

LABORATORY NOTEBOOK

Tissue Culture

4/1/06
01

GENENTECH, INC.

Celltrion v. Genentech
IPR2017-01374
Genentech Exhibit 2006

11162



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NOTEBOOK NO. 11162
ISSUED TO John Ridgway
ON Feb 20 19 90
DEPARTMENT Cell Genetics 435
RETURNED 19

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

—SCIENTIFIC NOTEBOOK CO.—
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Genentech Laboratory Notebook Procedures

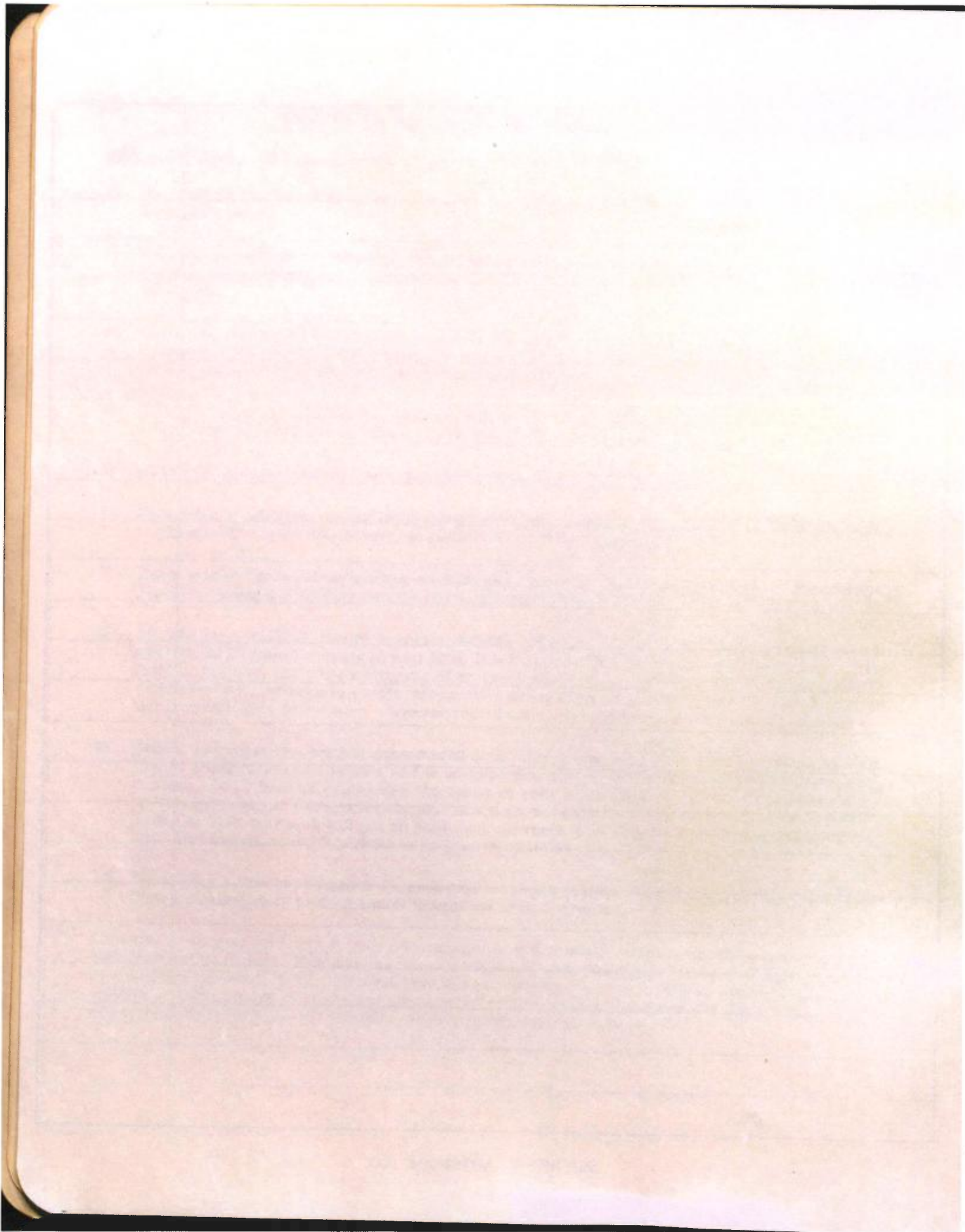
Important steps for maintenance of your notebooks:

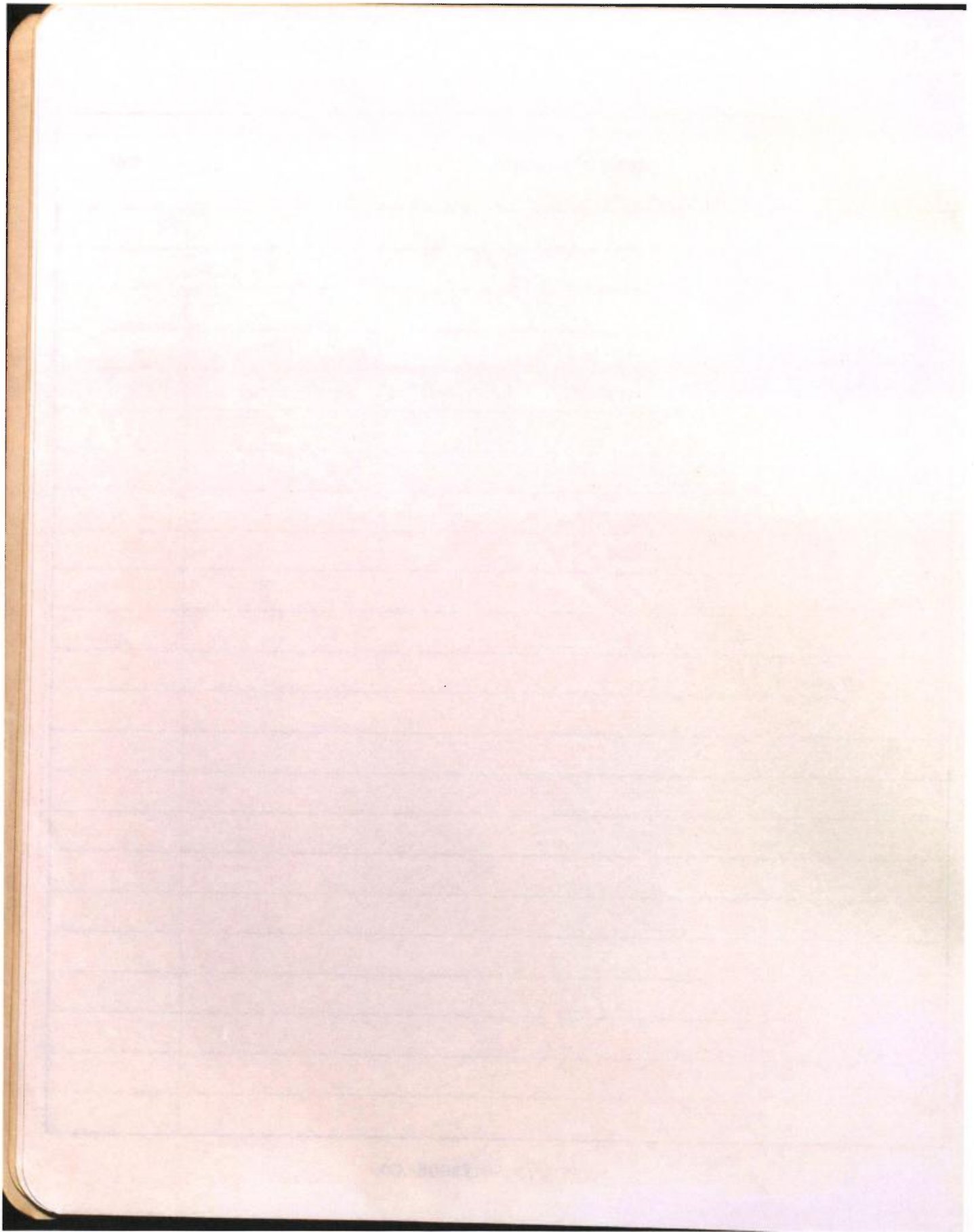
- 1) Use dark permanent ink to record all of your work in your notebook on a daily basis. Sign and date each day's entries.
- 2) Permanently affix all attachments without covering any other entries. Please attach copies of any computer data entered into your computer files. These data are considered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

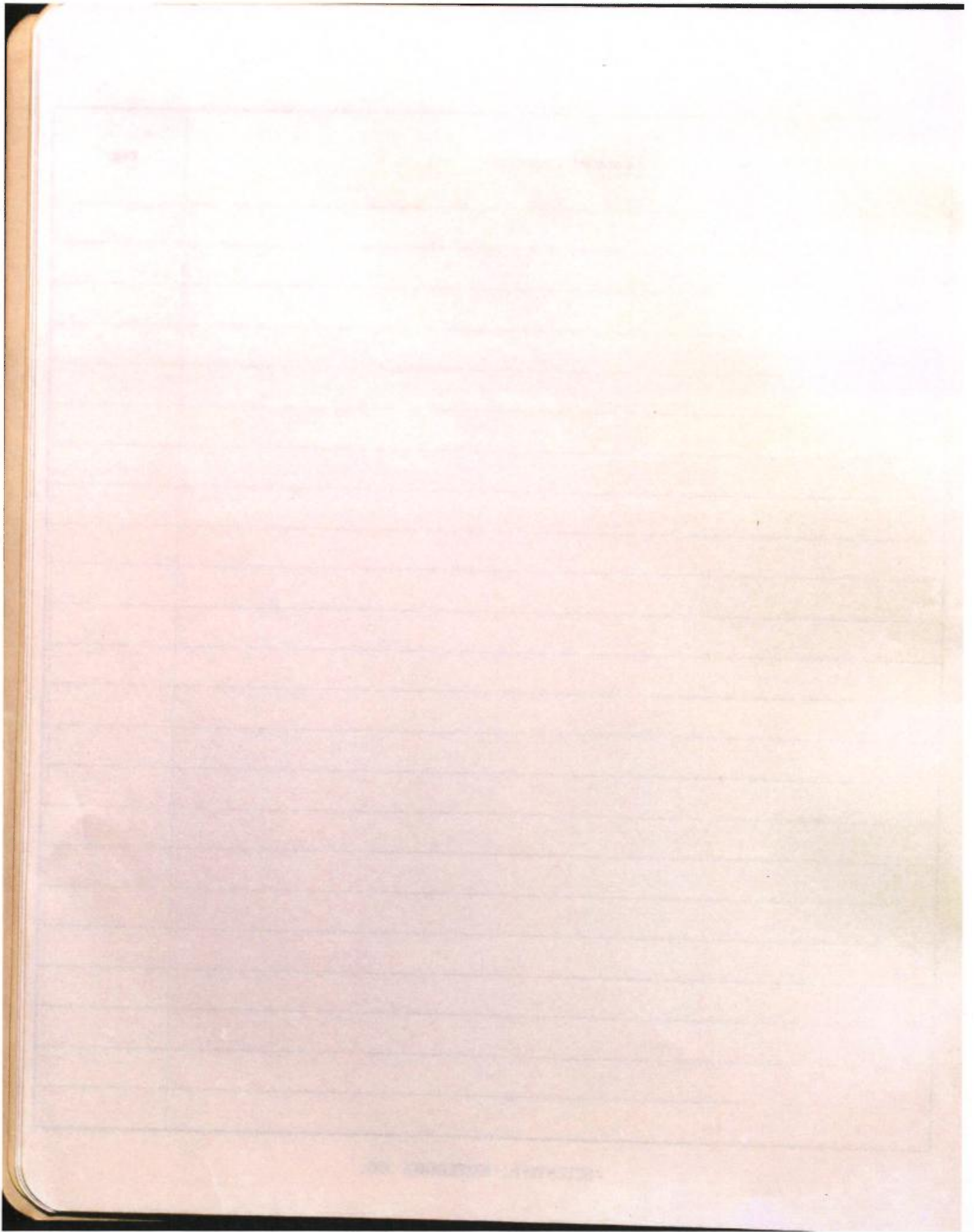
Genentech's success, and with it your own prosperity and research support, depends upon the quality and timing of your work. This may require that we prove what you did and when you did it in the face of a contrary challenge. Such challenges have and will continue to arise in judicial proceedings, in 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.

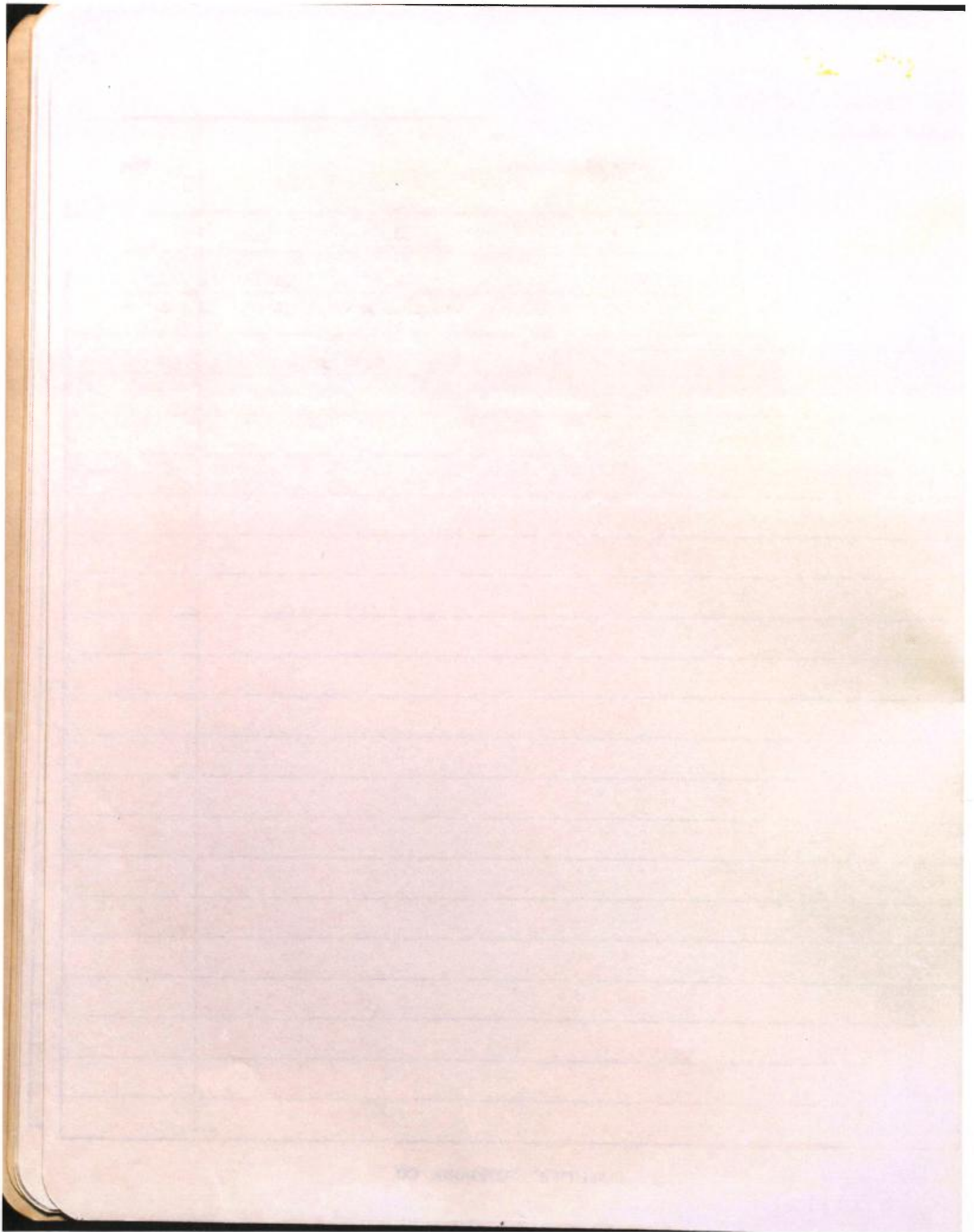
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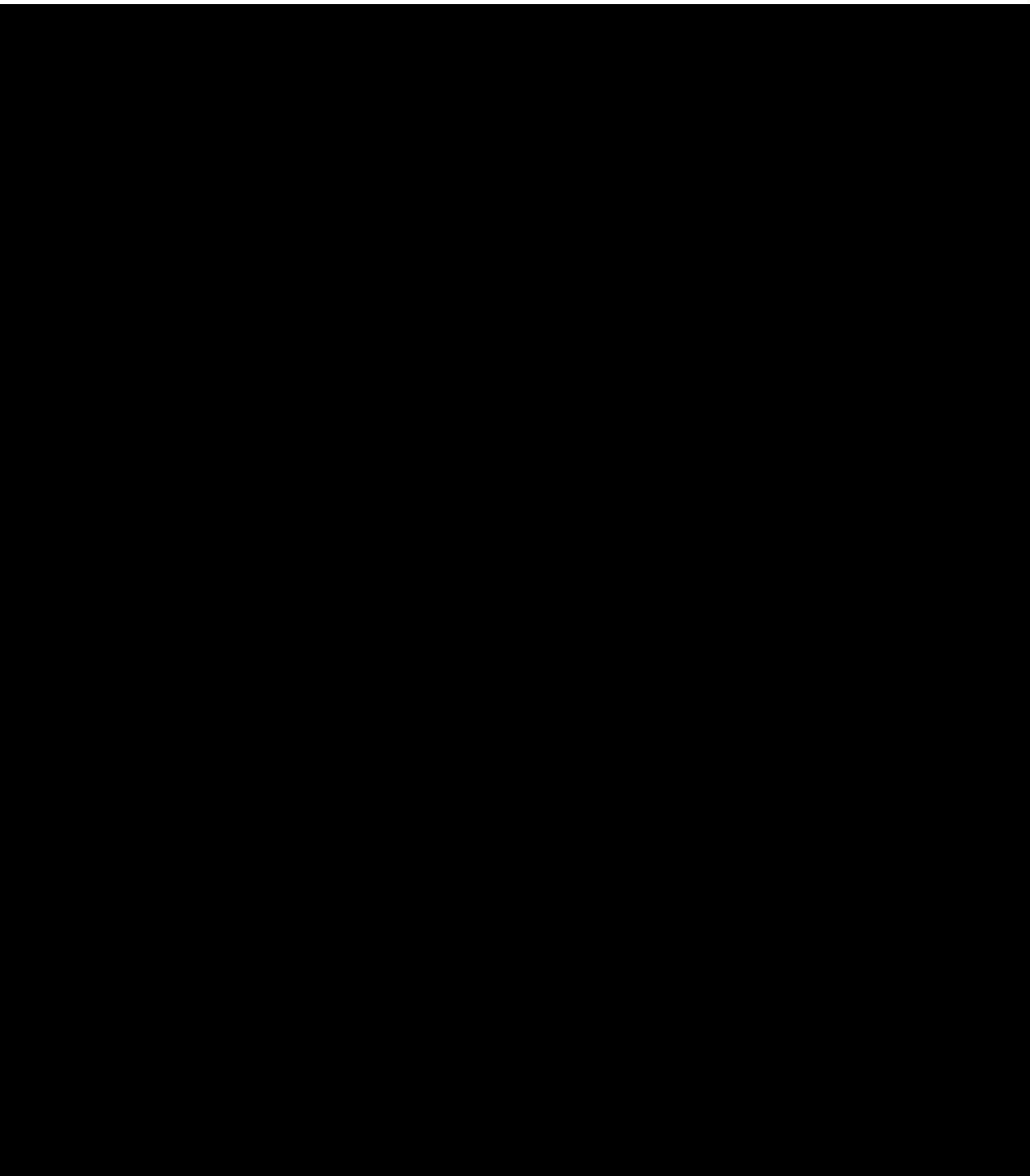
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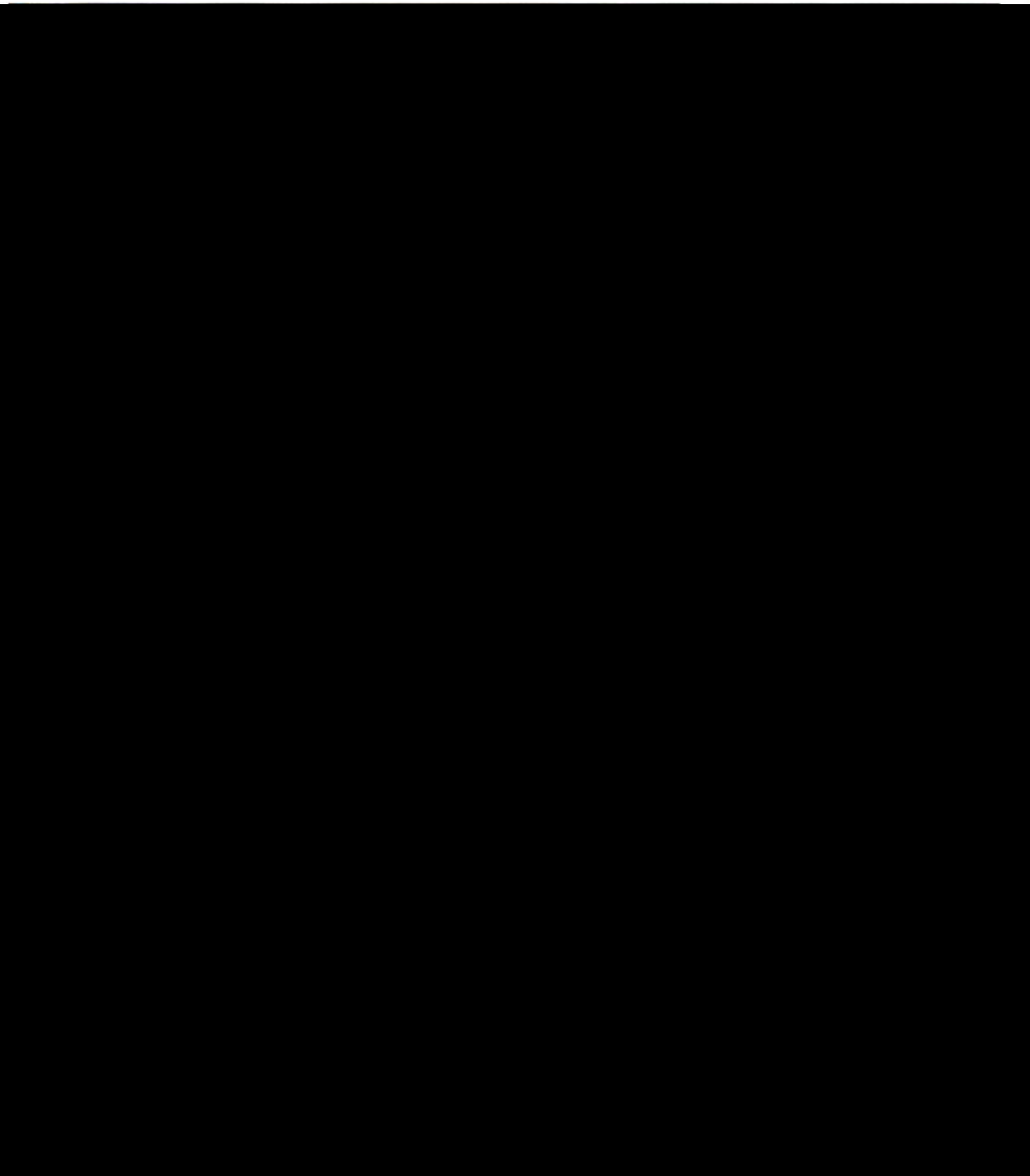




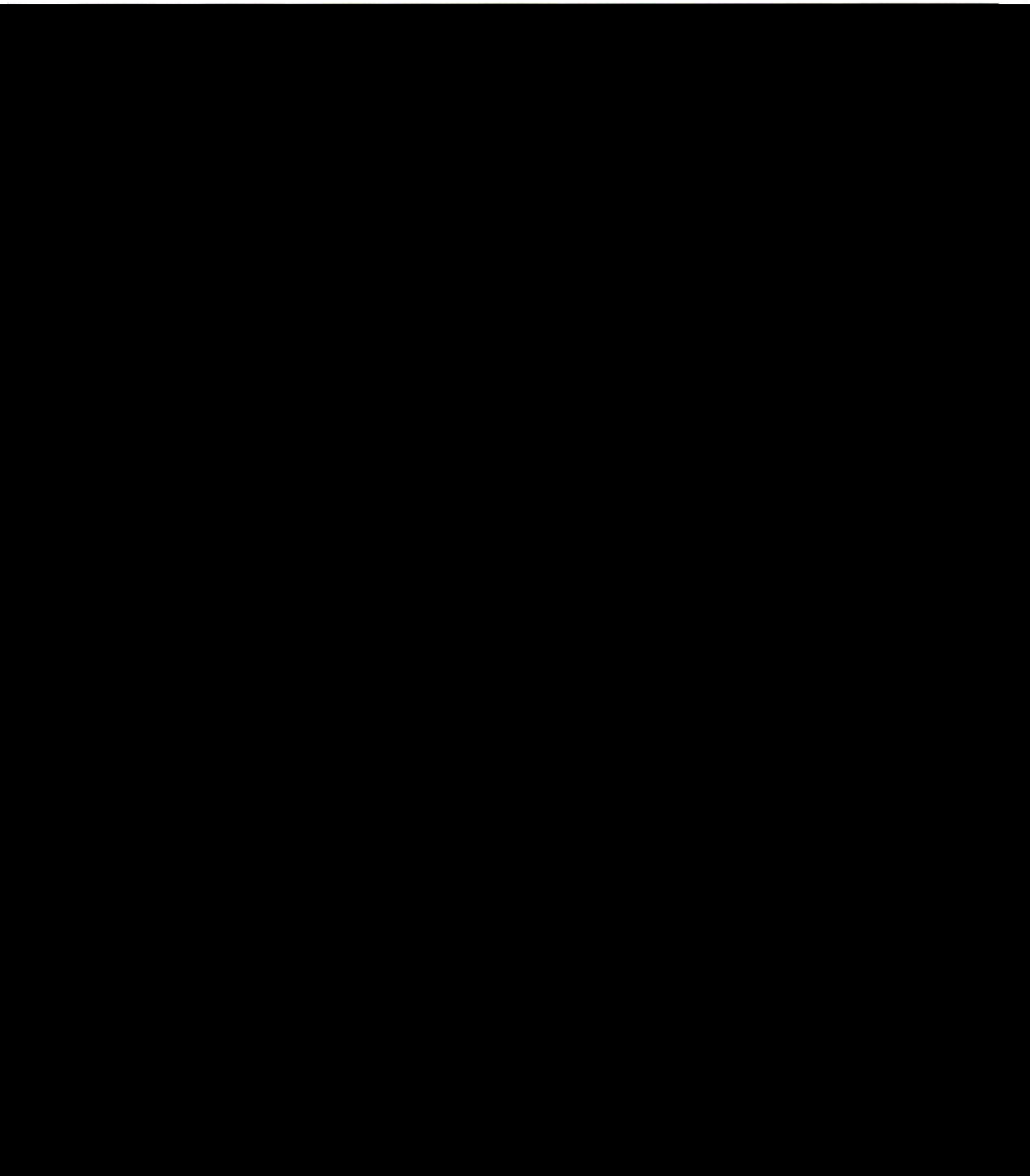




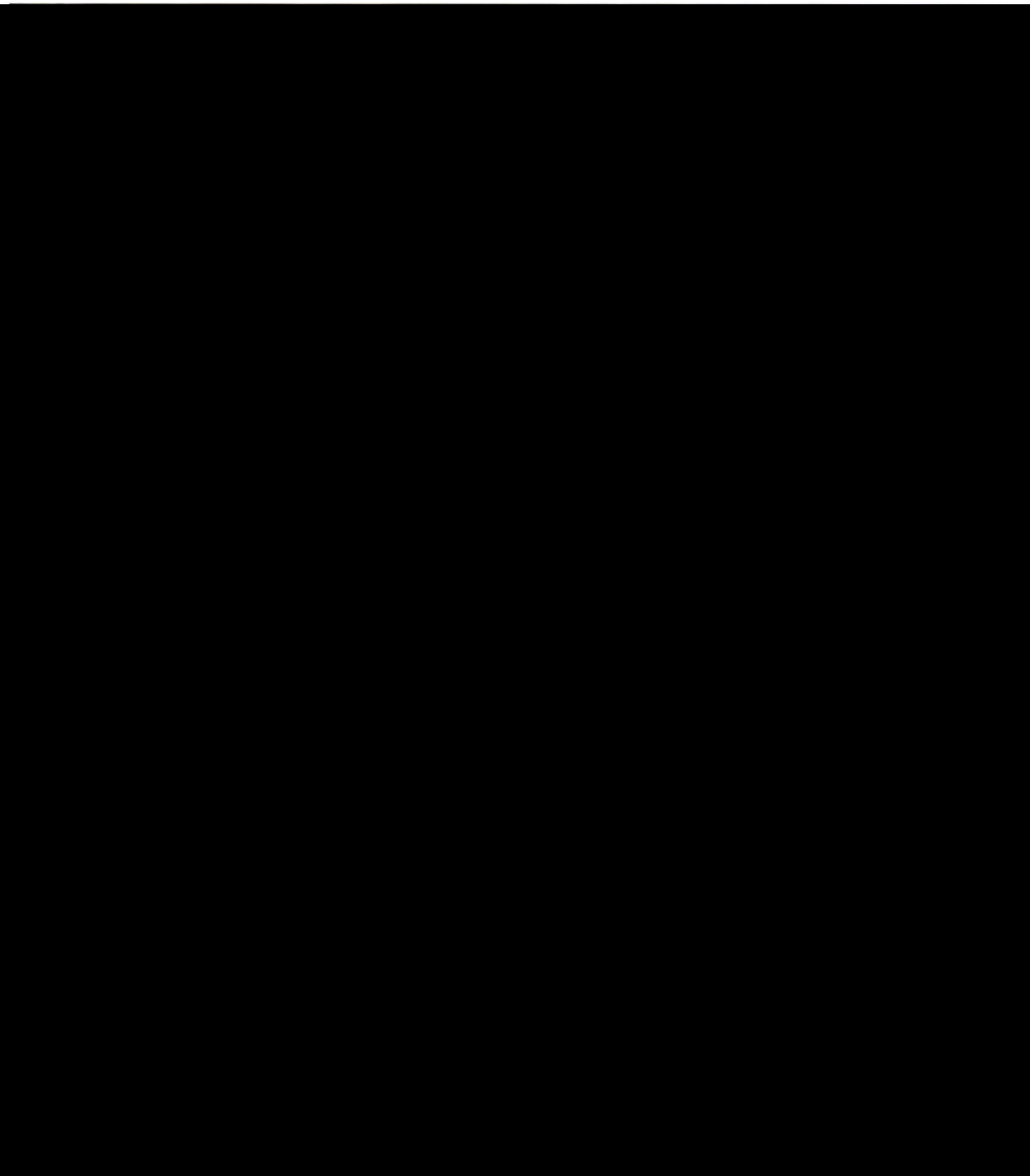
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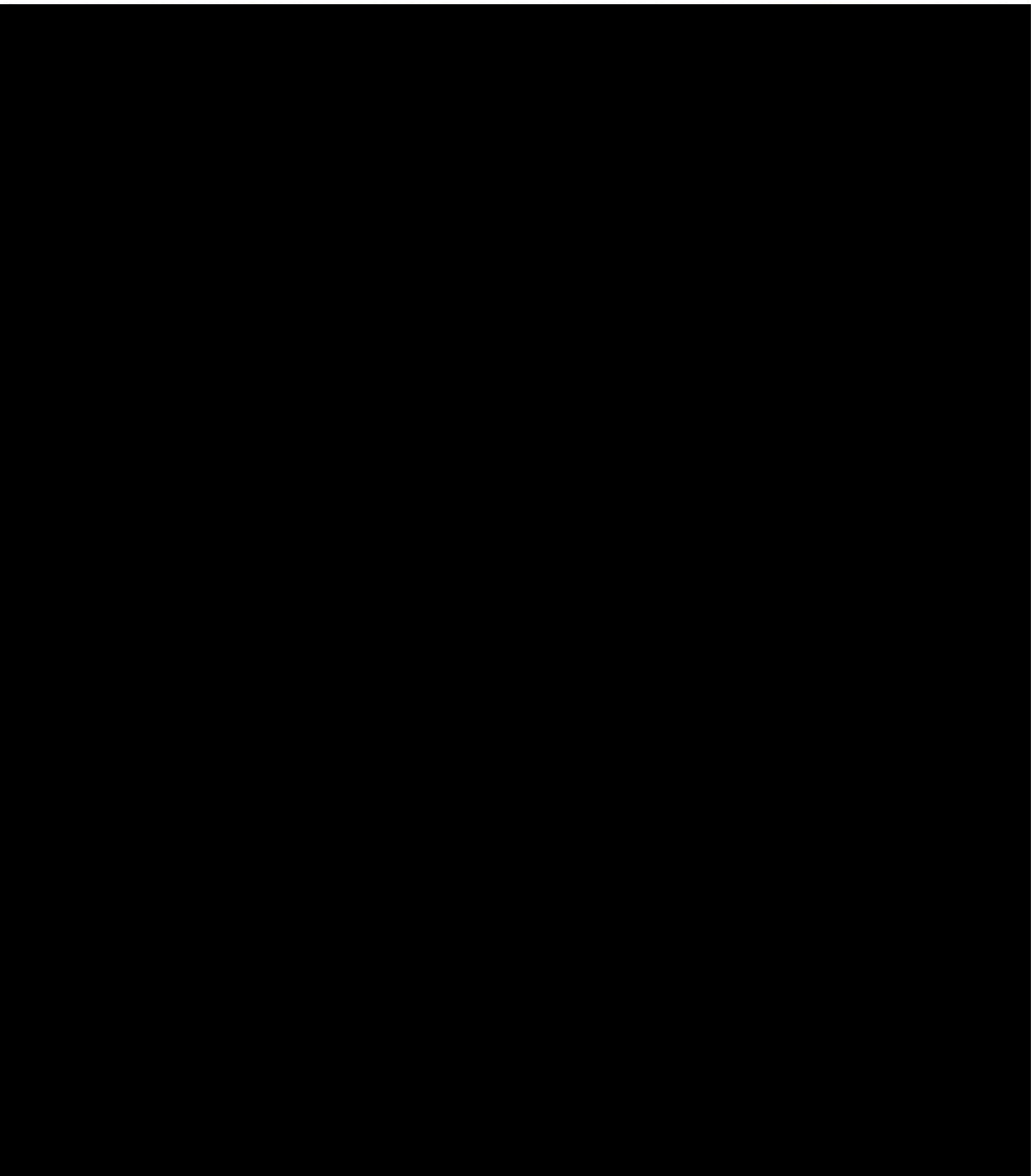
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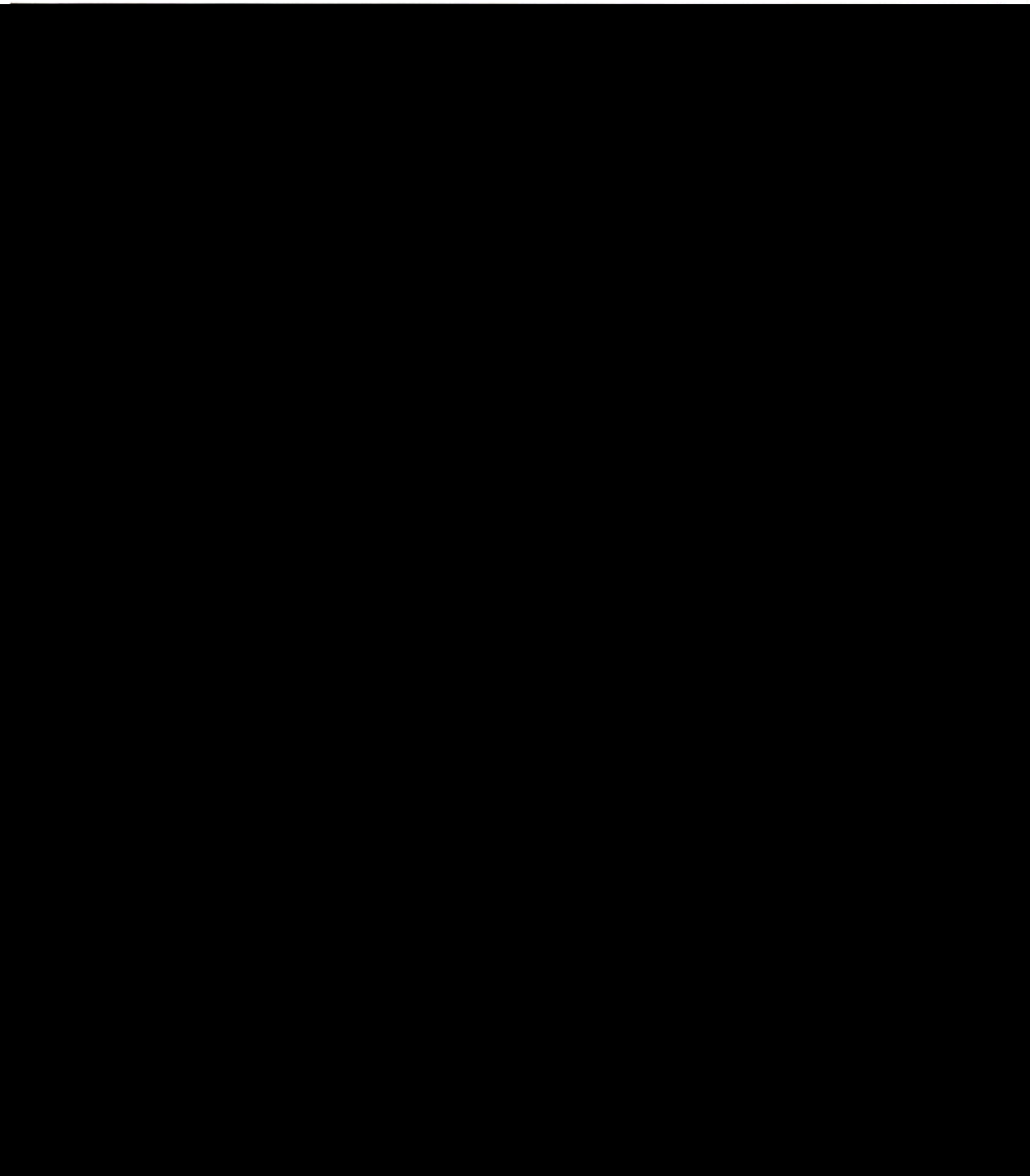
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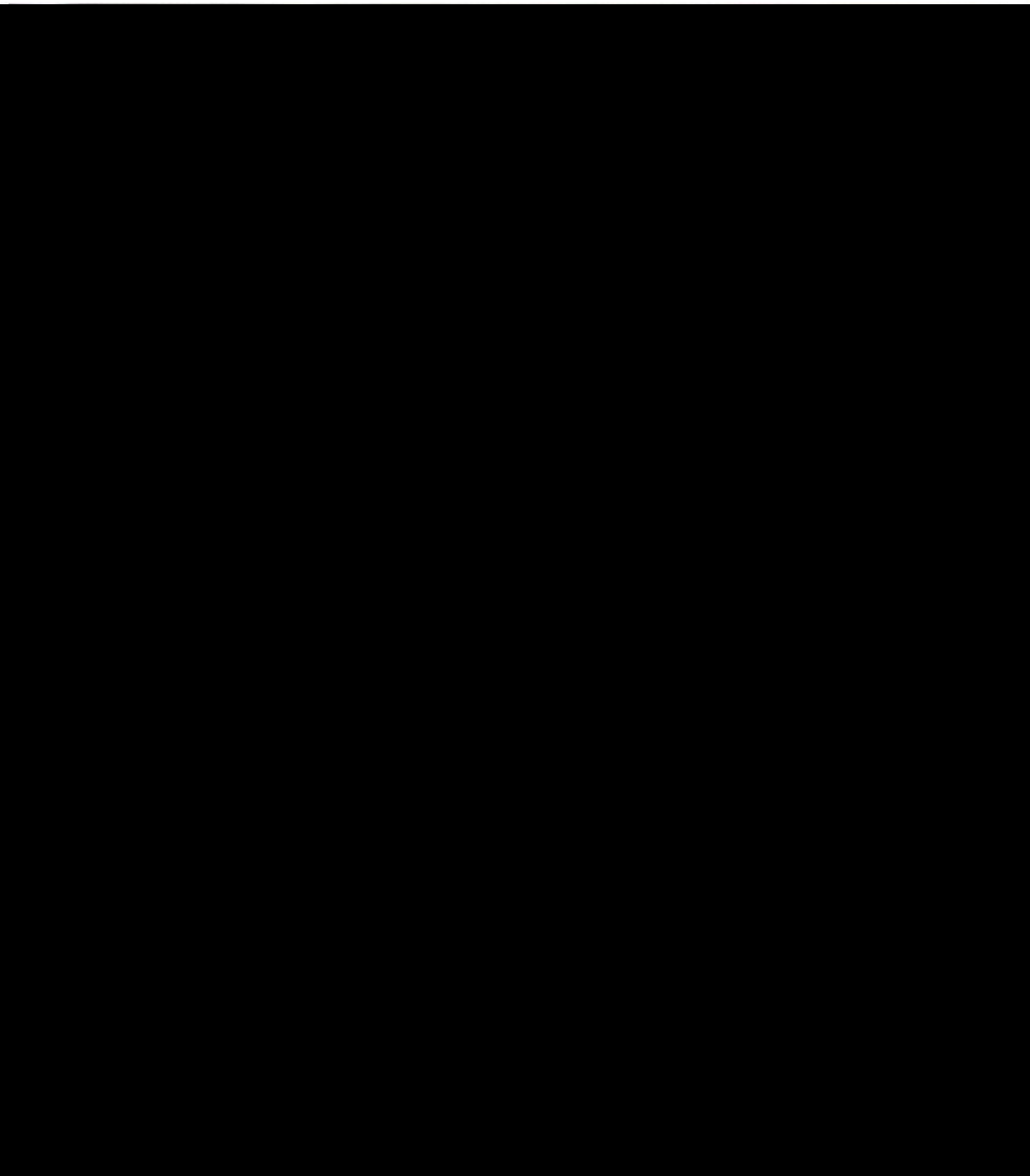
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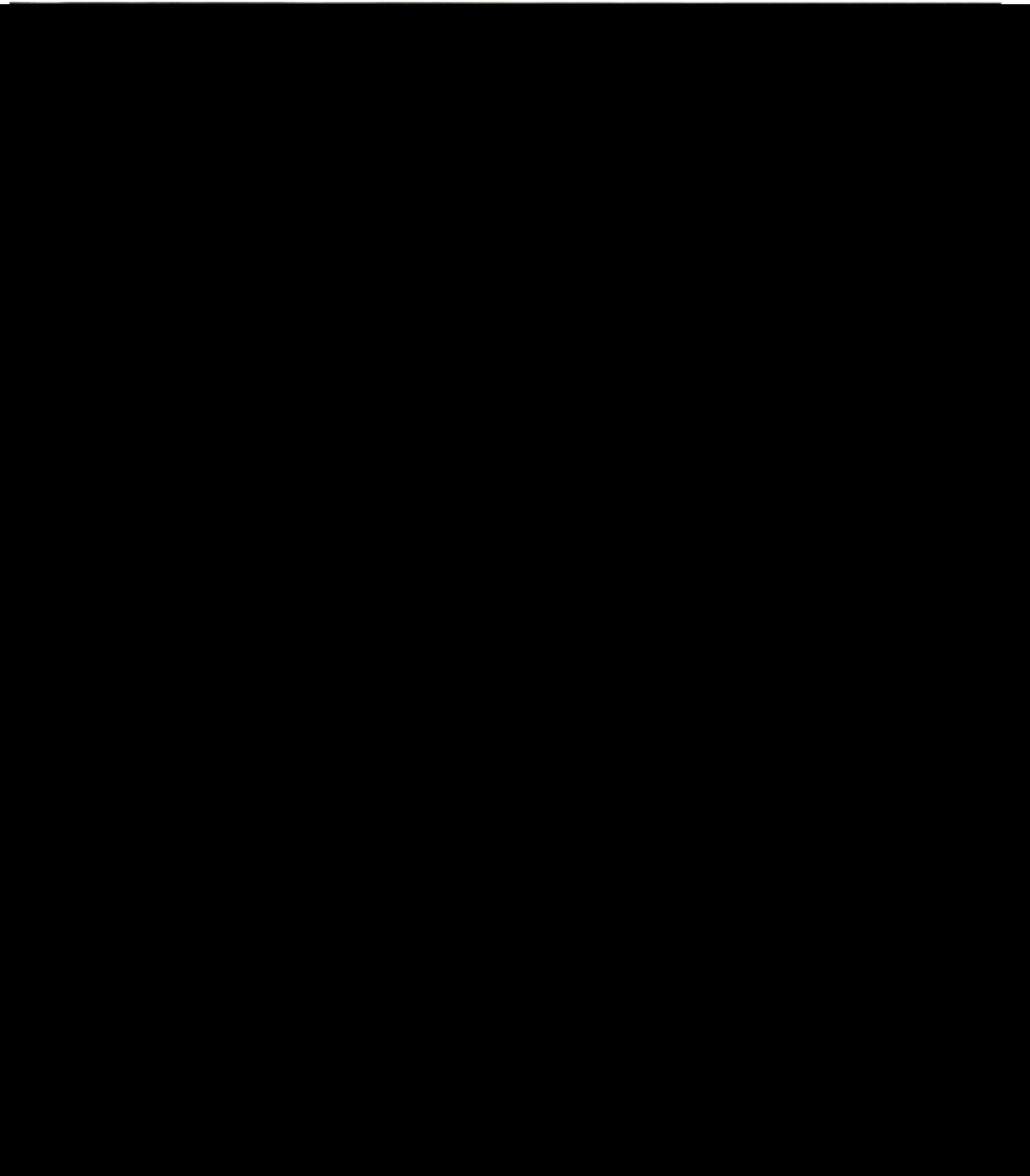
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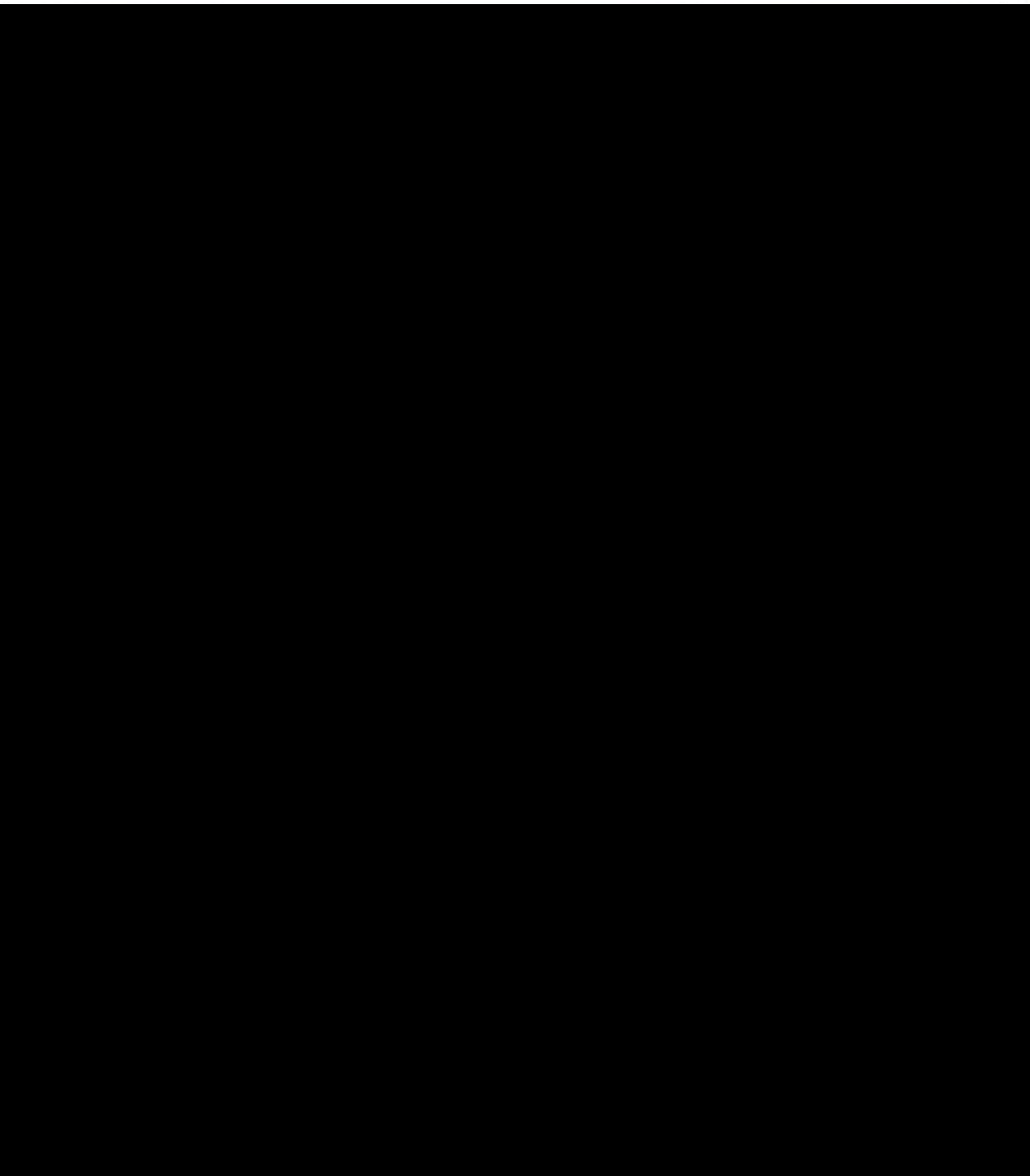
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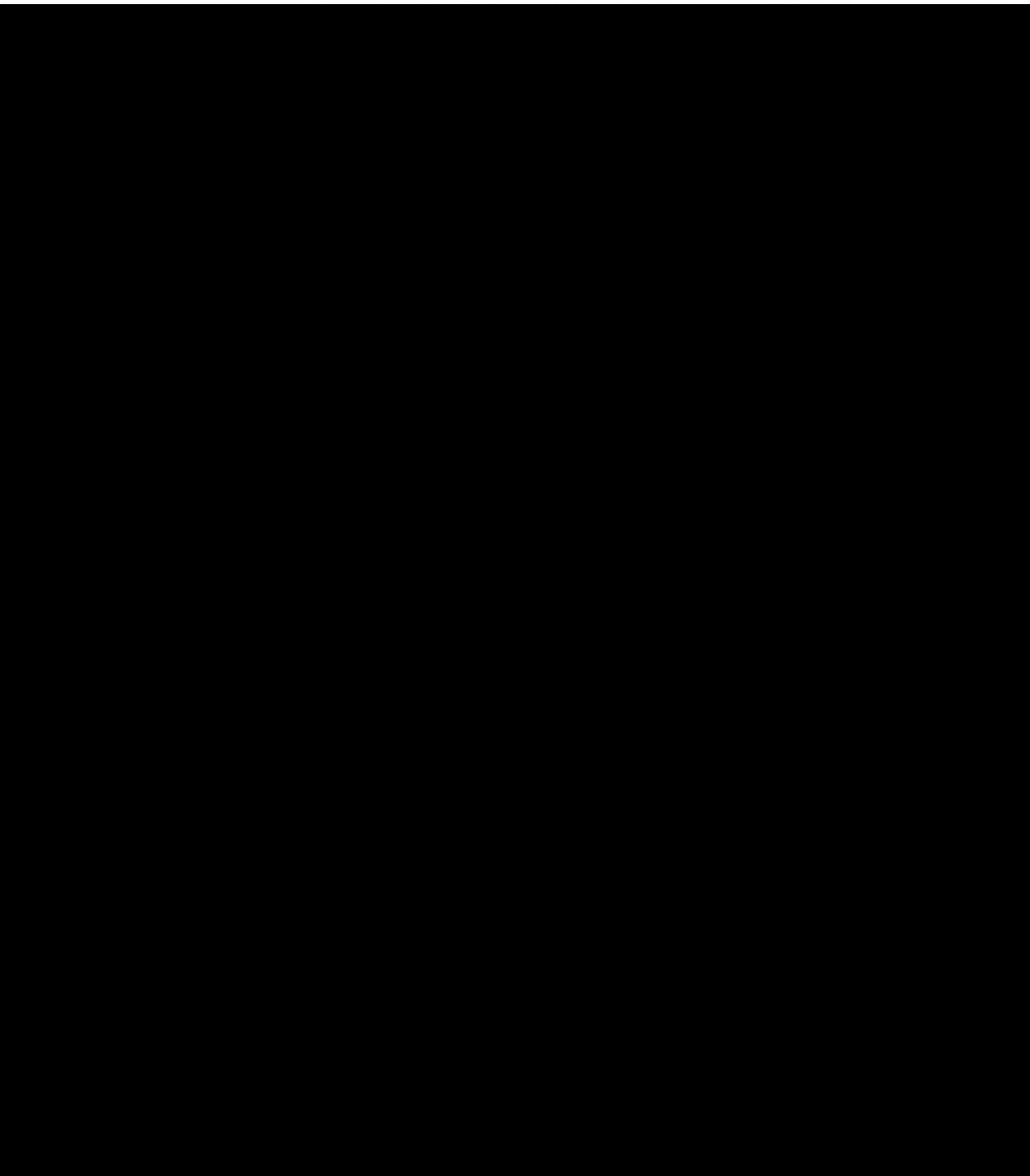
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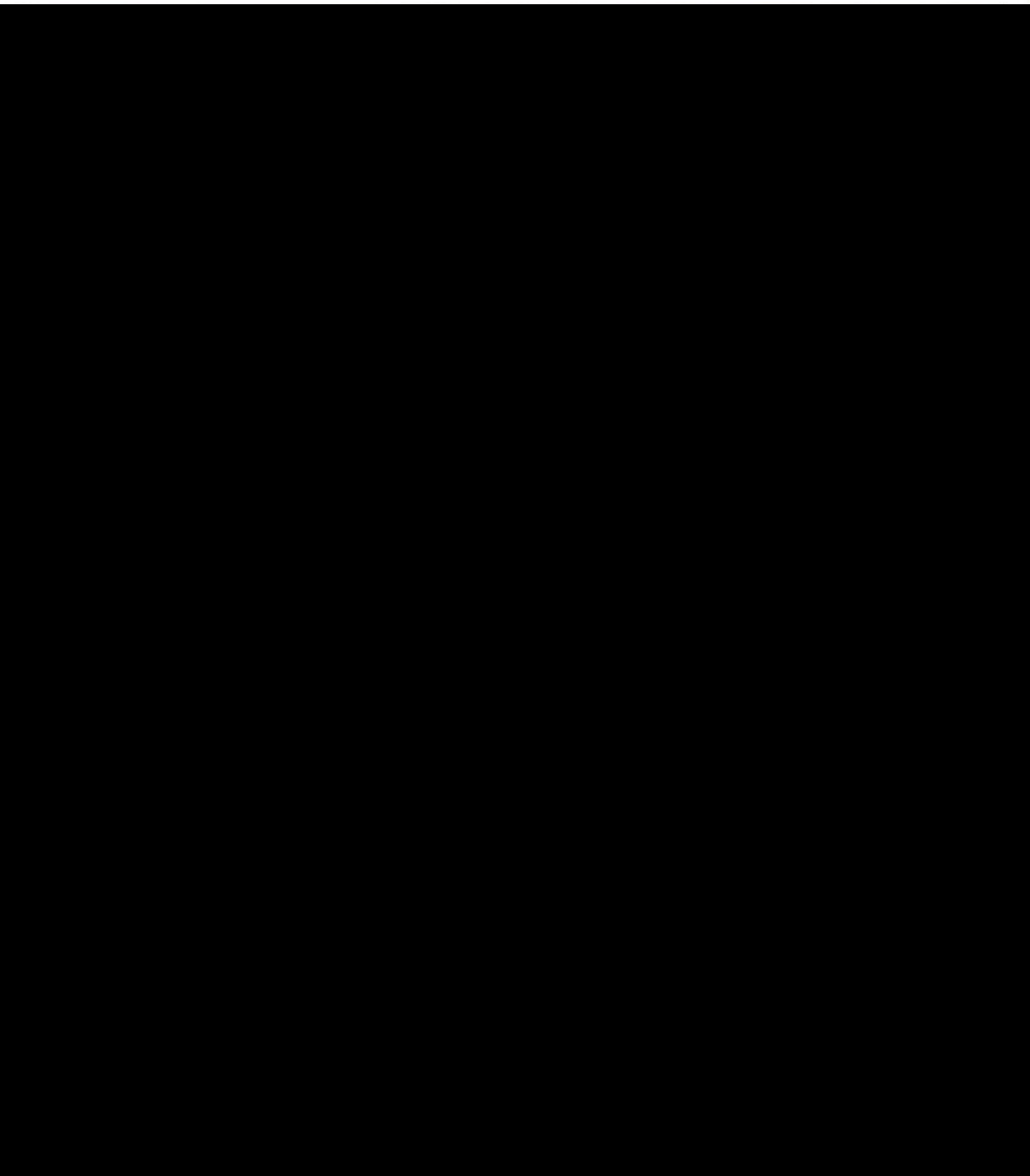
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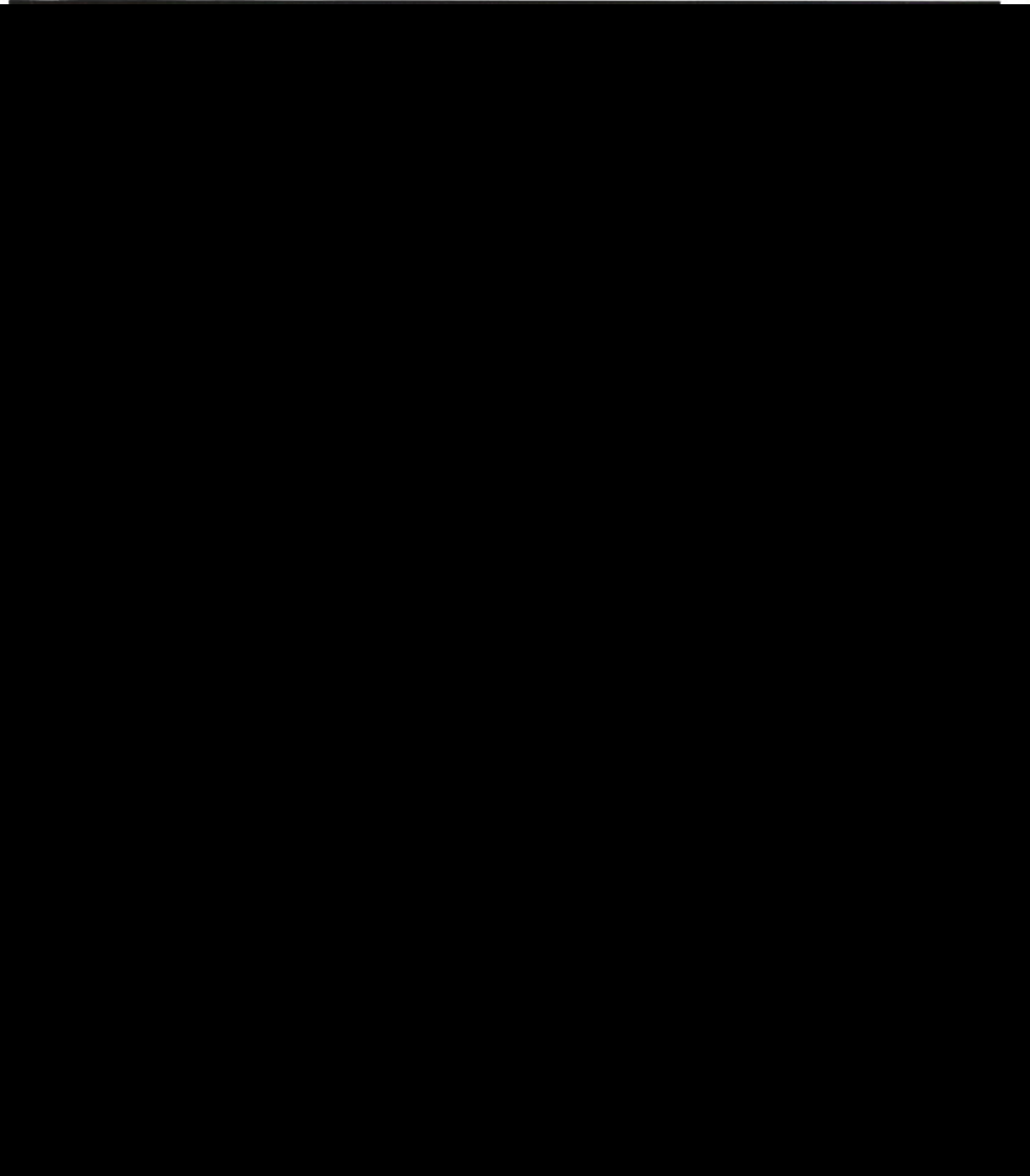
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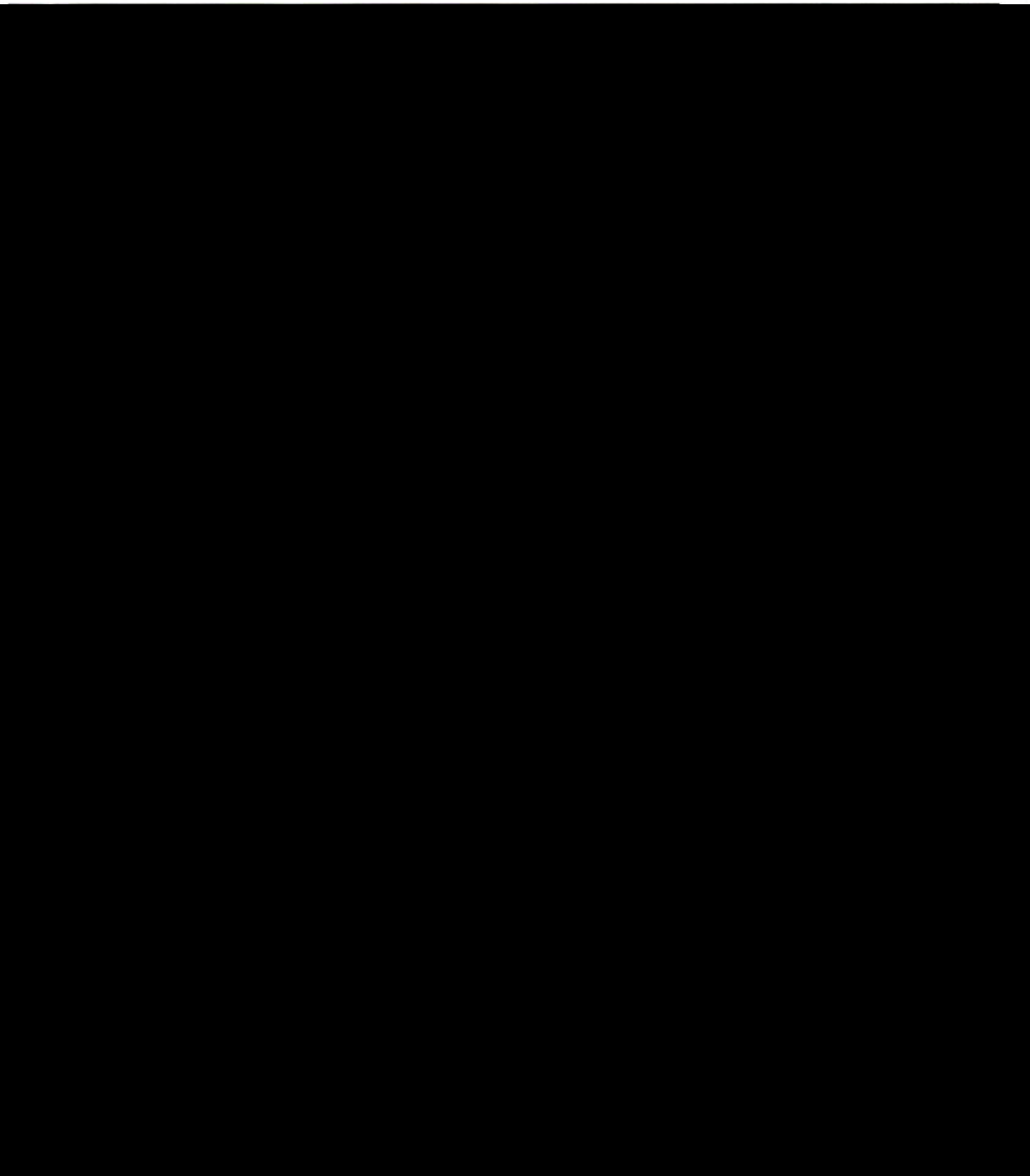
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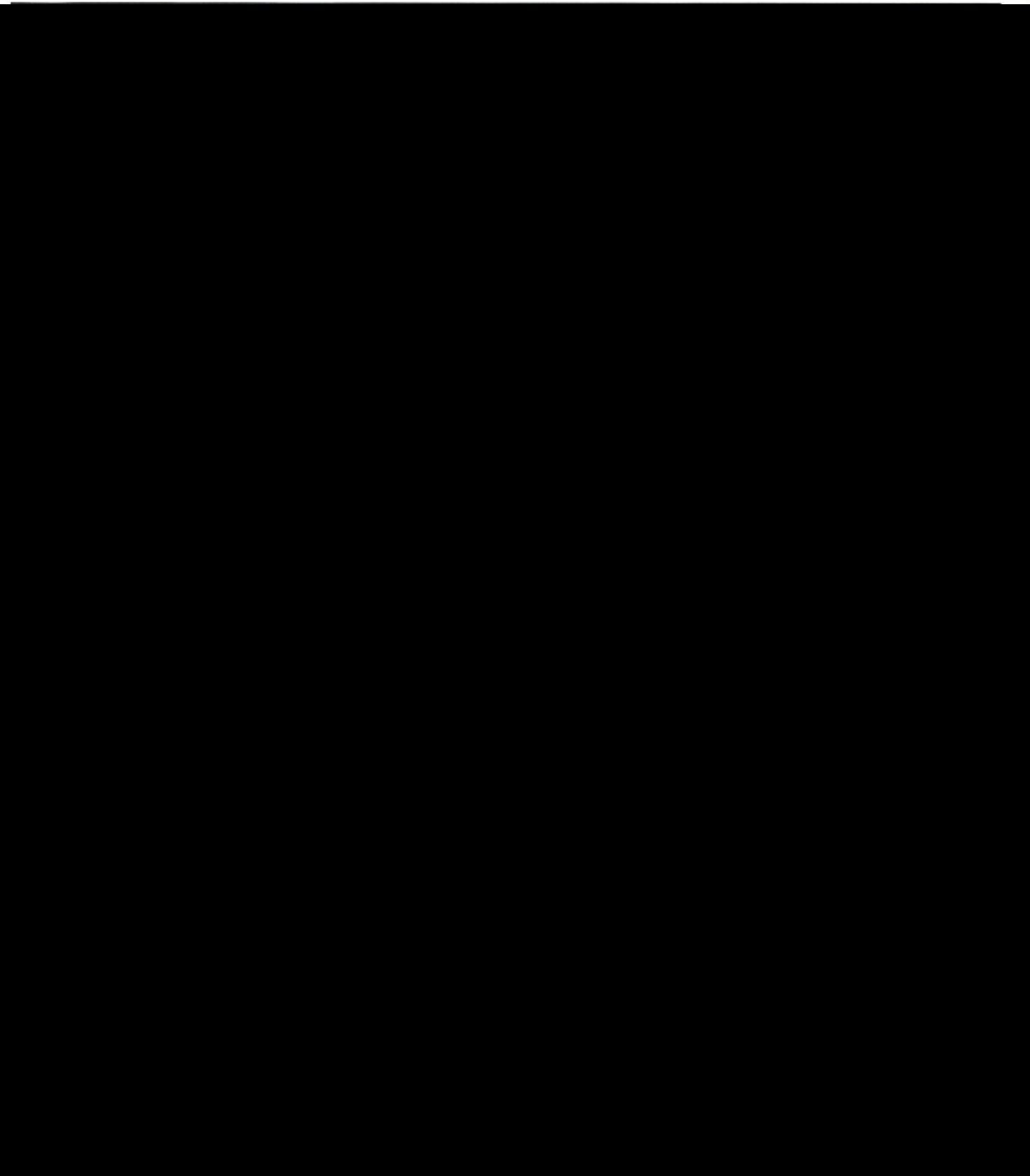
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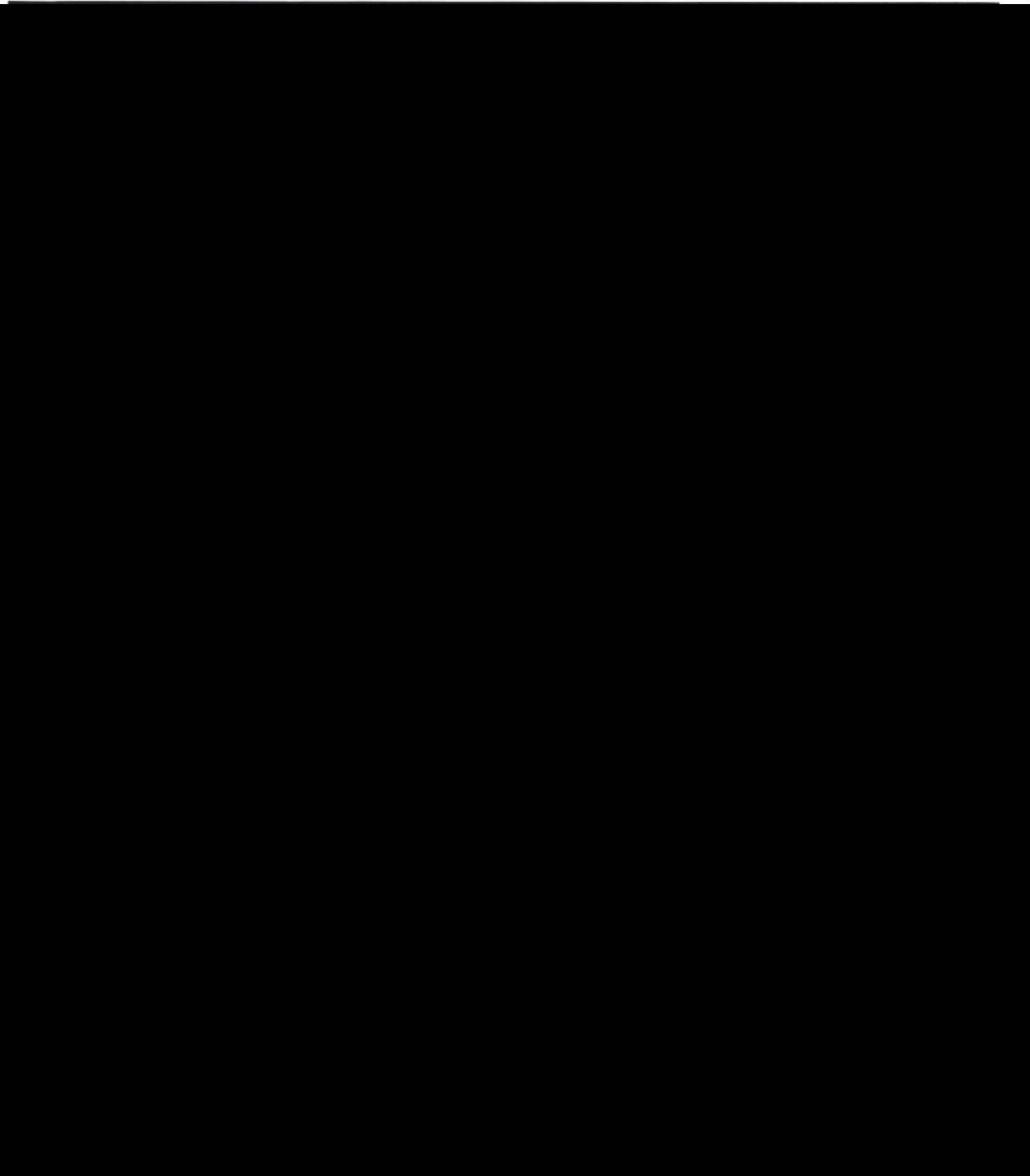
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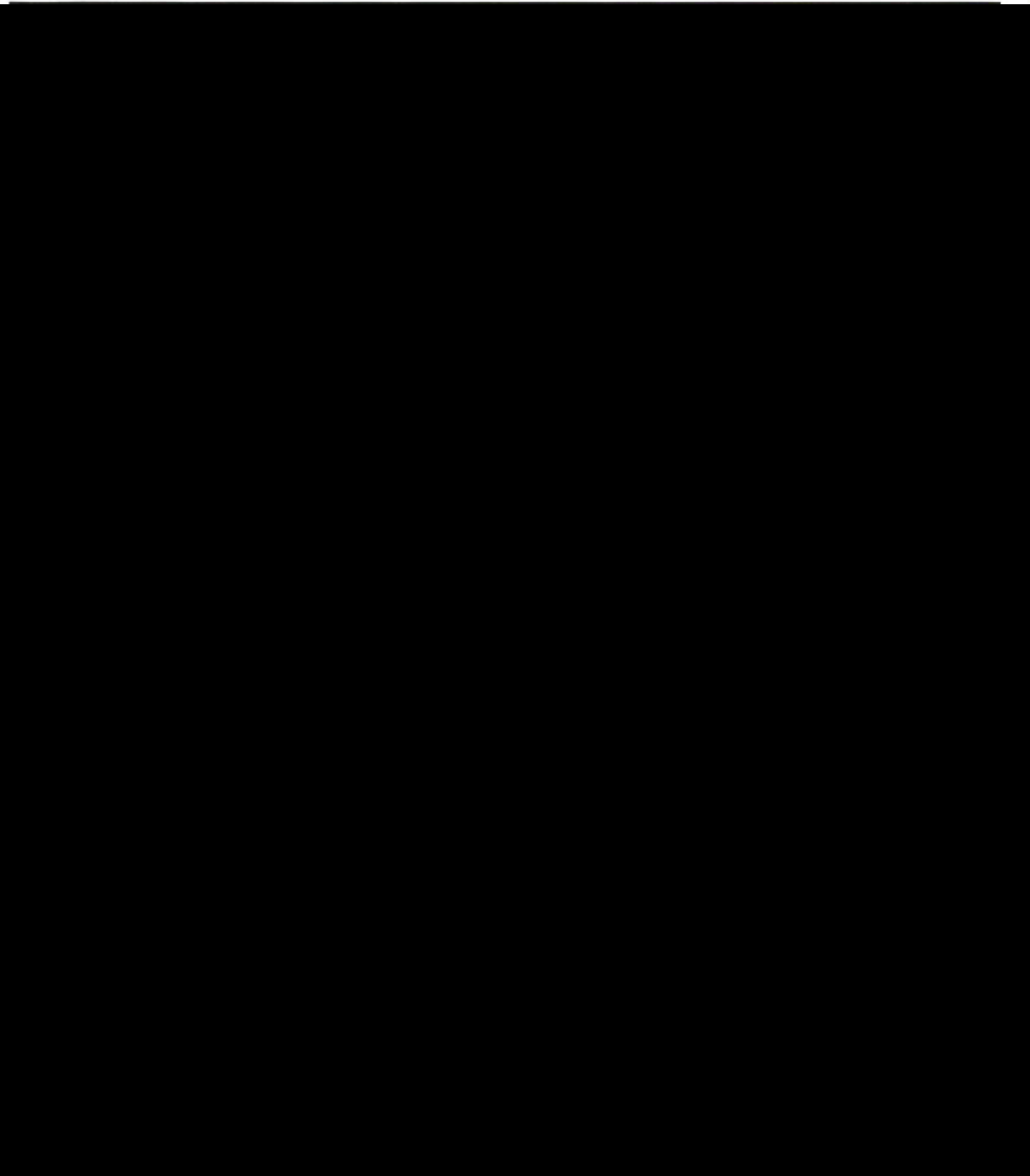
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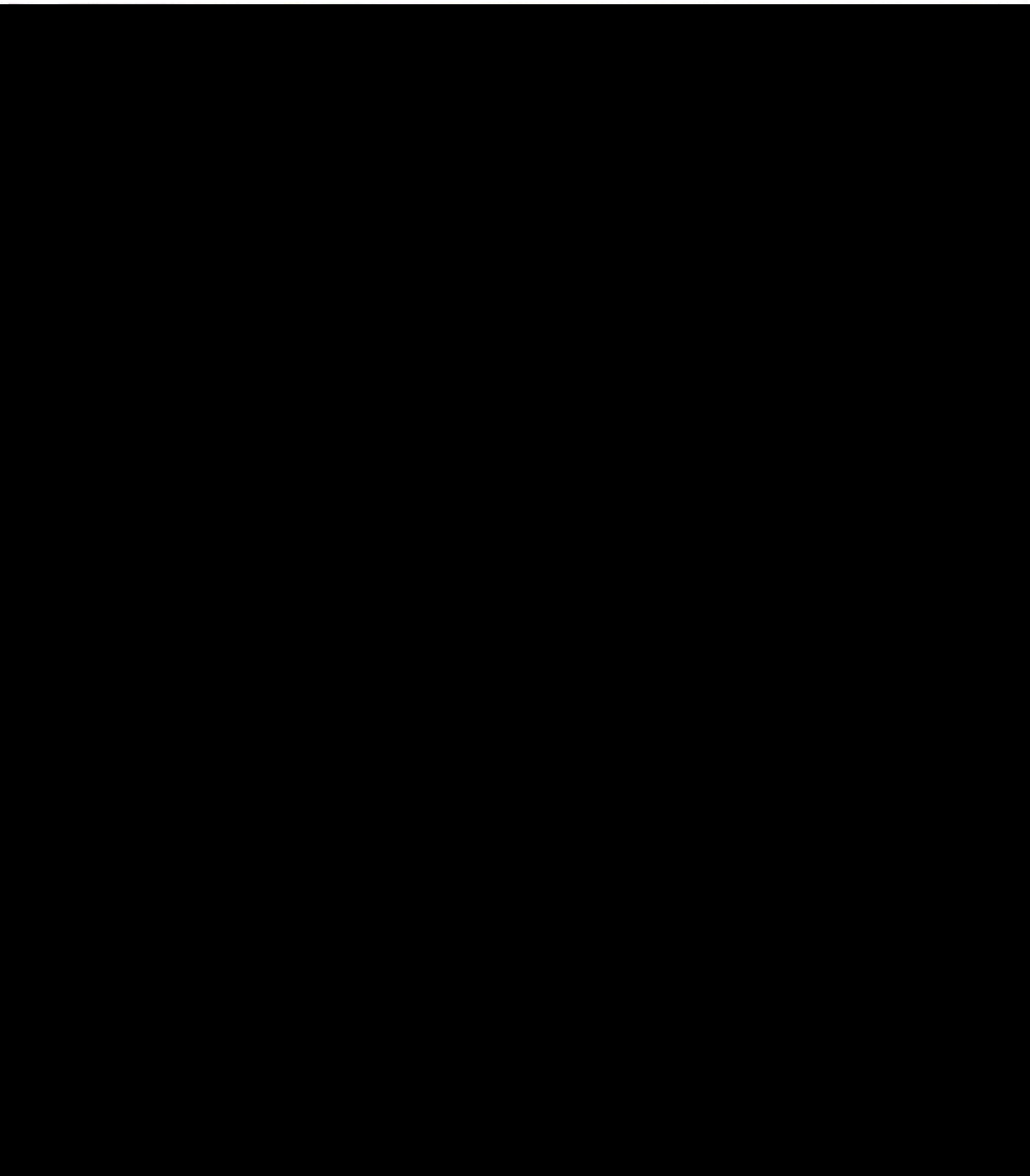
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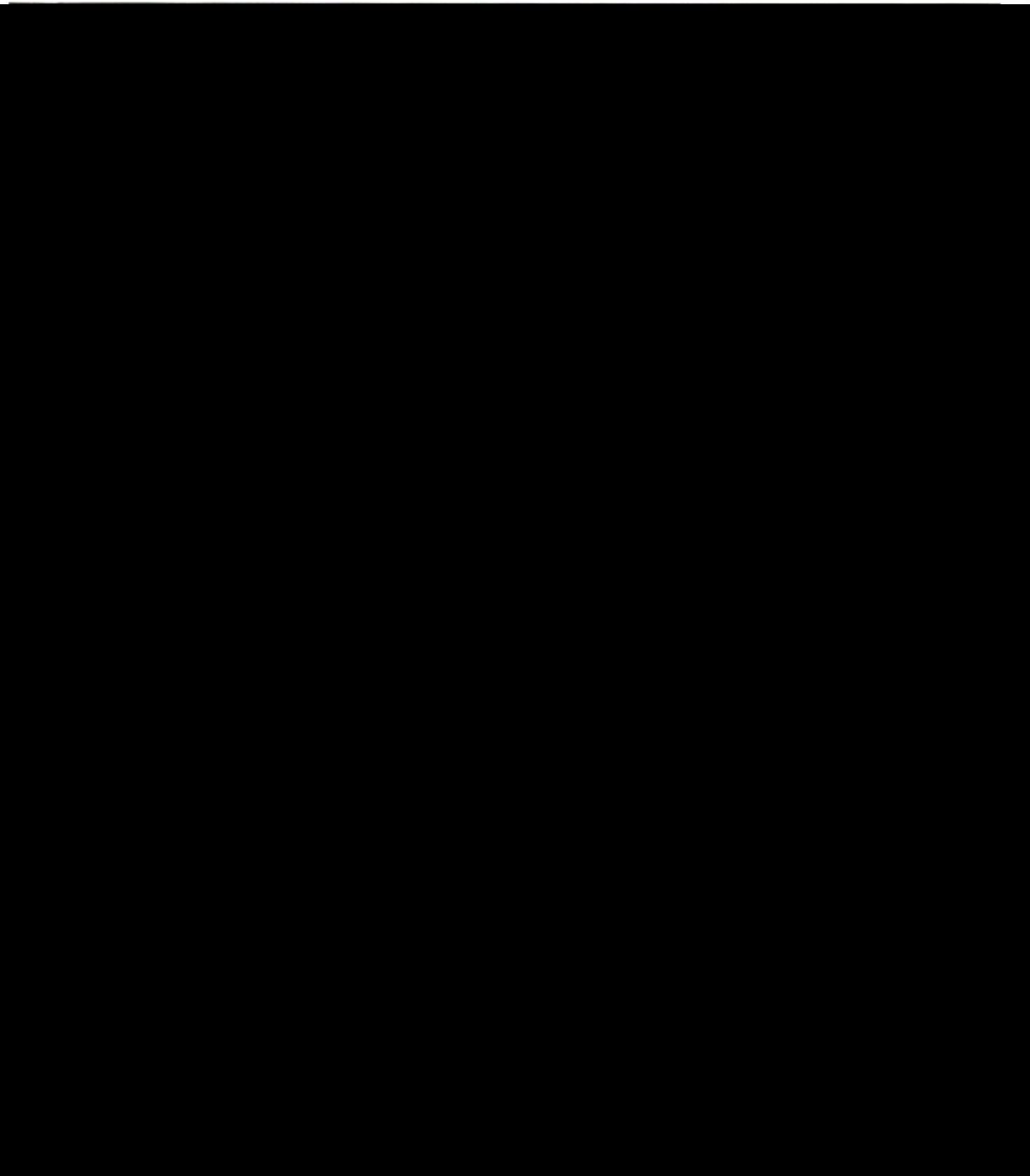
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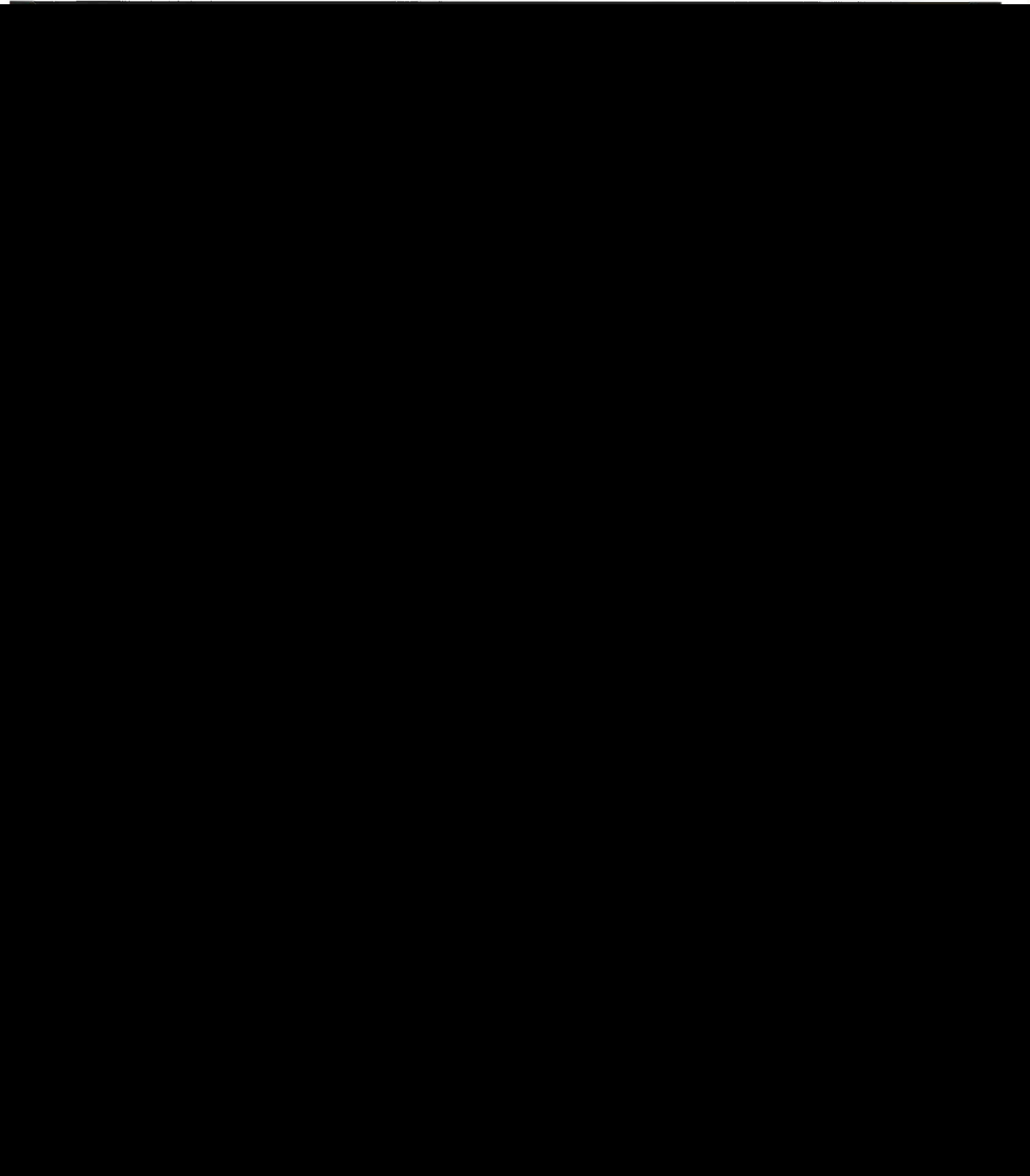
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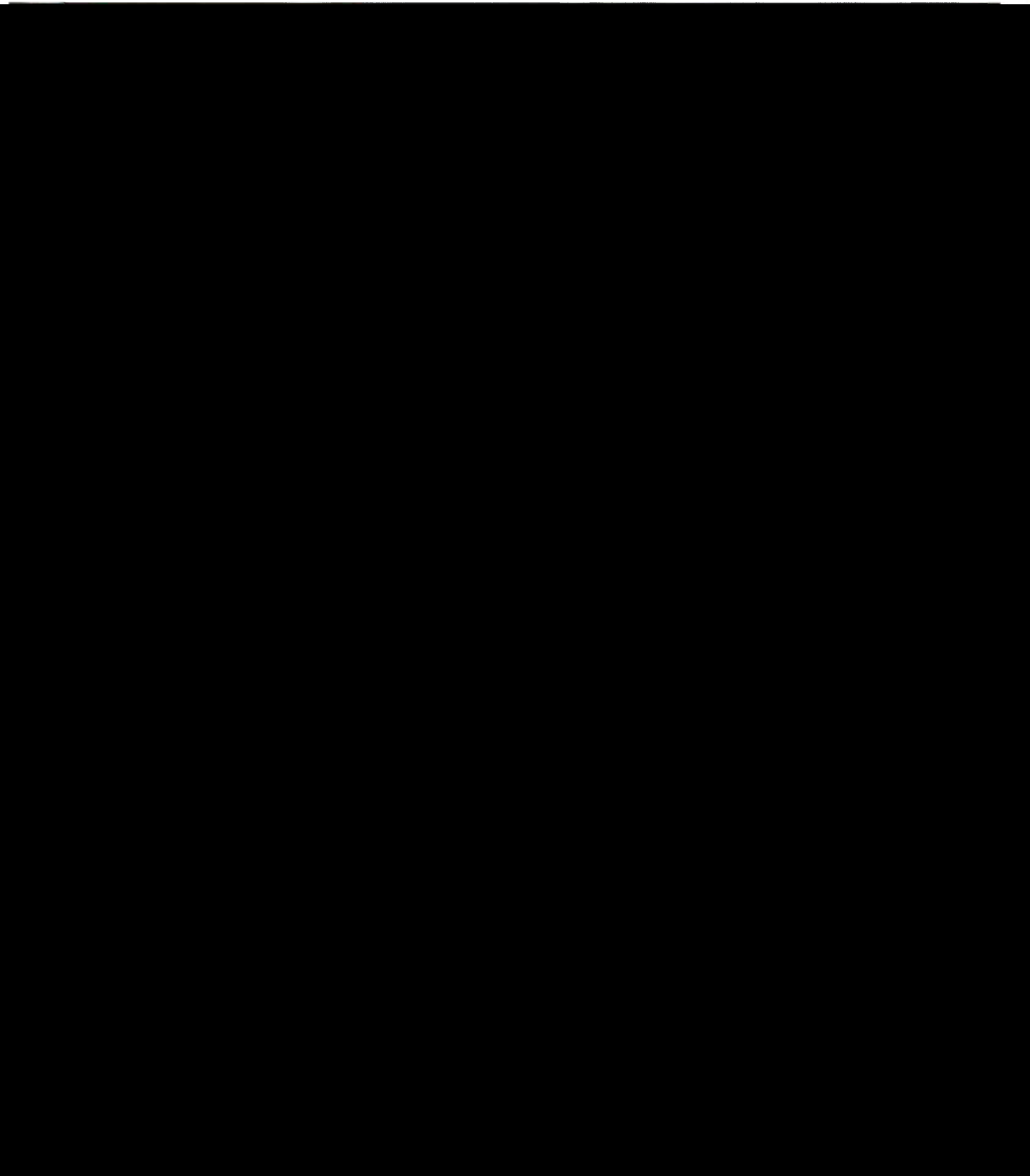
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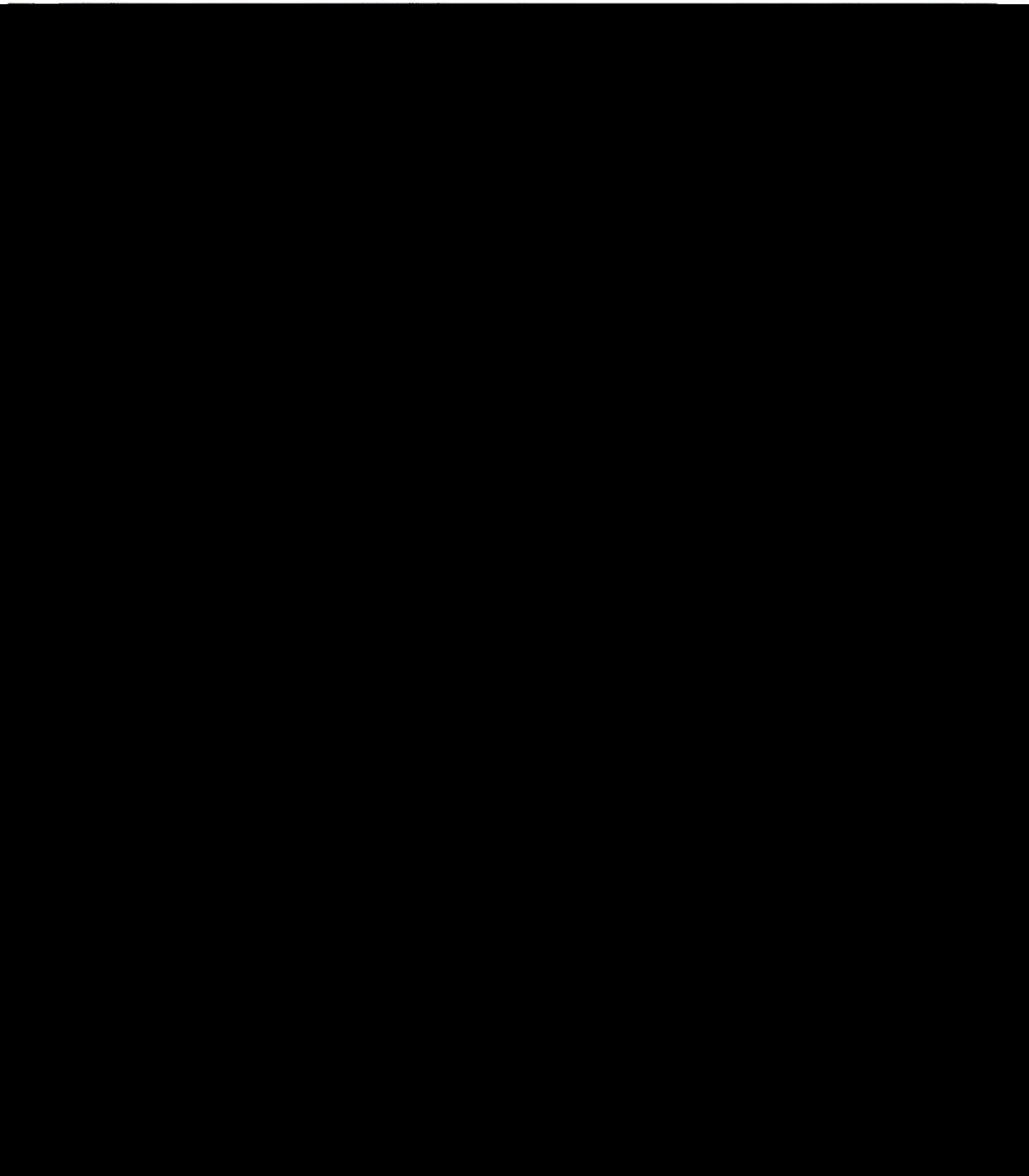
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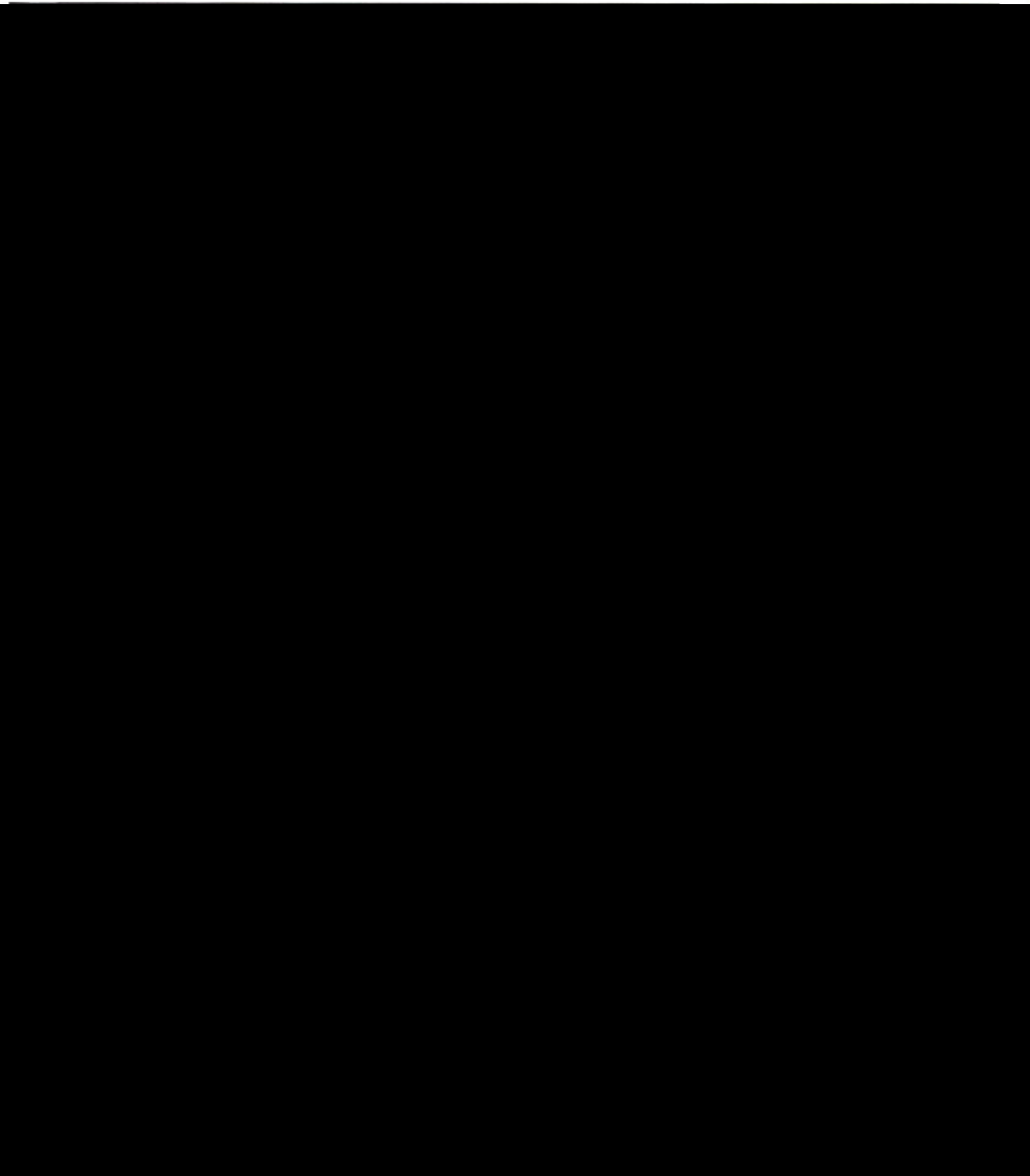
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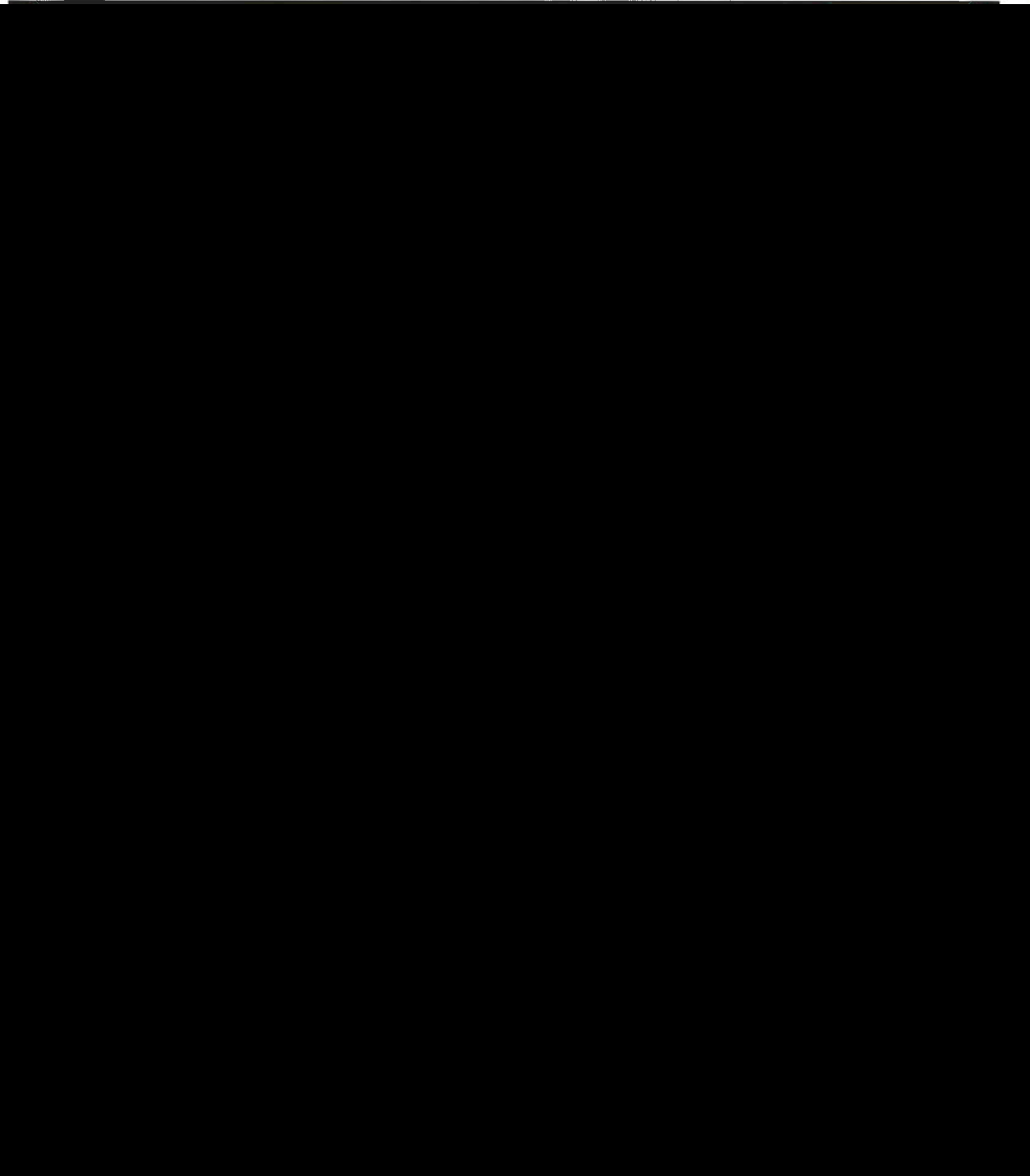
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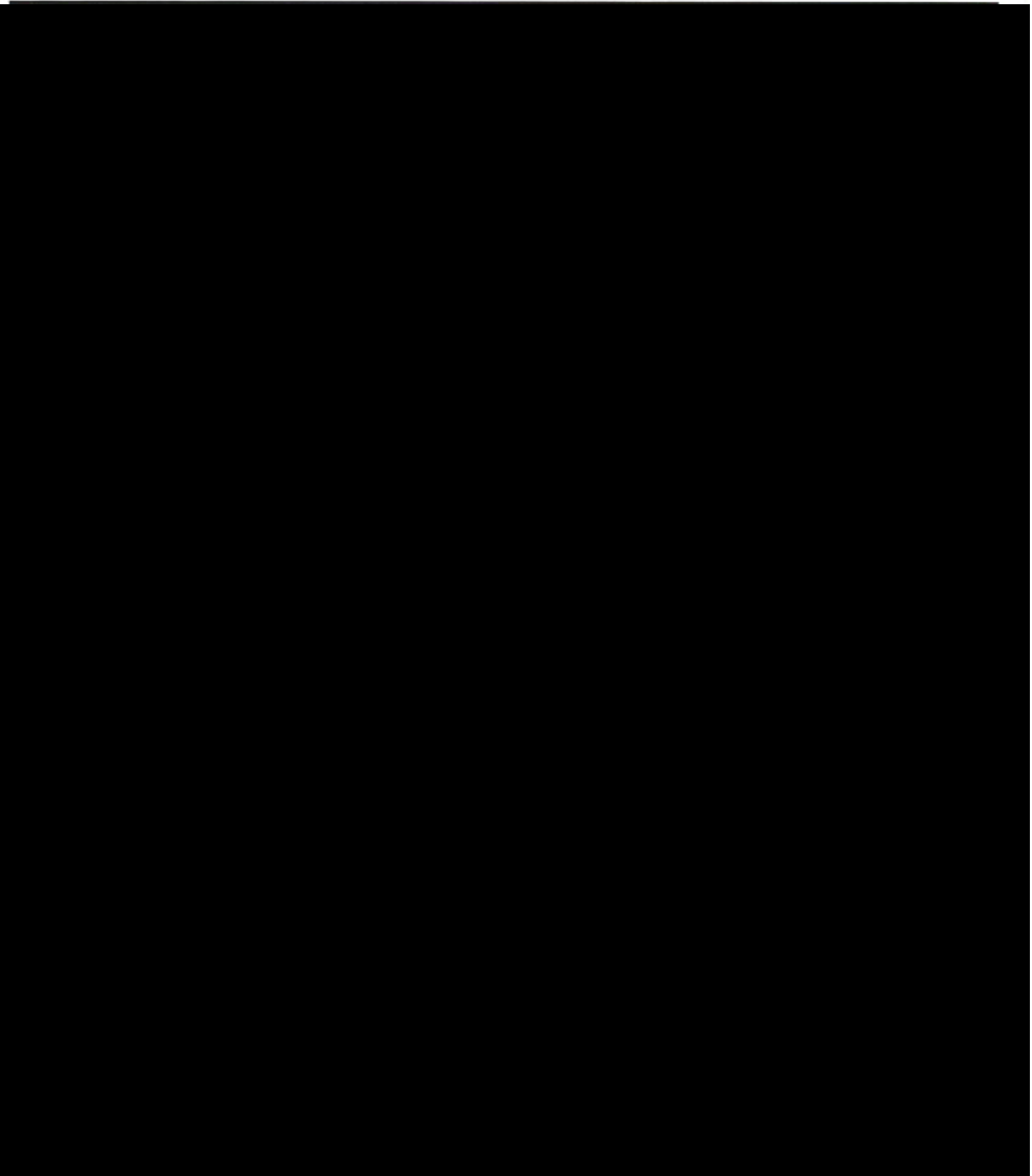
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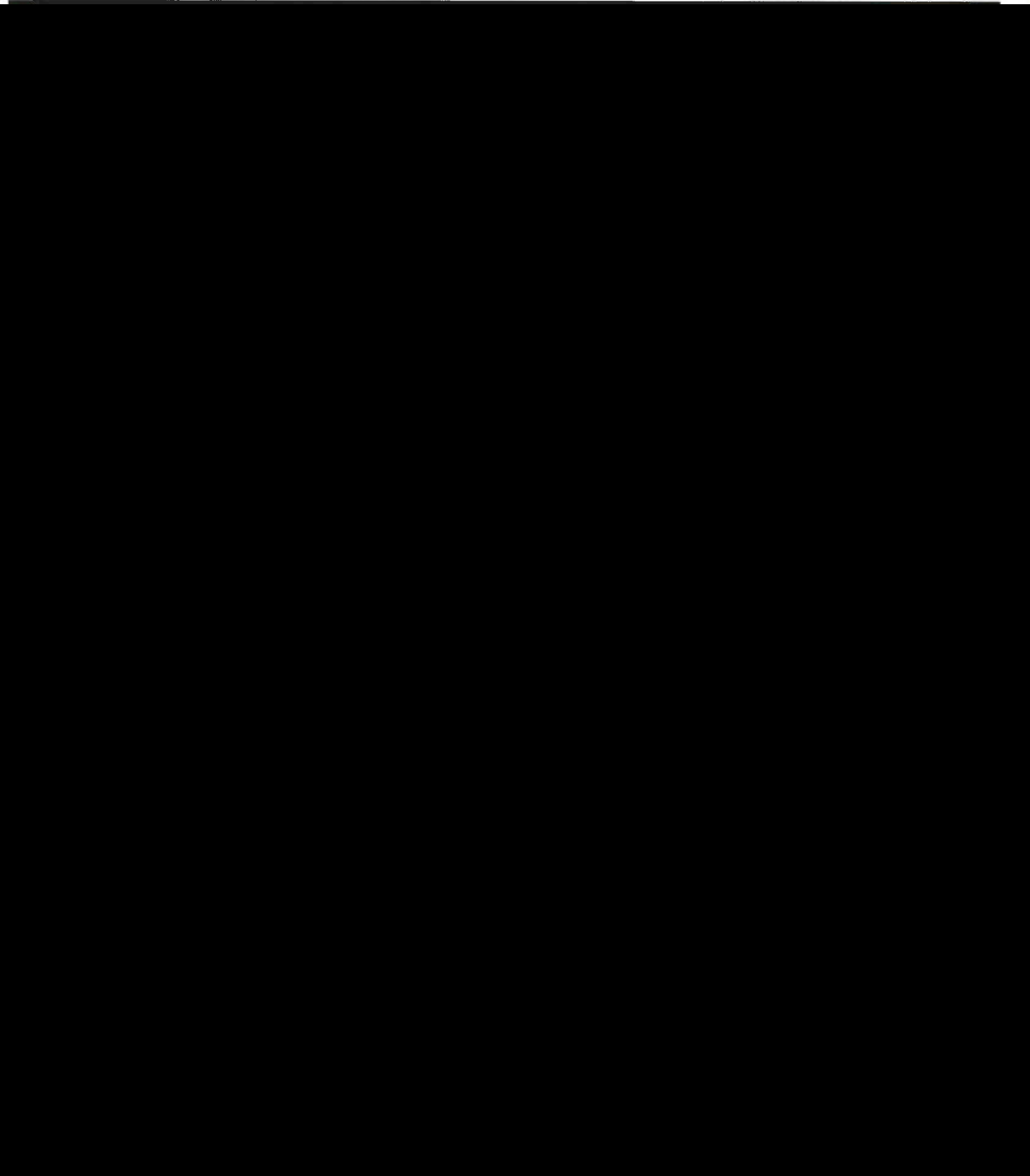
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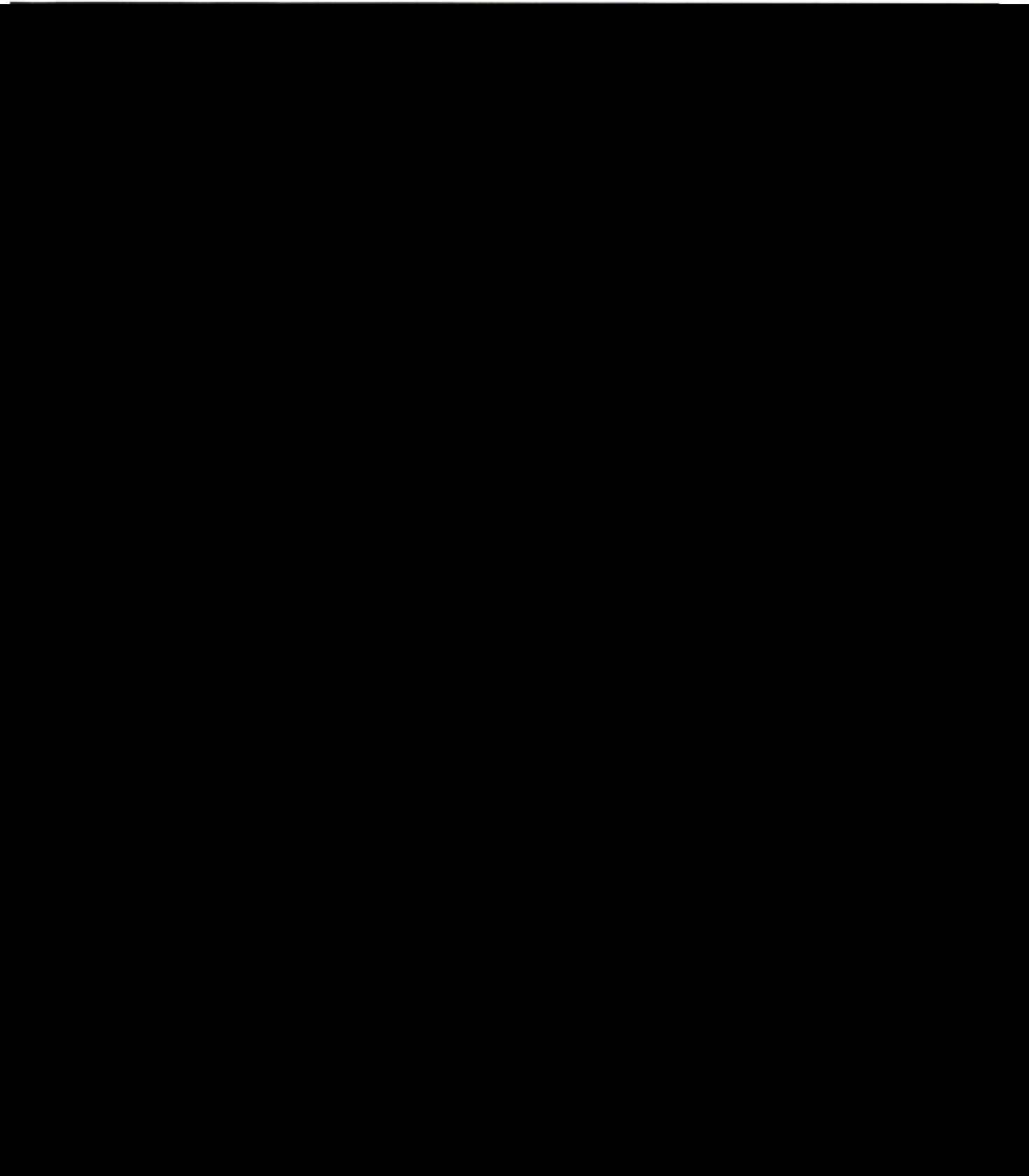
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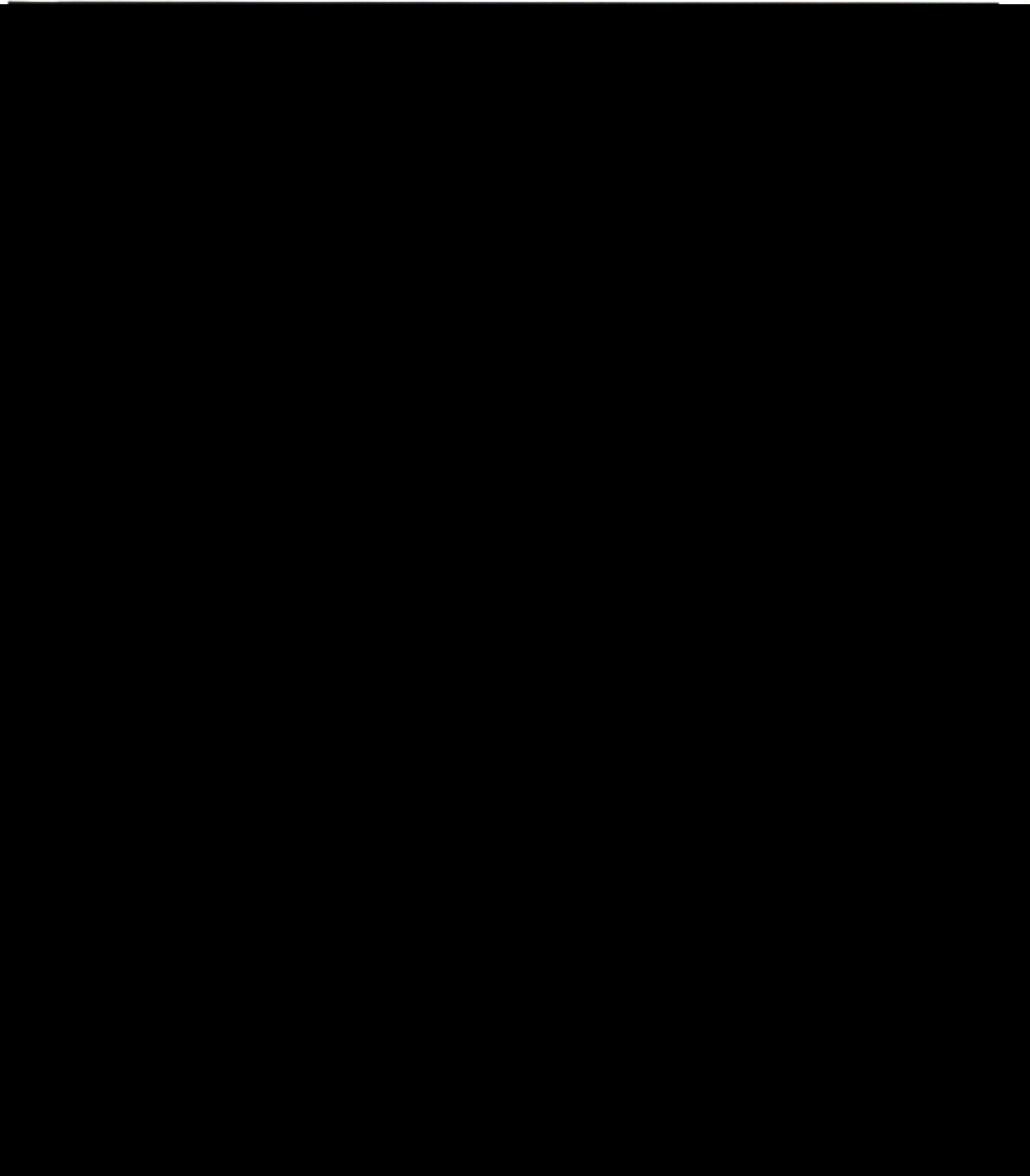
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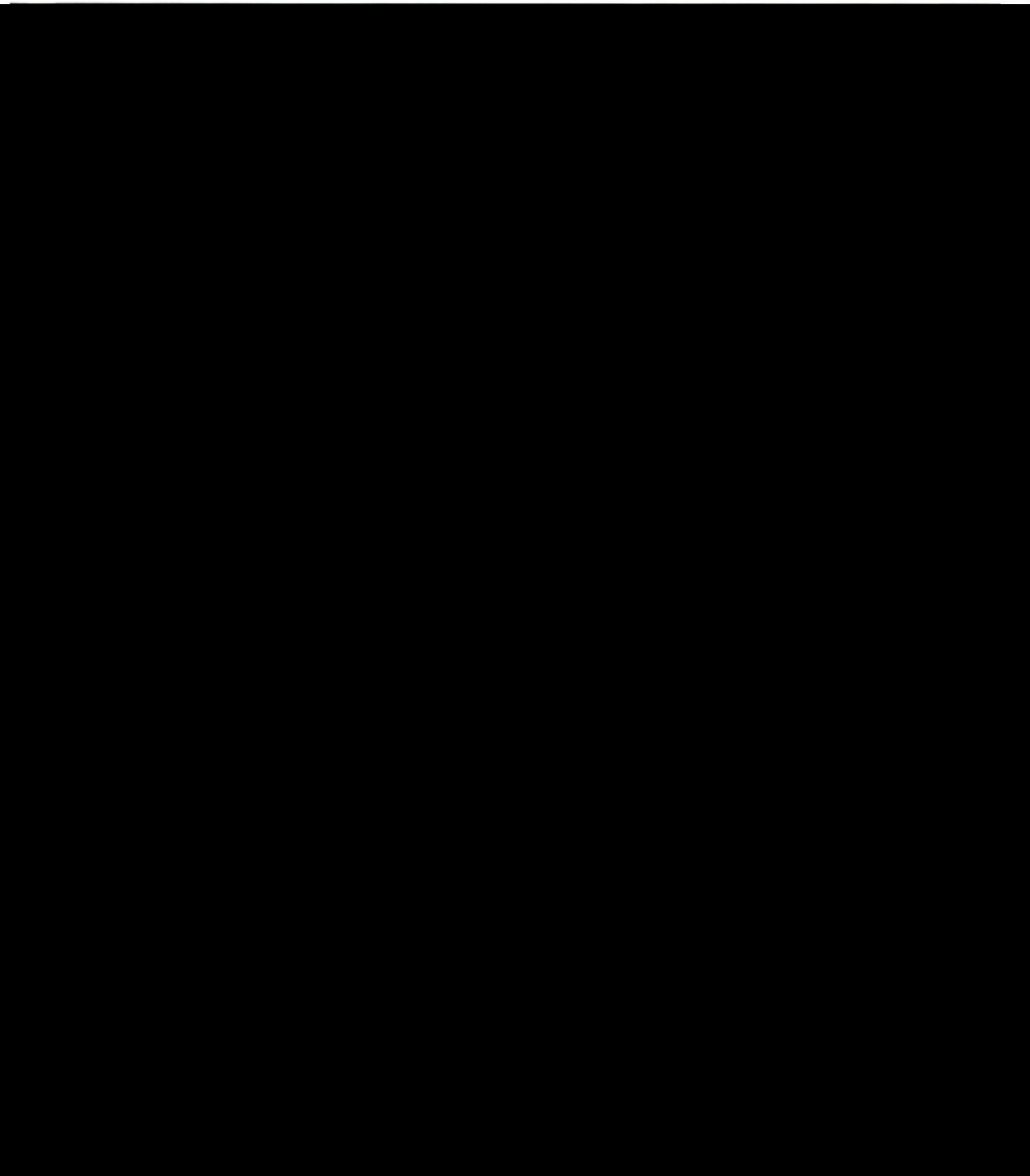
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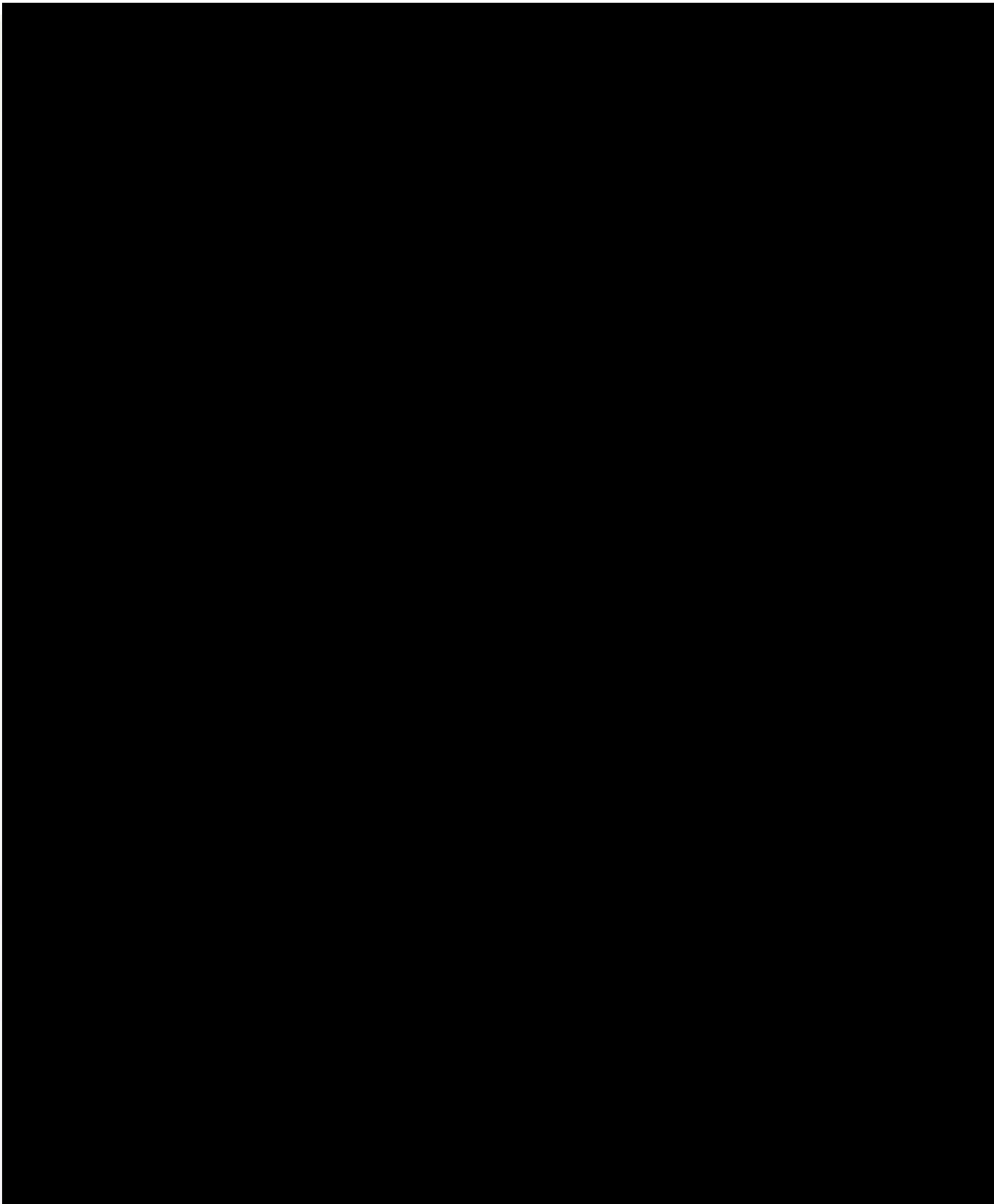
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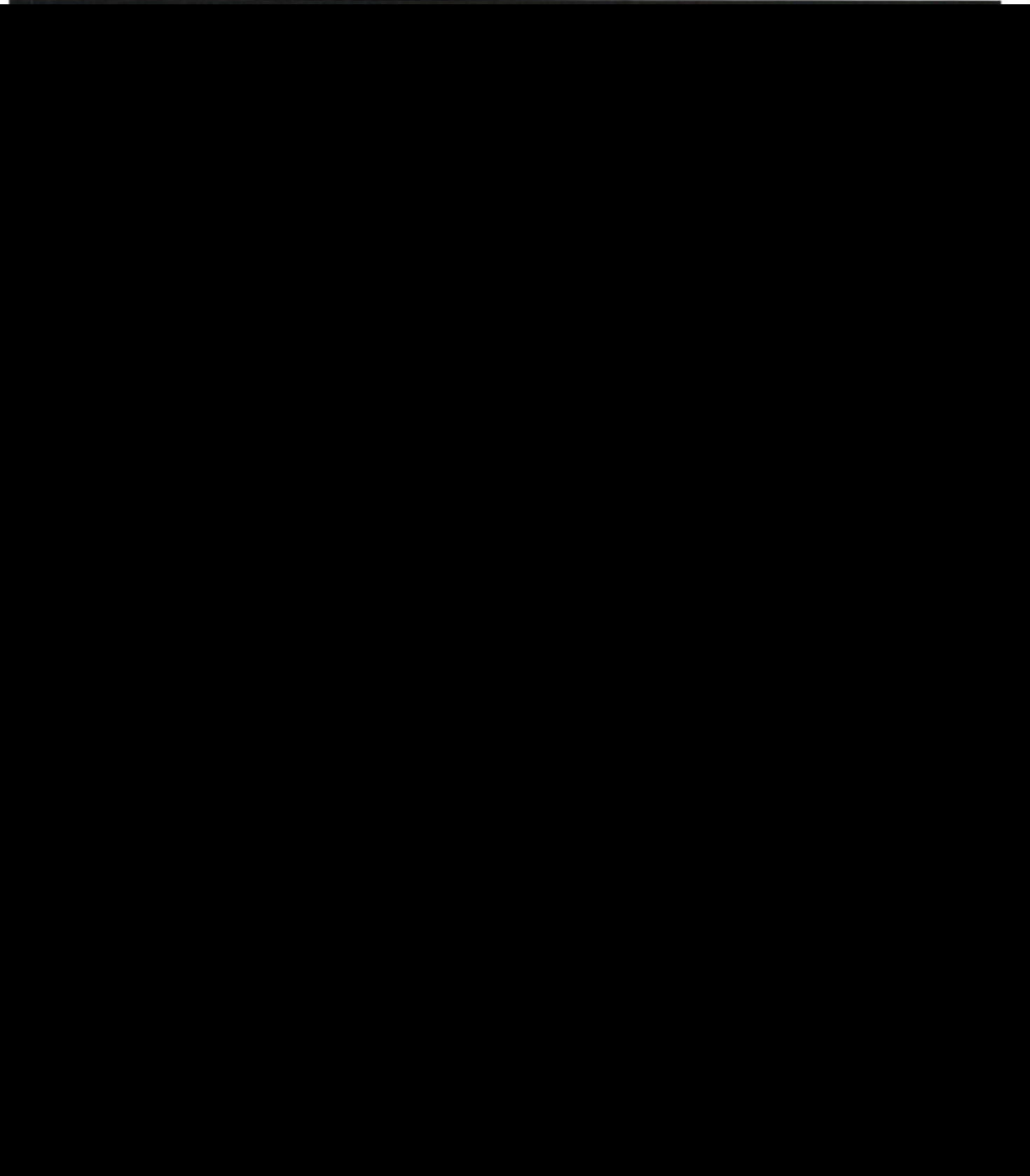
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Witnessed & Understood by me,

[Handwritten Signature]

Date

6/1/90

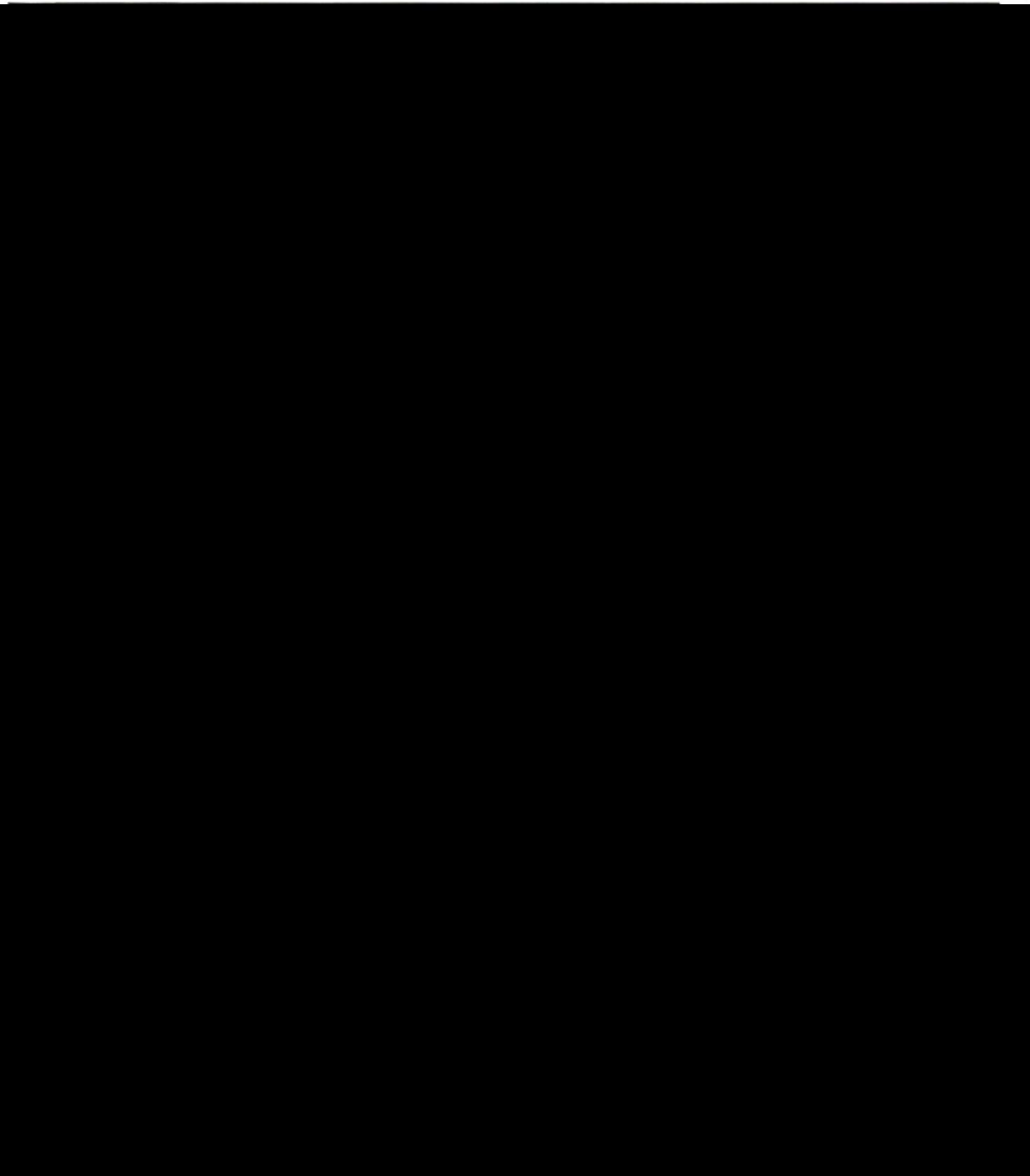
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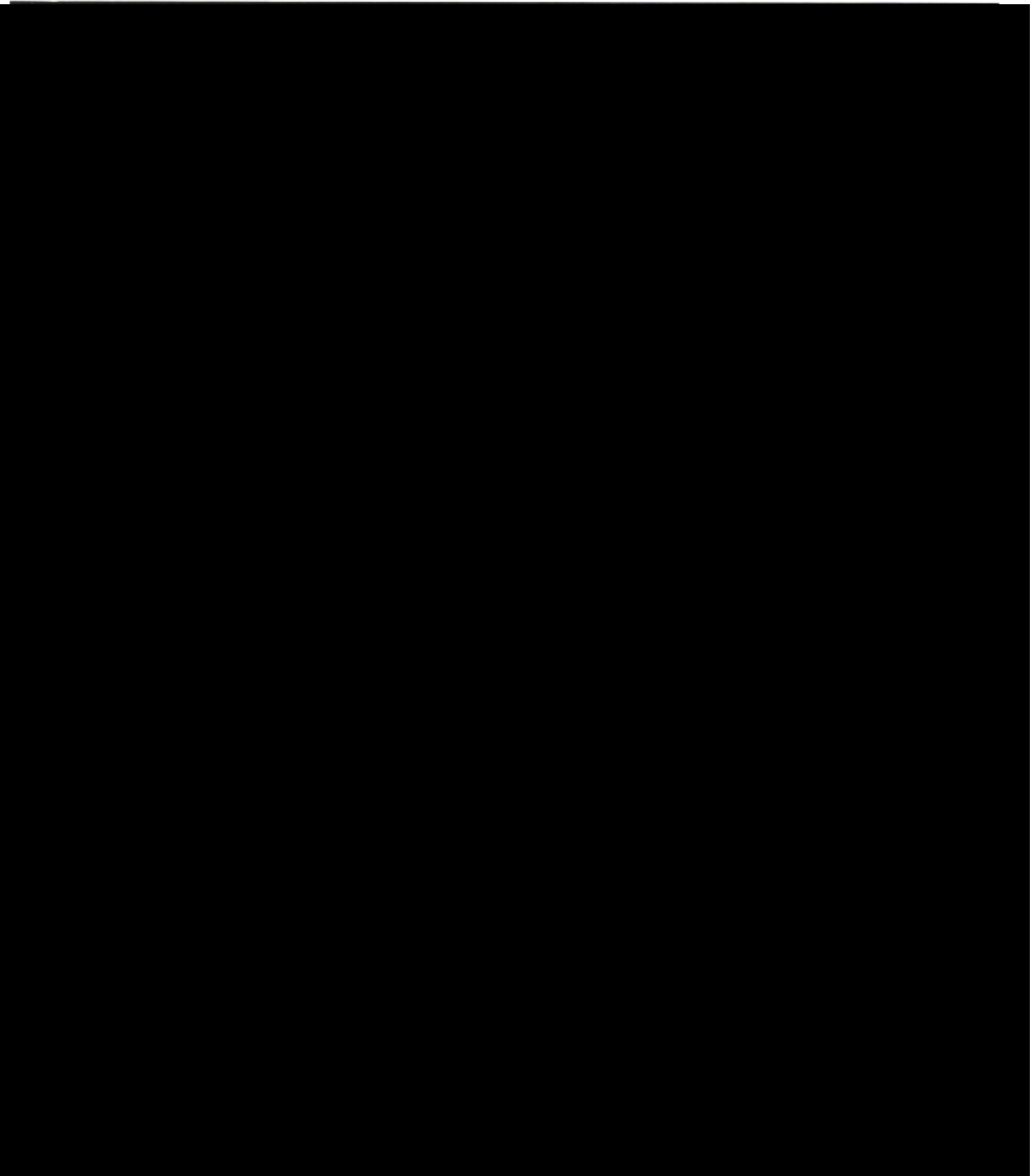
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5/23/92



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		Recorded by	



Witnessed & Understood by me, <i>[Signature]</i>	Date <i>6/1/90</i>	Invented by <i>John Redgen</i> Recorded by	Date <i>5/23/90</i>	To Page No. _____
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Humanized 405 (wild type) H+L

TLE

293 λ transient Mutant/He 405

Project No. _____

Book No. _____

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5/9/90 1) pA5 Heavy ^{I_gH} Hu405 : 5.5. pA5. hu4d5hc
clone 2/1 DNA prep 3.38 μ g/ λ

2) pA3 Light Hu405-K1 : 5.5. pA3. hu4d5hc
clone 7 DNA prep 2.74 μ g/ λ

4 x 100 μ m² dishes : 10 μ g/dish x 4 = 40 μ g DNA total
in 4 ml

DNA ratios 4:4:2, pA5: pA3: ALVA 0.88 μ g/ λ .

16 μ g pA5 \div 3.38 = 4.7 ml

16 μ g pA3 \div 2.74 = 5.8 ml

8 μ g ALVA \div 0.88 = 9 ml

DNA	%OTE	CaCl ₂	2x Hepar
pA5 - 4.7 ml	1.8 ml	200 μ l	2.0 ml
pA3 - 5.8 ml			
ALVA - 9 ml			

Feed cells 10AM

PM on 1: PM

Shake 4: PM Repeat w/ 12% FBS plate

5/10/90 10AM Change to Serum Free P504 + Ins + Glu.
Plate very near, conflu. 1 plate split 1 \rightarrow 4. Sealed w/ Reg 10% FBS
medium until cells settle 4pm. Change 4 plate back to Serum Free + Ins + Glu.

5/11/90 Fri. Collect media Sup + re pl. all plates

To Page No. 39

Witnessed & Understood by me,

Glynn A. Coy

Date

6/1/90

Invented by

John R. ...

Recorded by

Date

5/23/90

Project No. _____

Book No. _____

TITLE 4D5 2.6 in MTX

From Page No. _____

5/10/90 Count $56,860 \times 40 = 2,274,400$ /ml

Seed at 5×10^7 use 0.25 ml./plate

MTX at $2.5, 10, 20 \mu M$

Froze rest of cells 2 vials at 1×10^7 cells each

labeled

4D5. 2.6
JK

5/14 fl

5/29

To Page No. _____

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

[Signature]

6/1/90

[Signature]

5/22/90

om Page No. _____

5/9/90 Only 1 colony was produced by 10 μ M MTX so try again w/ less.
 Thaw 1 vial from 4/17/90, into 3T 100s. Cells look great 79% viability.
 Wait for recovery. Seed at 5 x 10⁵ cells/100² dish (Re-fuse 2 vials)
 Use 2, 5, 10, 20 μ M MTX. + 0 μ M MTX Control

5/10/90 Trypsin 1 plate resuspended in 10 ml.
 Count 0.5 ml in 9.5 ml PBS = 20x dil machine count 0.5 ml
 12,674 x 20 (dil) x 2 = 506,960 cells/ml
 Use 1 ml/plate + 9 ml 4% X02 + Glu (no GHT)

Stock 1000 μ M MTX.

2 μ M : $\frac{2\mu M}{1000\mu M} \times 10,000\text{ul} = 20\text{ul}$ of 1000 μ M MTX

5 μ M : $\frac{5}{1000} \times 10000 = 50\text{ul}$ of 1000 μ M MTX

10 μ M : use 100 ul 1000 μ M MTX

20 μ M : use 200 ul 1000 μ M MTX

50 μ M : use 500 ul 1000 μ M MTX

There are 2.16 cells not 2.6

5/14 Feb
 5/26 Feb

To Page No. _____

Witnessed & Understood by me, <i>[Signature]</i>	Date 6/1/90	Invented by <i>[Signature]</i>	Date 5/22/90
		Recorded by	

From Page No. _____

5/10/90 Transfection p35.

table 1 100µl dish, 1 293 CTR

2.5µl label x 2 = 5µl label

Use 100µCi/µl cys, met ³⁵S.

= 500µCi cys, met ³⁵S

Use, Amersham label at conc of 1µCi/100µl.

500µCi/100µl = 5µl

10µCi/µl

Use 50µl each of cys, met ³⁵S in 5µl cys, met - mix.

See p. 51 for I.P. of Amers.

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Witnessed & Understood by me, *[Signature]*

Date 6/1/90

Invented by *[Signature]*
Recorded by *[Signature]*

Date 5/23/90

TITLE Humanized 405 "wild type L+H" 293 trans.

Project No. _____

Book No. _____

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From Page No 35

5/14/90 Collected Sups from Fri 5/11 - Mon 5/14.
20ml unsplit cells + 40ml 1→4 split cells.

Transient Humanized:

Assayed: IgG (Human) + Her-2 Binding (ECD)

Unsplit 5/11	5.3 µg/ml	7.45 µg/ml	30hrs
Unsplit 5/14	7.1 - 7.7 µg	12.5 µg	65hrs
1→4 split 5/11	1 µg	1.44 µg	24hrs
1→4 split 5/14	1.35 µg	2.24 µg	65hrs

Control: 2.16 CHIMERA (Mouse Variable + Human Constant)
1.11 µg 1.92 µg

The humanized looks very similar to the Chimera and the transfection was very good.

Other: IgG Her-2 Binding

ASSAY RESULTS ARE NOT CORRECTED FOR DILUTION

NO.	IDENTITY PRODUCT CODE/STEP NO.	DILUTION	µg/ml	NO.	IDENTITY PRODUCT CODE/STEP NO.	DILUTION	µg/ml
1	1 unsplit 5/11/90	1/10	280	11	1→4 split 5/11/90	1/10	280
2	"	1/100	28.9	12	"	1/100	28
3	"	1/1000	5.3	13	"	1/1000	7.45
4	2 unsplit 5/14/90	1/2	210	14	"	1/2	210
5	"	1/10	7.1	15	"	1/10	7.7
6	"	1/100	7.7	16	"	1/100	12.5
7	3 1→4 5/11/90	1/10	118.6	17	"	1/10	1.44
8	"	1/100	11.85	18	"	1/100	2.24
9	"	1/1000	1.185	19	"	1/1000	2.24
10	4 1→4 5/14/90	1/2	600	20	"	1/2	600
11	"	1/10	120	21	"	1/10	120
12	"	1/100	12	22	"	1/100	12
13	5 1→4 2.16	1/2	1050	23	"	1/2	1050
14	"	1/10	105	24	3 thank you		
15	"	1/100	10.5	25			
16				26			
17				27			
18				28			

To Page No. _____

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

[Signature]

6/1/90

[Signature]

5/23/90

From Page No. _____

5/15/90 405 China 2.16 : Froze 3 vials from 2 100 disk
Labeled "405-2.16 China"

Results of 405-2.16 ELISAs
and 293 km of 405 for Mike Shepard

Genentech, Inc. (in blue)

ASSAY: He IgG ELISA

SAMPLES SUBMITTED BY: John R. Klein PROJECT NO. _____ PRODUCT _____

EXTENSION: 1107 DATE SUBMITTED: 5/10/90 DATE TO BE ASSAYED: 5/10/90

GMP BIOHAZARD
 OLP TOXIC
 RADIO NONE OF THE ABOVE
 MAIL RESULTS CALL WHEN READY
 * RESULTS NOT PICKED UP BY END OF DAY WILL BE MAILED.

Sample Matrix: He IgG NOTES: _____
 Anticoagulant (if used): ELISA
 Storage Conditions: Ambient 2-8°C -10°C or Below
 Other: _____

NO.	IDENTITY PRODUCT CODE/STEP NO.	DILUTION	R
1	405 2.16 5/10	1/10	2
2	"	1/50	2
3	"	1/100	2
4	"	1/200	2
5	"	1/400	2
6	293 405 5/10	1/10	2
7	"	1/50	2
8	"	1/100	2
9	"	1/200	2
10	"	1/400	2
11	293 405 5/7/90	1/10	31
12	"	1/50	32
13	"	1/100	33
14	"	1/200	34
15	"	1/400	35
16			36

Genentech, Inc. (in blue)

ASSAY: Her-2 Binding

SAMPLES SUBMITTED BY: John R. Klein PROJECT NO. _____ PRODUCT _____

EXTENSION: 1107 DATE SUBMITTED: 5/10/90 DATE TO BE ASSAYED: 5/10/90

GMP BIOHAZARD
 OLP TOXIC
 RADIO NONE OF THE ABOVE
 MAIL RESULTS CALL WHEN READY
 * RESULTS NOT PICKED UP BY END OF DAY WILL BE MAILED.

Sample Matrix: _____ NOT
 Anticoagulant (if used): _____
 Storage Conditions: Ambient 2-8°C -10°C or Below
 Other: _____

NO.	IDENTITY PRODUCT CODE/STEP NO.	DILUTION
1	405 2.16	1/10
2	"	1/50
3	"	1/100
4	"	1/200
5	"	1/400
6	293 405 5/10	1/10
7	"	1/50
8	"	1/100
9	"	1/200
10	"	1/400
11	293 405 5/7/90	1/10
12	"	1/50
13	"	1/100
14	"	1/200
15	"	1/400
16		

Her-2
ECS binding.

To Page No. _____

Witnessed & Understood by me,

John R. Klein

Date

6/1/90

Invested by

John R. Klein

Recorded by

Date

5/23/90

From Page No. _____

5/10/90 Submitted Seps from of 4DS Humanized + Chimera Ab to the bio assay.

Dilutions made to 0.32, 0.16, 0.08, 0.04 $\mu\text{g}/\text{ml}$ of ECD binding Ab, based on two less than 2 binding sites

4 Samples submitted:

1) 4DS chimera: mouse Variable region w/ the Constant region done 2.16. Starting conc 4.24 $\mu\text{g}/\text{ml}$

First dilution to 0.32 $\mu\text{g}/\text{ml}$ made as follows

$$4.24(x) = .32(5\text{ml}) \quad x = .377\text{ml}$$

$$377\text{ml of } 2.16 \text{ conc} + 4623\text{ml diluent} = 5\text{ml at } 0.32 \mu\text{g}/\text{ml}$$

2) 4DS chimera from 293 transient expression at 2.5 $\mu\text{g}/\text{ml}$

$$2.5x = .32(5) \quad x = 0.640\text{ml} \quad \text{QS} \rightarrow 5\text{ml}$$

3) 4DS humanized: mouse CDR w/ the Variable region backbone and the Constant region

293 trans exp 5.3 $\mu\text{g}/\text{ml}$ IgG, 7.45 $\mu\text{g}/\text{ml}$ ECD binding.

$$7.45(x) = .32(10) \quad x = .430\text{ml} \quad \text{QS} \rightarrow 10\text{ml}$$

4) 4DS humanized: 293 transient expression. 2.24 $\mu\text{g}/\text{ml}$ ECD

$$2.24(x) = .32(5) \quad x = 0.714\text{ml} \quad \text{QS} \rightarrow 5\text{ml diluent}$$

For all samples $\times 4$, use 1:4 serial dilutions to 0.04 $\mu\text{g}/\text{ml}$

Results p42, 43

To Page No. _____

Witnessed & Understood by me,

Glynnis W. Uy

Date

6/1/90

Invented by

[Signature]

Recorded by

Date

5/16/90

42 Genentech, Inc.
 Genentech, Inc.
 Genentech, Inc.
 Genentech, Inc.
 Genentech, Inc.

ASSAY REQUEST/REPORT FORM

GRAY TO BE COMPLETED BY ASSAY SERVICES	
A.S. EXPT NO. 11006-88	DATE
A.S. TECH. M.C	TEST PROCEDURE

ASSAY: *Anti Her-2 Bioassay*

SAMPLES SUBMITTED BY <i>John Ridgway</i>		PROJECT NO.	PRODUCT I.D.	COST CENTER NO. <i>435</i>	TIME OF REPORT
EXTENSION <i>X1107</i>	DATE SUBMITTED <i>5/16/90</i>	DATE TO BE ASSAYED <i>5/17/90</i>		NUMBER SUBMITTED	DATE
CHECK ONE OF THE FOLLOWING:					TIME
ASSAY STATUS AND SPECIFICATIONS					INIT.

<input type="checkbox"/> GMP	<input type="checkbox"/> BIOHAZARD	<input type="checkbox"/> Nonqualified	Control Value				
<input type="checkbox"/> GLP	<input type="checkbox"/> TOXIC HAZARD	<input type="checkbox"/> Qualified/Nonvalidated	Expected A	B	C	D	
<input type="checkbox"/> RADIO ACTIVE	<input checked="" type="checkbox"/> NONE OF THE ABOVE	<input type="checkbox"/> Validated	Obtained A	B	C	D	
<input type="checkbox"/> MAIL RESULTS*	<input type="checkbox"/> CALL WHEN READY	Acceptable Range					

* RESULTS NOT PICKED UP BY END OF DAY WILL BE MAILED.

Sample Matrix:	NOTES: <i>Dilutions in ug/ml of ECA binding activity, from Wai kei Her-2 binding elisa.</i>
Anticoagulant (If used):	
Storage Conditions: <input type="checkbox"/> Ambient <input type="checkbox"/> 2°-8°c <input type="checkbox"/> -10°c or Below	
<input type="checkbox"/> Other:	

ASSAY RESULTS ARE NOT CORRECTED FOR DILUTION

NO.	IDENTITY PRODUCT CODE/STEP NO.	DILUTION	4DB conc (ug/ml)	% Inhib CTRL	NO.	IDENTITY PRODUCT CODE/STEP NO.	DILUTION		
1	405 Chimera	0.32	.30	53	21				
2	Clone 2.16	0.16	.16	74	22				
3		0.08	.08	88	23				
4	↓	0.04	4.025	-	24				
5	405 Chimera	0.32	.10	84	25				
6	trans.out 293	0.16	4.025	-	26				
7		0.08	4.025	-	27				
8	↓	0.04	4.025	-	28				
9	405 humanized	0.32	4.025	-	29				
10	trans.out 293 #1	0.16	4.025	-	30				
11		0.08	4.025	-	31				
12	↓	0.04	4.025	-	32				
13	405 humanized	0.32	4.025	-	33				
14	trans.out 293 #4	0.16	4.025	-	34				
15		0.08	4.025	-	35				
16	↓	0.04	4.025	-	36				
17	293 media ctrl	1/5	4.025	-	37				
18	sp.12 media ctrl	1/5	4.025	-	38				
19					39				
20					40				

John Ridgway 5/23/90

TITLE

4D5 hybridoma standard for Bio Assay

Project No. _____

Book No. _____

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HHG

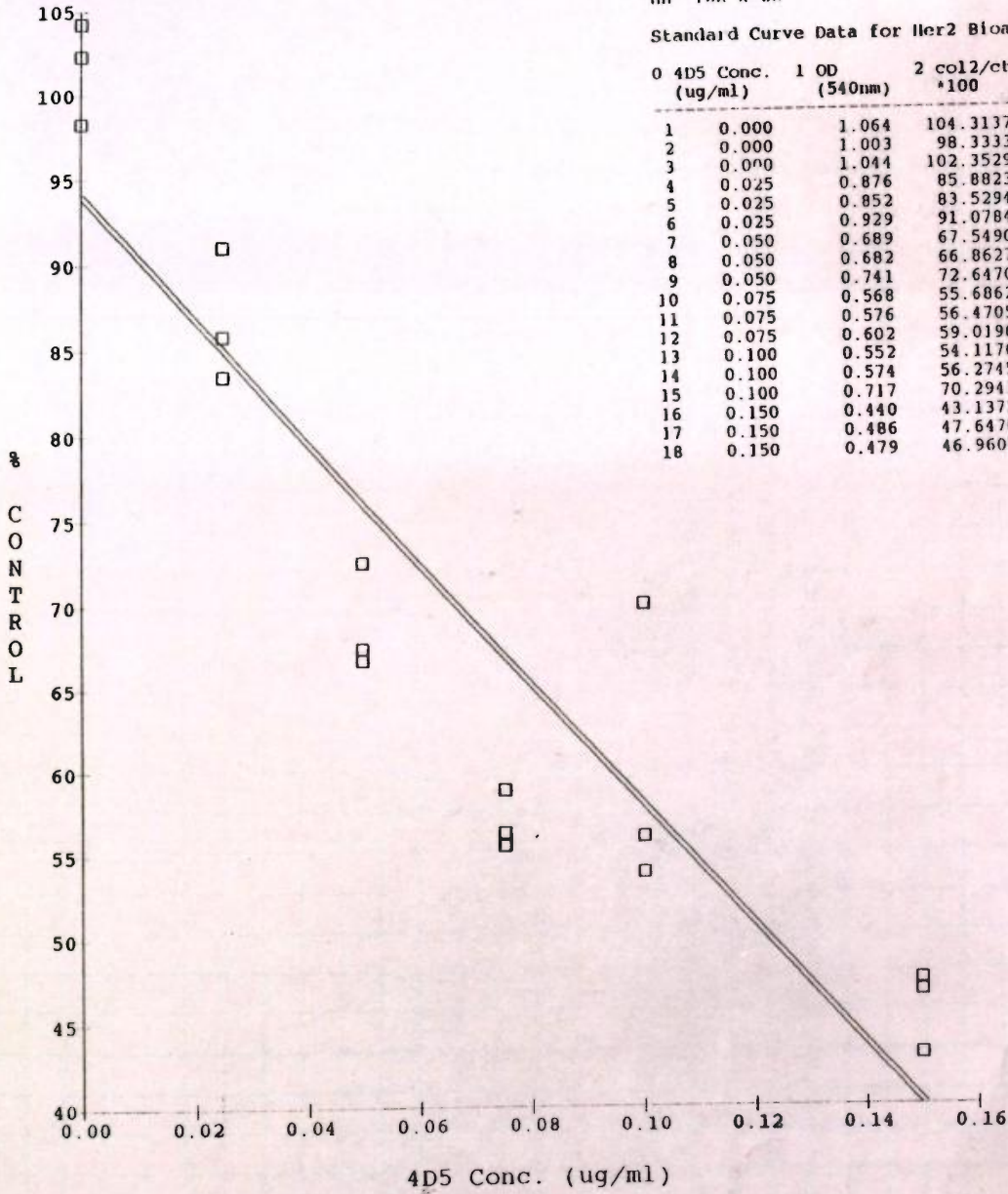
21-MAY-90 16:53 Page 1

4D5 Standard Curve

III 1BR x 2C

Standard Curve Data for Her2 Bioassay 11006-88

0	4D5 Conc. (ug/ml)	1 OD (540nm)	2 col2/ctrl *100
1	0.000	1.064	104.313725
2	0.000	1.003	98.333333
3	0.000	1.044	102.352941
4	0.025	0.876	85.882353
5	0.025	0.852	83.529412
6	0.025	0.929	91.078431
7	0.050	0.689	67.549020
8	0.050	0.682	66.862745
9	0.050	0.741	72.647059
10	0.075	0.568	55.686275
11	0.075	0.576	56.470588
12	0.075	0.602	59.019608
13	0.100	0.552	54.117647
14	0.100	0.574	56.274510
15	0.100	0.717	70.294118
16	0.150	0.440	43.137255
17	0.150	0.486	47.647059
18	0.150	0.479	46.960784



□ col2/ctrl *100
 ----- $-359.084967 * X + 94.058824$

To Page No. _____

Witnessed & Understood by me,

Date

Invented by

Date

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6/1/90

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5/23/91

From Page No. _____

5/23/90 Bioassay Done 5/17 - 5/22 to be repeated w/ Sample concentrations based on IgG elisa data not ECA binding

Sample 1: 405 chimera Stable line 2.16. Sup from 5/6/90

(p.40) By IgG Elisa: 4.5 ug/ml.

Dilute to 1.28 ug/ml

$$4.5(x) = 1.28(5ml)$$

$$x = 1.42$$

$$1.42 ml sup + 3.58 ml ~~sup~~ diluent = 5mls at 1.28 ug/ml$$

Dilute 1:2 For Conc. .64, .32, .16, .08, .04 ug/ml

Sample 2: 405 chimera From 293 trans 3/9/90 2.5 ug/ml IgG
make dilutions ~~2.5~~ 1.25 \rightarrow .04 ug/ml

Sample 3: Human 405 - Version (a) From 293 trans 5/14/90 sup 39.
By IgG Elisa: 7.1 ug/ml

Dilute to 2.56 ug/ml

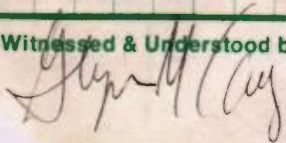
$$7.1(x) = 2.56(5ml)$$

$$x = 1.8ml$$

$$+ 3.2 ml diluent = 2.56 ug/ml$$

make ~~2.56~~ 2.56 \rightarrow .04 ug/ml dil.

Witnessed & Understood by me,

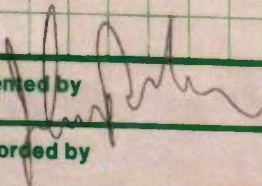


Date

6/11/90

Invented by

Recorded by



Date

6/11/90

To Page No. _____



Project No. _____

TITLE _____

Book No. _____

45

From Page No. _____

[Large grid area for data entry]			
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To Page No. _____

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

46

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

A large grid of graph paper, approximately 30 columns wide and 60 rows high, enclosed in a green border. The grid is intended for recording data or observations during an experiment or project.

Witnessed & Understood by me, _____

Date _____

Invented by _____

Date _____

Recorded by _____

To Page No. _____

From Page No. _____

5/23/90 All Samples in 1/10, 1/100, 1/1000 dil

Samples: ^{1000ug/ml} 1) 4AS 2.6 10uM MTX - started 5/10/90

2) 4AS 2.6 20uM MTX - "

3.3ug/ml 3) 4AS 2.6 10uM MTX clone selected from Amp started 4/18

3.4ug/ml 4) Same as #3 - 3-4 day culture.

1.9ug/ml 5) 4AS 2.16 5uM MTX - started 5/10/90.
- this culture looks partially selected - too many clones

600ug/ml 6) 4AS 2.16 10uM MTX - 5/10 - in complete killing

200ug/ml 7) 4AS 2.16 20uM MTX - 5/10

4ug/ml 8) 4AS 2.16 No MTX > 5 day sup.

3.6ug/ml 9) 4AS 2.16 5/6/90 used in Bioassay

1.7ug/ml 10) ~~4AS~~ 4AS 293 trans 3/9/90 used in Bio Assay

7.3ug/ml 11) Humanized 4AS pA3, pA5 293 trans from 5/14 #2 used in Bio Assay

290 ug/ml 12) 4AS 2.16 O.N. on cells in 100mm dish to test if all are still producing Ab. (whole cells)

- | | | | | | | |
|-------|--------------|-----|----------|------------------------------|---------------|---------------------|
| 570ug | 10uM MTX Amp | 13) | 4AS-2.6 | - 10 ⁵ start seed | Pa Cell Assay | 3 days old culture. |
| 1.3ug | | 14) | 4AS-2.6 | - 10 ⁶ | " | " |
| 2ug | | 15) | 4AS-2.16 | - 10 ⁵ | " | " |
| 4.5ug | | 16) | 4AS-2.16 | - 10 ⁶ | " | " |

Results next page

To Page No. _____

Witnessed & Understood by me,

John Rubin

Date 6/15/90

Invented by

John Rubin

Date

6/15/90

Recorded by

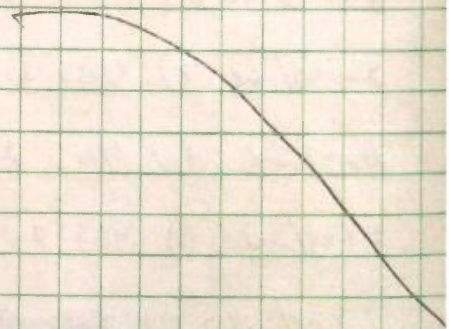
From Page No. 47

Results.

- 1.) 100 ng/ml
- 2.) 0
- 3.) 3.3 ug/ml
- 4.) 3.4 ug/ml
- 5.) 1.9 ug/ml
- 6.) 600 ng/ml
- 7.) 200 ng/ml
- 8.) 4 ug/ml
- 9.) 3.6 ug/ml
- 10.) 1.7 ug/ml
- 11.) 7.3 ug/ml
- 12.) 290 ng/ml
- 13.) 570 ng/ml
- 14.) 1.3 ug/ml
- 15.) 2 ug/ml
- 16.) 4.5 ug/ml

Per Cell Assay

- a
- b
- c
- d



To Page No. _____

Witnessed & Understood by me,

[Signature]

Date

6/15/90

Invented by

[Signature]

Recorded by

Date

6/15/90

405 2.6 10µM MTX Amp

Project No. _____

TITLE Per Cell Assay 2.16 405 non Amp.

Book No. _____

49

From Page No. _____

5/26/90 ~ Noon

Cell Counts: $\frac{1}{200}$ dil \times 0.5 ml count = 2951

2.16 \rightarrow $2951 \times 400 = 1.18 \times 10^6$ cells/ml

$\frac{5 \times 10^5}{1.18 \times 10^6} = 0.42$ ml/dish

Setup 2 dishes 1) 5×10^5 , 2) 1×10^6

2.6 (10µM): $\frac{1}{200}$ dil, $\frac{1}{2}$ ml count: 3037

$3037 \times 400 = 1.21 \times 10^6 = 0.41$ ml/dish

Setup 2 dishes 1) 5×10^5 , 2) 1×10^6

5/27/90 Cell Counts:

a) 2.6 (10µM) - 5×10^5 seed - $\frac{1}{100}$ dil = 1034 \times 10 ml.
 $\frac{1034 \times (2 \times 100 \times 10 \text{ ml})}{200} = 2.068 \times 10^6$ cells

b) 2.6 (10µM) - 1×10^6 seed - $\frac{1}{100}$ dil = 2500 \times count.
 $2500 \times 2000 = 5 \times 10^6$ cells

c) 2.16 - 8×10^5 seed - $\frac{1}{100}$ dil = 1288 \times 10 ml.
 $1288 \times 2000 = 2.576 \times 10^6$ cells

d) 2.16 - 1×10^6 seed - $\frac{1}{100}$ dil = 2575 \times 10 ml.
 $2575 \times 2000 = 5.15 \times 10^6$ cells.

Results

a) 2.6 (10µM): 0.09 Picoquants \cdot cell $^{-1}$ day $^{-1}$
b) 2.6 (10µM): 0.38 " " "
c) 2.16 : 0.36 " " "
d) 2.16 = 0.39 " " "

Forgot 10x factor (10ml of Sup)

To Page No. _____

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[Signature]

Date

6/15/90

Invented by

[Signature]

Recorded by

Date

6/15/90

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2.6 10mM MTX
2.16 No MTX

a) 2.6 10mM MTX 5×10^5 Seal : $570 \mu\text{g/ml} \times 10 \text{ ml} = 5.7 \mu\text{g}$ IgG

$5.7 \mu\text{g} = 5,700,000 \text{ pg} / 2.068 \times 10^6 \text{ cells} \cdot 3 \text{ days} = 0.9 \text{ pg/cell} \cdot \text{day}$

b) 2.6 10mM MTX 1×10^6 Seal : $= 0.8 \text{ pg/cell} \cdot \text{day}$

c) 2.16 5×10^5 Seal $= 2.6 \text{ pg/cell} \cdot \text{day}$

d) 2.16 1×10^6 Seal $= 2.9 \text{ pg/cell} \cdot \text{day}$

To Page No. _____

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[Signature]

Date

6/15/90

Invented by

Recorded by

[Signature]

Date

6/15/90

Humoral

TITLE I.P of ³⁵S 293 405 PA3,5

Project No. _____

Book No. _____

51

From Page No 38

5/30/90 Second run of Cell + Supers

- a) 293 ctn cells
- b) " " Supers
- c) 293 405 PA3,5 cells
- d) " " Supers

Incubated w/ 3ul of Rd Human H+L IgG 30 min → 1 hr
 Wash 4x with PBS - 50ul - 30 min
 Washes as per I.P. protocol

Resuspended in 80ul Sample buffer

Run 10ul/lane Reduced + Non-Reduced. See NB 10840 p72

To Page No. _____

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[Signature]

Date

6/15/90

Invented by

[Signature]

Recorded by

Date

6/15/90

From Page No. _____

6/1/90 Concern that old Sups from 293 transfections of ^{Chinese} 405H+L are degrading the Ab, resulted in these exp

ASSAY RESULTS ARE NOT CORRECTED FOR DILUTION

NO.	IDENTITY PRODUCT CODE/STEP NO.	DILUTION	405H+L (µg/ml) 2000	NO.	IDENTITY PRODUCT CODE/STEP NO.	DILUTION	405H+L (µg/ml) 2000
1	405 2.16 5h26	1:25	2.204	21	None 405	5.5	2.204
2		0.64	.379	22	"	0.55	.329
3		0.32	.167	23	"	0.25	.166
4		0.16	.112	24	# 22 in #5 h26	0.55	.194
5	293 Chinese 1:3	—	2.025	25	# 23 "	0.25	.146
6	#5 w/0.14 & 2.16		.199	26			
7	Hu #1 (293)	1:2	2.025	27			
8	" #2	1:2	2.025	28			
9	" #3	1:2	2.025	29			
10	" #4	1:2	2.025	30			
11	293 Chinese (panel)	1/10	.347	31			
12		1/20	.345	32			
13		1/40	.356	33			
14		1/80	.345	34			
15		1/160	.357	35			
16	#11 in #5 h26	1/2	.071	36			
17	#12 "	1/2	2.025	37			
18	#13 "	1/40	2.025	38			
19	#14 "	1/80	2.025	39			
20	#15 "	1/160	2.025	40			

RETURN TO REQUESTOR

Samples 1-4 Good Clts 2.16 material.
 5 - Bad Sups - No activity
 6 - 2.16 Spike is inhibited by 293 Sups
 7-10 Humanized transients - No activity perhaps due to degradation
 11-15 Ab made in 293 cells - purified - works fine.
 16-20. 293 Sups totally inhibits Ab.

To Page No. _____

Witnessed & Understood by me, _____

Date _____

Invented by _____

Date _____

Recorded by _____

From Page No. _____

6/1/90 1 Confluent mouse 175 C⁴ into 2 Roller bottles
w/ 2 mouse media ~ 1:60 split
+ 10µm Hepes

6/4/90 Cells good - ~ 70-80% Confluent.

Change to Serum Free PS24 + Insulin + Glc
250 µl / bottle w/ 10µm Hepes

6/7/90 Harvested both bottles Submitted En IgG Assay.
Re pl w/ same media as above.

Results IgG - 1 µg/l - or 1 µg/ml

6/13/90 Many more cells in Sup.
Remove the media - ~~Stop~~ Spin down cells (6.5 pellet)
Remove media to clean containers

Submit both 6/7 and 6/13 harvest to Greg Blank
for purification on Sephadex A Column.
6/7 Purified: 113 µg/ml

IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS			ASSAY RESULTS ARE NOT	
NO.		DILUTION		
1	2.16 6/7/90	1/5	4.25	1.41
2	Transfert 6/8/90	1/5	4.025	
3	Purified 6/5/90	1/5	4.025	
4				
5				

Bio Assay results
of 2.16 roller bottle
material. 1/5 dil is ~ 0.2 µg/ml
∴ it works as well as 4A5
at this low concentration.

~~Submit~~ IgG slide results for R.B 6/13/90 harvest - 240 µg/ml

To Page No. _____

Witnessed & Understood by me,

[Signature]

Date

6/15/90

Invented by

[Signature]

Recorded by

Date

8/10/90

From Page No. _____

Feb 1990 9:15 ~ 70% confluent.

6/5/90 Make more transient Chinese and purify on Part A right after harvest.

4 100 dishes using 10 mg/dish in 1 ml

40mg DNA ~ 4 vials.

L chain pdH158 = 0.4mg/ml $(4.5 \times 4) \div 0.4 = 45 \text{ ml}$
 H chain pdH160 = 1.3mg/ml $(4.5 \times 4) \div 1.3 = 13.8 \text{ ml}$
 helper AdVA = 0.25mg/ml $1 (4) \div 0.25 = 16 \text{ ml}$
75ml DNA

Ratio of 4.5 : 4.5 : 1
 (158) : (160) : AdVA

DNA	1/10 TE	Cache	2x Hepes
75ml total.	1.725ml	200ml	2.0ml

pt on 12:15
 shock at 5:15

6/6/90 AM took sup sample and changed media to S.F.

6/7/90 Collected S.F. media from 2 of 4 plates
 labeled: Chinese 293 12 hrs.

6/8/90 Collected S.F. from remaining 2 plates
 labeled Chinese 293 48 hrs.

Slown Assay for IgG - Results = Zero

Stained plate
 w/ R2H IgG H+L ?
 Results: + ?
 CTR 293 - -

To Page No. _____

Witnessed & Understood by me,
[Signature]

Date
 6/15/90

Invented by
[Signature]
 Recorded by

Date
 8/10/90

From Page No. _____

6/14/90

- plasmids:
- 1) pA3 - Hu HAS-K₁ (Light) - L_a 2.74 µg/µl
 - 2) pA5 - Hu HAS-H (Heavy) - H_a 3.38 µg/µl
 - 3) pA3 + Hu HAS-K₁ - Light - L_b - 1.3 µg/µl (3-b-14)
 - 4) pA3 + Hu HAS-K₁ - Light - L_c - 4.7 µg/µl (3-c-14)
 - 5) pA5 + Hu HAS-H - Heavy - H_b - 2.0 µg/µl (5-b-4)
 - 6) pA5 + Hu HAS-H - Heavy - H_c - 3 µg/µl (5-c-4)
 - 7) pdH158 - L chain chimeras - 0.4 µg/µl
 - 8) pdH160 - H chain chimeras - 1.3 µg/µl
 - 9) Ad₀ 0.25 µg/µl

DNA	1/0TE	CaCl ₂	2x Hepes
X _{in} 1: Humanized L _a + H _a 1ul - L _a 1ul - H _a 4ul Ad ₀	225ul	25ul	250ul
X _{in} 2: L _b + H _b 1ul - L _b 1ul - H _b 4ul Ad ₀			
X _{in} 3: L _c - L _c H _c - L _c Ad ₀ - 4ul			
X _{in} 4 L _b - 3ul H _a - 1ul Ad ₀ - 4ul			
X _{in} 5 L _c - 1ul H _a - 1ul Ad ₀ - 4	X _{in} 6 L _c - 1ul H _c - 1ul Ad ₀ - 4	X _{in} 7 L _b - 3ul X _{in} 8 L _c - 1ul	

5 µg DNA total in 0.5 µl total

put on 12:45
Shook at 4:30

Re Feed 10% FBS w/ media

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To Page No. _____

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

6/15/90

[Signature]

8/10/90

From Page No. _____

6/15 Am. Many cells floating in most dishes

- take samples of Sups from 1-6 and
give to Tye Elise and Bio Assay

- Stain # 7, 8 for L chain

Refer to S.F. media
+ Insulin

Staining Results # 7 xfer: L chain version b - prep 3-b-14
Staining was extremely low - next to nothing

8 L chain version c - prep 3-c-7
Stained very well.

Note: DNA for # 7 (3-b-14) ~~was~~ had very little supercoiled
compared to # 8 (3-c-7)

See DNA gel for these and others run run Centri
MB 10840 p 77

6/17/90 Removed S.F. media (keep) Spin out ~~cells~~ cells.
Submit to Tye Elise on Monday (6/18)

6/18/90
Results: # by Xfer SupSS.

ASSAY RESULTS			
NO.	IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS	DILUTION	ng/ml
1	#1	1/10	133.2
2		1/100	14.6
3	↓	1/1000	1.4
4	#2	1/10	>180
5		1/100	36.9
6	↓	1/1000	3.9
7	#3	1/10	>180
8		1/100	59.2
9	↓	1/1000	5.6

10	#4	1/10	102.0
11		1/100	11.2
12	↓	1/1000	1.0
13	#5	1/10	>180
14		1/100	29.8
15	↓	1/1000	3.1
16	#6	1/10	>180
17		1/100	80.3
18	↓	1/1000	8.3
19			
20			

48hr Sups

RETURN

To Page No. _____

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[Signature]

Date

6/15/90

Invented by

[Signature]

Recorded by

Date

8/12/90

From Page No. _____

6/19/90 Fed Cells 10AM

Repeat from p 55

2 100 dishes each = 2ml prot w/ 20ug A1/A4

1) Repeat xfas #1

#1	DNA	Yus TE	Cells	2x Hepes
	PA3-La - 1/2 ml	0.9 ml	100ml	1ml
	PA5-Ha - 0.3 ml			
	AdVA - 1/2 ml			

2) Repeat xfas #2

	PA3-La - 1/2 ml	0.9	100ml	1ml
	Hb - 0.5 ml			
	AdVA - 1/2 ml			

3) Repeat xfas #3

	PA3-La - 1/2 ml	0.9 ml	100ml	1ml
	Hc - 1/2 ml			
	AdVA - 2ml			

Get on 2:15

Shock at 6 AM (prot look o.k. 1. light but fine)

6/20/90 Changed to S.F. media w/ Insulin. AM

6/21/90 AM Harvested sup from 4 plates - Repeat w/ SF, 1 plate, stained other. See prot A Purification

Stained w/ R+H(H+L) Ab 1:500 on 9AM

xfas:

- 1) Slightly - but definitely some.
- 2) Stained well
- 3) Fair Not as well as #2

To Page No. _____

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9/1/90

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Date

9/1/90

Project No. _____

Book No. _____

TITLE Myo plasma test. Results

From Page No. _____

6/20/90

Submitted 4DS dilution 2.6 2.16
to Mycoplasma test on 6/4/90

ASSAY REQUEST/REPORT FORM

Genentech, Inc.

ASSAY: Mycoplasma

SAMPLES SUBMITTED BY <u>John Ridgway</u>		EXTENSION <u>1107</u>	MAILBIN # <u>40</u>	COST CENTER <u>435</u>
DATE SUBMITTED <u>6/4/90</u>	DATE TO BE ASSAYED <u>6/5/90</u>	<input checked="" type="checkbox"/> MAIL RESULTS* <input type="checkbox"/> CALL WHEN READY <input type="checkbox"/> ELEC. MAIL (Log in: <u>JBBK</u>) <small>*Results not picked up by end of day will be mailed</small>		
SAMPLE MATRIX <u>CHO cell sup w/ 1:1 FBS</u>		ANTICOAGULANT (IF USED)	<input type="checkbox"/> GMP <input type="checkbox"/> GLP <input type="checkbox"/> RADIO <small>*Specify type and amount</small>	
SAMPLES OF HUMAN ORIGIN? <input checked="" type="checkbox"/> NO <input type="checkbox"/> YES IF YES, IDENTIFY SOURCE: _____ (SPECIFY TISSUE)				

NOTES:

ASSAY STATUS AND SPECIFICATIONS

<input type="checkbox"/> NON-QUALIFIED	CONTROL VALUE EXPECTED	A _____	B _____	C _____
<input type="checkbox"/> QUALIFIED/ NON-QUALIFIED	CONTROL VALUE OBTAINED	A _____	B _____	C _____
<input type="checkbox"/> VALIDATED	ACCEPTABLE RANGE: _____			

NOTES:

ASSAY RESULTS ARE NOT CORRECTED FOR

NO.	IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS	DILUTION	RESULT	NO.	IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS
1	4DS 2.6	—	NEG	21	
2	4DS 2.16	—	NEG	22	
3				23	
4				24	
5				25	
6				26	
7				27	
8				28	

To Page No. _____

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[Signature]

Date

9/7/90

Invented by

Recorded by

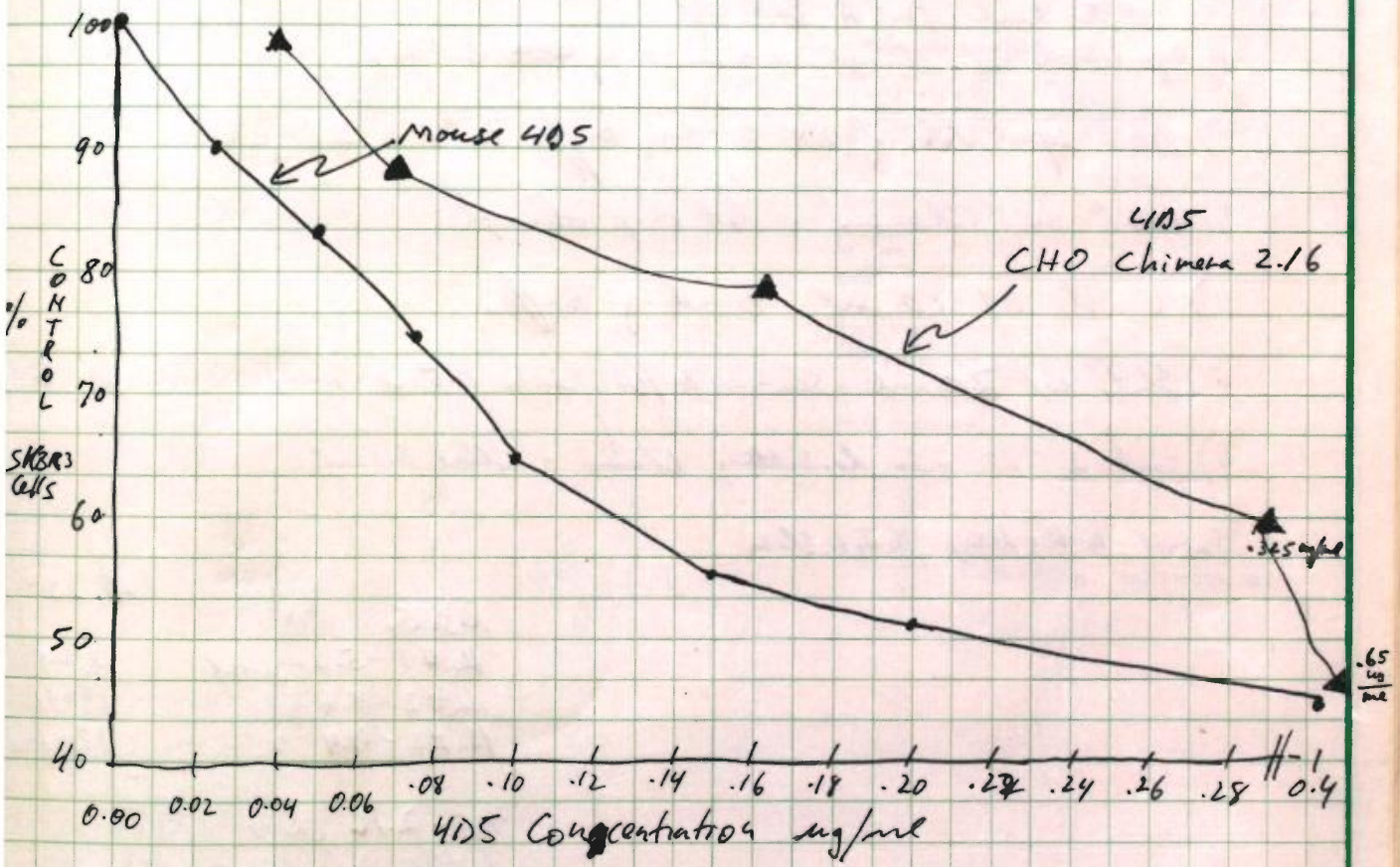
[Signature]

Date

8/6/90

From Page No. _____

6/20/90 4D5 CHO 2-16 stable
Purified from Roller Bottle.



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Date

8/10/90

From Page No. _____

6/21/90 Harvested Sups from 293 xbr 6/21 p57
3 xbr of 20 ml each in S.F. media.

0.5-1ml Prot A bed Vol

- 1) Equilibrate w/ 10ml 1X binding ~~the~~ buffer.
- 2) Add equal Vol of 2X binding buffer to ea. sample.
- 3) Load on column - let drip through.
- 4) Wash w/ 10ml binding buffer.
- 5) Elute w/ 20ml elution buffer into 0.5ml 1M Tris-HCl pH 8.0
- 6) Load 2.5 ul onto de-salting column - collect 3.5ml.

Submit to Bio Assay, the IgG Elisa

NOV 11/11/90 Hts #1, 2, 3 see NB "some cells" 6/17/90 Transfer

6/21/90 the IgG Elisa

results: IgG Elisa **Ab 280**
Hts #1 - 5.2 ug/ml 23 ug/ml
Hts #2 - 8.6 " 18 ug/ml
Hts #3 - 4.4 " 18 ug/ml

ASSAY RESULTS ARE NOT CORRECTED FOR DILUTION			
NO.	IDENTIFY EACH SAMPLE ORIGIN AND/OR INDICATE COMPONENTS	DILUTION	ng/ml
1	Hts #1 Purified	1/10	>180
2	293 6/21/90	1/100	49.5
3	↓	1/1000	5.2
4	↓	1/10000	< 0.5
5	Hts #2 Purified	1/10	>180
6	293 6/21/90	1/100	85.2
7	↓	1/1000	8.6
8	↓	1/10000	< 0.5
9	Hts #3 Purified	1/10	>180
10	293 6/21/90	1/10 ²	43.2
11	↓	1/10 ³	4.4
12	↓	1/10 ⁴	< 0.5
13	405 2-16	1/10	>180
14	Roller Bottle	1/10 ²	>180
15	Purified	1/10 ³	113.2
16	↓	1/10 ⁴	11.9
17	405 2.6-a	1/10	5.4
18	10 MTX	1/100	0.55
19	↓	1/1000	< 0.5
20	405 2.6-b 10 MTX	1/10	>180

405-2.16 clone Roller
Bottle 3 day growth 113 ug/ml
(Purified on Prot A column)
yield ~ 1 mg/liter.

To Page No. _____

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Date

9/7/90

Invented by

[Signature]

Date

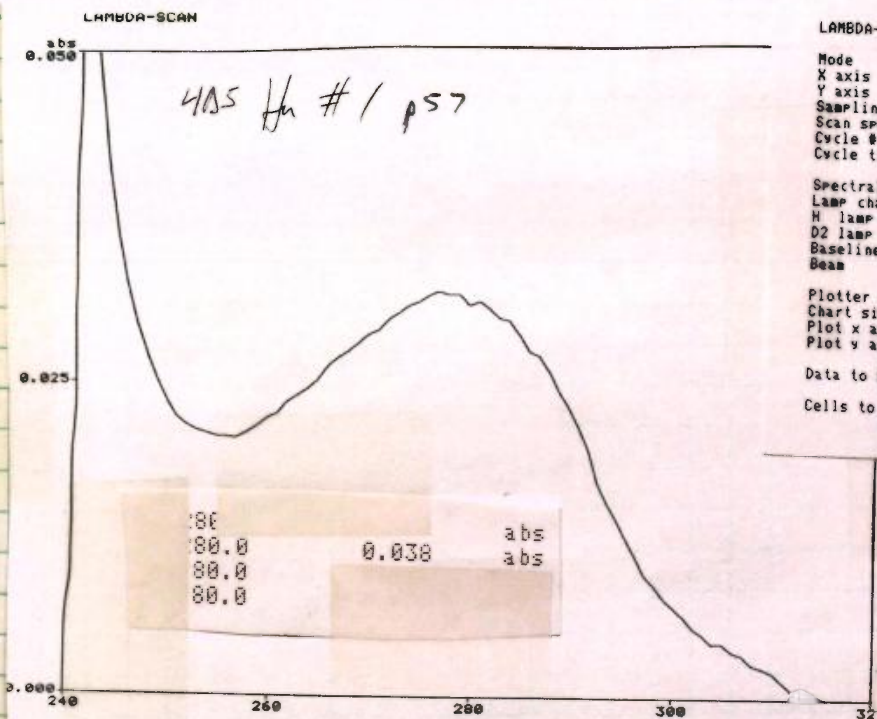
8/10/90

Recorded by

From Page No. _____

6/20/90

Concentration after purification and Concentration 20 ml → 3.5 ml



LAMBDA-SCAN PARAMETER

Mode abs
 X axis min * max 240*320 nm
 Y axis min * max 0.000*0.040 abs
 Samplings interval 1.000 nm
 Scan speed 120 nm/min
 Cycle # 1
 Cycle time 0.1 min
 Spectral bandwidth 2.0 nm
 Lamp change 340 nm
 H lamp on
 D2 lamp on
 Baseline corr off
 Beam double
 Plotter mode off
 Chart size 20 cm
 Plot x axis incr off
 Plot y axis incr off
 Data to RS 232C off
 Cells to measure 4

KONTRON UVIKON 860

Date: 21.06.90 Time: 17:08 Operator: ...
 Sample Identification:

LAMBDA-FIX PARAMETER

Mode abs
 Wavelength 280.0 nm
 Integration time 5.0 sec
 Cycle # 1
 Cycle time 0.1 min
 Spectral bandwidth 2.0 nm
 Lamp change 340 nm
 H lamp on
 D2 lamp on
 Beam double
 Plotter mode off
 Data to RS 232C off
 Cells to measure 4

Formula of Conversion using Coefficient Extinction
 For W.T. 405 hybridoma Ab.

$1.65 \text{ OD}_{280} = 1 \text{ mg/ml protein}$

1st Run #1

$0.038 \text{ Abs} / 1.65 = 0.023 \text{ mg/ml}$

$\frac{0.7}{23 \text{ mg/ml}}$

By IgG Slope: 5.2 mg/ml

To Page No. _____

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Date

9/7/90

Invented by

[Signature]

Recorded by

Date

8/10/90

From Page No. _____

6/22/90 Repeat of Amp by MTX at

10ng/ml MTX : yield 3 colonies

To 6 Elisa

- a) 50ng/ml
- b) 2ng/ml
- c) 360ng/ml

See copy of Assay p 60.

Results: No amplification.

To Page No. _____

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8/10/90

From Page No. _____

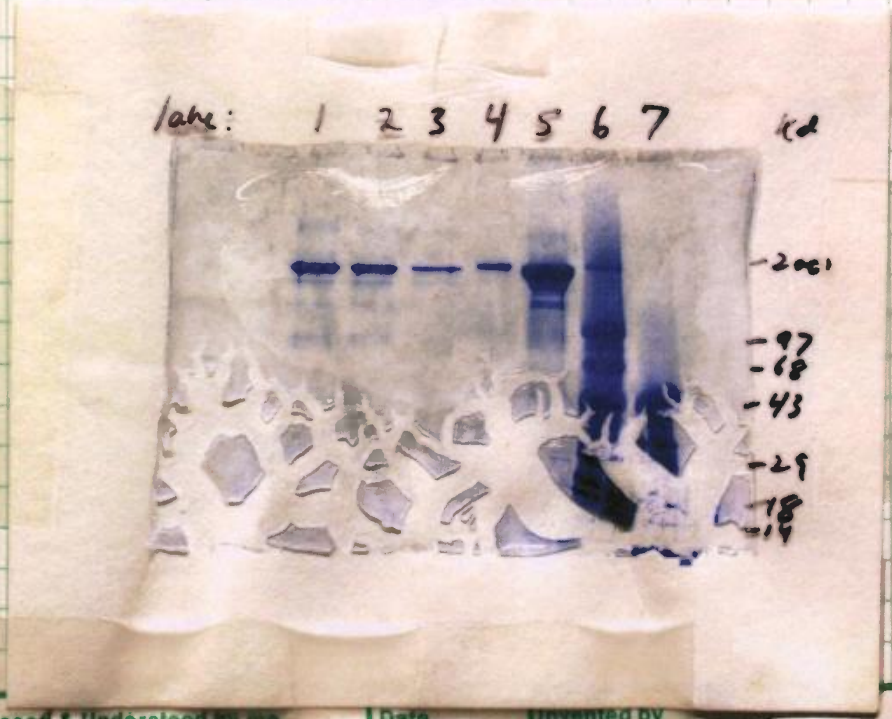
6/25/90 Precipitated 1 ml of Ab from P60.

These were purified on protein A + conc. to IgG level
except Elisa P6!

1 ml Ab + 4 ml Acetone. 15 min ppt time.
Spin in glass tube 10 min 10X RPM

run on 4-20% Grad gel pre casted
(Suspended in ~~to~~ 10 ml SDS buffer + 10 ml
2x Red buff)

- Lanes) 1) Var 1 \approx 5 μ g (Run Non Reduc)
- 2) Var 2 \approx 8.6 μ g
- 3) Var 3 \approx 4.4 μ g
- 4) YAS Chimera - 10 ml of .113 mg/ml \approx 1 μ g
- 5) From YAS - \approx 10 μ g
- 6) High MW 7 μ l
- 7) Low MW 7 μ l



Results:
Ab proteins of all
3 Var and Chimera
is intact at time
of Bio Assay

To Page No. _____

Witnessed & Understood by me.
Steph M. Gray

Date
9/7/90

Invented by
Recorded by *Steph M. Gray*

Date
8/10/90

From Page No. _____

6/26/90 Transfused 8 T-bags w/ Hm Ven #1 (PA3, PA5)

$\frac{0.5 \text{ ml ppt}}{60 \text{ plate}} \times 8 = 4 \text{ ml ppt.}$

$10 \text{ mg DNA/ml} \times 4 \text{ ml} = 40 \text{ mg DNA total} + \text{ALVA}$

$20 \text{ mg PA3 } 2.74 \text{ mg/ml} = 7.3 \text{ ml}$

$20 \text{ mg PA5 } 3.38 \text{ mg/ml} = 6 \text{ ml}$

$4 \text{ mg ALVA } 0.8 \text{ mg/ml} = 5 \text{ ml}$

DNA	^{125}I TE	Calc	2X Heparin
7.3 ml PA3	1.8 ml	0.200 ml	2 ml
6 ml PA5			
5 ml ALVA			

ppt on 12:35
Shook at 4:30

To Page No. _____

Witnessed & Understood by me,

[Signature]

Date

9/7/90

Invented by

[Signature]

Recorded by

Date

8/10/90

From Page No. 64

6/27/90 To transfered 293 cells. Change media to S.F. DMEM, F12 -cys, -met media for 30 mins.

Add same S.F. media w/ ³⁵S, met + cys for 2 hrs.

Cells don't look great but continue anyway.

~~8~~ 8 dishes x 2ml each = 16ml

Use 150ul each cys + met ³⁵S 2ml/dish

2hr pulse Label on 1 PM
off 3 PM - wash 2x replace 2ml PBS + PMS

Samples Chase

Time 0hr (3PM) Sup 2ml (Label original)
Cells 1ml 1% Triton + 5min lysis

1 hr (4PM) Sup 2ml (PMS) 4hr pulse - O.N. chase
Cells 1ml. Remove sups - wash 2x PBS then lyse w/ Tris + lysis

2hr (5PM) Sup 2ml
Cell 1ml

3hr (6PM) Sup 2ml
Cell 1ml

5hr (8PM) Sup 2ml
Cell 1ml

O.N. chase Sup 2ml
Cell 1ml

To Page No. 66

Witnessed & Understood by me, [Signature]

Date 9/7/90

Invented by [Signature]
Recorded by _____

Date 8/10/90

From Page No. 65

7/5/90 Immuno Precipitate the Supers and Cells collected

600ul of Supers	} 3ul of RdH IgG	20'
300ul of Cells		

↓
50ul of Staph A cells (washed)

↓
Resuspended in 40ul SAS sample buffer

Run on 4-20% Gradient gels

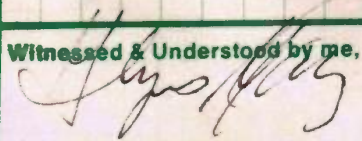
Gel 1 Lanes)	1)	2hr Pulse	HR-0	Supers	3.0ul
	2)	"	HR-0	Cells	
	3)	"	HR-1	Supers	
	4)	"	HR-1	Cells	
	5)	"	HR-2	Supers	
	6)	"	HR-2	Cells	
	7)	"	HR-3	Supers	
	8)	"	HR-3	Cells	
	9)	¹⁴ C MW higher - 15ul.			

Gel 2 Lanes)	1)	2hr Pulse	chase	Supers	3.0ul
	2)	"	5hr	Cells	
	3)	"	overnight	Supers	
	4)	"	"	Cells	
	5)	4hr pulse	o.n chase	Supers	
	6)	"	"	Cells	
	7)	¹⁴ C Markers			

Results: See gel Autorad in File (Kinetics of Ab secretion)

To Page No. _____

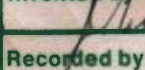
Witnessed & Understood by me,



Date

9/7/90

Invented by



Date

9/6/90

Recorded by

From Page No. _____

See p 55 for the Key

6/27/90 xbr #4 Lb + Ha Ha = 3.38 ug/2
 xbr #5 Lc + Ha Hc = (5-6-4) 3 ug/2
 xbr #6 Lc + Hc Lb = (3-6-8) 2 ug/2
 Lc = (3-6-7) 4.7 ug/2
 AdVA = 2.2 ug/2

3 100µl dists/xbr = 30ug DNA in 3ml

xbr #	DNA	1/10 TE	CaCl ₂	2x Hepes
# 4	Ha - 4.5 ml Lb - 7.5 ml AdVA - 2 ml	1.35 ml	150 ml	1.5 ml
# 5	Ha - 4.5 ml Lc - 3.2 ml AdVA - 2 ml	1.35 ml	150 ml	1.5 ml
# 6	Hc - 5 ml Lc - 3.2 ml AdVA - 2 ml	1.35 ml	150 ml	1.5 ml

put on 5 pm
 8:20 pm Feed w/ 10% FBS

6/28/90 Change media to S.E. P504 + Insulin
 6/29/90 AM Collected 30 ml of each xbr and purified by PstA
 Desalted ~~into~~ into 3.5 ml PBS
 Submitted to Bio Assay and the IgG Elisa.

To Page No. _____

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Date

Invented by

Date

Recorded by

9/7/90

[Signature]

8/20/90

Hm IgG Results

Project No. _____

Book No. _____

TITLE 405 Humanized Var #4, 5, 6

68

From Page No. _____

7/5/90 Purified Ab submitted to Hm IgG Elisa

Results.

#4: 16.6 ug/ml
 #5: 8.5 ug/ml
 #6: 6.0 ug/ml

ACCEPTABLE RANGE

Hm Var #4, 5, 6 Post Purified 3rd Sep → 2.5.
 i-PAT

ASSAY RESULTS ARE NOT CORRECTED FOR DILUTION

NO.	IDENTIFY EACH SAMPLE (GIVE REACTOR NAME COMPONENTS)	DILUTION	OD	NO.	IDENTIFY EACH SAMPLE (GIVE REACTOR NAME COMPONENTS)	DILUTION	OD
1	#4	1/10	2180	21			
2		1/100	55.3	22			
3		1/1000	16.6	23			
4	#5	1/10	2180	24			
5		1/100	11.6	25			
6		1/1000	3.5	26			
7	#6	1/10	2180	27			
8		1/100	10.7	28			
9		1/1000	6.0	29			
10				30			
11				31			

7/5/90 bel to Abase Protein.
 Precipitated 1 ml of each above w/ 4 ml of Acetone.
 Resuspended in ~ 25 ml SAS bel buffer
 Run on 4-20% gradient gel.

- (lanes)
- 1) #4
 - 2) #5
 - 3) #6
 - 4) Prestained BCL markers High (2ml diluted to 10 in buffer)
 - 5) " " " " " " " "

Results See bel p71
 #4 had some more degradation than other, but not too much
 #5, 6 have v. little degradation.

To Page No. 71

Witnessed & Understood by me:

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Date

9/7/90

Invented by

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Recorded by

Date

8/10/90

TITLE Bio Assay Results of 4D5 Humanized Versions #1, 2, 3

Project No. _____

Book No. _____

69

From Page No. _____

6/28/90

6/22/90 Assay

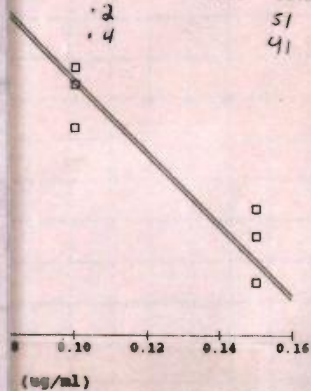
Standard Curve

T 18R x 2C

4D5 Standard Curve for Her2 Assay 11568-69

0 4D5 Conc. (ug/ml) 1 OD (540 nm) 2 %Control

1	0.000	1.345	95.661451
2	0.000	1.497	106.472262
3	0.000	1.376	97.866287
4	0.025	1.223	86.984353
5	0.025	1.435	102.062589
6	0.025	1.278	90.896159
7	0.050	1.076	76.529161
8	0.050	1.195	84.992888
9	0.050	1.132	80.512091
10	0.075	0.990	70.412518
11	0.075	1.039	73.897582
12	0.075	1.096	77.951636
13	0.100	0.890	63.300142
14	0.100	0.965	68.634424
15	0.100	0.944	67.140825
16	0.150	0.699	49.715505
17	0.150	0.756	53.769559
18	0.150	0.790	56.187767



To Page No. _____

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9/7/90

Invented by

[Signature]

Recorded by

Date

8/10/90

TITLE Bio Assay Results of 4D5 Humanized Versions #1, 2, 3

Project No. _____
Book No. _____

69

From Page No. _____

6/28/90

6/22/90 Assay

Concomitant 4D5 hybridoma	Subm. (H ₂ O)	±2 [eldest cells see]	SK-003 % CTIC	Complement
H ₂ O Var #1 [5.2 ug/ml]	1/10	0.26 mg/ml	56	0.136
	1/20	0.13	70	0.092
	1/40	0.065	80	0.06
	1/80	0.0325	89	0.03
H ₂ O Var #2 [8.6 ug/ml]	1/10	0.43	56	0.136
	1/20	0.215	67	0.101
	1/40	0.11	82	0.054
	1/80	0.055	100	
H ₂ O Var #3 [4.4 ug/ml]	1/10	0.22	66	0.104
	1/20	0.11	79	0.063
	1/40	0.055	85	0.044
	1/80	0.028	90	0.028
Cbl/Var #1 113 ug/ml	1/10	5.65	42%	
	1/100	0.565	39%	
	1/200	0.283	48%	
	1/400	0.141	63%	0.1135
	1/800	0.07	75%	0.075
	1/1600	0.035	83%	0.05

Witnessed & Understood by me, [Signature]

Date 9/7/90

Invented by [Signature]
Recorded by _____

Date 8/9/90

To Page No. _____

TITLE Bio Assay Results of 4D5 Humanized Versions #1, 2, 3

Project No. _____
Book No. _____

69

From Page No. _____

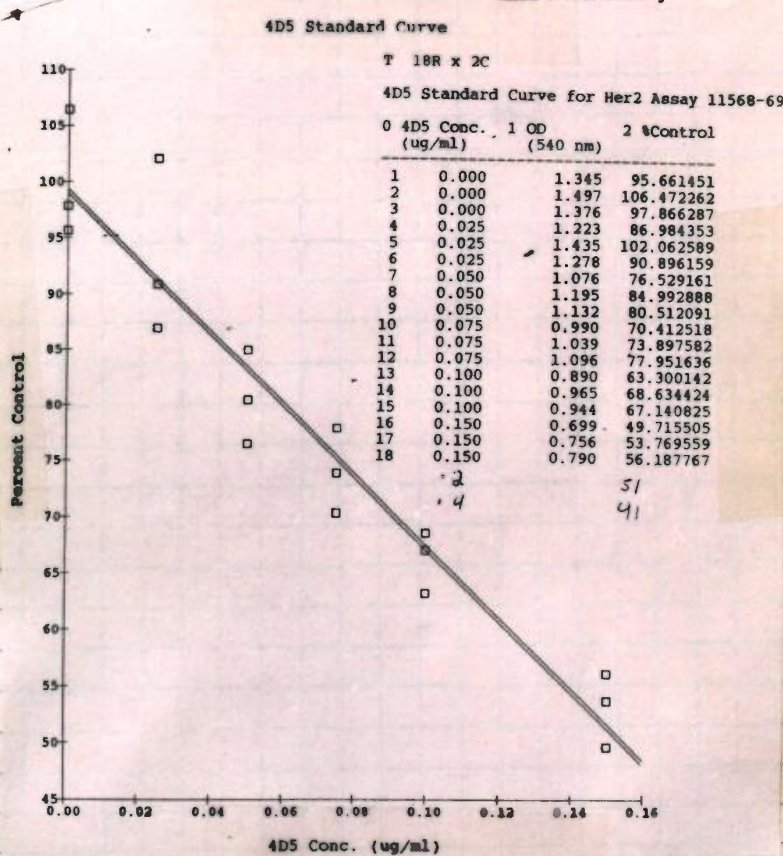
6/28/90

6/22/90 Assay

Ver #1 = W.T. humanized La + Ha
Ver #2 = La + Hb (A78(79)L)
Ver #3 = La + Hc (V102(109)Y)

Bio Assay Data

NO.	IDENTIFY EACH SAMPLE (GIVE ANIMAL NAME COMPONENTS)	DILUTION	OD	% CONTROL
1	4D5 Var #1 (293)	1:0	56	.271
2	(5.2ug/ml)	1:20	70	.168
3		1:40	80	.120
4		1:80	89	.103
5		1:160	93	.101
6		1:320	96	.101
7	4D5 Var #2 (293)	1:0	56	.271
8	(8.6ug/ml)	1:20	67	.302
9		1:40	82	.107
10		1:80	87	.101
11		1:160	90	.101
12		1:320	99	.101
13	4D5 Var #3 (293)	1:0	61	.268
14	4.4ug/ml	1:20	79	.186
15		1:40	85	.168
16		1:80	90	.156
17		1:160	94	.101
18		1:320	96	.101
19	4D5 2.16 ug/ml	1:0	48	.813
20	1/3 2.16 ug/ml	1:10	39	.615
21		1:200	48	.101
22		1:400	63	.209
23		1:800	75	.151
24		1:1600	83	.101
25				



Sigs are diluted 1:2 when added to SKBR3 cells. So [4D5] read off Standard Curve is multiplied 2x for comparison to unknown.

To Page No. _____

Witnessed & Understood by me, [Signature] Date 9/7/90 Invented by [Signature] Date 8/0/90
Recorded by _____

Project No. _____

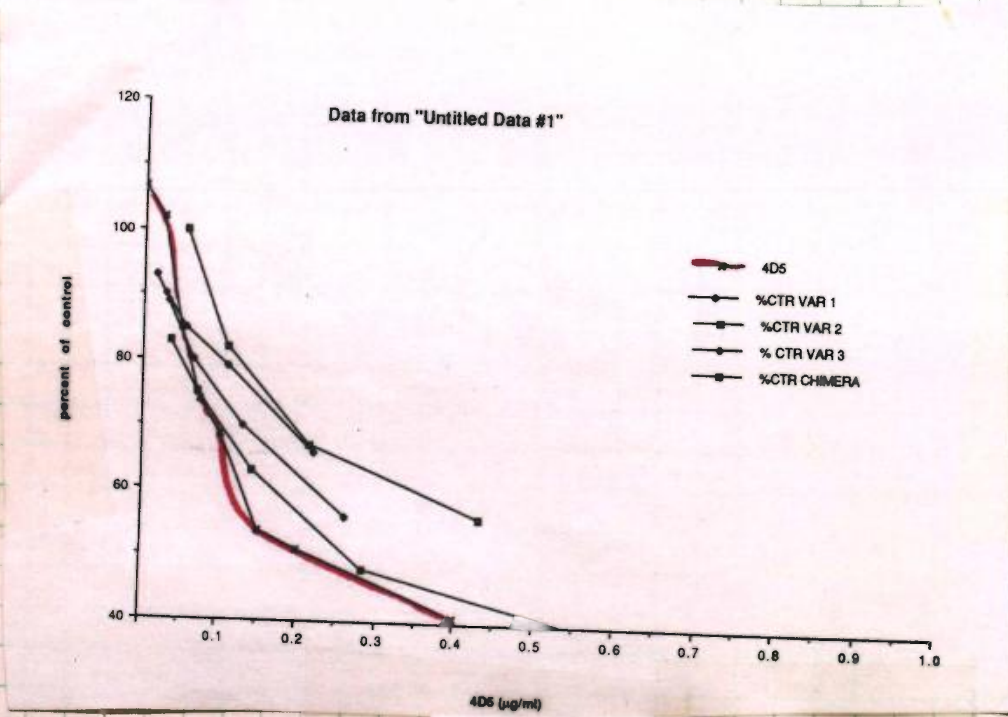
Book No. _____

TITLE Graph of Bio Assay Data For Hc#1, 2, 3

From Page No. 69

6/29/90

From 6/29/90 Assay



Data from p 69

Variants of Humanized 4D5 (Expt # 310)

Heavy Chain

Version	Clone Name	Mutagenic Primer	Restriction Site Site Changes	Amino Acid Replacements
b	1/1	H7	removes PstI	A78(79)L
c	2/3	H8	None	V102(109)Y

Light Chain

Version	Clone Name	Mutagenic Primer	Restriction Site Site Changes	Amino Acid Replacements
b	3/2	L8	removes BglII	R66G
c	4/3	L7	removes XhoI	E55Y

CDR 3
143

The Kabat/Wu residue numbers are given first and then the absolute residue in brackets (only different for the heavy chain where there are insertions).

Interesting Combination of Light and Heavy Chains
(See Len Presta's rationale for why these variants were constructed)

Var	VL	VH	
1	a	a	"wild-type" humanized 4D5
2	a	b	
3	a	c	
4	b	a	
5	c	a	
6	c	c	

Witnessed & Understood by me,

[Signature]

Date

9/7/90

Invented by

[Signature]

Date

8/20/90

Recorded by

TITLE Bio Assay Results for Her2# 4, 5, 6

Project No. _____
Book No. _____

71

From Page No. _____

7/6/90

K. 7/6/90

See p 68 ↓

~~Her2 IgG Et stain Problem~~

Protein Gel of Her2 Var 4, 5, 6
pp by acetone

NO.	IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS	DILUTION	ASSAY RESULTS ARE NO	
			Conc ^{ug/ml}	% OR
1	#4 16.6ug/ml	1/4	4.15	59
2		1/8	2.075	64
3		1/16	1.04	67
4		1/32	0.52	70
5	#5 8.5ug/ml	1/4	2.125	55.8
6		1/8	1.063	59
7		1/16	0.53	61
8		1/32	0.266	69.5
9	#6 6.0ug/ml	1/4	1.5	42
10		1/8	0.75	58.6
11		1/16	0.375	64.9
12		1/32	0.187	74.3



Data

Her2Bio#11568-88

7/5/90

4D5 Std Curve	Media Only	0.025 ^{ug/ml}	0.05	0.075	0.1	0.15	0.2	0.4	0.8 Ctrl
OD @ (540nm)	1.149	1.088	0.929	0.944	0.707	0.651	0.554	0.581	0.51
	1.12	1.029	0.93	0.884	0.814	0.709	0.623	0.565	0.524
Mean	1.187	1.035	0.925	0.902	0.838	0.668	0.659	0.623	0.532
SD	0.034	0.032	0.003	0.031	0.070	0.030	0.053	0.030	0.011
% Proliferation	100.0	91.2	80.6	79.0	68.3	58.7	53.1	51.2	45.3

John Sample	1	2	3	4	5	6	7	8	9	10
	0.659	0.768	0.717	0.793	0.592	0.687	0.674	0.747	0.3	0.64
	0.683	0.71	0.813	0.787	0.66	0.651	0.707	0.811	0.501	0.665
	0.695	0.734	0.784	0.843	0.675	0.7	0.722	0.844	0.675	0.721
Mean	0.679	0.737	0.771	0.808	0.642	0.679	0.701	0.801	0.492	0.675
SD	0.018	0.029	0.049	0.031	0.044	0.025	0.025	0.049	0.188	0.041
% Proliferation	58.9	64.0	67.0	70.1	55.8	59.0	60.9	69.5	42.7	58.6

John	11	12
	0.623	0.757
	0.627	0.729
	0.71	0.758
Mean	0.653	0.748
SD	0.049	0.016
% Proliferation	64.9	74.3

To Page No. _____

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9/5/90

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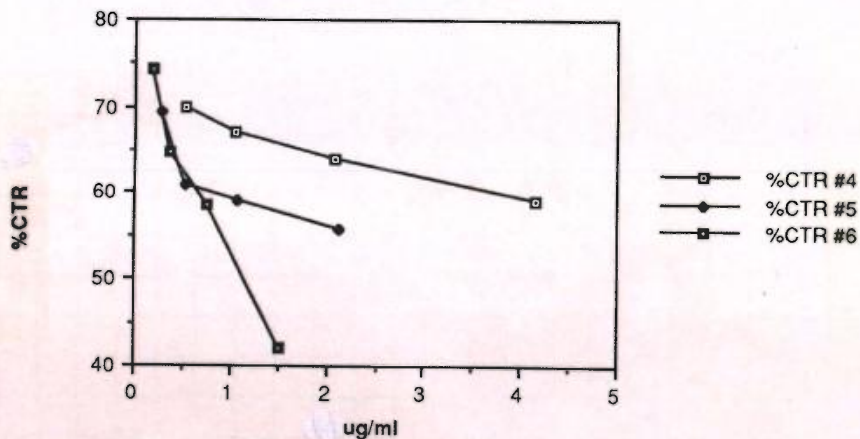
Date

8/6/90

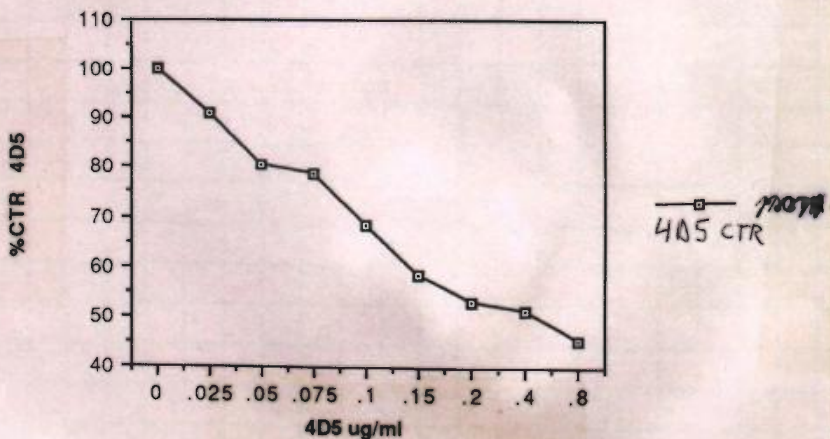
From Page No. 71

7/6/90

Data from "Data Hu 4d5 Var #4,5,6"



Data from "Data Hu 4d5 Var #4,5,6"



405 CTR

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9/10/90

From Page No. _____

For MTX Amplification repeat try.

7/9/90 A) Thawed 1 vial 4DS 2.16 from 5/15/90 into
 1-100mm dish 1% FBS + Glc

B) Thawed 1 vial 4DS 2.6 from 5/10/90 1×10^7 cells
 into 1-100mm dish 1% FBS + Glc

C) Also thawed 4DS 51.6 and 4DS 45.6
 frozen 4/17 and 4/12 respectively.

Remember to refreeze some of each of these 4 clons back

7/10/90 2.6, 51, 45 are very confluent \rightarrow split

7/11/90 Setup 2.6, 45.6, 51.6, 2.16 in 10, 15, 25 μ M MTX

Media 1% XDE + Glc (NO GHT) 5×10^5 cells/100mm dish

0.4ml of 10,000M MTX in 400ml = 10 μ M MTX

0.6ml " " " = 15 μ M MTX

1.0ml " " " = 25 μ M MTX

Cell Counts:

1/40
 2.6 19151 = 7.66×10^5 cells/ml \rightarrow use 0.65 ml/dish
 45.6 22700 = 9×10^5 cells/ml \rightarrow use 0.55 ml/dish
 51.6 27580 = 1.1×10^6 cells/ml \rightarrow use 0.5 ml/dish
 2.16 8426 = 3.4×10^5 cells/ml \rightarrow use 1.4 ml/dish

7/12/90 Cells look O.K.
 7/13/90 Cells OK - Not fed

Froze cells
 2 Vials each
 2.6 2.45, 2.51
 each vial = 100mm² dish

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7/12/90

From Page No. 73

7/12/90 Stained 2.6, 45.6, 51.6 to see if their expression level is related to % of cells expressing Ab in whole population.

Results:

- 2.6 - all cells stain v. well.
- 45.6 ~~45.6~~ _{PK} - ~ 50% cells stain moderately.
- 51.6 - all cells stain moderately.

(Stained w/ 1° - Cappel R+H (Pg 6 H+L)) 1/500 3hrs
 2° Dabo p-S&R IgG) 1/250 3hrs

7/16 - Fed Cells

7/17/90 - Cells not selecting - Not killing fast enough to ^{prevent growth} ~~prevent growth~~
 Perhaps the levels of MTX are not ↑ enough,
 Repeat w/ higher levels.

7/20/90 25uM is selecting some. Do assay at 40, 60uM MTX

8/3/90 Clones of 51.6 in 25uM MTX - None in 40

Clones of 45.6 in 25, 40, 60 uM MTX.

Picked 50 of 45.6 from 60uM MTX

Tripsinized rest from 60uM MTX replated in 100, 250, 500 uM MTX
 " " 40uM MTX " " " "

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Date

8/10/90

From Page No. _____

7/10/90 Purified Abs in PBS at conc of

Pre concentrated []

Using the Centricon microconcentrators
the volume was reduced ~~to~~ ^{by} ~4X

Centricon-10 used (10,000 MW cutoff).

- 1) 5 ug/ml
- 2) 8.6
- 3) 4.4
- 4) 16.6
- 5) 8.5
- 6) 6.0

Protein concentrations above (pre concentrated) were determined by IgG Elisa. This Elisa was over estimating by ~50% because the standard was off by ~50%. The new Elisa is corrected by the standard used is the chimera 2.16 CHO. This was amino acid analyzed.

These 6 were submitted to the Hu IgG Elisa and 405 ECD binding assay.

7/10/90

- Results:
- Var #1 - 12 ug/ml
 - " 2 - 15 ug/ml
 - " 3 - 9 ug/ml
 - " 4 - 31.4 ug/ml
 - " 5 - 7 ug/ml
 - " 6 - 8.8 ug/ml

Using the Chimera 2.16 as standard at 5 ug/ml by amino acid analysis. Previously as 113 ug/ml by O.A 250. This accounts for the protein conc of the Var not increasing 4X with concentration by 4X.

Same [] results for IgG and ECD binding assays



- Protein (ul of Hu 405 Ab - 10ul of each sample at above concentration (conc))
- 1 - Var #1
 - 2 - Var #2
 - 3 - Var #3
 - 4 - Var #4
 - 5 - Var #5
 - 6 - Var #6
 - 7 - 405 Chimera - 10ul of 50ug/ml

To Page No. _____

Glynn J. Coy

9/7/90

Recorded by

phd/rdg

Date

8/6/90

From Page No. _____

7/13/90 Submitted all 6 Hu 4DS Variants & Bio Assay w/ the chimeric as well.

Conc'd material used as per p 77. ~~Using the~~ Protein concentrations determined by Tg6 Elm using the Chimeric Standard newly analyzed by a.a. analysis.

Submitted to Bio Assay at [] as follows:

1.6, 0.8, 0.4, 0.2, 0.15, 0.1, 0.05 $\mu\text{g/ml}$.

	Ab	diluent	
$CV = CV$ $1.6(1) : 12 \times$	#1 = 133 μl Ab	+ 867 μl dil	= 1.6 $\mu\text{g/ml}$
$1.6(1) : 15(\times)$	#2 = 107 μl Ab	+ 893 μl	= 1.6 $\mu\text{g/ml}$
$1.6(1) : 9$	#3 = 177 μl	+ 823 μl	= 1.6 $\mu\text{g/ml}$
$1.6/31.4$	#4 = 51 μl	+ 949 μl	= 1.6 $\mu\text{g/ml}$
$1.6/7$	#5 = 229 μl	+ 771 μl	= 1.6 $\mu\text{g/ml}$
$1.6/8.8$	#6 = 182 μl	+ 818 μl	= 1.6 $\mu\text{g/ml}$

Calculations used to get first dilution of 1.6 $\mu\text{g/ml}$.

Samples submitted 1-49, in order of Var 1-6, Chimeric is according to concentrations.
Sample #1 4DS Var #1 1.6 $\mu\text{g/ml}$
#49 Chimeric 4DS 2.16, 0.05 $\mu\text{g/ml}$

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9/7/90

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8/10/90

Recorded by

30' pulse → chase

TITLE 293 xfm of 405 H₂O w.t. Var for Kinetics Labeling

Project No. _____

Book No. _____

77

From Page No. _____

7/16/90 Fed cells at 9:AM (split 7/5)

9-60³ dishes for xfm

0.5 ml of DNA prep x 9 = 4.5 ml of prep at 10 µg/ml = 45 µg total

Ratio of ~ DNAs 5:5:2
H + L + AdVA

H chain (PA5) = 3.38 µg/ml

L chain (PA3) = 2.74 µg/ml

AdVA = 2.2 µg/ml

5/12 x 45 = 18.7 µg / 3.38 = 5.6 ml of PA5

18.7 µg / 2.74 = 6.8 ml of PA3

2/12 x 45 = 7.5 / 2.2 = 3.4 AdVA

<u>DNA</u>	<u>1/10 TE</u>	<u>Cells</u>	<u>2x Hepes</u>
5.6 ml of PA5	2025 µl	225 µl	2.25 ml
6.8 ml of PA3			
3.4 ml of AdVA			

prep 4 min on 12:00
Shake at 4 PM.

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9/7/90

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8/12/90

From Page No. 77

7/17/90 TO transfected cells. Add ³⁵S met + Cys to dishes for 30 min. (Pulse)

8 dishes x 1.5 ml label = 12 ml label

No preincubation w/ Cys⁻, met⁻ media.

12 ml label: 1/2, 1/2, 1/2 Cys, met + ³⁵S Cys, met.

100 µCi/ml Cys, met = 1200 µCi each

CTR plate: 293 not xln w/ Ab

Label is 10 µCi/ml: so 120 µl of ³⁵S Cys + met each

Pulse 30 min - Wash label off w/ P204 1x. Repeat w/ P204 ~~2x~~ ^{5.0}

11:57 AM	0 - Take Supers + Cells (1 ml TRI x 1 ml 1% + 5 ml P204)	(p. 7.2)
12:15 PM	1 hr - Supers + 1 ml Cells	
1:15 PM	2 hr	
2:15 PM	3 hr	
3:15 PM	4 hr	
4:15 PM	5 hr	
5:15 PM - 6 PM	6 hr	
10:45 AM	13 hrs	+ CTR plate harvested at 23 hr Sup + Cells

7/18/90 Immuno precipitate Supers 1.5 ml and Cells 300 µl
 (To compare w/ the last Krebs exp p65 - need to ppt same proportion of Supers 2/3 of 5 ml = 1.5)

- used 50:50 mix of R4H IgG(Fc) and R4H + kappa chain - 4 ml / tube.

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Witnessed & Understood by me,

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8/10/90

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From Page No. _____

7/17/90 Transient Transfection: 293 cells w/ Hn 4A5 w.t. 3-100 dishes
 Collect Seps:

- Dishes 1) 24hrs, 48hrs, 72hrs
 2) 48hrs, 72hrs
 3) 72hrs

100-3 dishes = 3ml prot at 10mg/ml DNA = 30mg DNA

Ratio 5:5:2
 H - C - DNA
 H Chemi (PA5) : 3.38mg/2
 C Chem (PA3) = 2.74mg/2
 AdVA = 2.2 "

DNA	1/10 TE	CaCl ₂	2x tubes
5ml Hchemi (PA5)	1350ul	180ul	1.5mls
6ml Cchem (PA3)			
3ml AdVA			

prot on 1:30

7/18/90 AM change media to all 3 plates to SE + Inducible (bleb)
 7/19/90 Cells still look fine ~90-95% confluent, v. few floatings.
 Removal media from plate 1 - 0-24hrs - refreshment some
 7/20/90 Cells looking more granular + dark, gaps appear, as cells clump - few floatings.
 media removed plate 1 - 24-48hrs - refresh
 plate 2 - 0-48hrs refresh.
 Parity samples from 7/19-20/90 on Stage A cells today.
 7/21/90 Still few cells floating, though somewhat more unhealthy looking.
 (AM) Removal media plate 1 - 48-72, plate 2 48-72, plate 3 - 0-72hrs. Refresh
 7/23/90 Kept media plate 1 72-120hrs.

To Page No. 80

Witnessed & Understood by me, <i>Glynn P. King</i>	Date 9/7/90	Invented by <i>John Rodgers</i>	Date 8/6/90
		Recorded by	

From Page No. 29

7/24/90 Samples 1-6 as follows were purified on Staph A Gels on Saturday 7/21

Samples:

- #1 plate 1 0-24hrs
- #2 plate 1 24-48hrs
- #3 plate 1 48-72hrs
- #4 plate 2 0-48hrs
- #5 plate 2 48-72hrs
- #6 plate 3 0-72hrs

chr 15 at start of Serum Free P504 + Insulin. (Day after xps)

Samples 10ul each.

A - 7th sample was taken on Monday 7/23

#7 plate 1, 72-120hrs

Samples were purified on Staph A cells to 2.5mls, buffer exchanged on PD10 Sephadex G-25M Columns to PBS 3.5-4mls, then concentrated in Amicon-30 (3,000mw cutoff) microconcentrators to ~900ul-1.0ml

7/25/90 Samples submitted to Igt Slits - For total proteins.

ECD binding - a whole the Igt std } Comparison for
to the Fab std } degradation CTR

Conjugate

- (a - G + the Fc-HRP)
- (b - G + the Fab-HRP)

Submitted Samples:

Fresh Concentrated Samples 1-7 - diluted 1/100, 1/1000, 1/10,000 (5, 6, 7 ~~are~~ purified + conc from 9mls)

Sups prior to anything (purifying etc) 1-7 diluted 1/10, 1/100, 1/1000 (1, 2, 3, 4 diluted 1/3 w/ 2x binding buffer) (5, 6, 7 - sup straight from dish)

Sample # 6 after buffer exchange / before concentration dil 1/100, 1/1000, 1/10,000 (4.0ul total)

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Witnessed & Understood by me,

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Date

9/17/90

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Date

8/10/90

From Page No. 78

7/18/90 I.P. of sample done, Resuspended in 35-40ul of SDS sample buffer.

Run on 4-20% Gradient Gels. - 70ml/lanes.

- Gel 1 : Lane
- 1) T=0hrs Sup
 - 2) " Cells
 - 3) T=1hrs Sup
 - 4) " Cells
 - 5) T=2hrs Sup
 - 6) " Cells
 - 7) Blanks
 - 8) ¹⁴C Lys markers
 - 9) Prestained HPLC markers

- Gel 2 : Lane
- 1) T=3hrs Sup
 - 2) " Cells
 - 3) T=4hrs - Sup
 - 4) " - Cells
 - 5) T=5hrs Sups
 - 6) " Cells
 - 7) Blanks
 - 8) " " "
 - 9) ¹⁴C Lys markers
 - 10) Prestained HPLC

- Gel 3) Lane:
- 1) T=6hrs Sup
 - 2) " Cells
 - 3) T=23hrs Sups
 - 4) " Cells
 - 5) 295 CTAs 23hrs Sups
 - 6) " " Cells
 - 7) Blanks
 - 8) " " "
 - 9) ¹⁴C Lys markers
 - 10) Prestained HPLC markers

To Page No. _____

Witnessed & Understood by me,

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Date

9/17/90

Invented by

[Signature]

Date

8/10/90

Recorded by

From Page No. 76

7/19/90 Concentrations of Ab (IgG) ^{submitted} ~~will~~ will be adjusted before calculating/working up data from bio assay.

IgG concentrations by elisa for the Human 4A5 Variant were determined with the chimeric 4A5 as the standard. This gives false readings (I think now) because the ~~the~~ values derived using ~~the~~ a 'humanized 4A5 as standard' are different.

The humanized 4A5 "wild type" made in ECL is probably a better standard to use in determining the IgG conc. of humanized Ab's made in ~~the~~ ~~the~~ mammalian cells.

Samples were submitted for Bio Assay ^(hr-2) on 7/13/90 based upon [IgG] using the 4A5 chimera as standards. This Bio Assay data will be used but adjusted for higher IgG concentrations.

Each dilution submitted will be multiplied by a factor ~~the~~ developed ~~for~~ from the [IgG] data on p83.

Var #1: $21.3/12.1 = 1.76$ ∴ all dilutions for Var #1 multiplied by 1.76 before plotting [] to % CTR curve

Var #2 $27/15.1 = 1.79$

Var #3 $15.3/9 = 1.7$ Use Ave of 1.7 as factor to increase to correct conc.

Var #4 $5.5/3.2 = 1.72$ Divide submitted conc by 2 before graphing as the true conc. seen by SKBR3 cells

Var #5 $11.8/7.1 = 1.66$

Var #6 $14.2/8.8 = 1.61$

To Page No. _____

Witnessed & Understood by me,

Shirley M. [Signature]

Date

9/7/90

Invented by

John [Signature]

Recorded by

Date

8/10/90

From Page No. _____

7/19/90 Values for Ig G Conc for Humanized 405 Variants 1-6 are higher using the Humanized Ab as standard.

(g.aaa)

John Ridgway

7-12-90

	Chimeric Std.		Humanized Std.		Date in ng/ml
	IgG Total Protein	HER2 ECD	IgG	HER2 ECD	
1	>100	>100	>100	>100	
2	12.1	11.9	21.3	21.6	
3	1.2	1.2	1.8	1.9	
4	>100	>100	>100	>100	
5	15.1	13.9	27.0	25.4	
6	1.6	1.6	2.4	2.5	
7	>100	73.7	>100	>100	
8	9.0	8.7	15.3	15.5	
9	0.8	0.8	1.2	1.4	
10	>100	>100	>100	>100	
11	31.4	30.2	64.9	59.0	
12	3.2	3.4	5.5	5.6	
13	82.6	59.5	>100	>100	
14	7.1	6.6	11.8	11.3	
15	0.7	0.7	1.0	1.2	
16	>100	86.2	>100	>100	
17	8.8	8.5	14.2	15	
18	0.8	0.9	1.3	1.5	

Key:

1	Hu 405 Var #1	1/100 dil	10	Hu 405 Var #4	1/100 dil
2	"	1/1000	11	"	1/1000
3	"	1/10,000	12	"	1/10,000
4	Hu 405 Var #2	1/100 dil	13	Hu 405 Var #5	1/100
5	"	1/1000	14	"	1/10,000
6	"	1/10,000	15	"	1/10,000
7	Hu 405 Var #3	1/100 dil	16	Hu 405 Var #6	1/100
8	"	1/1000	17	"	1/1000
9	"	1/10,000	18	"	1/10,000

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Date

8/10/90

From Page No. 74

7/20/90 Setup again 5×10^5 cells/plate
at 40 and 60 μ M MTX

Cell Counts:

45.6 - 13,541 $\times 40 = 5.42 \times 10^5$ /ml - use 0.92 ml/dish
51.6 - 10,726 $\times 40 = 4.29 \times 10^5$ /ml - use 1.17/dish
2.16 - 4458 $\times 40 = 178320$ /ml - use 2.8 ml/dish.

8/9/90 45.6 population of ~~the~~ clones at 250 μ M MTX, 500 μ M MTX
shows no IgG

51.6 clone in 60 μ M MTX IgG.

<input type="checkbox"/> VALIDATED		ACCEPTABLE RANGE	100 ng/ml - 1.4.
NOTE: <u>CHIMERIC 4DS</u>			
ASAP RESULTS ARE NOT			
NO.	IDENTIFY EACH SAMPLE ORIGIN AND/OR URICAC COMPONENTS	DILUTION	ng/mL
1	51.6 60 μ M MTX	1/10	28.4
2	↓	1/100	3.4
3	↓	1/1000	< STD
4	45.6 250 μ M MTX	1/10	< STD
5	↓	1/100	< STD
6	↓	1/1000	< STD
7	45.6 500 μ M MTX	1/10	< STD
8	↓	1/100	< STD
9	↓	1/1000	< STD

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Date 9/7/90

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Date 9/9/90

From Page No. 80

Use 415 w.t. 293 transient Exp

7/26/90 Total mg IgG %⁶
 plate 4: (post purity) IgG 0-24hrs 24-48hrs 48-72hrs 72hr total 72-120hr
 13.5mg + 12.7mg + 11.9mg = 38mg 38mg

(pre-purity) Supp 18mg + 22.5mg + 42mg = 82mg yield 46% Ave.
 (IgG) 25mg 33mg 58mg 116

plate 2: post 0-24hrs 0-48hrs 48-72hrs 72hr total
~~pre~~ purity — 40mg 22mg = 62mg

Supp 69.5mg 30mg = 93 yield ~ 66%

plate 3: pur. final conc 0-72hrs 50mg total IgG yield ~ 60%

plate 1 Supp 84mg total IgG
 72-120hrs - 37.5mg total pur. final yield ~ 52%
 45 Supp

% ~~ECN~~ ECN binding IgG to total IgG as function of Age of Culture
 Q: do the longer culture times (longer periods between ~~off~~ refreshing) result in a greater % Ab not binding to ECN.

24hr culture	ECN	IgG	After Purification % ECN of IgG	Before Purification % ECN of total IgG
Plate 1 Sample 1 (0-24)	13.2	13.5	98%	89% 75%
PL 2 Samp 2 (24-48)	11.2	12.7	88%	72%
PL 1 Samp 3 (48-72)	11.2	11.9	94%	65% 76%
PL 2 Sam 4 (0-48hr)	36.5	35.5	103%	80% 75%
PL 2 Sam 5 (48-72)	17.7	18.1	97%	90% 80%
PL 3 Sample 6 (0-72hrs)	44.8	50.8	88%	66%
PL 1 Sample 7 (72-120hrs)	36	45	80%	85% 79%

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9/7/90

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Date

9/10/90

From Page No. 85

7/26/90

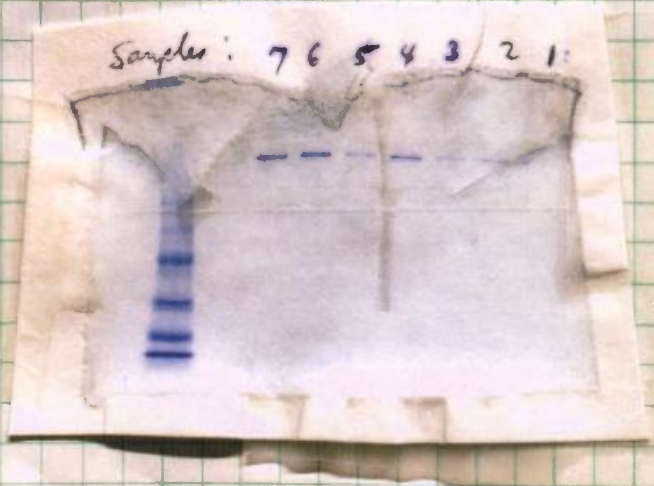
Her 4MS Fab elisa
SCD Her 2 binding

HER2				HERP			
ASSAY RESULTS ARE NOT CORRECTED FOR DILUTION							
NO.	IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS	DILUTION	HER2	HERP	NO.	IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS	DILUTION
1	#1 pure/conc	1/100	7100	7100	21	#7 pure/conc	1/100
2	"	1/100	13.2	13.5	22	#1 on sup 1/dil	1/100
3	"	1/1000	1.1	1.5	23	"	1/1000
4	#2 pure/conc	1/100	7100	7100	24	"	1/1000
5	"	1/1000	11.2	12.7	25	#2 on sup 1/dil	1/100
6	"	1/100	7100	7100	26	(little hyd)	1/100
7	#3 pure/conc	1/100	7100	7100	27	(round)	1/1000
8	"	1/1000	11.2	11.9	28	#3 on sup 1/dil	1/100
9	"	1/100	7100	7100	29	(chump/dil)	1/100
10	#4 pure/conc	1/100	7100	7100	30	"	1/1000
11	"	1/100	36.5	35.5	31	#4 on sup 1/dil	1/100
12	"	1/100	3.4	4.4	32	"	1/100
13	#5 pure/conc	1/100	7100	7100	33	"	1/1000
14	"	1/100	17.7	18.1	34	#5 on sup 1/dil	1/100
15	"	1/100	1.5	2.8	35	"	1/100
16	#6 pure/conc	1/100	7100	7100	36	"	1/1000
17	"	1/100	46.5	45.6	37	#6 on sup 1/dil	1/100
18	"	1/100	4.3	5.6	38	"	1/100
19	#7 pure/conc	1/100	7100	7100	39	"	1/1000
20	"	1/1000	7100	36.4	40	#7 on sup 1/dil	1/100

NO.	HER2	HERP
1	34.4	
2	8.8	
3	0.9	
4	69.3	
5	8.0	
6	0.7	
7	71.5	
8	7.8	
9	0.7	
10	>100	
11	23.1	
12	2.5	
13	99.0	
14	11.7	
15	1.1	
16	>100	
17	29.2	
18	3.0	
19	>100	
20	24.3	
21	2.5	
22	69.3	
23	9.0	
24	0.9	
25	>100	
26	12.0	
27	1.2	
28	>100	
29	20.4	
30	2.4	
31	>100	
32	21.5	
33	2.5	
34	>100	
35	16.9	

ng/ml results.

41	#7 on sup 1/dil	1/100	44.2	45.6
42	"	1/1000	4.0	6.0
43	#6 beta conc	1/100	11.3	>100
44	4ms	1/100	6.3	13.8
45	"	1/1000	0.7	1.8



Non Reducing -
4-20% Grad prot gel
of Samples 1-7 showing
intact Ab

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TITLE

Kd Analysis of 4D5 Hu Ab

Project No. _____

Book No. _____

87

From Page No. _____

8/8/90 Kd analysis by cell binding assay using SKBR3 cells and competition w/ 125I 4D5 Ab.

Assay Done by Niels Dna

Control 4D5 hybridoma Ab : Kd 3.5 nM

4D5 Hu Var #1 _____ Kd 2.66 nM

4D5 Hu Var #4 _____ Kd 2.77 nM

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8/10/90

From Page No. _____

7/31/90 4 100 plates each = 40ug DNA each Var + AdVA

Var 1 VL PA3(a) + VH PA5(a)
 Var 2 PA3(a) + Hb (5-b-4)
 Var 3 PA3(a) + Hc (5-c-4)
 Var 4 Lb (3-b-8) + PA5(a)

DNA's:
 PA3 - wild type Humanized L-chain 2.74ug/μl
 PA5 - W.T Hu H-Chain 3.38ug/μl
 Hb (5-b-4) - Ver b of Hu H-Chain clone (5-b-4) 2.0ug/μl
 Hc (5-c-4) - Ver C of Hu H chain clone (5-c-4) 3.0ug/μl
 Lb (3-b-8) - Ver b of Hu L-Chain clone (3-b-8) 2.0ug/μl
 AdVA - 2.2ug/μl

4 plates x 10ug DNA/plate = 40ug total in 4ml

Ratio H & L: AdVA → for 40ug use 20 20 8ug
 H + L + AdVA

Var#	DNA	% TE	25m CaCl ₂	2X Hepes
1	PA3 7.3ul PA5 6.0ul AdVA 4ul	1.8mls	200ul	2mls
2	PA3 - 7.3ul (5-b-4)Hb - 10ul AdVA - 4ul	1.8mls	200ul	2mls
3	PA3 - 7.3ul (5-c-4)Hc - 7ul AdVA - 4ul	1.8mls	200ul	2mls
4	(3-b-8)Lb - 10ul (PA5)Ha - 6.0ul AdVA - 4ul	1.8mls	200ul	2mls

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Date 9/7/90

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Date 8/10/90

From Page No. 88

7/31/90 Cells refed at 9:30 AM - Some plates have cells seen -
 mtDNA on 10:45 - 1:00 - evenly spaced.

New batch of 2x4gpa + 2.5m GCLs

Let ppt go 7 min:

#1 -	7.5 min	- more more granular but O.K.
#2 -	7	- " "
#3 -	4	- light ppt but O.K.
#4 -	4 min	- light " "

Oxygen shock 5:30 - 6 PM

8/1/90 changed to P504 + Zn + Glu (Serum Free)

8/3/90 Collected media 40ml ea Var 1-4. Refed with same.

8/5/90 Collected 40 ml from each Var 1-4 refed.

8/7/90 Collected 40 ml from each - discarded plates.

Supes were collected at 48 hr intervals over 6 days.

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8/12/90

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From Page No. _____

8/1/90 4 plates 293 cells each - Fed 9AM 10% FBS.
Seeded light ~ 30-40% confluent.

	VL		VH
Var 5	C (3-C-7) 4.7ug/l	+	a (PAS) 3.38ug/l
Var 6	C (3-C-7)	+	c (5-C-4) 3.0ug/l
Var 7	b (3-b-8) 2.0ug/l	+	e (PA13) 3.0ug/l clone 1/6 Paul Carter
Var 8	b (3-b-8)	+	d (PA14 clone 3/2) 0.16ug/l

AdVA 2.2ug/ml.
Prepare 4 ml each with ~ 10ug/ml total DNA each
ea. L-20ug $\frac{1}{8}$ H-20ug AdVA 8ug

Var	DNA's	$\frac{1}{100}$ TE	2.5m CaCl ₂	2X HEPES
#5	3-C-7 - 5ml PAS - 6.9ml AdVA - 4ml	1.8ml	200ml	2ml
#6	3-C-7 - (5ml) 5-C-4 - (7.8ml) AdVA - 4ml	1.8ml	200ml	2ml
#7	3-b-8 - 11.7ml PA13 - 7.8ml AdVA - 4ml	1.8ml	200ml	2ml
#8	3-b-8 11.7ml PA14 150ml AdVA 4ml	1.635ml	200ml	2ml

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8/12/90

TITLE 293 *Sp* of *Hu* Vol 5-8

Project No. _____

Book No. _____

91

From Page No. _____

8/1/90 let ppt go 7 min each

ppt on 12:30 Shocks 5-6pm Feed w/ 10% FBS

8/2/90 remove 10% FBS. Feed w/ 7504 + Ins. Glu

8/4/90 Collected 40 ml each Var 5-8

8/6/90 Collected 40 ml each Var 5-8

8/7/90 Collected 40 ml ea. Var 5-8.

120 ml collected over 5 days for each Var.

All samples purified on protein A column and
concentrated by 30K MW cut off centrifuge tubes

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Date

8/12/90

From Page No. _____

8/8/90 Igt Elisa submitted for untreated sups of Var 1-8 and final purified + Conc samples.

405. Hra Var 1-8 Final Yield:	µg/ml Igt by Elisa	total µg Igt Protein
#1 : 2.75mls	x 60.5 µg/ml	= 166 µg
#2 : 2.75mls	x 229 µg/ml	= 630 µg
#3 : 3.0mls	x 54.5 µg/ml	= 163 µg
#4 : 3.25mls	x 34 µg/ml	= 110 µg
#5 : 2.75mls	x 30.75 µg/ml	= 84 µg
#6 : 3.0mls	x 39 µg/ml	= 118 µg
#7 : 2.5mls	x 71.25 µg/ml	= 178 µg
#8 : 2.5mls	x 42 µg/ml	= 105 µg

Starting Sups 120ml
for Var 1-8

	Total Possible Protein	% Yield
#1 : 3.2 µg/ml x 120 mls = 382	382 x 100 = 43%	
#2 : 9 µg/ml x 120 ml = 1080	1080 x 100 = 58%	
#3 : 2.6 µg/ml x 120 ml = 314	314 x 100 = 52%	
#4 : 4.5 µg/ml x 120 ml = 544	544 x 100 = 20%	
#5 : 2.3 µg/ml x 120 ml = 276	276 x 100 = 30%	
#6 : 2.5 µg/ml x 120 ml = 306	306 x 100 = 38%	
#7 : 2.75 µg/ml x 120 ml = 330	330 x 100 = 54%	
#8 : 1.9 µg/ml x 120 = 228	228 x 100 = 46%	

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9/7/90

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Date

8/10/90

TITLE *Fig to Elson of Human and 405 Var 1-8*

Project No. _____

Book No. _____

93

From Page No. _____

8/8/90

Assay done 8/8/90

ASSAY RESULTS ARE NOT CORRECTED FOR DILUTION					
NO.	IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS	DILUTION	ng/mL	NO.	IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS
1	Var 1 Pure + Conc	1/100	>STD	21	Var 6 Pure + Conc
2		1/100	54.2	22	
3		1/10 ⁴	6.7	23	
4		1/10 ⁵	<STD	24	
5	Var 2 Pure + Conc	1/10 ²	>STD	25	Var 7 Pure + Conc
6		1/10 ⁵	>STD	26	
7		1/10 ⁴	20.8	27	
8		1/10 ⁵	2.5	28	
9	Var 3 Pure + Conc	1/10 ²	>STD	29	Var 8 Pure + Conc
10		1/10 ⁵	44.5	30	
11		1/10 ⁴	6.5	31	
12		1/10 ⁵	<STD	32	
13	Var 4 Pure + Conc	1/10 ²	>STD	33	Var 1 Combined on Sup
14		1/10 ³	27.8	34	
15		1/10 ⁴	4.0	35	
16		1/10 ⁵	>STD	36	Var 2 Sup
17	Var 5 Pure + Conc	1/10 ²	>STD	37	
18		1/10 ³	25.5	38	
19		1/10 ⁴	3.6	39	Var 3 Sup
20		1/10 ⁵	<STD	40	

NO.	IDENTITY	DILUTION	ng/mL
41	Var 3 Sup	1/10 ³	2.7
42	Var 4 Sup	1/10	>STD
43		1/10 ²	39.7
44		1/10 ³	5.1
45	Var 5 Sup	1/10	>STD
46		1/10 ²	14.4
47		1/10 ³	3.1
48	Var 6 Sup	1/10	>STD
49		1/10 ²	16.9
50		1/10 ³	3.4
51	Var 7 Sup	1/10	>STD
52		1/10 ²	24.1
53		1/10 ³	3.1
54	Var 8 Sup	1/10	>STD
55		1/10 ²	16.9
56		1/10 ³	2.1

*Pure + Conc samples are the final material.
Used Ave of 1/10³ and 1/10⁴ dilutions.*

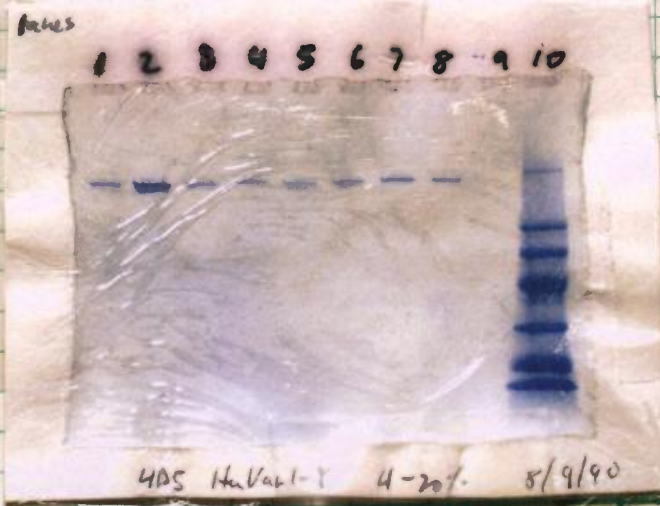
*8/9/90
Sup are raw material as is off the dishes to calculate yield.*

Run on 4-20% Gradient Gel No. 10.

10ml of each Var.

15ml of #5

- Lanes):*
- 1 Var 1
 - 2 " 2
 - 3 " 3
 - 4 " 4
 - 5 " 5
 - 6 " 6
 - 7 " 7
 - 8 " 8
 - 9 Blank
 - 10 BSL High MW



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8/7/90

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Date

8/6/90

No. _____

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8/9/90 Samples taken during the purification process of the 1st Var 1-8 are assayed to find which step the loss occurs.

NO.		IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS	DILUTION	ng/ml	Corrected	NO.		IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS	DILUTION	ng/ml	Corrected
1	2	Var 1 8/3 Sup	1/10	2100	2100	21	22	Var 4 8/3 elu	1/1000	2.5	2.5
3	4	Var 1 8/3 elu	1/100	20.5	20.5	23	24	Var 4 8/5 Sup	1/10	>100	>100
5	6	(by passed prot A)	1/1000	2.1	2.4	25	26	Prot A by pass	1/100	44.2	39.9
7	8	Var 1 8/3 elu	1/10	4.5	4.5	27	28	Var 4 8/7 Sup	1/100	9.0	3.8
9	10	elut off prot A	1/100	2.5	2.5	29	30	Var 4 8/7 elu	1/100	10.0	2.5
11	12	Var 1 8/3 2 nd elu	1/1000	21.0	21.0	31	32	Prot A by pass	1/100	2.5	2.5
13	14	off Prot A	1/100	26.0	21.0	33	34	Var 4 8/7 elu	1/10	8.3	3.7
15	16	Var 1 8/3 sup from filter conc	1/100	12.0	10.5	35	36	Filter concentration	1/100	1.9	2.5
17	18	Var 4 8/3 Sup	1/10	4.5	4.5	37	38	Sup Var 5 8/4	1/1000	4.5	4.5
19	20	Prot A by pass	1/100	20.5	0.6	39	40	Var 5 elu 8/4	1/10	25.9	19.4

NO.	IDENTITY	DILUTION	ng/ml	Corrected
41	Var 5 elu 8/4	1/100	2.5	2.5
42	Var 5 Sup 8/6	1/10	>100	>100
43	Var 5 elu 8/6	1/100	2.0	1.3
44	Var 5 elu 8/6	1/100	2.5	2.5
45	Var 5 elu 8/6	1/100	2.5	2.5
46	Var 5 elu 8/6	1/100	2.5	2.5
47	Var 5 elu 8/6	1/100	2.5	2.5
48	Var 5 elu 8/6	1/100	2.5	2.5
49	Var 5 elu 8/6	1/100	2.5	2.5
50	Var 5 elu 8/6	1/100	2.5	2.5
51	Var 5 elu 8/6	1/100	2.5	2.5
52	Var 5 elu 8/6	1/100	2.5	2.5
53	Var 5 elu 8/6	1/100	2.5	2.5
54	Var 5 elu 8/6	1/100	2.5	2.5
55	Var 5 elu 8/6	1/100	2.5	2.5
56	Var 5 elu 8/6	1/100	2.5	2.5
57	Var 5 elu 8/6	1/100	2.5	2.5
58	Var 5 elu 8/6	1/100	2.5	2.5
59	Var 5 elu 8/6	1/100	2.5	2.5
60	Var 5 elu 8/6	1/100	2.5	2.5

Var #1 as example 8/3 collection of 40 ml
 Raw sups = 2.4 ug/ml x 40 ml = 96 ug total IgG
 all bound to prot A column (elu pass thru 4.5)

Ab eluted off column 3 ml = 21 ug/ml x 3 ml = 63 ug total recovery,
 the loss at this step is 35%. It is either not coming off the column or it is denatured by the low pH of the elution buffer so that it is not picked up by the elisa.

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 Glynn H. Cox

Date
 9/7/90

Invented by
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Date
 8/9/90

TITLE

Cell of 2.16 Prebank vs Un Banked

Project No. _____

Book No. _____

95

From Page No. _____

9/7/90 /chlat Cells 355

Pen Padlock & Nylon Padlock

1) (over)

1) Non Banked Prod line - Cells

2) " " - Sigs

3) Prebanked - Cells

4) " " - Sigs

5) DP-12 - Cells CTRs

6) " " - Sigs

7) H m/w stls

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9/7/90

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9/25/90 PRK Ha 405 H+L chain (one plasmid) Var#6 5-C-4-H
3-C-3-L
2.2mg/2

HAMAR 0.25mg/2

	DNA	1/10TE	CaCl ₂	2XHEPES
1. HAMAR only	40 HAMAR	450ml	50ml	0.5 500ml
2. HAMAR prkVar 6	5ml prkVar 6 5ml HAMAR	440ml	50ml	500ml
3. prkVar 6 +ALVA	5ml prkVar 6 1ml ALVA	450	50ml	500ml
4) PA10 1/5 PA9 9	50ml PA9 56ml PA10 3ALVA	800ml	100ml	1ml

mt on 11:30

9/27/90 Split into 10% FBS + Colchicine range, 2ng-14ng/ml

10/2/90 all cells have died. Control 293 cells died at 2ng/ml level. Promote on Hamar plasmid not strong enough in 293 cells.

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10/2/90

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Date

10/5/90

Recorded by