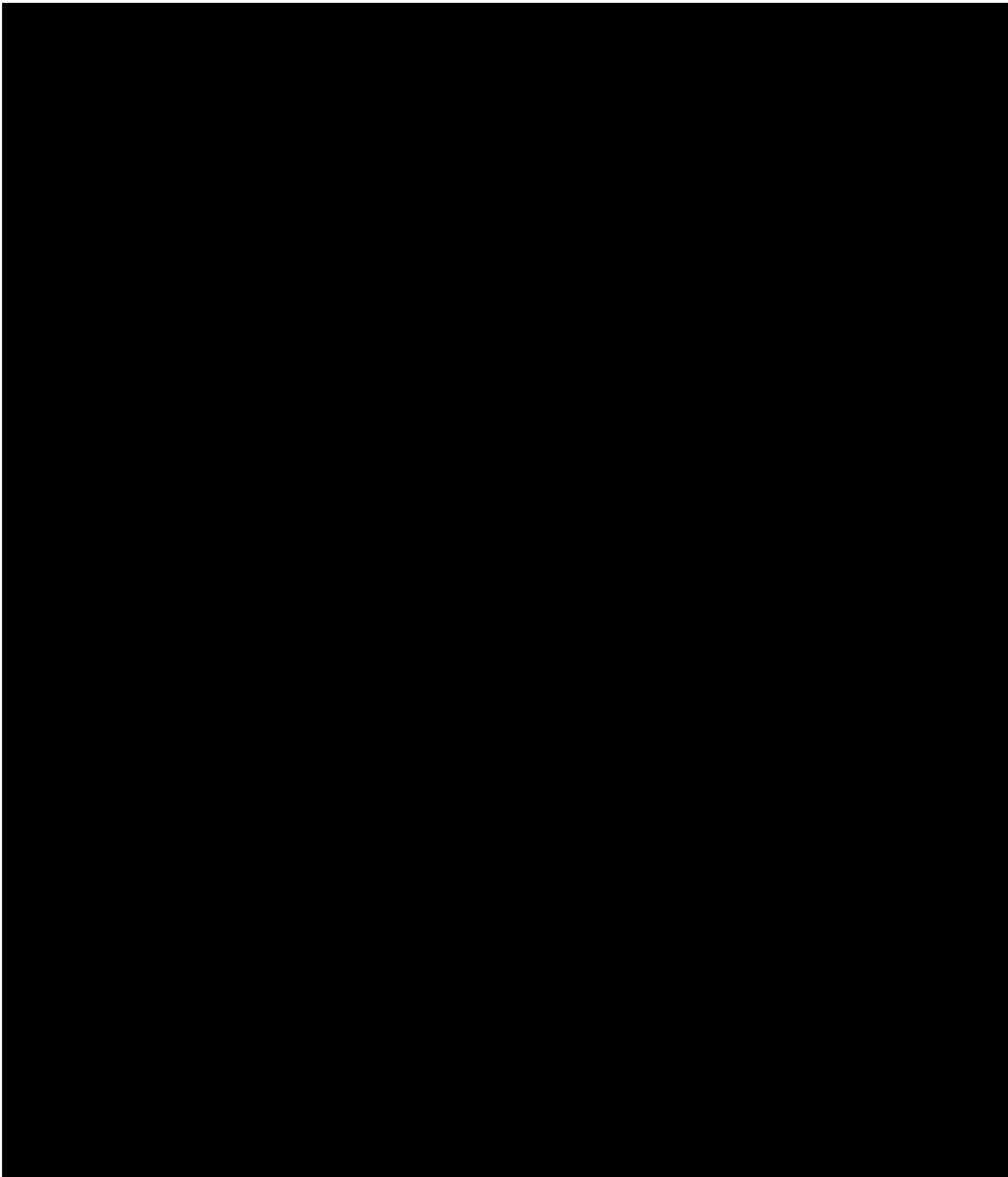
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Date

1/22/90

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Witnessed & Understood by me,

*Deymann A*

Date

*26.1.90*

Invented by

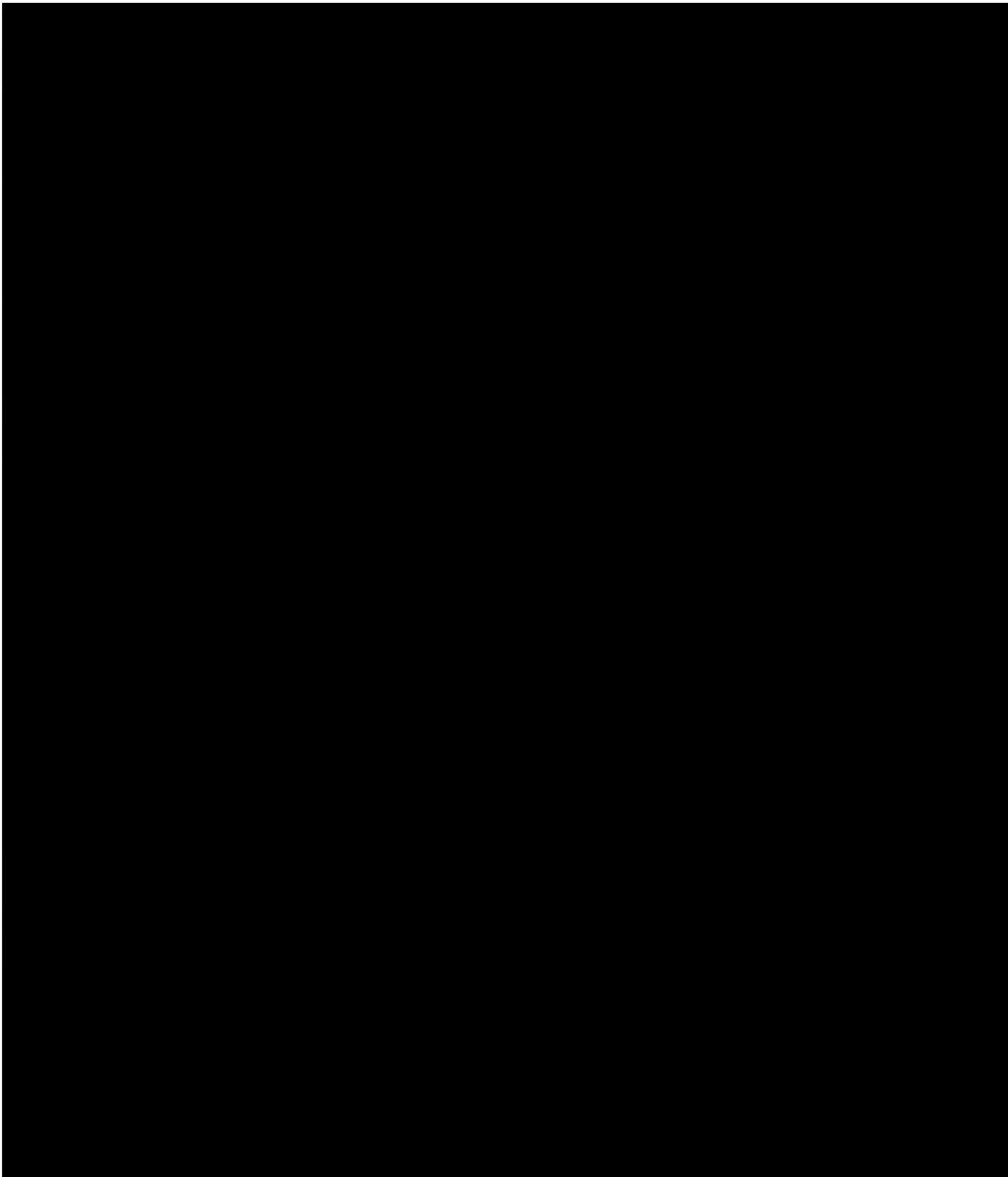
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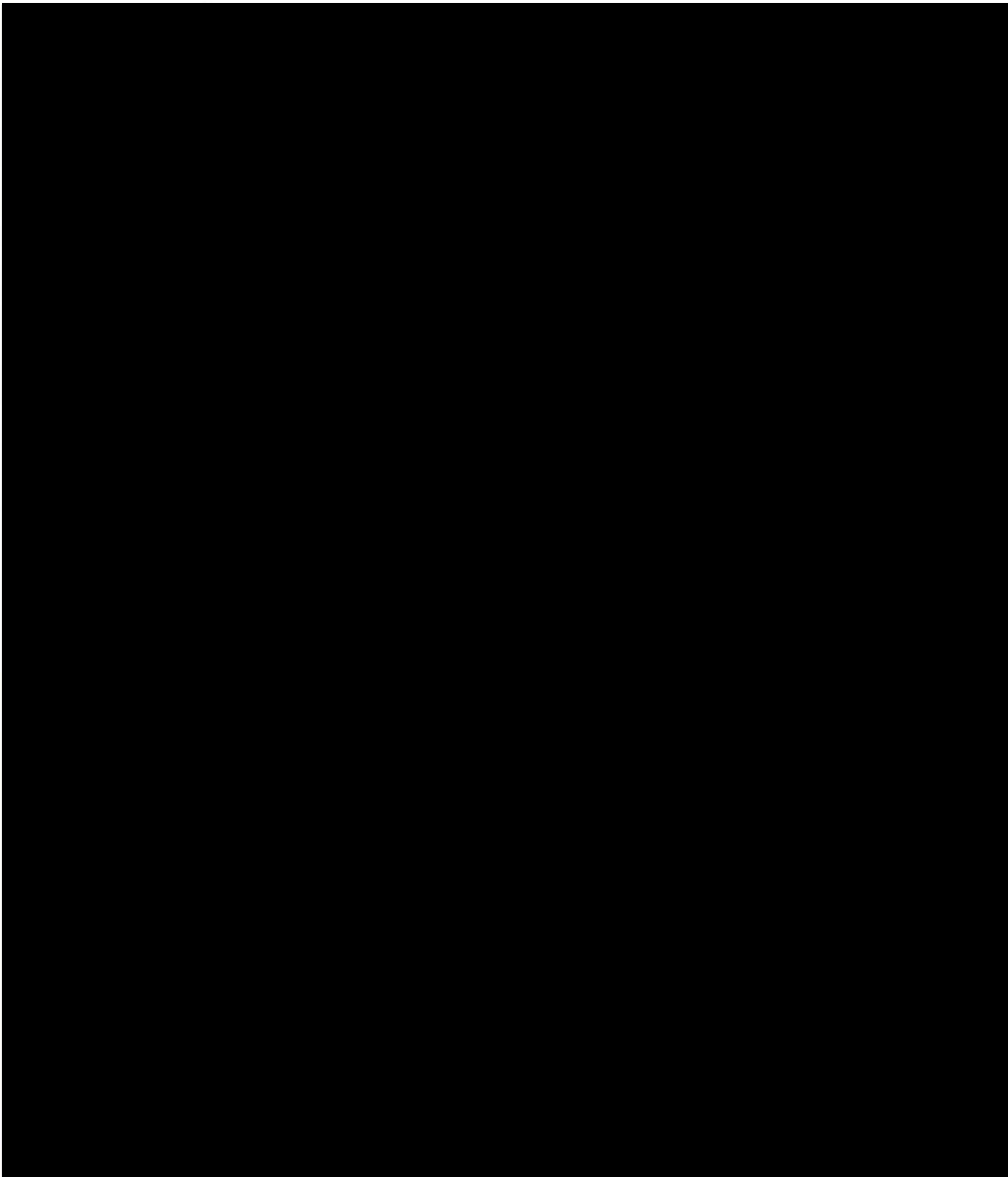
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*Daymond*

Date

26.1.90

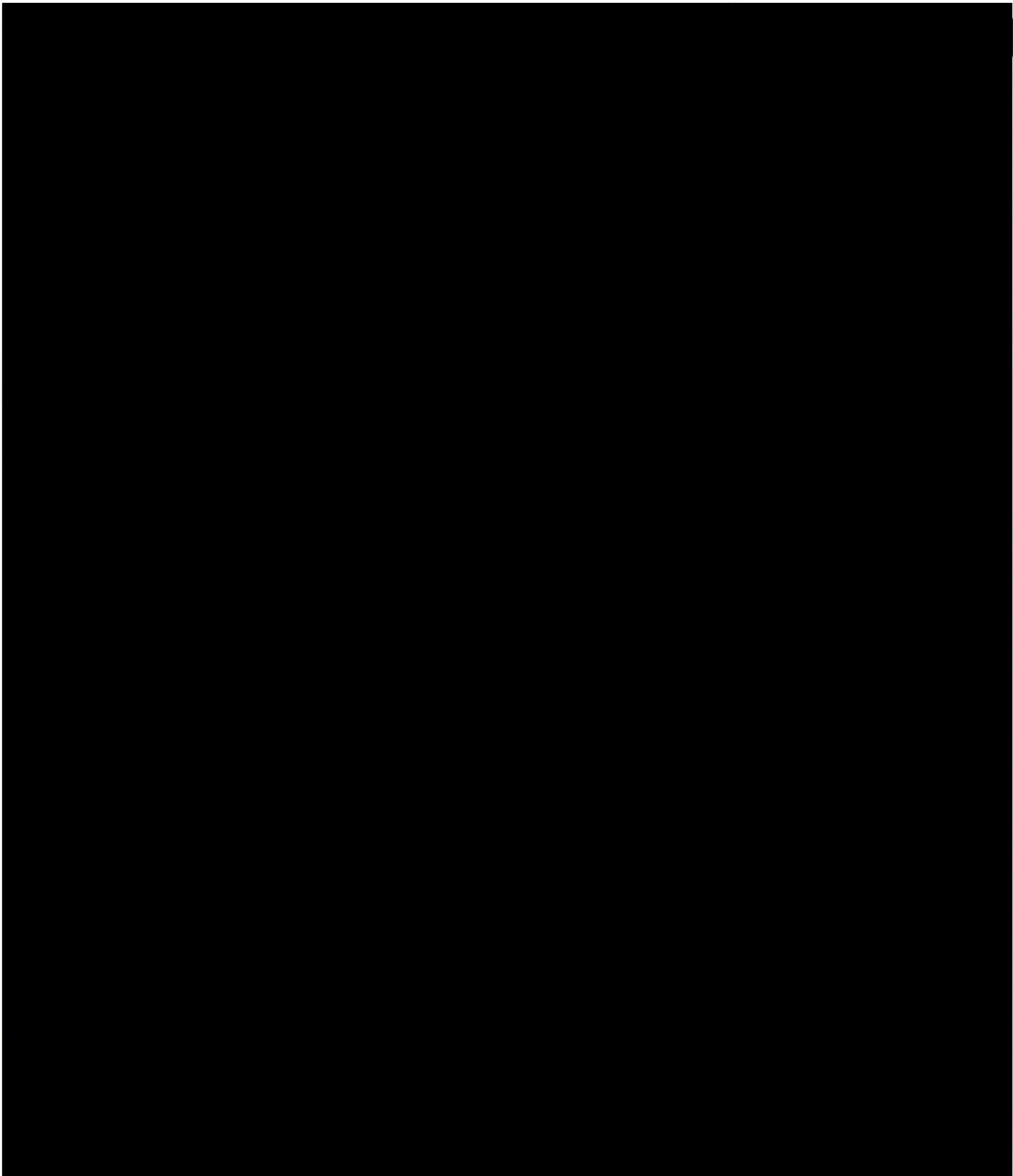
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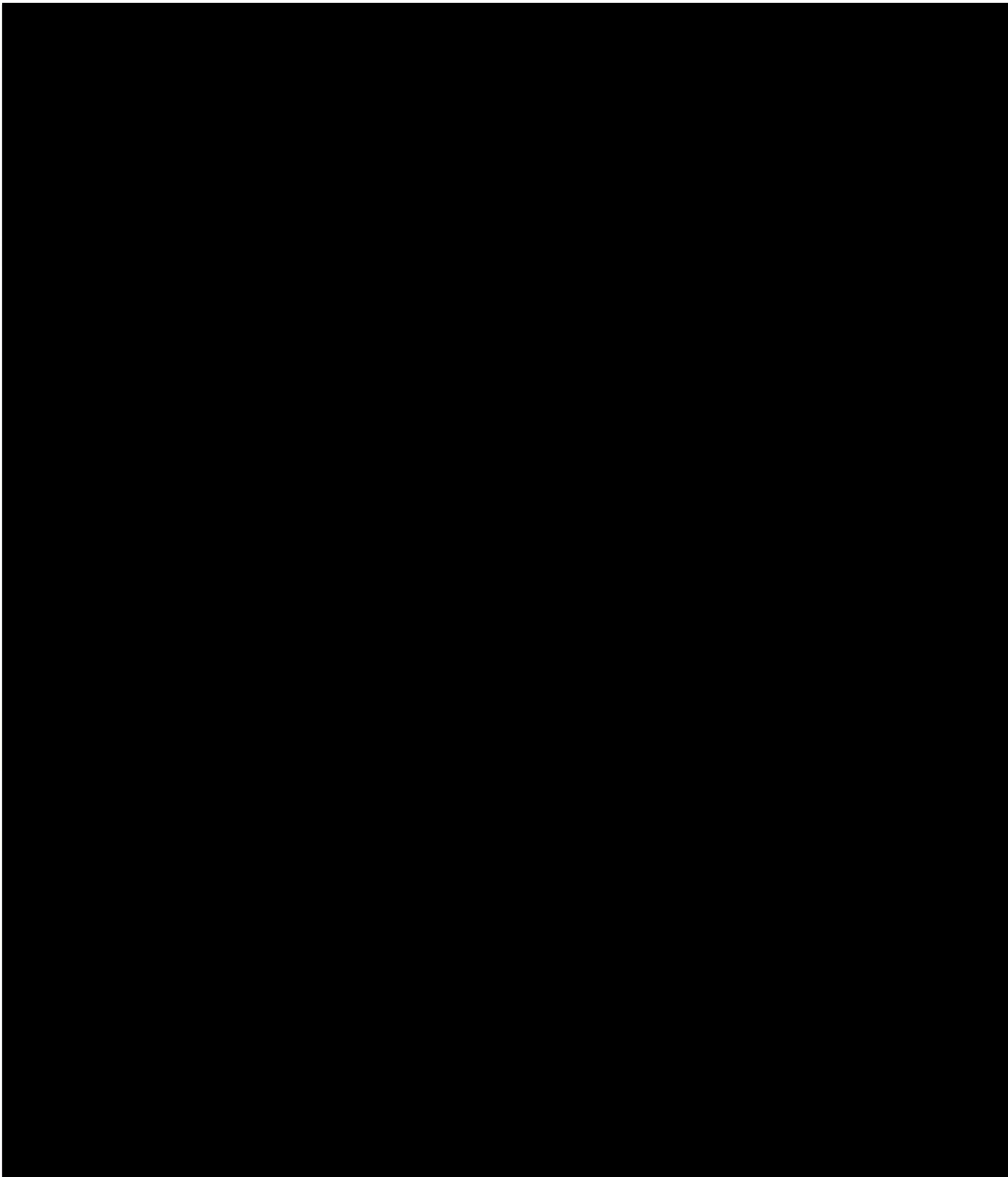
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1/23/90



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*Daymanth*

Date

*26.1.90*

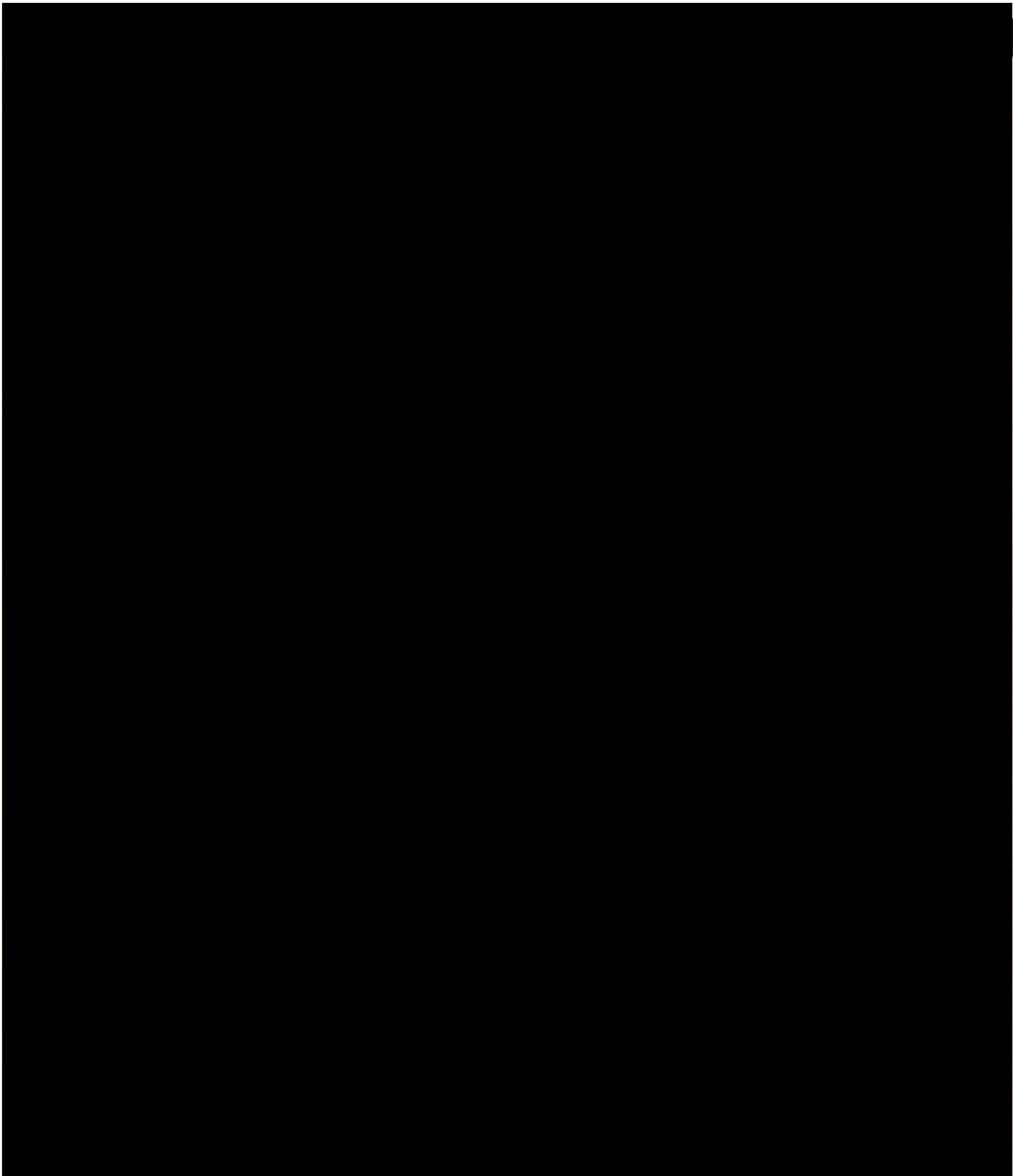
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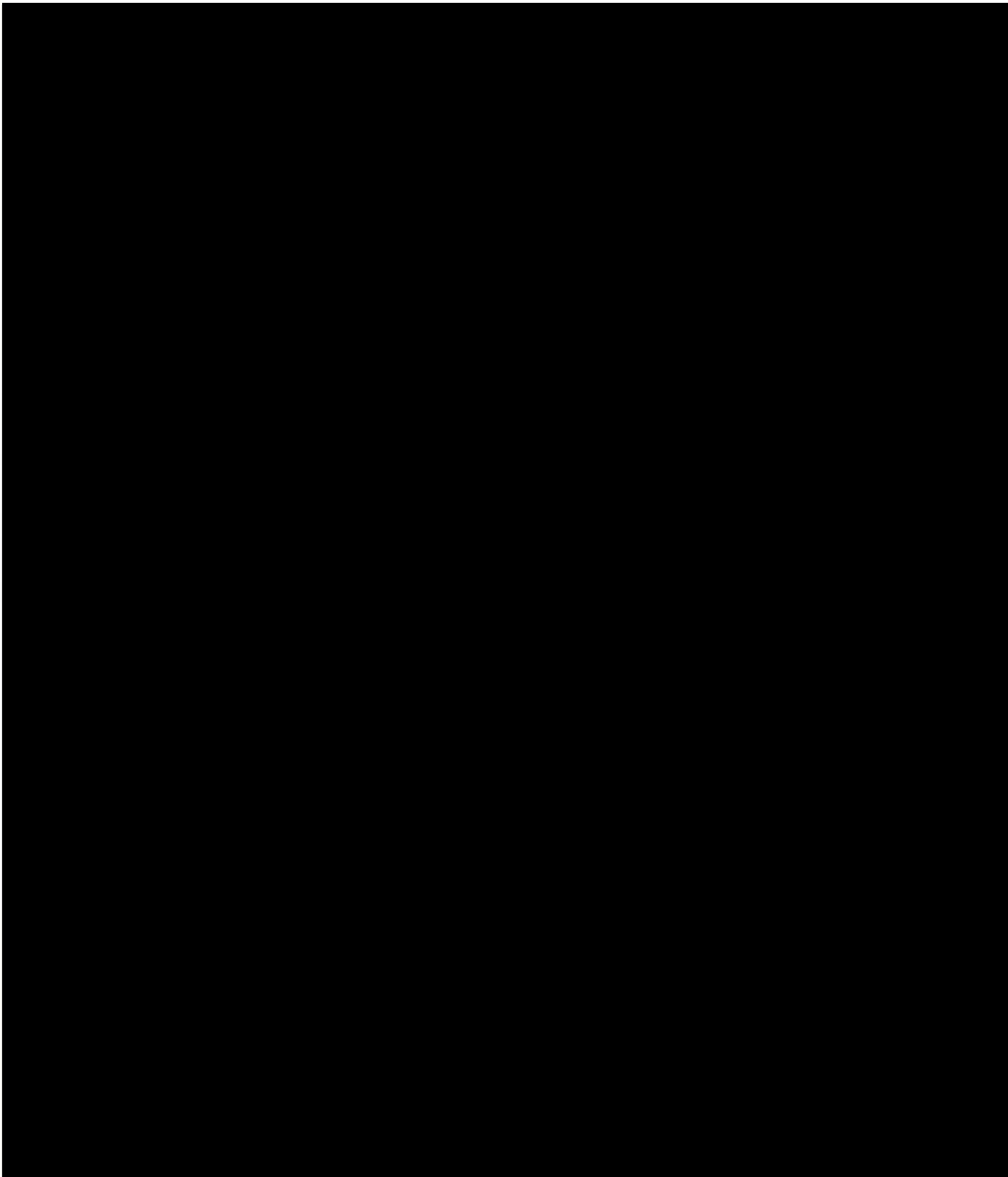
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Date

*1/24/90*



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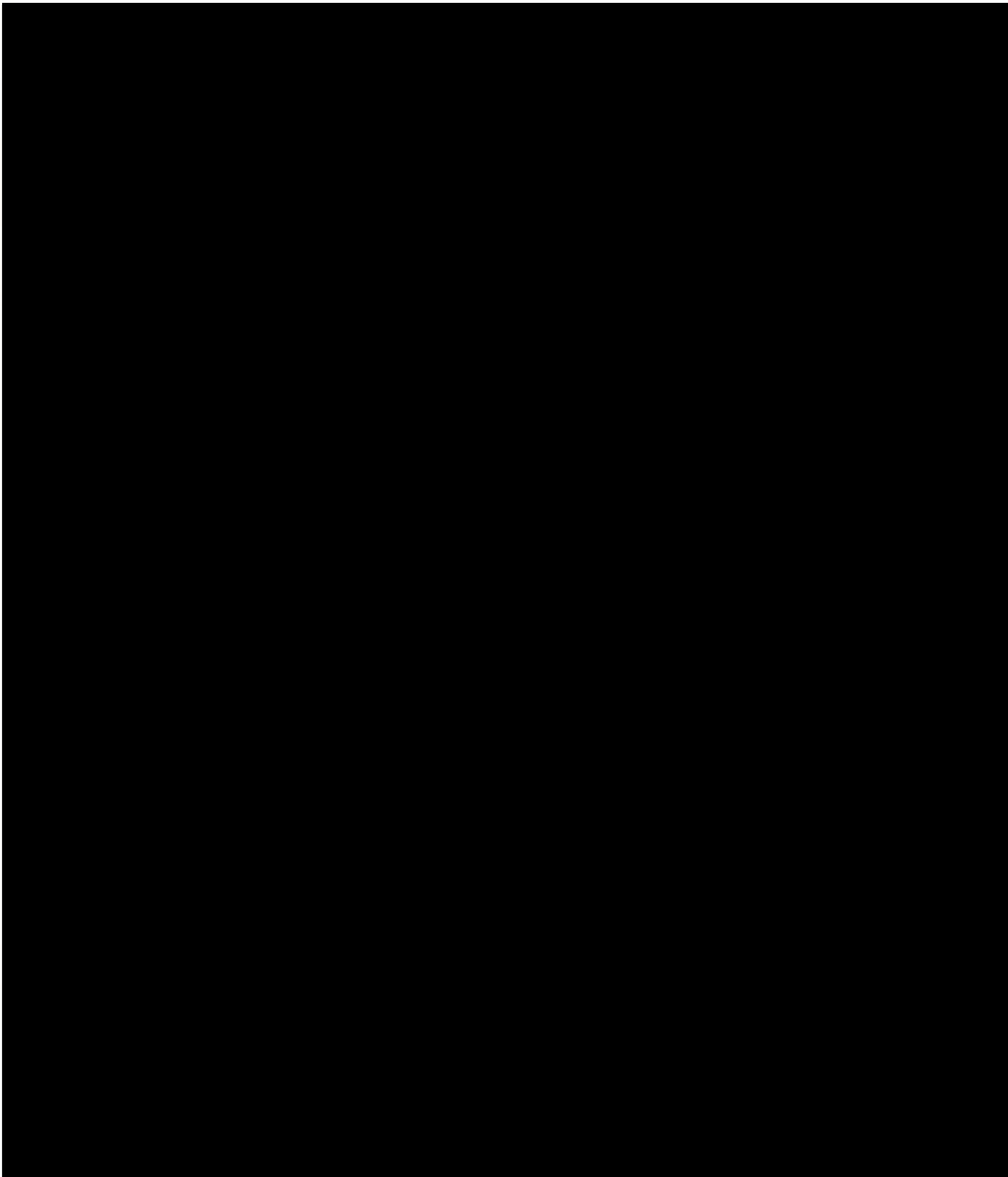


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*Daymen*

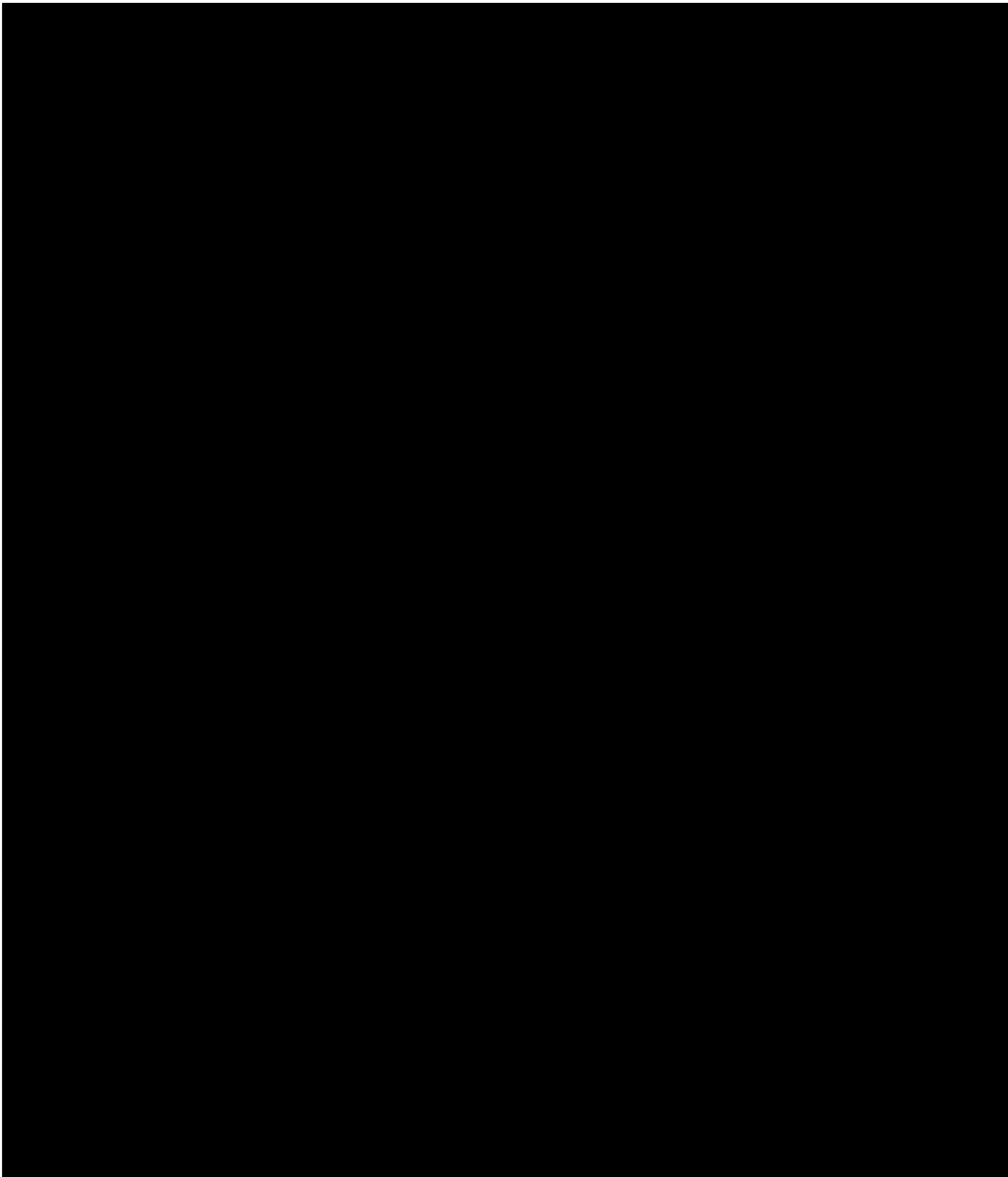
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Date  
*1/24/90*



Witnessed & Understood by me, <i>Dayman</i>	Date <i>26.1.90</i>	Invented by <i>John Redman</i>	Date <i>1/25/90</i>	To Page No.
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Witnessed & Understood by me,

*D. J. Martin*

Date

*13.4.80*

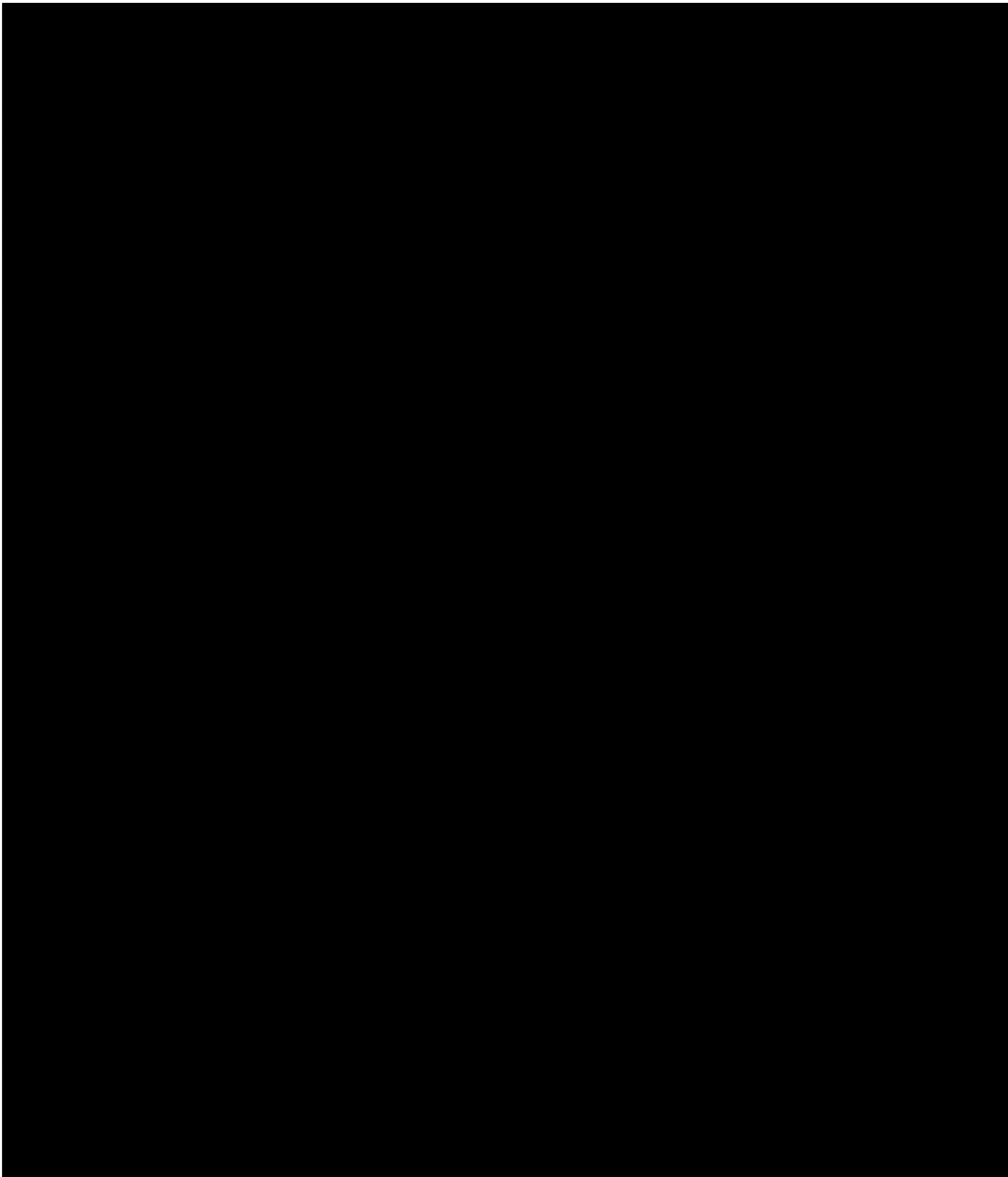
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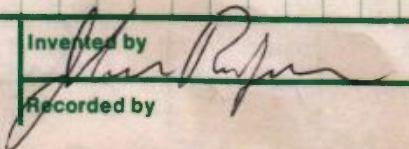
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*John R. ...*

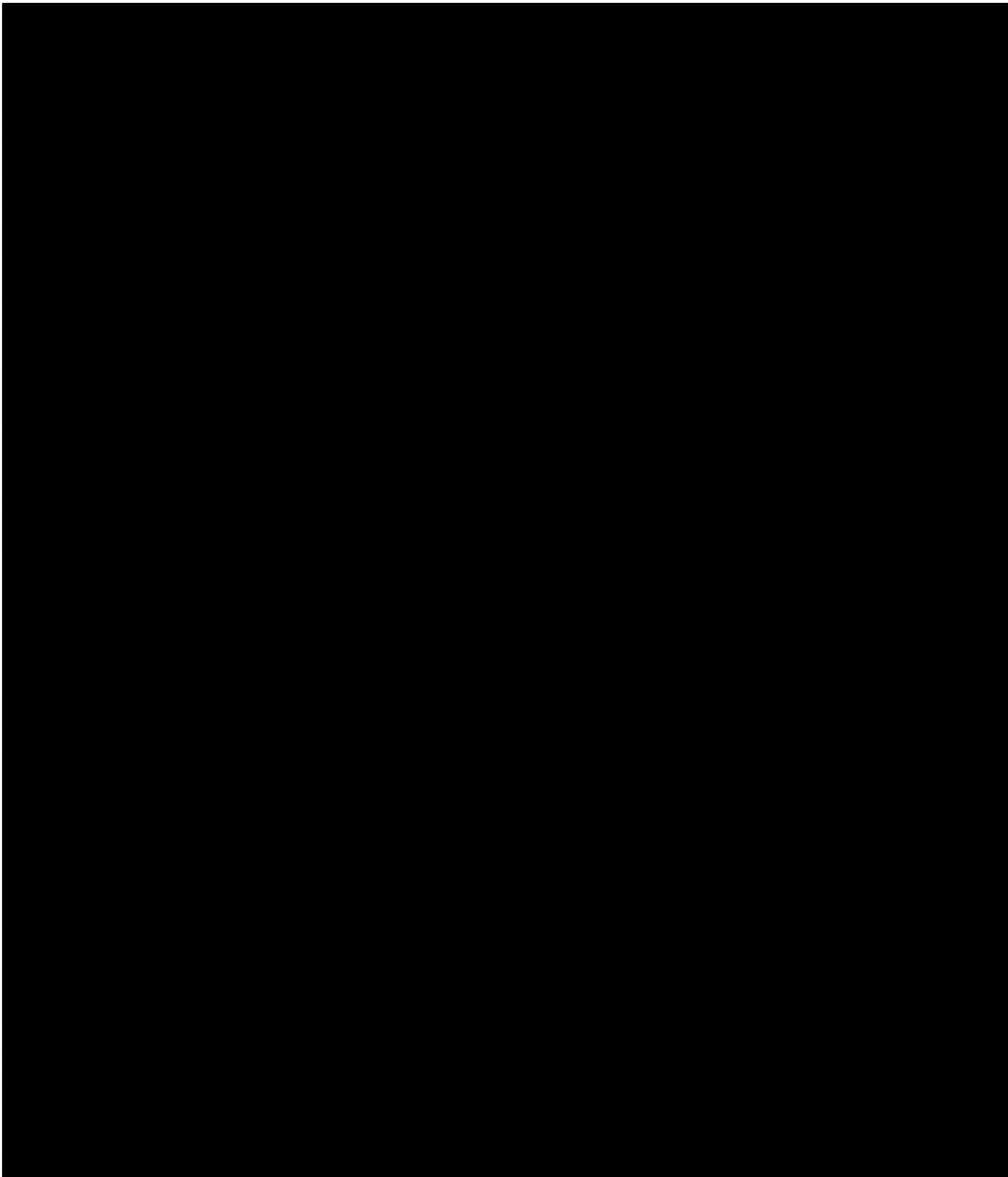
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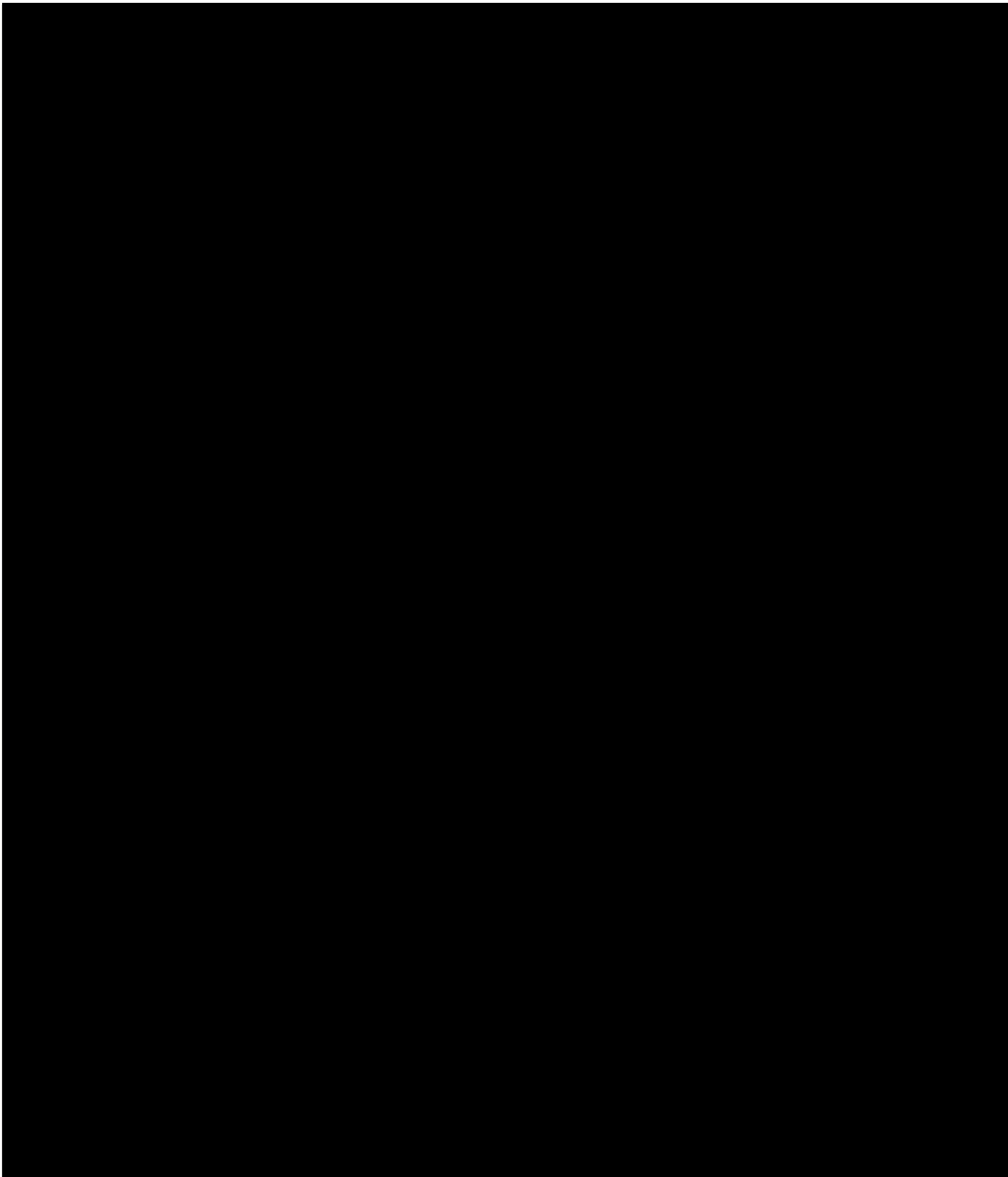


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			Recorded by		





Witnessed & Understood by me, DajmanitA	Date 13.4.90	Invented by [Signature]	Date 24/90
		Recorded by	



Witnessed & Understood by me,

Dajmanisa

Date

13.4.90

Invented by

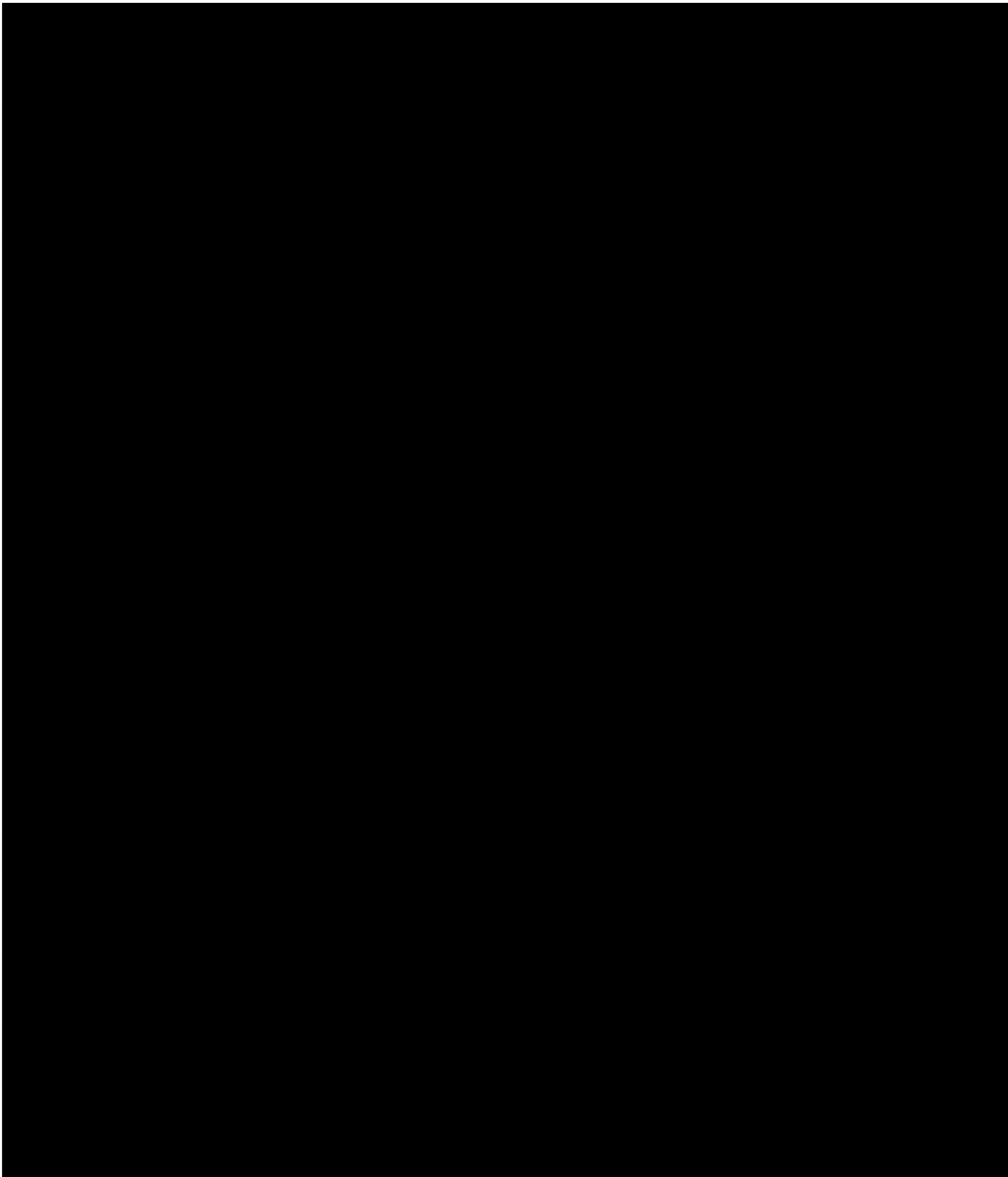
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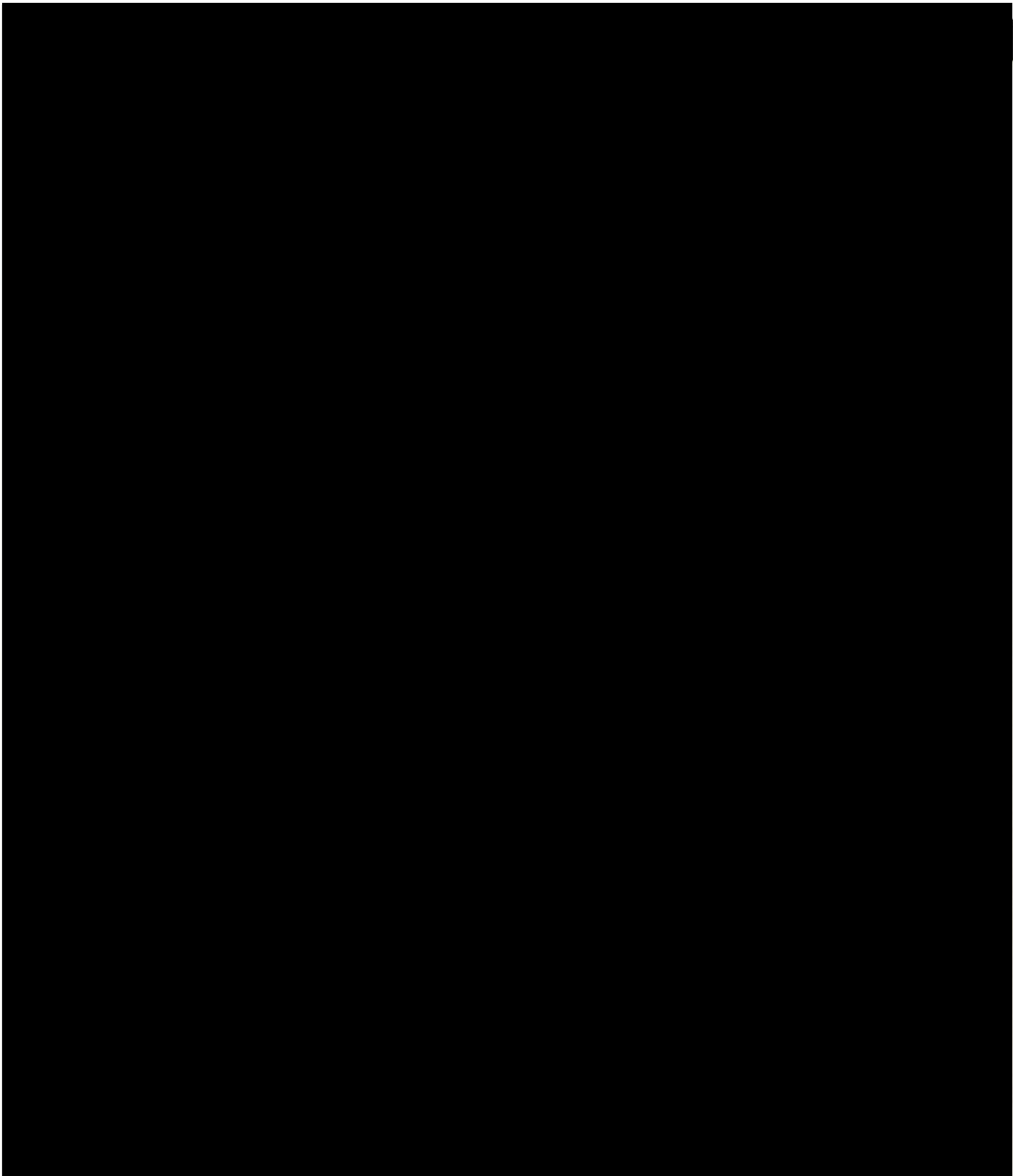
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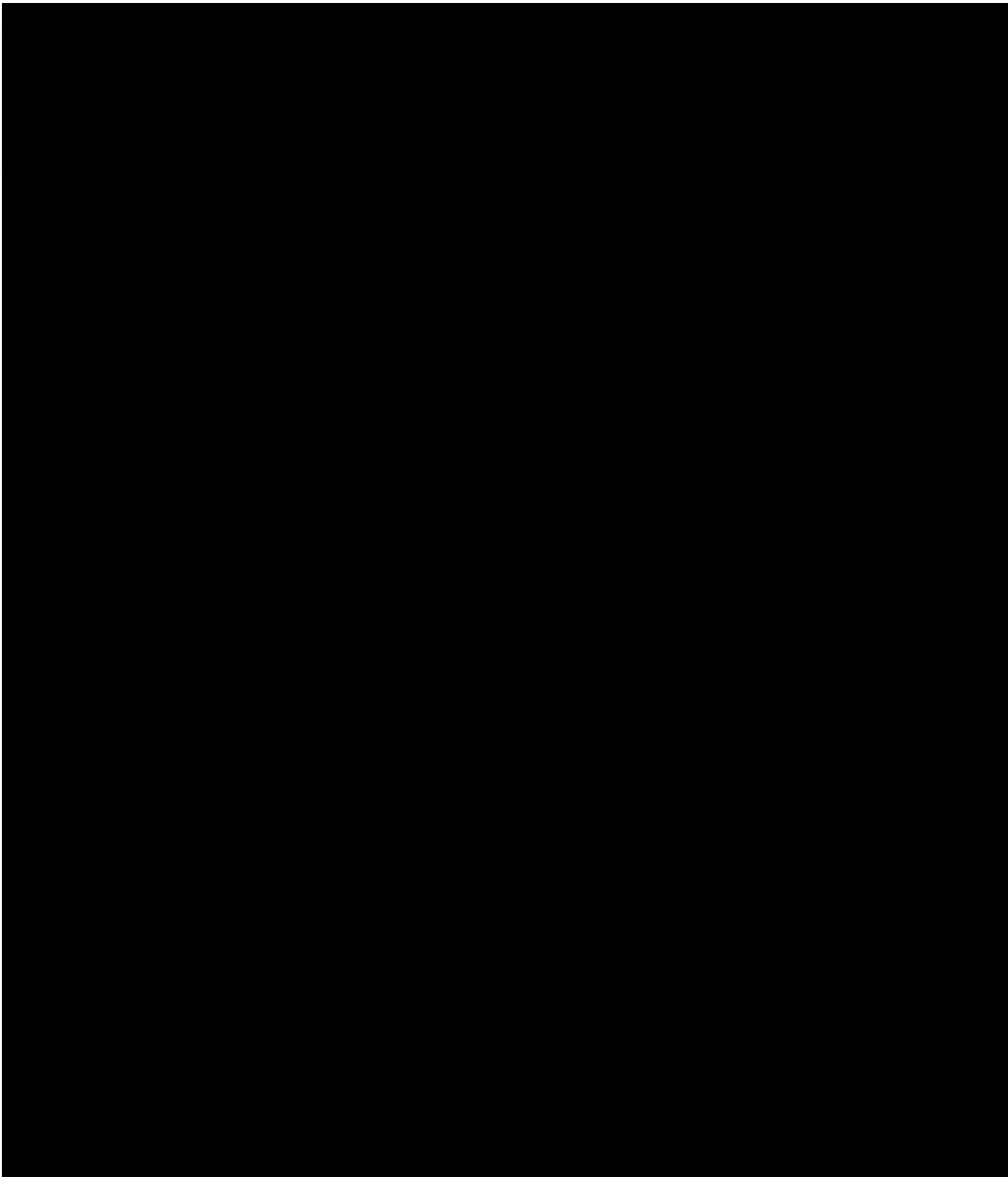
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Witnessed & Understood by me, <i>O. J. Manantla</i>	Date <i>13.4.90</i>	Invented by <i>[Signature]</i>	Date <i>4/6/90</i>
		Recorded by	



Witnessed & Understood by me, Dalmarin A	Date 13.4.90	Invented by <i>[Signature]</i>	Date 2/5/90	To Page No. _____
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Witnessed & Understood by me,

*Daymanik*

Date

*13.4.90*

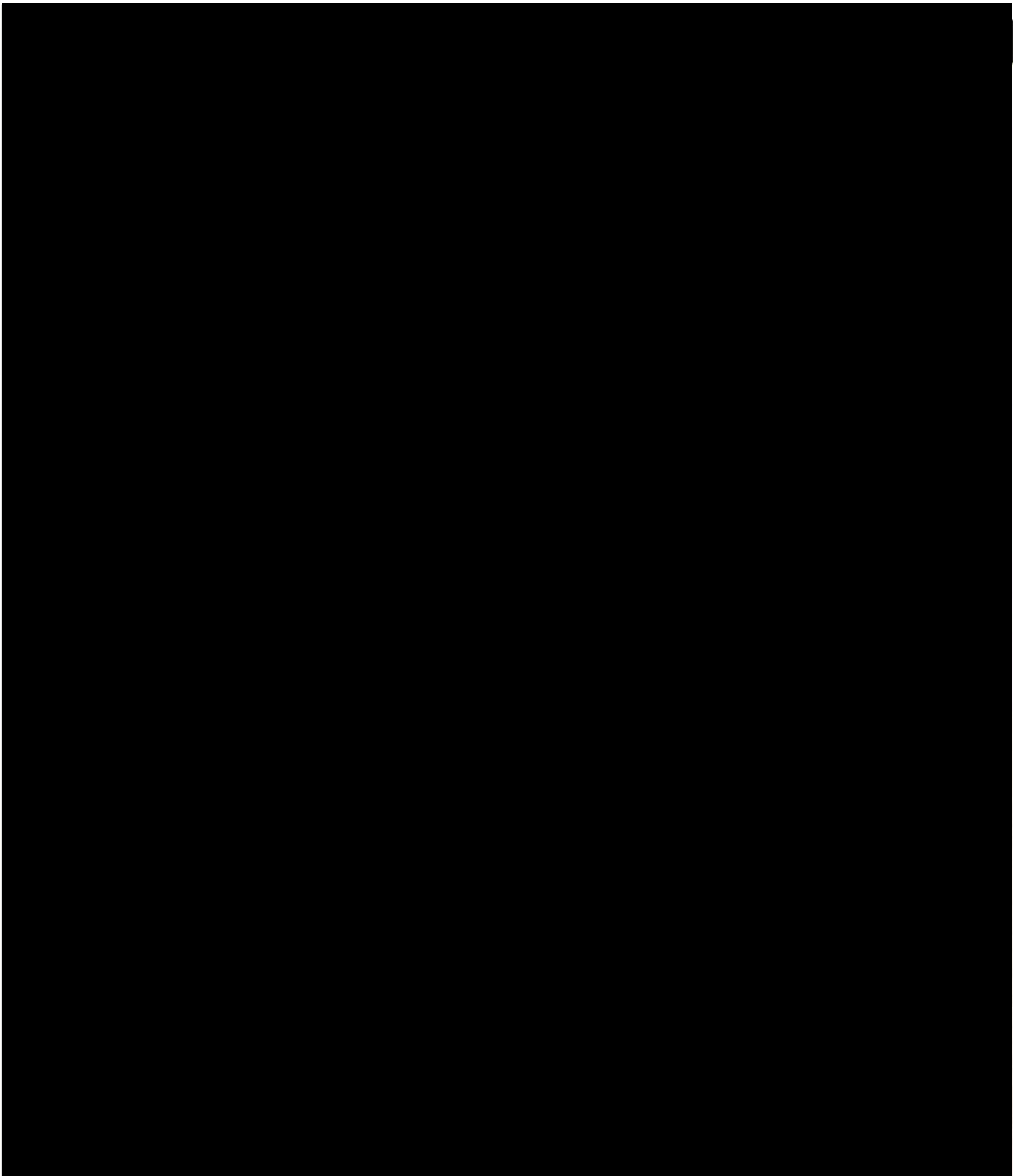
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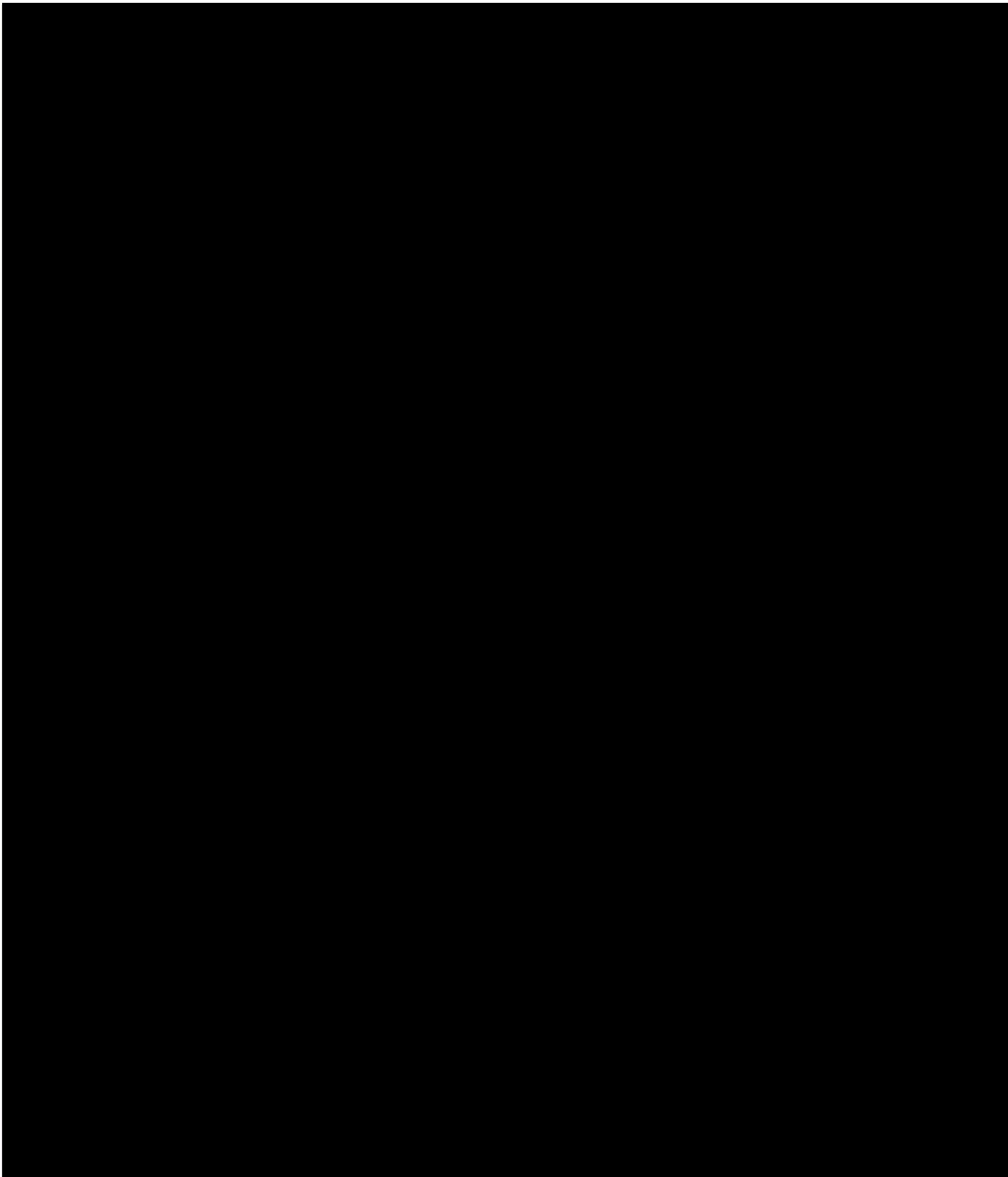
*[Signature]*

Date

*4/6/97*



Witnessed & Understood by me, <i>Daulmani</i>	Date <i>13.4.90</i>	Invented by <i>[Signature]</i>	Date <i>2/6/90</i>	To Page No. _____
		Recorded by		



Witnessed & Understood by me,

Jaymanth

Date

13.4.90

Invented by

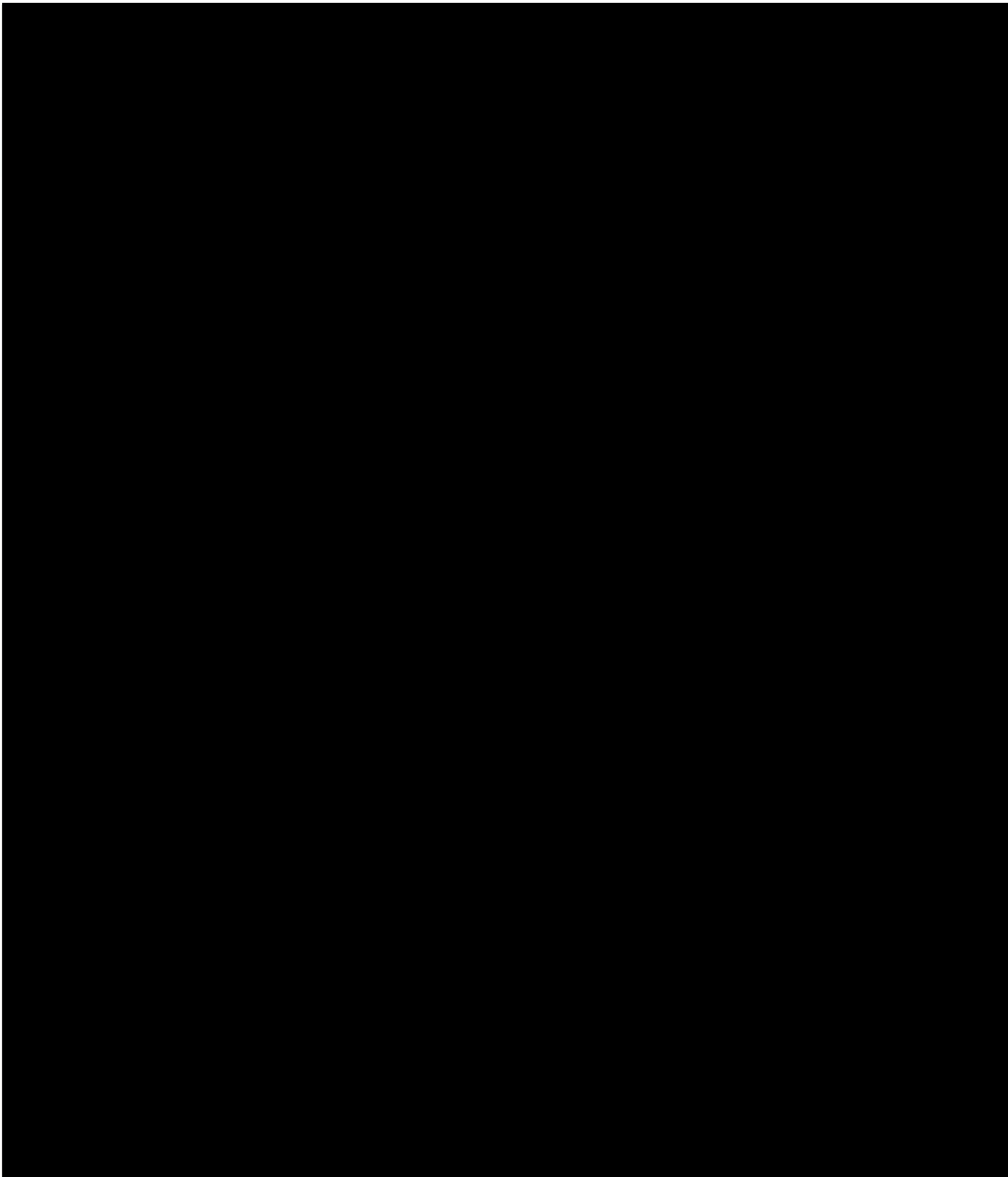
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Date

2/7/90

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Witnessed & Understood by me,

Dalman/A

Date

13.4.90

Invented by

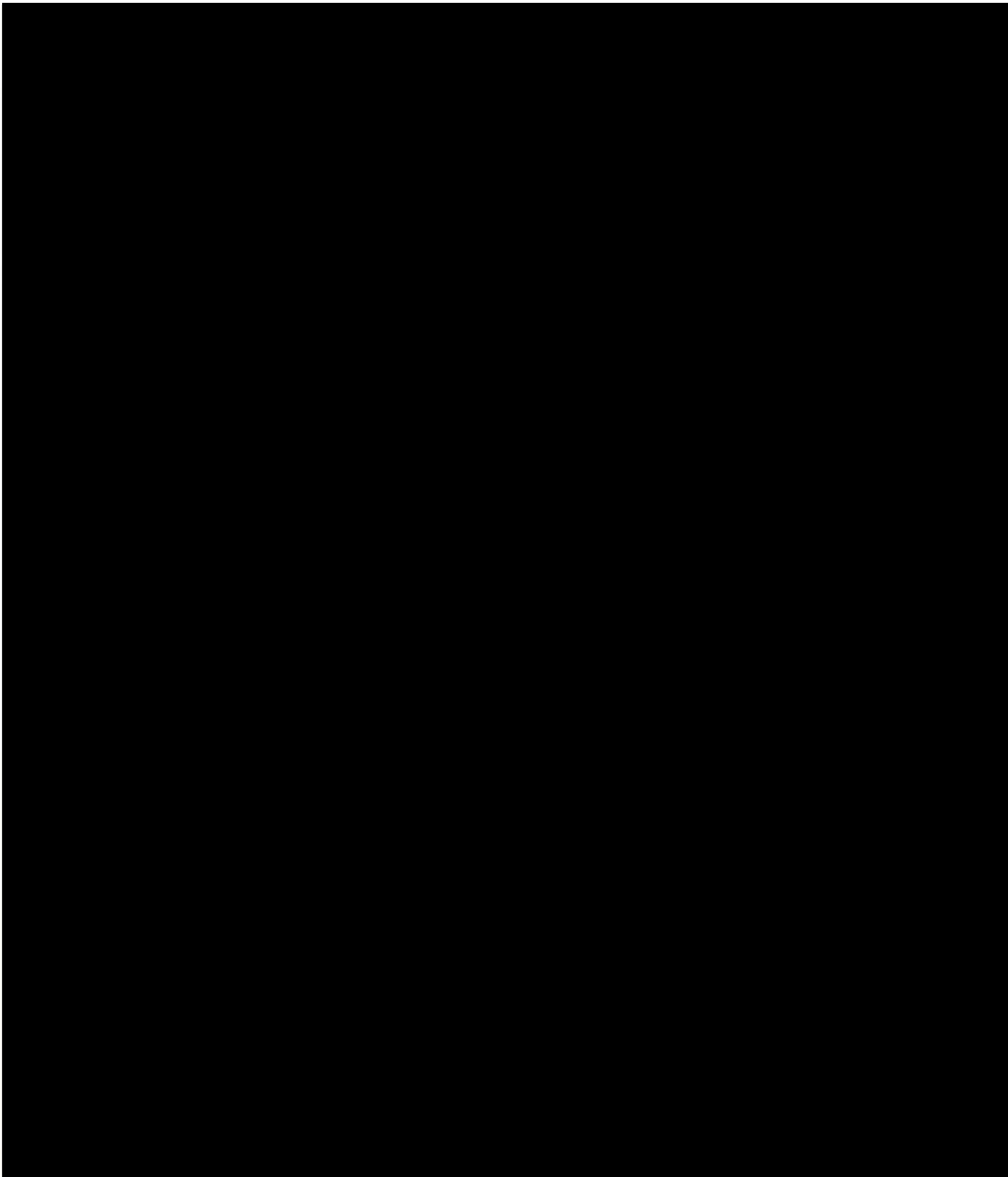
Recorded by

*John Rodriguez*

Date

2/8/90





Witnessed & Understood by me,

*Dalman A*

Date

*13.4.90*

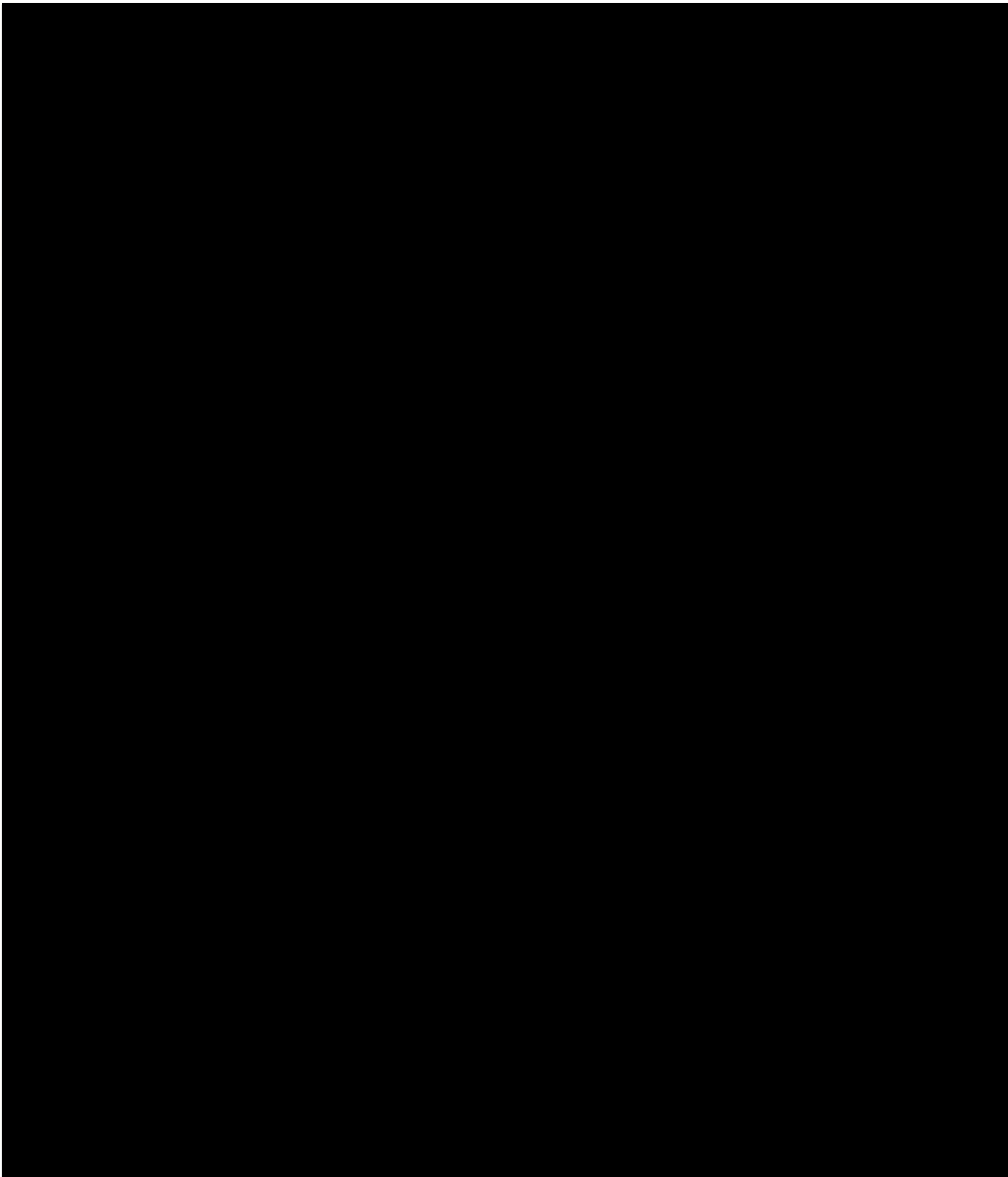
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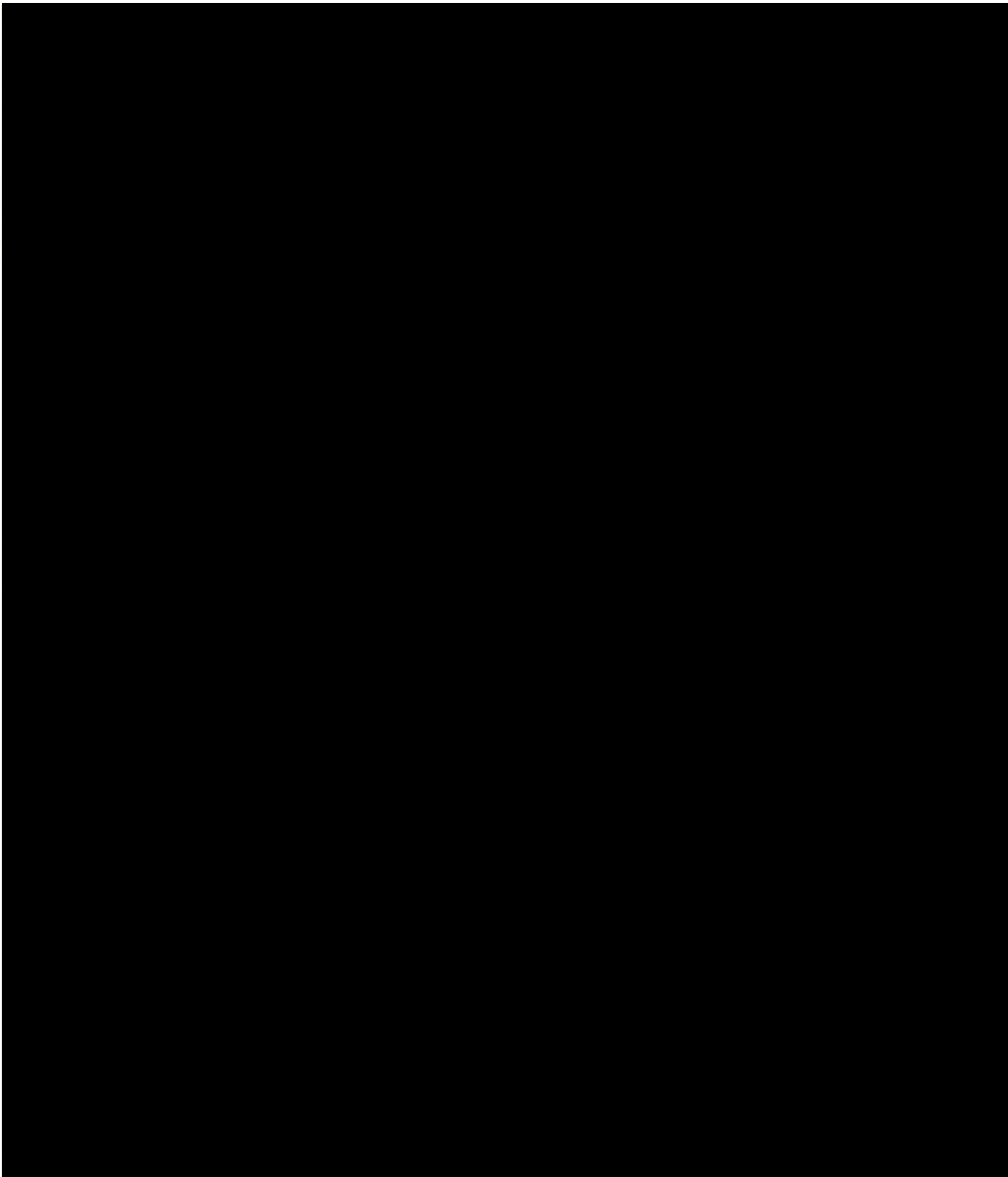
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Date

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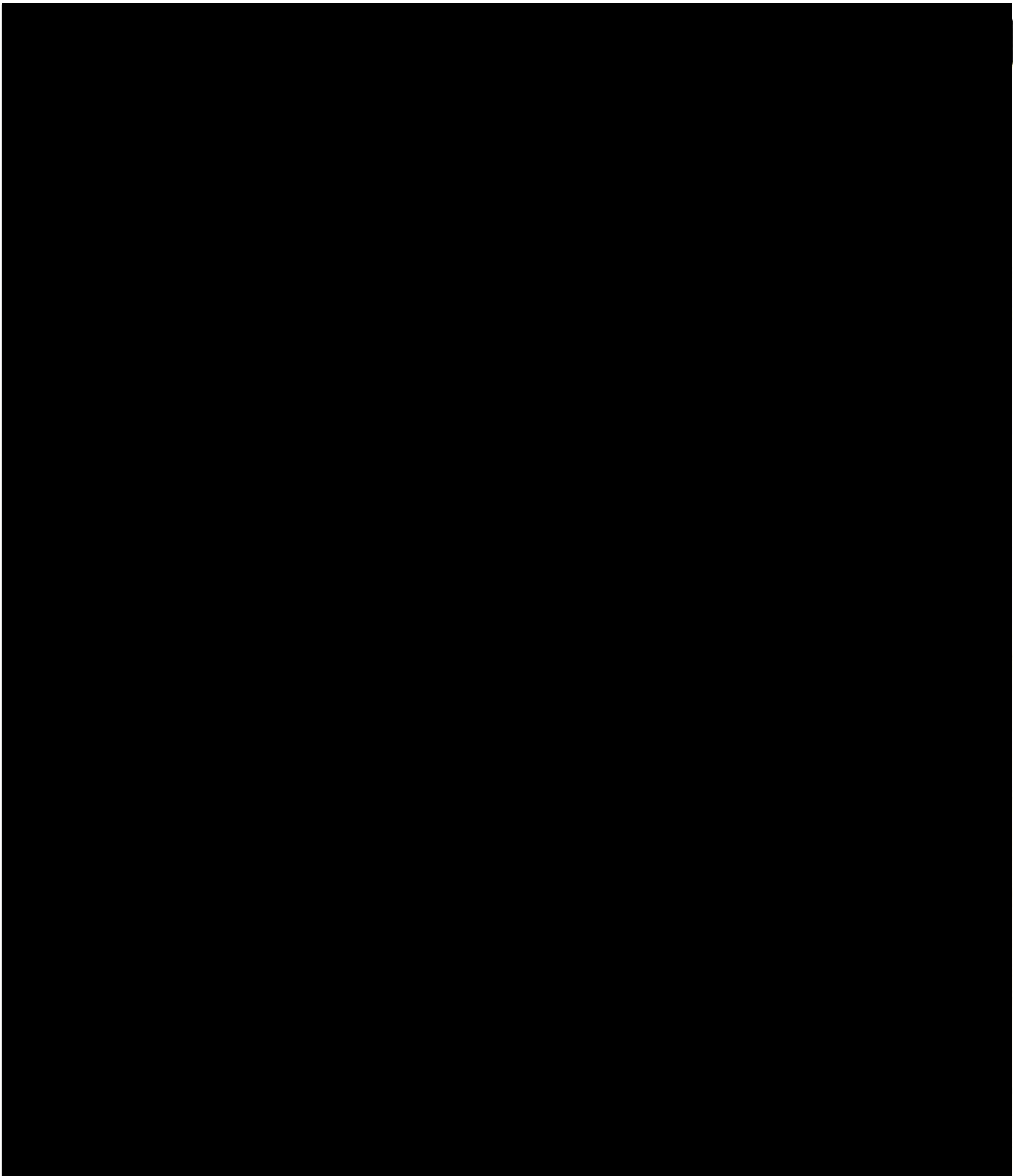
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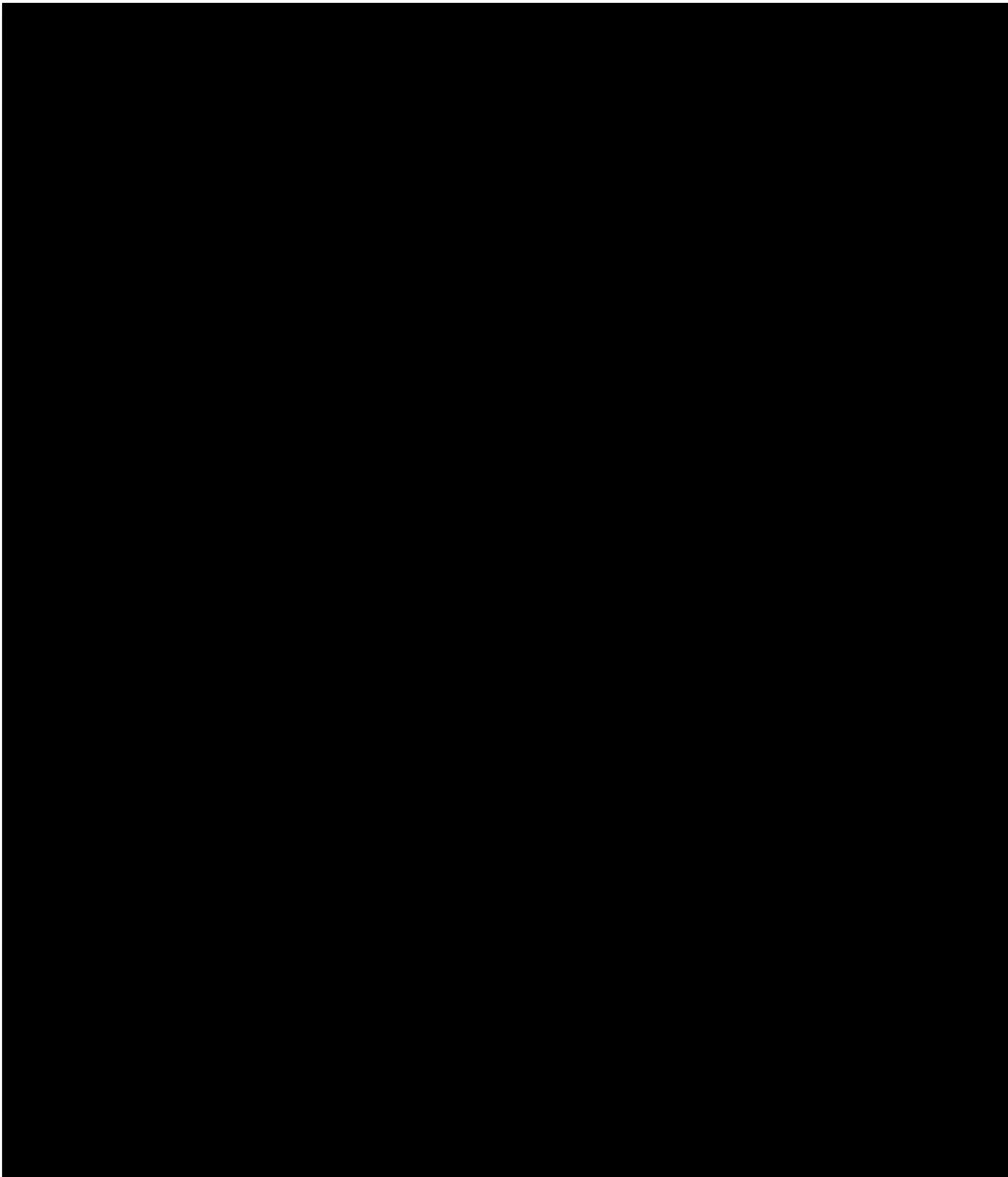
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Witnessed & Understood by me,

*Daymanth*

Date

*13.4.90*

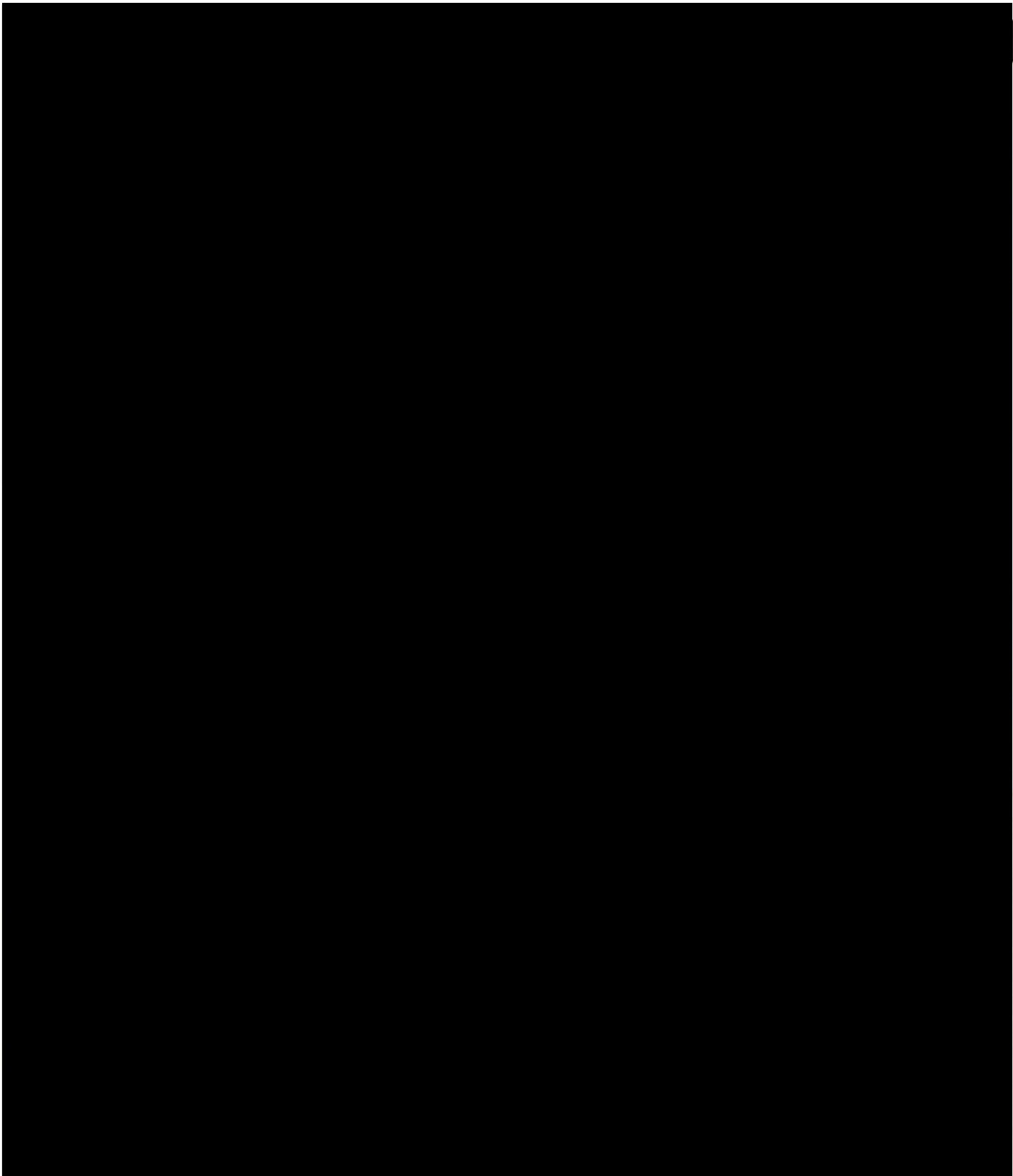
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Date

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Witnessed & Understood by me,

*Dan Maniff*

Date

*13.4.90*

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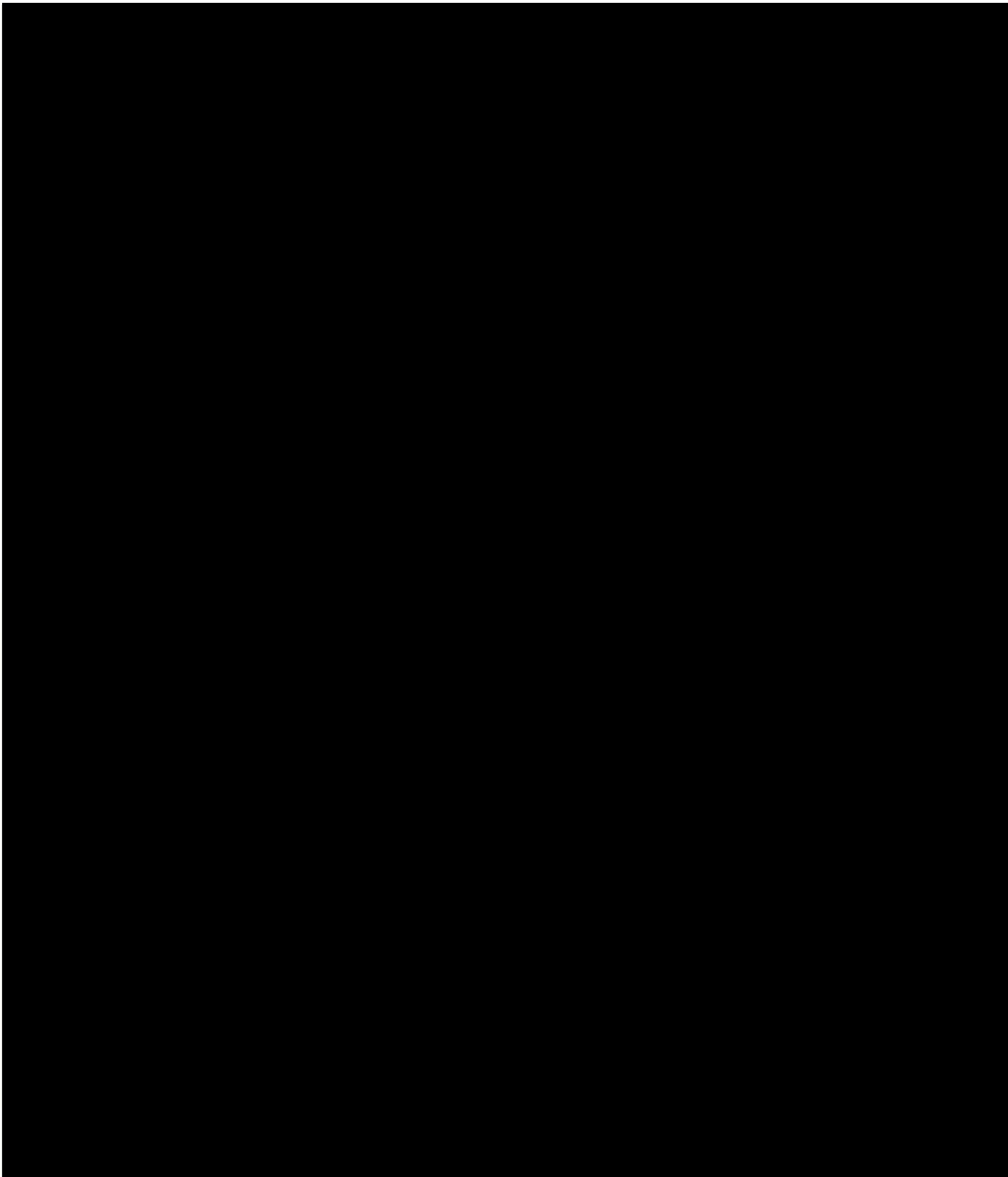
Recorded by

*John Ridge*

Date

*2/9/90*

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Witnessed & Understood by me,

Dayman *AS*

Date

12.4.90

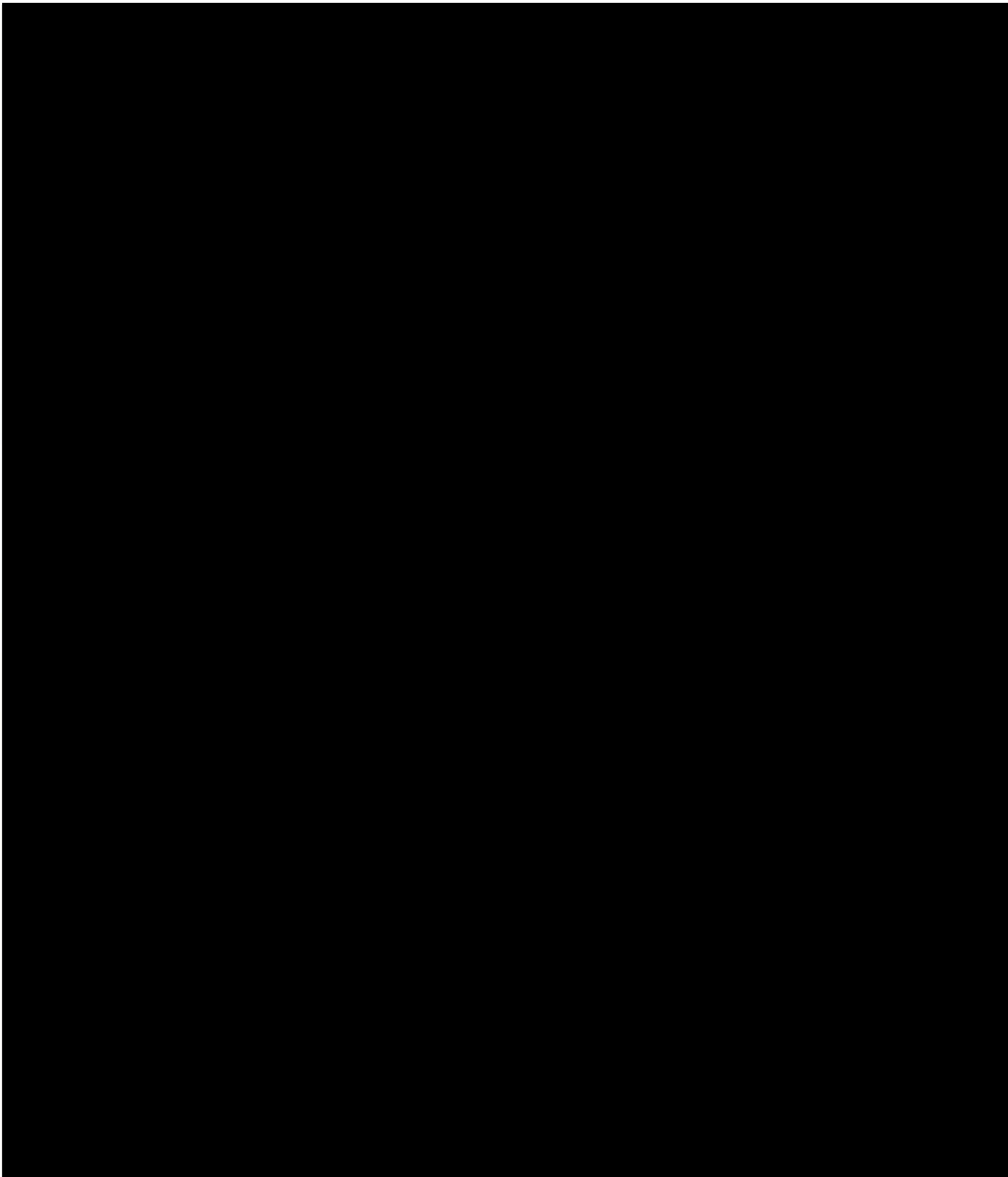
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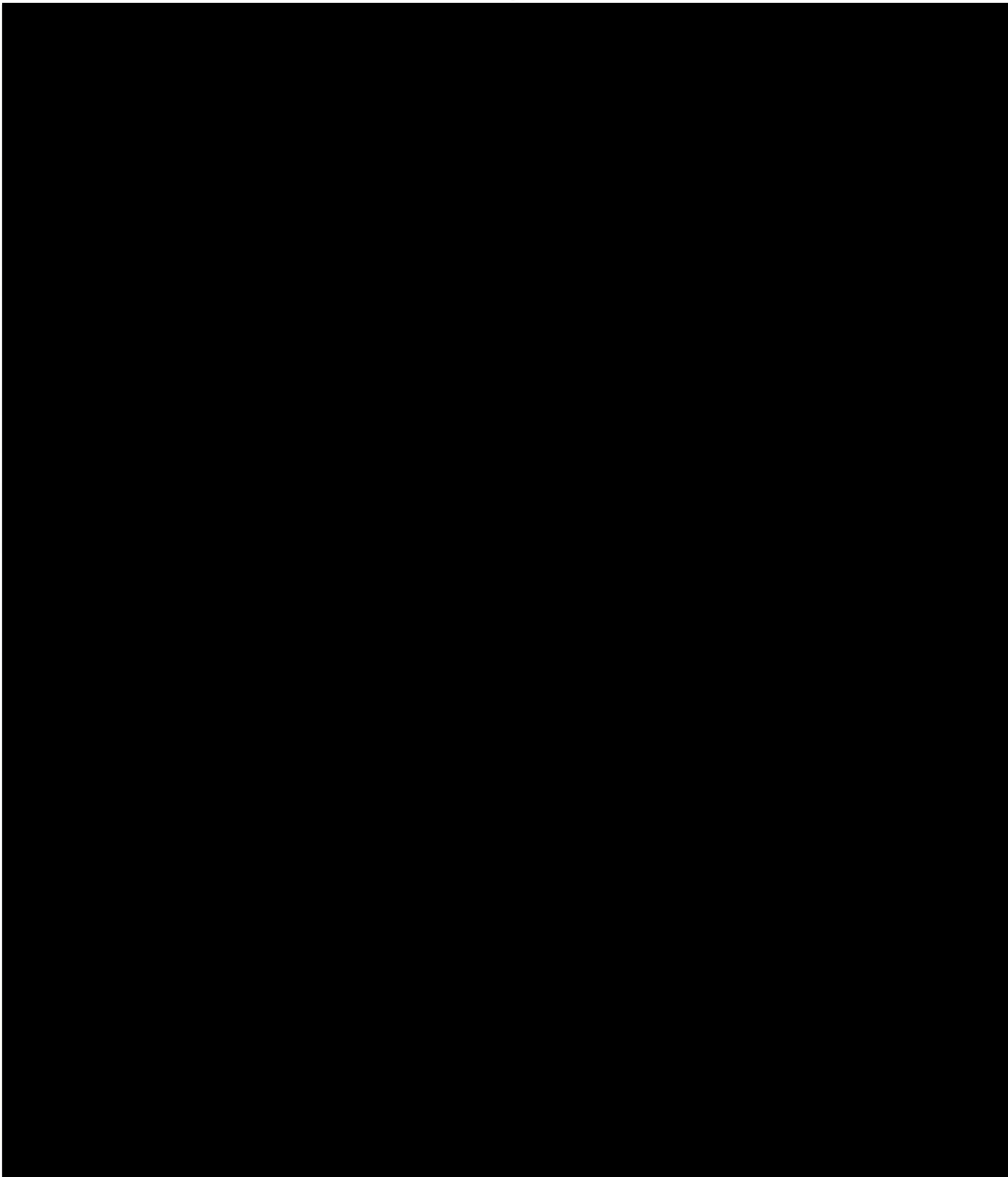
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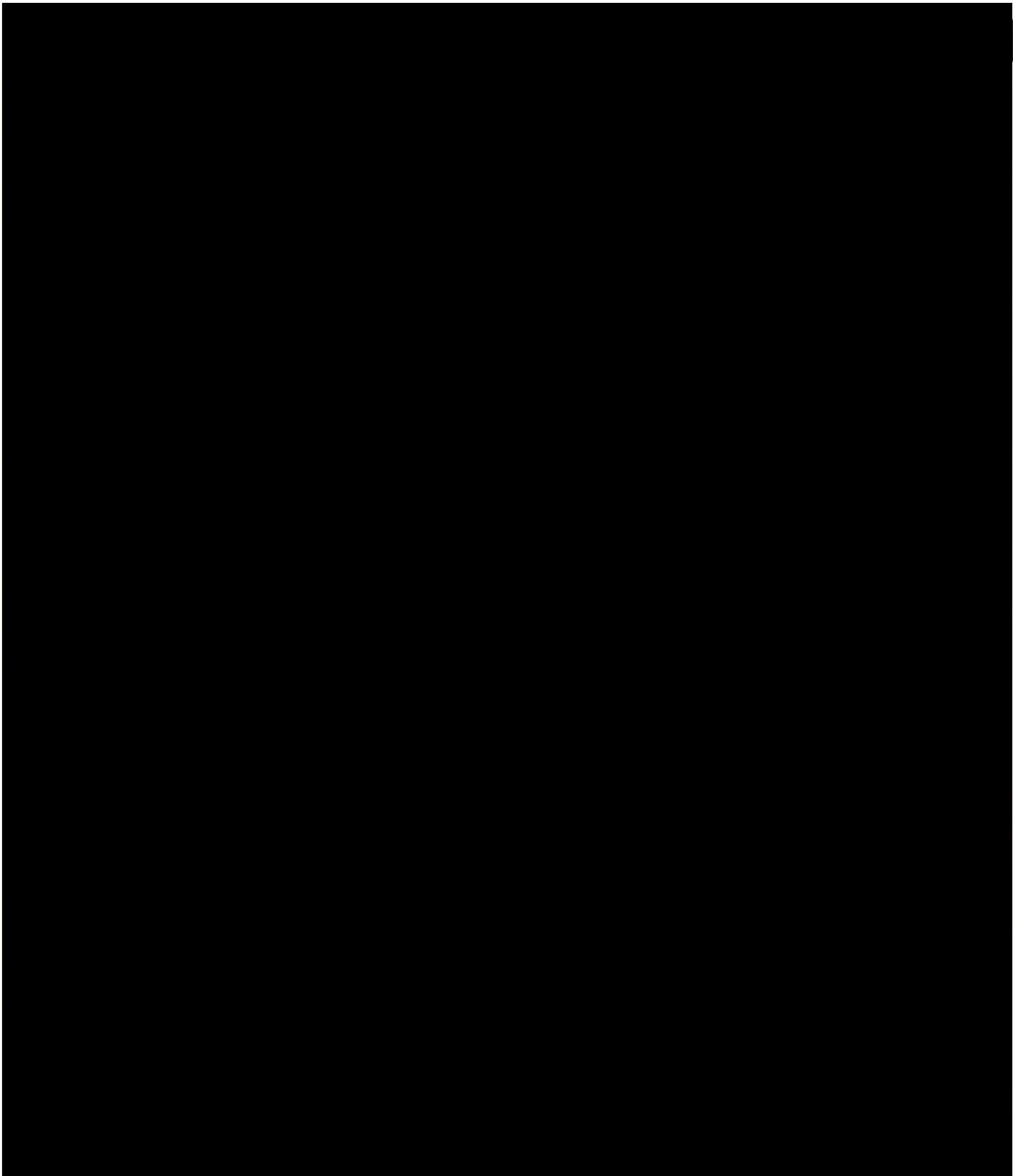


Witnessed & Understood by me, <i>Darman A</i>	Date <i>13.4.80</i>	Invented by <i>John Ridge</i>	Date <i>3/9/90</i>	To Page No. _____
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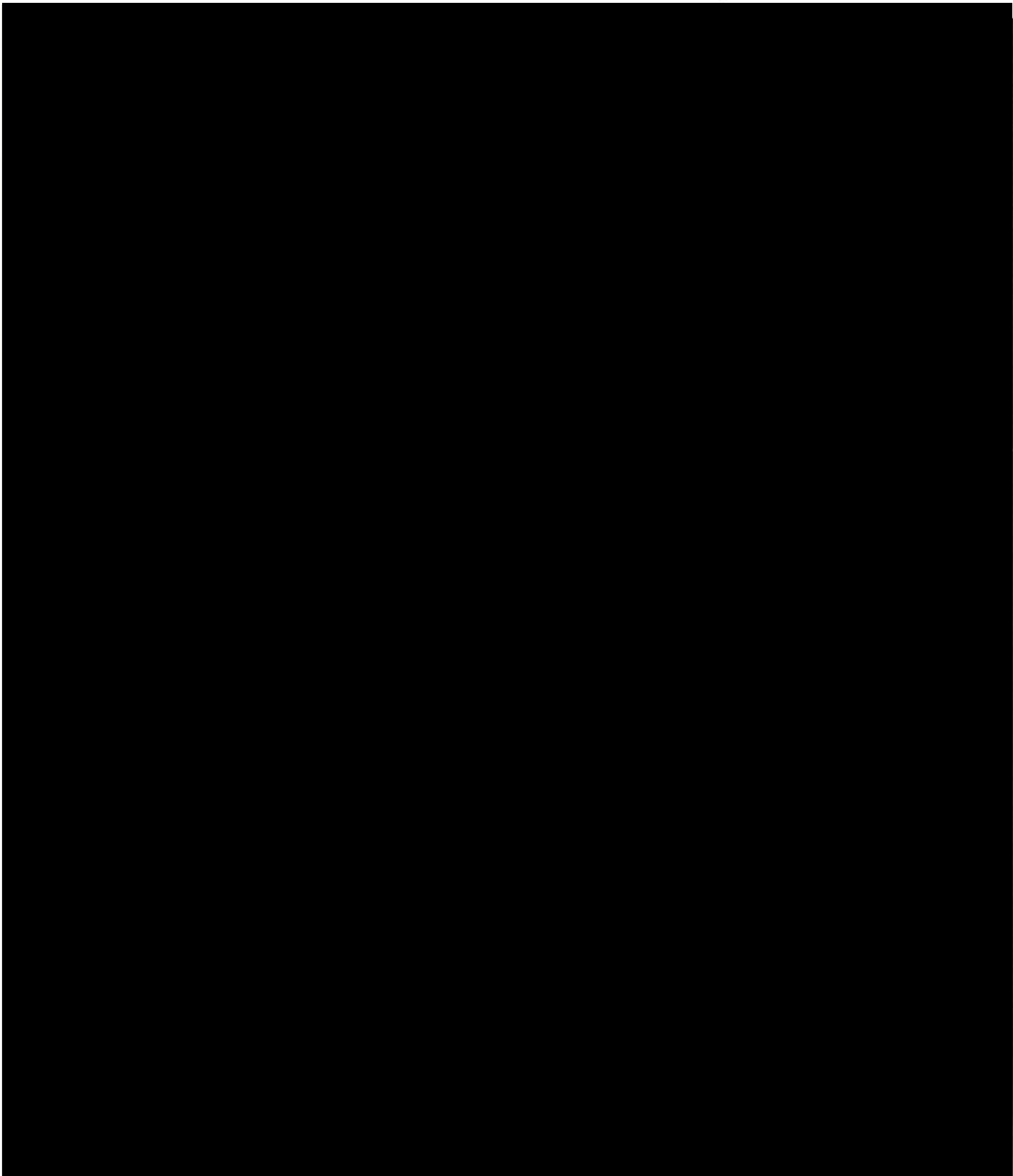




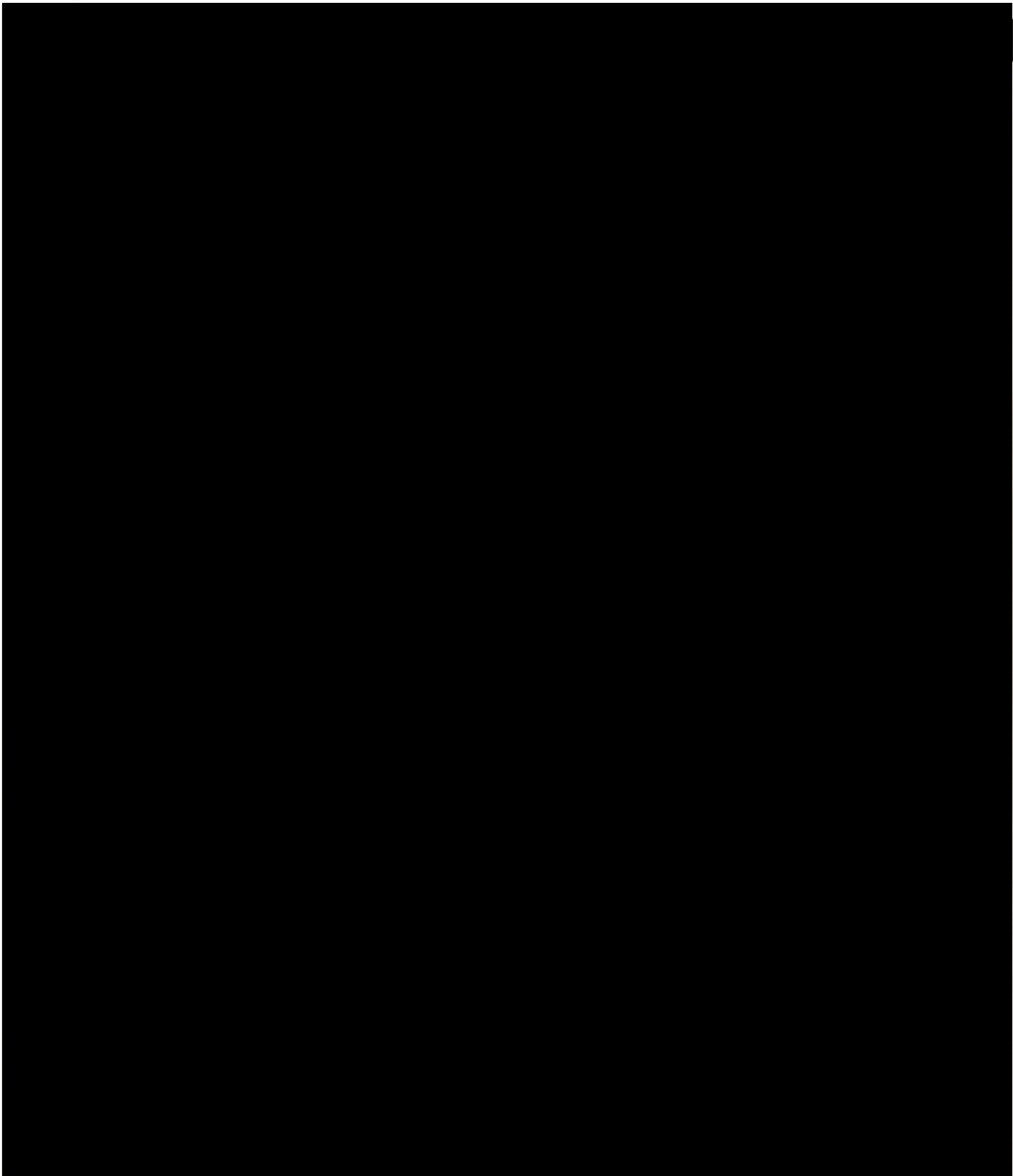
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Witnessed & Understood by me,		Date	Invented by	Date	To Page No. _____
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Witnessed & Understood by me,

Dad/mairek

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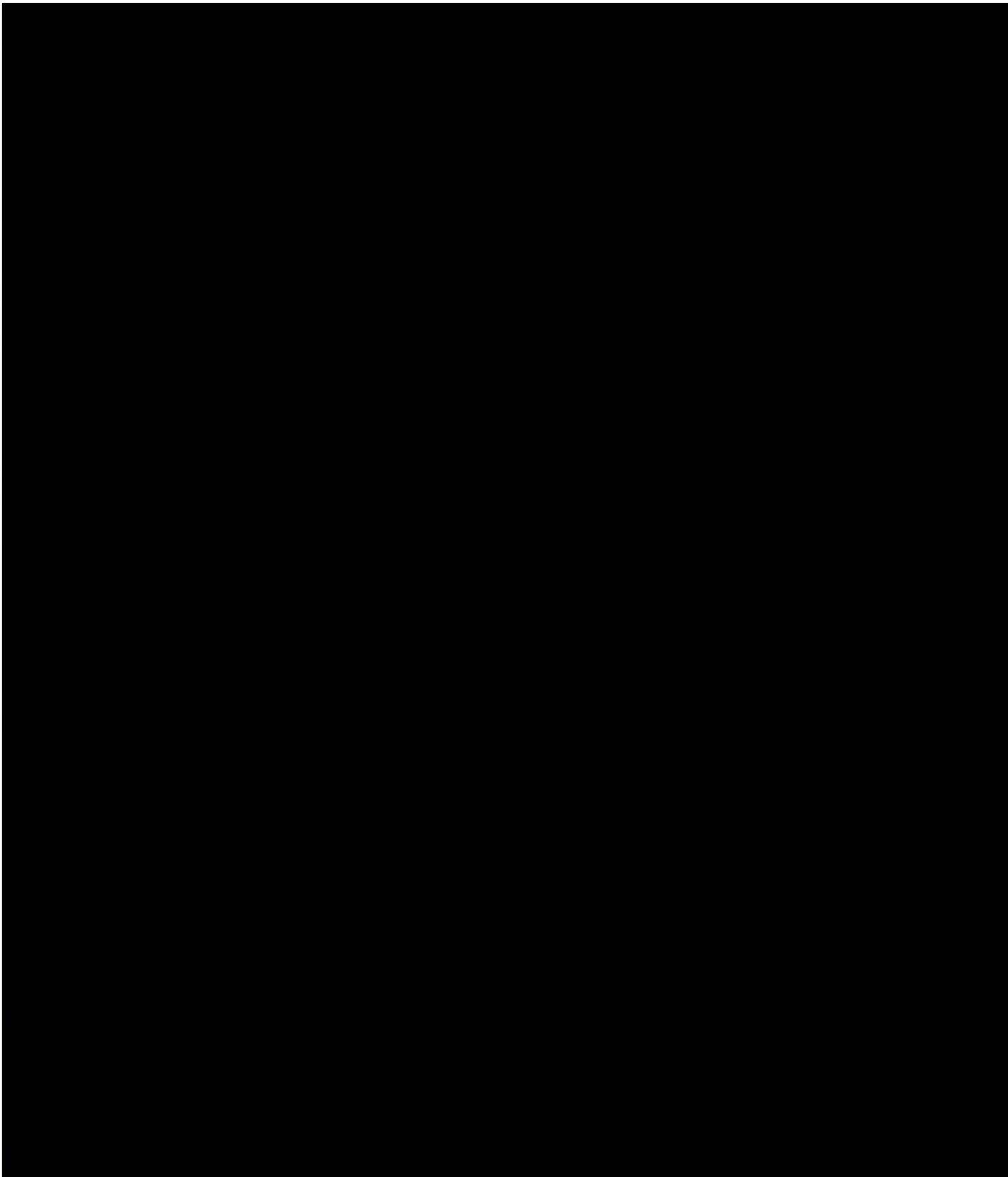
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*John Ridgway*

Date

4/17/80

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Witnessed & Understood by me,

*Daymanis A*

Date

*13.4.20*

Invented by

Recorded by

*Frank Ryan*

Date

*4/13/20*

Witnessed & Understood by me,

Dadymanis

Date

13.4.90

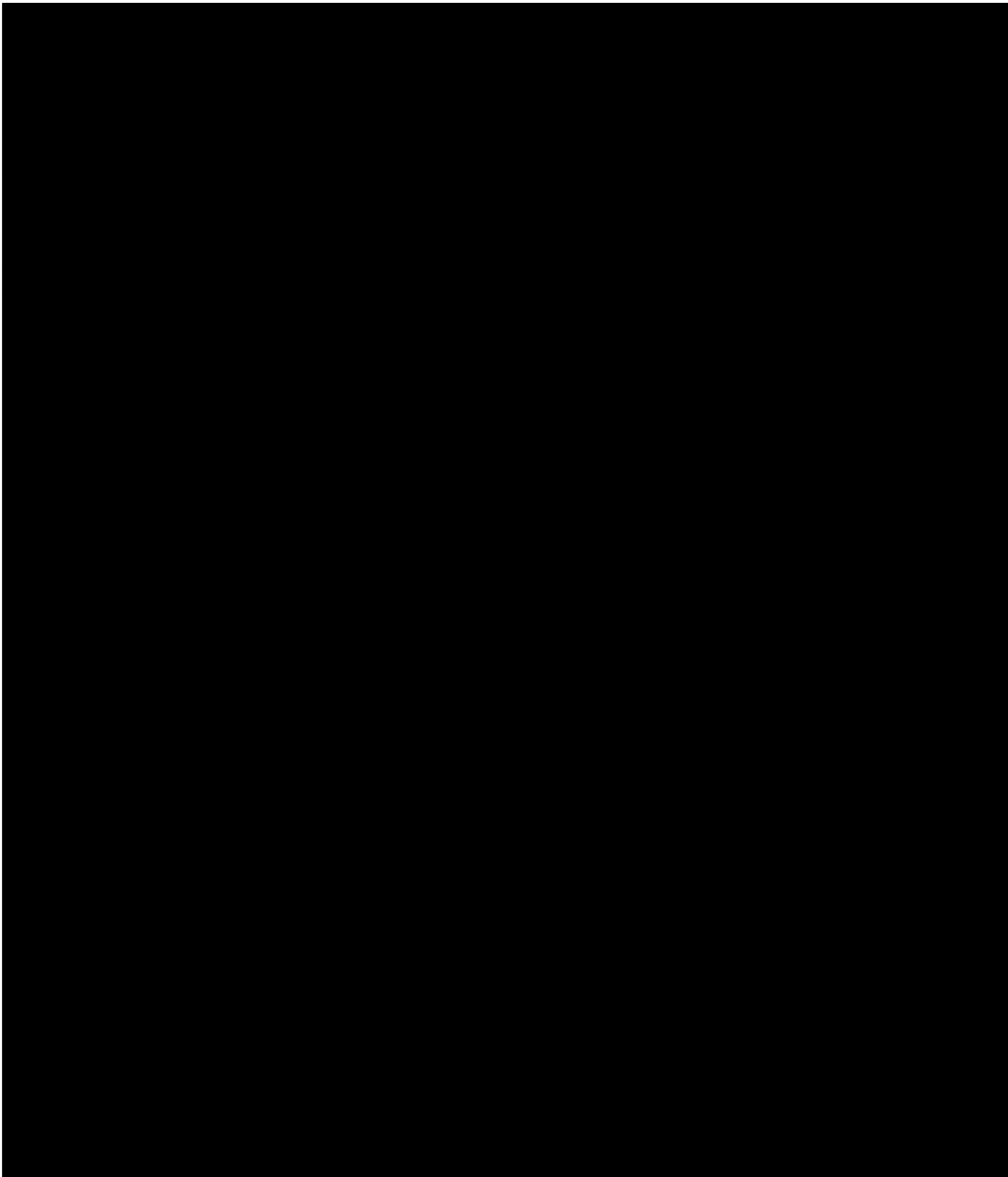
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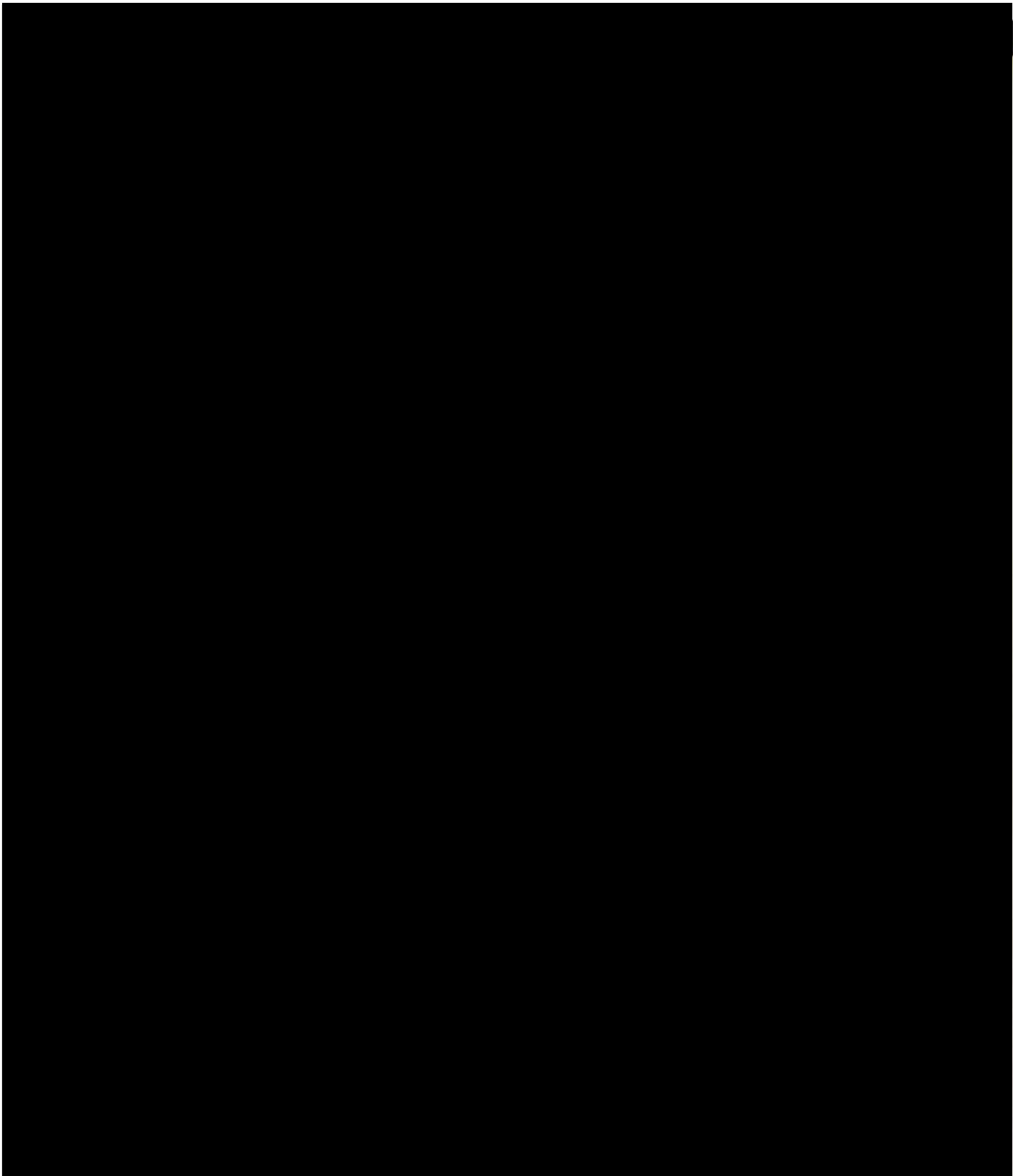
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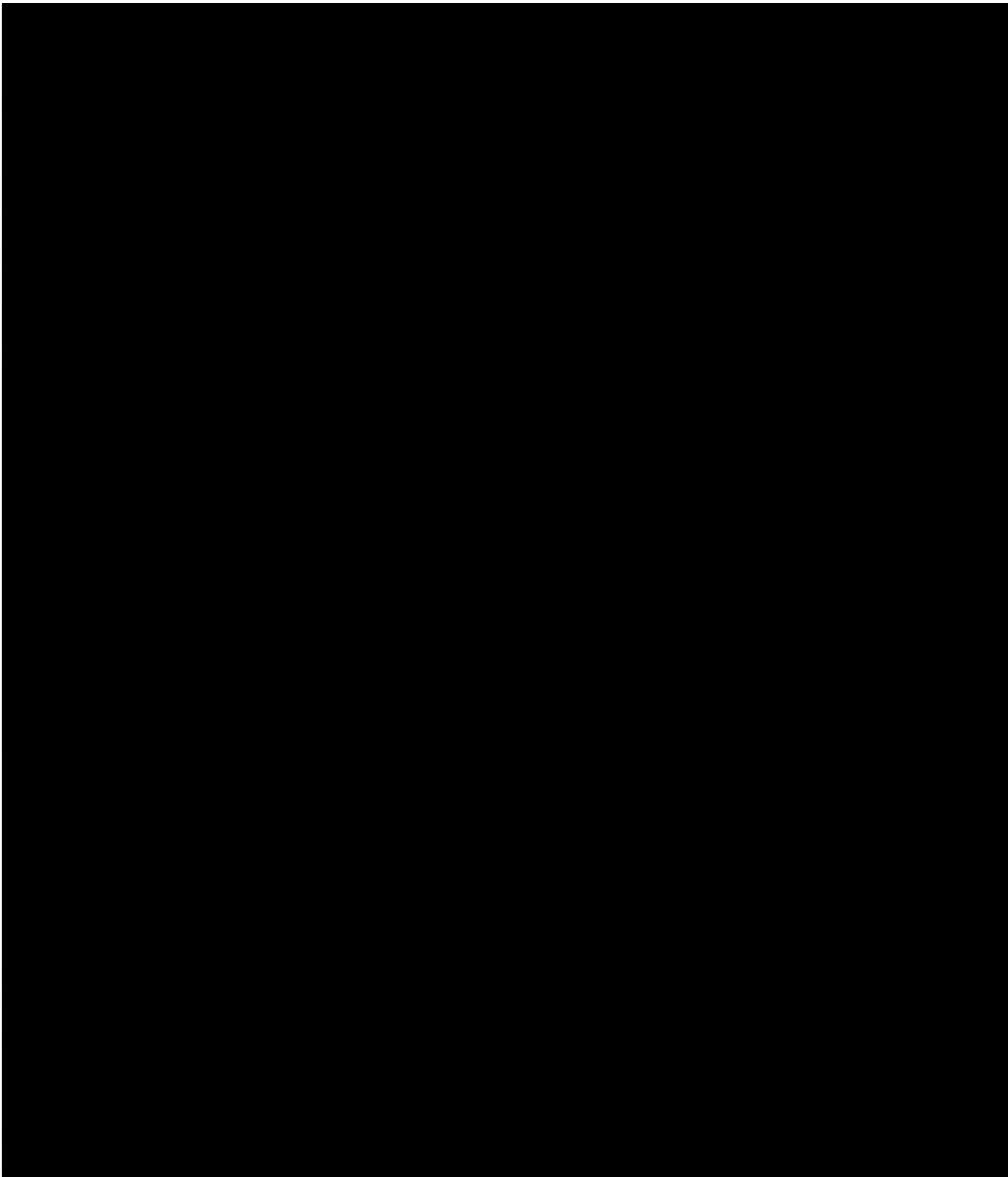


Witnessed & Understood by me, Dachman A	Date 13.4.90	Invented by <i>[Signature]</i>	Date 4/17/90	to Page No. _____
		Recorded by <i>[Signature]</i>		

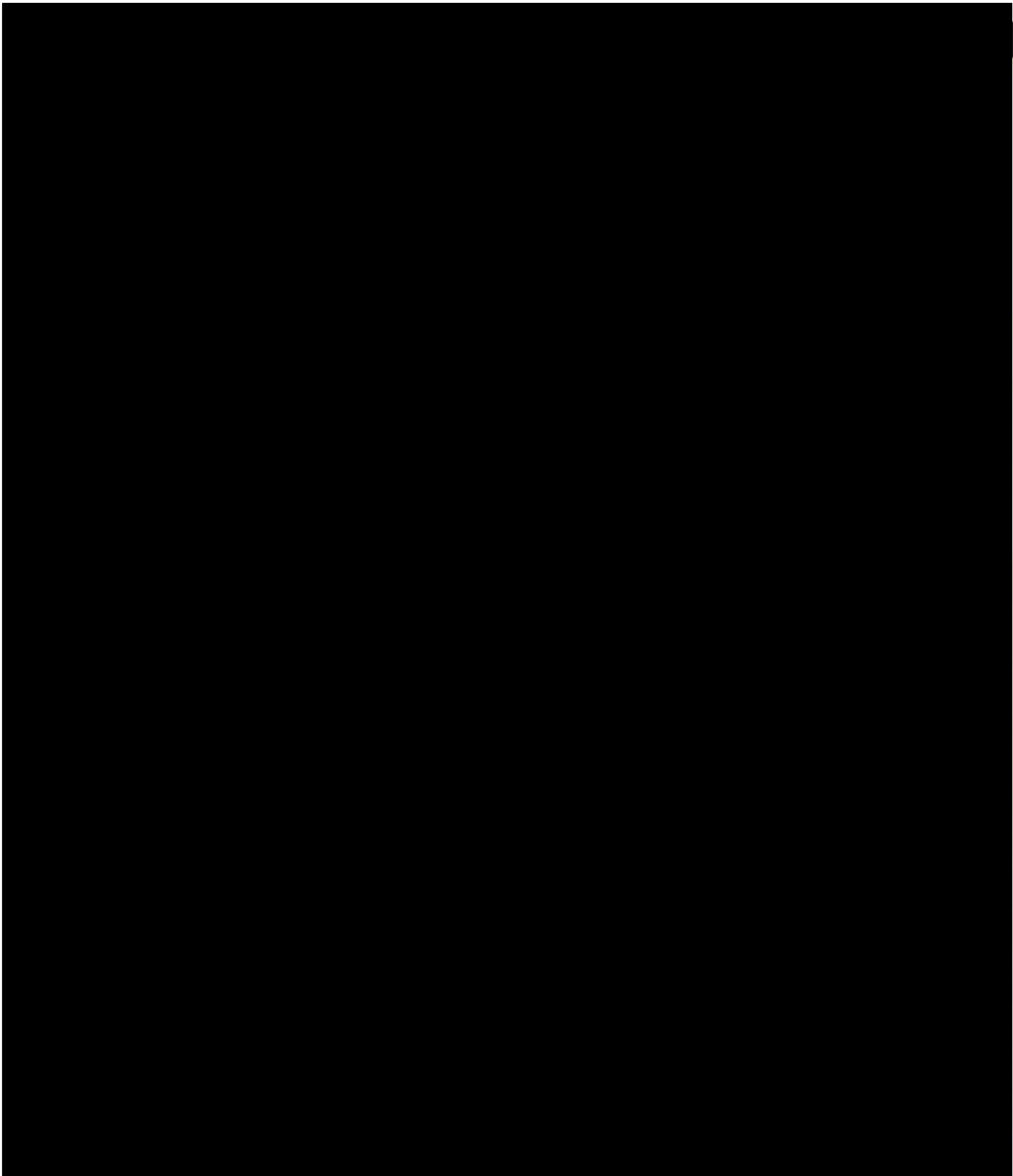


Witnessed & Understood by me,		Date	Invented by	Date	To Page No.
Deelyman		13.4.90	<i>[Signature]</i>	4/13/90	
			Recorded by		





Witnessed & Understood by me, Dayman	Date 13.4.20	Invented by [Signature]	Date 4/9/20
		Recorded by	



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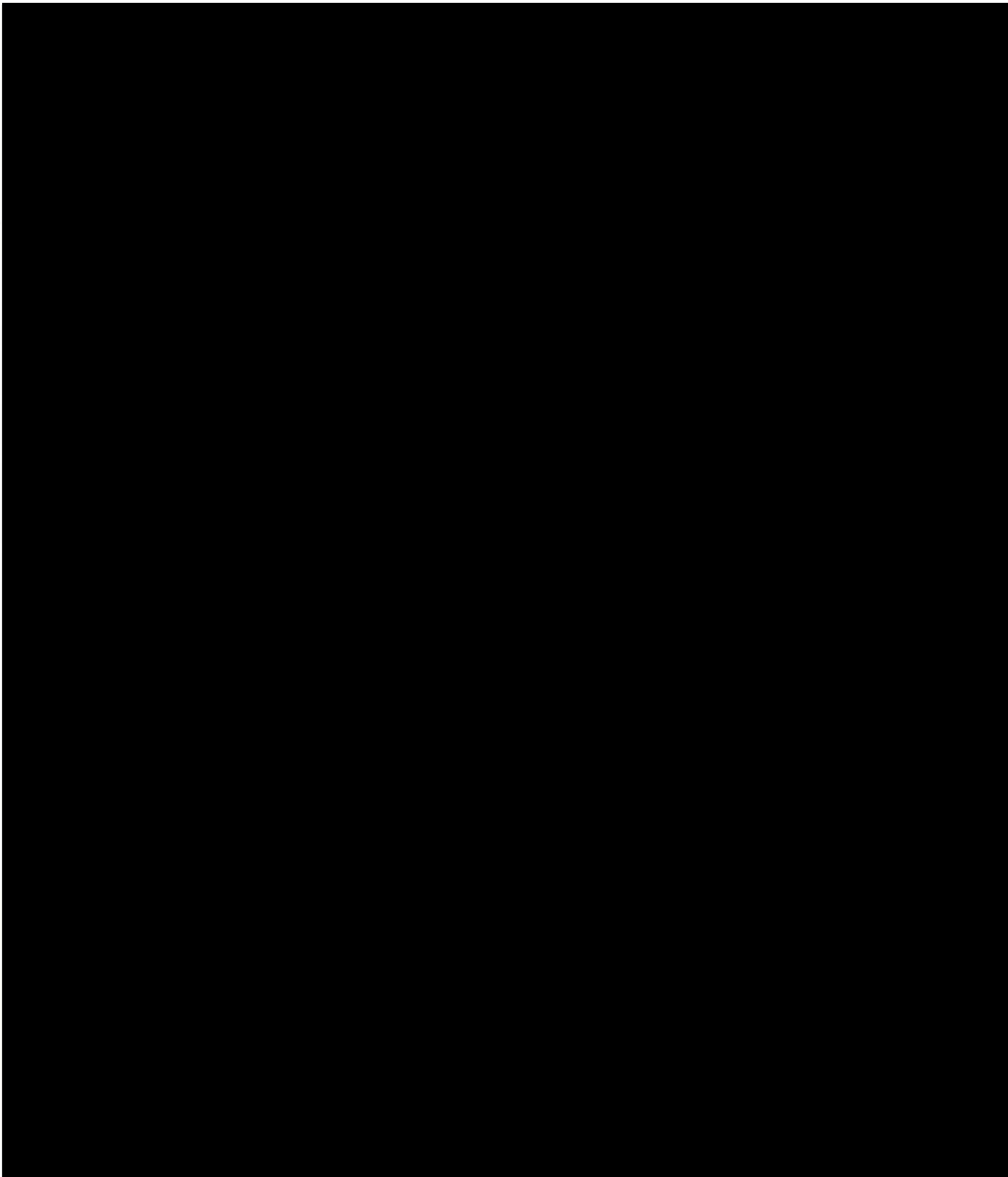
Date

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Date

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4/10/90



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Date

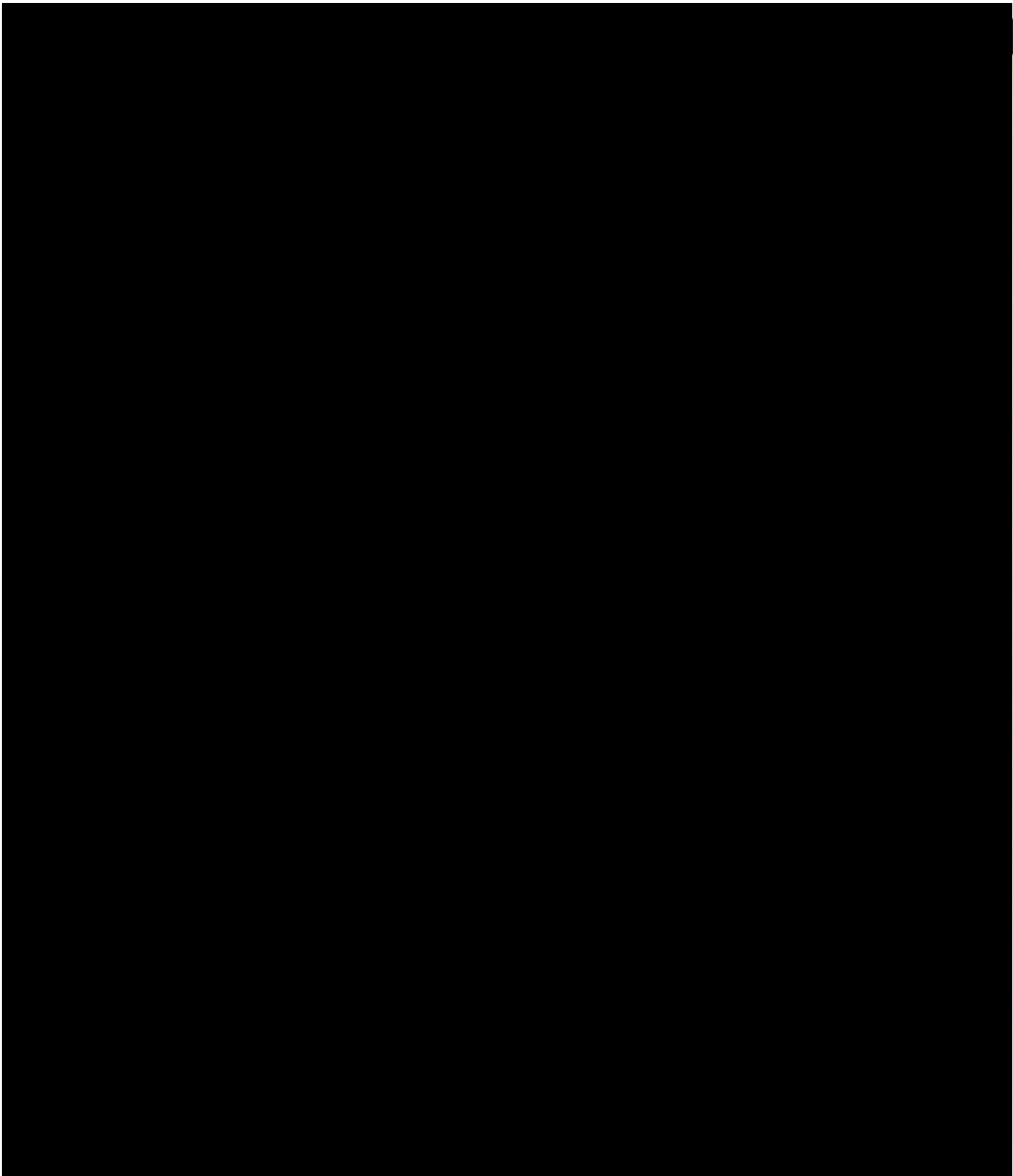
Invented by

Recorded by

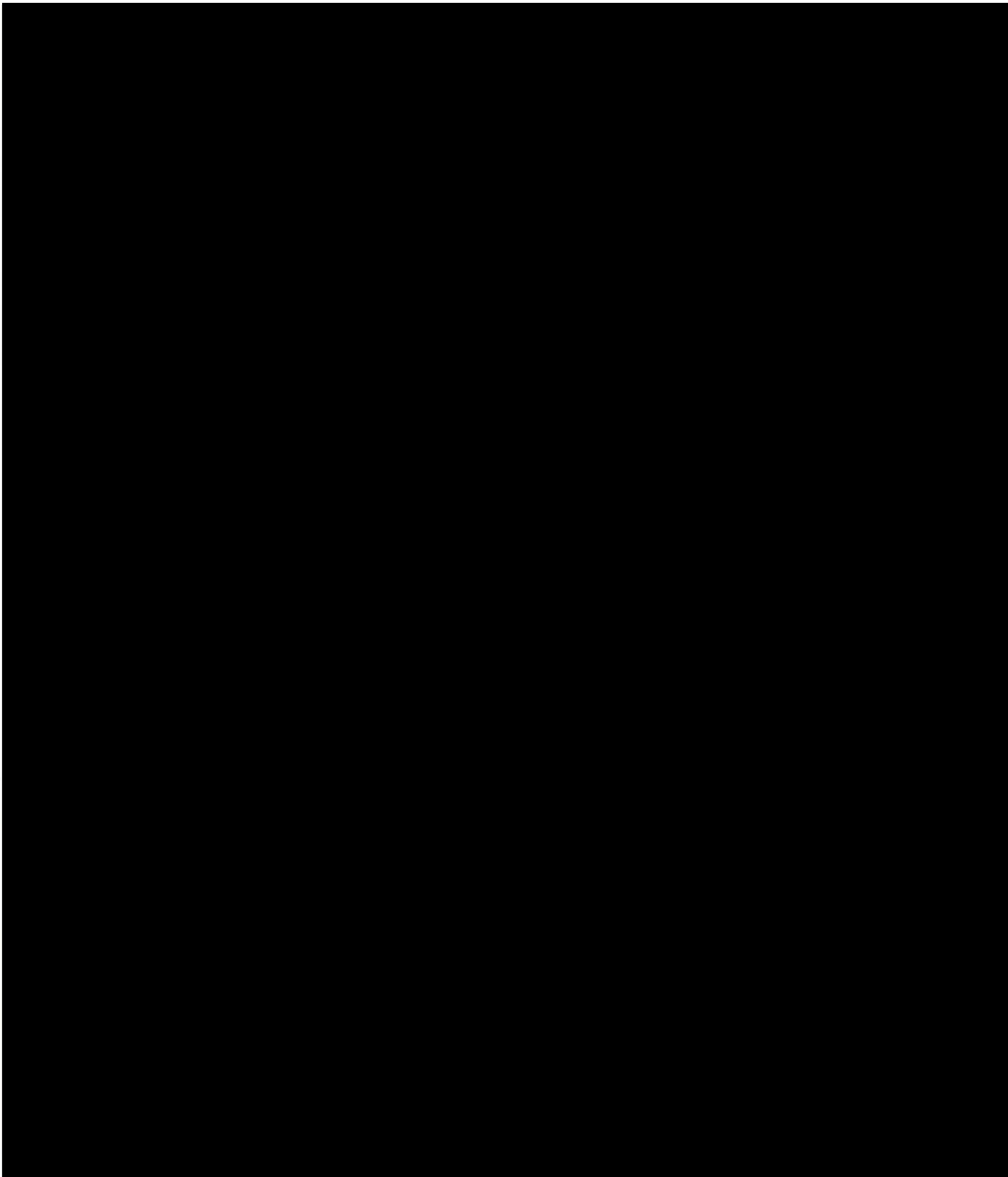
Date

*[Handwritten signature]*

4/10/97



Witnessed & Understood by me, Dalyman	Date 13.4.90	Invented by <i>[Signature]</i>	Date 4/13/91
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Witnessed & Understood by me,

Dayman *DA*

Date

13.4.90

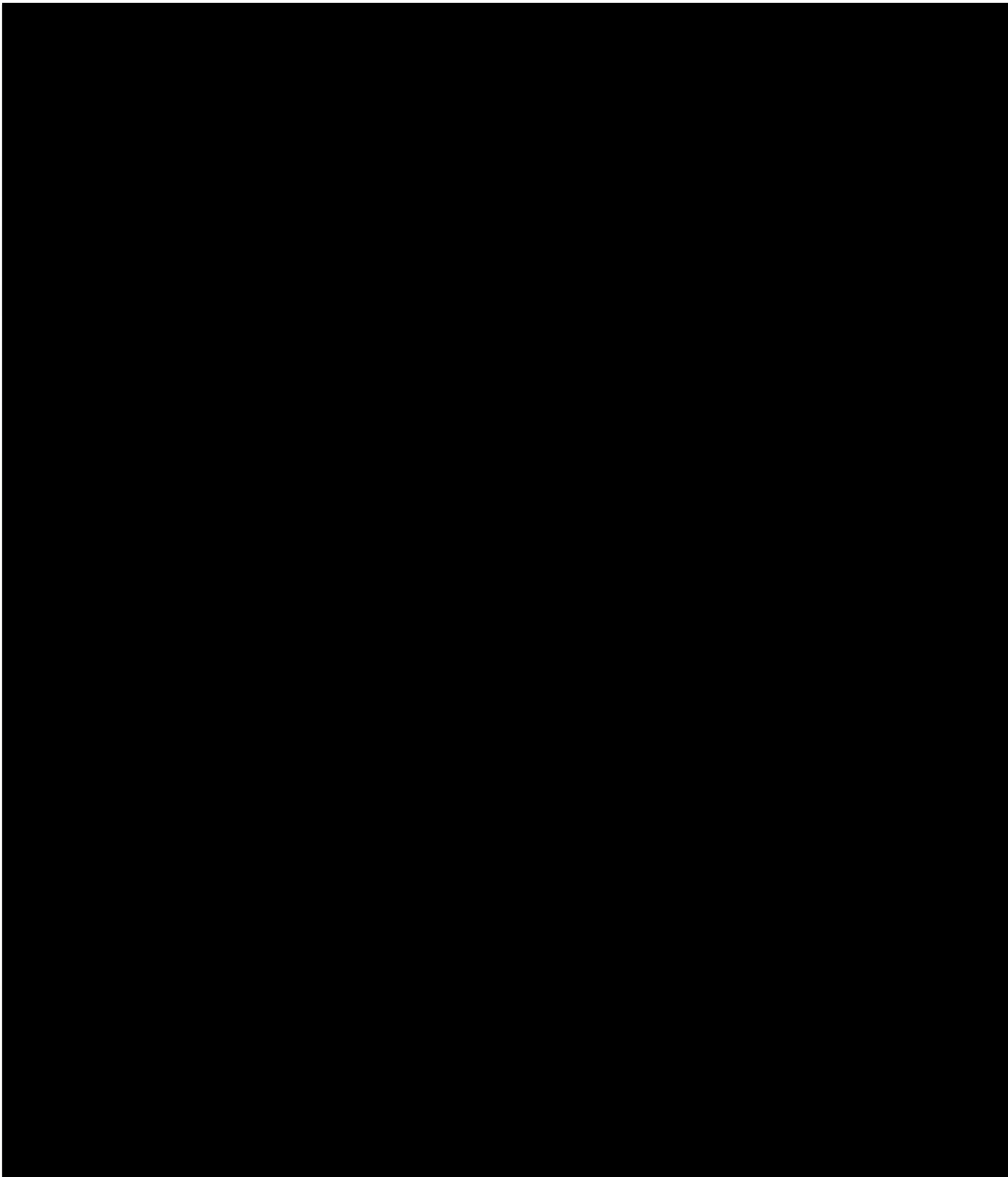
Invented by

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Date

4/13/90



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*Daisymanoff*

Date

*13.4.90*

Invented by

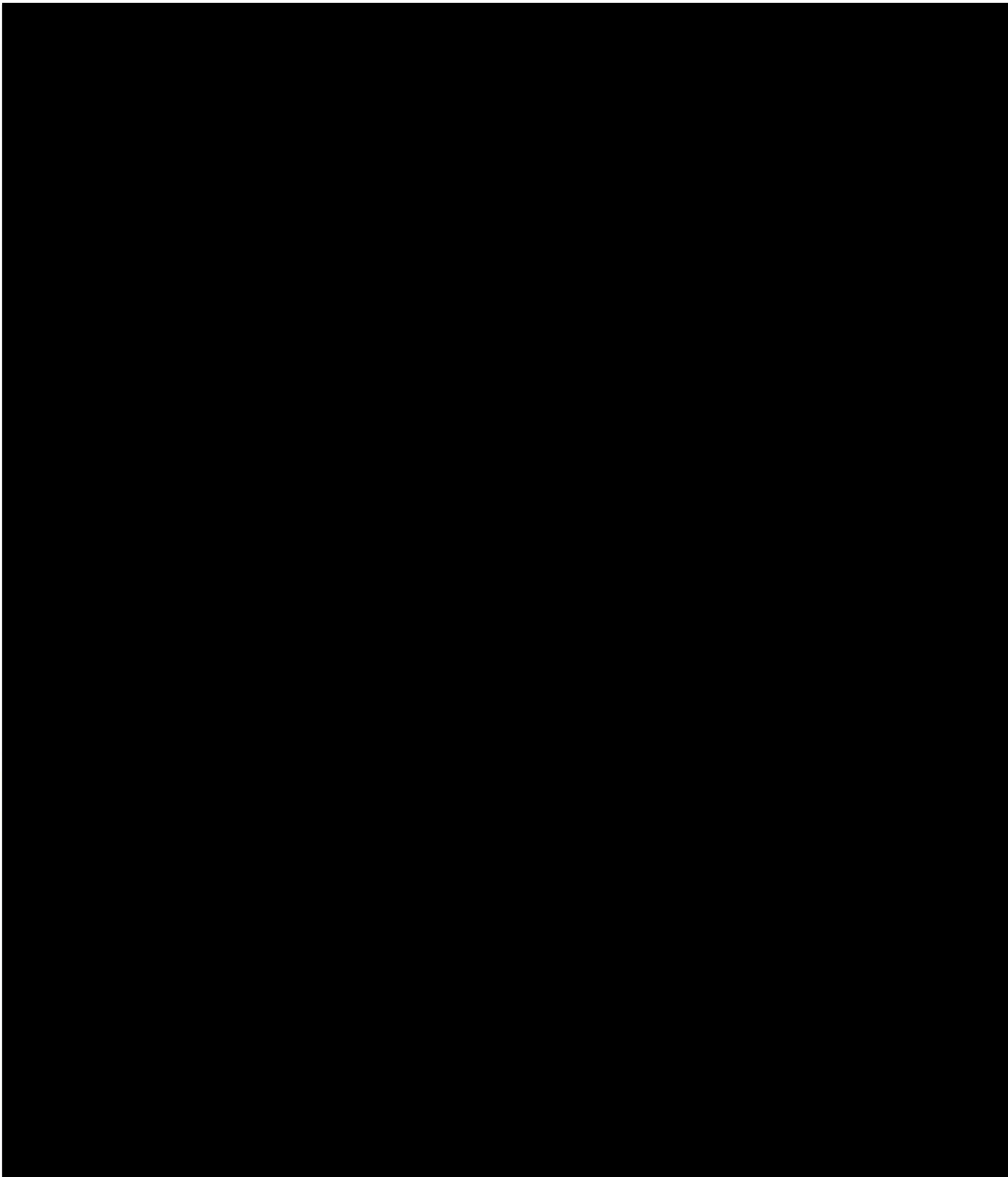
Recorded by

*[Signature]*

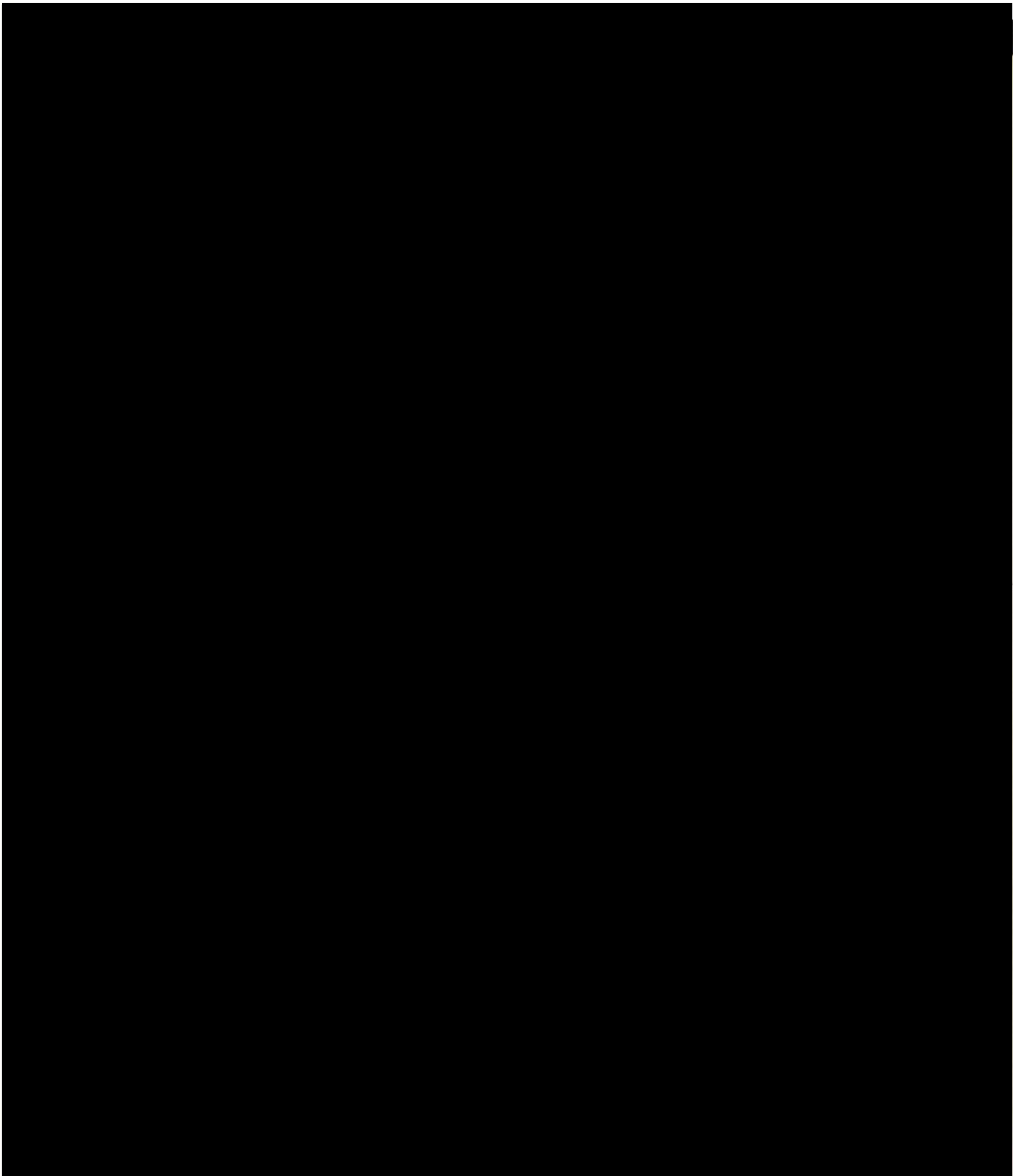
Date

*4/14/91*

To Page No.



Witnessed & Understood by me, <i>Amen</i>	Date 1-7-80	Invented by <i>[Signature]</i>	Date 5/22/91
		Recorded by	



Witnessed & Understood by me,

*Amir A*

Date

*1-7-90*

Invented by

*[Signature]*  
Recorded by

Date

*5/2/90*



From Page No. \_\_\_\_\_

5/15/90 The first variant of humanized 4A5 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(b)] w/ ApaI and PvuII → ~350bp 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/ Rit and AccI → insert VL-b frag ~550

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

cut clone 4/3 Rit-AccI Insert VL-c → frag ~550.

To Page No. 62

Witnessed & Understood by me,

*Bomanifk*

Date

1-7-90

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Recorded by

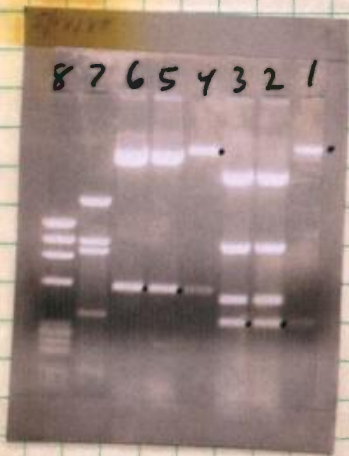
Date

5/22/90

From Page No. 61

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

- 1) PAS - PvuII + ApcI
- 2) Clone 1/1 VH(B) - PvuII - ApcI
- 3) Clone 2/3 VH(C) - PvuII - ApcI
- 4) PAS - Rfl - AccI
- 5) Clone 3/2 VL(B) - Rfl - AccI
- 6) Clone 4/2 VL(C) - Rfl - AccI
- 7) PBR722 w/ BstXI
- 8) ~~EX174~~ HaeIII



Should be only 1 band at 365

lane 1) Vector PAS - has faint bands - possibly contamination or pvuII site not deleted? (Recheck Vector)

Cut out bands next to dots for constructions

Witnessed & Understood by me,

*SmainA*

Date

1.7.90

Invented by

Recorded by

*John Redger*

Date

5/22/90

To Page No. \_\_\_\_\_

From Page No. \_\_\_\_\_

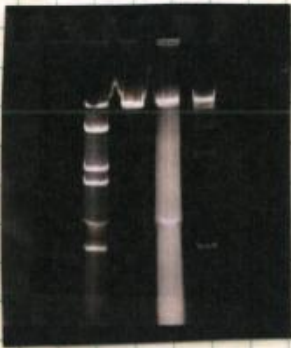
5/17/90

Lanes) 1) pAS - PvuII + ApaI (left over from same digest run on lane 1 of Agar gel p62.)

2) #316 clone 41 (pAS mini screen) PvuII (forgot to use PvuII)

3) pAS - PvuII

4) PBR BstNI



Results: 1) pAS - PvuII has contamination for other lanes.

2) No apparent multi cuts from PvuII - could it be the ApaI

5/19/90 Do more complete digests

- 1) pAS - PvuII
- 2) pAS - ApaI
- 3) pAS - PvuII + ApaI
- 4) #316 PvuII
- 5) #316 ApaI
- 6) #316 PvuII + ApaI
- 7) PBR 322 = BstNI



Results: pAS Vector is O.K.  
PvuII and ApaI are unique.

To Page No. \_\_\_\_\_

Witnessed & Understood by me,

*Omarina*

Date

1.7.90

Invented by

Recorded by

Date

5/22/90

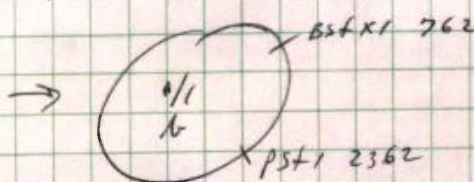
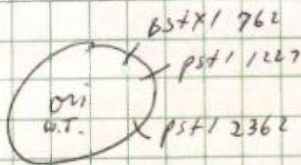


From Page No. \_\_\_\_\_

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 sites at 1227 and 2362  
BstXI site at 762



Vector cloning  
w/o insert, then  
cut w/ pst2 + BstXI  
work for 365 bp.

Frags  
1135  
465

frags  
1600

1600 - 365 = 1235



Lane 1	pAS VH(b) 1/1	-1
2	"	-2
3	"	-3
4	"	-4
5	pAS Wild type (a)	
6	PBR pSTM 1 marker	

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

To Page No. \_\_\_\_\_

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Date

5/21/90

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE Verification of Hummerid 405 PAS VH(b) 1/1

From Page No. 65

5/22/90

Mix: Screen #4

cut again w/ pst1 + BstXI

1° pst1 37°C

2° BstXI 65°C

Also pvtI + ApeI - look for 365bp

Use marker  $\Phi$ X174 w/ HaecIII See p. 68

PAS VH(c) 2/3 done - has no changes in site  
cut w/ pvtI + ApeI to show insert w/ in  
this sequence to show correct bp seq.  
(should be O.K. since background was low)

See gel p.68, 70

To Page No. \_\_\_\_\_

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*Maria A*

Date

1-7-90

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Date

5/22/90

From Page No. \_\_\_\_\_

5/24/90 Vec cut from Soft Agar (RD - Acet) p 62  
 Inset VL (A) clone 3/2 Soft Agar.  
 VL (C) clone 4/3

Lig Vec dil 1/10 - Result: Vec control background too high  
 Vec CTR colonies same # as lig.

Cut more Vec. 100 ul RX Acet - 6hrs  
 RD - overnight.  
 B.I. Salt Acet - A  
 RD - B



Cut out Vec Band.  
 Lig use Vec 1/100 dil.

+ 3ul undiluted Inset A, C

Result: No. Vec Background.  
 Inset Lig ~ 50 colonies.

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

To Page No. \_\_\_\_\_

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*main*

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1-7-90

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5/24/90

From Page No. 67

5/22/90 6% Axy gel.

- Lanes 1)  $\lambda$  - HindIII markers
- DNA map #2) pAS VH(c) clone 2/3 #1 - ApaI + PvuII
- #3) " " #2 - " "
- #4) pAS VH(b) clone 1/1 #4 - pstI + BstXI (p65 #4)
- #5) " " - PvuII + ApaI
- #6) pA3 VL(a) clone 3/2 #1 - BglII + HindIII lig 5/10/90
- #7) " " #4 - " "
- #8) " " #9 - " "
- #9) " " #11 - " "
- #10) pA3 wildtype original Vec - BglII + HindIII
- #11)  $\lambda$  - HindIII markers
- #12) OX174 HindIII
- #13) PBR BstXI

Results:

- Lane: 1) markers
- 2) VH(c) #1 has insert -  
Seq this one
- 3) VH(c) #2 - has homolog
- 4) VH(b) #4 pst-BstXI frag is over 1350  
So it is 1600. (No pst site)
- 5) VH(b) #4 has insert.
- 6)
- 7) VL(b) suggests no BglII site
- 8)
- 9)
- 10) Control for 6-9 has BglII site.

$\therefore$  pAS VH(b) #4 is correct

Human and 4AS H+L Variants



To Page No. \_\_\_\_\_

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From Page No. \_\_\_\_\_

5/23/90 G/Accy Gel

(lanes)

- 1)  $\Phi$ X174 Ibae III
- 2) pA3(b) #9 RII+AccI - 570bp whole insert.
- 3) pA3(A) #9 BspI+HindIII - NO bands.
- 4) pA3(b) #11 RII-AccI - 570bp
- 5) pA3(b) #11 BspI+HindIII - No bands.
- 6) pA3(c) #7 RII-AccI - 570bp. ~~X~~ DNA prep.
- 7) pA3(c) #7 Xho-H<sub>3</sub> - No bands.
- 8) pA3(c) #10 Acc-RII - 570.
- 9) pA3(c) #10 Xho-H<sub>3</sub> - No bands.
- 10)  $\Phi$ X174 Ibae III
- 11) PBC BSM1



Results: pA3(b) #9, 11 have no inserts,  
pA3(c) #7, 10 are correct!

To Page No. \_\_\_\_\_

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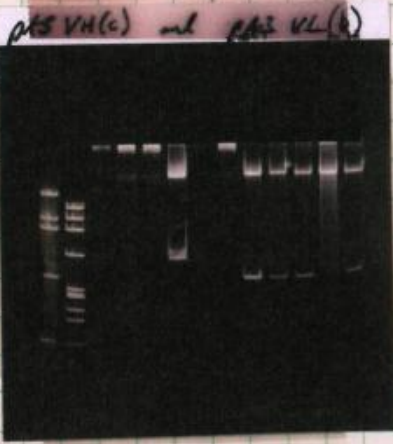
*[Signature]*

Date  
5/23/90

From Page No. \_\_\_\_\_

5/23/90 6/1 Acc-1

- Lanes: 1) PAS C #1 Agat + pVATII (Repeat from p67)
- 2) " C #3 " "
- 3) " C #4 " \* used this for Prep
- 4) " C #6 " "
- 5) PAS Vec Ctr Agat + pVATII
- 6) PA<sup>3</sup> VL(b) #6 AccI + BstII 570
- 7) " #7 " "
- 8) " #8 " "
- 9) " #10 " "
- 10) " #4 " "
- 11) " #1 " "
- 12) Markers  $\Phi$ X174 HaeIII
- 13) Markers pBR322 BamHI



Results: Lanes 1)

- 3) PAS(c) Insert present
- 4) Sub 2 of these

8) PAS(b) Insert there cut to show absence of BstII site.

To Page No. \_\_\_\_\_

Witnessed & Understood by me,

Dominic PA

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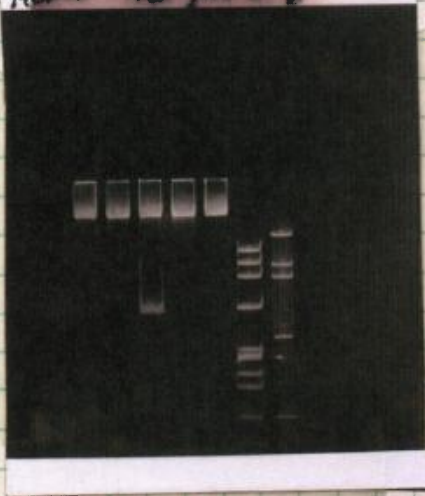
TITLE PA3 VL(b)

From Page No. \_\_\_\_\_

5/23/90 6% Acyl.

- Lanes) 1) PA3 VL(b) #13 From ligation 5/22/90 p67 1/100 ucc  
AccI + RII - 570 bp. frag
- 2) " " BglII - H<sub>3</sub> - H<sub>2</sub> frag.
- 3) PA3 VL(b) #14 AccI + RII 570 bp - ~~2~~ - DNA prep
- 4) " " BglII - H<sub>3</sub>
- 5) PA3 VL(b) #8 See p 70. BglII + H<sub>3</sub>  
It has the AccI - RII frag.
- 6) ~~OK~~ HaeIII
- 7) PBR BseNI

Gen. L #405 PA3 VL(b)



Results: PA3 VL(b) #14 is good  
PA3 VL(b) #8 is also good.

To Page No. \_\_\_\_\_

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Date

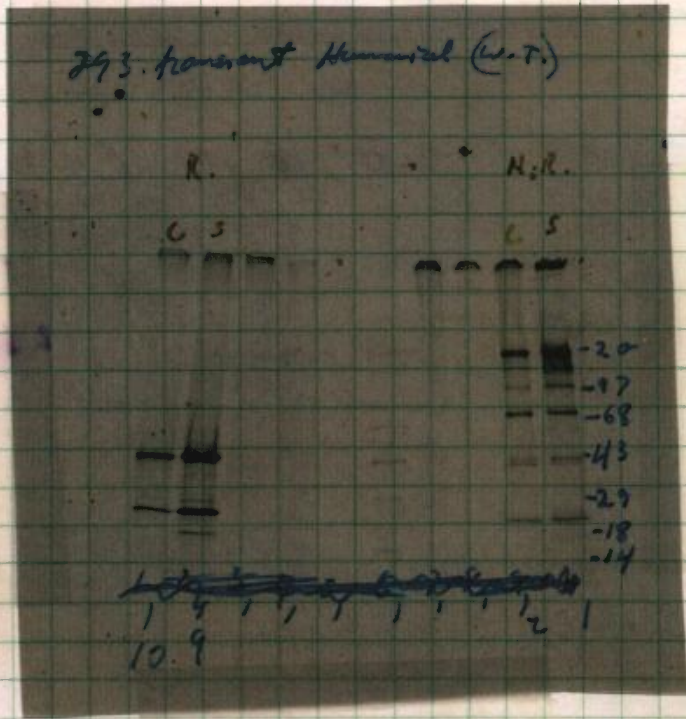
5/23/90

From Page No. NB 11162 p 51 → I.P. as usual.

5/30/90  
Lanes

4-20% precat mini gradient

- 1) 4D5 Sups - H.R.
- 2) Cells - H.R.
- 3) 293 Sups - H.R.
- 4) Cells - H.R.
- 5) 14C mouse Higgs
- 6) Prestained mouse Higgs
- 7) 293 Sups - Reduced.
- 8) Cells - "
- 9) 4D5 Hu pA3,5 - Sups - Red.
- 10) " - Cells - Red.



To Page No. \_\_\_\_\_

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Date

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6/4/90

From Page No. \_\_\_\_\_  
 6/4/90

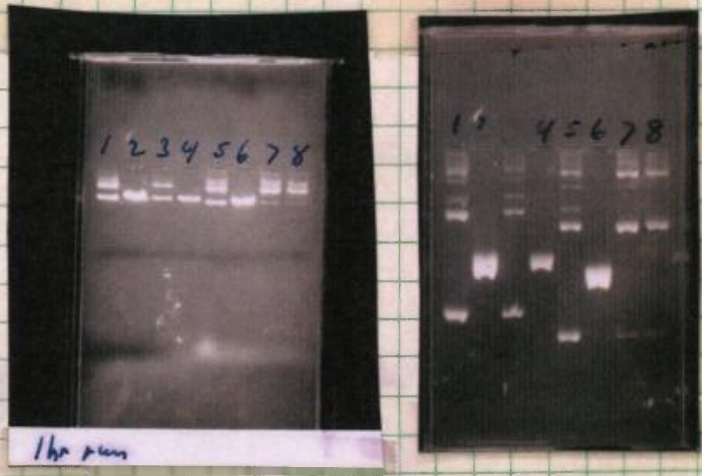
Hu 4DS DNA #	260.0	280.0	A <sub>260</sub> /A <sub>280</sub>
L (3-b-14) 1	0.7967	0.4231	1.882
L (3-c-7) 2	0.9493	0.5047	1.880
H (5-b-4) 3	0.3877	0.2054	1.887
H (5-c-4) 4	0.6207	0.3294	1.884

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 in long plane 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



To Page No. 75

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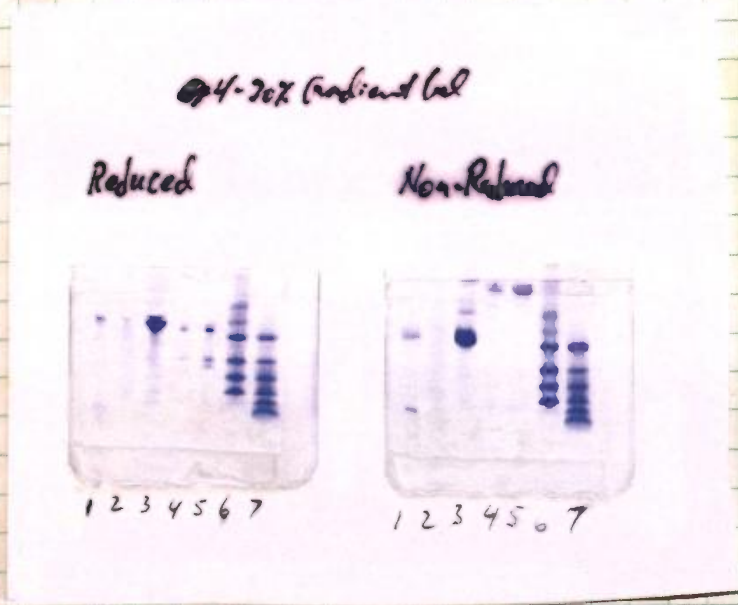
Date 6-7-90

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Date 6/15/90

From Page No. \_\_\_\_\_

5/30/90



- lanes) 1) 293 UAS chimera (3/9/90) - Inactive on Bio Assay  
50ul of Sup + 10ul protein buffer
- 2) 293 Humanized UAS tube #2 (5/14/90) - Inactive on Bio Assay.  
50ul Sup + 10ul buffer.
- 3) 2.16 stable chimera 5/6/90 - Bio Active. 50ul Sup + 10ul buffer
- 4) 293 UAS chimera - purified on prot A column. 20ul + 5ul buffer
- 5) UAS hybridoma (monoclonal) purified
- 6) ~~BRL~~ BRL prestained markers. (Highs)
- 7) BRL " " (Lows)

To Page No. \_\_\_\_\_

Witnessed &amp; Understood by me,

Date

6.7.90

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Date

6/15/90

From Page No. 73

6/5/90 the 1% agar gel of 3-b-14 runs out and then out, didn't look good.

No R1 cut and little was supercoiled.

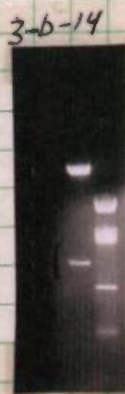
No Diagnostics cuts

6% Acry gel. Inl in 50% R1.

Lanes

- 1) AccI + R1 - ~~●~~ - 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/ PstI
- 6) X174 Haett

Results: #1 lane for 570bp is very faint. ~~the~~  
The other cuts are o.k.



Cut again w/ AccI + R1, using RE cut buffer & for AccI, add 50mM Salt before R1.

- labeled middle letter
- 1) 3-b-14 DNA AccI + R1
  - 2) PBR w/ PstI

To Page No. \_\_\_\_\_

Witnessed & Understood by me,

*[Signature]*

Date

6.7.8

Invented by

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*[Signature]*

Date

6/15/90

From Page No. \_\_\_\_\_

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



Previous gel w/ 3-b-14 run

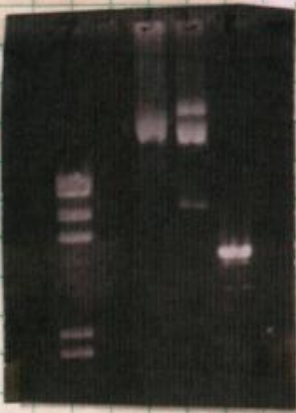
- lane 1) R1 cut once - linear
- 2) linear cut

Question - is this band marked on 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) linear cut
- 3) topoisomerase - Relaxes DNA
- 4) X Hind III markers



Run at 100V 2hrs No SFR

To Page No. \_\_\_\_\_

Witnessed & Understood by me,

*Amanda*

Date

6-7-90

Invented by

Recorded by

*[Signature]*

Date

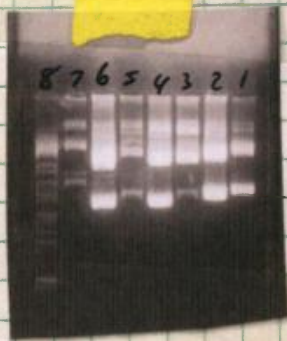
6/15/90



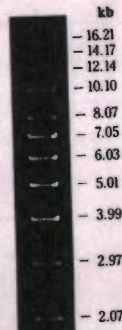
From Page No. \_\_\_\_\_

6/14/90 1% Agarose 1mg/ml ETBR TBE buffer

- lanes 1) Hm 405-H 5-b-4
- 2) Hm 405-H 5-C-4
- 3) Hm 405-L 3-b-14
- 4) Hm 405-L 3-C-7
- 5) Chimera 405-L dH158 (dennis' prep 4mg/ml 2.5.90)
- 6) dH158 " (JR prep 0.4mg/ml)
- 7) Chimera 405-H dH160 (dennis' prep 1.3mg/ml)
- 8) Super Coiled ladder marker.



upercoiled DNA Ladder



Supercoiled DNA Ladder  
0.2 µg/lane  
0.9% agarose gel  
containing 2 µg/ml ethidium bromide.

lane #3: Hm LC 3-b-14 is pure  
#5: dH158 (dennis prep is pure)  
#7: dH160 ( " " )

To Page No. \_\_\_\_\_

Witnessed & Understood by me,

*MmanuA*

Date

6.7.90

Invented by

Recorded by

Date

6/15/90

From Page No. \_\_\_\_\_

6/18/90 1/1. Agar gel w/ 10x one ETRR 1.6 Gel.

Lanes:

- 1) AdVA prep 0.884g/ml
  - 2) AdVA prep 0.25mg/ml
  - 3) Ha 4D5L1chan PA3 (Paul Carter prep) Ha
  - 4) Ha 4A5-Hchan PA5 ( " ) Ha
  - 5) Ha 4A5L1chan Version 1 (3-6-14) Ha
- 6/18/90 prep (Sample before banding)



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



- 1) old prep
- 2) new prep
- 3) markers

Results: Lack of good % supradial is not due to prep failure.  
 Made up 3-6-8 (different clone of same <sup>Construct</sup>)

To Page No. \_\_\_\_\_

Witnessed & Understood by me,

*BmanifA*

Date

6-7-90

Invented by

Recorded by

*[Signature]*

Date

7/6/90

TITLE Plasmid Preps, ALVA, Hu 3-b-8 405

Project No. \_\_\_\_\_  
Book No. \_\_\_\_\_

79

From Page No. \_\_\_\_\_

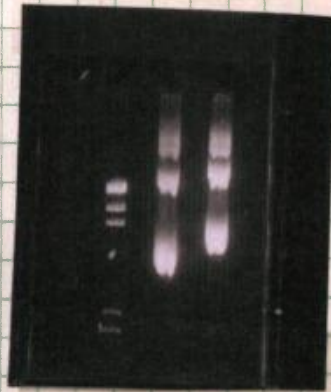
6/27/90 Prepared 1L prep of ALVA and 405 Hu 3-b-8

Banded in CsCl 1 time.

Run 7ul of each direct from Quik Seal Cartridge tube.  
w/ CsCl and EtBr still in it.

1% Agar gel uncut plasmid.

- Lanes) 1) 3-b-8 - Light Chain  
2) ALVA  
3)  $\lambda$  H<sub>III</sub> marker



Appears that there is good supercoiled DNA.

delete some of each 1/100  
take 26000 for cone

SH	260.0	SH	21
ALVA	0.4376	x5 =	2.2 ng/ul
3-b-8	0.3911	x5 =	2 ng/ul

To Page No. \_\_\_\_\_

Witnessed & Understood by me,

*[Signature]*

Date

6.2.90

Invented by

Recorded by

*[Signature]*

Date

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	coming soon (hopefully)
pA14			d	

1/100 pA9 1 0.6844 x5 = 3.422ug/ml  
 " pA10 2 0.6393 x5 = 3.2ug/ml  
 " pA13 3 0.5944 x5 = 3.0ug/ml



DNA prep uncut for Supercoiled

PA9 clone 2 → DNA hot  
 PA10 clone 1/4 → good for xfa  
 No good supercoiled!  
 PA13 - Heavy chain - e is fine

To Page No. \_\_\_\_\_

Witnessed & Understood by me,

*Carlman*

Date

10.8.90

Invented by

Recorded by

*Plasmid Preps*

Date

8/10/90

TITLE plasmid Prep pA9, pA10, pA13, pA14

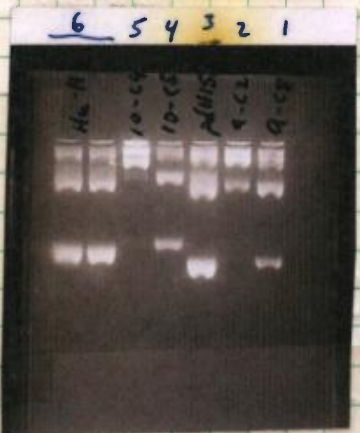
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Book No. \_\_\_\_\_

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From Page No. \_\_\_\_\_

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

	Expt #	Clare	[DNA]	Vd mL
pA9	340	8	8/12 0.21	1.0
pA10	339	1/5	0.18	1.0
pA14	347	3/2	0.16	1.0



1% Agarose 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% Agarose 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,  
Deufman

Date  
10.8.90

Invented by  
[Signature]  
Recorded by

Date  
8/10/90

From Page No. \_\_\_\_\_

For stable lines in CCHO and myelomas

8/20/90 405 Humanized Var 5 and 6 are chosen to make stable lines. The existing plasmids are pRR based and without selectable markers.

For best xpr 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 (PAS)
Var 6	C (3-C-7)	C (5-C-4)

Strategy for construction:

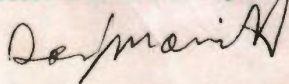
Light chain (3-C-7) used in both Var 5 and 6, is in pRR vector with a restriction map based on dh158 (chicken light)

Heavy chains PAS and 5-C-4 are in pRR vectors based on dh160 map. (heavy chicken).

- 1) Make pSV1 based 3-C-7 light chain:  
Cut pRR 3-C-7 w/ R<sub>1</sub> + H<sub>3</sub> to remove L-chain only.  
Insert it into R<sub>1</sub>-H<sub>3</sub> of pSV16BAD. Name: pSV1 Light chain 3C7
- 2) Make pSV1 based PAS, 5-C-4 heavy chains,  
Cut out H chains from pRR versions → insert to R<sub>1</sub>-H<sub>3</sub> of pSV16BAD.  
Name: pSV1 PAS and pSV1 5-C-4.
- 3) Remove pSV1 L-chain 3-C-7 transcription unit w/ PvuII and insert into blunt end KPMI site of pSV1 PAS and pSV1 5-C-4. Name: pSV1 Var 5 and pSV1 Var 6 respectively.

To Page No. \_\_\_\_\_

Witnessed &amp; Understood by me,



Date

12.10.98

Invented by



Date

8/20/90

Recorded by

TITLE Const of pSV1 Var 5 and Var 6 JhFr.

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

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From Page No. \_\_\_\_\_

8/21/90 Cut Light chain pRK 3C7 w/ R<sub>1</sub>-H<sub>3</sub> in Sift Agar 1%.

Should be 710 bp

Marker is pBR322 - BstXI

Cut out at attas



Ligation: R<sub>1</sub>-H<sub>3</sub> Frag of pRK 3C7 w/  
R<sub>1</sub>-H<sub>3</sub> Vec of pSV1.6B Ad.

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.

569 bp - all correct!



To Page No. \_\_\_\_\_

Witnessed & Understood by me,

*Daymond*

Date

12.10.90

Invented by

*JhFr*

Recorded by

Date

8/24/90

From Page No. 83

8/27/90 Cut pRK PAS and S-C-Y Heavy chains  
w/ H<sub>3</sub>+R<sub>1</sub> ~1450bp. each..

0) PAS H<sub>3</sub>+R<sub>1</sub>



H chain only

Probes of X174 H<sub>3</sub>III, & H<sub>3</sub>III

Run in Soft Agar, Cut out at dlt

Do ligations w/ H-chain H<sub>3</sub>-R<sub>1</sub>  
w/ pSV16B A & R<sub>1</sub>-H<sub>3</sub>

SVI SCY, SVI PAS xba cut



all correct

8/30/90

8/30/90 MiniScreens of  
SVI SCY and SVI PAS  
cut w/ Xba ~1685bp

Lanes 1-3: SVI SCY  
4-5: SVI PAS  
7: OX H<sub>3</sub>III

all correct!

To Page No. \_\_\_\_\_

Witnessed & Understood by me,

*Darmanik*

Date

12.10.90

Invented by

*Jim Rubin*  
Recorded by

Date

8/27/90



TITLE psu1/Var 5 and psu1/Var 6 w/dhfr

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

85

From Page No. \_\_\_\_\_

9/3/90 Cut psu1 (3-C+7) light chain transcription unit out w/ pvuII



~ 1588bp frag

Use this insert in both psu1/Var 5 and 6

9/5/90 Cut Vec SUI PAS (Heavy chain for SUI Var 5) with KPN1.  
Blunt by chew back w/ T4 DNA Pol I → phosphate  
Gel Purify in Soft Agar

Set up ligation: Vector - SUI PAS KPN1-blunted (above) H chain  
Insert - psu1 3C7 - puvII 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.

SUI Var 5 H+L

To Page No. \_\_\_\_\_

Witnessed & Understood by me,

Danmanick

Date

26.10.90

Invented by

Recorded by

Date

9/7/90

From Page No. 85

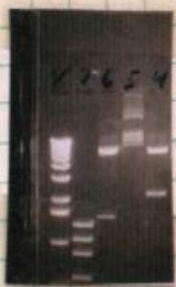
9/10/90 pSV1 Var 5 4 min: success

2 possible orientations, Digest w/ Hind III

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



#3 is correct.



Test further digests

- #4 - R1 - ~ 2257 - yes
- #5 - KpnI - 562 - (NO!)
- #6 - H3 - 1519 - yes
- #7 - ~~OX174~~ w/ Hind III
- #8 - UK15 ladder

pSV1 Var 5 KpnI



Lane 2 - ~~OX174~~ Hind III  
3 - SV1 Var 5 - KpnI  
yes!

he cut!

To Page No. \_\_\_\_\_

Witnessed & Understood by me,

OajmanidA

Date

26.10.90

Invented by

Recorded by

Date

9/10/90

From Page No. \_\_\_\_\_

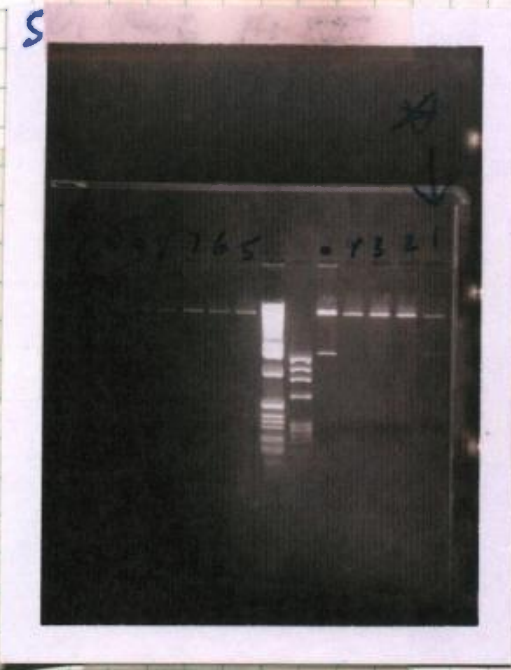
9/11/90 Prepare vector heavy chain for Var 6 (SV1-SC4)  
 Cut w/ KPMI - blunt w/ T<sub>4</sub> DNA pol I. → phosphatase  
 Gel purify -

Ligate Vec - KPMI blunted (SV1-SC4)  
 Ins - 1588 bp ~~SV1~~ fragment of SV1 (3C-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 correct.  
 • is SV1/Var 6 control number.  
 We have pSV1/Var 6 H+L - probably.



Better picture!  
 Same gel.

To Page No. \_\_\_\_\_

Witnessed &amp; Understood by me,

Dorfman

Date

26.10.90

Invested by

Recorded by

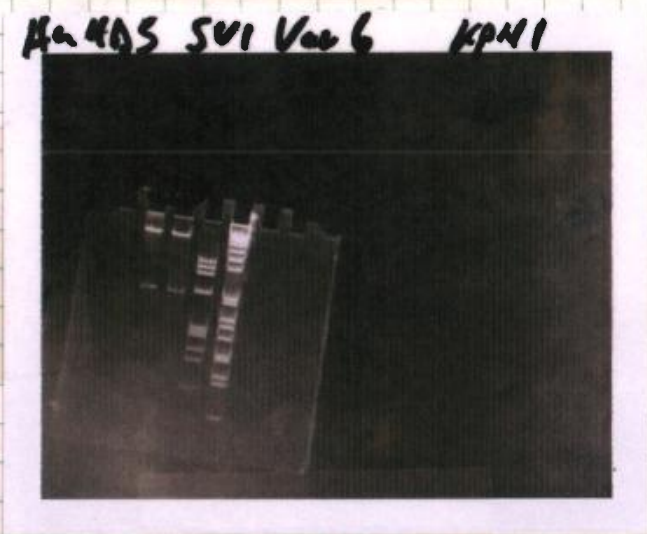
*[Signature]*

Date

7/14/90

From Page No. \_\_\_\_\_

9/17/90 Check pSV1 405 Var 6 HPL w/ KPM1 cont.  
Should be ~ 560



Correct!

Test pSV1 Var 6 w/ R1 ~ 2257



Lane 1) R1 - Correct!

New plasmid Formal name:  
pSV16BS del.d. 4d5V6

1/8/92

To Page No. \_\_\_\_\_

Witnessed & Understood by me,

Darfman SA

Date  
26.10.90

Invented by

Recorded by

Date  
9/17/90

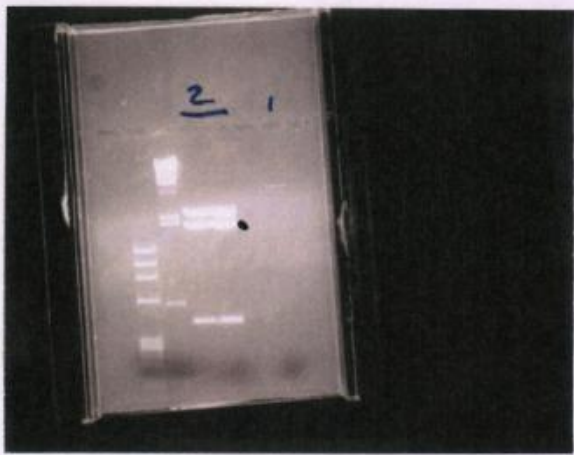
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9/18/90 Combine the pRK vectors for 4D5 the light chain Var C and pRK 4D5 the heavy chain Var C on one plasmid. This is the 4D5 Var 6 in pRK vector.

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  
phosphatase.

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

Soft Agar Gel 1%

To Page No. \_\_\_\_\_

Witnessed &amp; Understood by me,

Danymanish

Date

26.10.90

Invented by

Recorded by

Date

9/17/90

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE Const pRK the 405 Var 6

From Page No. \_\_\_\_\_

9/19/90 Ligation.

Vpc - pRK 504 vprN Hunted.

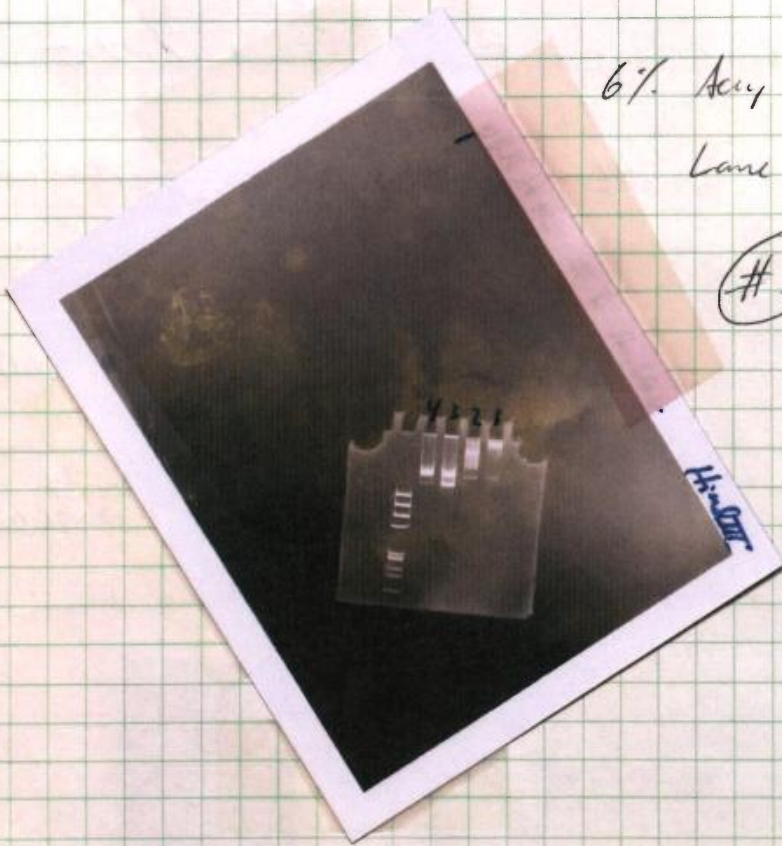
Ins - pRK 2056 bp Frag of pRK 3-C-7.

9/21/90 Mini Screens Out w/ Hind III ~ 2030

6% Acry Gel.

Lane 3 } is correct product  
4 }

#3



To Page No. \_\_\_\_\_

Witnessed & Understood by me.

Dahymani A

Date

26.10.90

Invented by

Recorded by

*[Handwritten Signature]*

Date

9/21/90

From Page No. \_\_\_\_\_

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



Lane 1) #3 KpNI ~ 562 ✓ o.k.  
 2) #3 HindIII ~ 2032 ✓ o.k.  
 4) #3 AccI ~ 1962 ✓ o.k.  
 3) #3 R<sub>1</sub>-H<sub>2</sub> - 1482 ✓  
 1320 ✓ o.k.  
 710 ✓

5) #6 No band  
 6) #6 No band  
 7) #6 No band  
 8) #6 No band

9) 0x174 HindIII  
 10) 1K6 ladder  
 11) 2 HindIII

the #3 is good

To Page No. \_\_\_\_\_

Witnessed & Understood by me,

Dadmanis

Date

26.10.90

Invented by

Recorded by

Date

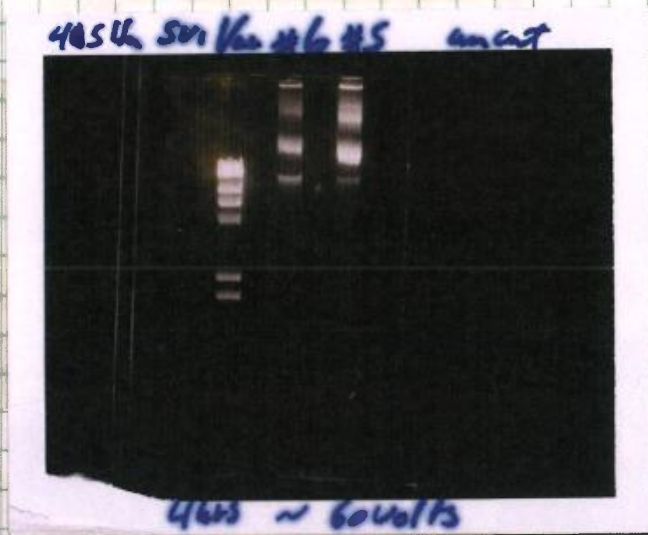
9/21/90

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9/24/90 Qiagen prep

SUI Var 5 and Var 6

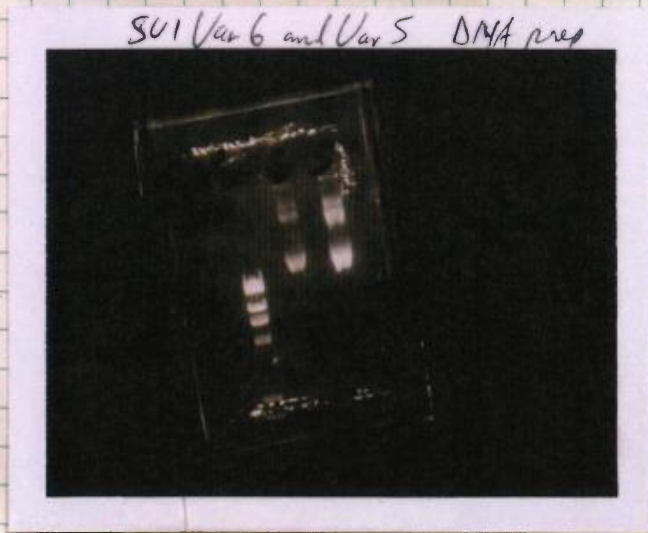
Not too good



9/25/90

the above DNA banded CSCI

Much better



amcat DNA

To Page No. \_\_\_\_\_

Witnessed & Understood by me,

Dalymanista

Date

26.10.90

Invented by

Recorded by

[Signature]

Date

9/24/90



TITLE

DNA prep

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

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From Page No. \_\_\_\_\_

9/25/92

pRK Var 6 L155 the H+L Oligon prep.



pRK Var 6 DNA prep

run out DNA

To Page No. \_\_\_\_\_

Witnessed & Understood by me,

*Oshmani*

Date

26.10.92

Invented by

Recorded by

*[Signature]*

Date

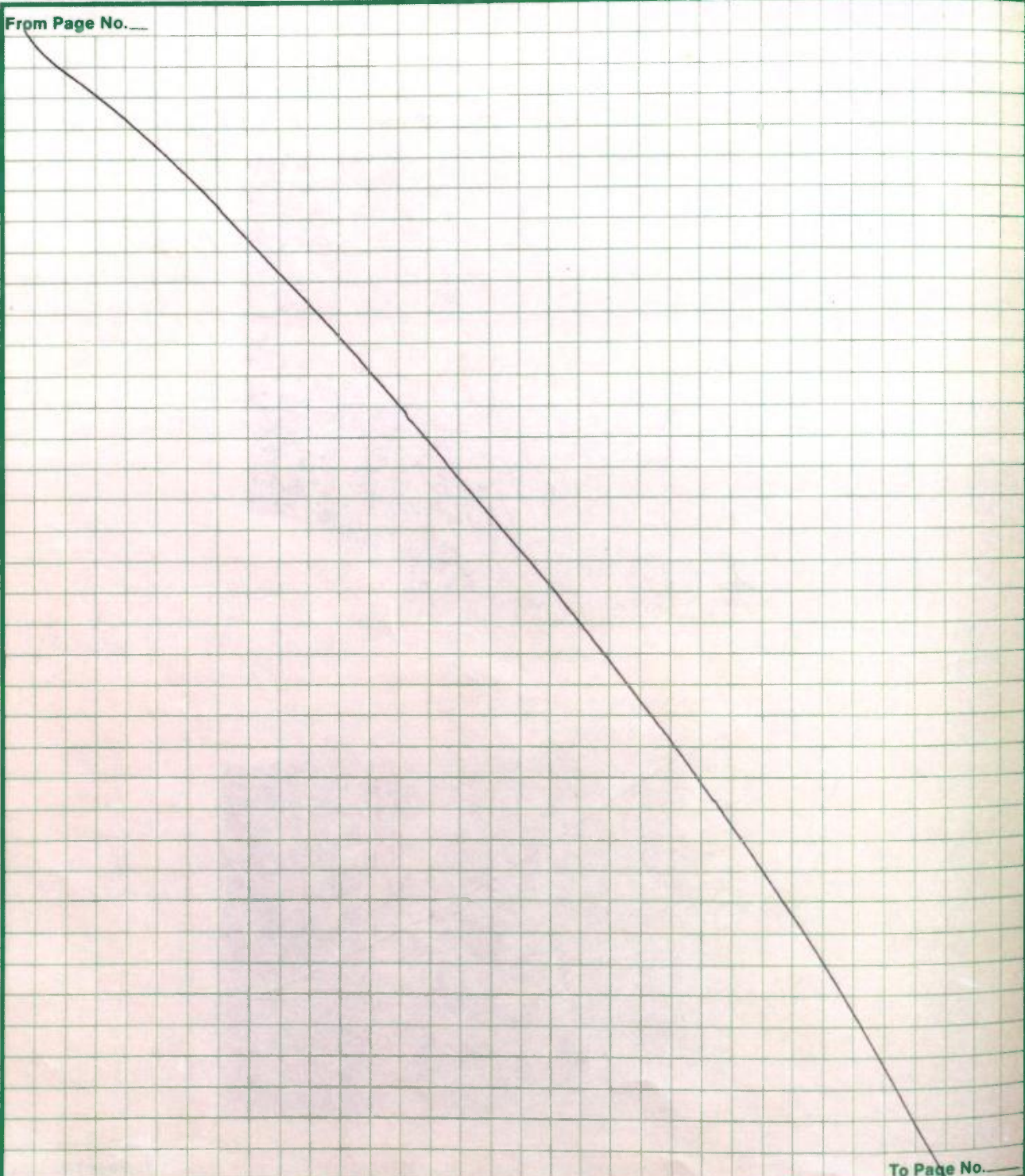
9/25/92

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		Recorded by	

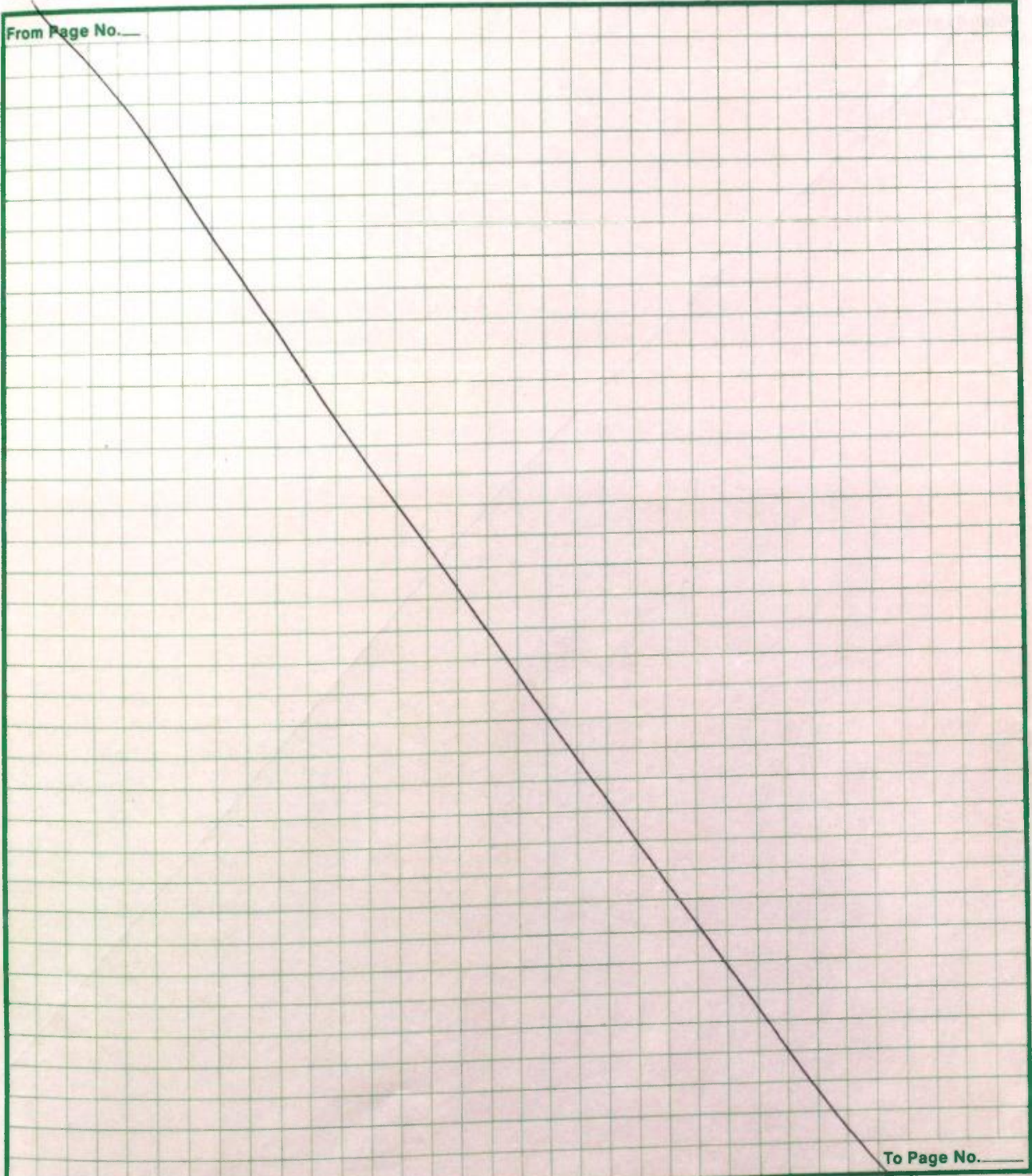
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Witnessed & Understood by me, \_\_\_\_\_

Date \_\_\_\_\_

Invented by \_\_\_\_\_

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Recorded by \_\_\_\_\_

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

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Witnessed & Understood by me, \_\_\_\_\_

Date \_\_\_\_\_

Invented by \_\_\_\_\_

Date \_\_\_\_\_

Recorded by \_\_\_\_\_