LABORATORY NOTEBOOK

Tissue Culture 2/30/04

111162

GENENTECH, INC.

Celltrion v. Genentech
IPR2017-01374
Genentech Exhibit 2006

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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 Cazares on March 1, 1991 pages #1-96

—SCIENTIFIC NOTEBOOK CO.—
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P.O. BOX 238
STEVENSVILLE, MI 49127
616-429-8285

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.

Table of Contents	Page

SCIENTIFIC NOTEBOOK CO.

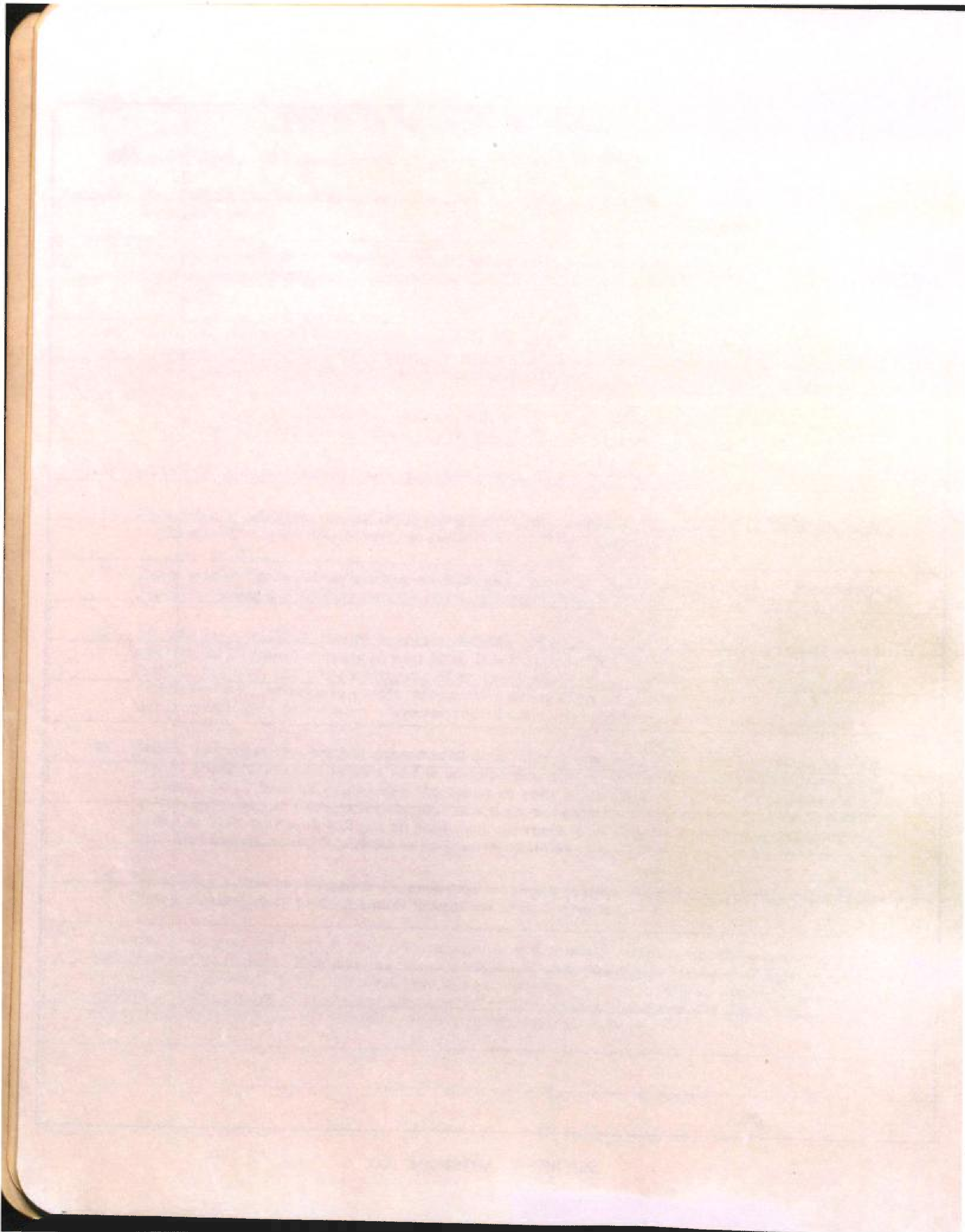


Table of Contents	Page

SCIENTIFIC NOTEBOOK CO.

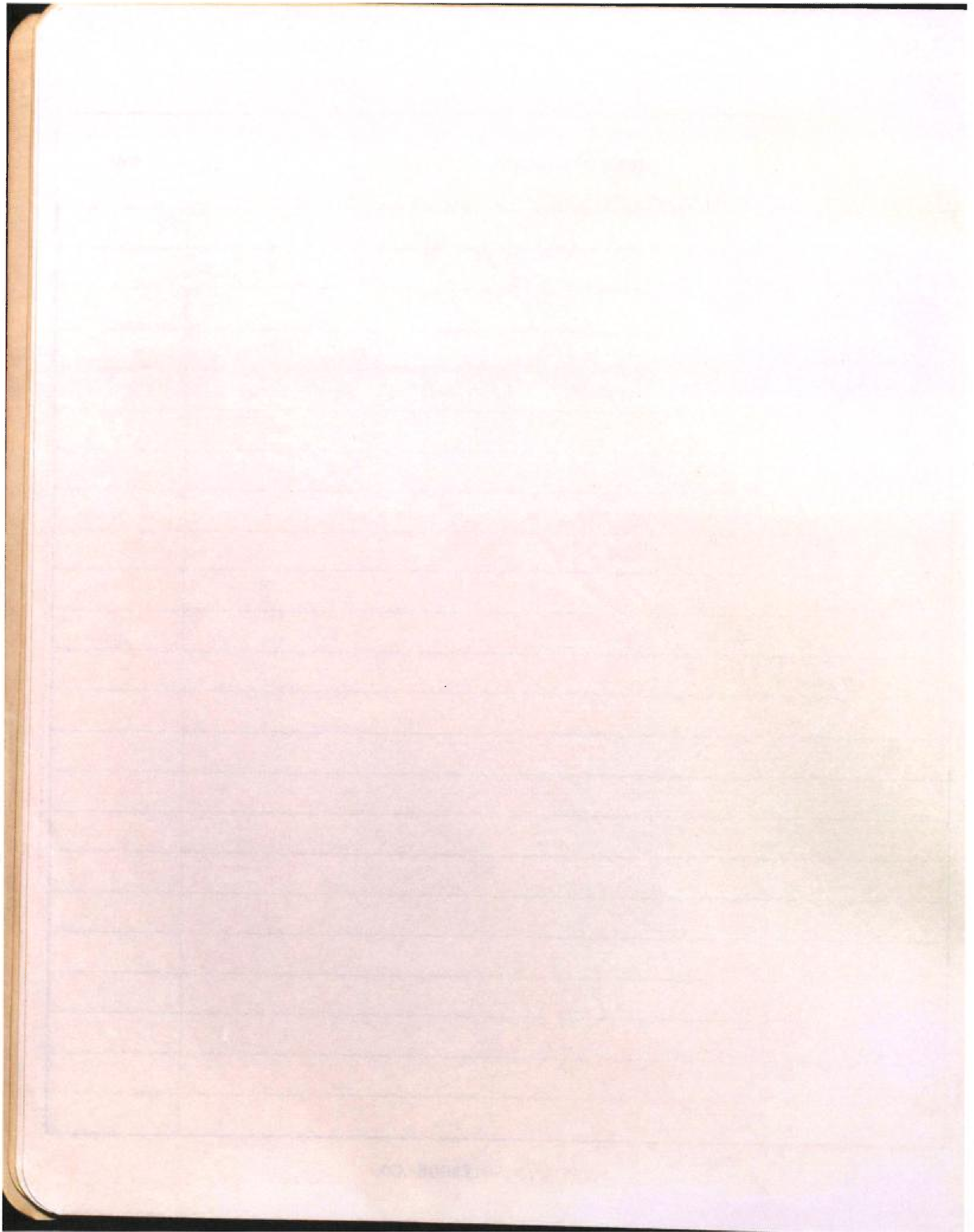


Table of Contents	Page

SCIENTIFIC NOTEBOOK CO.

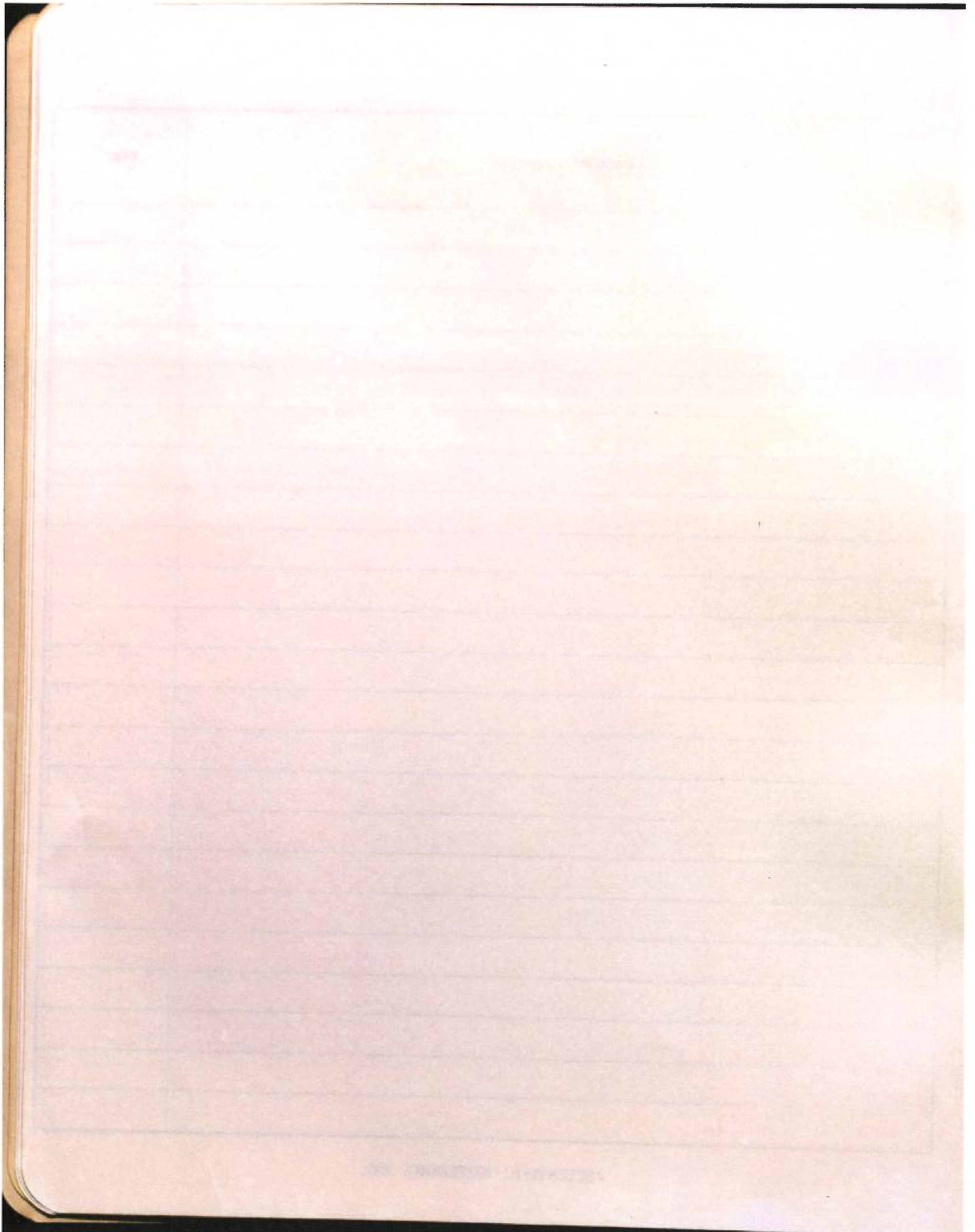
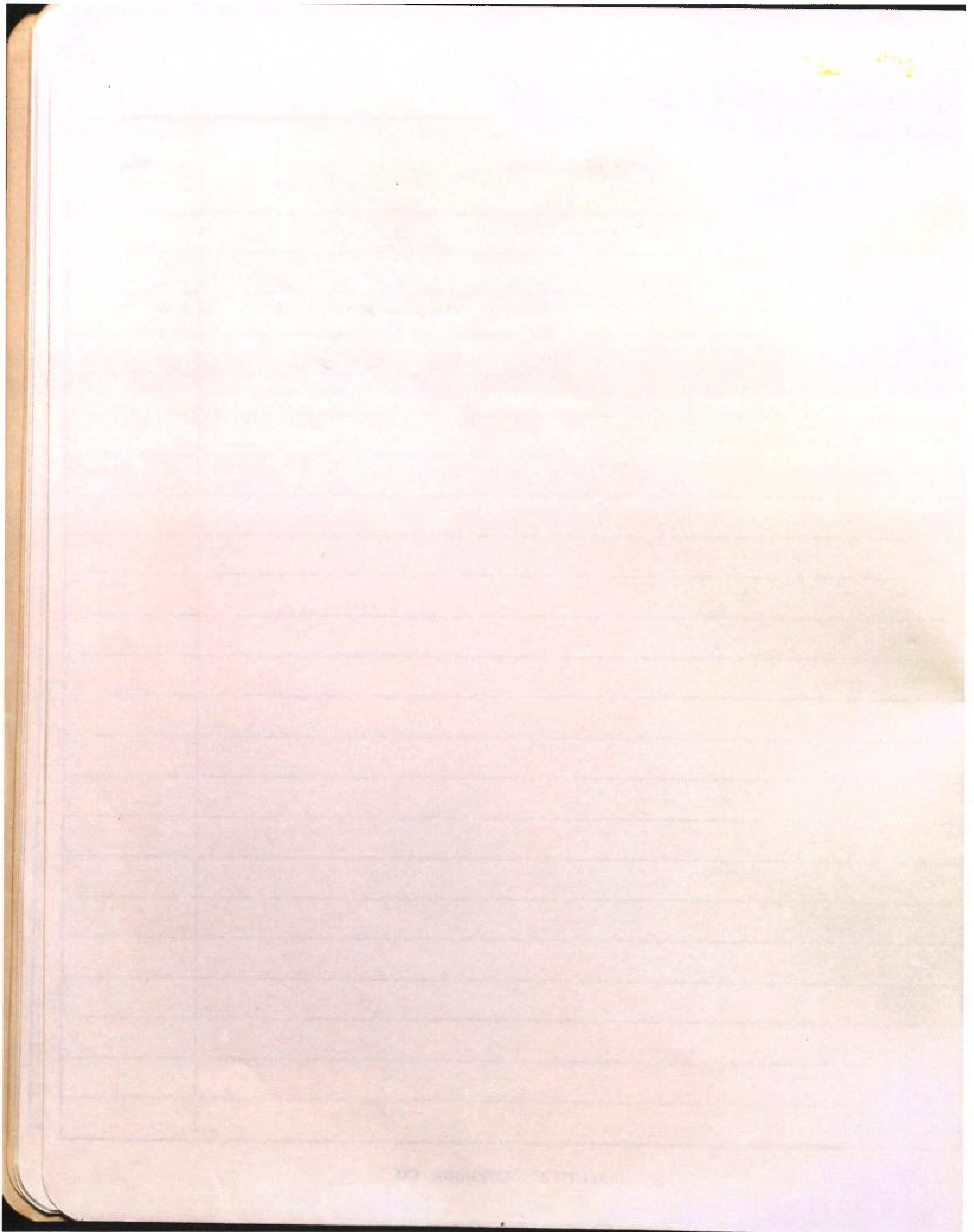
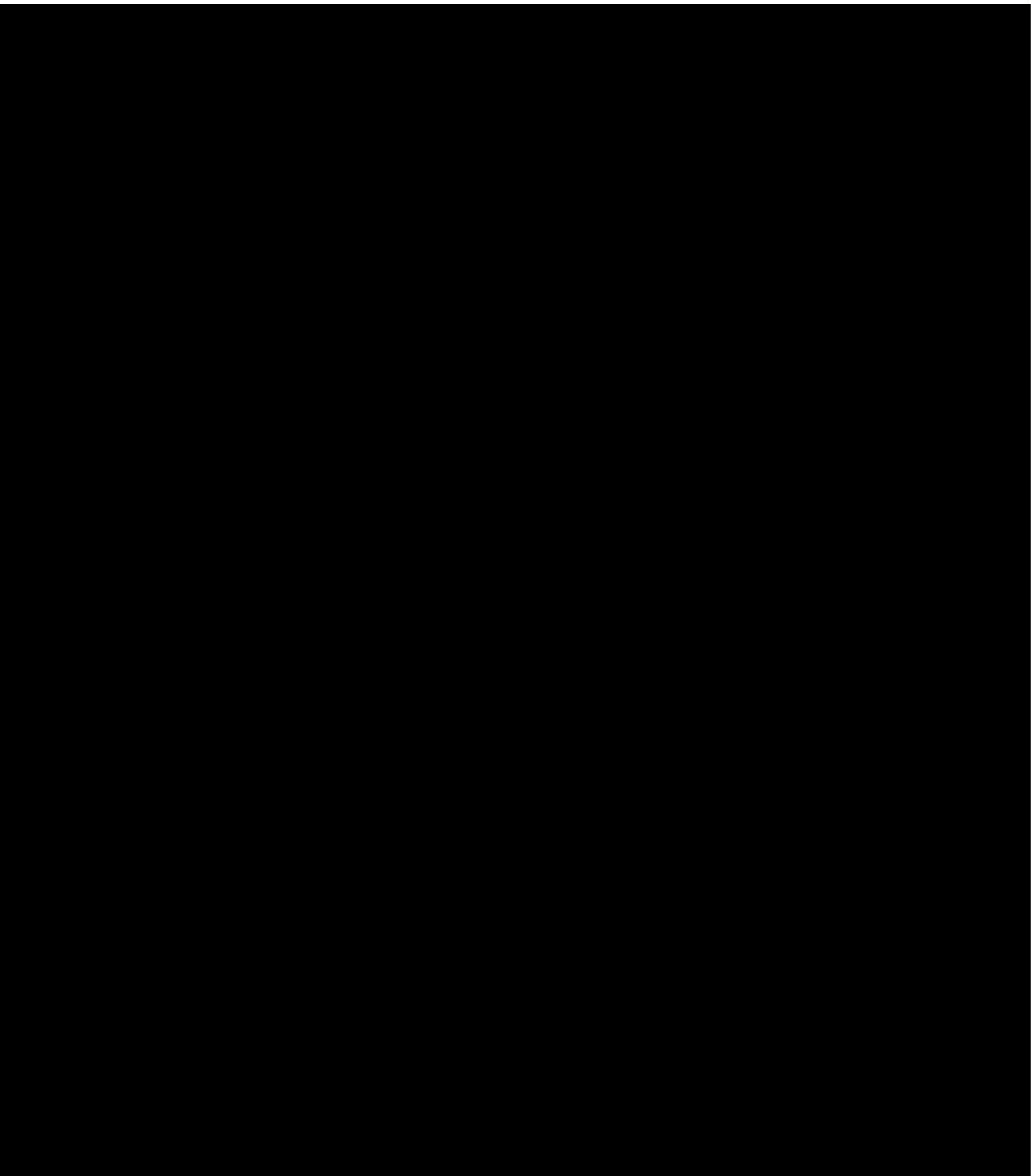


Table of Contents	Page

SCIENTIFIC NOTEBOOK CO.





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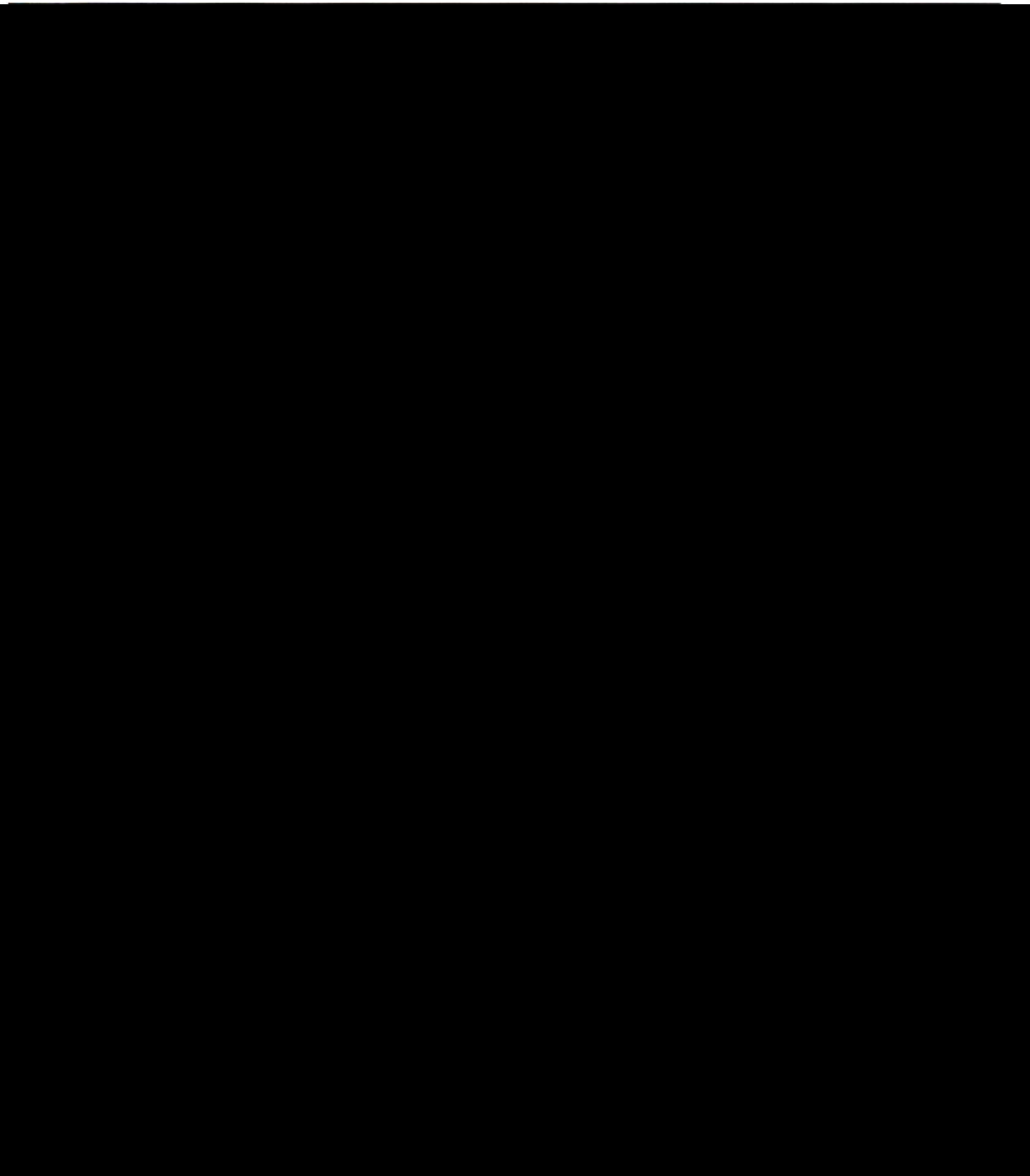

To Page No.____

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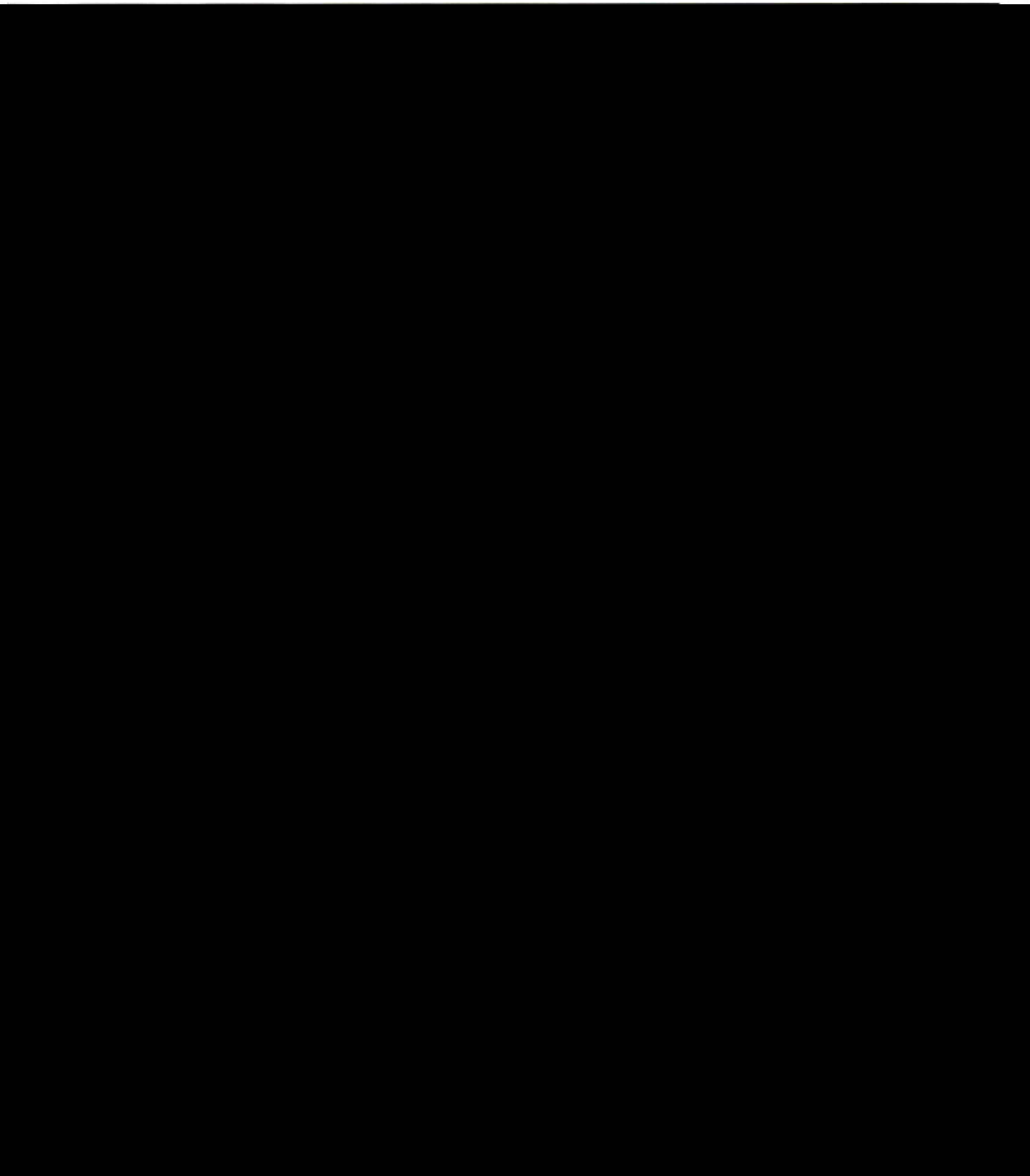

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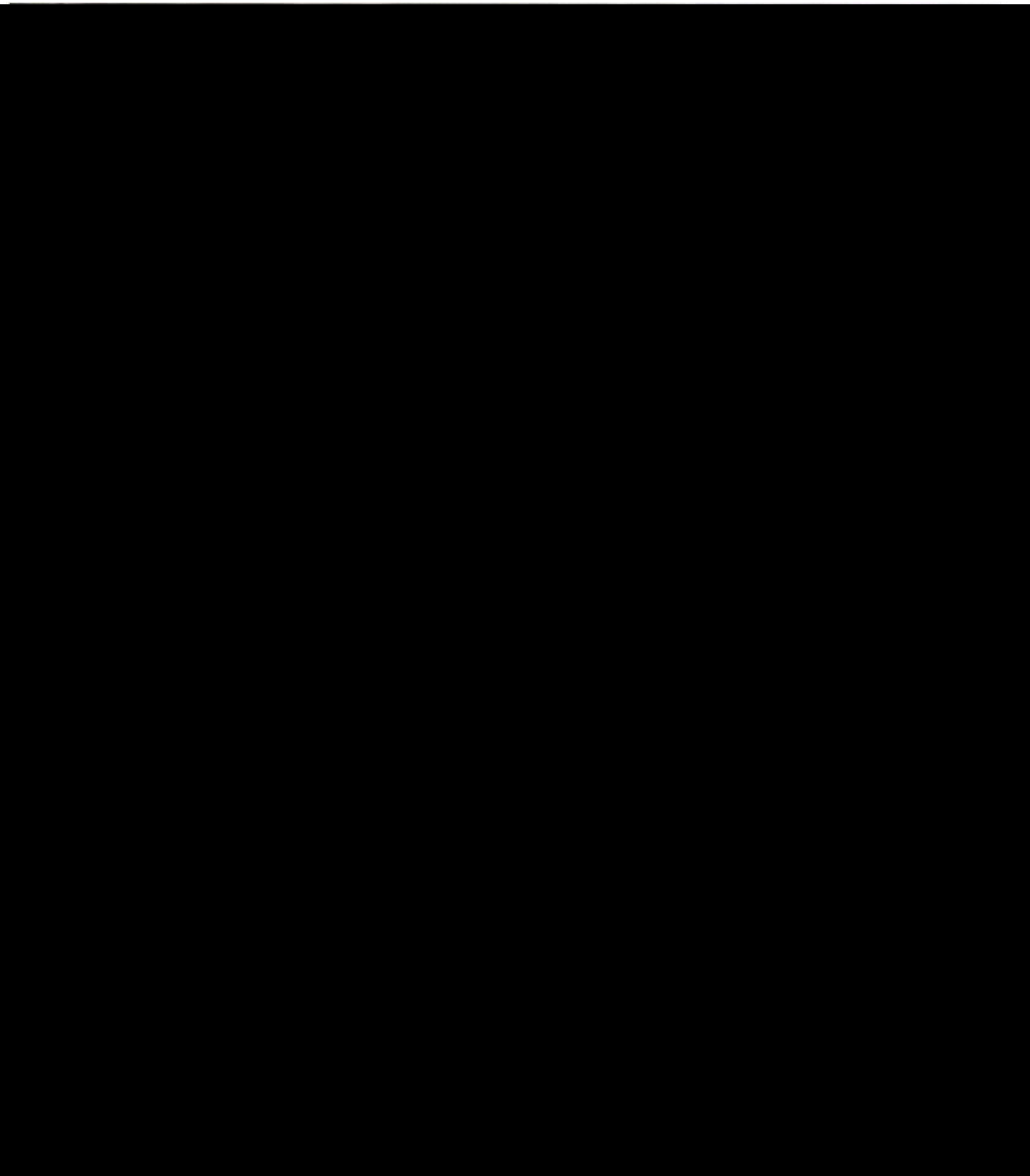

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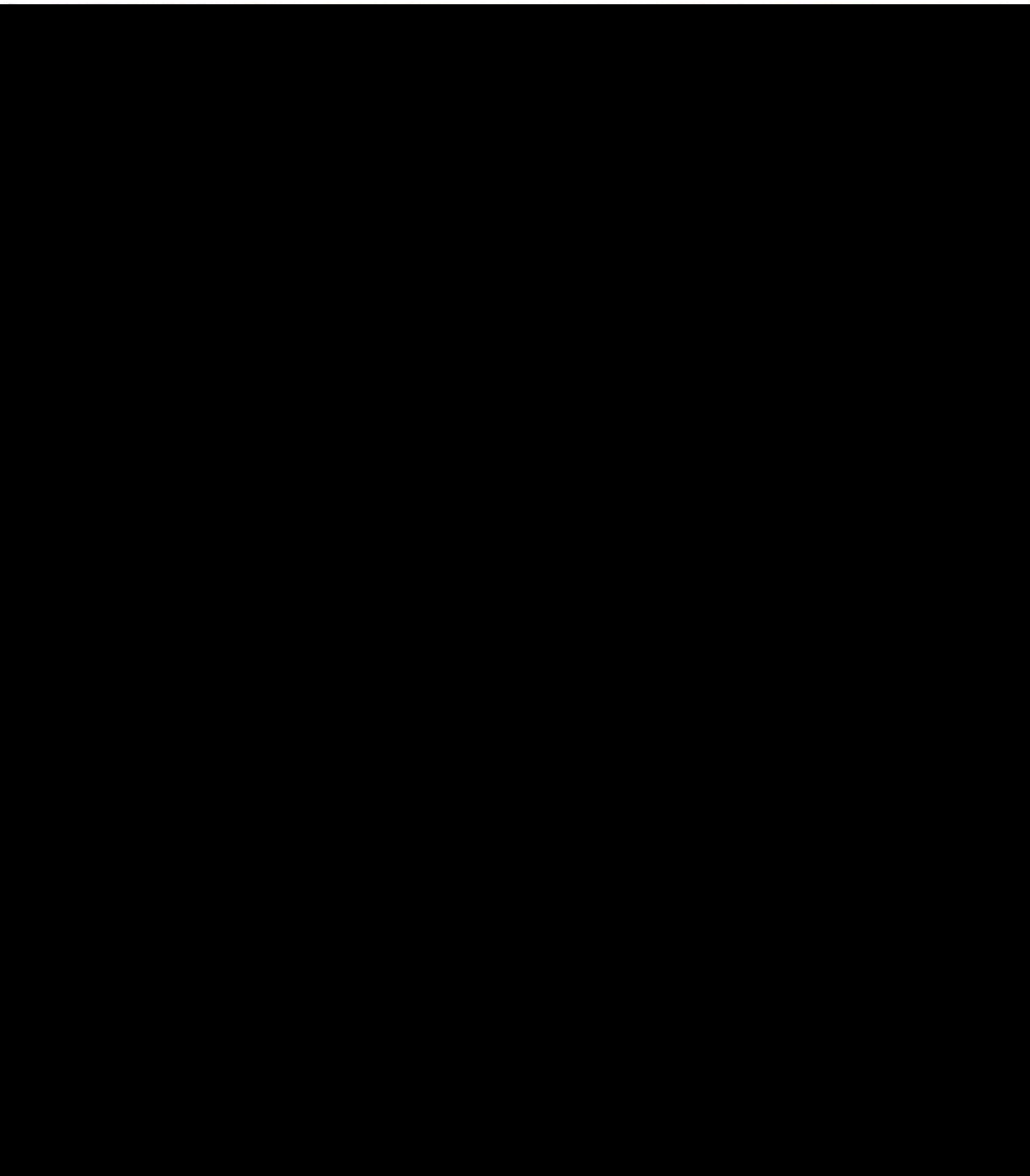

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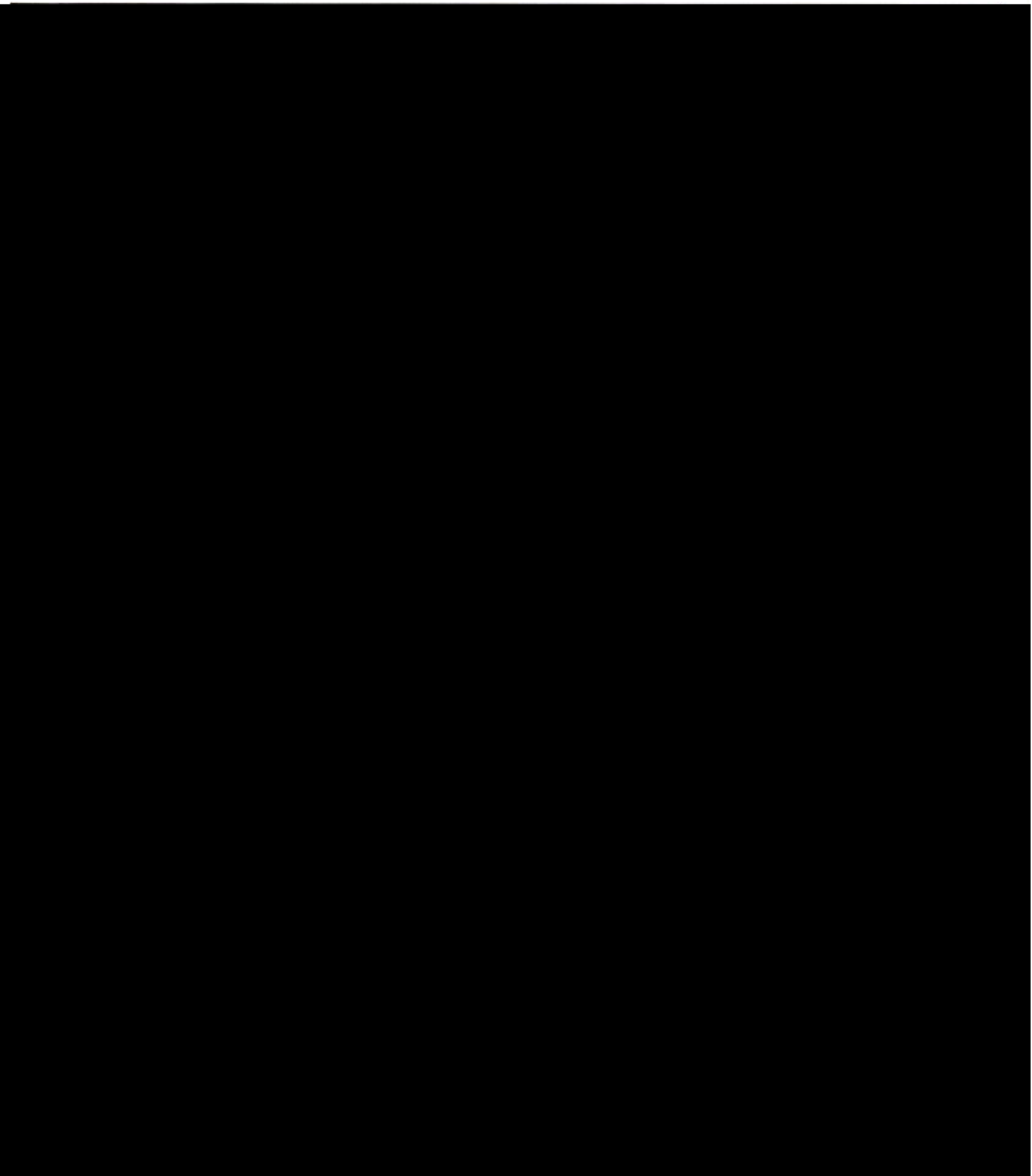

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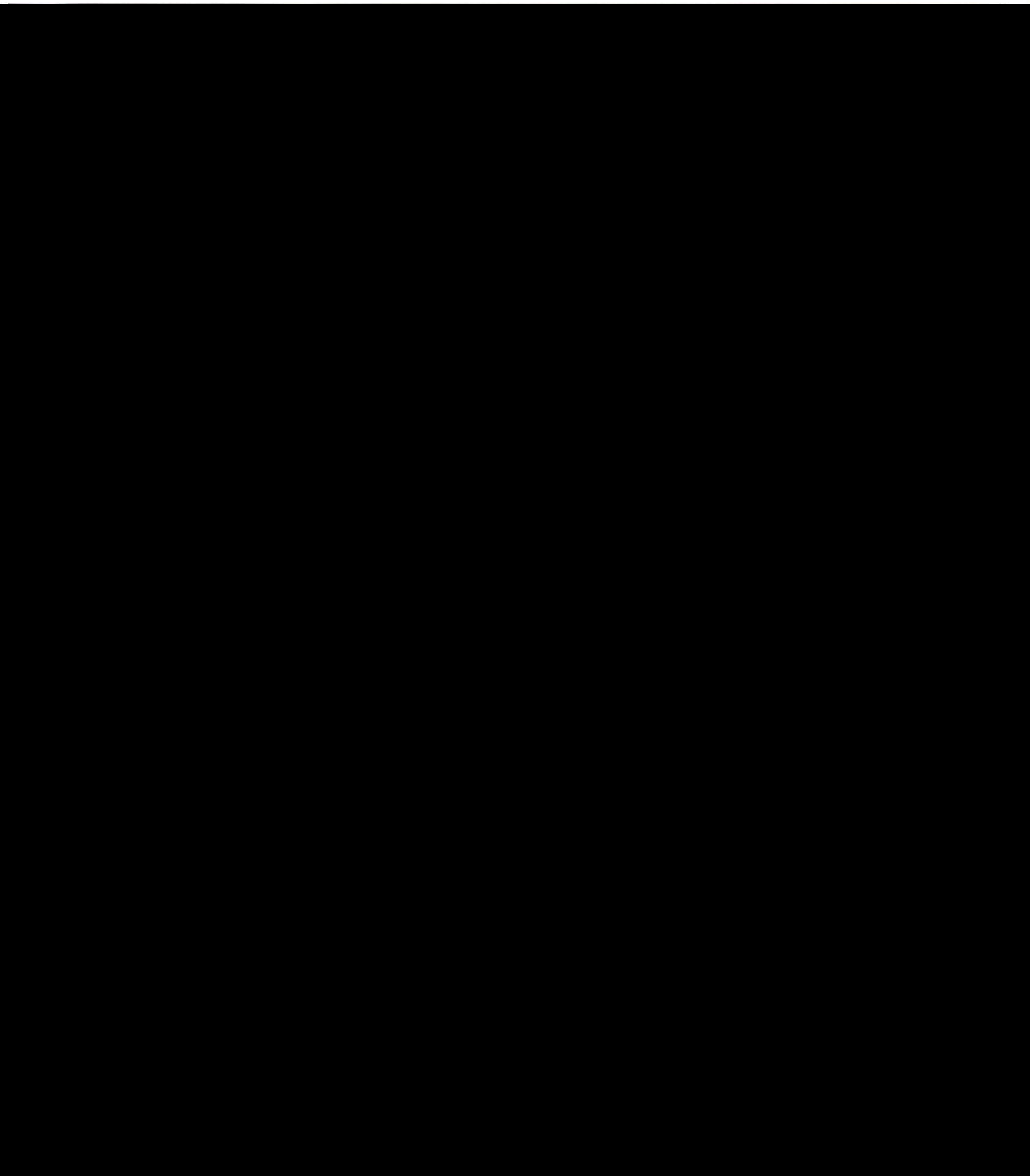

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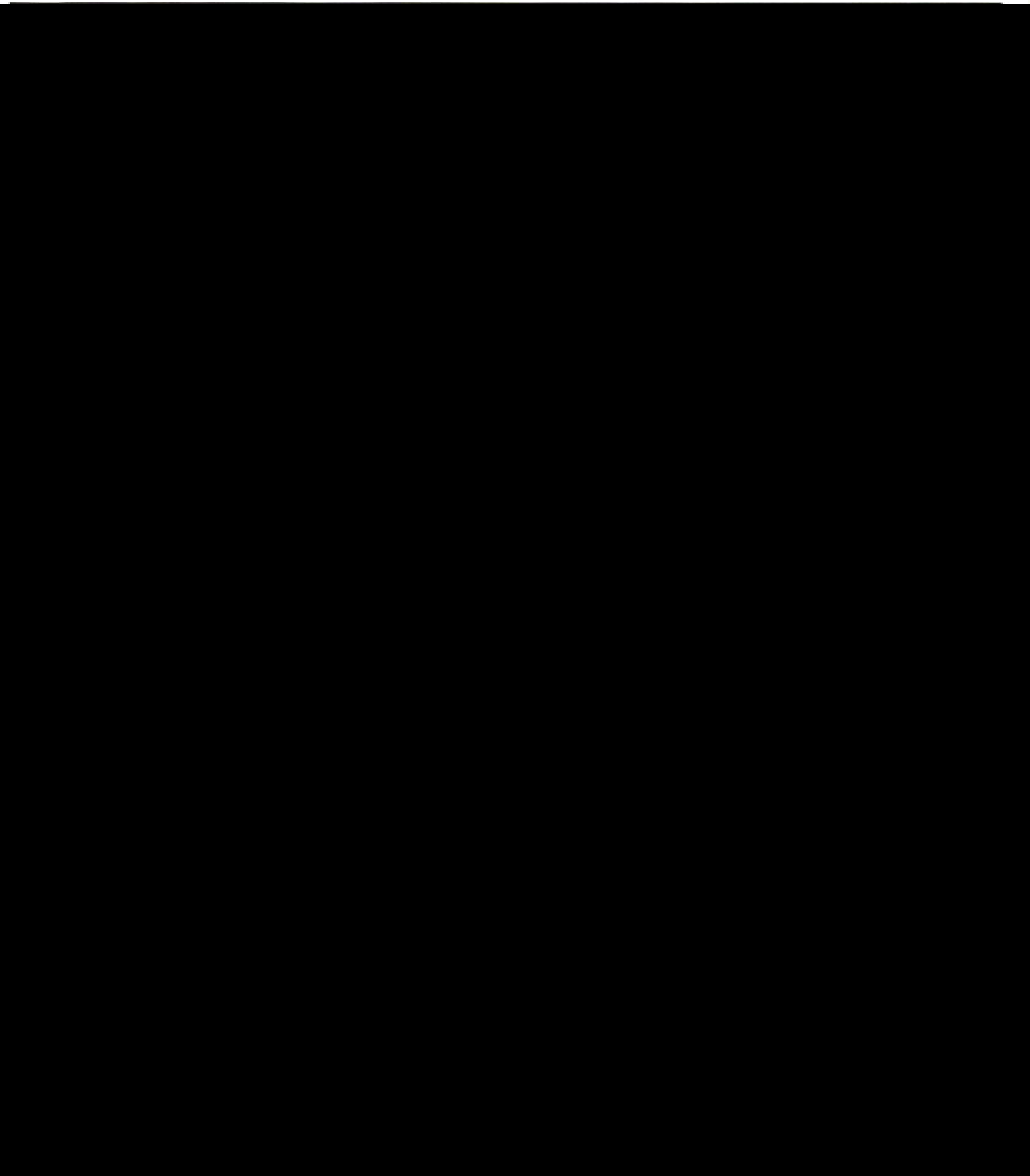

To Page No.

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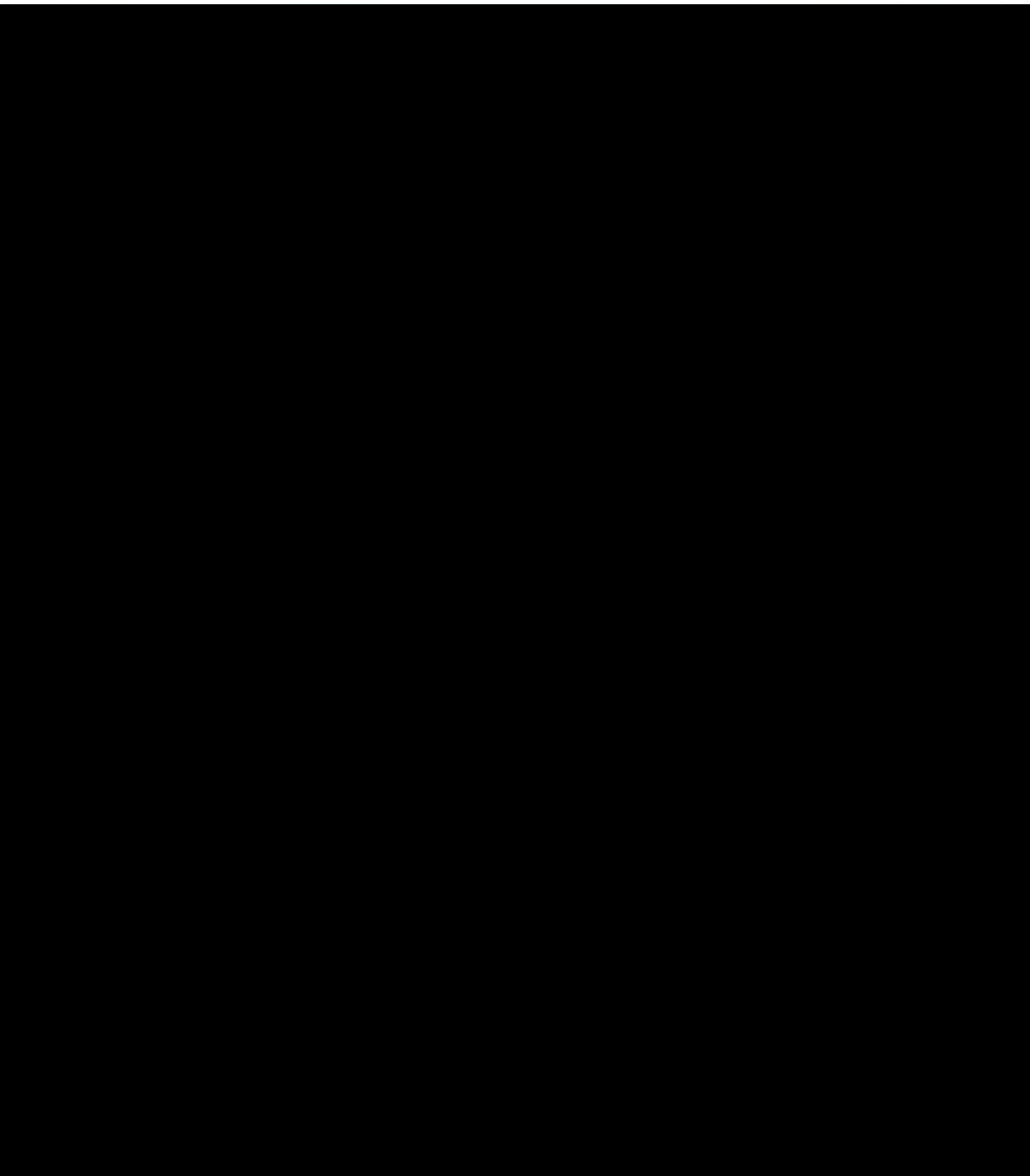

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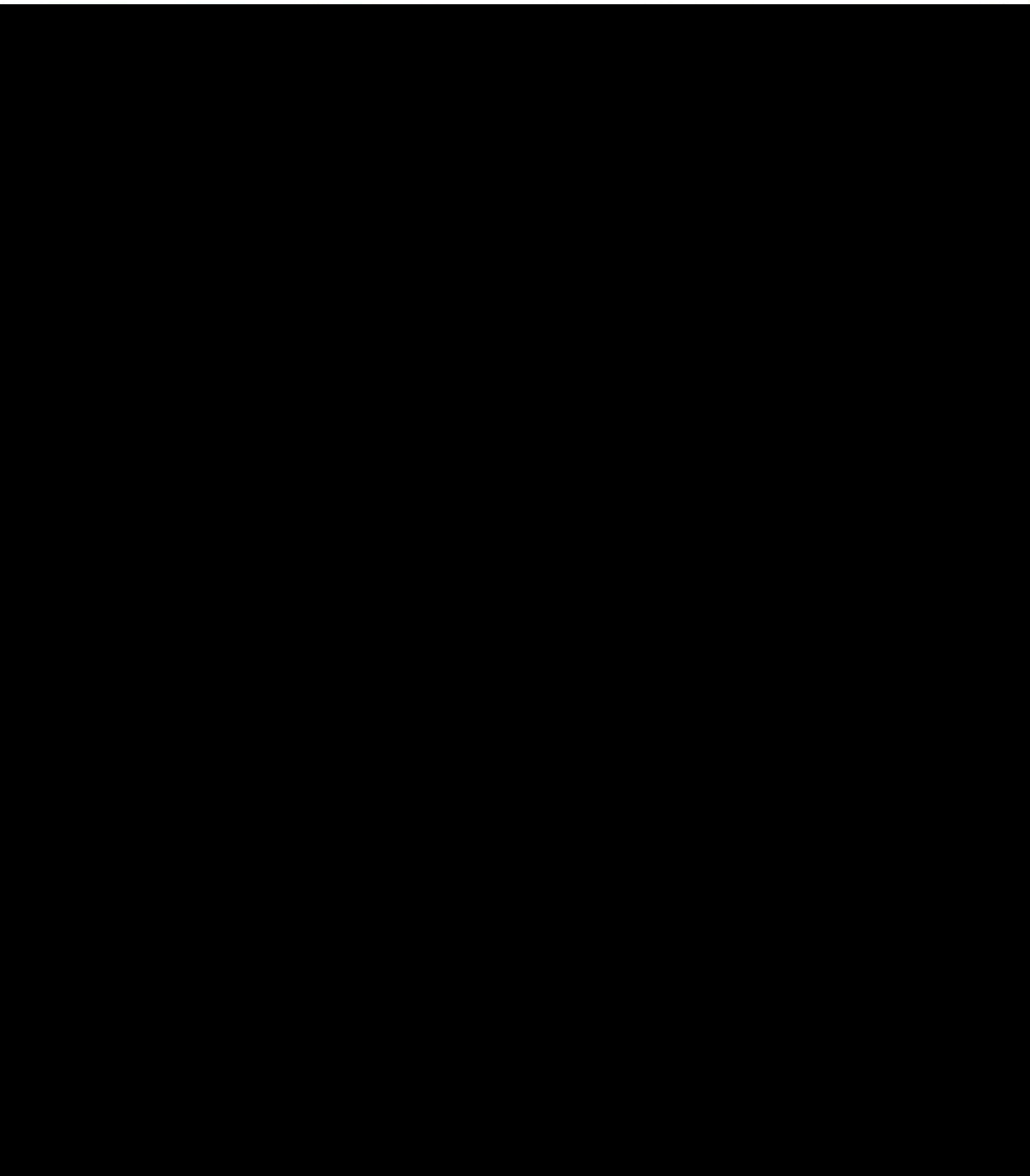

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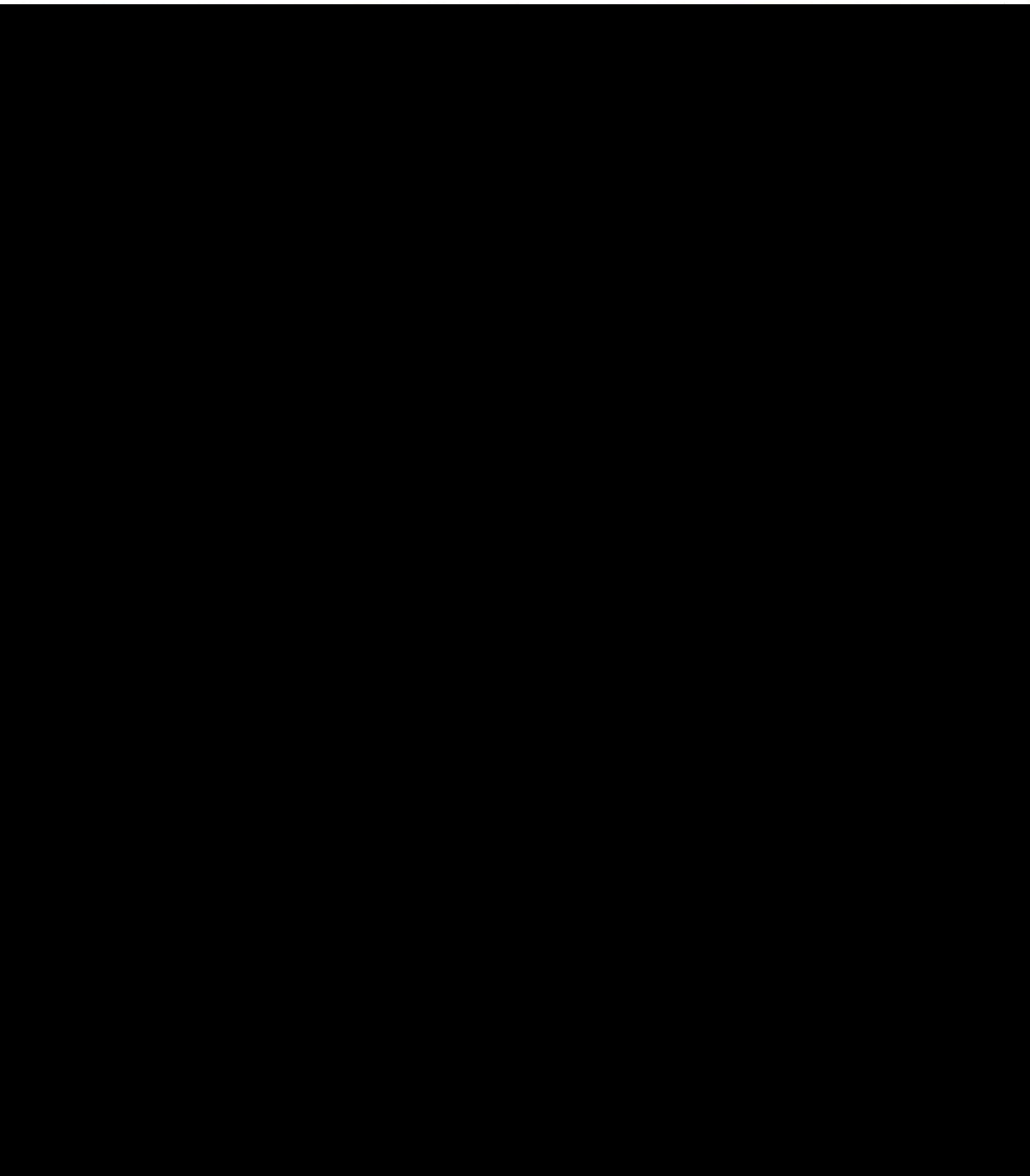

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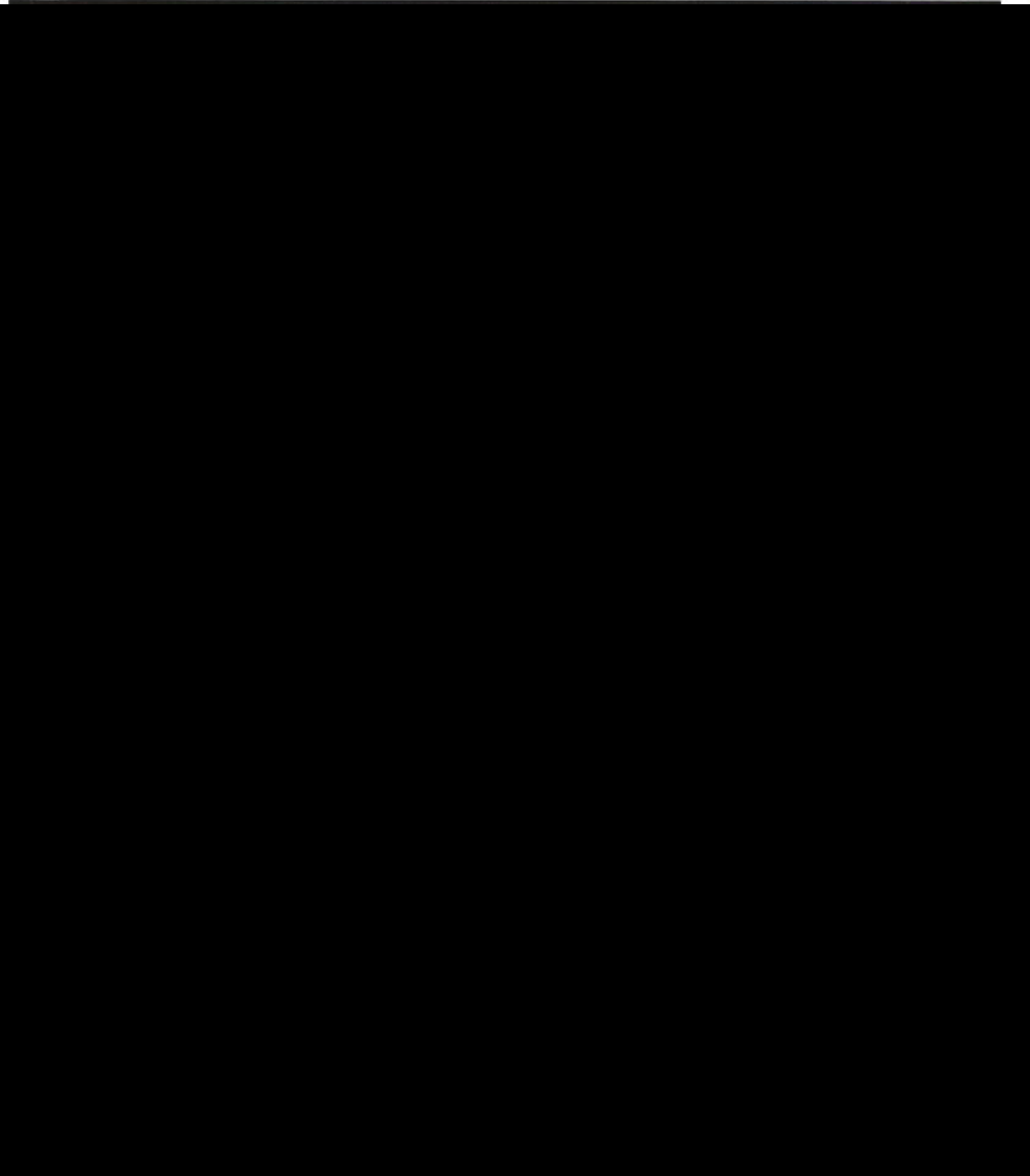

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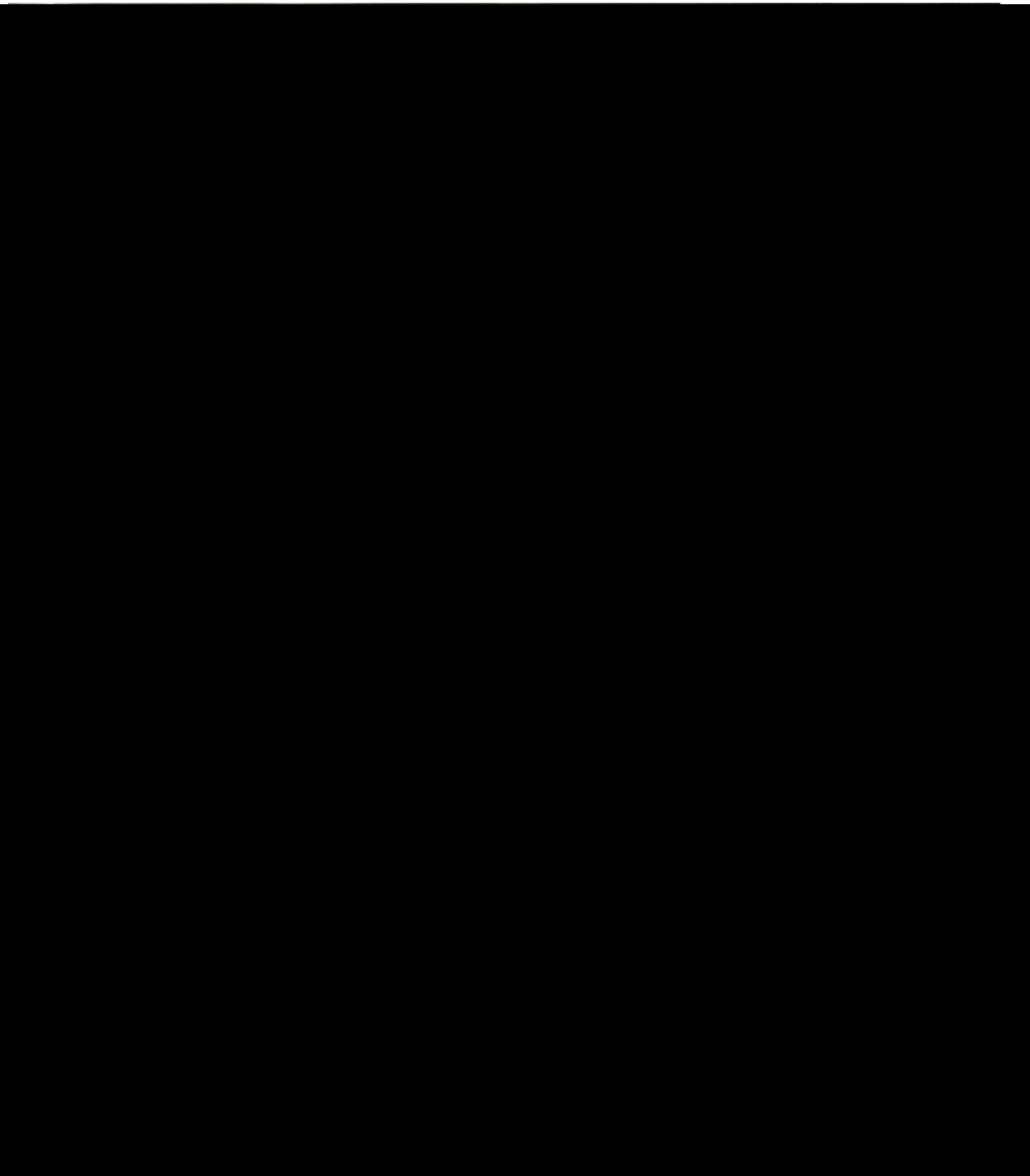

To Page No.____

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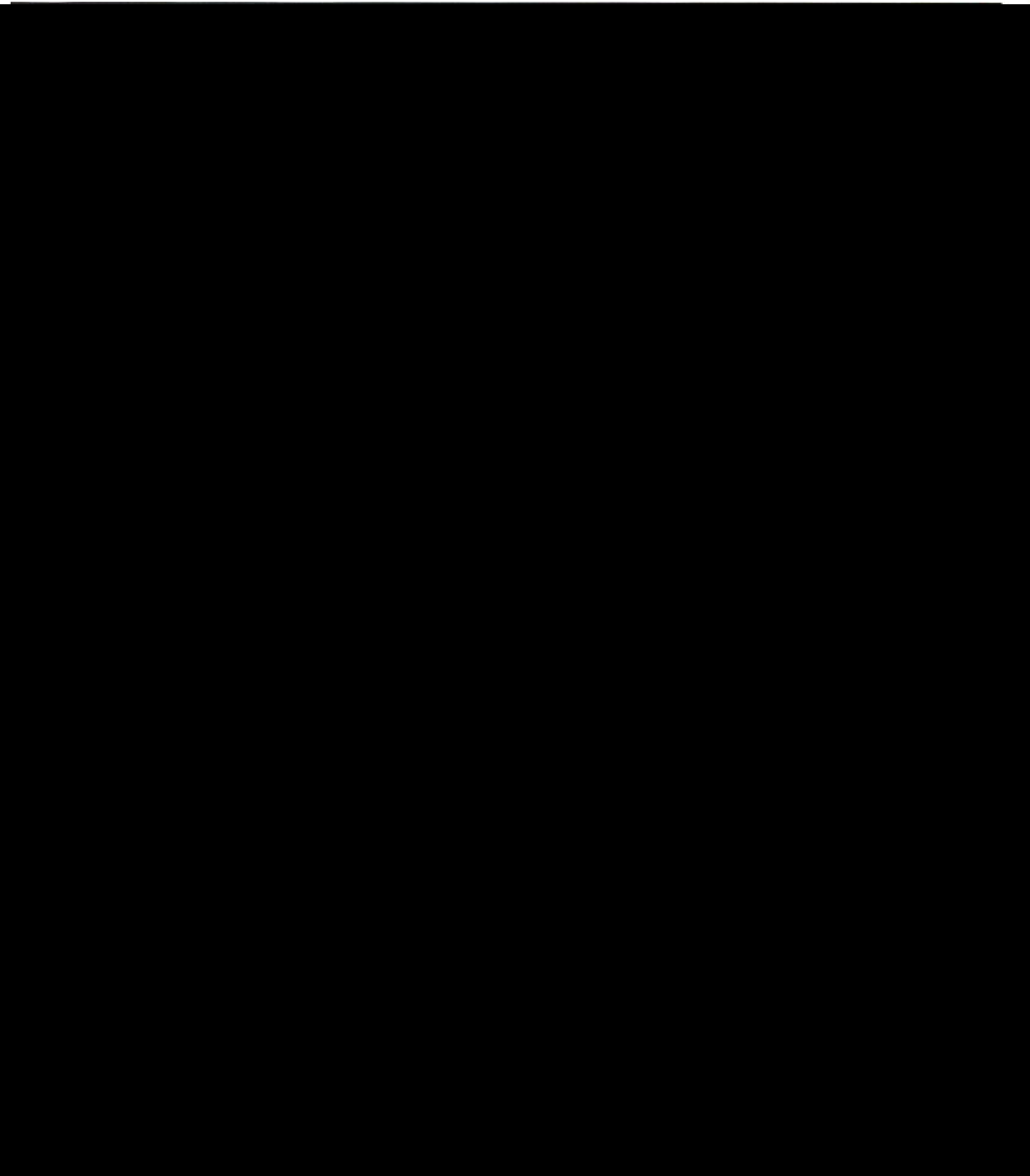

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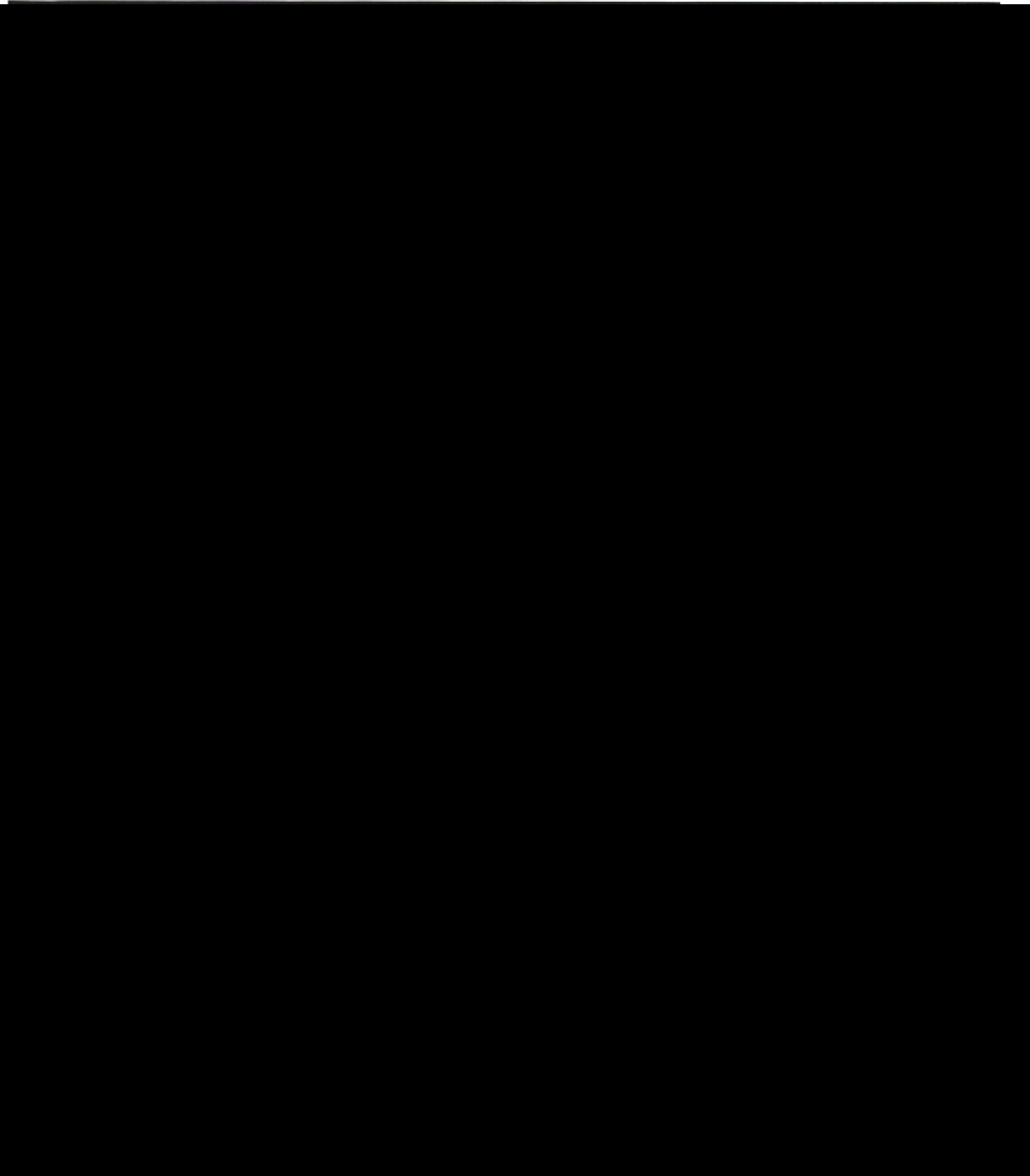

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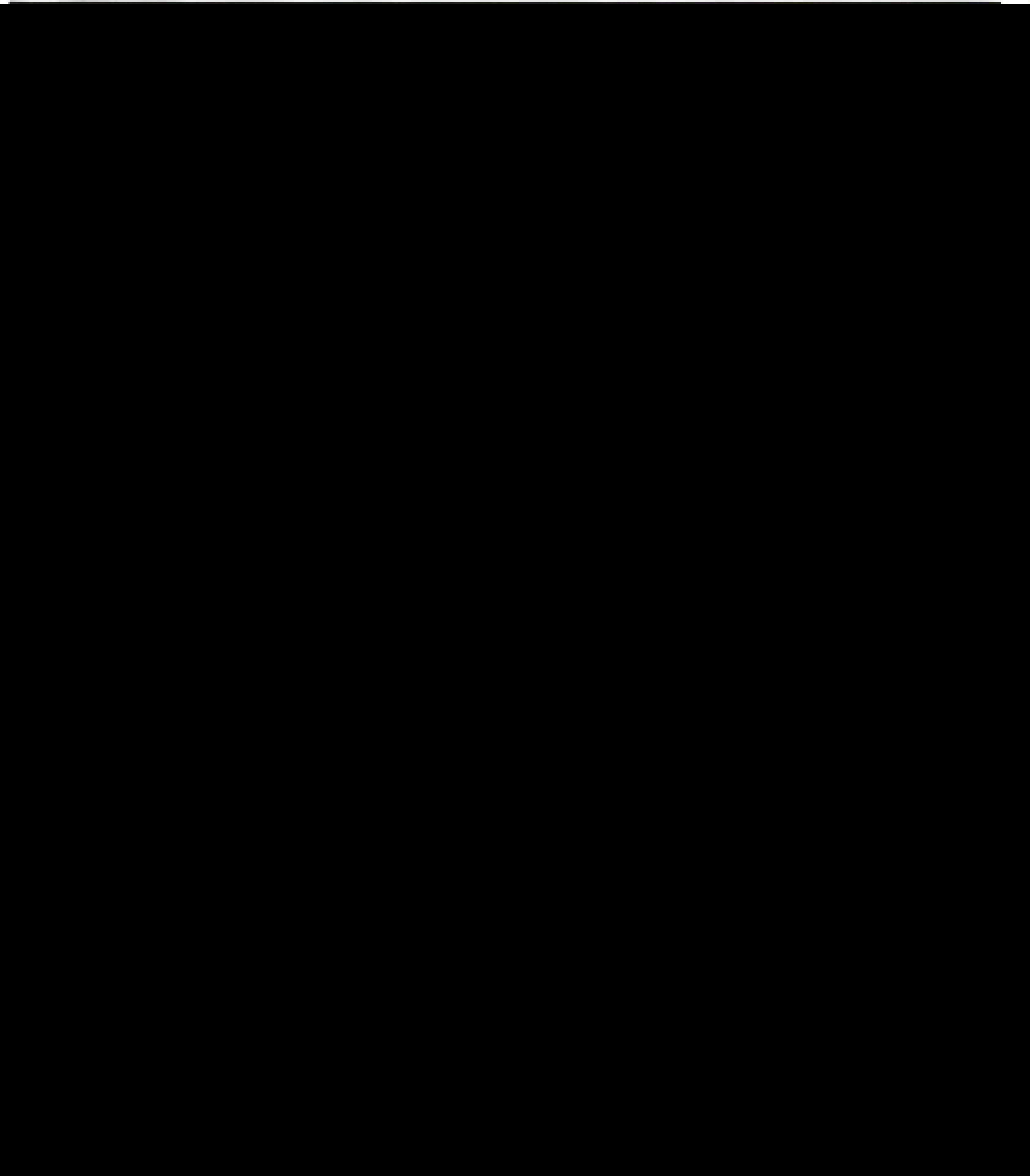

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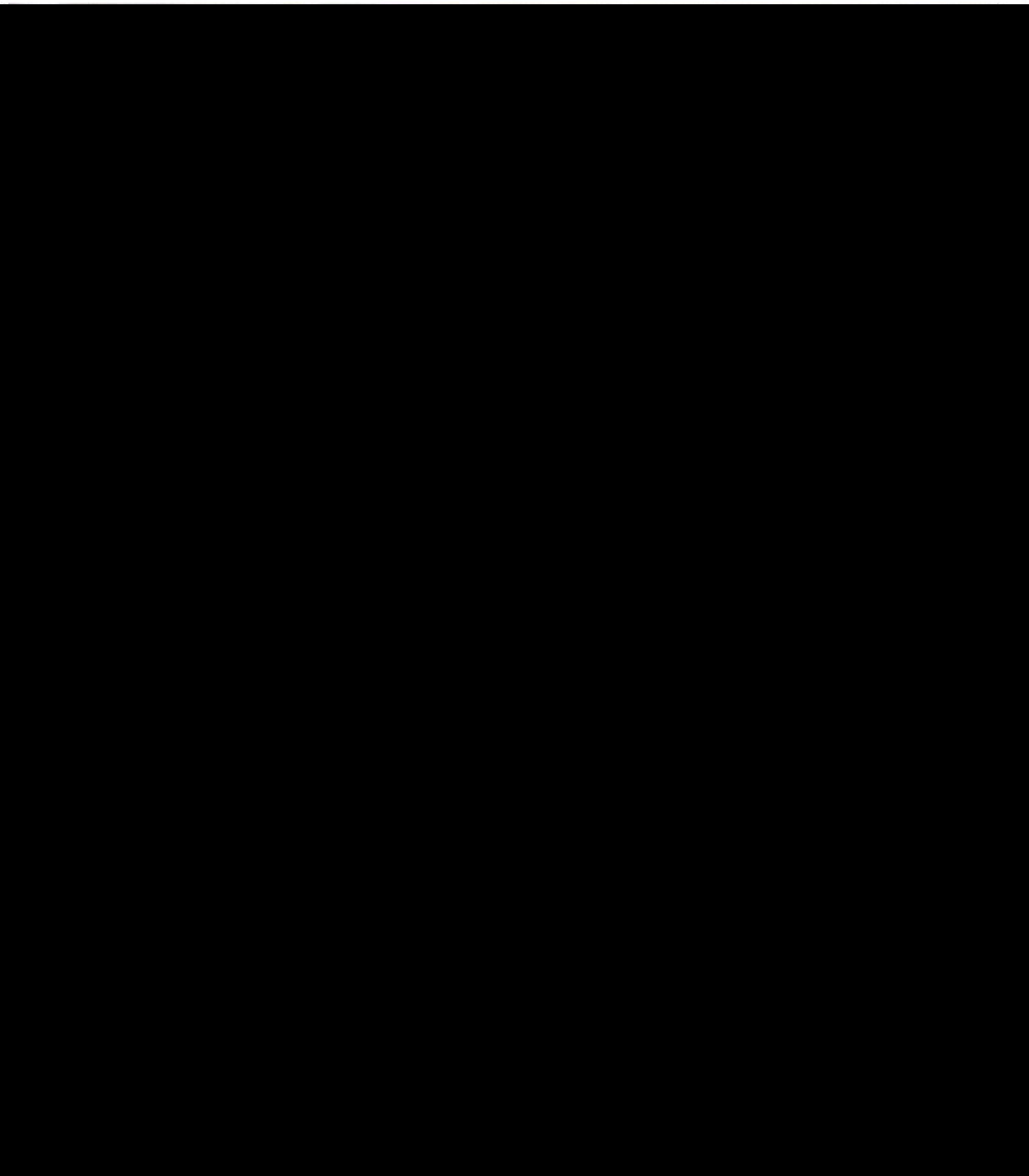

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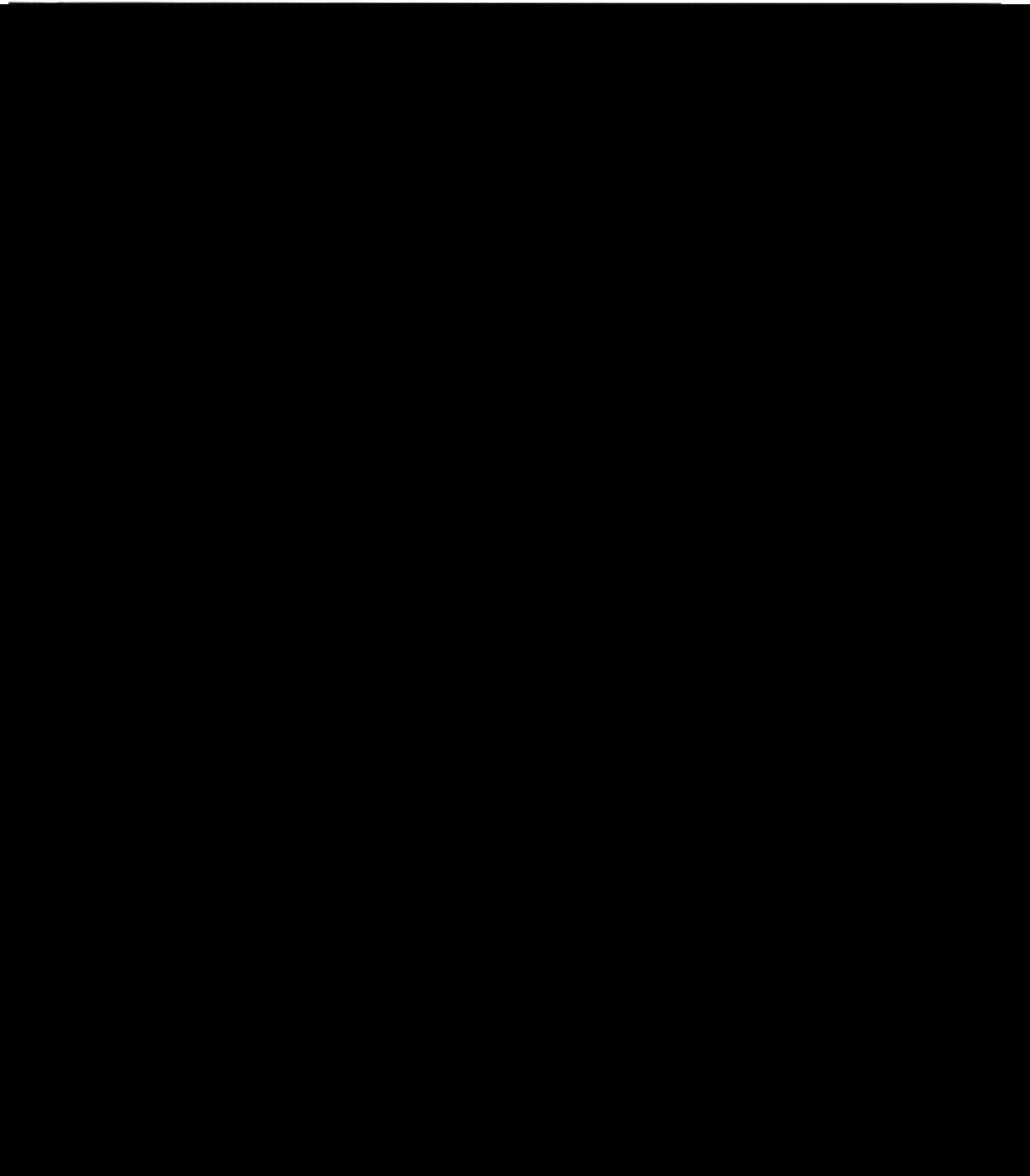

To Page No.

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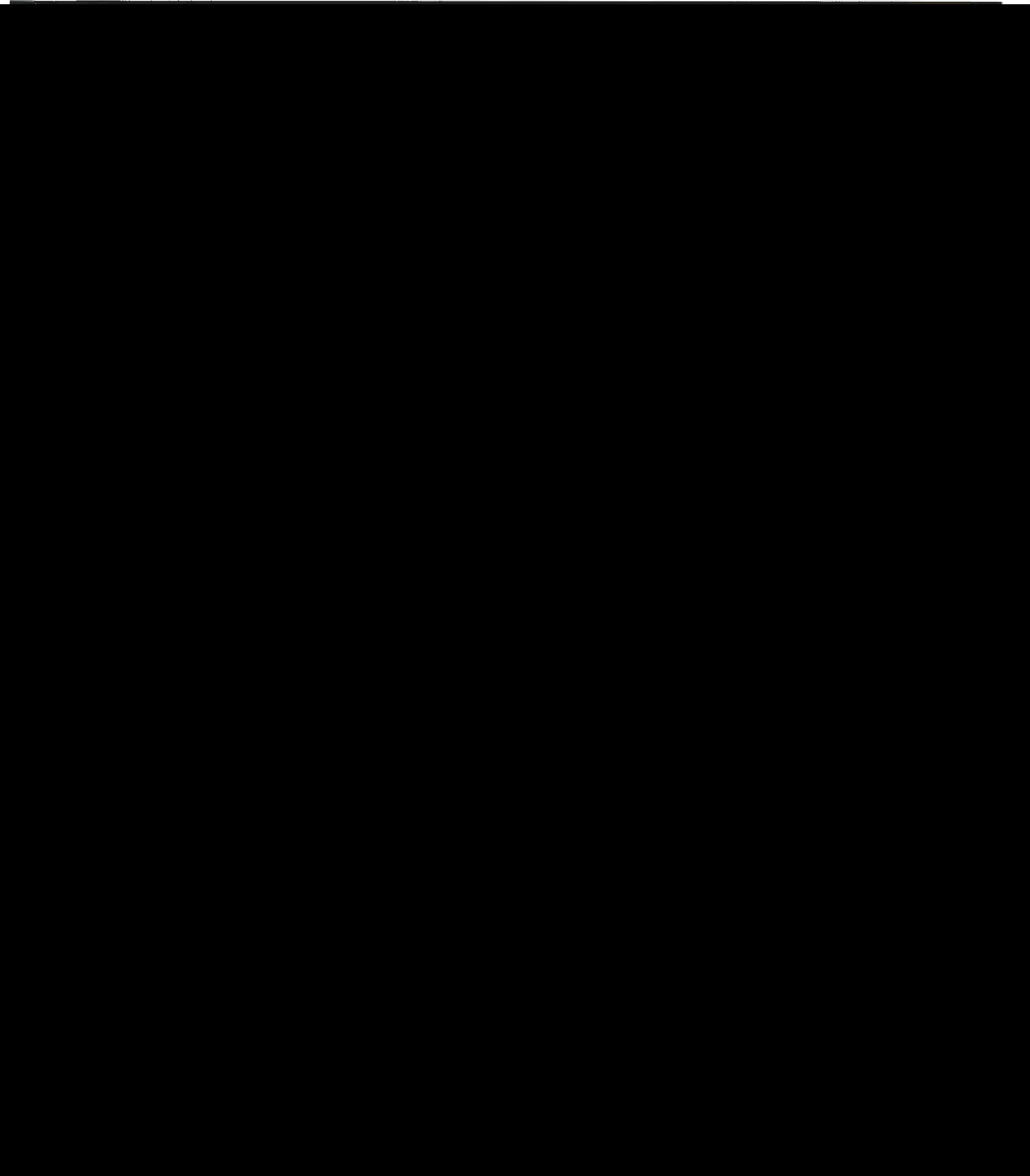

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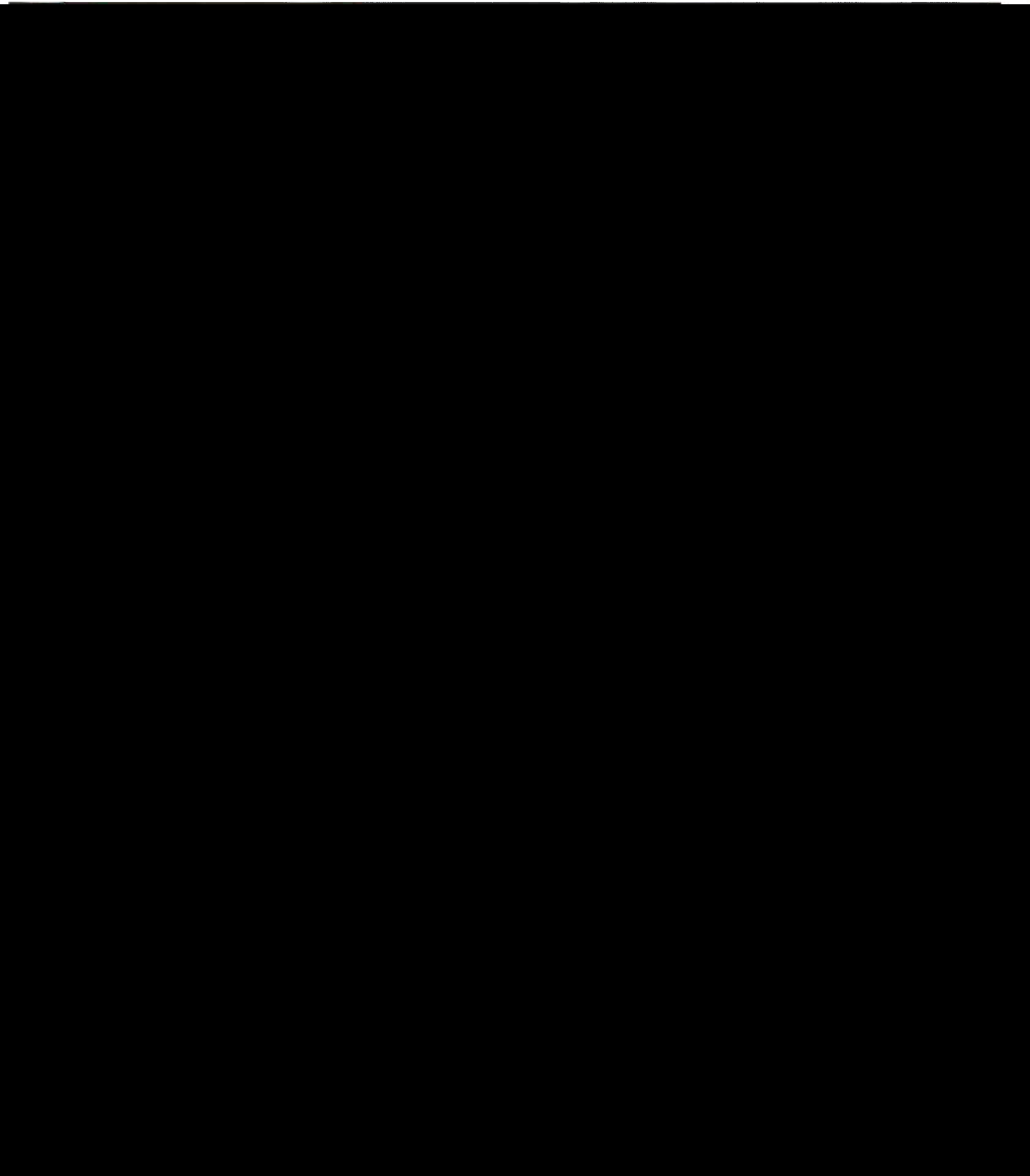

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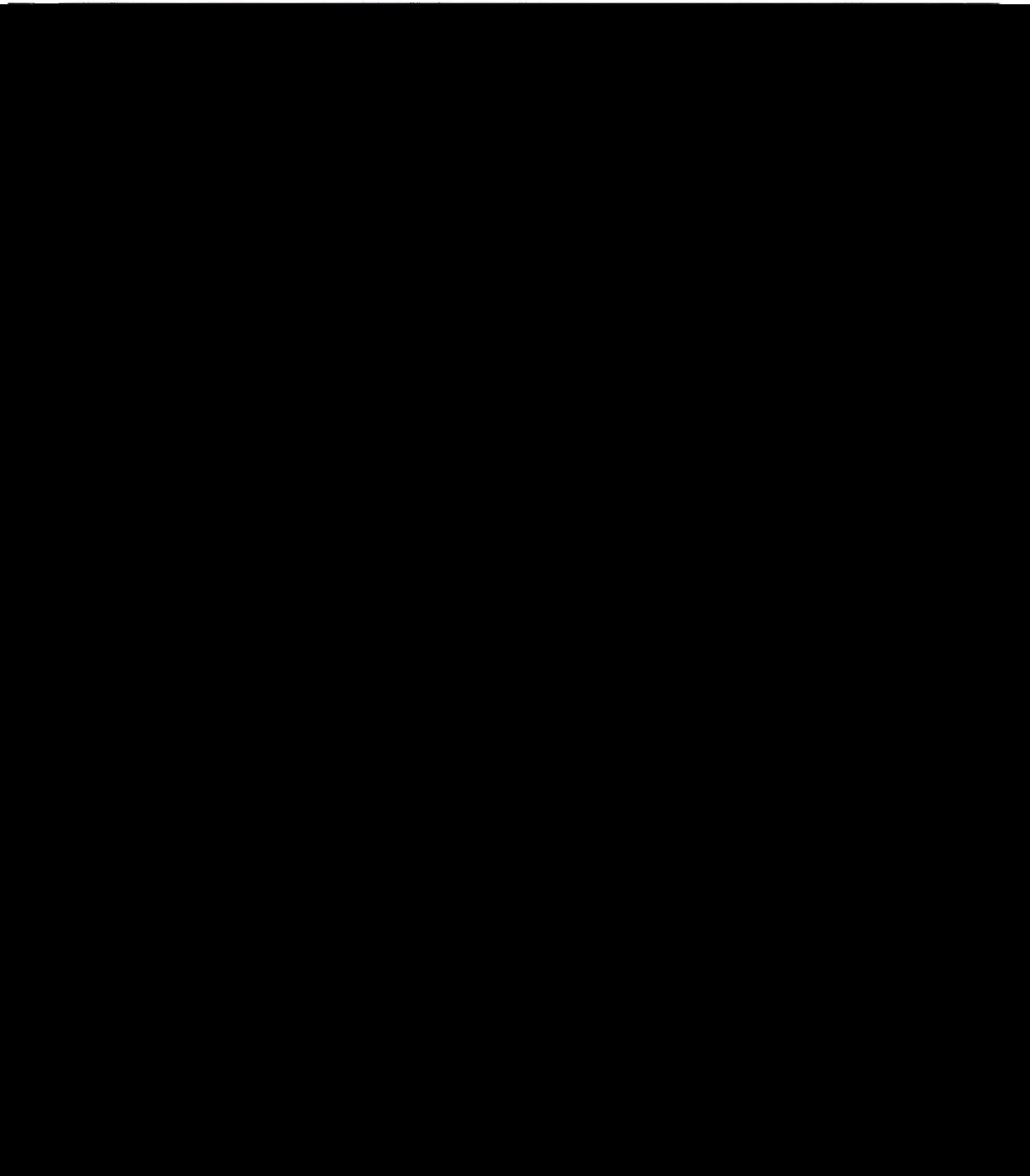

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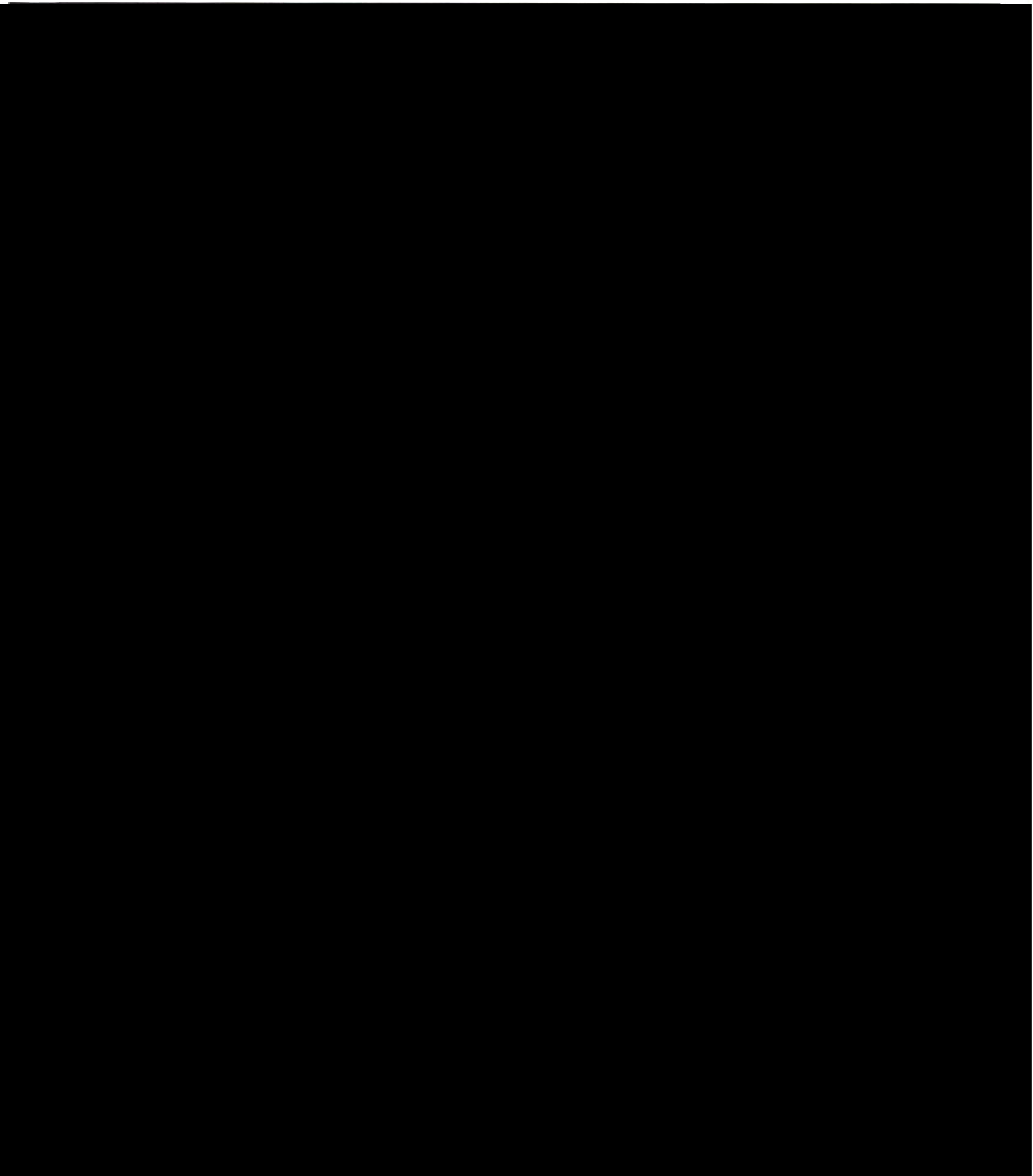

To Page No.

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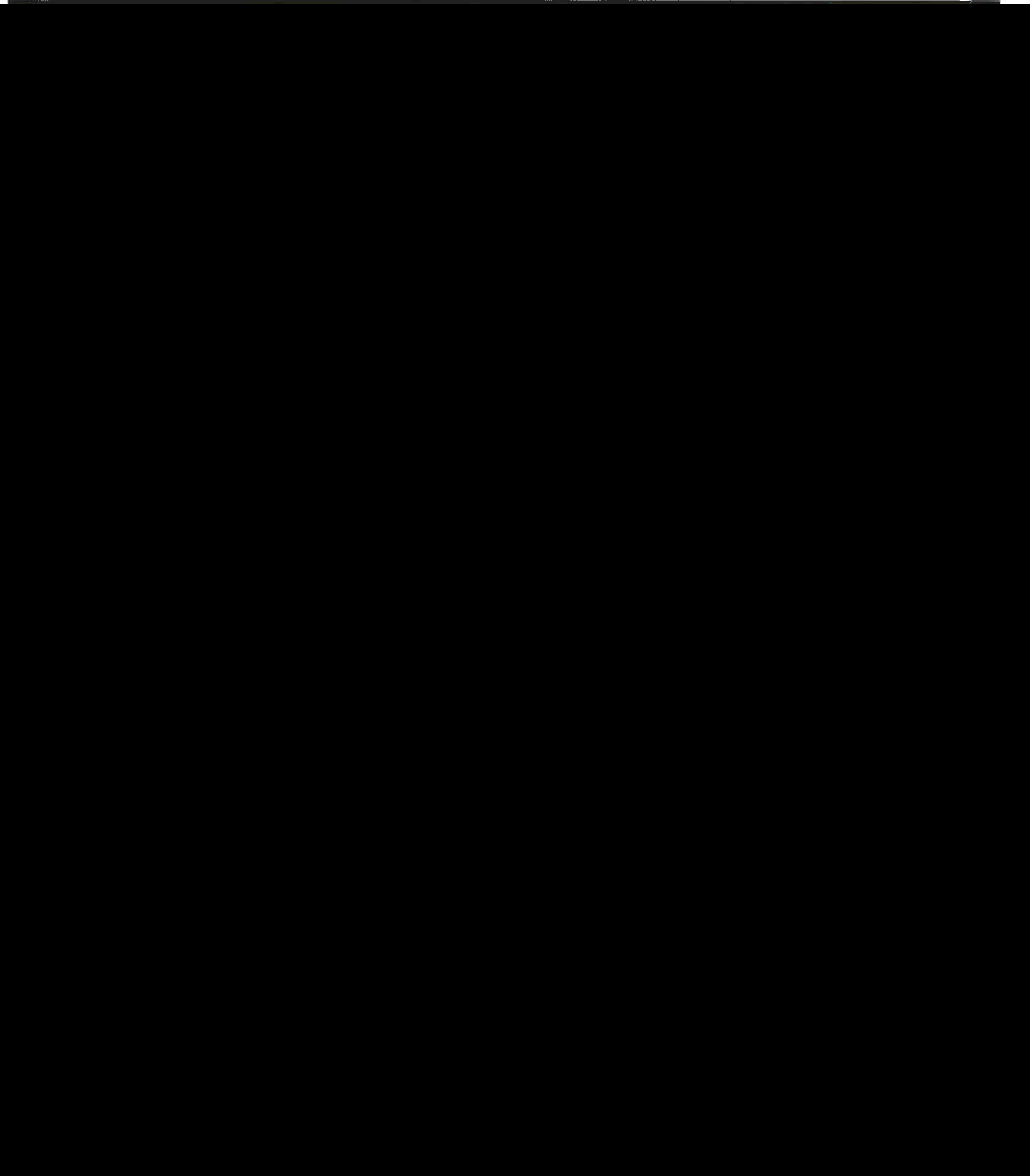

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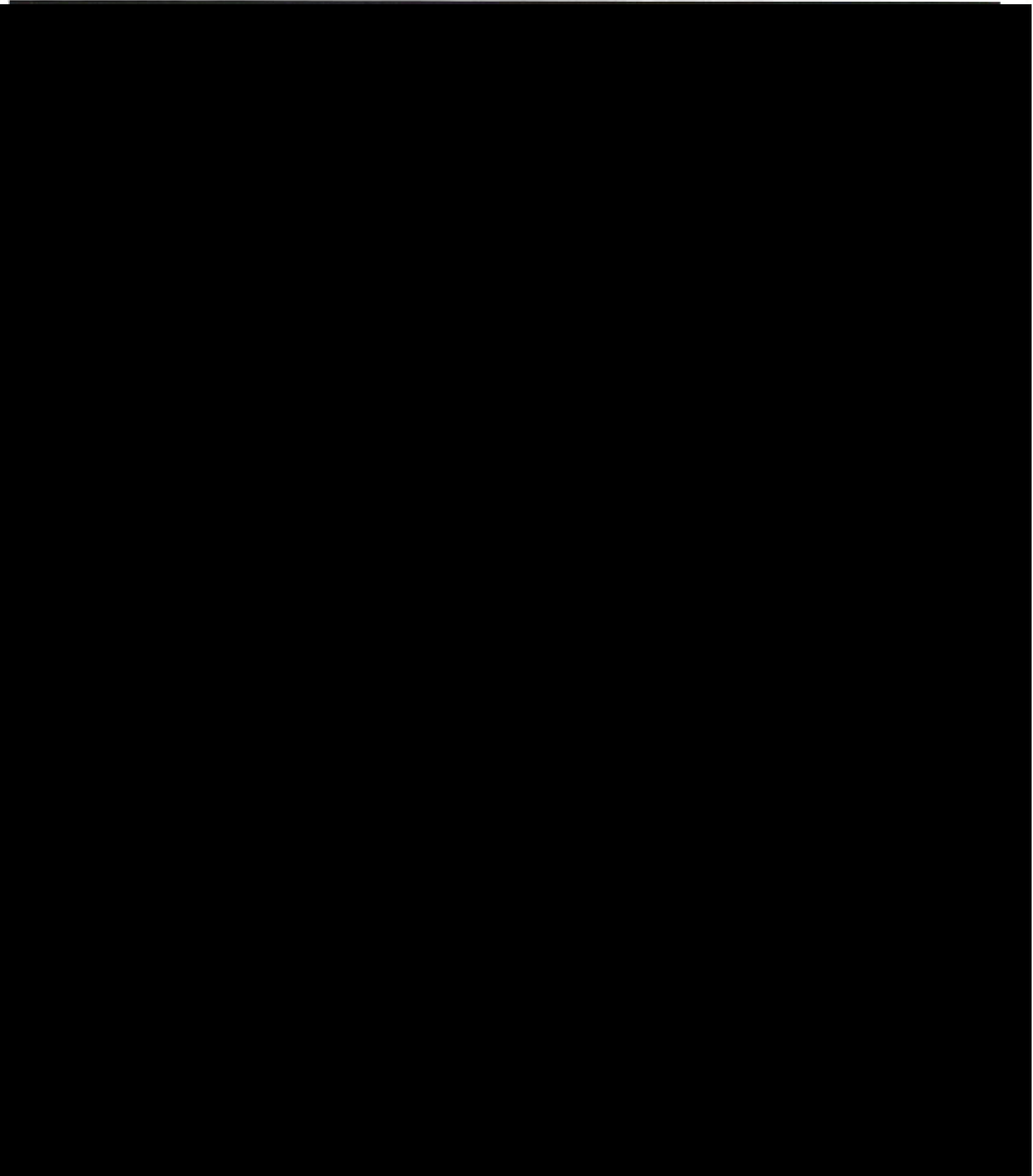

To Page No.____

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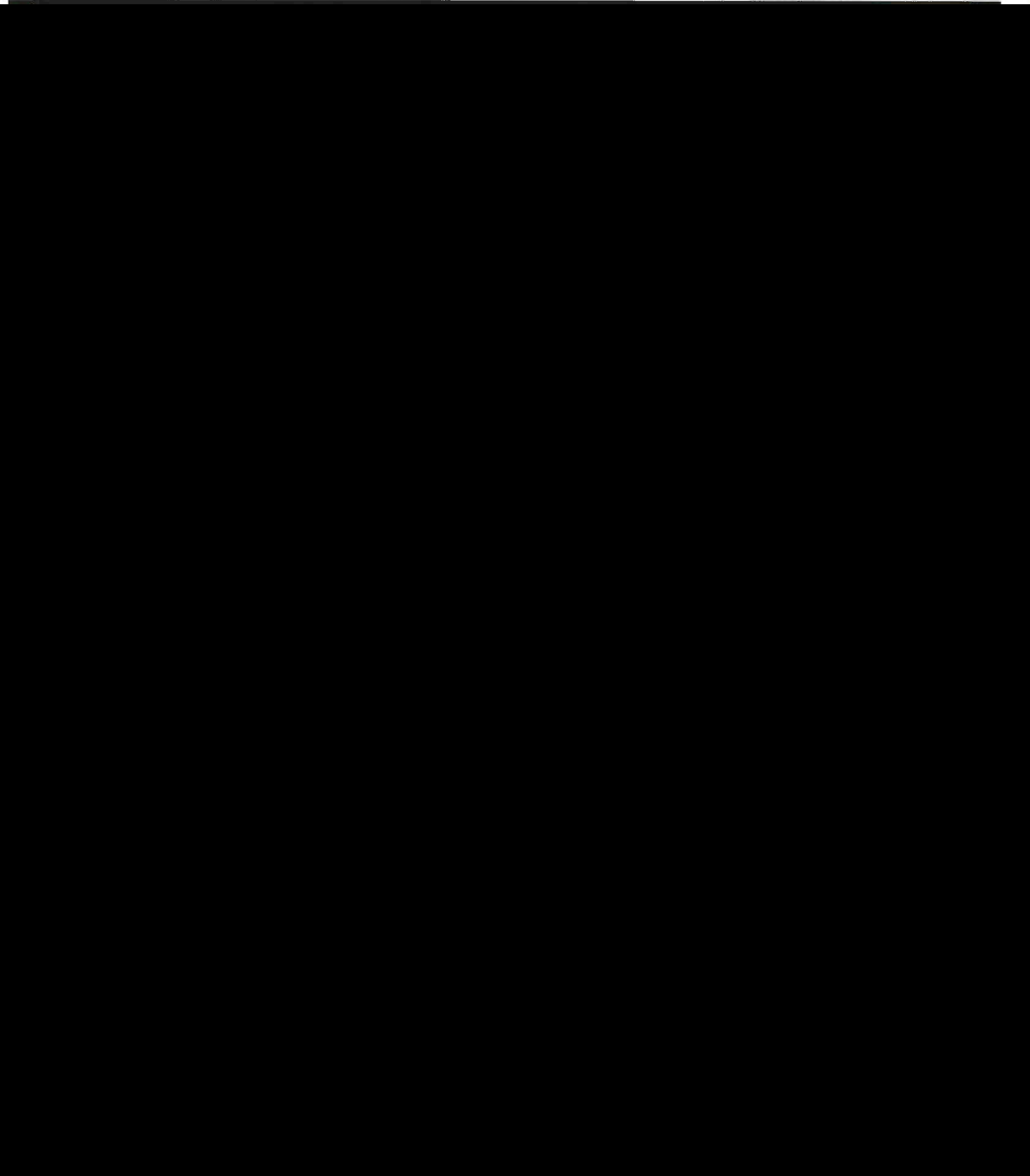

To Page No.____

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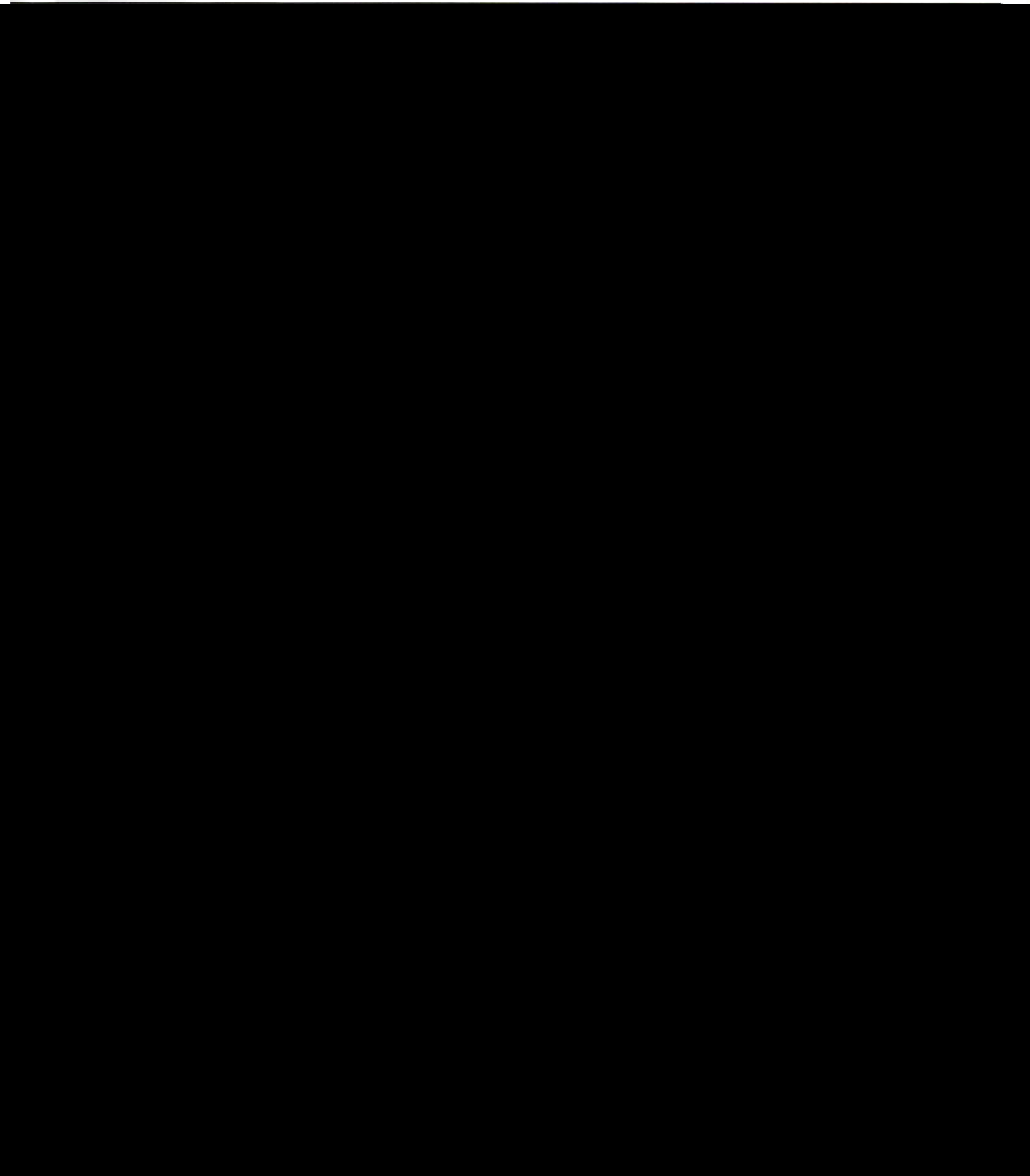

To Page No.

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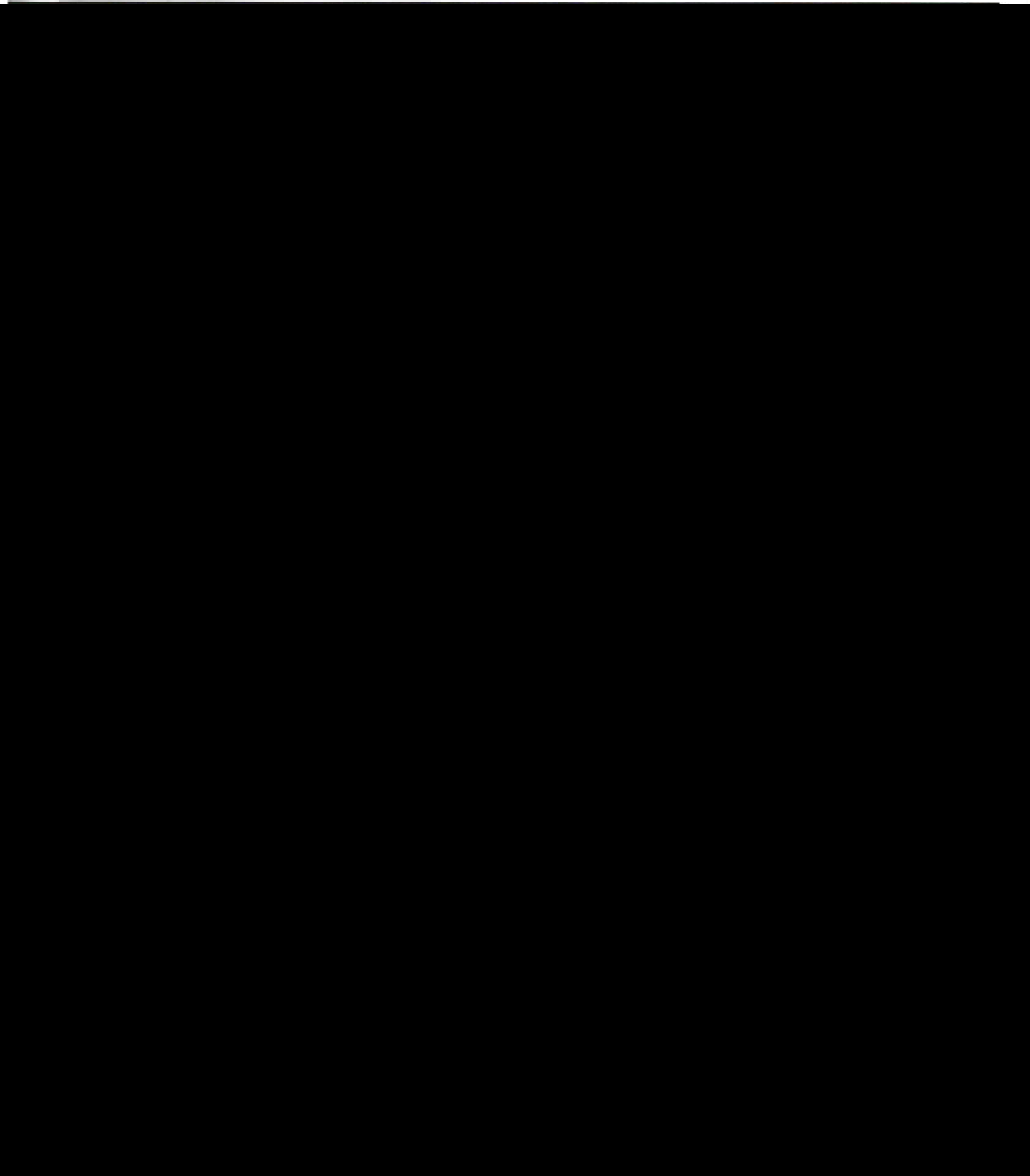

To Page No.

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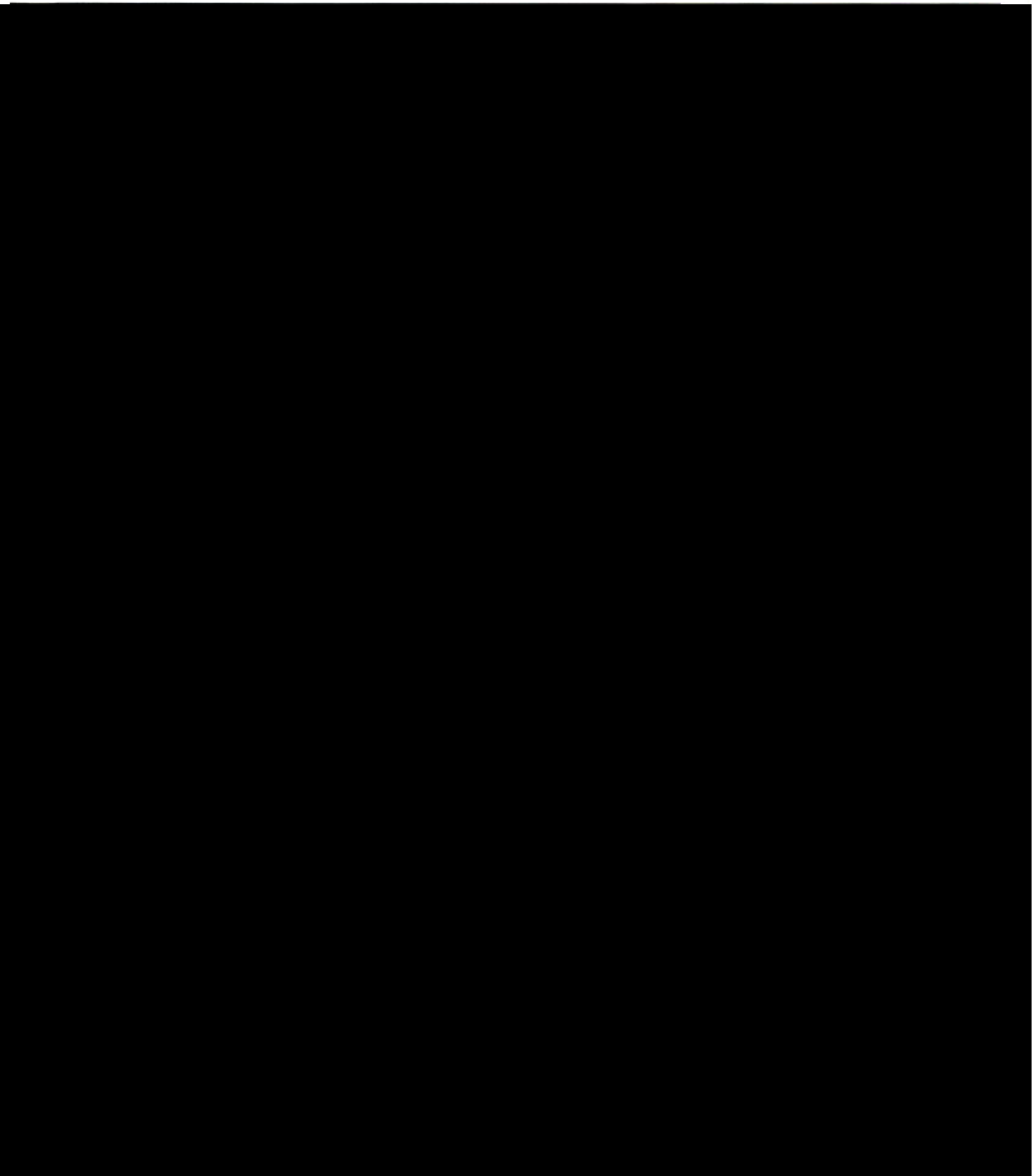

To Page No.

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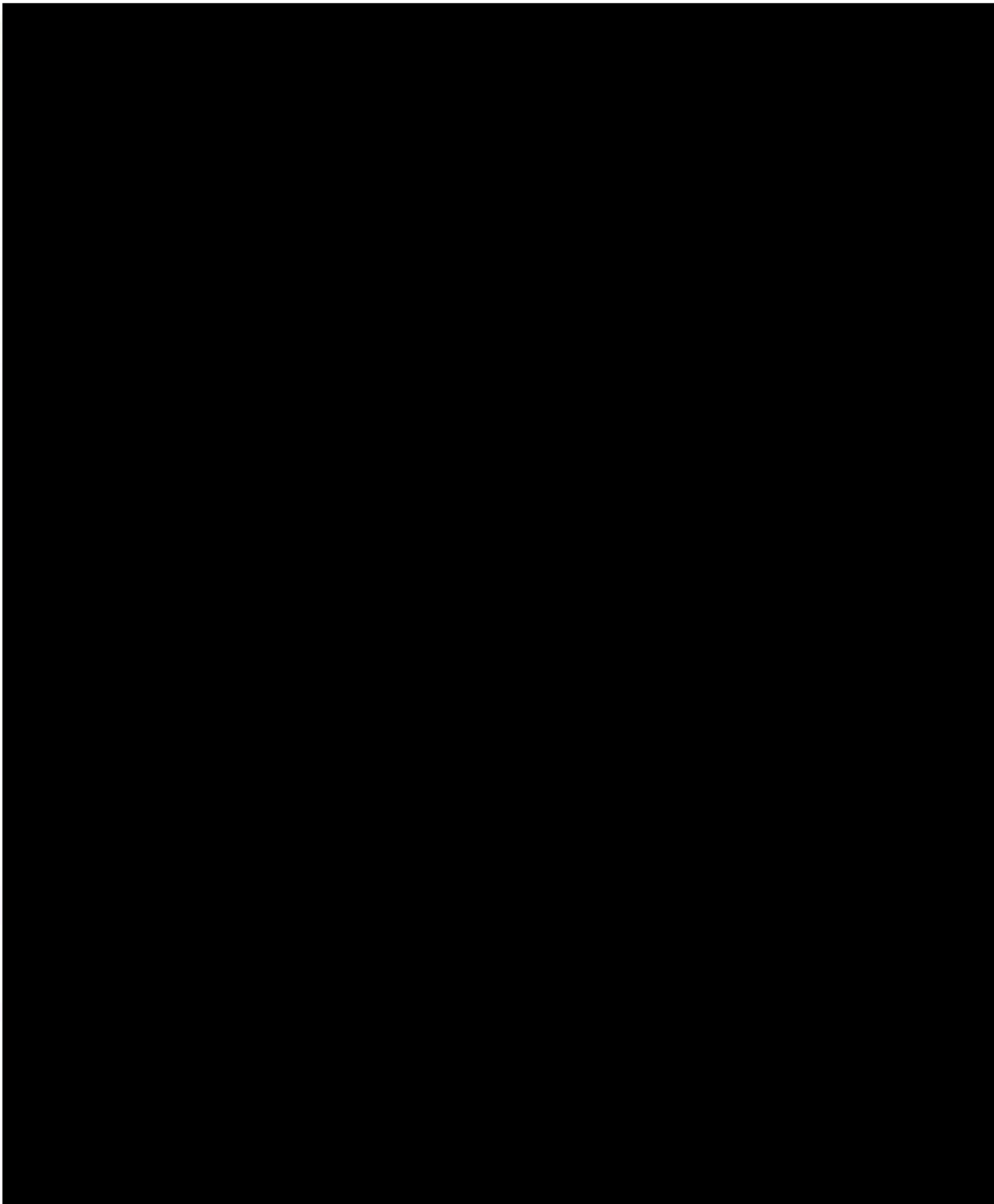

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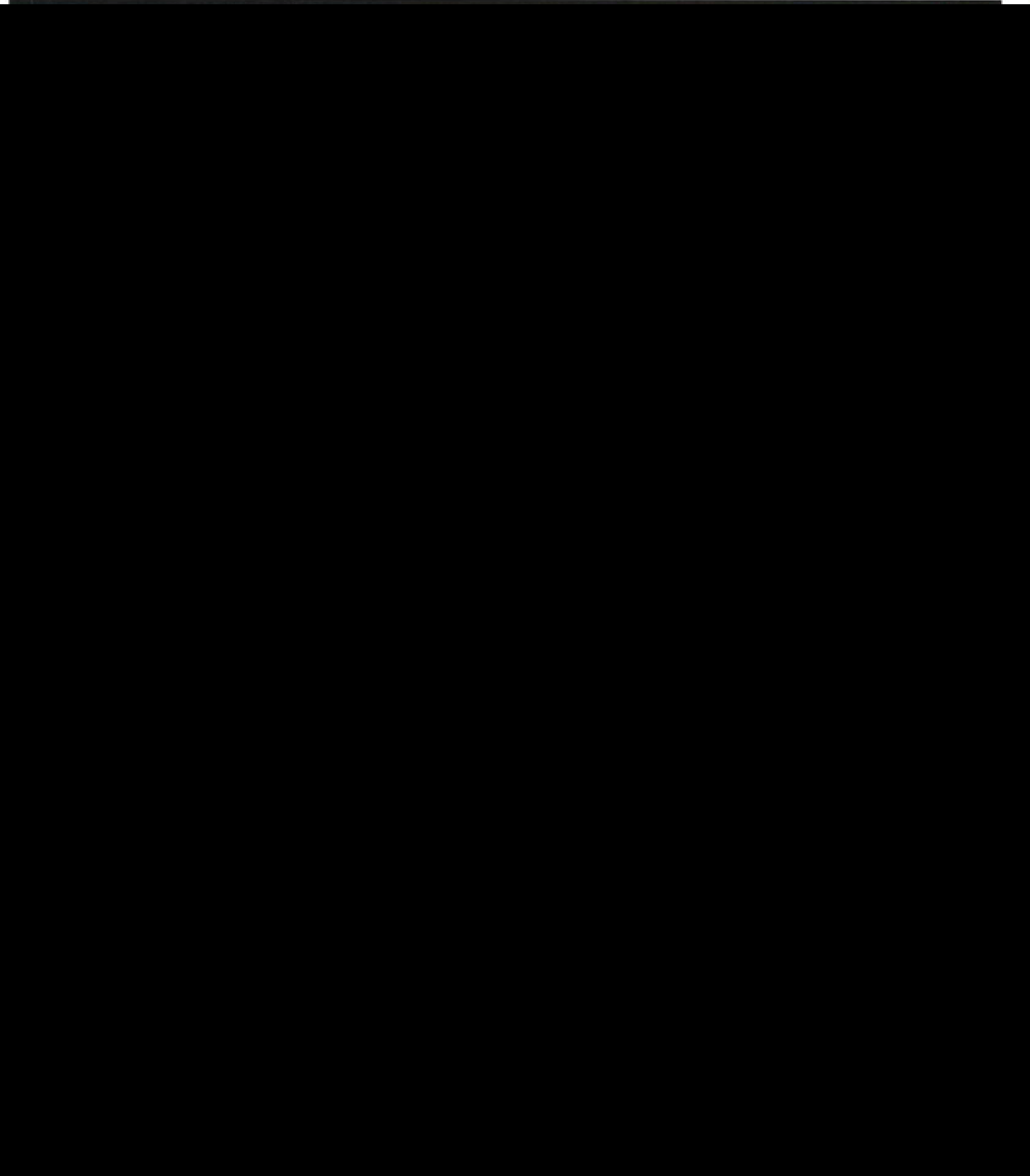

To Page No.____

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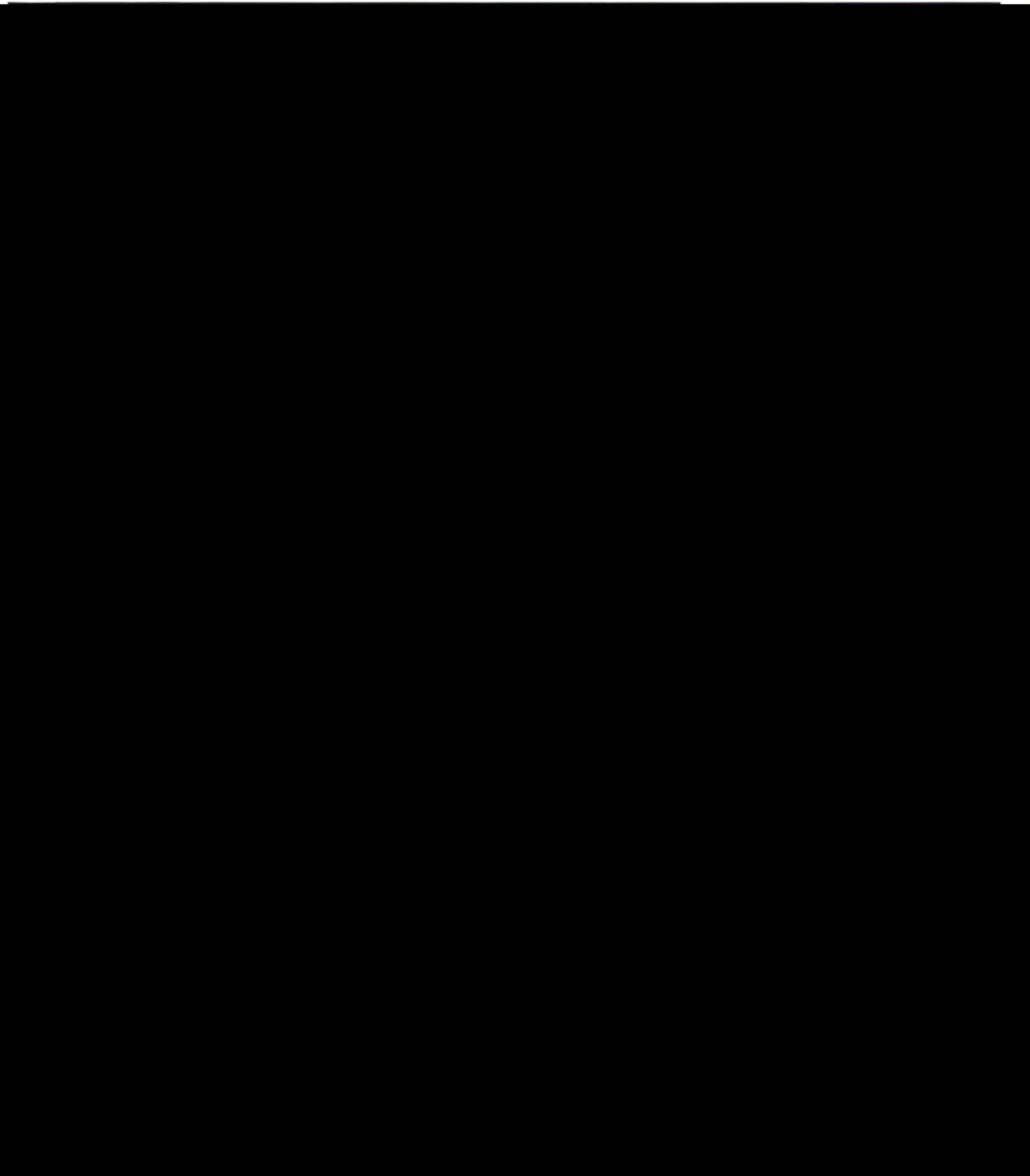

To Page No.

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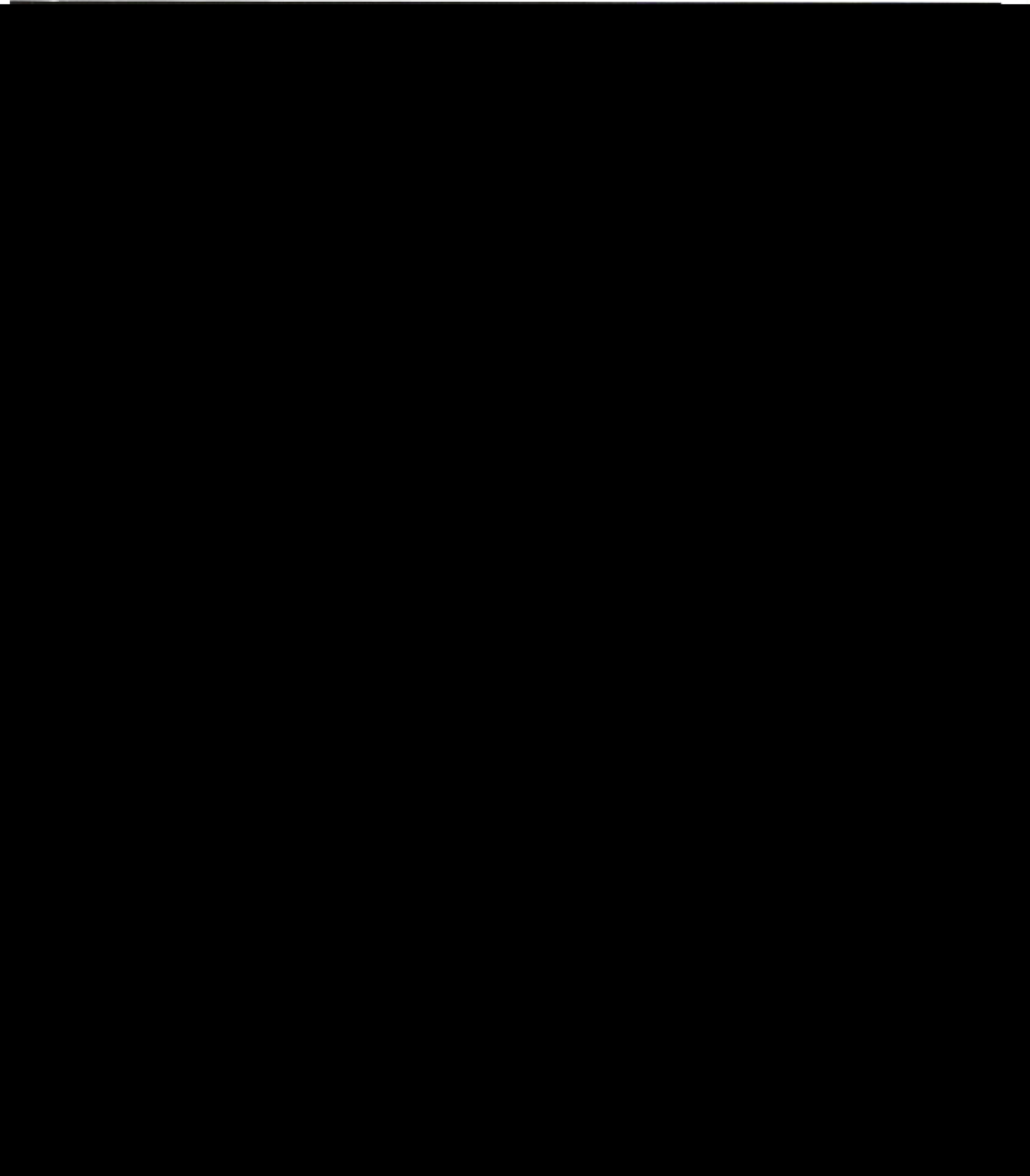


To Page No. ____

Witnessed & Understood by me,	Date 6/1/90	Invented by	Date 5/23/90
		Recorded by	

Humanized 4D5 (wild type) H+L

TLE 293 xfn transient Mutant Hu 4D5

Project No.

Book No.

From Page No.

5/9/90 1) pA5 Heavy IgG1 Hu4D5 : SS.pRK5.hu4d5hc
Clone 2/1 DNA prep 3.38 γ/λ

2) pA3 Light Hu4D5-K1 : SS.pRK5.hu4d5lc
clone 7 DNA prep 2.74 γ/λ

4 × 100 mm² dishes : 10 μg/dish × 4 = 40 μg DNA total in 4 ml

DNA ratio 4:4:2, pA5 : pA3 : AdVa 0.88 μg/μl

16 μg pA5 ÷ 3.38 = 4.7 μl

16 μg pA3 ÷ 2.74 = 5.8 μl

8 μg AdVa ÷ .88 = 9 μl

DNA	1/10 TE	CaCl2	2X Hepes
pA5 - 4.7 μl pA3 - 5.8 μl AdVa - 9 μl	1.8 ml	200 μl	2.0 mls

Fed cells 10AM
ppt on 1: pm
Shock 4: pm Refeed w/ 10% FBS media

5/10/90 10AM Change to Serum Free PSO4 + Ins + Glu.
Plate very nearly confluent. 1 plate split 1→4. Seeded w/ Reg 10% FBS media until cells settle. 4pm. Change 4 plate back to Serum Free, Ins + Glu
5/11/90 Fri Collected media Sup + re fed. all plates

To Page No. 39

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	6/1/90	[signature]	5/23/90
		Recorded by	

Project No.
Book No.
TITLE 4D5 2.6 in MTX

From Page No.

5/10/90 Count 56,860 × 40 = 2,274,400 /ml.

Seed at 5×10⁵ use 0.25 ml / plate

MTX at 2, 5, 10, 20 nM

Froze rest of cells 2 vials at 1×10⁷ cells each

labeled [4D5. 2.6 JR 5/29]

5/14 fed

To Page No.

Witnessed & Understood by me, [signature] Date 6/1/90

Invented by [signature] Date 5/23/90

Recorded by

chimeric 4D5

TITLE Thaw DP-12 4D5 (2.6) for MTX amp. Project No.____ Book No.____

2.16 MTX Amp.

37

From Page No.__

5/9/90 Only 1 colony was produced by 10nM MTX so try again w/ less.

Thaw 1 vial from 4/17/90, into 37T1500. Cells look great > 90% viability.

(Re-freeze 2 vials)

Wait for recovery. Seed at 5×10^5 cells/100² dish

Use 2, 5, 10, 20nM MTX + 0nMTX Control

5/10/90 Trypsinize 1 plate resuspend in 10 ml.

Count 0.5ml in 9.5ml PBS = 20× dil machine counts 0.5ml

12,674 × 20 (dil) × 2 = 506,960 cells/ml

Use 1ml/plate + 9ml 4% XDZ + Glu (no GHT)

Stock 1000nM MTX.

2nM : $\frac{2nM}{1000nM}$ × 10,000ul = 20ul of 1000nM MTX

5nM : 5/1000 × 10,000 : 50ul of 1000nM MTX

10nM : use 100ul 1000nM MTX
20nM : use 200ul 1000nM MTX
50nM : use 500ul 1000nM MTX

there are 2.16 cells not 2.6

5/14 Fed
5/26 Fed

To Page No.____

Witnessed & Understood by me. [signature] Date 6/1/90

Invented by [signature] Date 5/27/90

Recorded by

Project No.____
Book No.____ TITLE ^{35}S labeling of 293 for 4DS pA35

From Page No.__

5/10/90 Transfection p35.

Label 1 100mm dish, 1 293 CTR

2.5ml label x 2 = 5ml label

Use 100uCi/ml cys, met ^{35}S.

= 500uCi cys, met ^{35}S

Using Amersham label at conc of 1mCi/100ul.

500uCi/10uCi/ul = 50ul 10uCi/ul

Use 50ul each of cys, met ^{35}S in 5ml cys⁻, met⁻ medium

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

To Page No. 51

Witnessed & Understood by me, [signature] Date 6/1/90 Invented by [signature] Recorded by Date 5/23/90

TITLE Humanized 4D5 "wild type L+H" 293 trans. Project No. Book No. 39

From Page No 35

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

Transient Humanized:

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

	IgG (Human)	Her-2 Binding (ECD)	
Unsplit 5/11	5.3 ug/ml	7.45 ug/ml	30 hrs
Unsplit 5/14	7.1 - 7.7 ug	12.5 ug	65 hrs
1→4 split 5/11	1 ug	1.44 ug	24 hrs
1→4 split 5/14	1.35 ug	2.24 ug	65 hrs

Control: 2.16 CHIMERA (Mouse Variable + Human Constant)

1.11 ug   1.92 ug

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

Other: IgG					Her-2 Binding				
NO.	IDENTITY PRODUCT CODE/STEP NO.	DILUTION	ng/ml		NO.	IDENTITY PRODUCT CODE/STEP NO.	DILUTION	ng/ml	
1	1 unsplit 5/11/90	1/10	>10		21			>28	
2	"	1/100	50.9		22			>28	
3	"	1/1000	5.3		23			7.45	
4	2 unsplit 5/14/90	1/10	>10		24			>28	
5	"	1/100	71.1		25			>28	
6	"	1/1000	[illegible]		26			12.5	
7	3 1→4 5/11/90	1/10	112.6		27			>28	
8	"	1/100	11.5		28			[illegible]	>28
9	"	1/1000	<.5		29			1.44	
10	4 1→4 5/14/90	1/10	[illegible]	135.2	30			>28	
11	"	1/100	[illegible]	13.1	31			[illegible]	>28
12	"	1/1000	[illegible]	1.35	32			2.24	
13	5 CHIM 2.16	1/10	[illegible]	111.5	33			>28	
14	" 5/14/90	1/100		11.35	34	thank you		>28	
15	"	1/1000		[illegible]	35			1.92	
16					36				
17					37				
18					38				

ASSAY RESULTS ARE NOT CORRECTED FOR DILUTION

To Page No.

Witnessed & Understood by me, [signature] Date 6/1/90

Invented by [signature] Date 5/23/90

Recorded by

Project No.

Book No.

TITLE Froze 4D5.2.16 Cells

From Page No.

5/15/90 4D5 chimera 2.16 : Froze 3 vials from 2 100 dish
Labeled "4D5-2.16 chimera"

Results of 4D5-2.16 Elisa's
and 293 sup of 4D5 for Mike Shepard

Genentech, Inc.

ASSAY: Hu IgG Elisa

SAMPLES SUBMITTED BY	PROJECT NO.	PRODUCT
John Ridgway		
EXTENSION 1107	DATE SUBMITTED 5/10/90	DATE TO BE ASSAYED 5/10/90

Sample Matrix: Hu IgG

Anticoagulant (If used): Elisa

NOTES:

NO.	IDENTITY PRODUCT CODE/STEP NO.	DILUTION
1	4D5 2.16 5/4/90	1/10
2	"	1/50
3	"	1/100
4	"	1/1000
5	"	1/10,000
6	293 4D5 5/4/90	1/10
7	"	1/50
8	"	1/100
9	"	1/1000
10	"	1/10,000
11	293 4D5 5/7/90	1/10
12	"	1/50
13	"	1/100
14	"	1/1000
15	"	1/10,000
16		

Genentech, Inc.

ASSAY: Her-2 Binding

SAMPLES SUBMITTED BY	PROJECT NO.
John Ridgway	
EXTENSION 1107	DATE SUBMITTED 5/10/90

NO.	IDENTITY PRODUCT CODE/STEP NO.	DILUTION
1	4D5 2.16	1/10
2	"	1/50
3	"	1/100
4	"	1/1000
5	"	1/10,000
6	293 4D5 5/4/90	1/10
7	"	1/50
8	"	1/100
9	"	1/1000
10	"	1/10,000
11	293 4D5 5/7/90	1/10
12	"	1/50
13	"	1/100
14	"	1/1000
15	"	1/10,000
16		

Her-2 ECD binding.

To Page No.

Witnessed & Understood by me, [signature] Date 6/1/90

Invented by [signature] Recorded by Date 5/23/90

TITLE Bio Assay w/ SKBR3 Cells. Project No.____ Book No.____

From Page No.__

5/10/90 Submitted Sups ~~[illegible]~~ of 4D5 Humanized + Chimeric Ab to the bioassay.

Dilutions made to 0.32, 0.16, 0.08, 0.04 ug/ml of ECD binding Ab, based on Wai Lee's Her-2 binding ELISA

4 Samples submitted:

1) 4D5 chimera: Mouse Variable region w/ Hu Constant region. Clone 2.16. Starting Conc 4.24 ug/ml

First dilution to 0.32 ug/ml made as follows

4.24(x) = .32(5 ml) x = .377 ml

377 ml of 2.16 clone + 4623 ml diluent = 5 ml at 0.32 ug/ml.

2) 4D5 chimera from 293 transient expression at 2.5 ug/ml

2.5 X = .32(5) X = 0.640 ml QS → 5 ml

3) 4D5 humanized: Mouse CDR w/ Hu Variable region framework and Hu Constant regions

293 trans exp 5.3 ug/ml IgG, 7.45 ug/ml ECD binding.

7.45(x) = .32(10) X = .430 ml QS → 10 ml

4) 4D5 humanized: 293 transient expression. 2.24 ug/ml ECD
2.24(X) = .32(5) X = 0.714 ml QS → 5 ml diluent.

For all samples ~~#~~ 4, use 1:2 serial dilutions to 0.04 ug/ml.

results p42, 43

To Page No.____

Witnessed & Understood by me, [signature] 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. 4006-88	DATE
A.S. TECH. M.C	TEST PROCEDURE

ASSAY: Anti Her-2 Bioassay

SAMPLES SUBMITTED BY	PROJECT NO.	PRODUCT I.D.	COST CENTER NO.	TIME OF REPORT
John Ridgway			435	DATE
EXTENSION: X1107 / DATE SUBMITTED: 5/16/90	DATE TO BE ASSAYED: 5/17/90		NUMBER SUBMITTED	TIME / INIT.

CHECK ONE OF THE FOLLOWING:

- ☐ GMP ☐ BIOHAZARD
- ☐ GLP ☐ TOXIC HAZARD
- ☐ RADIO ACTIVE ☑ NONE OF THE ABOVE
- ☐ MAIL RESULTS* ☐ CALL WHEN READY

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

ASSAY STATUS AND SPECIFICATIONS

- ☐ Nonqualified — Control Value Expected A ____ B ____ C ____ D ____
- ☐ Qualified/Nonvalidated — Obtained A ____ B ____ C ____ D ____
- ☐ Validated

Acceptable Range

Sample Matrix:

Anticoagulant (If used):

Storage Conditions: ☐ Ambient ☐ 2°-8°c ☐ -10°c or Below

☐ Other:

NOTES: Dilutions in ug/ml of ECD binding activity, from Wai Lee's Her-2 binding elisa.

ASSAY RESULTS ARE NOT CORRECTED FOR DILUTION

NO.	IDENTITY PRODUCT CODE/STEP NO.	DILUTION	4D5 Conc (ug/ml)	% Inhib. CTR	NO.	IDENTITY PRODUCT CODE/STEP NO.	DILUTION		
1	4D5 Chimera	0.32	.30	53	21				
2	Clone 2.16	0.16	.16	74	22				
3		0.08	.08	88	23				
4	↓	0.04	<.025	--	24				
5	4D5 Chimera	0.32	.10	84	25				
6	transient 293	0.16	<.025	-	26				
7		0.08	<.025	-	27				
8	↓	0.04	<.025	-	28				
9	4D5 humanized	0.32	<.025	-	29				
10	transient 293 #1	0.16	<.025	-	30				
11		0.08	<.025	-	31				
12	↓	0.04	<.025	-	32				
13	4D5 humanized	0.32	<.025	-	33				
14	transient 293 #4	0.16	<.025	-	34				
15		0.08	<.025	-	35				
16	↓	0.04	<.025	-	36				
17	293 media CTR	1/5	<.025	-	37				
18	Dp.12 media CTR	1/5	<.025	-	38				
19					39	[signature] 5/23/90			
20					40				

TITLE 4D5 hybridoma standard for BioAssay

Project No.______
Book No.______

HHG

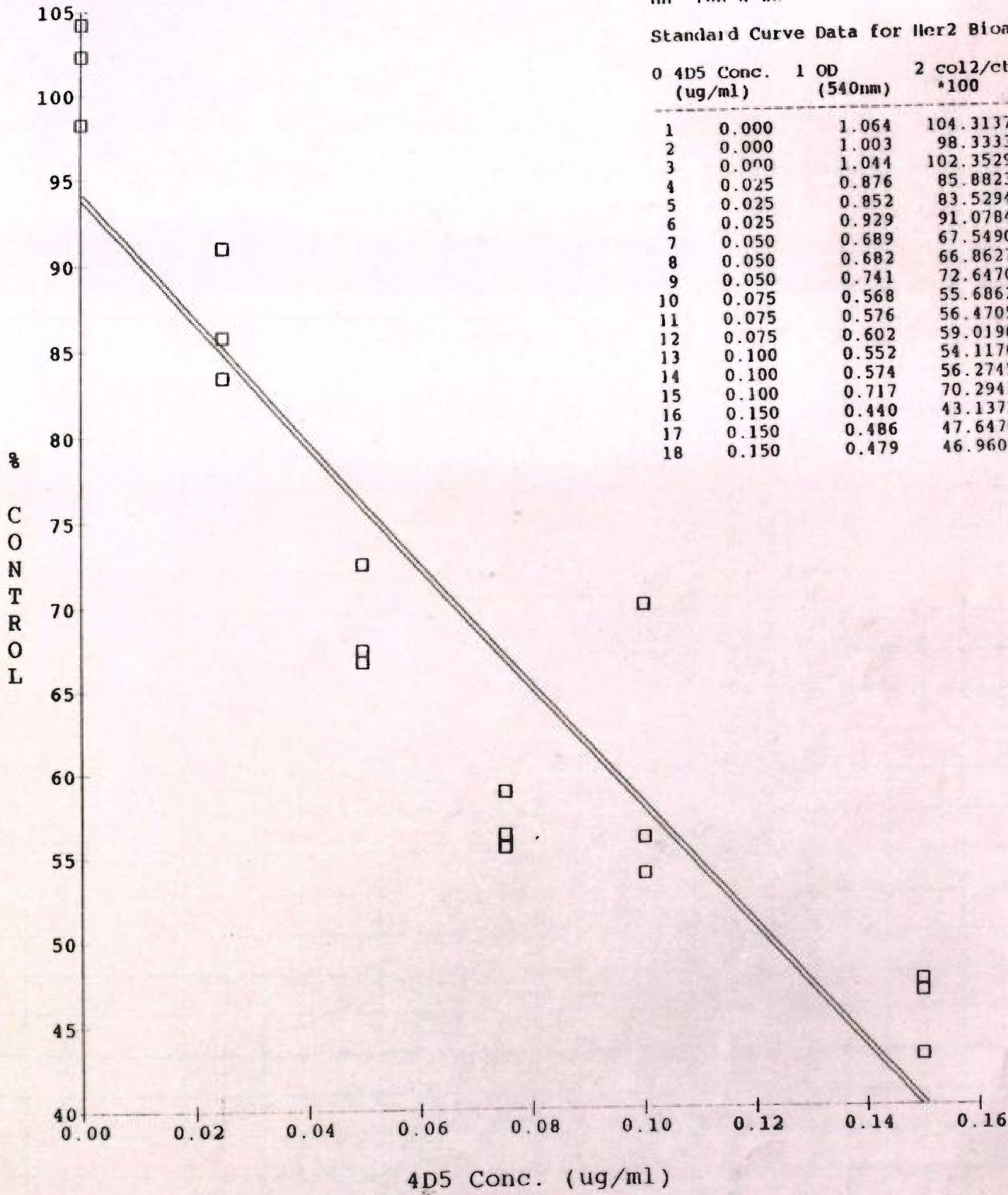
21-MAY-90 16:53 Page 1

4D5 Standard Curve

HH 18R 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: 6/1/90

Invented by / Recorded by: Date: 5/23/90

Project No.______
Book No.______ TITLE BioAssay Hu 4D5 VS Chimera Repeat.

From Page No.__

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

Sample 1: 4D5 chimera Stable line 2.16. Sup from 5/6/90

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

Dilute to 1.28ug/ml 4.5(x) = 1.28(5ml)
x = 1.42
1.42 ml Sup + 3.58 ml diluent
= 5mls at 1.28ug/ml

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

Sample 2: 4D5 chimera From 293 trans 3/9/90 2.5ug/ml IgG
make dilutions 1.25 → .04ug/ml

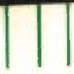
Sample 3: Humanized 4D5 - Version(a) From 293 trans 5/14/90 sup39.
By IgG Elisa : 7.1ug/ml

Dilute to 2.56ug/ml 7.1(x) = 2.56(5ml)
x = 1.8ml + 3.2 ml diluent
= 2.56ug/ml

make 2.56 → .04ug/ml dil.

Witnessed & Understood by me, [signature] Date 6/1/90 Invented by [signature] Recorded by Date 6/1/90

To Page No.__



Project No.______

TITLE______ Book No.______

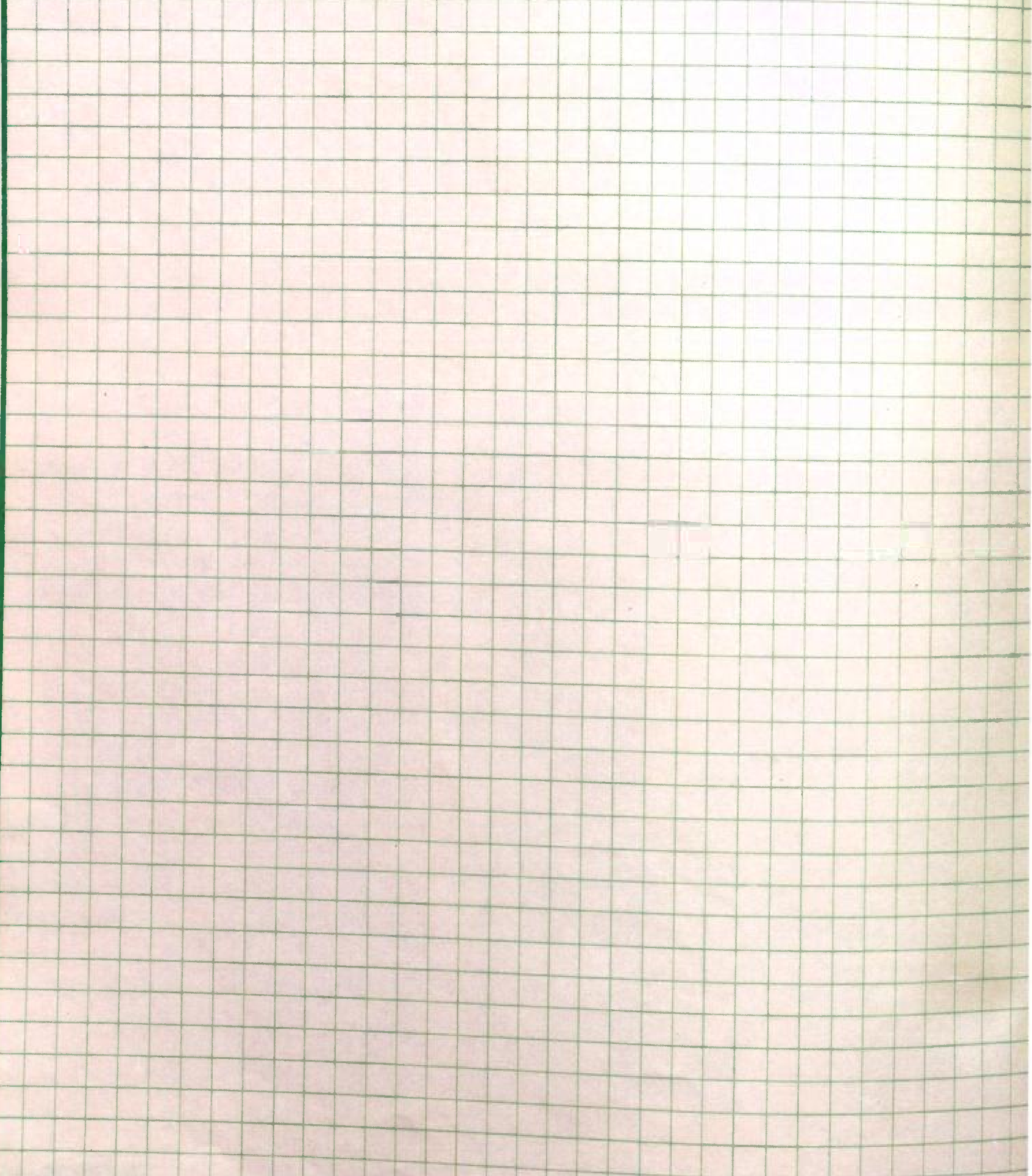
From Page No.__

To Page No.____

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by	

Project No._______
Book No._______ TITLE _______

From Page No.___



Witnessed & Understood by me,	Date	Invented by	Date	
		Recorded by		

To Page No.___

TITLE Fgb Elisa of MTX Amp + others Project No.____ Book No.____

From Page No.__

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

Samples: 100ng/ml 1) 4D5 2.6 10nM MTX - Started 5/10/90

2) 4D5 2.6 20nM MTX - "

3.3ug/ml 3) 4D5 2.6 10nM MTX clone selected from Amp started 4/18

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

1.9ug/ml 5) 4D5 2.16 5nM MTX - started 5/10/90.
- this culture looks partially selected - too many clones

600ng/ml 6) 4D5 2.16 10nM MTX - 5/10 - incomplete killing

200ng/ml 7) 4D5 2.16 20nM MTX - 5/10

4ug/ml 8) 4D5 2.16 No MTX >5day sups.

3.6ug/ml 9) 4D5 2.16 5/6/90 used in Bioassay

1.7ug/ml 10) ~~[illegible]~~ 4D5 293 trans 3/9/90 used in BioAssay

7.3ug/ml 11) Humanized 4D5 pA3, pA5 293 trans from 5/14 #2
used in BioAssay

290ng/ml 12) 4D5 2.16 O.N. on cells in 100mm dish
to test if cells are still producing Ab. (older cells)

10nM MTX Amp
570ng → 13) 4D5-2.6 - 10^5 start seed for Cell Assay 3days old culture.
1.3ug → 14) 4D5-2.6 - 10^6 " " "
2ug → 15) 4D5-2.16 - 10^5 " " "
4.5ug 16) 4D5-2.16 - 10^6 " " "

Results next page →

To Page No.____

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

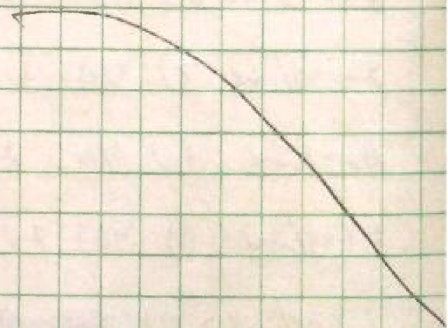
Project No.
Book No.
TITLE IgG Elisa Results from p47

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

4DS 2.6 10uM MTX Amp

TITLE Per Cell Assay 2.16 4DS non Amp.

Project No.

Book No.

From Page No.

5/26/90 ~Noon

Cell Counts: 1/200 dil × 0.5 ml count = 2951

2.16 → 2951 × 400 = 1.18×10^6 cells/ml

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

Set up 2 dishes 1) 5×10^5, 2) 1×10^6

2.6 (10 MTX): 1/200 dil, 1/2 ml count: 3037

3037 × 400 = 1.21×10^6 = 0.41 ml/dish

Set up 2 dishes 1) 5×10^5, 2) 1×10^6

5/29/90 Cell Counts:

a) 2.6 (10 MTX) - 5×10^5 seed - 1/100 dil = 1034 × 10 mls.
1034 × (2 × 100/2000 × 10 mls) = 2.068×10^6 cells

b) 2.6 (10 MTX) - 1×10^6 seed - 1/100 dil: 2500 × counts.
2500 × 2000 = 5×10^6 cells

c) 2.16 - 5×10^5 seed - 1/100 dil: 1288 × 10 mls.
1288 × 2000: 2.576×10^6 cells

d) 2.16 - 1×10^6 seed - 1/100 dil = 2575 × 10 mls.
2575 × 2000 = 5.15×10^6 cells.

Results

a) 2.6 (10 MTX): 0.89 Picogram. cell^{-1} day^{-1}
b) 2.6 (10 MTX): 0.88 " "
c) 2.16 : 0.26 " "
d) 2.16 = 0.29 " "

Forgot 10× factor (10 ml of Sup)

To Page No.

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

Invented by [signature] Date 6/15/90

Recorded by

Project No.
Book No.
TITLE Per Cell Assay Results

From Page No.

2.6 10nM MTX
2.16 No MTX.

a) 2.6 10nM MTX 5x10^5 Seed : 570ng/ml x 10ml = 5.7ug IgG

5.7ug = 5,700,000pg / 2.068x10^6 cells · 3days = 0.9pg/cell·day

b) 2.6 10nM MTX 1x10^6 Seed: = 0.8pg/cell.day.

c) 2.16 5x10^5 Seed = 2.6pg/cell.day

d) 2.16 1x10^6 Seed = 2.9pg/cell.day

To Page No.

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

TITLE I.P of ^{35}S 293 4DS pA3,5 Humanized

Project No.______
Book No.______

From Page No. 38

5/30/90 500 ul ea of Cell + Sups

a) 293 CTR cells
b) " " Sups
c) 293 4DS pA3,5 cells
d) " " Sups

Incubated w/ 3 ul of Rab Human H+L IgG 30 min → 1 hr
Staph A cells - 50 ul 30 min
Washed as per I.P. protocol

Resuspended in 80 ul Sample buffer

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

To Page No.______

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

Invented by [signature] Date 6/15/90

Recorded by

Project No.

Book No.

TITLE Bio Assay Spike Exp w/ 293 Sups

From Page No.

6/1/90 Concern that older Sups from 293 transfections of Chimeric 4DS4+L are degrading the Ab, resulted in this exp

ug/ml

ASSAY RESULTS ARE NOT CORRECTED FOR DILUTION

NO.	IDENTITY PRODUCT CODE/STEP NO.	DILUTION	4DS CONC (ug/ml)	% CTRL
1	4DS 2.16 stable	1.28	>.2(1.4)	44.3
2		0.64	.379	51.2
3		0.32	.267	65.4
4		0.16	.112	84.9
5	293 chimeric 1:1	—	<.025	94.9
6	#5 w/0.64 of 2.16		.198	74.0
7	Hu #1 (293)	1:2	<.025	109
8	" #2	1:2	<.025	162
9	" #3	1:2	<.025	95
10	#4	1:2	<.025	154
11	293 chimeric (purif)	1/10	.347	55
12	↓	1/20	.345	56
13		1/40	.356	54
14		1/80	.345	56
15		1/160	.357	67
16	#11 in #5 buffer	1/10	.071	90
17	#12 "	1/20	<.025	98
18	#13 "	1/40	<.025	99
19	#14 "	1/80	<.025	[illegible]
20	#15 "	1/160	<.025	[illegible]

ug/ml

NO.	IDENTITY PRODUCT CODE/STEP NO.	DILUTION	ug/ml	% CTRL
21	Mouse 4DS	5.5	>.20(1.4)	41
22	"	0.55	.329	57
23	"	0.25	.266	66
24	#22 in #5 buffer	0.55	.199	77
25	#23 "	0.25	.146	81
26				
27				
28				
29				
30				
31				
32				
33				
34				
35				
36				
37				
38				
39				
40				

73 (12/86)

RETURN TO REQUESTOR

Samples 1-4 Good CHO 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 / Recorded by	Date
		[signature]	8/10/90

TITLE 4D5-2.16 chimera Roller Bottle

Project No.
Book No.

From Page No.

6/1/90 1 Confluent [illegible] 175 cm² into 2 Roller bottles w/ 200ml media ~1:10 split + 10mM Hepes

6/4/90 Cells good - ~70-80% Confluent.
Change to Serum Free PSO4 + Insulin + Glu
250 ml / bottle w/ 10mM Hepes

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

Results IgG - 1mg/l - or 1ug/ml

6/13/90 Many more cells in Sup.
Removed the media - Spun down cells (big pellet)
Removed media to clean container

Submitted both 6/7 and 6/13 harvest to Greg Blank for purification on Staph A Column.
6/7 Purified : 113ug/ml

NO.	IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS	DILUTION	4D5 Conc ug/ml
1	2.16 [illegible] 6/7/90	1/5	.215
2	~~Transient 6/8/90~~	~~1/5~~	~~<.025~~
3	~~Purified 6/7/90~~	~~1/5~~	~~<.025~~
4			
5			

ASSAY RESULTS ARE NOT

Bio Assay results of 2.16 roller bottle material. 1/5 dil is ~0.2ug/ml ∴ it works as well as 4D5 at this low concentration.

IgG Elisa results for R.B 6/13/90 harvest - 240ng/ml

To Page No.

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

Invented by [signature] Date 8/1/90

Recorded by

54 Project No. Book No. TITLE 293 transient Chimeric 4D5 H+L

From Page No.

Fed Cells 9:15 ~70% Confluent.

6/5/90 Make more transient chimera and purify on Prot A right after harvest.

4 100 dishes using 10 ug/dish in 1 ml.

40ug DNA in 4 mls.

Lchain pdH158 = 0.4ug/ul (4.5)(4) ÷ 0.4 = 45ul
Hchain pdH160 : 1.3ug/ul (4.5)(4) ÷ 1.3 = 13.8ul
helper AdVA = 0.25ug/ul 1 (4) ÷ 0.25 = 16ul
75ul DNA

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

DNA	1/10TE	CaCl2	2x Hepes
75ul total.	1.725 mls	200ul	2.0 mls

ppt 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: Chimeric 293 24hrs.

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

Slisa Assayed for IgG - Result : Zero

Stained plate w/ RaH IgG H+L. ?
Result: +
Ctr 293 - —

To Page No.

Witnessed & Understood by me, [signature] Date 6/15/90 Invented by [signature] Recorded by Date 8/10/90

293 Transient 4D5 transfections

TITLE ~~Chimeric~~ + Humanized

Project No.______
Book No.______

From Page No.__

6/14/90

plasmids:
1) pA3 - Hu 4D5-K1 (Light) - La 2.74 ug/λ
2) pA5 - Hu 4D5-H (Heavy) - Ha 3.38 ug/λ

3) pA3 - Hu4D5K1 - Light - Lb - 1.3 ug/λ (3-b-14)
4) pA3 - Hu 4D5K1 - Light - Lc - 4.7 ug/λ (3-c-[illegible])

5) pA5 - Hu 4D5-H - Heavy - Hb - 2.0 ug/λ (5-b-4)
6) pA5 - Hu 4D5-H - Heavy - Hc - 3 ug/λ (5-c-4)

7) pdH158 - L chain chimera - 0.4 ug/λ
8) pdH160 - H chain chimera - 1.3 ug/λ
9) AdVa 0.25 ug/ul

DNA	1/10 TE	CaCl2	2x Hepes
Xfn 1. Humanized La + Ha 1 ul - La 1 ul - Ha 4 ul AdVa	225 ul	25 ul	250 ul
Xfn 2: La + Hb 1 ul - La 1 ul - Hb 4 ul AdVa			
Xfn 3: La - 1 ul Hc - 1 ul AdVa - 4 ul			
Xfn 4 Lb - 3 ul Ha - 1 ul AdVa - 4 ul			

5 ug DNA total in 0.5 ml total

Put on 12:45
Shock at 4:30

Re feed 10% FBS w/ media

Xfn 5 Lc - 1 ul
Ha - 1 ul
AdVa - 4

Xfn 6 Lc - 1 ul
Hc - 1 ul
AdVa - 4

Xfn 7 Lb - 3 ul
Xfn 8 Lc - 1 ul

To Page No. 56

Witnessed & Understood by me. [signature] Date 6/15/90

Invented by / Recorded by [signature] Date 8/10/9[illegible]

Project No.______
Book No.______ TITLE 293 Trans Xfn / Humanized

From Page No.__

6/15 Am. Many cells floating in most dishes
- take Samples of Sups from 1-6 and give to IgG Elisa and BioAssay
- Stain #7, 8 for L chain

Refed w/ S.F. media + Insulin

Staining Results #7 Xfn: L chain Version b - prep 3-b-14
Staining was extremely low - hard to notling

#8 L chain Version c - prep 3-c-7
Stained Very Well.

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

See DNA Gel of these and others run uncut NB 10840 p77

6/17/90 Removed S.F. media (Keep) Spin out cells.
Submit to IgG Elisa on Monday (6/18)

6/18/90
Results: # by Xfn Sups.

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			

RETUR

48hr Sups

To Page No.____

Witnessed & Understood by me,	Date	Invented by / Recorded by	Date
[signature]	6/15/90	[signature]	8/1/90

TITLE 293 Hu 4A5 Variant Xfns — Project No. — Book No. —

From Page No.

6/19/90 Fed Cells 10AM — Repeat from p 55

2 100 dishes each = 2ml ppt w/ 20ug DNA

1) Repeat Xfn #1

#1 DNA	1/10 TE	CaCl2	2X Hepes
pA3-La - 4ul pA5-Ha - 3ul AdVA - 4ul	0.9 ml	100ul	1ml.
2) Repeat Xfn #2 pA3-La - 4ul Hb - 5 ul AdVA - 4ul	0.9	100ul	1ml
3) Repeat Xfn #3 pA3-La - 4ul Hc - 4ul AdVa - 2ml	0.9 ml	100ml	1ml

Put on 2:15
Shocked at 6PM (ppt look O.K. light but Fine)

6/20/90 Changed to S.F. media w/ Insulin. AM
6/21/90 AM Harvested Sup from plates - Refed w/ SF. 1 plate, stained other.
See prot A Purification

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

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

To Page No.

Witnessed & Understood by me, [signature] Date 9/7/90 Invented by [signature] Recorded by Date 9/10/90

Project No.______
Book No.______
TITLE Myco plasma test. Results

From Page No.____

6/20/90 Submitted 4D5 chimera 2.6, 2.16 to mycoplasma test on 6/4/90

ASSAY REQUEST/REPORT FORM

Genentech, Inc.

ASSAY: Mycoplasma

SAMPLES SUBMITTED BY	EXTENSION	MAILBIN #	COST CENTER
John Ridgway	1107	40	435

DATE SUBMITTED: 6/4/90
DATE TO BE ASSAYED: 6/5/90
☑ MAIL RESULTS* ☐ CALL WHEN READY ☑ ELEC. MAIL (Log in: JBBR)
*Results not picked up by end of day will be mailed

SAMPLE MATRIX: CHO cell sup w/ 1% FBS
ANTICOAGULANT (IF USED):
☐ GMP ☐ GLP ☐ RADIO
*Specify type and amount: ___

SAMPLES OF HUMAN ORIGIN? ☑ NO ☐ YES IF YES, IDENTIFY SOURCE: ______ (SPECIFY TISSUE

NOTES:

ASSAY STATUS AND SPECIFICATIONS

☐ NON-QUALIFIED — CONTROL VALUE EXPECTED A ______ B ______ C ______
☐ QUALIFIED/NON-QUALIFIED — CONTROL VALUE OBTAINED A ______ B ______ C ______
☐ VALIDATED — ACCEPTABLE RANGE: ______

NOTES:

ASSAY RESULTS ARE NOT CORRECTED FOR

NO.	IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS	DILUTION	RESULT		NO.	IDENT ORIGIN AND
1	4D5 2.6	—	NEG		21	
2	4D5 2.16	—	NEG		22	
3					23	
4					24	
5					25	
6					26	
7					27	
8					28	

To Page No.____

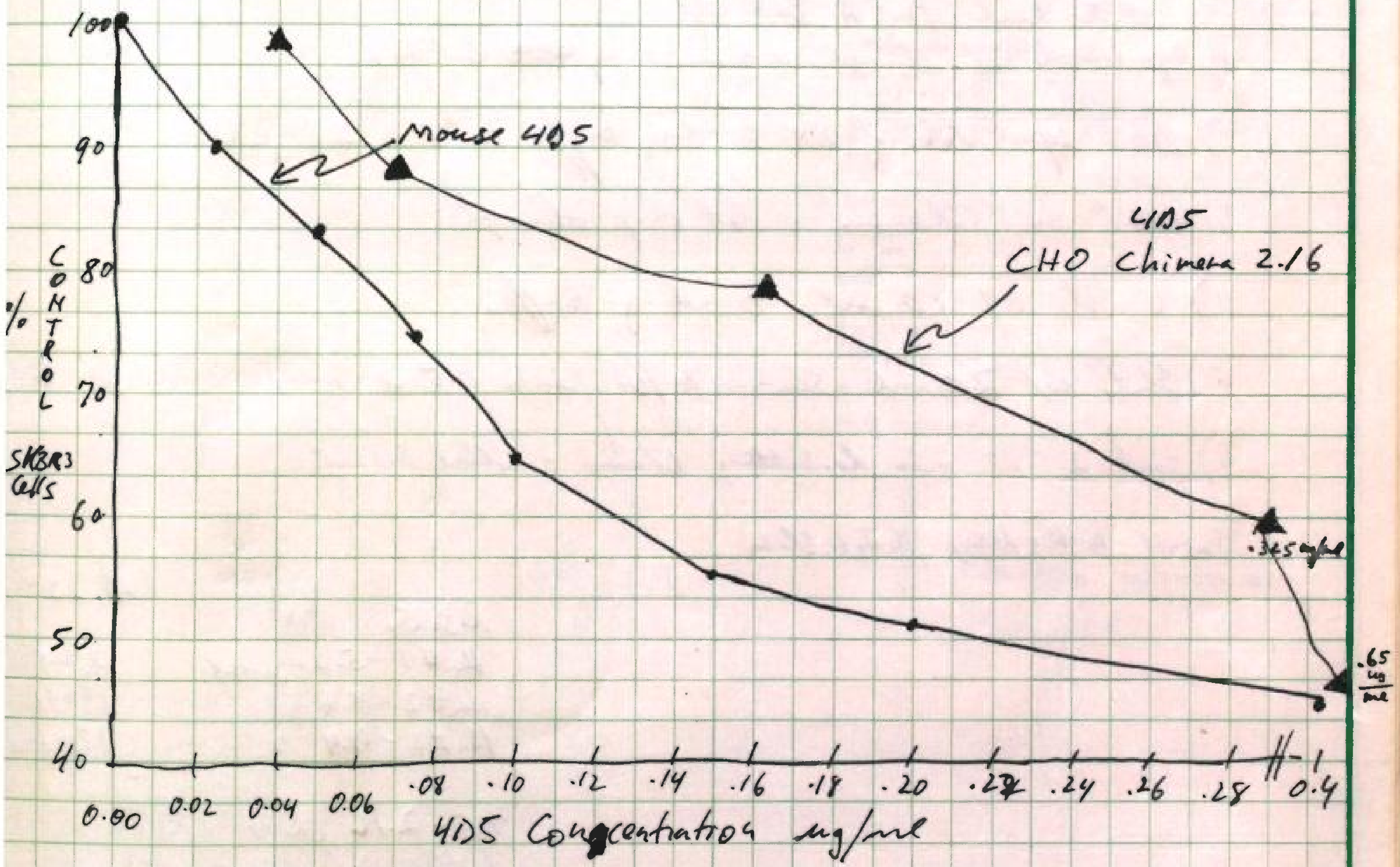
Witnessed & Understood by me,	Date	Invented by / Recorded by	Date
[signature]	9/7/90	[signature]	8/6/90

TITLE Her-2 Bio Assay SKBR3 Cells

Project No.______
Book No.______

From Page No.__

6/20/90 4D5 CHO 2.16 Stable
Purified from Roller Bottle.



To Page No.____

Witnessed & Understood by me, [signature] | Date 9/7/90 | Invented by [signature] | Recorded by | Date 8/10/90

Project No.
Book No.
TITLE Prot A Purification of Hu 4D5 Ab's

From Page No.

6/21/90 Harvested Sups from 293 xfn 6/11 p57
3 xfn w/ 20 ml each in S.F. media

0.5-1ml Prot A bed Vol
1) Equilibrate w/ 10 ml 1X binding ~~buf~~ buffer.
2) Add equal Vol of 2X binding buffer to ea. Sample.
3) Load on Column - let drip through.
4) Wash w/ 10 ml binding buffer.
5) Elute w/ 3.0 ml Elution buffer into 0.5 ml 1M Tris-HCl pH 8.0
6) Load 2.5 ml onto de-Salting Column - collect 3.5 ml.

Submit to Bio Assay, Hu IgG Elisa

NOTES: Hu #1, 2, 3 see NB "Tissue Culture" 6/19/90 Transfect

6/24/90 Hu IgG Elisa

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	DILUTION	ng/ml
1	Hu #1 Purified	1/10	>180		21	↓	1/100	20.6
2	293 6/21/90	1/100	49.5		22	↓	1/1000	20
3		1/1000	5.2		23	4D5 2.6-C	1/10	34.0
4	↓	1/10000	<.5		24	10 MTX	1/100	3.6
5	Hu #2 Purified	1/10	>180		25		1/1000	<.5
6	293 6/21/90	1/100	85.2		26			
7		1/1000	8.6		27			
8	↓	1/10^4	<.5		28			
9	Hu #3 Purified	1/10	>180		29			
10	293 6/21/90	1/10^2	[illegible]	43.2	30			
11		1/10^3	[illegible]	4.4	31			
12	↓	1/10^4	[illegible]	<.5	32			
13	4D5 2/6	1/10	>180		33			
14	Roller Bottle	1/10^2	>180		34			
15	Purified	1/10^3	113.2		35			
16	↓	1/10^4	11.9		36			
17	4D5 2.6-a	1/10	5.4		37			
18	10 MTX	1/100	.55		38			
19	↓	1/1000	<.5		39			
20	4D5 2.6-b 10 MTX	1/10	>180		40			

SERVICES

results: IgG Elisa

		Ab 280
Hu #1	5.2 ug/ml	23 ug/ml
Hu #2	8.6 "	18 ug/ml
Hu #3	4.4 "	18 ug/ml

4D5-2.16 Clone Roller Bottle 3 day growth 113 ug/ml (Purified on Prot A Column) yield ~ 1mg/liter.

To Page No.

Witnessed & Understood by me, [signature] Date 9/7/90

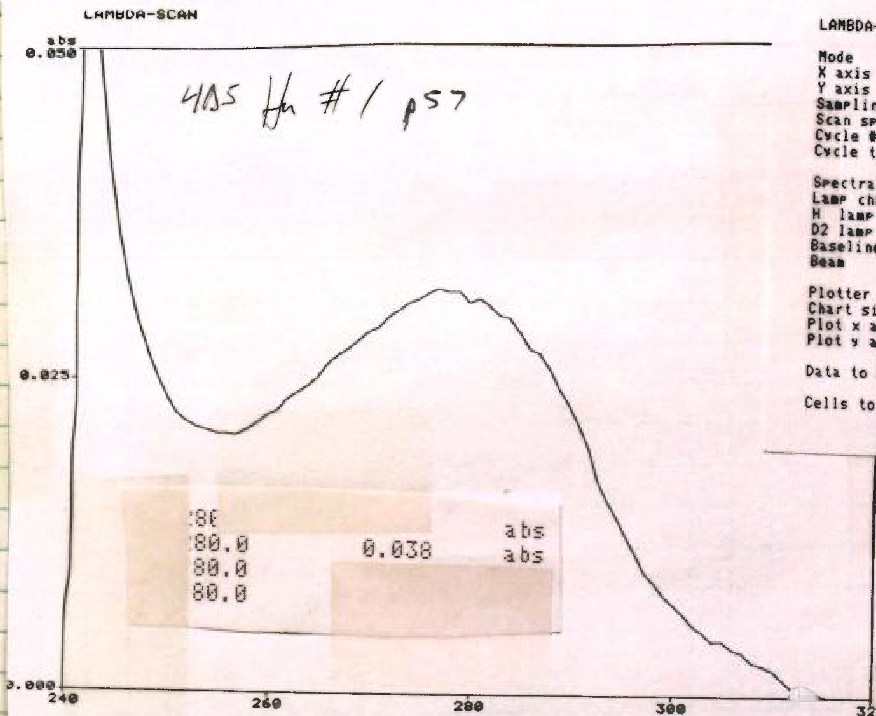
Invented by [signature] Recorded by Date 8/10/90

TITLE [Protein IgG] determination w/ Spectrophotometer Project No.______ Book No.______

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
Sampling 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. 4D5 hybridoma Ab.

$1.65\ OD_{280} = 1$ mg/ml protein

Hu Van #1

.038 Abs / 1.65 = .023 mg/ml

or

23 µg/ml

By IgG Elisa : 5.2 µg/ml

To Page No.____

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	9/7/90	[signature]	8/10/90
		Recorded by	

Project No.
Book No.
TITLE MTX Amplification of 2.6 4DS

From Page No.

6/22/90 Repeat of Amp by MTX at
10ng/ml MTX : yield 3 colonies

IgG Elisa a) 50ng/ml
b) 2ug/ml
c) 360ng/ml

See copy of Assay p 60.

Results: No amplification.

To Page No.

Witnessed & Understood by me,	Date	Invented by / Recorded by	Date
[signature]	9/7/90	[signature]	8/6/90

TITLE Protein gel of Hu Var 1, 2, 3 4D5

Project No.

Book No.

From Page No.

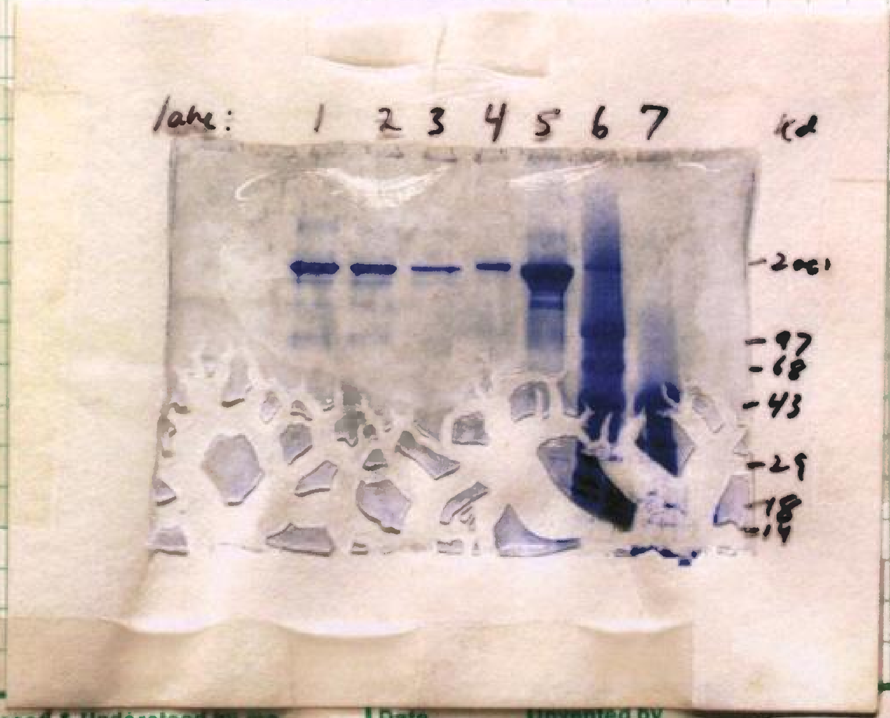
6/25/90 Precipitated 1ml of Ab from p60.

These were purified on ProtA + Conc. to IgG levels
accord. Elisa IgG!

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

run on 4-20% Grad gel precast
(Suspended in ~~[illegible]~~ fin 10ml SDS buffer + load
2x gel buffer

Lanes) 1) Var 1 ≃ 5µg (Run Non Reduced)
2) Var 2 ≃ 8.6µg
3) Var 3 ≃ 4.4µg
4) 4D5 Chimera - 10ml of .113mg/ml ≃ 1µg
5) Mouse 4D5 - ≃ 10µg
6) High MWM 7ml
7) Low MWM 7ml



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

To Page No.

Witnessed & Understood by me, [signature] Date 9/7/90

Invented by

Recorded by [signature] Date 9/10/90

Project No.
Book No.
TITLE Kinetics of 4D5 secretion exp

From Page No.

6/26/90 Transfect 8 T-60i w/ Hu V?? #1 (pA3, pA5)

0.5 ml ppt / 60 plate × 8 = 4 ml ppt

10 ug DNA/ml × 4 ml = 40 ug DNA total + AdVA

20 ug pA3 2.74 ug/ul = 7.3 ul
20 ug pA5 3.38 ug/ul = 6 ul
4 ug AdVA 0.88 ug/ul = 5 ul

DNA	1/10 TE	CaCl2	2X Hepes
	1.8 mls	0.200 ml	2 mls
7.3 ul pA3 6 ul pA5 5 ul AdVA.			

ppt on 12:35
Shocked 4:30

To Page No.

Witnessed & Understood by me, [signature] Date 9/7/?? Invented by [signature] Recorded by Date 8/10/90

TITLE Pulse Chase for Secretion Kinetics
Project No.
Book No.

From Page No. 64

6/27/90 To transfected 293 cells. Change media to S.F. DMEM + F12 − cys, − met media for 30 min.

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

Cells don't look great but continue anyway.

8 dishes x 2 ml each = 16 ml

Use 150 ul each cys + met ^{35}S. 2 ml / dish

2 hr Pulse Label on 1 PM
off 3 pm − wash 2x refeed 2 ml PSO4 + tms

Samples Chase
Time 0 hr (3PM) Sup 2 ml (Label original)
Cells 1 ml 1% Triton + 5 mM Hepes

1 hr (4pm) Sup 2 ml (PSO4)
Cells 1 ml.

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

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

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

O.N. chase Sup 2 ml
Cell 1 ml

4 hr pulse − O.N. Chase

Remove sups. Wash 2x PBS then lyse w/ Trix + Hepes

To Page No. 66

Witnessed & Understood by me, [signature] Date 9/7/90
Invented by [signature]
Recorded by
Date 8/6/90

Project No.
Book No.
TITLE Pulse Chase of 4DS Kinetics cont.

From Page No. 65

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

600ul of Sups } 3ul of R&H IgG 20'
300ul of Cells }

↓

50ul of Staph A cells (washed)

↓ washes

Resuspended in 40ul SDS sample buffer

Run on 4-20% Gradient gels

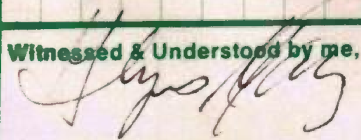
Gel 1 Lanes)
1) 2hr Pulse Hr-0 chase Sups 30ul
2) " Hr-0 Cells
3) " Hr-1 Sups
4) " Hr-1 Cells
5) " Hr-2 Sups
6) " Hr-2 Cells
7) " Hr-3 Sups
8) Hr-3 Cells
9) ^{14}C MW Highs - 15ul.

Gel 2 Lanes)
1) 2hr Pulse chase 5hr - Sups 30ul
2) " 5hr Cells
3) " overnight chase - Sups
4) " " Cells
5) 4hr Pulse O.N chase Sups
6) " " Cells
7) ^{14}C Markers

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

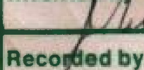
To Page No.

Witnessed & Understood by me,



Date 9/7/90

Invented by



Recorded by

Date 8/6/90

TITLE 293 Hu Var xfn #4, 5, 6

Project No.
Book No.

From Page No.

See p55 for xfn Key

6/27/90 xfn #4 Lb + Ha
xfn #5 Lc + Ha
xfn #6 Lc + Hc

Ha = 3.38 ug/λ
Hc = (5-c-4) 3ug/λ
Lb = (3-b-8) 2ug/λ
Lc = (3-c-7) 4.7ug/λ
AdVA = 2.2 ug/λ

3 100mm dishes / xfn = 30ug DNA in 3 ml

xfn #	DNA	1/10 TE	CaCl2	2x Hepes
#4	Ha - 4.5 ml Lb - 7.5 ul AdVA - 2 ml	1.350 ml	150 ul	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 AdVA - 2 ml	1.35 ml	150 ml	1.5 ml.

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

6/28/90 Change media to S.F. PSO4 + Insulin
6/29/90 AM Collected 30 ml of each xfn and purified by ProtA
Desalted into 3.5 ml PBS
Submitted to Bio Assay and Hu IgG Elisa.

To Page No.

Witnessed & Understood by me, [signature] Date 9/?/90 Invented by [signature] Recorded by Date 8/6/90

Hu IgG Results

Project No.______
Book No.______ TITLE 405 Humanized Var #4, 5, 6

From Page No.__

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

Hu Var #4, 5, 6 Prot A Purified 3 and Seq → 3.5
1 - PBS

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	DILUTION
1	#4	1/10	>180	21		
2	↓	1/100	155.3	22		
3		1/1000	16.6	23		
4	#5	1/10	>180	24		
5	↓	1/100	81.6	25		
6		1/1000	8.5	26		
7	#6	1/10	>180	27		
8	↓	1/100	60.7	28		
9		1/1000	6.0	29		
10				30		
11				31		

Results:

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

7/5/90 Gel of Above Protein.
Precipitated 1 ml of each above w/ 4 ml of Acetone.
Resuspended in ~25 ul SDS Gel buffer
Run on 4-20% Gradient Gel

(Lanes) 1) #4
2) #5
3) #6
4) Prestained BRL markers High (2ul diluted to 10 in buffer)
5) " Lows "

Results See Gel 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: [signature] Date 9/7/90
Invented by [signature] Date 8/20/90
Recorded by

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

Project No.

Book No.

From Page No.

6/28/90

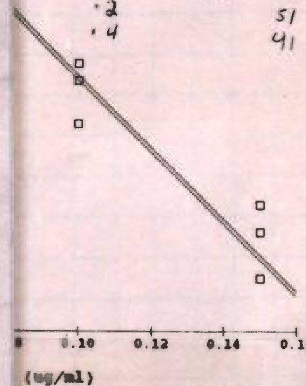
6/22/90 Assay

ard 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
	.2		51
	.4		41



To Page No.

Witnessed & Understood by me, [signature]

Date 9/7/9[illegible]

Invented by [signature]

Recorded by

Date 8/6/90

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

Project No.______
Book No.______

From Page No.___

6/28/90

6/22/90 Assay

	[Submitted]	÷2 [What cells see]	SKBR3 % CTR	[Comparable 4D5 hybridoma]
Hu Var#1 [5.2 ug/ml]	1/10	0.26 ug/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
	1/160	0.016	93	
Hu Var#2 [8.6ug/ml]	1/10	0.43 ug/ml	56	0.136
	1/20	0.215	67	0.101
	1/40	0.11	82	0.054
	1/80	0.055	100	
Hu Var#3 [4.4ug/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
CHIMERA 113ug/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

To Page No.___

Witnessed & Understood by me, [signature] Date 9/7/90

Invented by [signature] Date 8/6/90

Recorded by

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

Project No.

Book No.

From Page No.

6/28/90

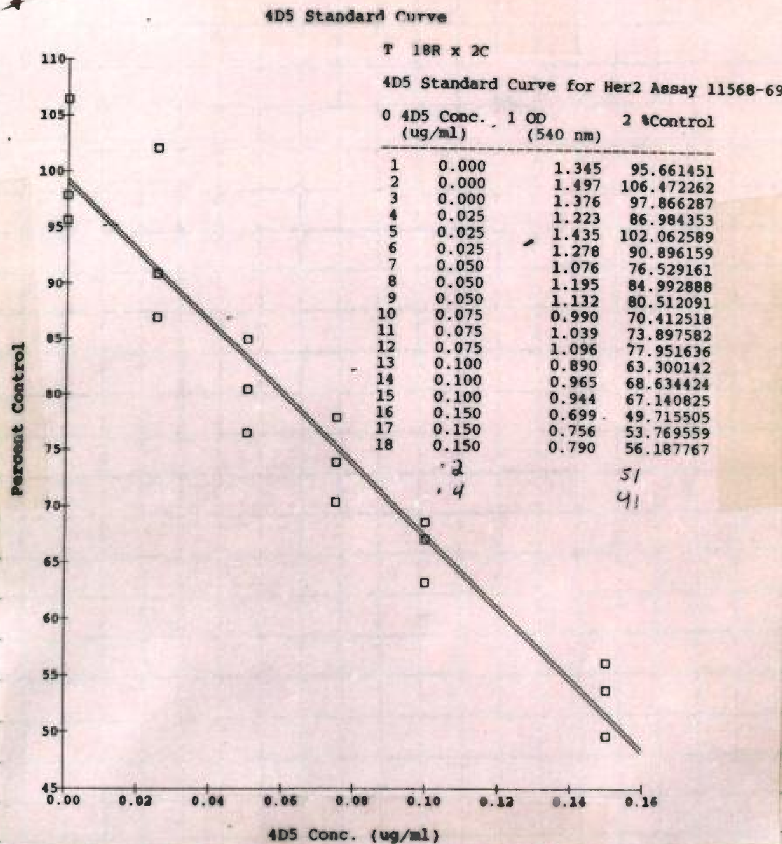
6/22/90 Assay

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

Bio Assay Data

ASSAY RESULTS ARE NOT

NO.	IDENTIFY EACH SAMPLE (GIVEN AND/OR UNIQUE COMPONENTS)	DILUTION	%Ctrl	ug/ml
1	Hu Var #1 (293)	1:10	56	.271
2	(5.2 ug/ml)	1:20	70	.18
3		1:40	80	.12
4		1:80	89	.06
5		1:160	93	LTS
6		1:320	96	LTS
7	Hu Var #2 (293)	1:10	56	.271
8	(8.6 ug/ml)	1:20	67	.20
9		1:40	82	.10
10		1:80	107	LTS
11		1:160	96	LTS
12		1:320	99	LTS
13	Hu Var #3 (293)	1:10	66	.20
14	4.4 ug/ml	1:20	79	.126
15		1:40	85	.08
16		1:80	90	.056
17		1:160	94	LTS
18		1:320	96	LTS
19	4D5 2.16 done	1:10	42	GTS
20	113 ug/ml	1:100	39	GTS
21		1:200	48	GTS
22		1:400	63	.297
23		1:800	75	.151
24		1:1600	83	.101
25				



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
	.2		51
	.4		41

Sups are diluted 1:2 when added to SKBR3 cells.
So [4D5] read off Standard Curve is multiplied 2X For Comparison to Unknowns

To Page No.

Witnessed & Understood by me, [signature] Date 9/7/90

Invented by [signature] Recorded by Date 8/6/90

Project No.

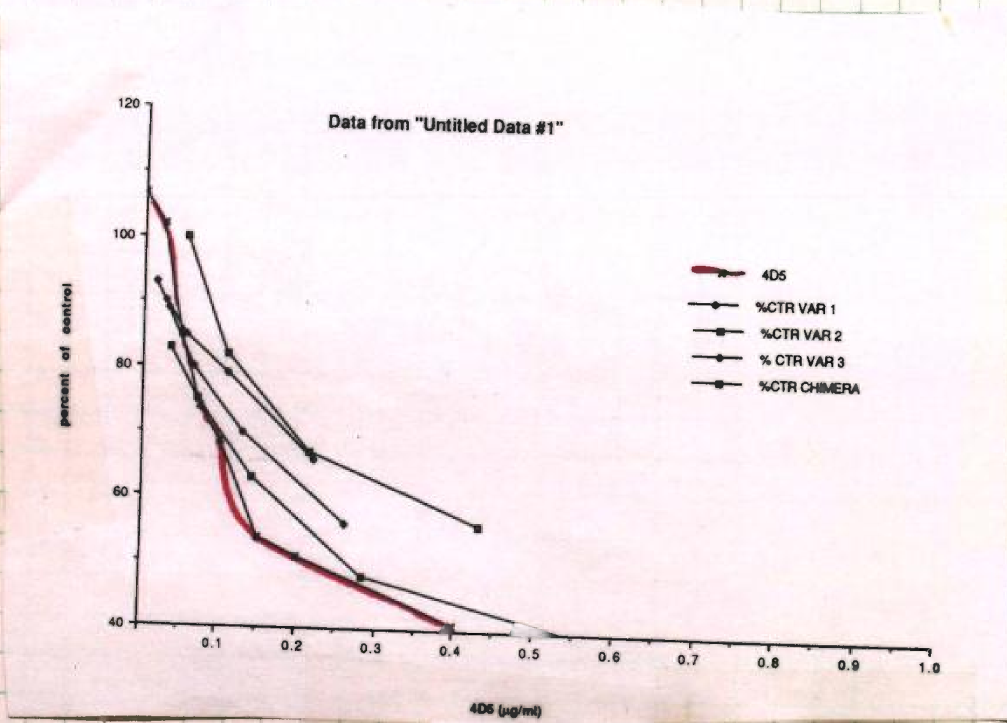
Book No.

TITLE Graph of BioAssay Data For Hu#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

CDRs 1+3

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] Recorded by Date 8/6/90

TITLE Bio Assay Results for HuVer #4, 5, 6 | Project No.______ | Book No.______

From Page No.___

7/6/90

~~HuIgG Elisa Protein [illegible]~~

JK. 7/6/90

See p 68 ↓

ASSAY RESULTS ARE NO[T]

NO.	IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS	DILUTION	Conc ug/ml	% CTR
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
13				

Protein Gel of Hu Ver 4,5,6 ppt by Acetone

Data

Her2Bio#11568-88 7/5/90

(ug/ml)

4D5 Std Curve	MediaOnly	0.025	0.05	0.075	0.1	0.15	0.2	0.4	0.8	Ctrl
OD 6 (540nm)	1.149	1.088	0.929	0.944	0.707	0.651	0.554	0.581	0.51	0.797
	1.12	1.029	0.93	0.884	0.814	0.709	0.623	0.565	0.524	0.812
	1.187	1.035	0.925	0.902	0.838	0.668	0.659	0.623	0.532	0.822
Mean	1.152	1.051	0.928	0.910	0.786	0.676	0.612	0.590	0.522	0.810
SD	0.034	0.032	0.003	0.031	0.070	0.030	0.053	0.030	0.011	0.013
%Proliferation	100.0	91.2	80.6	79.0	68.3	58.7	53.1	51.2	45.3	70.3
	#4				#5			#6		
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
	#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								

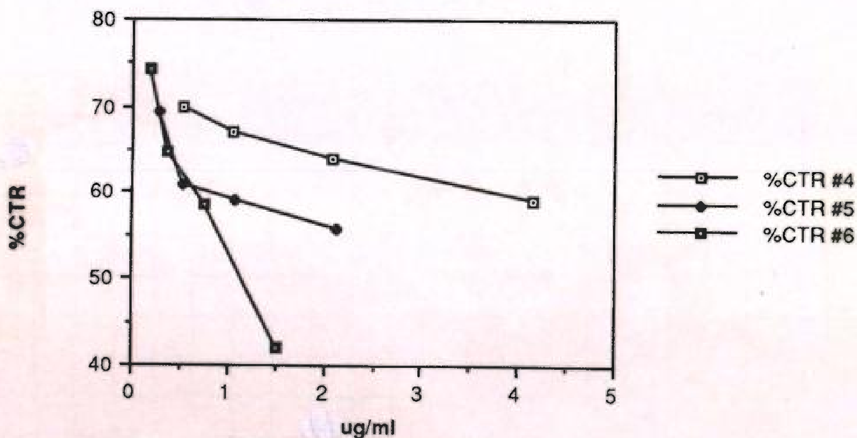
To Page No.____

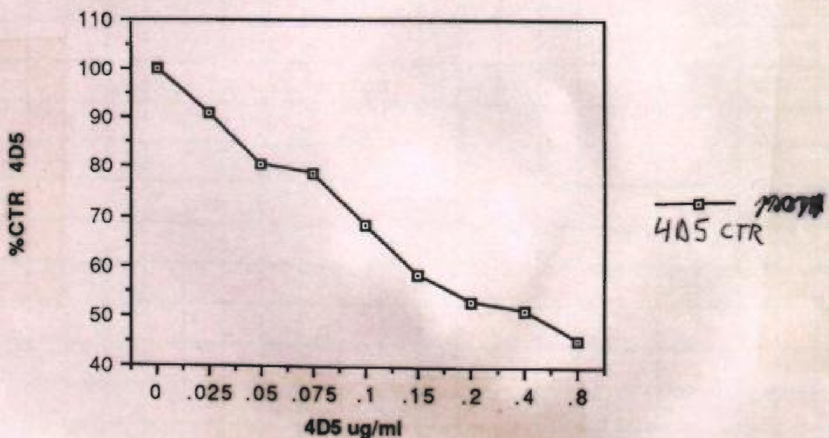
Witnessed & Understood by me,	Date	Invented by	Date
[signature]	9/7/90	[signature] John Ridgway	8/6/90
		Recorded by	

Project No.______
Book No.______ TITLE Bio Assay Results Hu 4D5 Var #4,5,6

From Page No. 71

7/6/90





To Page No.____

Witnessed & Understood by me, [signature] Date 9/7/90 Invented by [signature] Recorded by Date 9/10/90

TITLE Thaw 4DS 2.6 and 4DS 2.16 chimera

Project No.
Book No.

From Page No.

For MTX Amplification repeat try.

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

B) Thawed 1 vail 4DS 2.6 from 5/10/90 1x10^7 cells into 1-100mm dish 1% FBS + Glu

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 clones back

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

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

Media 1% XDZ + Glu (NO GHT) 5x10^5 cells/100mm dish

0.4ml of 10,000nM MTX in 400ml = 10nM MTX

0.6ml " " " = 15nM MTX

1.0ml " " " = 25nM MTX

Cell Counts:

	1/40	
2.6	19151	= 7.66x10^5 c/ml → use 0.65 ml/dish
45.6	22700	= 9x10^5 c/ml → use 0.55 ml/dish
51.6	27580	= 1.1x10^6 c/ml → use 0.5 ml/dish
2.16	8426	= 3.4x10^5 c/ml → 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 = 1 100mm dish

To Page No. 74

Witnessed & Understood by me, [signature] Date 9/7/90

Invented by [signature] Date 7/12/90

Recorded by

Project No.______
Book No.______ TITLE MTX Amp of 4D5 Chimera

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 - ~50% cells stain moderately.
51.6 - all cells stain moderately.

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

7/16 Fed Cells
7/17/90 - Cells not selecting - Not killing fast enough to prevent growth
Perhaps the levels of MTX are not ↑ enough,
Repeat w/ higher level?

7/20/90 25nM is selecting some. Do again at 40, 60nM MTX

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

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

Picked 50 of 45.6 from 60nM MTX

Trypsinized rest from 60nMTX replated in 100, 250, 500nM MTX
" " 40nMTX " " "

To Page No. 84

Witnessed & Understood by me, [signature] Date 9/7/90
Invented by [signature] Recorded by Date 8/10/90

Project No.
Book No.

TITLE Hu 4D5 Variants 1-6 Concentrated.

From Page No.

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

Pre Concentrated [ ]
1) 5 ug/ml
2) 8.6
3) 4.4
4) 16.6
5) 8.5
6) 6.0

Using the Centricon microconcentrators the volume was reduced ~4X

Centricon-10 used (10,000 mw Cutoff).

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 chimeric 2.16 CHO. this was amino acid analyzed.

These 6 were submitted to the Hu IgG Elisa and 4D5 ECD Binding Assay.

7/11/90
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

Same [ ] results for IgG and ECD Binding assays

Using the Chimeric 2.16 as standard at 58 ug/ml by amino acid analysis. Previously as 113 ug/ml by O.D 280. This accounts for the protein conc of the Var not increasing 4X with Concentration by 4X.

Protein Gel of Hu 4D5 Ab. - 10ul of each sample at above concentration

lane) 1 - Var #1
2 - Var #2
3 - Var #3
4 - Var #4
5 Var #5
6 Var #6
7 4D5 Chimeric - 10ul of 58ug/ml.

To Page No.

9/7/90 Recorded by [signature] Date 8/4/90

Project No.______
Book No.______ TITLE Hu 4D5 Var 1-6, BioAssay

From Page No.__

7/13/90 Submitted all 6 Hu 4D5 Variants to BioAssay w/ the chimeric as well.

Concentrated material used as per p 77. ~~[illegible]~~ Protein concentration determined by IgG Elisa using the Chimeric Standard newly analyzed by a.a. analysis.

Submitted to BioAssay at [ ] as follows:

1.6, 0.8, 0.4, 0.2, 0.15, 0.1, 0.05 ug/ml.

CV=CV		Ab	diluent	
1.6(1)=12 x	#1 =	133ul Ab	+ 867 dil	= 1.6 ug/ml
1.6(1)=15(x)	#2 =	107ul Ab	+ 893	= 1.6 ug/ml
1.6(1)=9	#3 :	177	+ 823	= 1.6 ug/ml
1.6/31.4	#4 =	51	+ 949	= 1.6 ug/ml
1.6/7	#5 =	229	+ 771	= 1.6 ug/ml
1.6/8.8	#6 =	182	+ 818	= 1.6 ug/ml

Calculations used to get first dilution of 1.6 ug/ml

Samples submitted 1-49, in order of Var 1-6, Chimera in decending concentrations.
Sample #1 4D5Var#1 1.6 ug/ml
#49 Chimeric 4D5 2.16, 0.05 ug/ml.

To Page No. 82

Witnessed & Understood by me, [signature] Date 9/7/90
Invented by [signature] Recorded by Date 8/20/90

30' pulse → chase

TITLE 293 xfn of 405 Hu w.t. Var for Kinetics labeling

Project No.____
Book No.____

77

From Page No.__

7/16/90 Feed cells at 9:AM (split 7/15)

9 - 60° dishes for xfn

0.5 ml of DNA ppt × 9 = 4.5 ml of ppt at 10 ug/ml = 45 ug total

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

H chain (PA5) = 3.38 ug/ml
L chain (PA3) = 2.74 ug/ml
AdVA = 2.2 ug/ml

5/12 × 45 = 18.7 ug / 3.38 = 5.6 ul of PA5
18.7 ug / 2.74 = 6.8 ul of PA3
2/12 × 45 = 7.5 / 2.2 = 3.4 AdVA

DNA	1/10 TE	CaCl2	2X Hepes
5.6 ul of PA5 6.8 ul of PA3 3.4 ul of AdVA	2025 ul	225 ul	2.25 ml

ppt 4 min on 12:00
Shock at 4 pm.

To Page No.____

Witnessed & Understood by me, [signature] Date 9/7/90

Invented by [signature] Recorded by Date 8/10/90

Project No.
Book No.
TITLE 293 pulse chase w/ the 4D5 (w.T.)

From Page No. 77

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

8 dishes × 1.5 ml label = 12 ml label

No preincubation w/ cys⁻, met⁻ media.

12 ml label: F12, DMEM w/o cys, met + ^{35}S cys, met.

100 uCi/ml cys, met : 1200 uCi each — CTR plate: 293 not xfn w/ Ab

label is 10 uCi/ul : so 120 ul of ^{35}S cys + met each
Rinse 1x w/ PBS before adding label
Pulse 30 min — Wash label off w/ PSO4 1x. Refeed w/ PSO4 5.0 ml.

11:15 Time 0 — take Sups + Cells (1 ml Tri X [illegible] 1% + 5 ul [illegible]) pg 7.2)
12:15 1 hr — Sup 5 ml + [illegible] cells
1:15 2 hr ✓ ✓
2:15 3
3:15 4 hr — ✓ ✓
4:15 5 hr ✓ ✓
5:15 - 6 hr — ✓ ✓
10 AM - 23 hrs ✓ ✓ + CTR plate harvested at 23 hr Sup + Cells

7/18/90 Immunoprecipitate Sups 1.5 ml and Cells 300 ul
(TO compare w/ the last Kinetics exp p65 - need to ppt same proportion of sups 6/20 of 5 ml = 1.5)

— used 50:50 mix of RAH IgG(Fc) and RAH α kappa chain - 4 ul / tube.

To Page No. 81

Witnessed & Understood by me, [signature] Date 9/7/90
Invented by [signature] Recorded by Date 8/10/90

TITLE Harvest Exp. 4D5 Hu Ab.

Project No.____
Book No.____

From Page No.__

7/17/90 Transient Transfection 293 cells w/ Hu 4D5 w.T. 3-100 dish
Collect Sups:

Dish 1) 24hrs, 48hrs 72hrs
2) 48hrs, 72hrs
3) 72hrs

100-3 dishes = 3 ml ppt at 10ug/ml DNA = 30 ug DNA

Ratio 5 : 5 : 2
H - L - AdVA

H chain (pAK5) : 3.38 ug/λ
L chain (pAK3) = 2.74 ug/λ
AdVA = 2.2 "

DNA	1/10 TE	CaCl2	2X Hepes
	1350ul	150ul	1.5 mls
5ul Hchain (pAK5)			
6ul Lchain (pAK3)			
3ul AdVA			
ppt on 1:30			

7/18/90 AM change media to all 3 plates to SF + Transferrin Glu
7/19/90 Cells still look fine ~90-95% confluent. V. few floating.
Removed media from plate 1 - 0-24hrs - refed 6ml Some
7/20/90 Cells looking more granular + dark, gaps appearing as cells clump - few floating.
Media removed plate 1: 24-48hrs - refed
plate 2: 0-48hrs refed.
Purify Samples from 7/19-20/90 on Staph A cols today,
7/21/90 Still few cells floating - though somewhat more unhealthy looking?
(AM) Removed media plate 1- 48-72, plate 2 48-72, plate 3 - 0-72hrs. Refed
7/23/90 Kept media plate 1 72-120hrs.

To Page No. 80

Witnessed & Understood by me, [signature] Date 9/7/90

Invented by [signature] Recorded by Date 8/6/90

Project No.______
Book No.______ TITLE Harvest Exp

From Page No. 79

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

0hr is at start of Serum Free PSO4 + Insulin. (Day after xfer)

Samples 10ml 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 Centricon-30 (30,000MW cutoff) microconcentrators to ~900ul-1.0ml

7/25/90 Samples submitted to IgG Elisa - For total protein.

ECD binding - a Whole Hu IgG std, b Hu Fab std } Comparison for degradation CHR

Conjugate
(a - Gt Hu Fc-HRP
b - Gt Hu Fab-HRP)

Submitted Samples:

Final Concentrated Samples 1-7 - diluted 1/100, 1/1000, 1/10,000
(5,6,7 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, 1/10^4
(4mls total)

To Page No. 85

Witnessed & Understood by me, [signature] Date 9/7/90

Invented by [signature] Date 8/6/90

Recorded by

TITLE Kinetics - Pulse Chase of 4D5 like Ab. Project No. Book No. 81

From Page No. 78

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

Run on 4-20% Gradient Gels. -20ul/lane.

Gel 1: Lane 1) T: 0hrs Sup
2) " Cells
3) T=1hrs Sup
4) " Cells
5) T=2hrs Sup
6) " Cells
7) Blank
8) 14C LMW markers
9) Prestained HMW 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 MWt lows
10) prestained HMW

Gel (3) Lane: 1) T=6hrs Sups
2) " Cells
3) T=23hrs Sups
4) " Cells
5) 293 CTRS 23hrs Sups
6) " Cells
7) Blanks
8)
9) 14C LMW markers
10) Prestained HMW markers

To Page No.

Witnessed & Understood by me, [signature] Date 9/7/90 Invented by [signature] Recorded by Date 8/10/90

Project No.
Book No.
TITLE Bio Assay Results Her-2

From Page No. 76

7/19/90 Concentrations of Ab (IgG) submitted will be adjusted before calculating/working up data from BioAssay.

IgG concentrations by elisa for the Human 4D5 Variant were determined with the chimeric 4D5 as the standard. This gives false readings (I think, now), because the values derived using a humanized 4D5 as standard are different.
The humanized 4D5 "wild type" made in E coli is probably a better standard to use in determining the IgG conc. of Humanized Ab's made in mammalian cells

Samples were submitted to BioAssay (Her-2) on 7/13/90 based upon [IgG] using the 4D5 chimera as standard. This Bioassay data will be used but adjusted for higher IgG concentrations.

Each dilution submitted will be multiplied by a factor developed 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 curves

Var #2 27/15.1 = 1.79

Use Ave of 1.7 a factor to increase to correct conc

Var #3 15.3/9 = 1.7

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, [signature] Date 9/7/90
Invented by [signature] Date 8/6/90
Recorded by

From Page No.__

7/19/90 Values for IgG Conc for Humanized 4D5 Variations 1-6 are higher using the Humanized Ab as standard.

7-12-90

John Ridgway

data in ng/ml

	(g.aaa) Chimeric Std.		Humanized Std.	
	IgG1 Total Protein	HER2 ECD	IgG1	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:

#	Sample	Dilution	#	Sample	Dilution
1	Hu 4D5 Var #1	1/100 dil	10	Hu 4D5 Var #4	1/100 dil
2	"	1/1000	11	"	1/1000
3	"	1/10,000	12	"	1/10,000
4	Hu 4D5 Var #2	1/100 dil	13	Hu 4D5 Var #5	1/100
5	"	1/1000	14	"	1/1000
6	"	1/10,000	15	"	1/10,000
7	Hu 4D5 Var #3	1/100 dil	16	Hu 4D5 Var #6	1/100
8	"	1/1000	17	"	1/1000
9		1/10,000	18	"	1/10,000

To Page No.____

Witnessed & Understood by me, [signature] Date 9/7/90 Invented by [signature] Recorded by Date 8/6/90

Project No.
Book No.
TITLE MTX Amp of ~~the 4D5 H~~ CHO 4D5 chimera clones

From Page No. 74

7/20/90 Set up again 5×10^5 cells/plate at 40 and 60nM MTX

Cell Counts:

45.6 — 13,541 ×40 = 5.42×10^5/ml — use 0.92 ml/dish

51.6 — 10,726 ×40 = 4.29×10^5/ml — use 1.17/dish

2.16 — 4458 ×40 = 178320/ml — use 2.8 ml/dish.

8/9/90 45.6 Population of ~~[illegible]~~ clones at 250nMTX, 500nMTX shows no IgG

51.6 clone in 60nM MTX <IgG.

☐ VALIDATED ACCEPTABLE RANGE: 100ng/mL – 1.4.

NOTES: CHIMERIC 4D5

ASSAY RESULTS ARE NOT ...

NO.	IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS	DILUTION	ng/mL
1	51.6, 60nMTX	1/10	28.4
2	↓	1/100	3.4
3		1/1000	<STD
4	45.6 250nMTX	1/10	<STD
5	↓	1/100	<STD
6		1/1000	<STD
7	45.6 500nMTX	1/10	<STD
8	↓	1/100	<STD
9		1/1000	<STD
10			

To Page No.

Witnessed & Understood by me, [signature] Date 9/7/90 Invented by [signature] Recorded by Date 8/10/90

TITLE Harvest Exp Results

Project No.______
Book No.______

From Page No. 80

Hu 4D5 w.T. 293 transient Exp

7/26/90 [Total ug IgG]

	IgG	0-24hrs		24-48hrs		48-72hrs		72hr total		72-120hr
plate 1:	(post purify)	13.5ug	+	12.7ug	+	11.9ug	=	38ug		38ug
(pre-purify)	Sups: (IgG)	~~18ug~~ 25ug	+	~~22.5ug~~ 33ug	+	~~42ug~~ 58ug	=	~~82ug~~ 116		yield 46% Ave.

		0-24hrs	0-48hrs	48-72hrs		72hr total	
plate 2:	post ~~purify~~ purify	—	40ug	22ug	=	62ug	
	Sups		~~63ug~~ 64.5	30ug	=	93	yield ~66%

0-72hrs

plate 3: Purified + Conc 50ug total IgG

yield ~ 60%

Sups 84ug total IgG

plate 1 72-120hrs - 37.5ug total purified — yield ~ 82%
45 Sups

% ~~[illegible]~~ ECD Binding IgG to total IgG as function of Age of Culture

Q: Do the longer culture times (longer periods between ~~[illegible]~~ refeeding result in a greater % Ab not binding to ECD.

24hr culture

	ECD	IgG	After Purification % ECD of IgG	Before Purification % ECD of total IgG
Plate 1 Sample 1 (0-24)	13.2	13.5	98%	~~89%~~ 74
PL 1 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 (0-48h)	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%	~~89%~~ 79%

To Page No.____

Witnessed & Understood by me, [signature] Date 9/7/90

Invented by [signature] Date 8/10/90

Recorded by

Project No.______
Book No.______
TITLE Harvest Exp Data

From Page No. 85

7/26/90

ASSAY RESULTS ARE NOT CORRECTED FOR DILUTION

NO.	IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS	DILUTION	HER2 (αFc HRP)	IgG
1	#1 pure/conc	1/100	>100	>100
2	"	1/1000	13.2	13.5
3	"	1/10000	1.1	1.5
4	#2 pure/conc	1/100	>100	>100
5	↓	1/1000	11.2	12.7
6	↓	1/10⁴	1.0	1.4
7	#3 pure/conc	1/100	>100	>100
8	↓	1/1000	11.2	11.9
9	↓	1/10⁴	[illegible] 2.2	[illegible] 2.2
10	#4 pure/conc	1/100	>100	>100
11	↓	1/10³	36.5	35.5
12	↓	1/10⁴	3.4	4.4
13	#5 pure/conc	1/100	>100	>100
14	↓	1/10³	17.7	18.1
15	↓	1/10⁴	1.5	2.8
16	#6 pure/conc	1/100	>100	>100
17	↓	1/10³	46.5	45.6
18	↓	1/10⁴	4.3	5.6
19	#7 pure/conc	1/100	>100	>100
20		1/1000	[illegible]	38.4
21	#7 pure/conc	1/10⁴	3.3	5.1
22	#1 ori sup 1/3 dil	1/10	>100	>100
23		1/100	13.0	14.6
24	↓	1/1000	1.2	1.9
25	#2 ori sup 1/3 dil	1/10	>100	>100
26	(1:1 to 16 stayed	1/100	17.0	17.4
27	↓ / cool)	1/1000	1.5	2.7
28	#3 ori sup 1/3 dil	1/10	>100	>100
29	clumped dilns	1/100	30.8	34.2
30	↓	1/1000	2.8	4.3
31	#4 ori sup 1/3 dil	1/10	>100	>100
32		1/100	35.1	43.8
33	↓	1/1000	3.0	4.2
34	#5 Ori sup neat	1/10	>100	>100
35		1/100	24.8	27.5
36	↓	1/1000	2.3	3.4
37	#6 Ori sup neat	1/10	>100	>100
38		1/100	59.5	85.1
39	↓	1/1000	5.4	8.4
40	#7 Ori sup neat	1/10	>100	>100
41	#7 Ori sup neat	1/100	44.2	45.6
42	↓	1/1000	4.0	6.0
43	#6 before conc	1/100	89.3	>100
44	4 mls	1/1000	1.3	13.8
45	↓	1/10,000	0.7	1.8
46				

ng/ml results.

Her 4D5 Fab elisa
ECD Her 2 binding

	ng/ml
1	84.4
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	24.8
21	2.5
22	69.3
23	9.0
24	0.9
25	>100
26	12.0
27	1.2
28	>100
29	20.0
30	2.4
31	>100
32	21.8
33	2.5
34	>100
	16.9

34	>100
35	39.0
36	4.0
37	>100
38	29.5
39	3.0
40	59.4
41	6.0
42	<.5
43	0.6
44	<.5
45.	<.5

Non Reducing - 4-20% Grad Prot Gel of Samples 1-7 showing intact Ab

To Page No.____

Witnessed & Understood by me, [signature] Date 9/17/90 Invented by [signature] Recorded by Date 8/6/90

TITLE Kd Analysis of 4D5 Hu Ab Project No.____ Book No.____

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 Dua

Control 4D5 hybridoma Ab : Kd 3.6 nM

4D5 Hu Var #1 ———— Kd 2.66 nM
4D5 Hu Var #4 ———— Kd 2.77 nM

To Page No.____

Witnessed & Understood by me, [signature] Date 9/7/90

Invented by [signature] Date 8/10/90

Recorded by

Project No.____
Book No.____ TITLE: 293 Xfer of Hu(Var) – 4

From Page No.__

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

	VL		VH
Var 1	pA3(a)	+	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/λ
- pA5 – W.T Hu H-Chain 3.38ug/λ
- Hb (5-b-4) – Ver b of Hu H-Chain clone (5-b-4) 2.0ug/λ
- Hc (5-c-4) – Ver c of Hu H chain clone (5-c-4) 3.0ug/λ
- Lb (3-b-8) – Ver b of Hu L-Chain clone (3-b-8) 2.0ug/λ

AdVA – 2.2ug/λ

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

Ratio H : L : AdVA 5 : 5 : 2 → for 40ug use H + L + AdVA 20 20 8ug

Var #	DNA	1/10 TE	2.5M CaCl2	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.

To Page No. 89

Witnessed & Understood by me, [signature] Date 9/7/90 Invented by [signature] Recorded by Date 8/10/90

TITLE 293 xfer of HuVar 1-4 Project No.____ Book No.____

From Page No. 88

7/31/90 Cells refed at 9:30AM - Some plates have cells un-evenly spaced.
ART DNA on 12:45 - 1:00
New batch of 2X Hepes + 2.5M CaCl2
Let ppt go 7min.

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

Glycerol Shock 5:30 - 6PM

8/1/90 Changed to PSO4 + Ins + Glu (Serum Free)

8/3/90 Collected media 40mls on 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 48hr. intervals over 6 days.

To Page No.____

Witnessed & Understood by me, [signature] Date 9/7/90 Invented by [signature] Recorded by Date 8/10/90

Project No.______
Book No.______ TITLE 293 Xfn of Hu Var 5–8

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/λ	+	a (PA5) 3.38ug/λ
Var 6	C (3-C-7)	+	c (5-C-4) 3.0ug/λ
Var 7	b (3-b-8) 2.0ug/λ	+	e (PA13) 3.0ug/λ clone 1/6 Paul Carter
Var 8	b (3-b-8)	+	d (PA14 clone 3/2) 0.16ug/λ

AdVA 2.2ug/ul.
Prepare 4ml each with ~ 10ug/ml total DNA each
ea. L- 20ug + H- 20ug AdVA 8ug

Var	DNAs	1/10 TE	2.5M CaCl2	2X HEPES
#5	3-C-7 - 5ul PA5 - 6.9ul AdVA - 4ul	1.8mls	200ul	2mls
#6	3-C-7-(5ul) 5-C-4-(7.8ul) AdVA-4ul.	1.8mls	200ul	2mls
#7	3-b-8 - 11.7ul PA13 - 7.8ul AdVA - 4ul	1.8 ml	200ul.	2ml
#8	3-b-8 11.7ul PA14 150ul AdVA 4ul	1.635 ml	200ul	2mls

To Page No.____

Witnessed & Understood by me, [signature] Date 9/7/90
Invented by [signature] Recorded by Date 8/20/90

TITLE 293 for ... Var 5-8 Project No.____ Book No.____

From Page No.__

8/1/90 let ppt go 7 min each

ppt on 12:30 Shock 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 Collect 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 centricon tubes.

To Page No.____

Witnessed & Understood by me, [signature] Date 9/7/90 Invented by [signature] Recorded by Date 8/10/90

Project No.
Book No.
TITLE Harvest + Purification of Hu Var 1-8.

From Page No.

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

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

Starting Sups 120 ml for ea Var 1-8

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

To Page No.

Witnessed & Understood by me, [signature] Date 9/5/90 Invented by [signature] Recorded by Date 8/10/90

TITLE IgG Elisa of Humanized 4D5 Var 1-8

Project No.

Book No.

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	DILUTION	ng/mL
1	Var 1 Pure + Conc	1/100	>STD	21	Var 6 Pure+Conc	$1/10^2$	>STD
2	↓	1/1000	54.2	22	↓	$1/10^3$	33.6
3	↓	$1/10^4$	6.7	23	↓	$1/10^4$	4.5
4	↓	$1/10^5$	<STD	24	↓	$1/10^5$	<STD
5	Var 2 Pure + Conc	$1/10^2$	>STD	25	Var 7 Pure+Conc	$1/10^2$	>STD
6	↓	$1/10^3$	>STD	26	↓	$1/10^3$	64.5
7	↓	$1/10^4$	20.8	27	↓	$1/10^4$	7.8
8	↓	$1/10^5$	2.5	28	↓	$1/10^5$	<STD
9	Var 3 Pure + Conc	$1/10^2$	>STD	29	Var 8 Pure+Conc	$1/10^2$	>STD
10	↓	$1/10^3$	44.5	30	↓	$1/10^3$	34.7
11	↓	$1/10^4$	6.5	31	↓	$1/10^4$	4.9
12	↓	$1/10^5$	<STD	32	↓	$1/10^5$	<STD
13	Var 4 Pure + Conc	$1/10^2$	>STD	33	Var 1 Combined our Sups.	1/10	>STD
14	↓	$1/10^3$	27.8	34	↓	1/100	27.8
15	↓	$1/10^4$	4.0	35	↓	$1/10^3$	3.6
16	↓	$1/10^5$	<STD	36	Var 2 Sups 8/3,5,7/90	1/10	>STD
17	Var 5 Pure+Conc	$1/10^2$	>STD	37	↓	1/100	91.1
18	↓	$1/10^3$	25.5	38	↓	1/1000	8.9
19	↓	$1/10^4$	3.6	39	Var 3 Combined Sups	1/10	>STD
20	↓	$1/10^5$	<STD	40		1/100	25.4

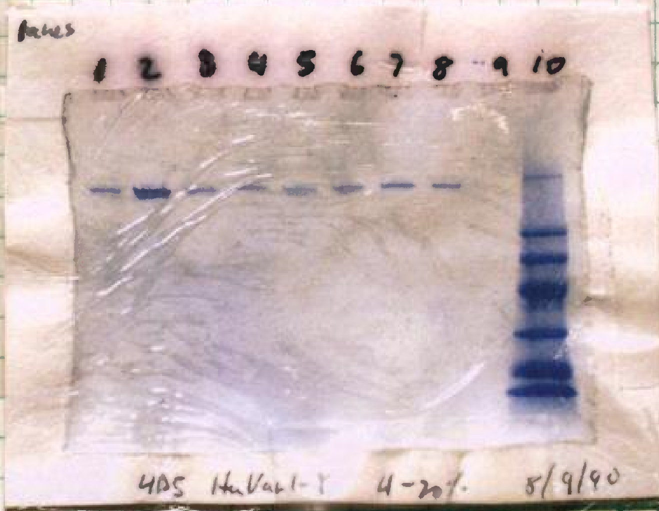
RETURN TO REQUESTOR

NO.	IDENTITY	DILUTION	ng/mL
41	Var 3 Sups	$1/10^3$	2.7
42	Var 4 Sups	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	↓	1/100	16.9
50	↓	1/1000	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 $1/10^3$ and $1/10^4$ dilutions.

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

Run on 4-20% Gradient Gel Non.Red.
10 ul of each Var.
15 ul of #5
Lanes: 1 Var 1
2 " 2
3 " 3
4 " 4
5 " 5
6 " 6
7 " 7
8 " 8
9 Blank
10 BRL High MWM



No.

Witnessed & Understood by me, Date 9/7/90

Invented by

Recorded by

Date 8/6/90

Project No.______
Book No.______

TITLE IgG Elisas to understand low yield.

From Page No.__

8/7/90 Samples taken during the purification process of the [illegible] Var 1-8 are assayed to find which step the loss occurs.

ng/ml

ASSAY RESULTS ARE NOT CORRECTED FOR DILUTION

NO.	IDENTIFY EACH SAMPLE ORIGIN AND/OR UNIQUE COMPONENTS	DILUTION	~~ng/ml~~	Correct
1	Var 1 8/3 Sup	1/10	>100	>100
2	↓	1/100	20.5	24.5
3	↓	1/1000	6.1	2.4
4	Var 1 8/3 elu	1/10	<.5	<.5
5	(by passed prot A)	1/100	<.5	<.5
6	↓	1/1000	<.5	<.5
7	Var 1 8/3 Purified elu	1/10	>100	>100
8	elu off prot A	1/100	>100	>100
9	↓	1/1000	26.6	21.0
10	Var 1 8/3 2° elu	1/10	67.3	70.3
11	off Prot A	1/100	12.0	6.5
12	↓	1/1000	<0.5	0.6
13	Var 1 Sup from	1/10	<.5	<0.5
14	filter Conc	1/100	<.5	<.5
15	↓	1/1000	<.5	<.5
16	Var 4 8/3 Sup	1/10	>100	>100
17	↓	1/100	51.0	47.7
18	↓	1/1000	9.6	4.5
19	Var 4 8/3 elu	1/10	0.9	6.0
20	Prot A bypass	1/100	<.5	0.6
21	Var 4 8/3 elu	1/1000	<.5	<.5
22	Var 4 8/5 Sup	1/10	>100	>100
23	↓	1/100	44.2	39.9
24	↓	1/1000	9.0	3.8
25	Var 4 8/5 elu	1/10	6.0	2.5
26	Prot A bypass	1/100	<.5	<.5
27	↓	1/1000	<.5	<.5
28	Var 4 8/7 Sup	1/10	>100	>100
29	↓	1/100	41.0	36.8
30	↓	1/1000	8.3	3.7
31	Var 4 8/7 elu	1/10	1.9	<.5
32	Prot A bypass	1/100	<.5	<.5
33	↓	1/1000	<.5	<.5
34	Var 4 Sup from	1/10	<.5	<.5
35	Filter Concentration	1/100	<.5	<.5
36	↓	1/1000	<.5	<.5
37	Sup Var 5 8/4	1/10	>100	>100
38	↓	1/100	25.[illegible]	19.4
39	↓	1/1000	4.9	1.8
40	Var 5 elu 8/4	1/10	5.4	2.2

ng/ml

NO	IDENTITY	DILUTION	~~ng/ml~~	Corrected
41	Var 5 elu 8/4	1/100	<.5	<.5
42	↓	1/1000	<.5	<.5
43	Var 5 Sup 8/6	1/10	>100	>100
44	↓	1/100	18.0	11.3
45	↓	1/1000	2.8	1.3
46	Var 5 elu 8/6	1/10	<.5	<.5
47	↓	1/100	<.5	<.5
48	↓	1/1000	<.5	<.5
49	Var 5 Filter	1/10	<.5	<.5
50	Conc elu (Sup)	1/100	<.5	<.5
51	↓	1/1000	<.5	<.5
52	Var 2 Final Pure	1/100	>100	>100
53	8/7/90	1/10³	>100	>100
54	↓	1/10⁴	26.8	19.5
55	↓	1/10⁵	5.4	1.9
56	Var 5 Final Pure	1/100	>100	>100
57	8/7/90	1/10³	32.7	25.3
58	↓	1/10⁴	6.7	2.4
59	↓	1/10⁵	<.5	<.5
60				

Var #1 as example 8/3 collection of 40 ml

Raw sups : 2.4 ug/ml × 40 ml = 96 ug total IgG

all bound to prot A column (elu pass thru <.5)

Ab eluted off column 3 mls : 21 ug/ml × 3 ml = 63 ug total recovery,

the loss at this step is 35%. It is either not comming off the column or it is denatured by the low pH of the elution buffer so that it isn't picked up by the elisa.

To Page No.____

Witnessed & Understood by me, [signature] Date 9/7/90

Invented by [signature] Date 8/9/90

Recorded by

TITLE Gel of 2.16 Prebank vs Un Banked

Project No.
Book No.

From Page No.

9/7/90 Ichlel Cells [35]S

Run Reduced + Non Reduced

lanes

1) Non Banked Prod line - Cells
2) " - Sups
3) Prebanked - Cells
4) " - Sups
5) DP-12 - Cells CTRS
6) " - Sups

7) H MW Stds

To Page No.

Witnessed & Understood by me, [signature] Date 9/7/90

Invented by [signature] Date 9/7/90

Recorded by

Project No.____
Book No.____
TITLE Stable 405 Hu Var 6 in 293 cells.

From Page No.__

9/25/90 pRK Hu 4D5 H+L chain (one plasmid) Var#6 5-C-4 – H
3-C-7 – L
2.2 μg/λ

HAMAR 0.25 μg/λ

	DNA	1/10 TE	CaCl2	2X HEPES
1. HAMAR only	40 HAMAR	450 μl	50 μl	500 μl
2. HAMAR pRK Var 6	5 μl pRK Var 6 5 μl HAMAR	440 μl	50 μl	500 μl
3. pRK Var 6 +ADVA.	5 μl pRK Var 6 1 μl ADVA	450	50 μl	500 μl
4) pA10 1/5 pA9 8	50 μl pA9 56 μl pA10 3 ADVA	800 μl	100 μl	1 ml

ppt on 1:30

9/27/90 Split into 10% FBS + Colchicine range, 2ng – 14ug/ml
10/2/90 all cells have died Control 293 cells died at 2ug/ml level. Promoter on Hamdr plasmid not strong enough in 293 cells.

To Page No.____

Witnessed & Understood by me, Date 10/24/90 ~~10/5/90~~
Invented by Date 10/5/90
Recorded by