UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

JANSSEN BIOTECH, INC., Plaintiff, v. AMGEN INC., Defendant.	C.A. No. 22-cv-01549-MN [redacted]

DECLARATION OF MATTHEW S. CROUGHAN, PH.D. IN SUPPORT OF JANSSEN'S MOTION FOR PRELIMINARY INJUNCTION

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I. INTRODUCTION

1. Plaintiff Janssen Biotech, Inc. ("Janssen") has retained me in the above-captioned litigation. I understand Defendant Amgen Inc. ("Amgen") has submitted an abbreviated Biologics License Application ("aBLA") seeking FDA approval of ABP 654,[1] a putative biosimilar of Janssen's STELARA® (ustekinumab) product. I submit this declaration in support of Janssen's motion for preliminary injunction, which I understand seeks to prevent Amgen from launching ABP 654 in the United States during the pendency of the ongoing lawsuit.

2. I have been asked to assess whether, in my opinion, Amgen infringes two Janssen patents: U.S. Patent No. 9,475,858 ("the '858 patent") and U.S. Patent No. 9,217,168 ("the '168 patent"). Together, I refer to these two patents as the "Asserted Manufacturing Patents."

3. In particular, the claims I was asked to consider are claim 33 of the '858 patent and claim 23 of the '168 patent (collectively, "the Asserted Manufacturing Claims").

II. SUMMARY OF OPINIONS

4. Having examined Amgen's aBLA as well as the Asserted Manufacturing Patents, I conclude that Amgen's manufacture of ABP 654 according to the methods set forth in its aBLA materials will infringe at least claim 33 of the '858 patent and claim 23 of the '168 patent.

5. I understand that the Asserted Manufacturing Patents were both duly issued by the U.S. Patent and Trademark Office (USPTO) and are each presumed valid. Should Amgen contest their validity, I understand that I will have an opportunity to respond.

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[1] I understand that Amgen has submitted [redacted] aBLAs to cover [redacted] ABP 654 to patients. [redacted] aBLAs have information in common for the manufacturing of the antibody. Accordingly, I will refer to them together as "the aBLA" or "Amgen's aBLA."

[redacted]

6. I have prepared this declaration based on the materials available to me, and I list in Appendix A the materials I considered.

III. QUALIFICATIONS

7. I am the sole proprietor of Matthew Shane Croughan, a Consulting Services Firm, where I consult on bioprocess development, scale-up, and manufacturing to over a hundred clients, including major biopharmaceutical companies. I have advised on projects involving, among other things, biopharmaceutical proteins including antibodies, vaccines, biofuels, cell and gene therapies, and cellular agriculture (meat grown from stem cells). As a consultant, I have also served as an expert witness and consultant for legal cases.

8. I am also the co-founder, CEO, and Managing Partner of Claymore Bio LLC. Claymore Bio LLC seeks to address shortfalls in the diagnosis and treatment of infectious disease by employing novel imaging technologies to find hidden infections and utilizing innovative strategies to treat viral and bacterial diseases.

9. I earned my B.S. in Chemical Engineering with High Honors from the University of California at Berkeley in 1983 and my Ph.D. in Chemical Engineering from the Massachusetts Institute of Technology in 1988.

10. After graduate school, I spent nearly a decade working at Genentech, where I served as a cell culture scale-up and mixing expert. I developed the first licensed, high-density, fed-batch animal cell culture process, a breakthrough platform technology now used throughout the industry for producing high-dose therapeutic antibodies and other proteins. I went on to become one of three group leaders in the first manufacturing sciences department in recombinant protein manufacturing. I then became chief scientist on the design team for the world's largest and most automated animal cell culture facility at the time, which became the standard antibody manufacturing platform at Genentech and nearly all antibody manufacturing worldwide.

[redacted]

11. After my time at Genentech, I later became the founding Professor of the bioprocessing program at Keck Graduate Institute (KGI) in Claremont, California, one of the largest graduate bioprocessing programs in the country. At KGI, I served as the Director of the Amgen Bioprocessing Center from 2006-2013. I created and taught five new courses at KGI. I also served as the advisor to 108 Masters in Biosciences (MBS) and Master of Science (MS) graduate students and two doctoral graduate students. I was honored to receive the Student Choice Faculty Award (Top Teaching Award) for 2012-2013 as well as the "Godfather of Bioprocessing Cup" by the Bioprocessing Class of 2013. Just recently, as part of the 25th anniversary of KGI, I received an award for being one of the top 25 people behind the success of the institute.

12. I note that Amgen Bioprocessing Center was named after Amgen in recognition of its $2 million dollar donation to KGI to fund the creation of the center, but the center was not affiliated with Amgen, Inc.

13. In total, I have over 35 years of experience in industry working or consulting on bioprocess development, scale-up, and manufacturing, as well as an additional 8 years of experience teaching on a part-time or full-time basis in this field.

14. I have published over 20 peer-reviewed publications in the bioprocess, scale-up, and manufacturing fields, including a first-author paper entitled: "*Hydrodynamic Effects on Animal Cells Grown in Microcarrier Cultures*." This publication was reprinted as one of the top 20 most cited papers ever published in the journal Biotechnology and Bioengineering, in a special 40th anniversary issue. I am also a co-inventor on patent application WO 2011/091350 A2 and issued U.S. Patent No. 8,470,552.

[redacted]

15. My curriculum vitae (CV) is attached as Appendix B. It summarizes my educational, industry, academic, and consulting experience. My CV also includes a comprehensive list of my publications and honors.

16. I am receiving compensation at my standard consulting rate of $600 per hour, plus reimbursements for reasonable expenses. My fee is not dependent on my opinions or on the outcome of this matter.

17. In the past four years, I have testified in one other matter: *Research Corp. Techns. Inc. v. Eli Lilly & Co.*, No. 16-191, before the United States District Court for the District of Arizona. I was retained as an expert by Eli Lilly. I filed an expert report and was deposed in July 2019.

IV. MATERIALS CONSIDERED

18. This declaration contains a statement of my present opinions and includes the bases and reasons therefor, and the data and other information which I have considered in forming these opinions.

19. In preparing my opinions in this matter and preparing this declaration, I have reviewed and considered the materials referenced in the body of this declaration, and also relied on my own knowledge, education, and experience.

20. A list of documents considered is attached as Appendix A.

V. LEGAL STANDARDS

21. I am not a lawyer. In forming my opinions, I have applied the legal standards that counsel provided to me, which are set forth below.

A. Claim Construction

22. I understand that claim terms are to be construed as they would be understood by a person of ordinary skill in the art of the invention, after review of the claim language, the

[redacted]

specification, and the prosecution history. I also understand that, unless a patentee defines a term differently in the patent specification, a claim term should be afforded its plain and ordinary meaning as it would be understood by a person having ordinary skill in the art at the time of the invention, taking into account the teachings of the claims themselves and the patent's specification and prosecution history. Accordingly, except where a particular claim term has been defined in the patent specification, either explicitly or by clear implication, I have applied the plain and ordinary meaning of the term.

B. Infringement

23. I understand that Plaintiffs bear the burden of proving patent infringement by a preponderance of the evidence. In other words, I understand Janssen must show that it is more likely than not that Amgen's ABP 654, its use, or the process by which it is made infringes the Asserted Manufacturing Claims. I understand Janssen must show that Amgen's ABP 654, its use, or the process by which it is made meets each and every claim limitation, properly construed, either literally or under the doctrine of equivalents. I further understand that at this early stage in the case the Court may consider whether Janssen is reasonably likely to meet its burden of showing infringement.

24. I understand that evaluating whether a product will infringe a patent involves a two-step analysis. The first step is determining the proper construction or meaning of the Asserted Manufacturing Claims. The second step is comparing the properly construed claims to the accused product, its use, or the process by which it is made. A party who performs the steps of a patented method in the United States can be found liable for infringement. A party who imports into the United States or offers to sell, sells, or uses within the United States a product which is made by a process patented in the United States can also be found liable for infringement. My analysis here

[redacted]

is focused on whether Amgen will perform (if ABP 654 is commercialized) the steps of the Asserted Manufacturing Claims.

VI. PERSON OF ORDINARY SKILL IN THE ART

25. I understand that patents are to be read from the perspective of a hypothetical or fictional "person of ordinary skill in the art" (or "POSA") at the time of the invention. I have been informed that for purposes of my analysis I should assume a time of invention based on the earliest effective filing date for the Asserted Manufacturing Patents—specifically, **July 8, 2011,** for the '858 patent and **March 14, 2013,** for the '168 patent.

26. In determining the characteristics and qualifications of a POSA at the time of each invention, I considered many factors. I reviewed the technology involved in the Asserted Manufacturing Patents as well as the skill and educational background of the named inventors and those who were working in the field at the time of the invention. I also considered the state of the prior art, including other scientific and patent publications regarding cell culture methods and process, issues encountered by those in the field and the rapidity of scientific progress in developing solutions to those problems. I also tried to imagine myself back in time (July 2011 for the '858 patent and March 2013 for the '168 patent) and evaluated the state of the art, the educational background and experience of those I worked with in the field at the time, and advanced cell culture methodologies during these time periods.

27. My opinion is that a POSA for the Asserted Manufacturing Patents would have a Ph.D. or M.S. (or equivalent) in chemical engineering or a closely related discipline combined with at least three years of practical experience in the field of cell culture and process development. Alternatively, a POSA could have a B.S. (or equivalent) in chemical engineering or a closely related discipline, but with at least five years of experience in the field.

[redacted]

VII. SCIENTIFIC BACKGROUND AND STATE OF THE ART

28. The asserted '858 patent claim (claim 33) generally relates to methods for producing recombinant antibodies in a cell culture medium containing a certain amount of arginine to influence levels of carboxyl terminal (C-terminal) variants.

29. The asserted '168 patent claim (claim 23) generally relates to methods of preparing recombinant proteins, including antibodies, in a cell culture medium containing a certain amount of putrescine to influence levels of glycan types.

30. In this Section VII, I explain generally the technical background relevant to these claims and my analysis.

A. Antibody Structure and Post-Translational Modifications

31. For the purposes of manufacturing, living cells are in essence little machines, producing proteins (such as antibodies) from template genetic material, e.g. DNA sequences.

32. The Asserted Manufacturing Patents relate to methods useful when using living cells to manufacture or produce antibodies.

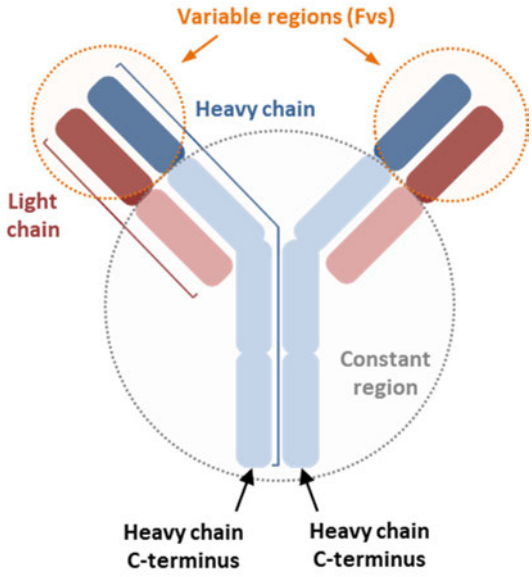
33. Antibodies are proteins and are made up of sequences of amino acids connected by peptide bonds (i.e., polypeptides). An antibody is a multimeric protein, meaning multiple polypeptide chains interact to form the complete antibody. The polypeptide chains that comprise an antibody each have a three-dimensional structure, and they interact to form the three-dimensional structure of the antibody as a whole.

34. In general, antibody structure is dictated by its sequence of amino acids (though as I will explain, post-translational modifications can play a role as well). The antibody amino acid sequence is determined by the nucleic acid (genetic) sequence that encodes the antibody. A cell expresses the antibody through a process called translation, where the cell's machinery "reads" the

[redacted]

nucleic acid sequence and assembles a polypeptide whose sequence of amino acids corresponds to the nucleic acid code.

35. The general, simplified structure of an antibody is a Y-shaped three-dimensional structure, as depicted below:



36. The Y-shaped structure of a typical antibody consists of two pairs of polypeptide chains: two identical "heavy" chains (shaded blue above) and two identical "light" chains (shaded red above). The heavy and light chains are so called because the heavy chain is longer—has more amino acids—than the light chain. The heavy and light chains themselves each contain a variable region domain starting at the **N-terminus** (the end of the polypeptide chain terminating in an ammonium (–$\mathbf{N}H_4$) group) connected to a constant region domain that extends to the **C-terminus** (the end of the polypeptide chain terminating in carboxylate (–$\mathbf{C}OO^-$) group.

37. While the structure and function of an antibody (or protein) is primarily determined by its amino acid sequence, the structure and function are also affected by other structural features. These additional structural features are often the result of changes to the antibody (or protein) that

[redacted]

occur after the amino acid sequence is synthesized during translation. Thus, they can be referred to generally as post-translational modifications.

38. Two types of post-translational modifications of interest here are removal of C-terminal lysine and glycosylation.

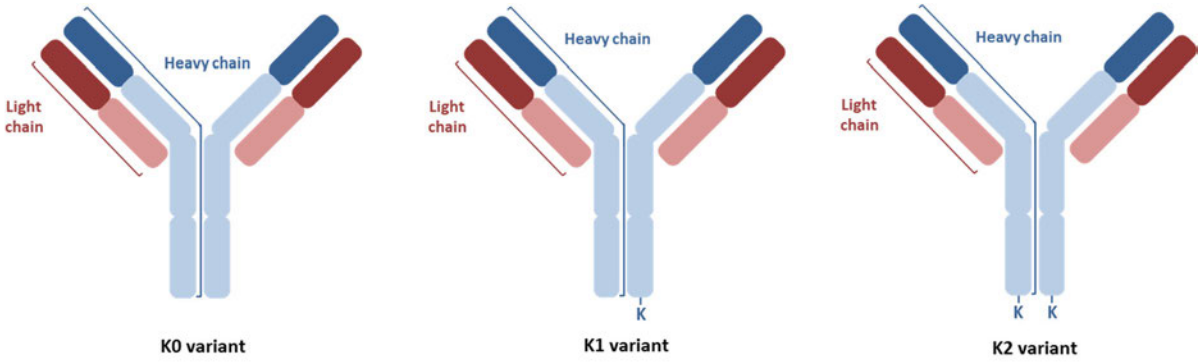
1. C-Terminal Variants

39. As I discussed above, the heavy and light chains that make up an antibody each have a C-terminus. In typical antibodies, the last amino acid at the C-terminus of each heavy chain is the amino acid lysine (commonly abbreviated "K"). In other words, when the antibody is initially synthesized by the cell, a lysine is attached at the end (C-terminus) of each heavy chains.

40. However, these C-terminal lysines can be removed, or "clipped," by enzymes that are present in cells or cell culture media. This can result in the removal of the lysine from one or both heavy chain C-termini.

41. In practice, such removal taking place across numerous antibodies results in a mixture of antibodies with zero, one, or two C-terminal lysines—called C-terminal variants. An antibody preparation will thus often contain a percentage of each variant listed below and illustrated in the figure below ("K" = lysine):

- K0 variant – antibody without a lysine at either C-terminus;
- K1 variant – antibody with a lysine at only one C-terminus; and
- K2 variant – antibody with a lysine at both C-termini.



[redacted]

42. In antibody manufacturing, the K0 variant is usually predominant. Because lysine is a positively charged and basic amino acid, variation in the number of lysine residues present will affect the overall charge and acidity profile of a given variant, as well as change the antibody's structure.

43. The different chemical properties amongst the K0, K1, and K2 variants facilitates their separation and also allows for characterization of the amount of each variant present in a sample. For example, cation exchange high performance liquid chromatography (CEX-HPLC) can separate antibody variants based on their charge profile. Antibodies passing through a CEX-HPLC column will separate into groups based on their charge profile and pass through the column (or "elute") at different times. The predominant variant(s) present in an antibody preparation are typically charge-neutral species and will elute as a "neutral" or "main" peak. Species having greater negative charge will elute as "acidic peaks." And species having greater positive charge will elute as "basic peaks." The K0 variant typically elutes with the main peak, whereas variants having extra positive charges, such as K1 and K2, will elute as basic peaks. In this way, CEX-HPLC can be used to assess the relative amounts of each C-terminal variant in an antibody population.

2. Glycosylation

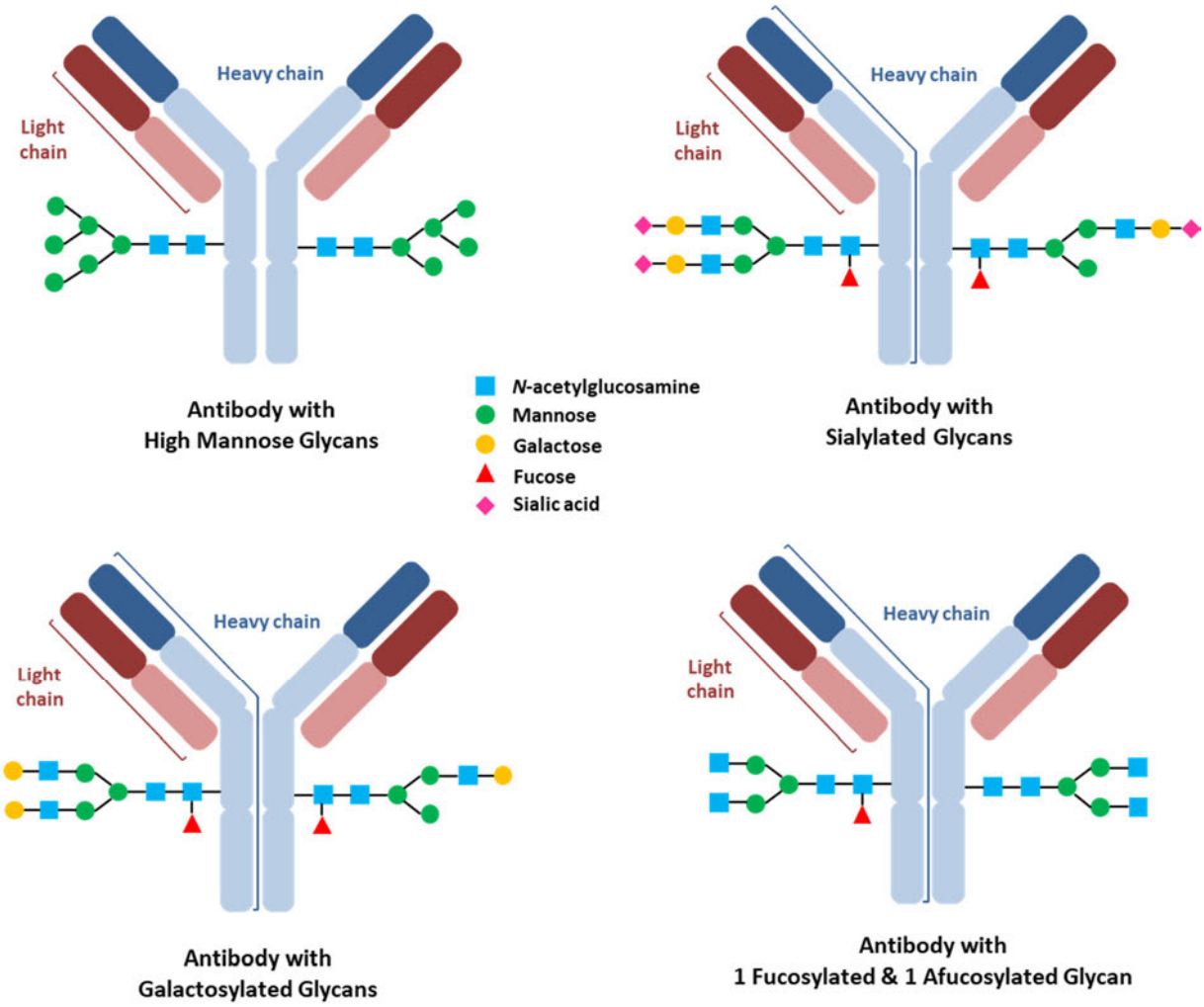
44. After translation, the cell may attach carbohydrate molecules called 'glycans' to certain amino acids in the antibody sequence. This process is called glycosylation, and the particular amino acids in a given antibody's sequence where glycans attach are called glycosylation sites. The attachment of glycan to the nitrogen atom of an asparagine (Asn) amino acid is called N-linked glycosylation. In a typical immunoglobulin (IgG) antibody, there is an

[redacted]

N-linked glycosylation site at a particular position (an asparagine residue) in each of the antibody heavy chains.

45. Glycans are made up of individual sugar units that are attached to a glycosylation site in different patterns. These sugars include fucose (Fuc for short), N-acetylglucosamine (GlcNAc), mannose (Man), galactose (Gal), and sialic acids (N-acetylneuraminic acid (NANA) or N-glycolylneuraminic acid (NGNA)). Different individual glycans can have different constituent sugars in different arrangements as well as different connections between sugars (e.g., galactose can be connected to N-acetylglucosamine as α-galactose or β-galactose).

46. Glycans are typically categorized based on the pattern in which their component sugars are arranged. There are four typical major glycan forms: high mannose glycans, sialylated glycans, galactosylated glycans, and fucosylated glycans. **"High mannose" glycans** are those having five or more terminal mannose sugars. Terminal refers to sugars that are attached at the terminal end of the glycan molecule, farthest away from the attachment to the asparagine molecule. **"Sialylated" glycans** are those having terminal sialic acid; **"galactosylated" glycans** are those with galactose sugars; **"fucosylated" glycans** are those that have a fucose sugar attached to the core N-acetylglucosamine sugar (whereas glycans lacking the fucose are "afucosylated"). The categories need not be mutually exclusive. For example, a glycan might be both galactosylated and fucosylated. However, a glycan cannot be both high mannose and sialylated. Exemplary glycans from the four major categories are schematically depicted below (note the "sialylated" and "galactosylated" glycans are both, in the example shown, also "fucosylated"):



47. Glycans can contribute to the structural stability of antibodies. Glycans can also affect how the body recognizes or interacts with an antibody. Thus, the complex structures of glycans can affect biological properties, including the safety and efficacy of a therapeutic antibody.

48. Glycan forms are also associated with important biological effects. For example, high mannose glycans cause antibodies to often be more rapidly cleared from the blood stream.[2] Thus, high mannose containing therapeutic antibodies may be less effective or require higher

[2] Andrew M. Goetze et al., *High-mannose Glycans on the Fc Region of Therapeutic IgG Antibodies Increase Serum Clearance in Humans*, GLYCOBIOLOGY 2011; 21(7): 949-959. *Available at*: https://doi.org/10.1093/glycob/cwr027.

[redacted]

dosing to achieve a desired therapeutic effect. Sialylated glycans, which are mutually exclusive to high mannose terminal glycans as described above, are thus often less rapidly cleared from the blood stream. Sialylated glycans have also been associated with anti-inflammatory effects.[3]

49. Even a population of otherwise identical antibodies may still differ in their glycans. To characterize the glycans present in a population of antibodies, a process called glycan mapping can determine which glycan forms are attached to an antibody or protein and their relative percentages. Glycan forms include categories such as fucosylated, sialylated, galactosylated, and/or high mannose, and a percentage of each glycan form can be determined for each such category of glycans. Thus, for an antibody of interest, a glycan profile or distribution can be determined.

B. Antibody Manufacturing

50. Therapeutic antibodies are produced using recombinant technology to modify cells and express the desired monoclonal antibody. To do this, a transgene—the nucleic acid sequence encoding the desired antibody's amino acid sequence—is assembled into a nucleic acid construct called an expression vector. A recombinant cell line is then prepared, by transfecting the cells with the expression vector, which causes the transgene and other nucleic acid segments from the expression vector to become incorporated into the cells' DNA.

51. In a process called cloning and selection, cells are then separated, grown, and tested for expression of the desired recombinant antibody to verify the success of the genetic modification. The clones that successfully express the desired antibody are selected for potential

[3] *See, e.g.*, Ravi Vattepu et al., *Sialylation as an Important Regulator of Antibody Function*, FRONTIERS IN IMMUNOLOGY 2022; 13: 818736. doi: 10.3389/fimmu.2022.818736

[redacted]

use in manufacturing. Several rounds of cloning and selection may be performed to find clones that express the desired antibody with preferred productivity and cell growth characteristics.

52. Once a recombinant cell line has been made and selected in this way, it can be grown and sustained to produce quantities of that particular encoded antibody. Recombinant cell lines are typically stored frozen in a cell bank until used for manufacturing.

53. To manufacture a recombinant antibody using such a cell line, a sample of cells from the recombinant cell line is thawed from the cell bank and the sample cultured (in one or more expansion steps) to go from a small quantity of cells to many cells. Once the sample has expanded by culturing to a sufficient volume and density of cells (i.e., sufficient total cell number), the cells are additionally cultured for several days or weeks to produce antibody. This final process step is often called the production culture, production stage, production bioreactor step, and/or N production bioreactor step. For a commercial process, the initial cell sample may be a vial containing just one to a few milliliters (mL) of cell culture, which must be expanded to fill a bioreactor with thousands of liters (L) of cell culture before production can begin in earnest. This entire process, starting from expansion through to the N production bioreactor, is often referred to as culturing the cells or the ”upstream” portion of the process.

54. The cell-liquid mixture in which the cells are expanded, and in which antibody is expressed, is called a cell culture. The solution itself, not counting the cells, is referred to as the cell culture medium. A commercial process might employ different cell culture media at different stages, such as one medium for expansion and another for production of the antibody—or it may use the same medium for multiple (or all) steps.

55. Typical upstream (i.e., cell culture) processes used for commercial antibody production are batch culture, fed-batch culture, and perfusion culture.

[redacted]

56. For a **batch culture process**, a bioreactor is charged with cell culture media and "inoculated" with a previously expanded cell culture. The cells are then cultured in the bioreactor without substantial quantities of additional components being added. Thus, the total culture volume remains largely constant. A batch process is typically stopped when antibody expression declines or cells stop growing or begin to die, at which point antibody is harvested.

57. A **fed-batch process** is similar to a batch process but involves adding substantial amounts of cell culture media or other nutrients at various times during the culture. These additions not only increase the culture volume and cell density, but also maintain a healthy culture for longer before harvest. Thus, fed-batch cultures are typically more productive than batch cultures and are commonly used for commercial antibody manufacturing. In a typical fed-batch culture process, the cells may occupy up to 10% of the total culture volume.

58. A **perfusion process** is a more complex setup involving a reactor that is continuously fed with cell culture media, and from which antibody is continuously harvested. A perfusion process can run longer than batch or fed-batch processes, but is more technically challenging to design and operate. Perfusion processes are rarely used for commercial antibody manufacturing.

59. Whichever production method is used, the antibody must be separated from the cells and cellular debris in a process called harvest. Harvest typically involves filtration, sometimes preceded by centrifugation, to provide a preparation of antibody in a cell-free medium.

60. Harvested antibody is purified in a series of steps that typically includes various forms of chromatography and filtration, as well as viral inactivation. Chromatographic and filtration steps remove unwanted components from the harvested cell culture fluid, such as antibody aggregates, unwanted antibody variants, antibody fragments, host cell proteins, and

[redacted]

viruses. Viral inactivation kills any virus that might be present. Purification may also involve mixing with other components to stabilize the antibody.

61. The end result of the commercial antibody production process (whichever method is used) is a solution of purified antibody. In commercial therapeutic antibody manufacturing, this purified antibody is referred to as the "drug substance." All the steps—from thaw to expansion to cell culture to harvest and purification—are considered part of drug substance manufacturing. Once recovered, the antibody drug substance is typically frozen for storage until it is formulated.

62. A drug substance must be formulated before it is ready for use by health care providers and patients. It must first be formulated into an approved dosage form, which is referred to as the "drug product." Antibody dosage forms are typically solutions for subcutaneous (s.c.) injection or intravenous (i.v.) infusion. Antibody formulation involves combining the drug substance with excipients that will stabilize the antibody in solution and facilitate administration. Drug product manufacturing for antibodies also involves filling the formulated solution into physical vials or syringes that will be sold for injection or infusion.

63. During an antibody drug manufacturing process, various measurements are typically performed to assess the properties of the antibody preparation. **C-terminal lysine variants** and the **glycan pattern** are two such properties that may be measured, but there are many more. These measurements may be performed during production, just prior to harvest, and/or during purification, and are often done on final drug substance, formulated drug product, or both. There may be particular targets set that these various properties must meet. If a certain property measured for the antibody preparation is inconsistent with the targets established for that preparation, it will typically not be immediately advanced or released to the next stage of processing, and may ultimately be rejected.

[redacted]

64. Cutoffs for the values of certain measured properties are sometimes referred to as "action limits," and properties that have an "action limit" and/or are considered important to drug quality are sometimes referred to as "critical parameters" or "critical quality attributes" (CQA). During design of the manufacturing process, the critical parameters, CQAs, and other important parameters are determined. As part of an activity called process validation, the manufacturing process is typically repeated to confirm that properties for such parameters fall within action limits or are otherwise consistent with the target values for each parameter.

VIII. THE '858 PATENT

65. The '858 patent issued on October 5, 2016. I have been asked to assume that the priority date of the '858 patent is July 8, 2011.

A. Overview of '858 Patent Disclosure

66. The '858 patent describes producing recombinant polypeptides involving culturing cells in a medium with specified amounts of "lysine, arginine, and/or agents that change intracellular pH" that do not adversely affect "cell growth, viability, or titer." Ex. A1, '858 patent at 19:49-62.

67. The '858 patent describes conditions in the cell culture medium which lead to an increase in the intracellular pH of cultured cells, such as cell media comprising about 1-50 g/L lysine, arginine, and/or histidine. *Id.* at 1:38-41.

68. The cell medium conditions described can influence C-terminal lysine variants in the manufacture of polypeptides (such as recombinant antibodies). *Id.* at 1:55-2:43.

69. The '858 patent addresses the issue that "C-terminal lysine or arginine residues are often absent in proteins isolated from mammalian cell cultures, even though their presence may be expected on the basis of gene sequence." *Id.* at 23:26-30. The patent teaches that this is often due to the activity of carboxypeptidases, such as carboxypeptidase B and carboxypeptidase H, which

[redacted]

are enzymes that hydrolyze the C-terminal amide bonds in polypeptides. *Id.* at 23:30-49. The patent teaches that lysine or arginine in the cell culture medium may work to ultimately attain a targeted level of C-terminal variants of polypeptides. *See, e.g.*, *id.* at 24:4-25.

70. The '858 patent further discloses how such methods are applied to manufacture of an antibody preparation in connection with making a biosimilar version of a reference product. *Id.* at 36:18-60.

B. The Asserted '858 Patent Claim

71. Claim 33 (which depends from claim 20) recites:

> **20.** A method of manufacturing a preparation of a recombinant antibody, comprising:
> culturing a cell in a medium comprising 2 g/L arginine to 8 g/L arginine under conditions in which the cell expresses a recombinant antibody;
> isolating the recombinant antibody, thereby producing a preparation of the recombinant antibody;
> and formulating the preparation into a drug product if the preparation meets a target value of C-terminal variants of the recombinant antibody, wherein the C-terminal variants differ in amino acid sequence only by the presence or absence of a lysine at their carboxyl termini.
>
> **33.** . . . wherein the cell is a CHO cell.

C. Claim Interpretation

72. In my analysis and in reaching my conclusions in this declaration, I have given the claim terms their plain and ordinary meaning as understood by a POSA in July 2011. I understand I will have the opportunity to respond to any proposed alternative claim interpretation offered by Amgen.

IX. THE '168 PATENT

73. The '168 patent issued on December 22, 2015. I have been asked to assume that the priority date of the '168 patent is March 14, 2013.

[redacted]

A. Overview of '168 Patent Disclosure

74. The '168 patent describes a method of producing recombinant protein involving culturing cells in a medium with a specified amount of putrescine to influence levels of glycans. '168 patent at 11:50-62. In an example in the '168 patent's specification, putrescine levels in the cell culture medium were varied between 0.17 mg/L and 0.52 mg/L. Ex. A2, '168 patent at 32:40-33:23.

75. The '168 patent describes that concentrations of putrescine in the culture medium may allow for the influence of the proportions of one or more mature glycans in the synthesis of polypeptides as part of making a biosimilar to a reference product. *See, e.g.*, *id.* at 1:33-61; 4:24-64. Glycan proportions discussed include fucosylated glycans, galactosylated glycans, high mannose glycans, and sialylated glycans. *Id.*

76. The '168 patent demonstrates exemplary methods whereby altering levels of putrescine in the cell culture medium of cultured Chinese hamster ovary (CHO) cells results in differing glycosylation levels of a model antibody produced by the CHO cells. For instance, fucosylated glycans, galactosylated glycans, and sialylated glycans **increased** with increasing levels of putrescine, whereas high mannose glycans **decreased** with increasing levels of putrescine, suggesting that putrescine may promote the maturation of glycans. *Id.* at 32:40-33:28.

B. The Asserted '168 Patent Claim

77. Claim 23 (which depends from claim 1, 10, 13, or 16) recites:

> **1.** A method of producing a recombinant protein preparation having a target value of one or more galactosylated glycans, high mannose glycans, and sialylated glycans, the method comprising:
> (a) providing a cell genetically engineered to express a recombinant protein;
> (b) culturing the cell in a culture medium comprising 0.1 mg/L to 10 mg/L putrescine under conditions in which the cell expresses the recombinant protein; and

[redacted]

(c) harvesting a preparation of the recombinant protein produced by the cell that meets the target value of the one or more of galactosylated glycans, high mannose glycans, and sialylated glycans,

wherein the target value of galactosylated glycans, or sialylated glycans is a level at least 10% higher than a level of galactosylated glycans, or sialylated glycans in a preparation produced by culturing the cell in the medium not comprising 0.1 mg/L to 10 mg/L putrescine; or

wherein the target value of high mannose glycans is a level at least 10% lower than a level of high mannose glycans in a preparation produced by culture the cell in the medium not comprising 0.1 mg/L to 10 mg/L putrescine.

23. … wherein the cell is a Chinese Hamster Ovary (CHO) cell.

78. I have been asked to consider claim 23 as it depends from claim 1.

C. Claim Interpretation

79. In my analysis and in reaching my conclusions in this declaration, I have given the claim terms their plain and ordinary meaning as understood by a POSA in March 2013. I understand I will have the opportunity to respond to any proposed alternative claim interpretation offered by Amgen.

X. JANSSEN'S STELARA®

80. Janssen manufactures and sells STELARA® for the treatment of plaque psoriasis, psoriatic arthritis, Crohn's disease, and ulcerative colitis. Ex. A3 at 1 (FDA labels for ustekinumab (STELARA®) 2022). The active ingredient, or drug substance, in STELARA® is ustekinumab. *Id.*

81. Ustekinumab is a recombinant antibody manufactured [redacted]

[redacted]

82. The properties of the STELARA® drug substance are in part due to the cell line in which the antibody is expressed. [redacted] These characteristics include a particular distribution of C-terminal variants and a particular glycan pattern.

83. The STELARA® drug substance has a [redacted]

84. The STELARA® drug substance has the following glycan levels: [redacted]

[redacted]

XI. AMGEN'S ABP 654 (BIOSIMILAR COPY OF STELARA®)

A. The ABP 654 Antibody

85. [redacted] "ABP 654 is a biosimilar to Stelara® (ustekinumab)." Ex. A12, Amgen_ABP654_000038766 at 38769 (§ 2.3.S – Quality Overall Summary, Drug Substance). The active ingredient and drug substance in ABP 654 is a recombinant antibody directed against the "the commonly shared p40 subunit of interleukin-23 and interleukin-12 cytokines." *Id.*

86. [redacted], "[b]oth ABP 654 and Stelara (ustekinumab) are recombinant human immunoglobulin isotype class G subclass 1 kappa monoclonal antibodies . . . ." Ex A13, Amgen_ABP654_000038570 at 38571 (§ 2.2 – Introduction). "Both ABP 654 and Stelara (ustekinumab) are manufactured by recombinant DNA technology." *Id.*

[redacted]

87. Amgen's manufacturing process for ABP 654 was developed [redacted]

[redacted]

[redacted]

88. [redacted]

[redacted]

B. Amgen's Cell Line

89. Amgen produces ABP 654 by [redacted]

[redacted]

90. [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

91. [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

C. Amgen's Cell Culture

92. [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

93. The cell culture process for ABP 654 involves multiple steps to [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

94. [redacted]

[redacted]

95. [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

96. [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

97. [redacted]

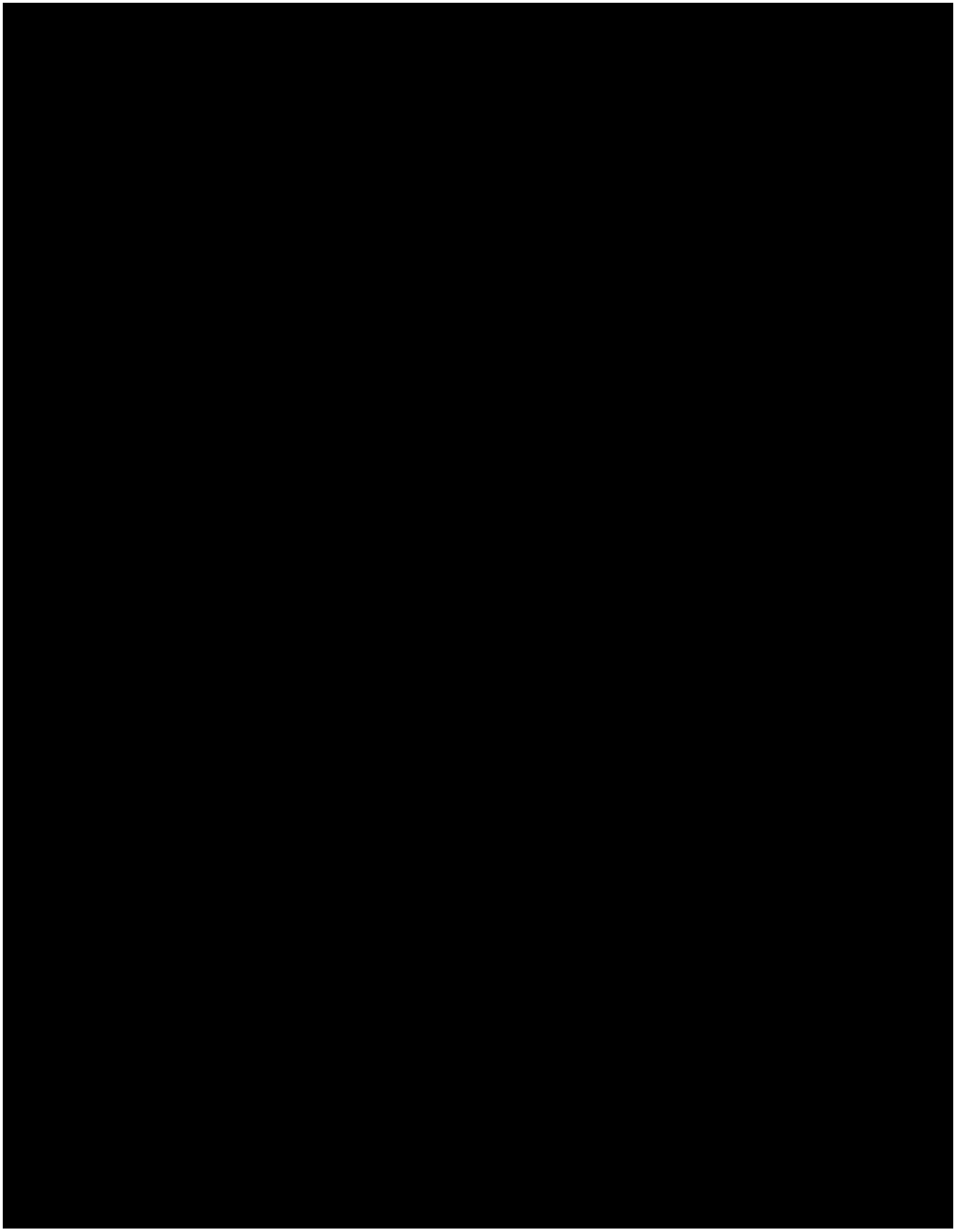
[redacted]

[redacted]

[redacted]

[redacted]

[redacted]



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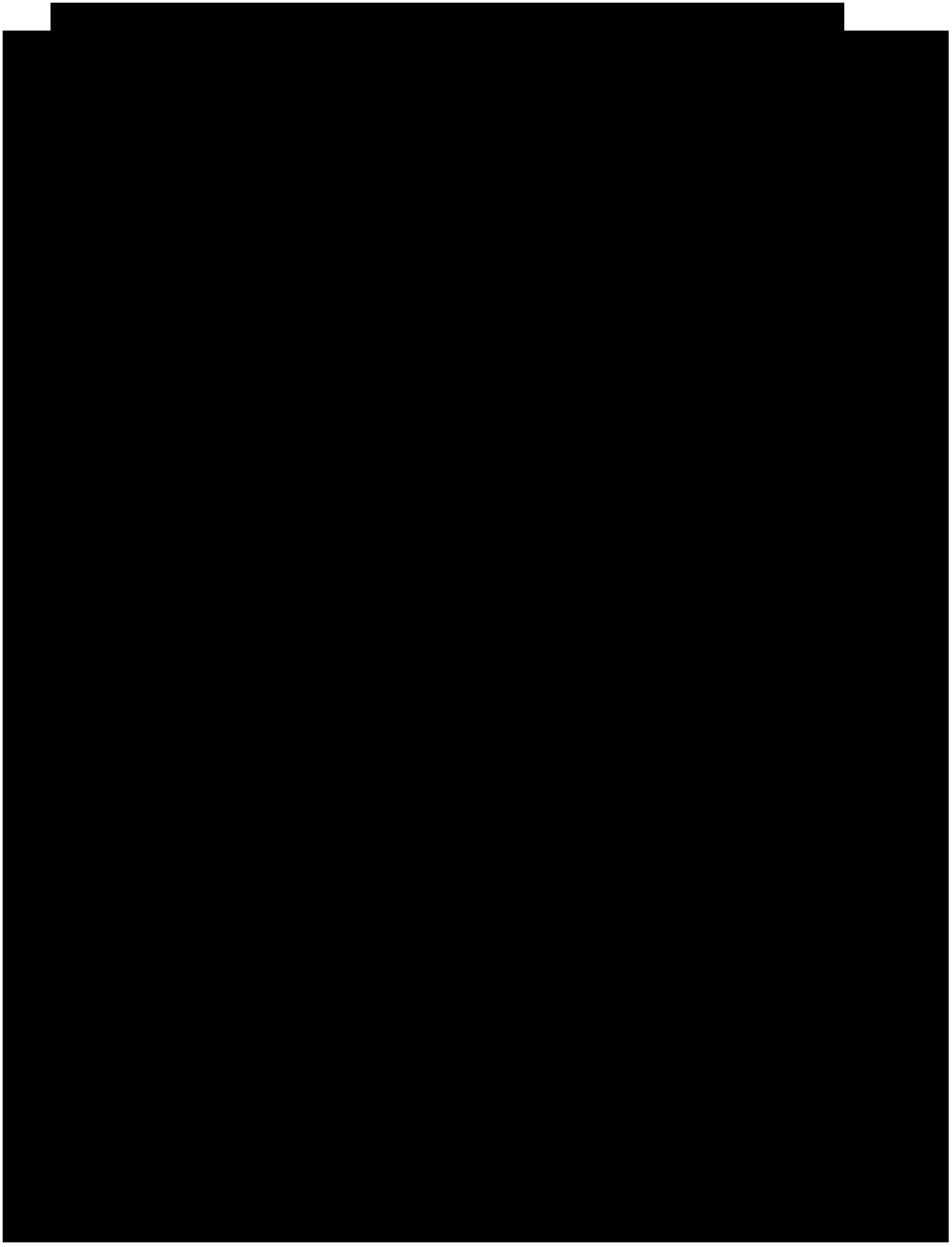

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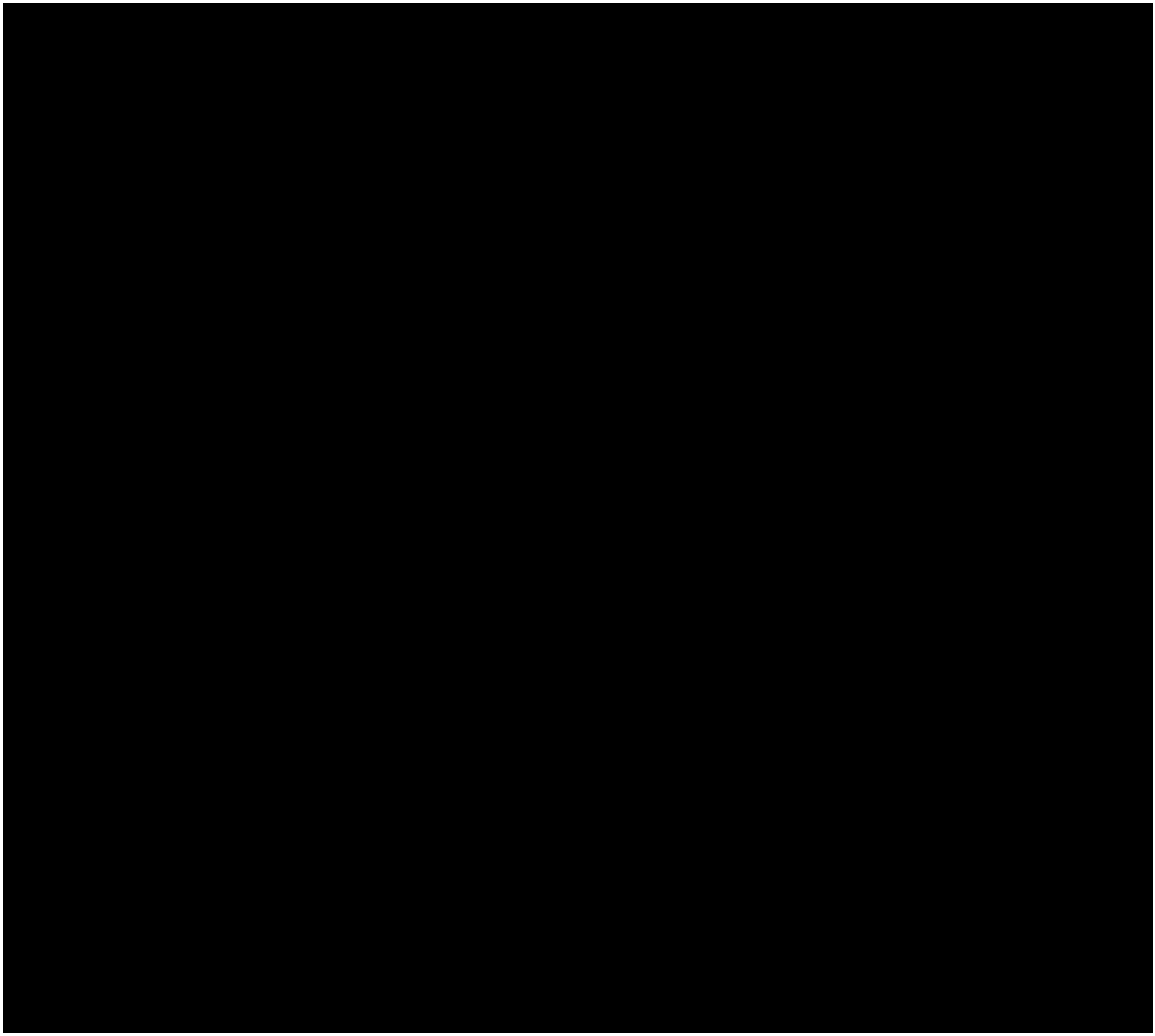

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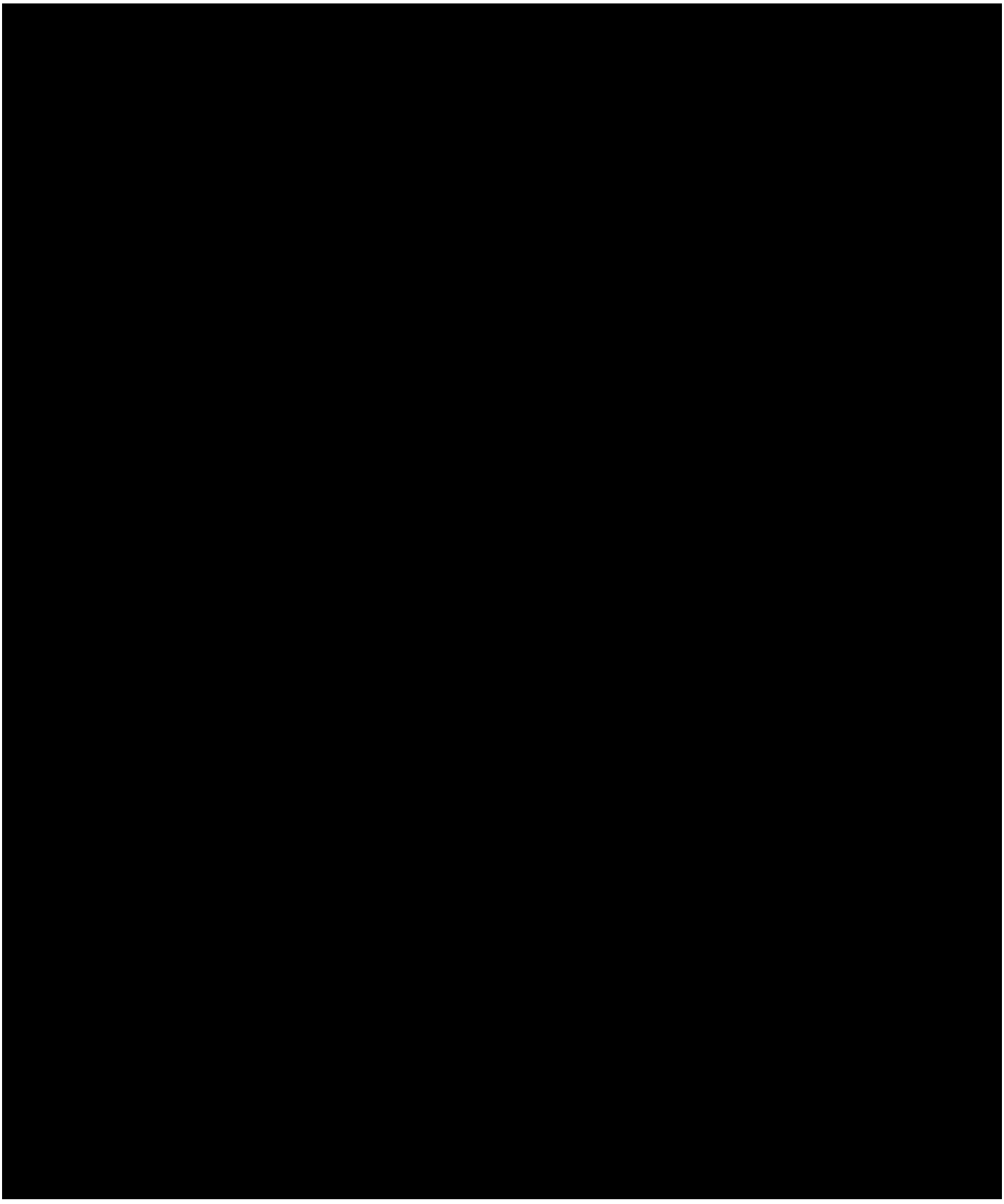
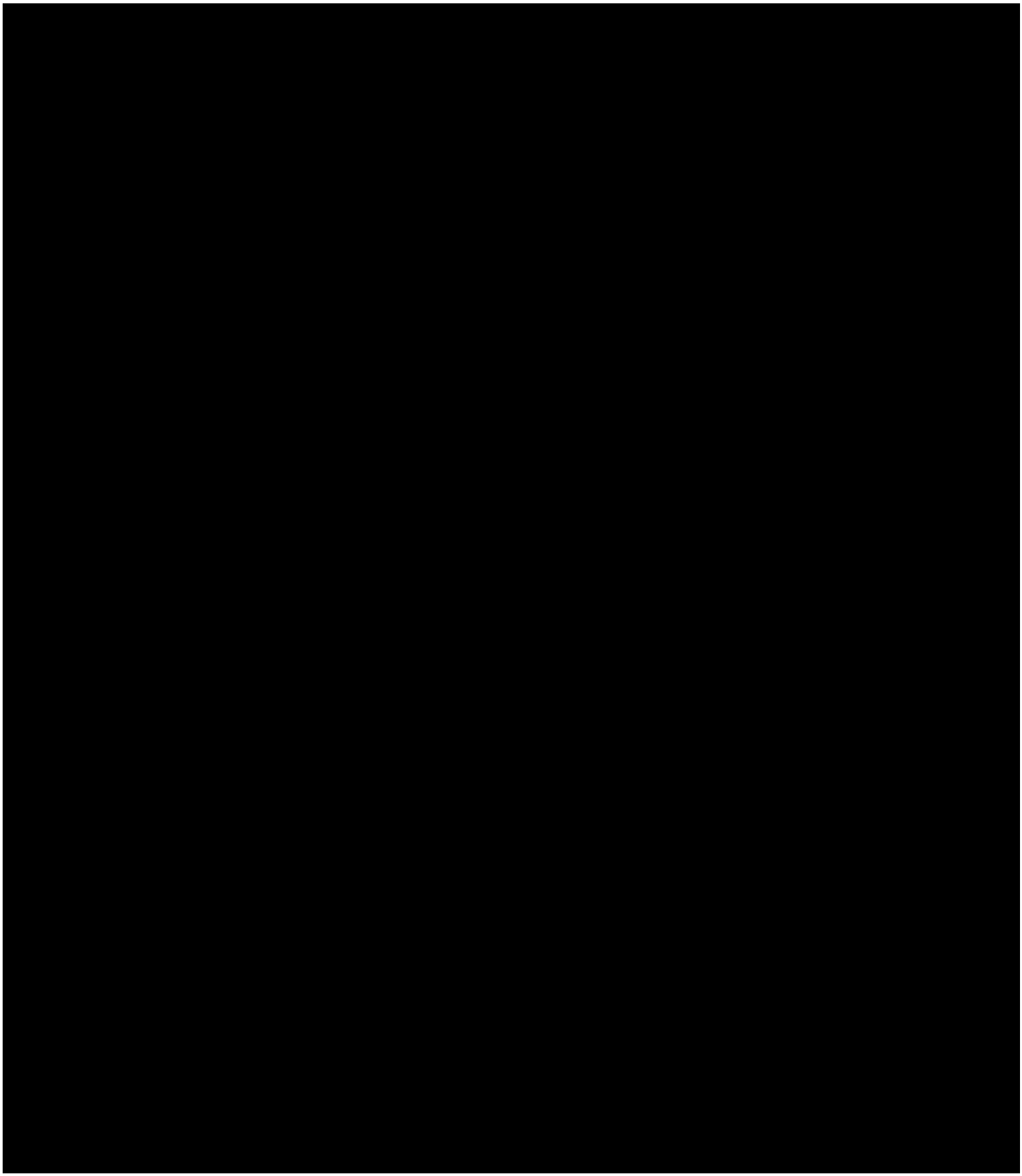
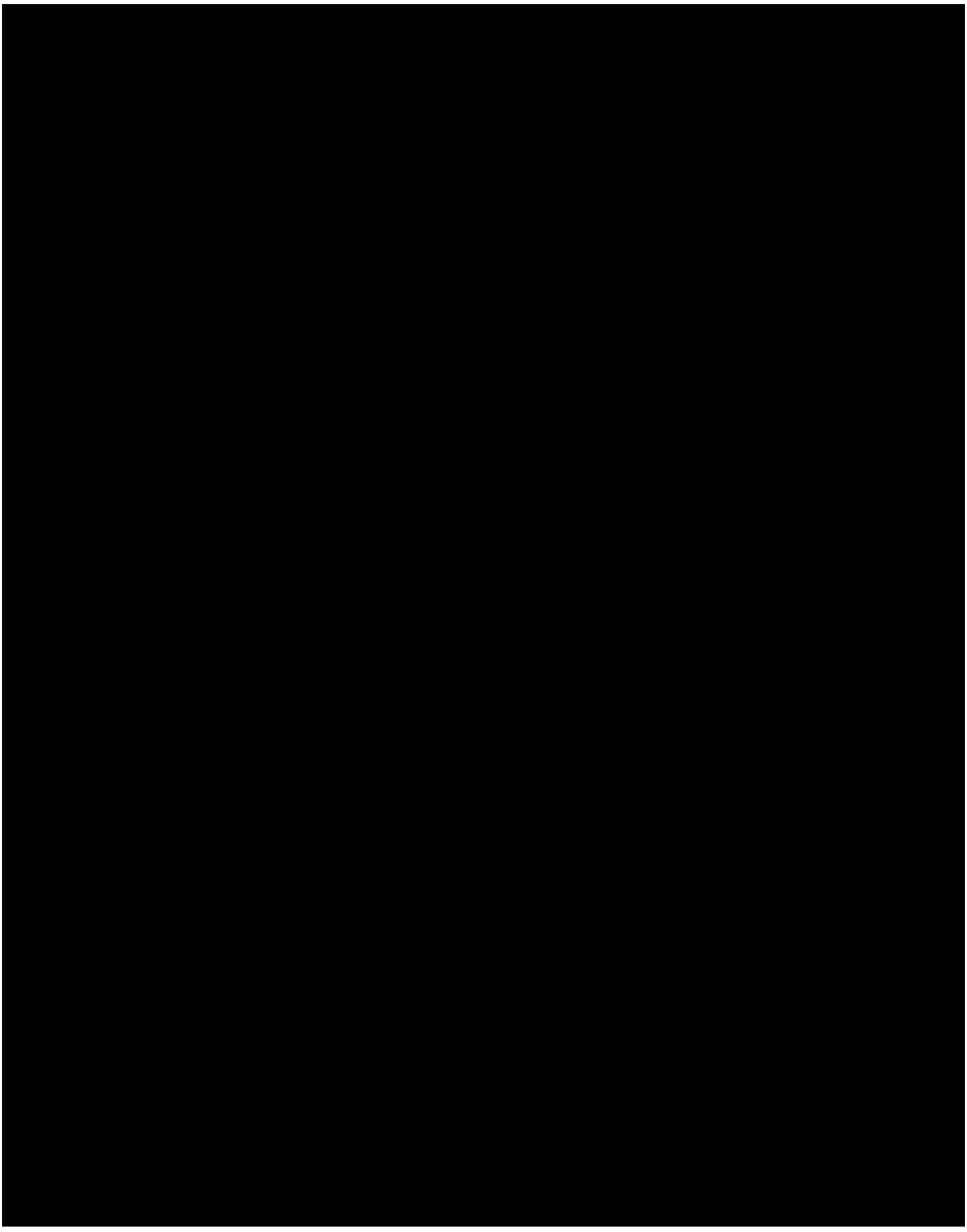
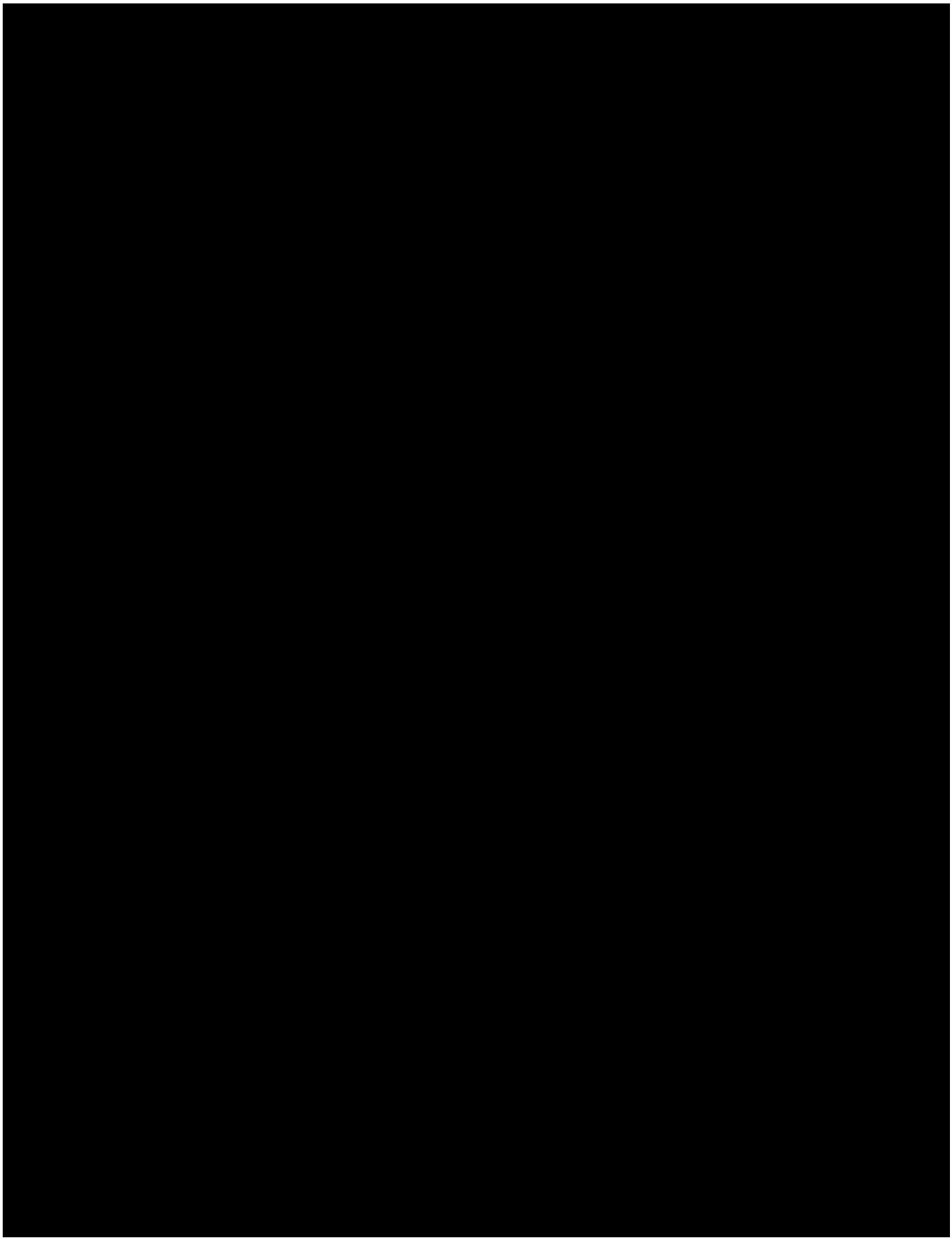






[redacted]

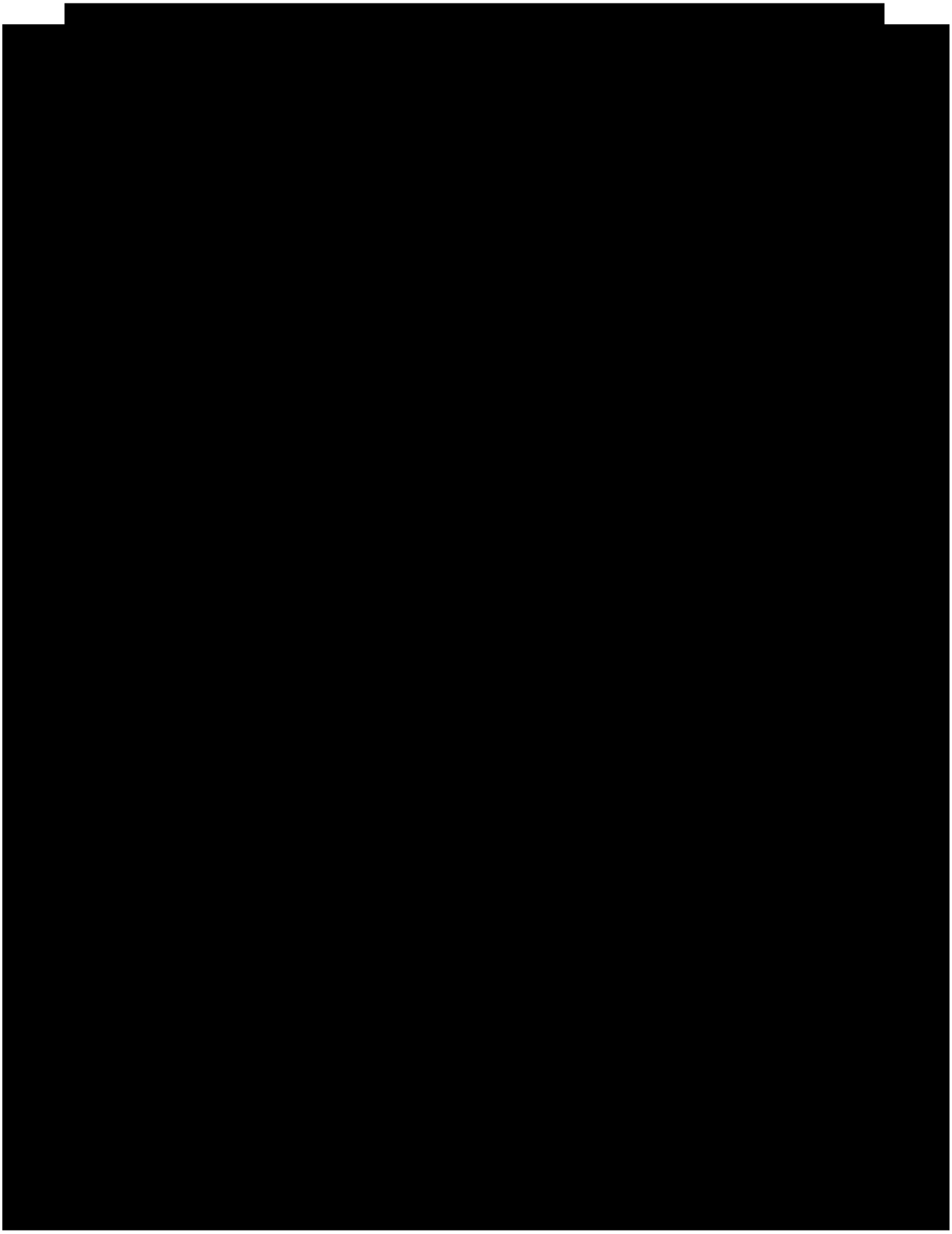
[redacted]



[redacted]

[redacted]

[redacted]



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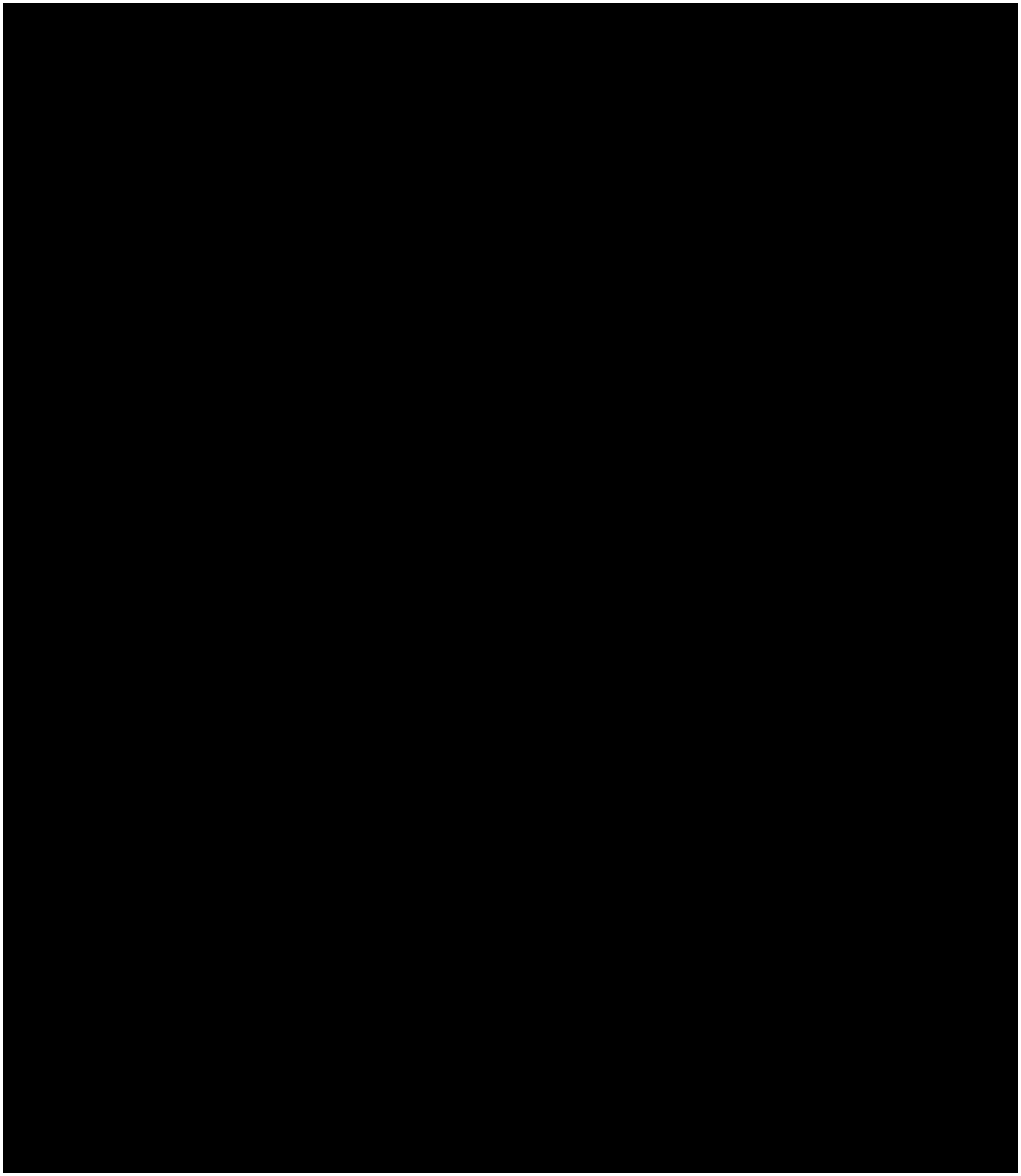


120. My analysis does not incorporate the very minor impact of culture sampling, by which small volumes of the culture are removed for testing. Accounting for sampling would, in

[redacted]

my conservative estimation, change the resulting arginine concentrations I have calculated by less than 1%.

121. Additionally, my analysis is based on the information available to me at this time from Amgen's aBLA concerning the cell culture process, [redacted]. I note that arginine is an amino acid that may be consumed or produced by cells, depending upon the culture conditions. I have considered the potential impact of arginine consumption and production during Amgen's production of ABP 654 and believe that any cellular consumption and production of arginine would cause only small changes in the arginine concentrations that would not be significant enough to change my conclusion that [redacted]

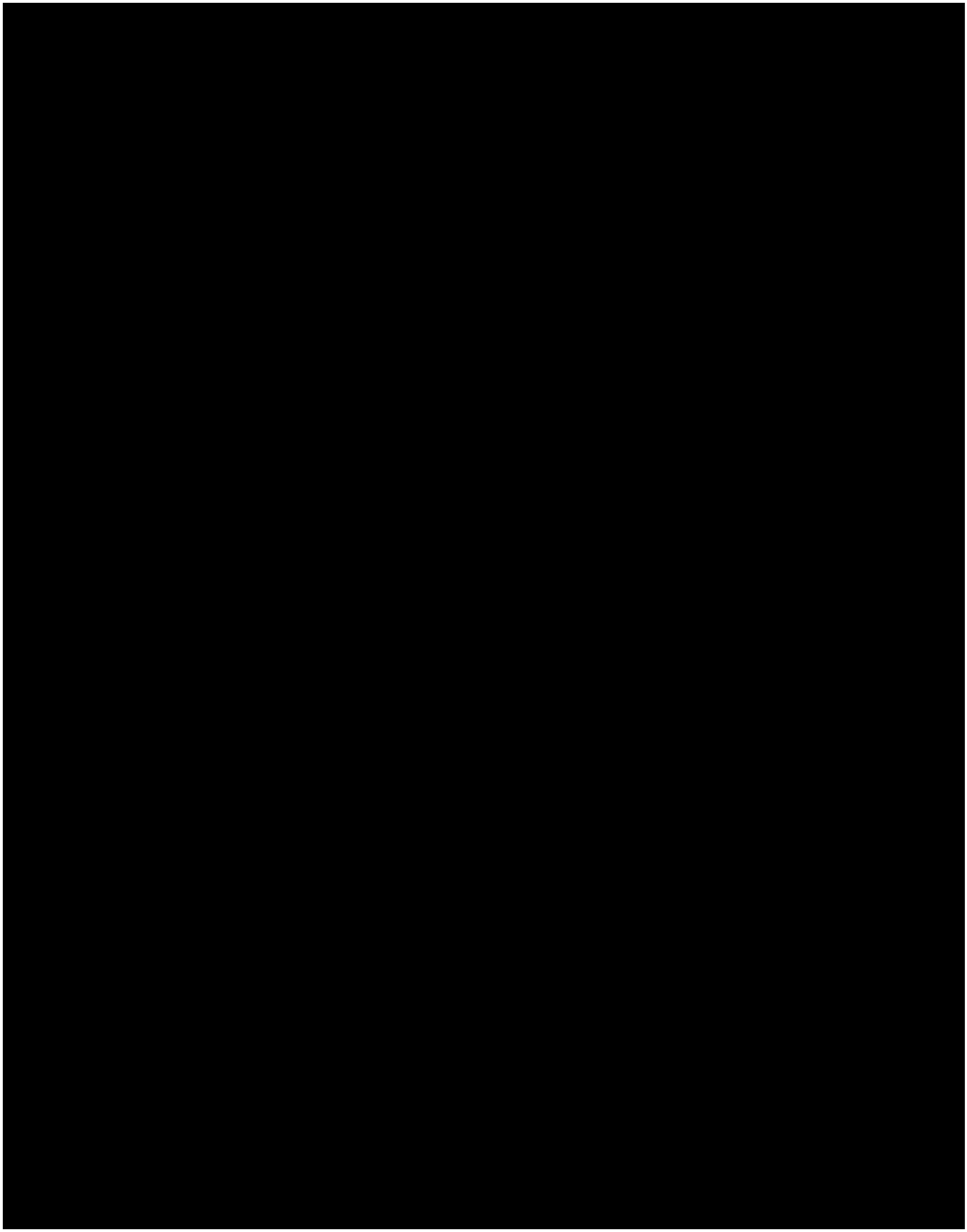
122. Amgen's aBLA also [redacted] Strictly speaking, to calculate the concentration of arginine in the culture medium immediately after the addition of production feed medium, one should use the total medium volume rather than the total culture volume. As mentioned previously, in some fed-batch cultures, the cells may occupy up to 10% of the total culture volume. In the Amgen production bioreactor, [redacted] Thus, the correction to total medium volume versus total culture volume could lead to a significant increase in arginine concentrations versus the estimates shown in the table above. This correction requires an estimate of the distribution of previously added arginine between the medium and cells. This correction also requires packed cell volume measurements at all steps of the culture analyzed above. Only the measurements on days 9, 10, and 11 were made available to me. Even without such corrections, [redacted]

[redacted]

3. Putrescine Concentration in Cell Culture Media

123. [redacted]

124. [redacted]



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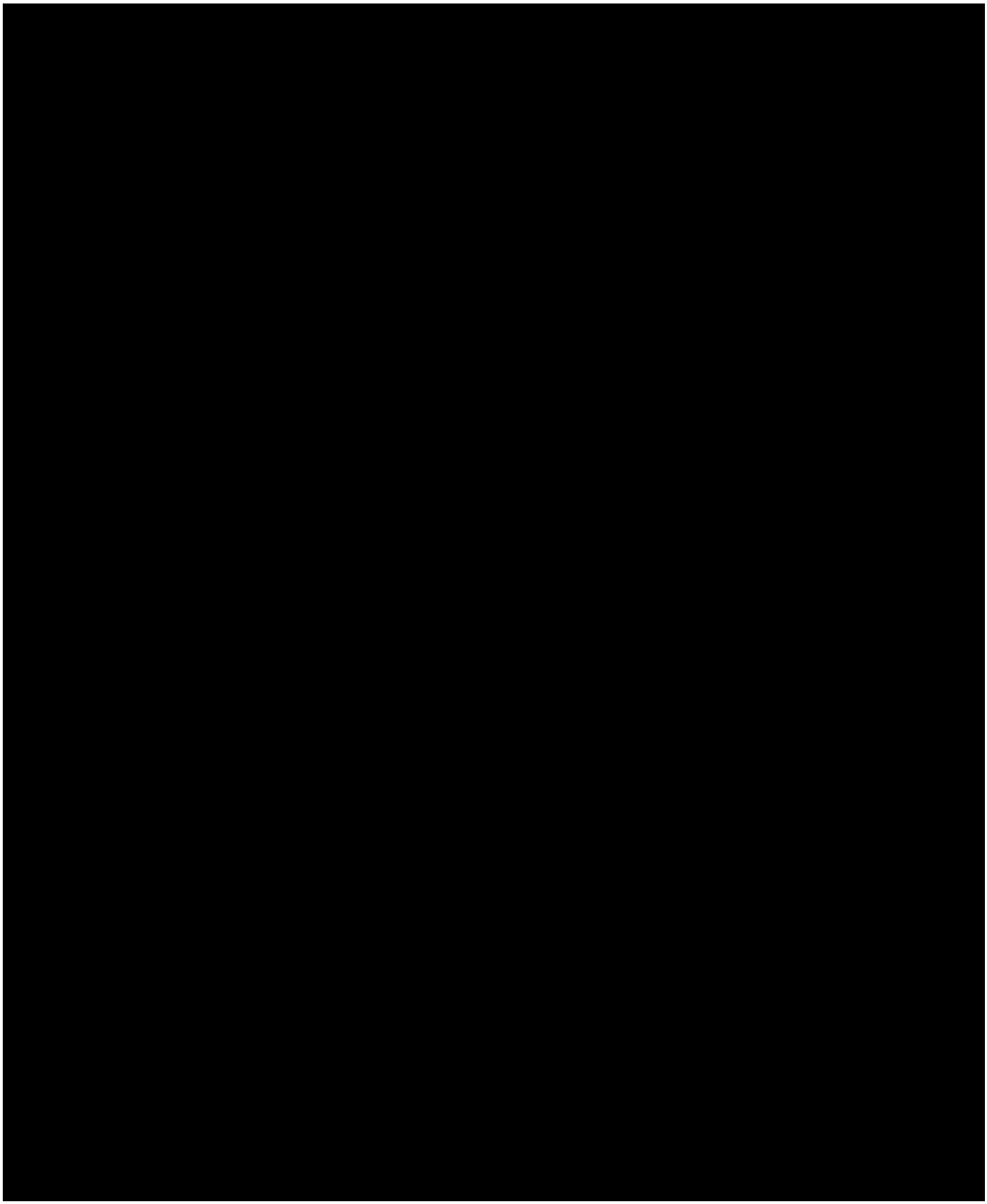


[redacted]

128. [redacted]

[redacted]

129. [redacted]

[redacted]

[redacted]

130. My analysis does not incorporate the very minor impact of culture sampling, by which small volumes of the culture are removed for testing. Accounting for sampling would, in my conservative estimation, change the resulting putrescine concentrations I have calculated by less than 1%.

131. Additionally, my analysis is based on the information available to me at this time from Amgen's aBLA concerning the cell culture process, which did not include measurements of putrescine concentration from the cell culture medium in the production bioreactor. I note that putrescine is a minor additive in cell cultures and not a nutrient that I would expect to be consumed or produced by cells in significant amounts. Accordingly, I believe that any cellular consumption and production of arginine would cause only small changes in the putrescine concentrations that would not be significant enough to change my conclusion that [redacted]

132. Amgen's aBLA [redacted]. Strictly speaking, to calculate the concentration of putrescine in the culture medium immediately after the addition of production feed medium, one should use the total medium volume rather than the total culture volume. As mentioned previously, in some fed-batch cultures, the cells may occupy up to 10% of the total culture volume. In the Amgen production bioreactor, [redacted] Thus, the correction to total medium volume versus total culture volume could lead to a significant increase in putrescine concentrations versus the estimates shown in the table above. This correction requires an estimate of the distribution of previously added putrescine between the medium and cells. This correction also requires packed cell volume measurements at all steps of the culture analyzed

[redacted]

above. Only the measurements on days 9, 10, and 11 were made available to me. Even without such corrections, [redacted]

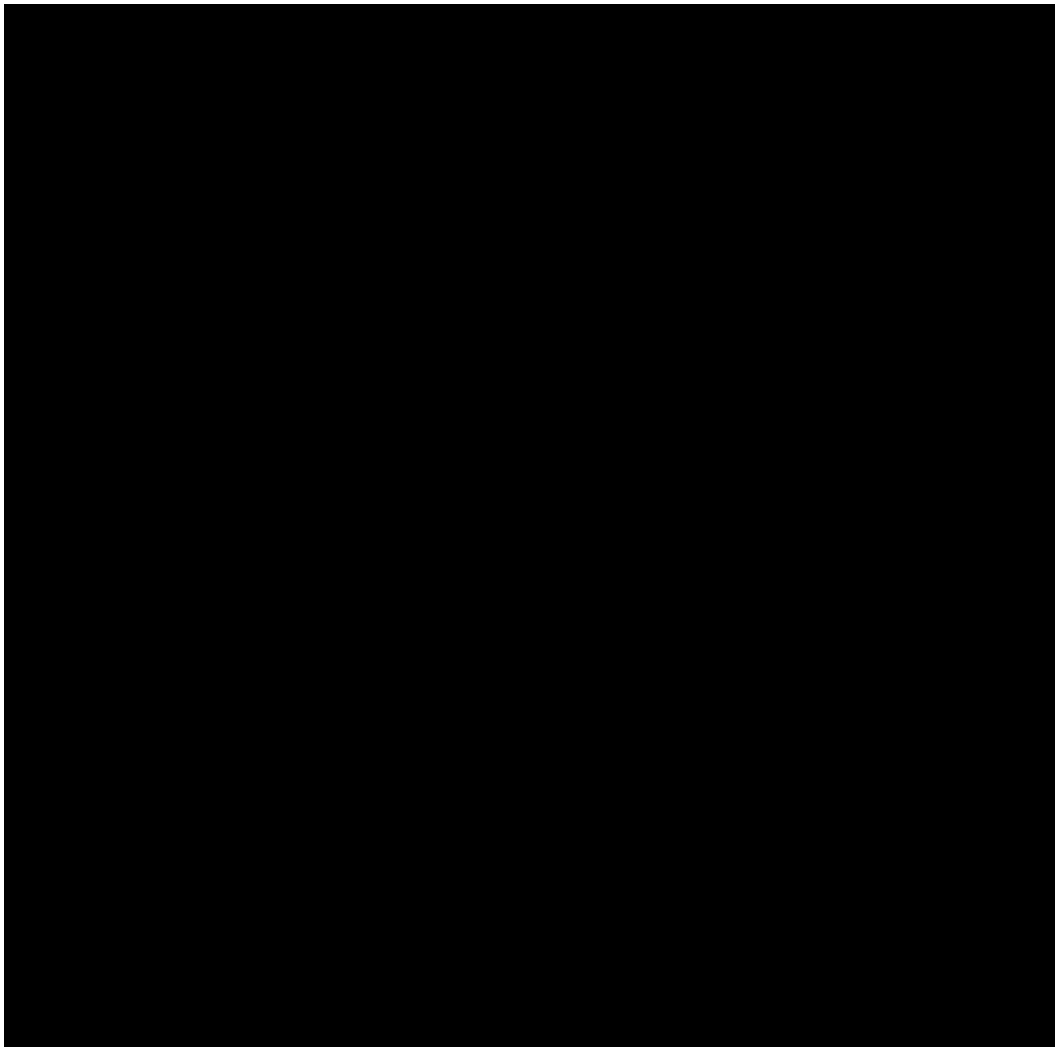
D. Amgen's Harvest and Purification of Drug Substance

133. [redacted]

[redacted]

[redacted]

[redacted]



[redacted]

[redacted]

E. Amgen's Formulation of Drug Product

136. [redacted]

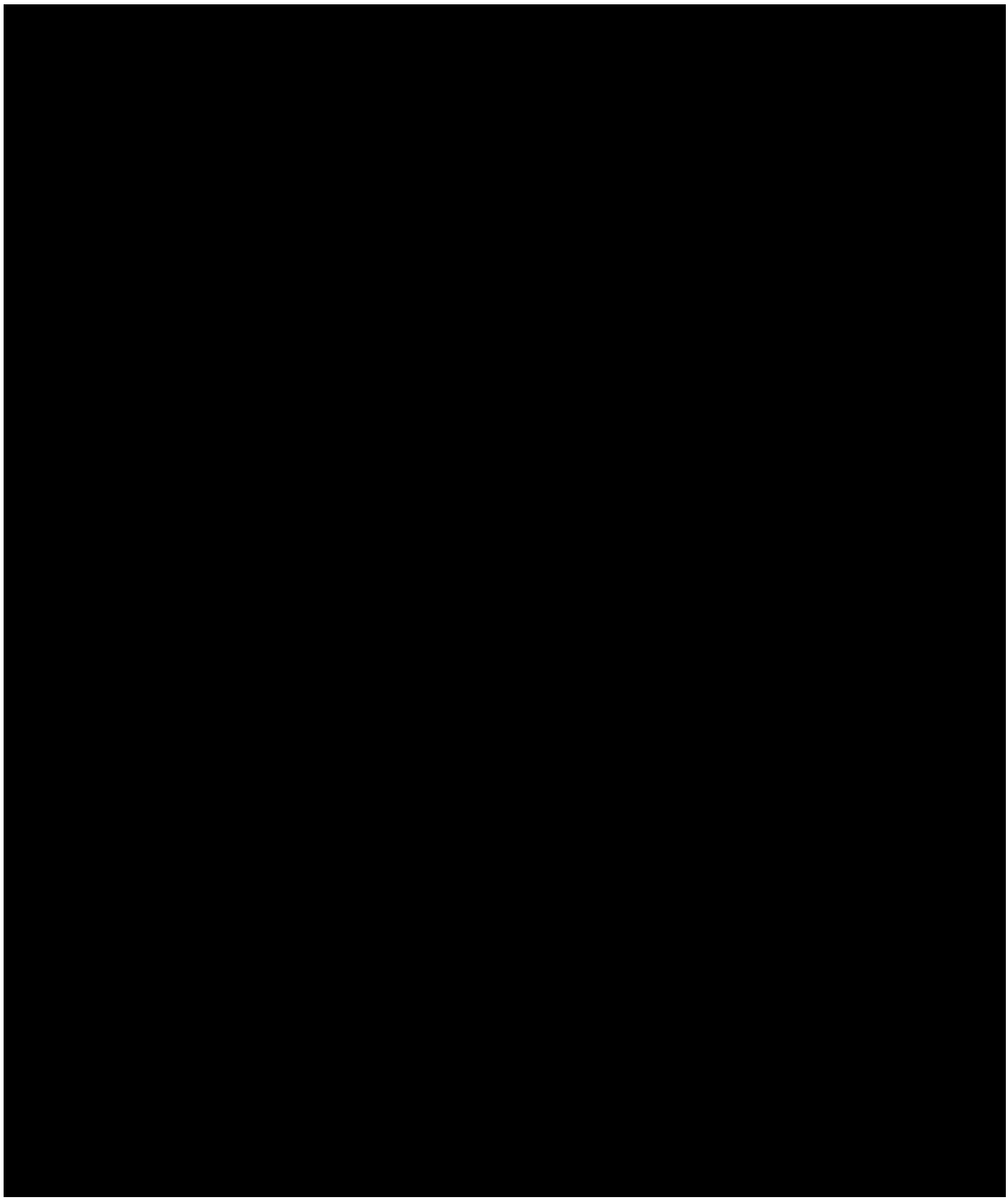
137. [redacted]

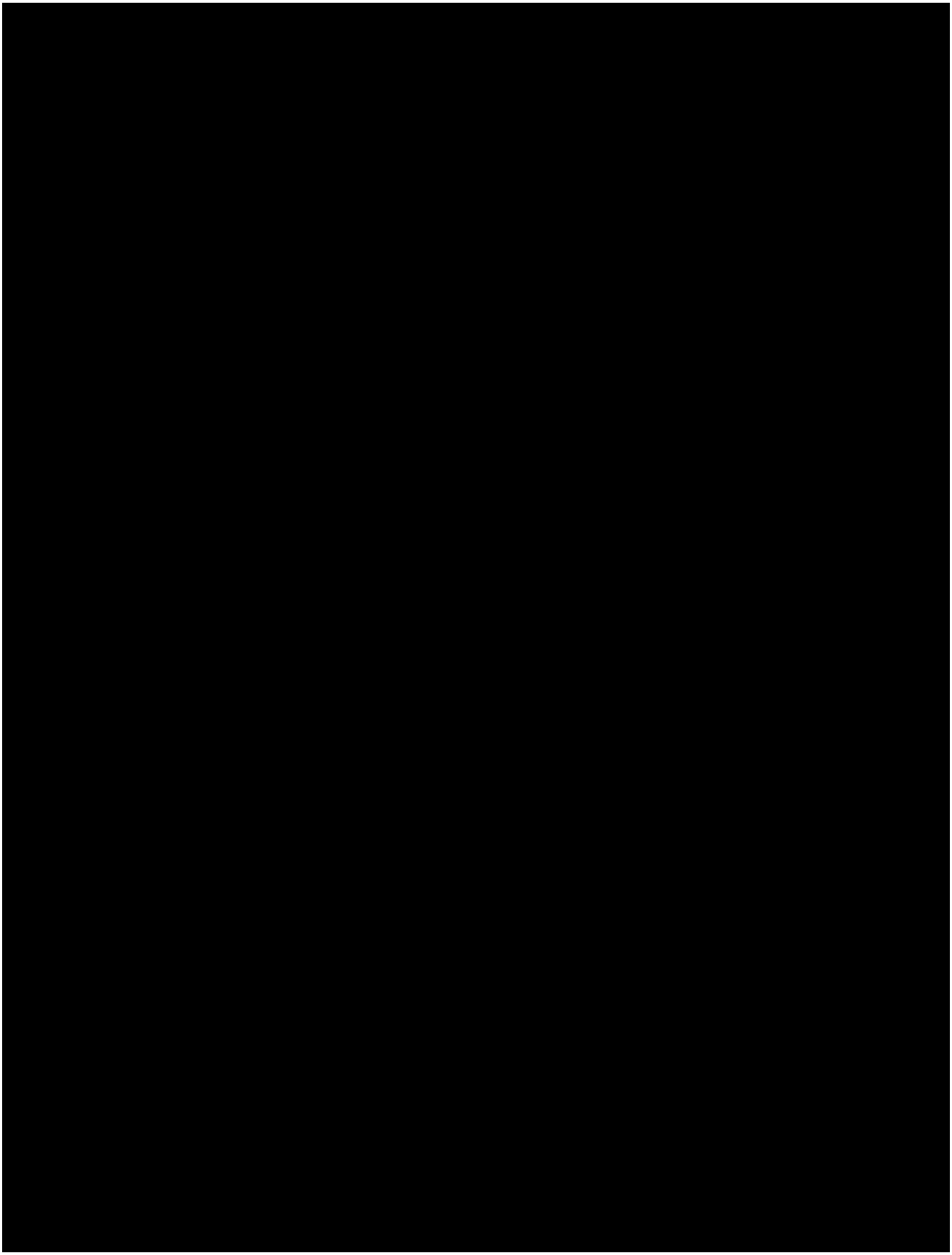
[redacted]

[redacted]

[redacted]

[redacted]





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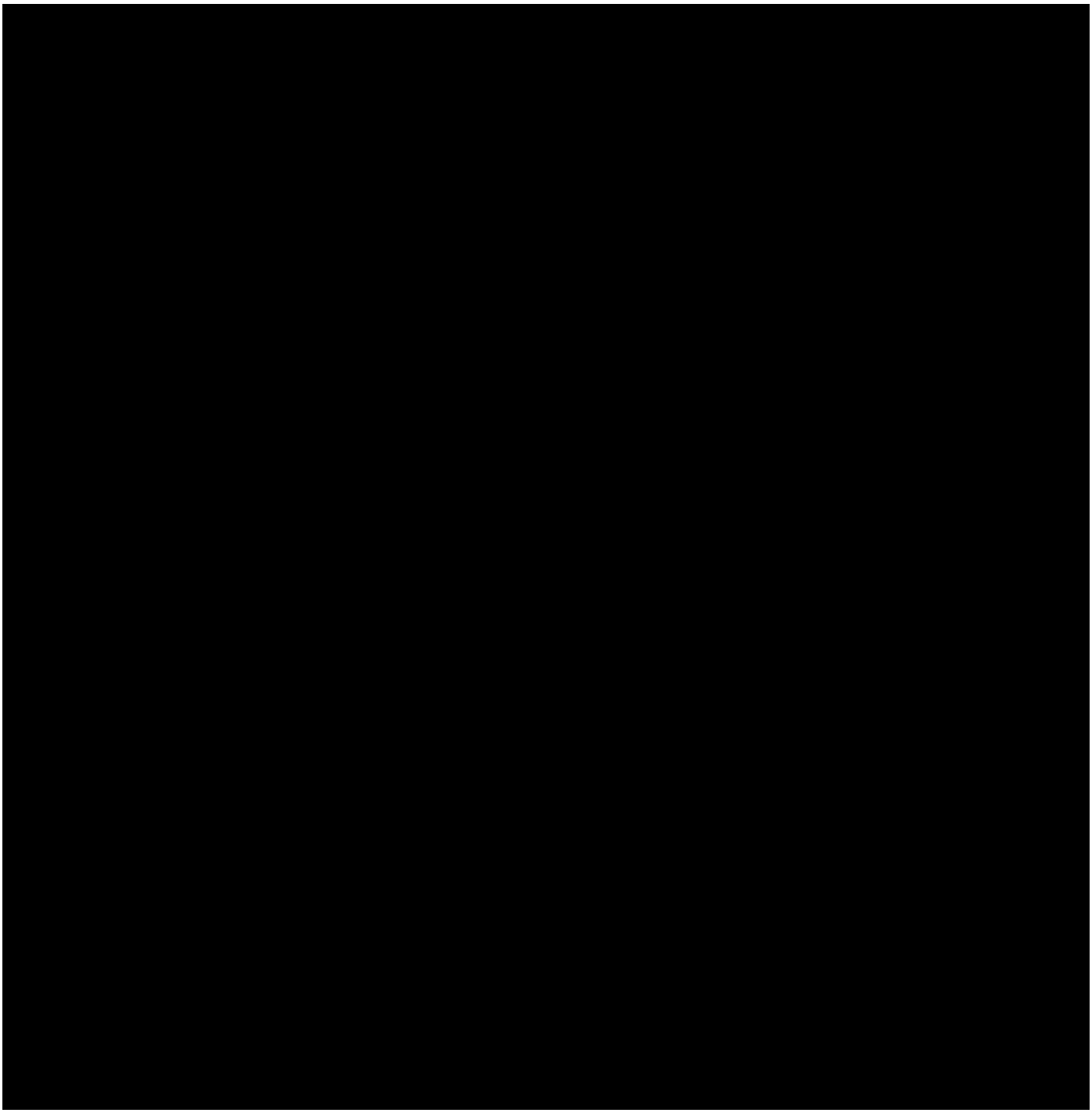
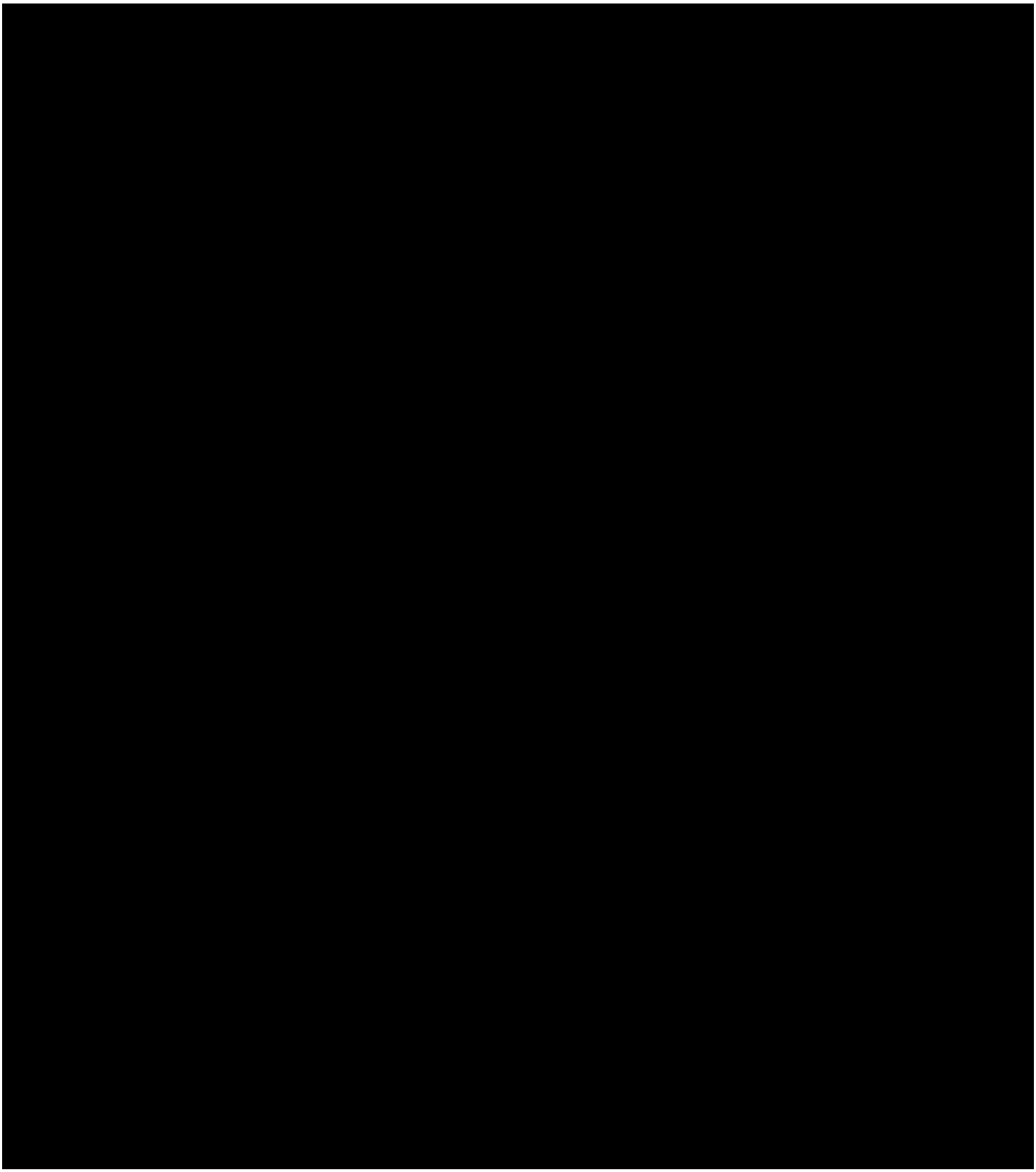


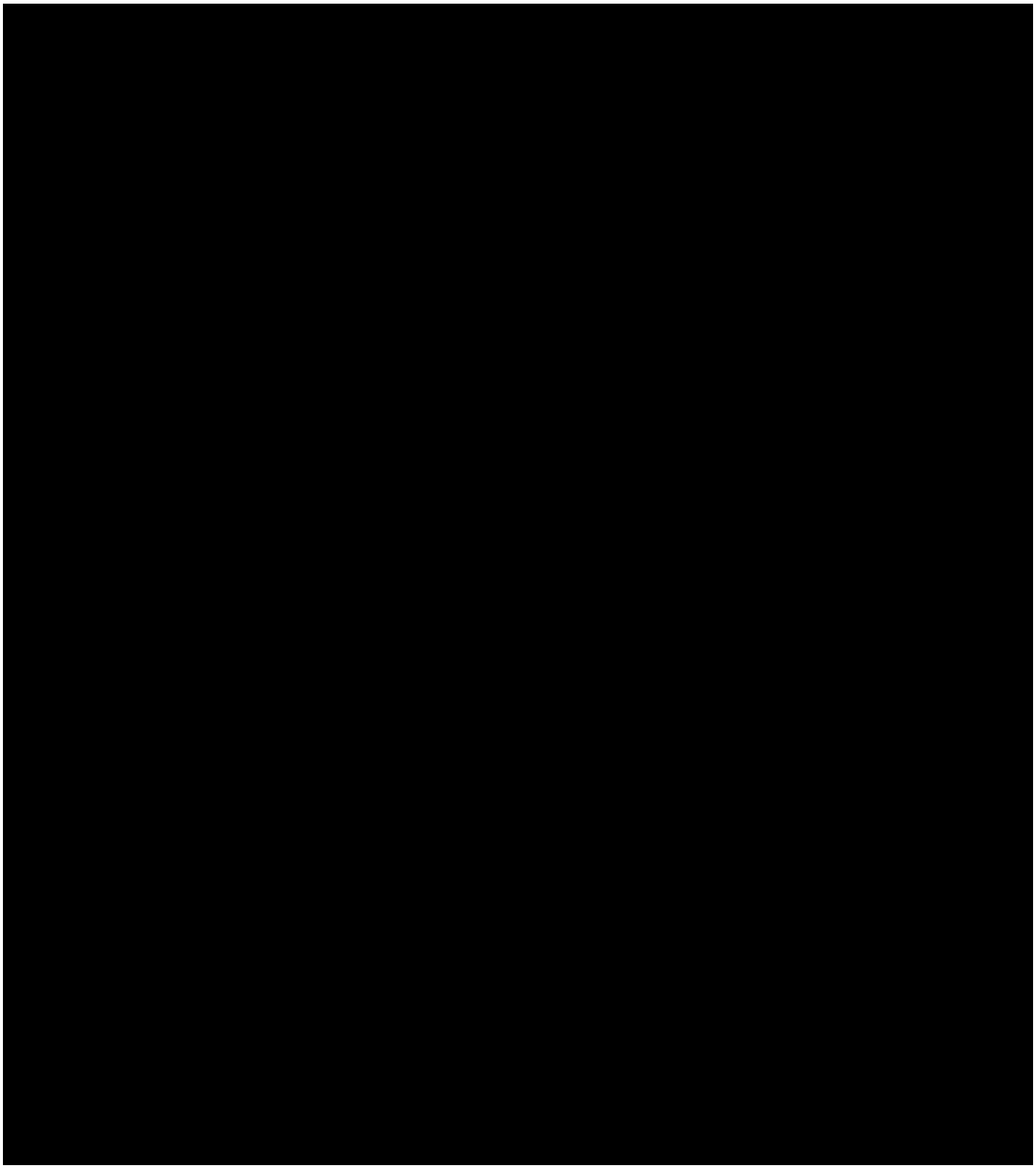
[redacted]

[redacted]

F. C-Terminal Variants: Amgen's Characterization and Targets

[redacted]





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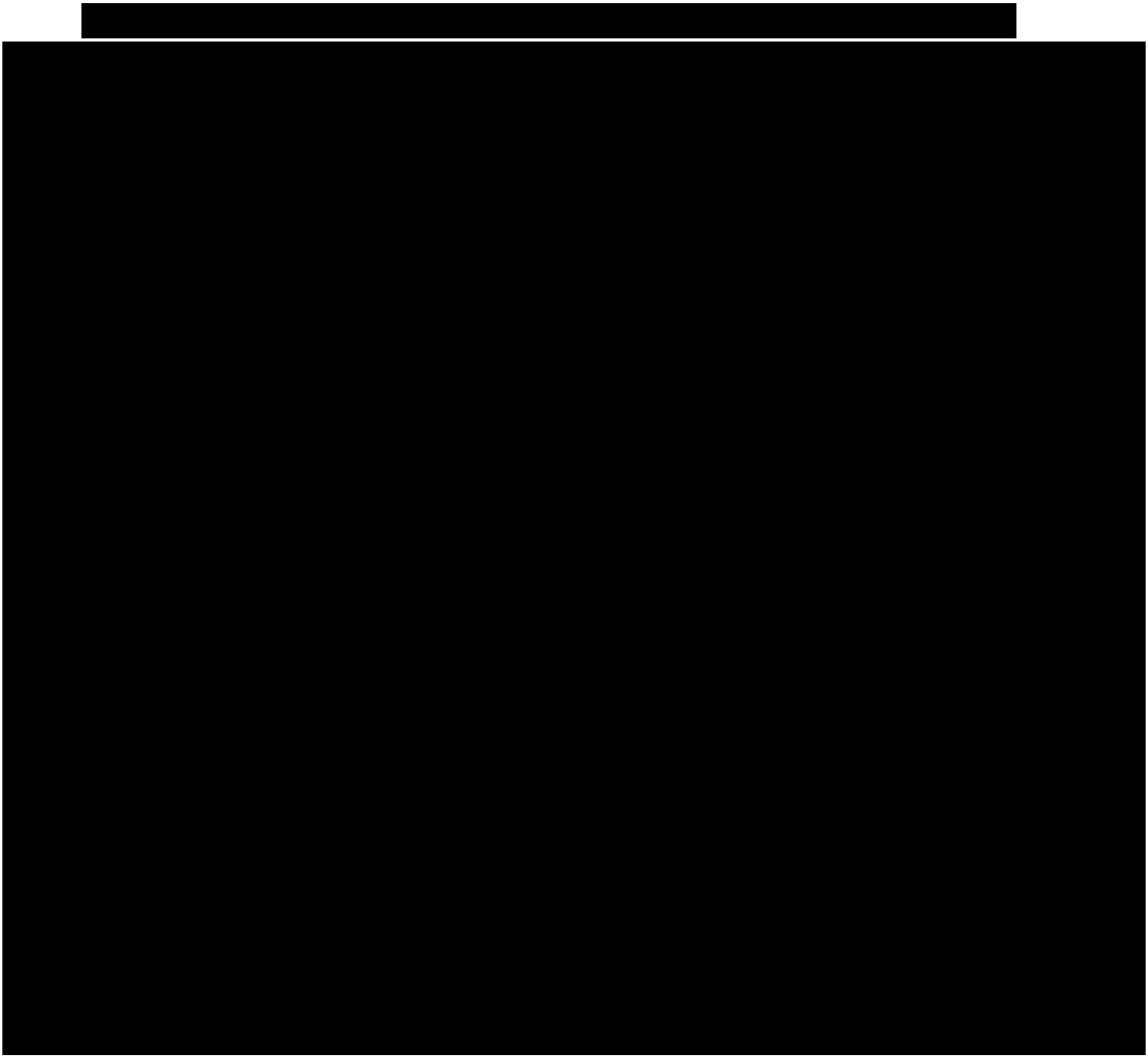
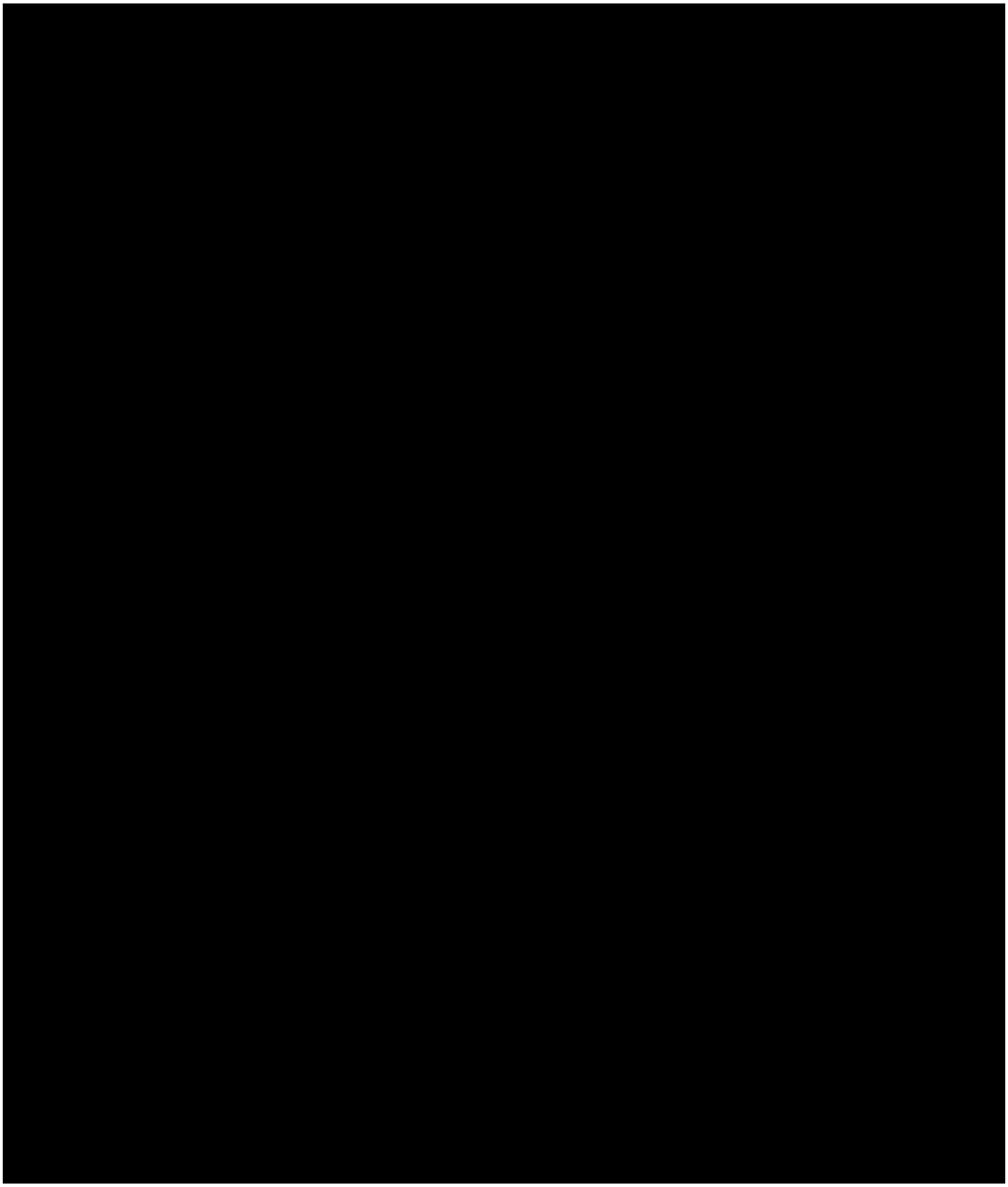
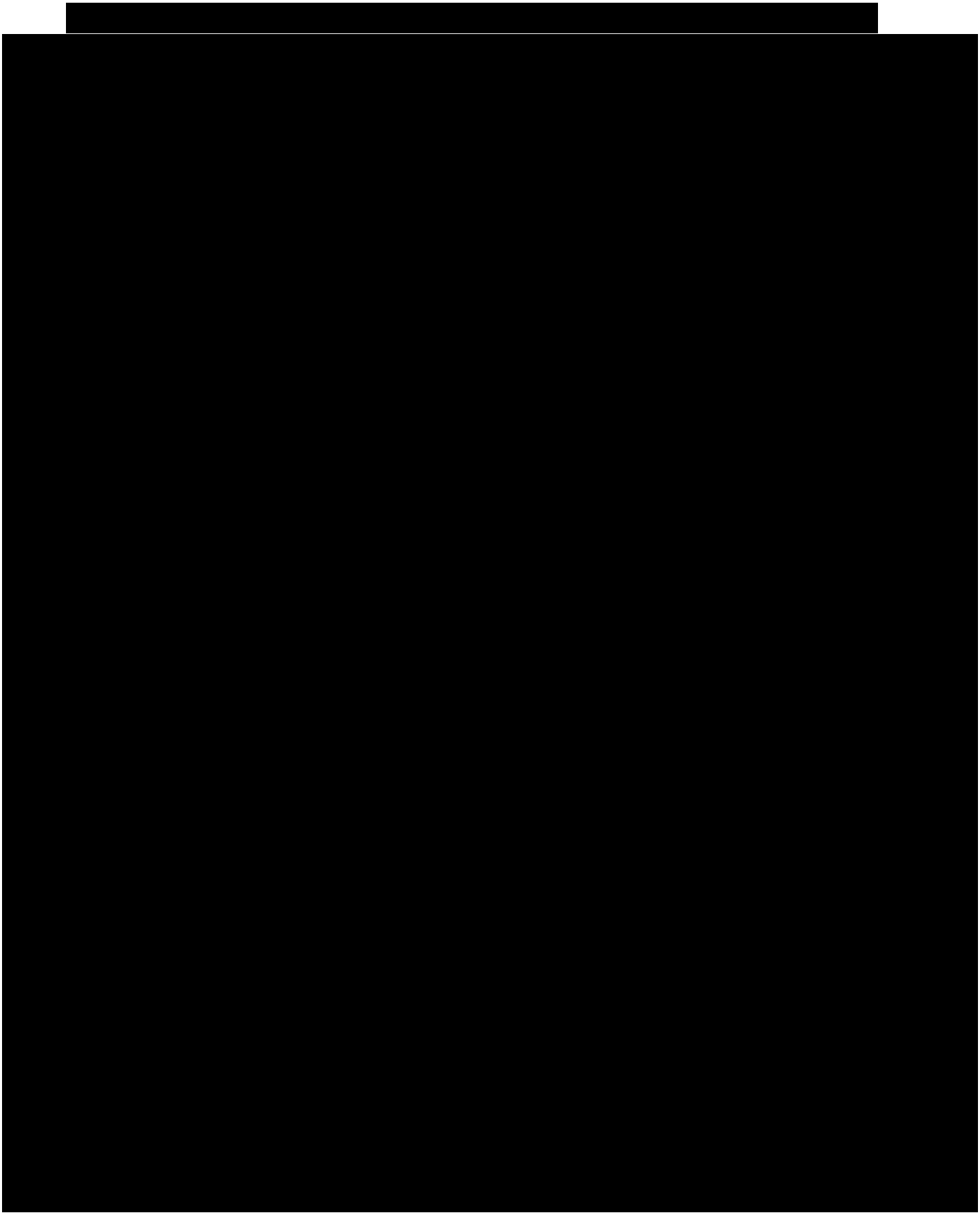
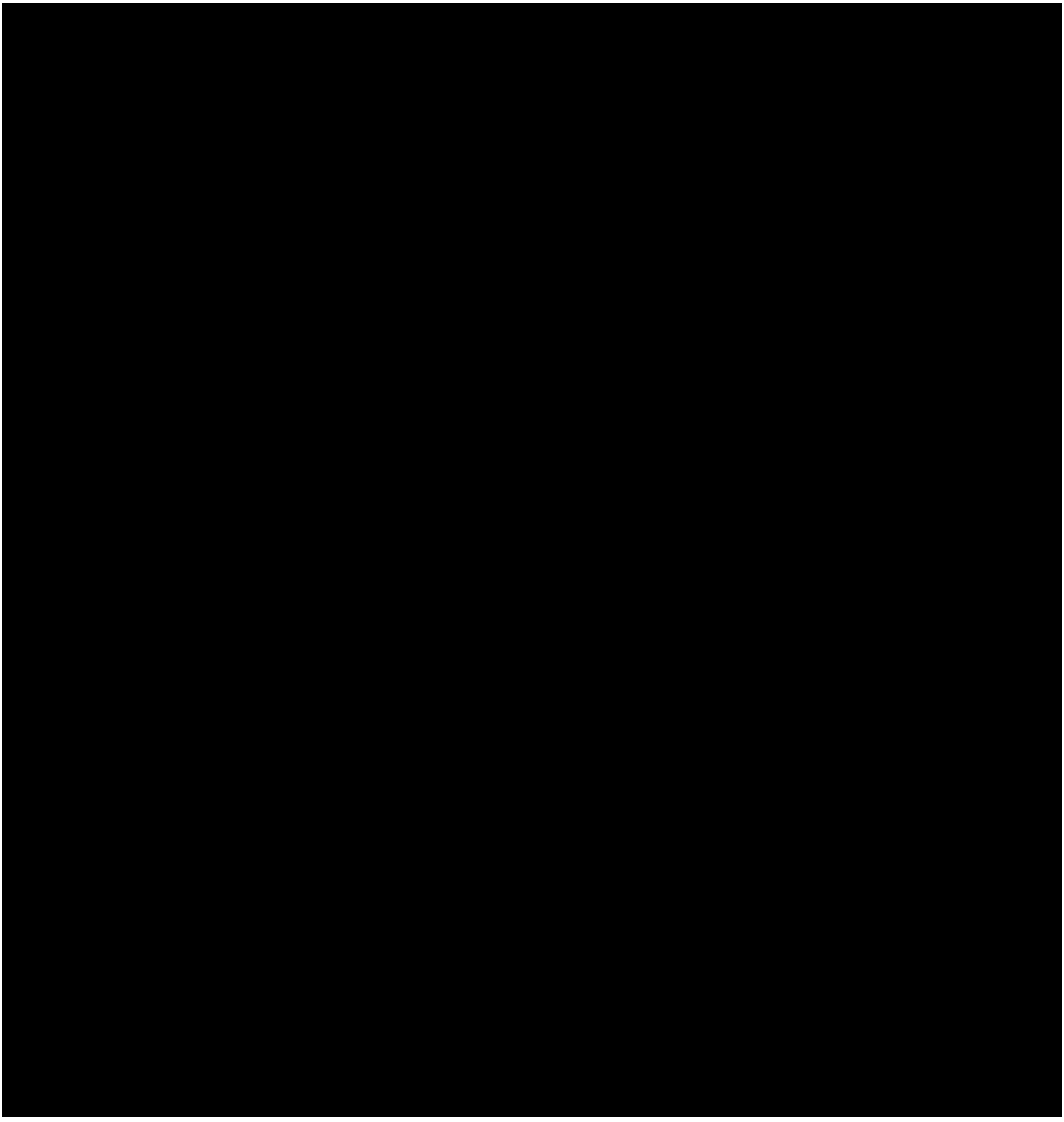








[redacted]

[redacted]

G. Glycans: Amgen's Characterization and Targets

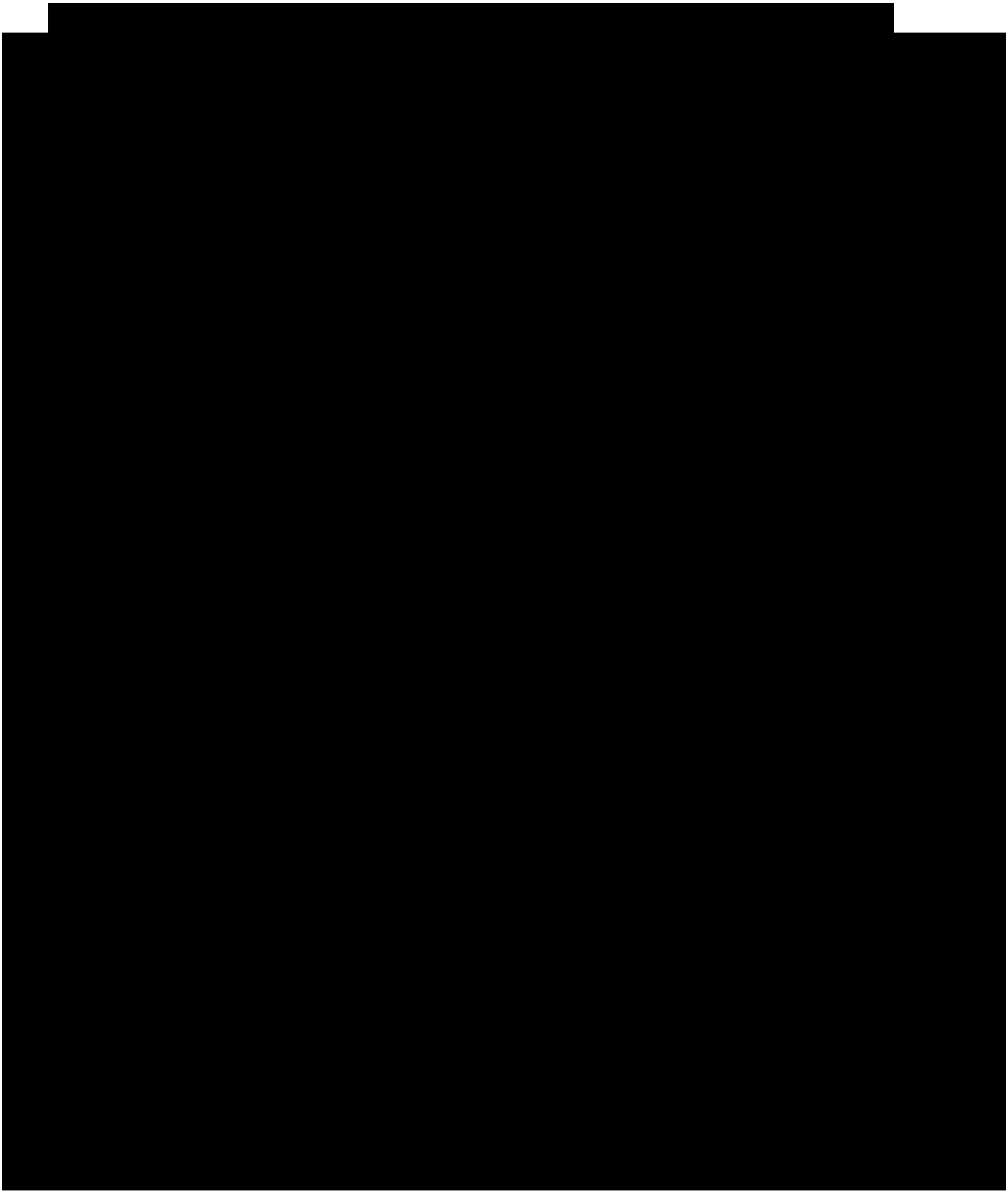
152. [redacted] As I explained in the

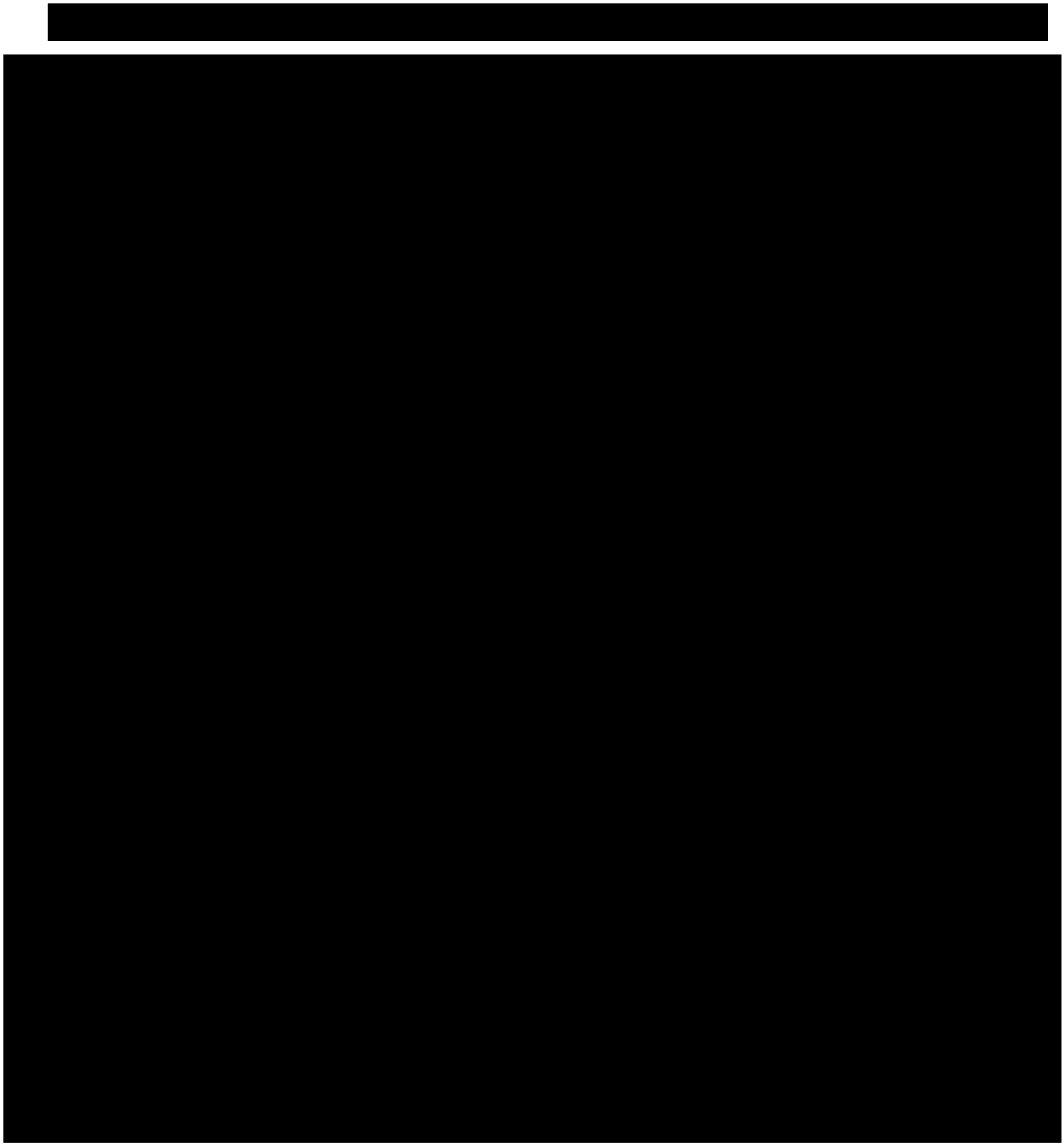
[redacted]

background discussion above in Section VII.A.2, a particular glycan cannot be both high-mannose and sialylated, meaning these categories are mutually exclusive.

[redacted]

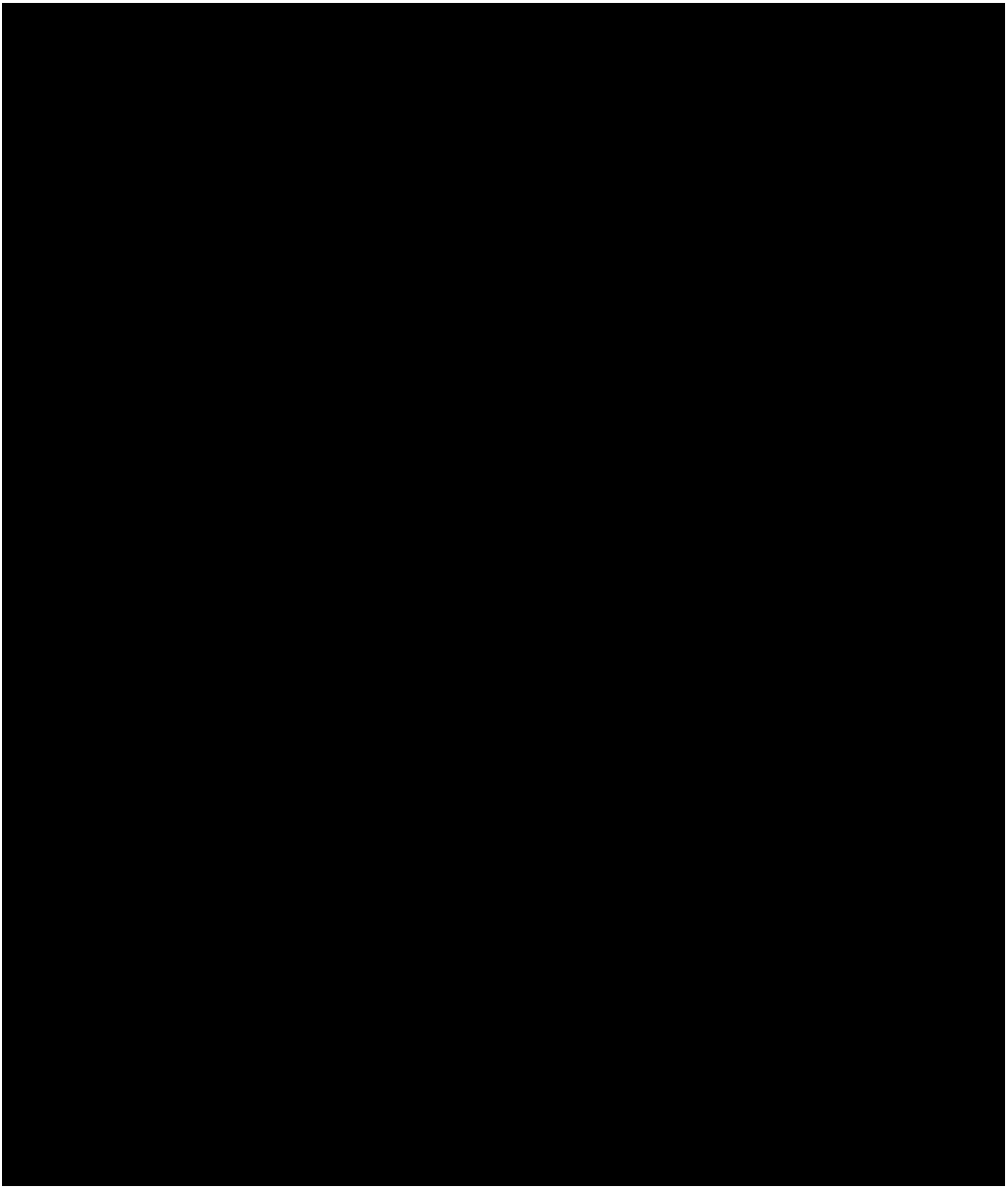
CONTAINS PLAINTIFF'S AND DEFENDANT'S CONFIDENTIAL INFORMATION

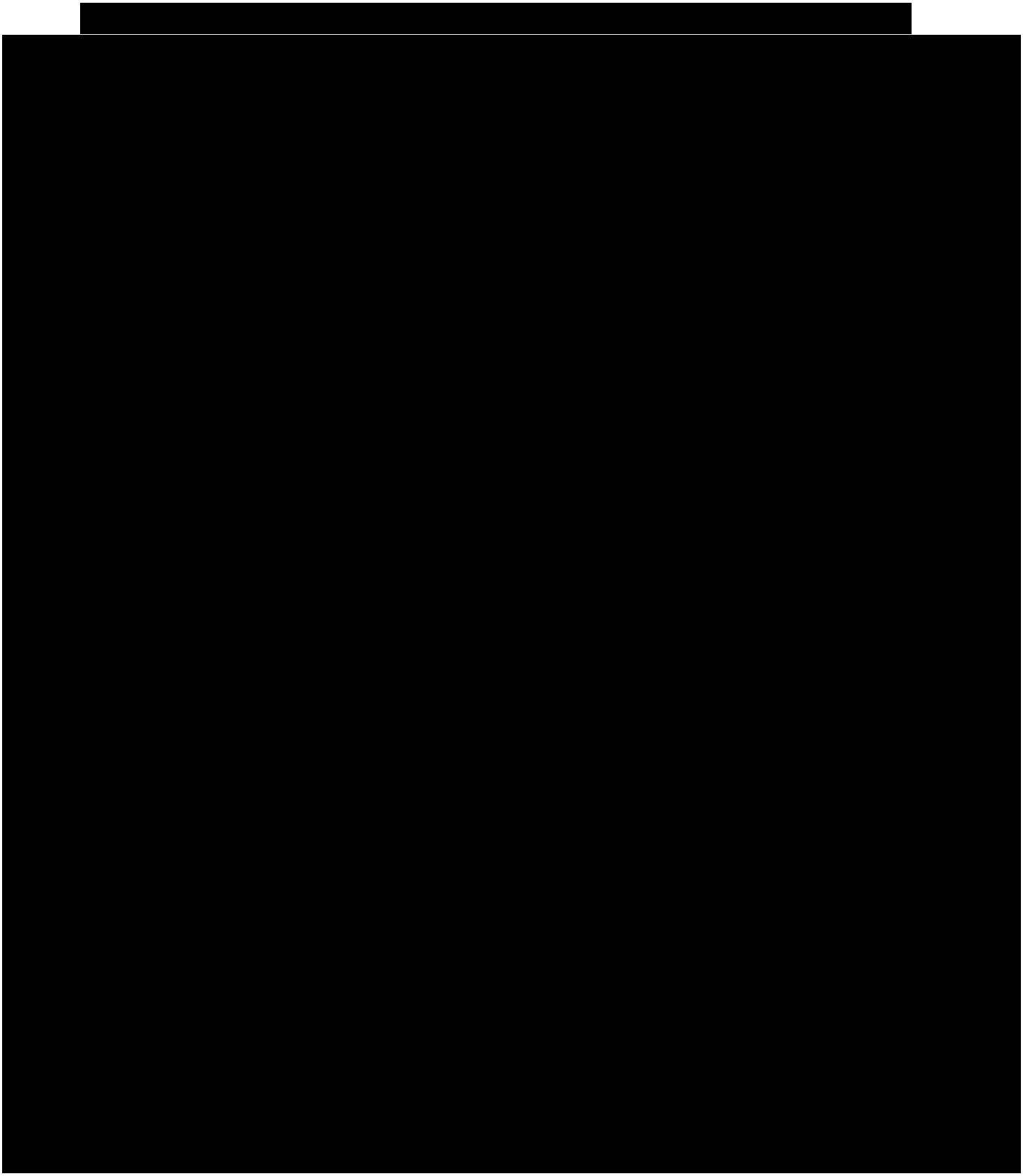


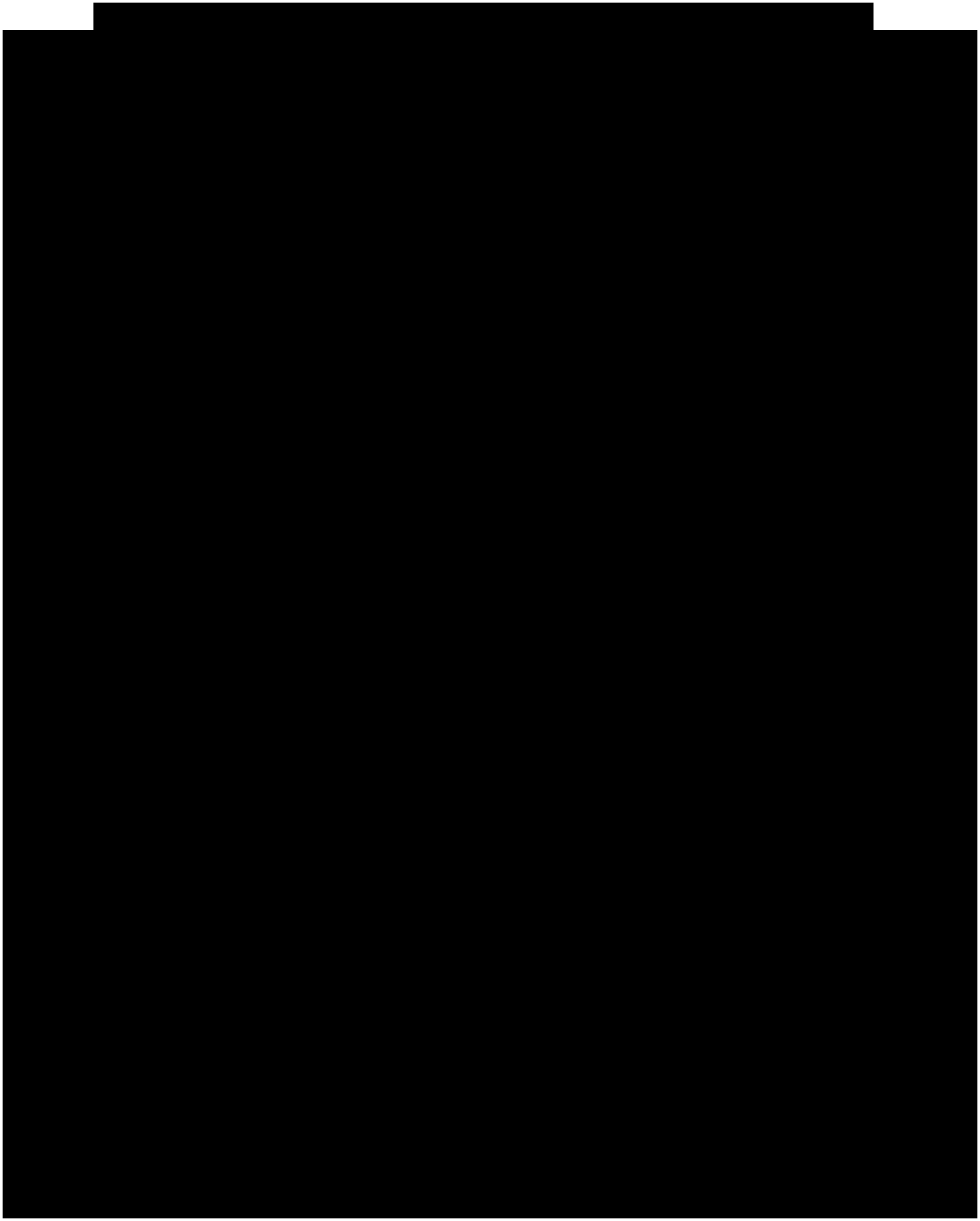


[redacted]

[redacted]

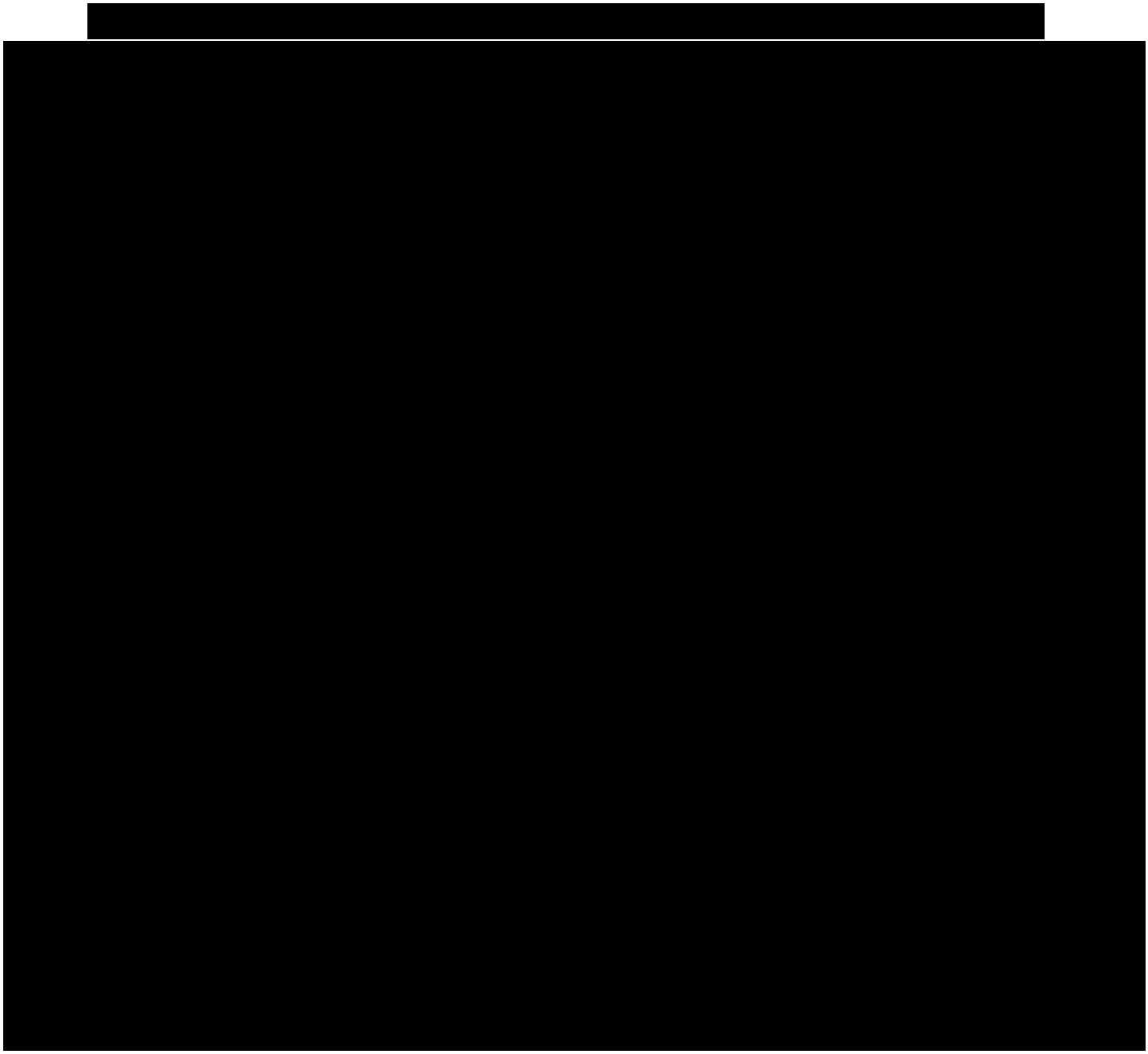


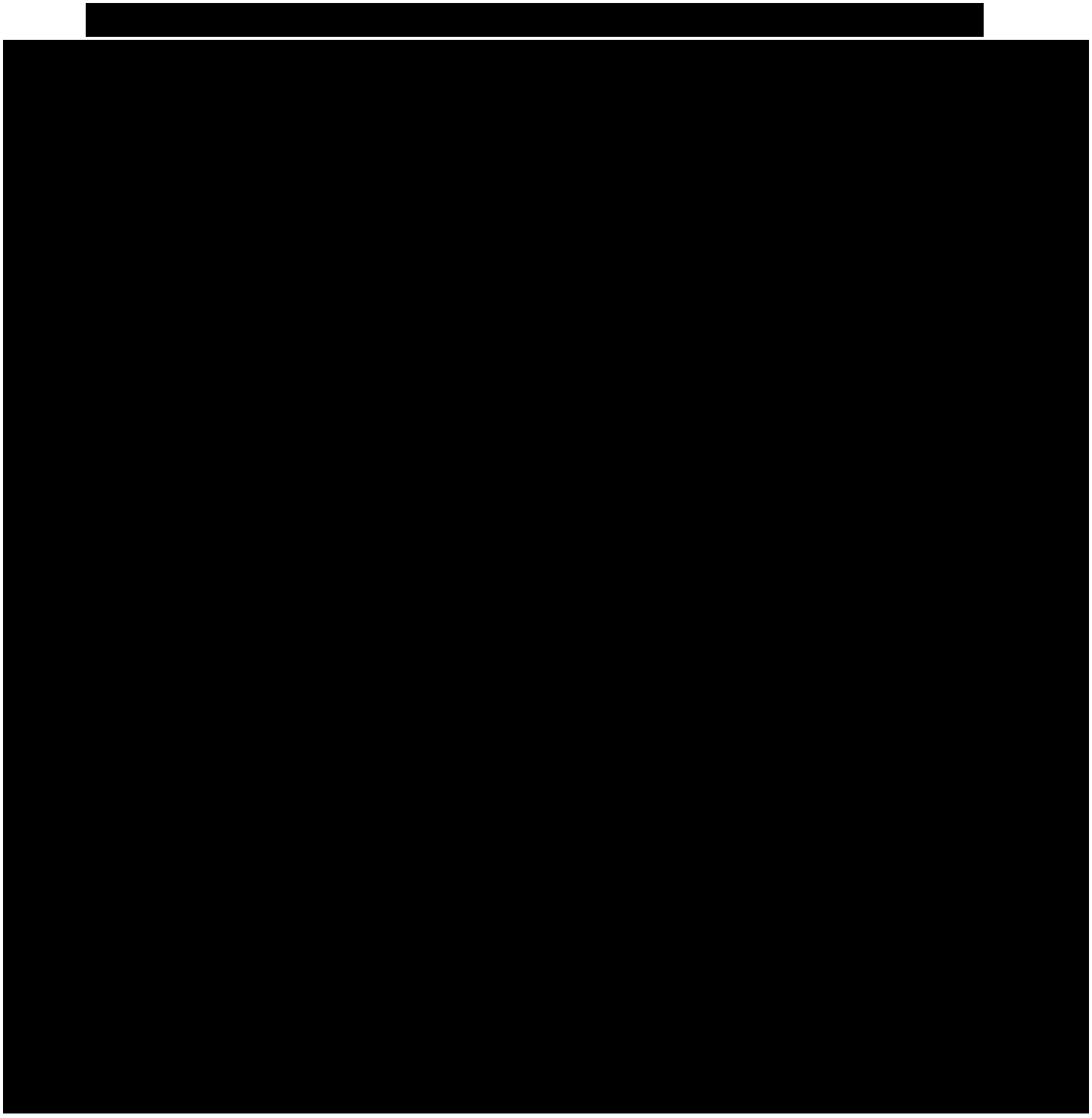


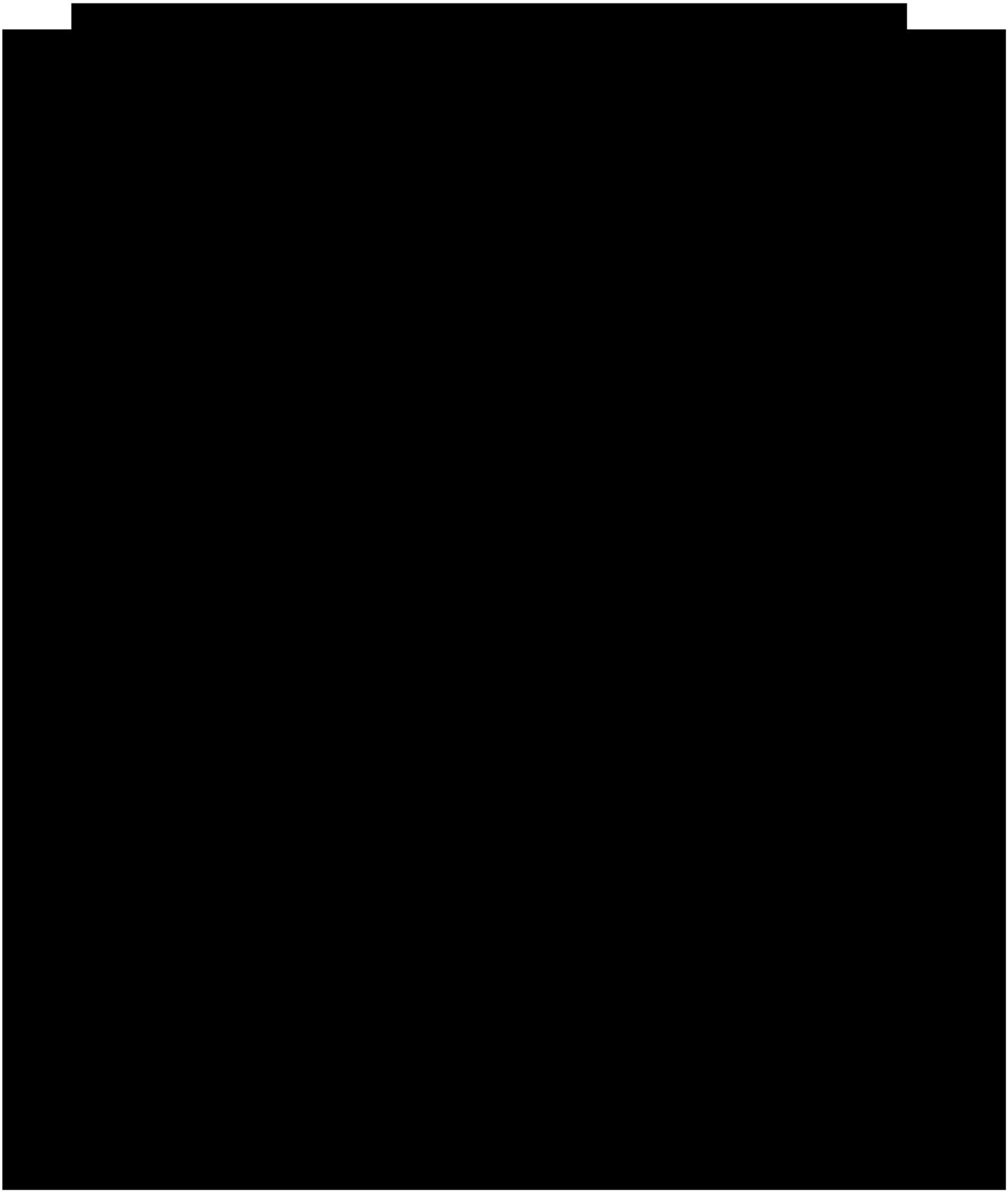


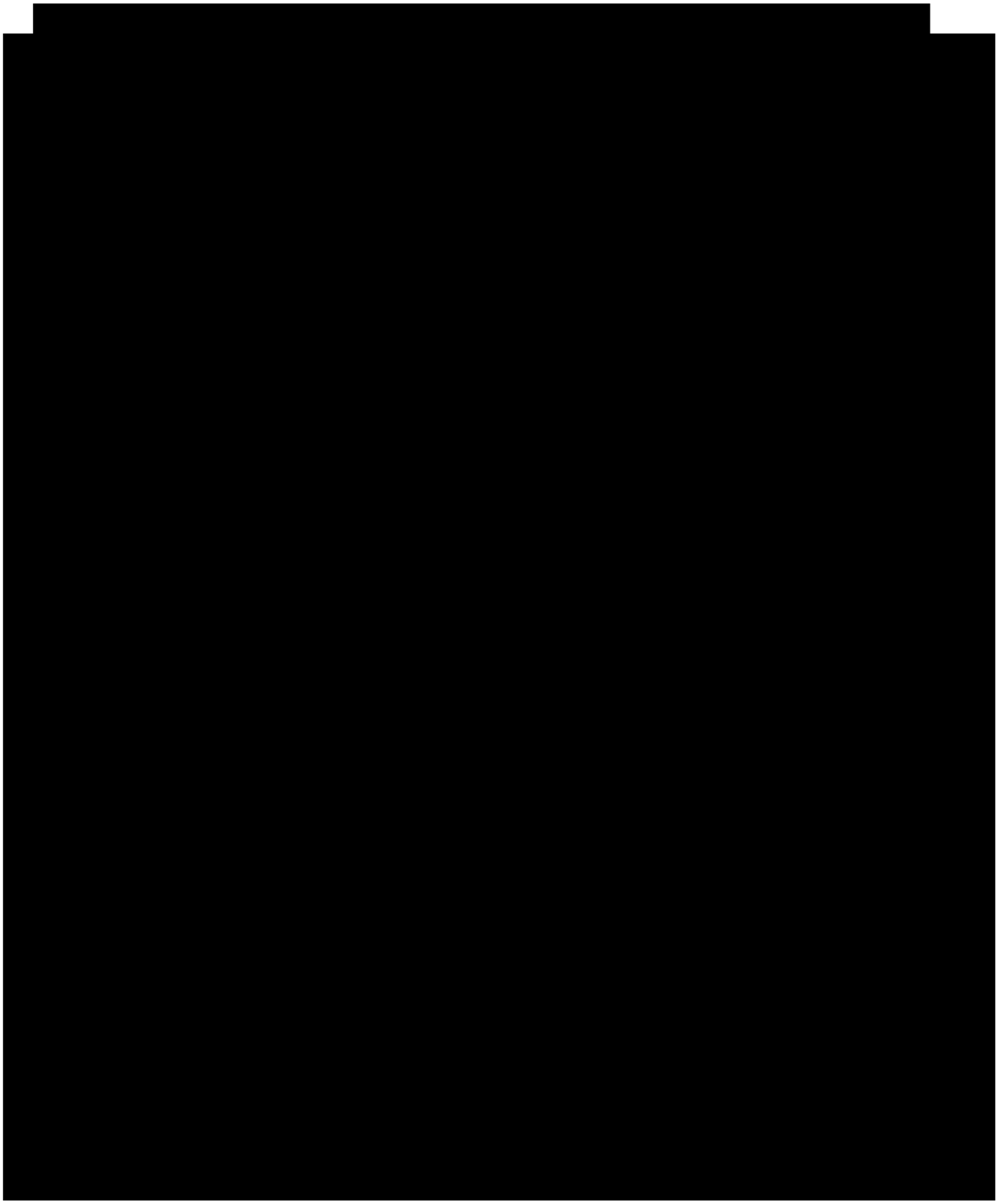
[redacted]

[4] As I explained above in Section VII.A.2, glycan forms can affect antibody properties. For instance, antibodies with high mannose glycans may be cleared more quickly than antibodies with sialylated glycans.









[redacted]

[redacted]

XII. AMGEN'S MANUFACTURING OF ABP 654 INFRINGES THE '858 PATENT

168. I have been asked to assess whether the process used to manufacture Amgen's ABP 654 will infringe claim 33 of the '858 patent. Based on my analysis, I conclude the process used to manufacture ABP 654 meets each and every limitation of, and thus infringes, claim 33 of the '858 patent.[5]

A. Amgen's Manufacturing of ABP 654 Meets the Limitations of Claim 33 of the '858 Patent

169. Claim 33 depends from claim 20 of the '858 patent, which reads:

> **20.** A method of manufacturing a preparation of a recombinant antibody, comprising:
> culturing a cell in a medium comprising 2 g/L arginine to 8 g/L arginine under conditions in which the cell expresses a recombinant antibody;
> isolating the recombinant antibody, thereby producing a preparation of the recombinant antibody;
> and formulating the preparation into a drug product if the preparation meets a target value of C-terminal variants of the recombinant antibody, wherein the C-terminal variants differ in amino acid sequence only by the presence or absence of a lysine at their carboxyl termini.

170. Claim 33 of the '858 patent reads:

> **33.** The method of claim **20**, wherein the cell is a CHO cell.

171. In the sections below, I set forth my analysis of how Amgen's manufacturing of ABP 654 meets each limitation of claim 33.

[5] See Section V.B.

[redacted]

1. "A method of manufacturing a preparation of a recombinant antibody, comprising:"

172. Amgen manufactures a preparation of ABP 654, which is a recombinant antibody. [redacted]

2. "culturing a cell in a medium comprising 2 g/L arginine to 8 g/L arginine under conditions in which the cell expresses a recombinant antibody;"

173. Amgen manufactures ABP 654 by [redacted]

174. As I describe above in Section XI.C (describing Amgen's Cell Culture), the cell culture process for ABP 654 involves multiple steps; [redacted]

175. As I set forth in my calculations in Section XI.C.2 above [redacted]

[redacted]

[redacted]

3. "isolating the recombinant antibody, thereby producing a preparation of the recombinant antibody; and"

177. [redacted]

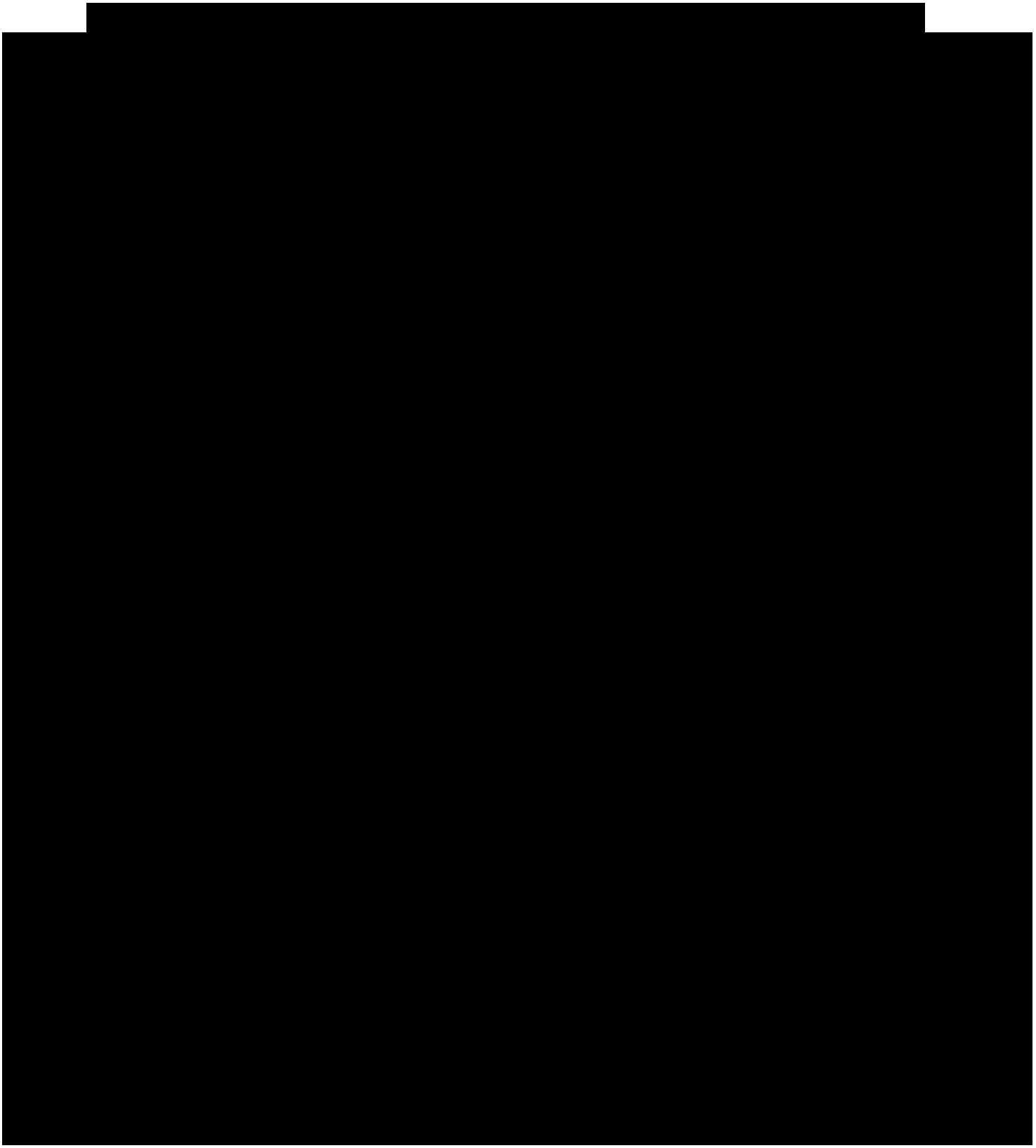
4. "formulating the preparation into a drug product if the preparation meets a target value of C-terminal variants of the recombinant antibody,"

178. [redacted]

[redacted]

179. [redacted]

180. [redacted]



[redacted]

[redacted]

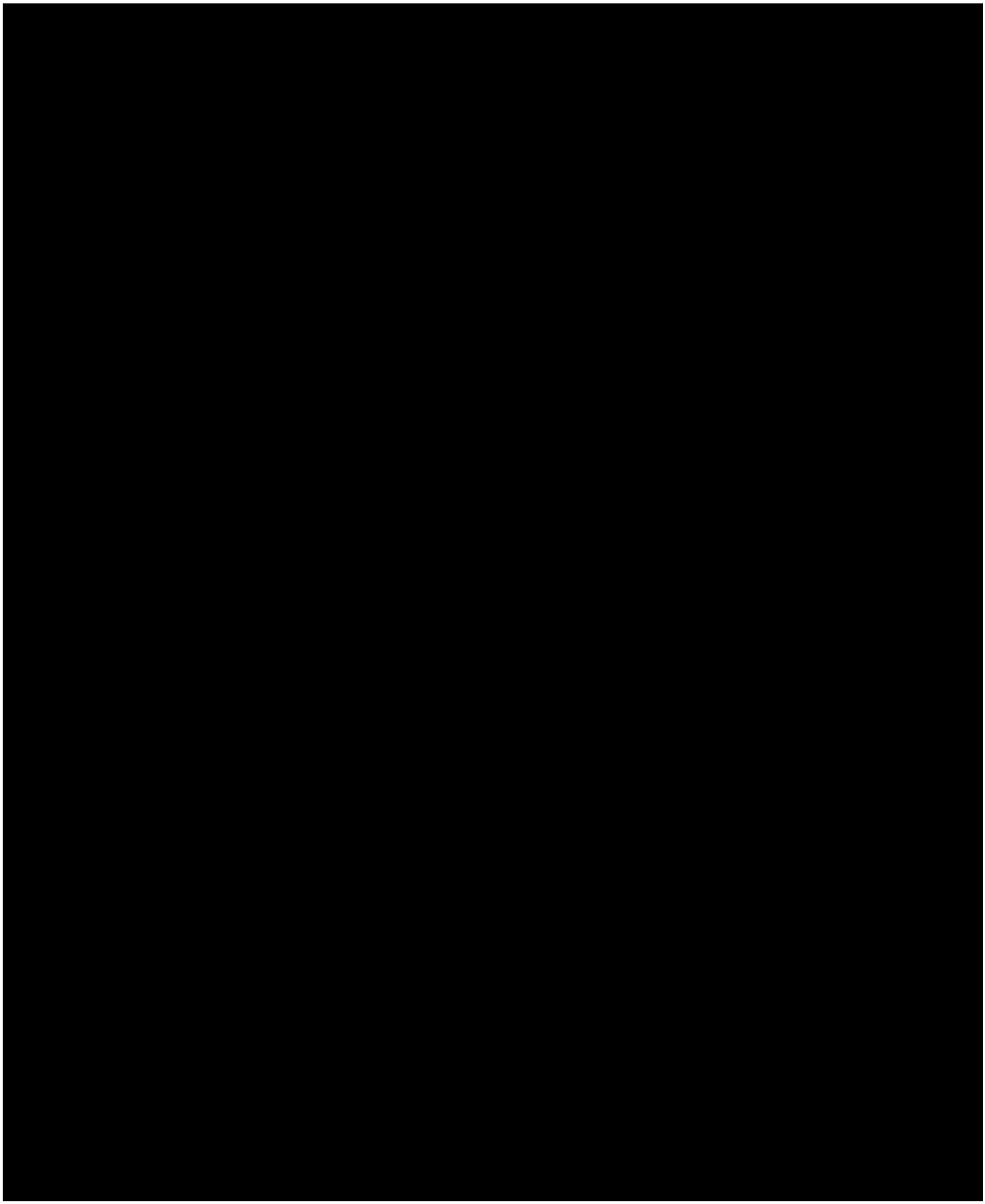
6 [redacted]

[redacted]

[redacted]

[7] [redacted]

[redacted]



[redacted]

[redacted]

n.

195. [redacted]

5. "wherein the C-terminal variants differ in amino acid sequence only by the presence or absence of a lysine at their carboxyl termini."

196. [redacted]

[redacted]

- [redacted]

[redacted]

197. [redacted]

198. [redacted]

6. "[The method of claim 20,] wherein the cell is a CHO cell."

199. [redacted]

B. Conclusion for '858 Patent

200. Based on the above analysis, I conclude that Amgen's manufacturing of ABP 654 performs each step of the claimed method, and thus meets each and every element of claim 33 of the '858 patent.

[redacted]

201. [redacted] I note that no subsequent processes in Amgen's manufacture of ABP 654, including the fill and finishing process, would materially change the ABP 654 product with respect to the claimed method.

XIII. AMGEN'S MANUFACTURING OF ABP 654 INFRINGES THE '168 PATENT

202. I have been asked to assess whether the process used to manufacture Amgen's ABP 654 infringes claim 23 of the '168 patent. Based on my analysis, I conclude the process used to manufacture ABP 654 meets each and every limitation of, and thus infringes, claim 23 of the '168 patent.

A. Amgen's Manufacturing of ABP 654 Meets the Limitations of Claim 23 of the '168 Patent

203. Claim 23 depends from claim 1 (or claims 10, 13, or 16) of the '168 patent, which reads:

> **1.** A method of producing a recombinant protein preparation having a target value of one or more galactosylated glycans, high mannose glycans, and sialylated glycans, the method comprising:
> (a) providing a cell genetically engineered to express a recombinant protein;
> (b) culturing the cell in a culture medium comprising 0.1 mg/L to 10 mg/L putrescine under conditions in which the cell expresses the recombinant protein; and
> (c) harvesting a preparation of the recombinant protein produced by the cell that meets the target value of the one or more of galactosylated glycans, high mannose glycans, and sialylated glycans,
> wherein the target value of galactosylated glycans, or sialylated glycans is a level at least 10% higher than a level of galactosylated glycans, or sialylated glycans in a preparation produced by culturing the cell in the medium not comprising 0.1 mg/L to 10 mg/L putrescine; or
> wherein the target value of high mannose glycans is a level at least 10% lower than a level of high mannose glycans in a

[redacted]

preparation produced by culture the cell in the medium not comprising 0.1 mg/L to 10 mg/L putrescine.

204. Claim 23 of the '168 patent reads:

23. The method of claim **1**, **10**, **13**, or **16**, wherein the cell is a Chinese Hamster Ovary (CHO) cell.

205. In the sections below, I set forth my analysis of how Amgen's manufacturing of ABP 654 meets each limitation of claim 23. As noted above in ¶ 78, I have been asked to consider claim 23 as it depends from claim 1.

1. "A method of producing a recombinant protein preparation …"

206. [redacted]

2. "… having a target value of one or more of galactosylated glycans, high mannose glycans, and sialylated glycans, the method comprising:"

207. [redacted]

[redacted]

3. "(a) providing a cell genetically engineered to express a recombinant protein;"

211. [redacted]

[redacted]

[redacted]

[redacted]

4. ***"(b) culturing the cell in a culture medium comprising 0.1 mg/L to 10 mg/L putrescine under conditions in which the cell expresses the recombinant protein; and"***

212. [redacted]

[redacted]

[redacted]

213. [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

214. [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

215. [redacted]

5. "(c) harvesting a preparation of the recombinant protein produced by the cell …"

216. [redacted]

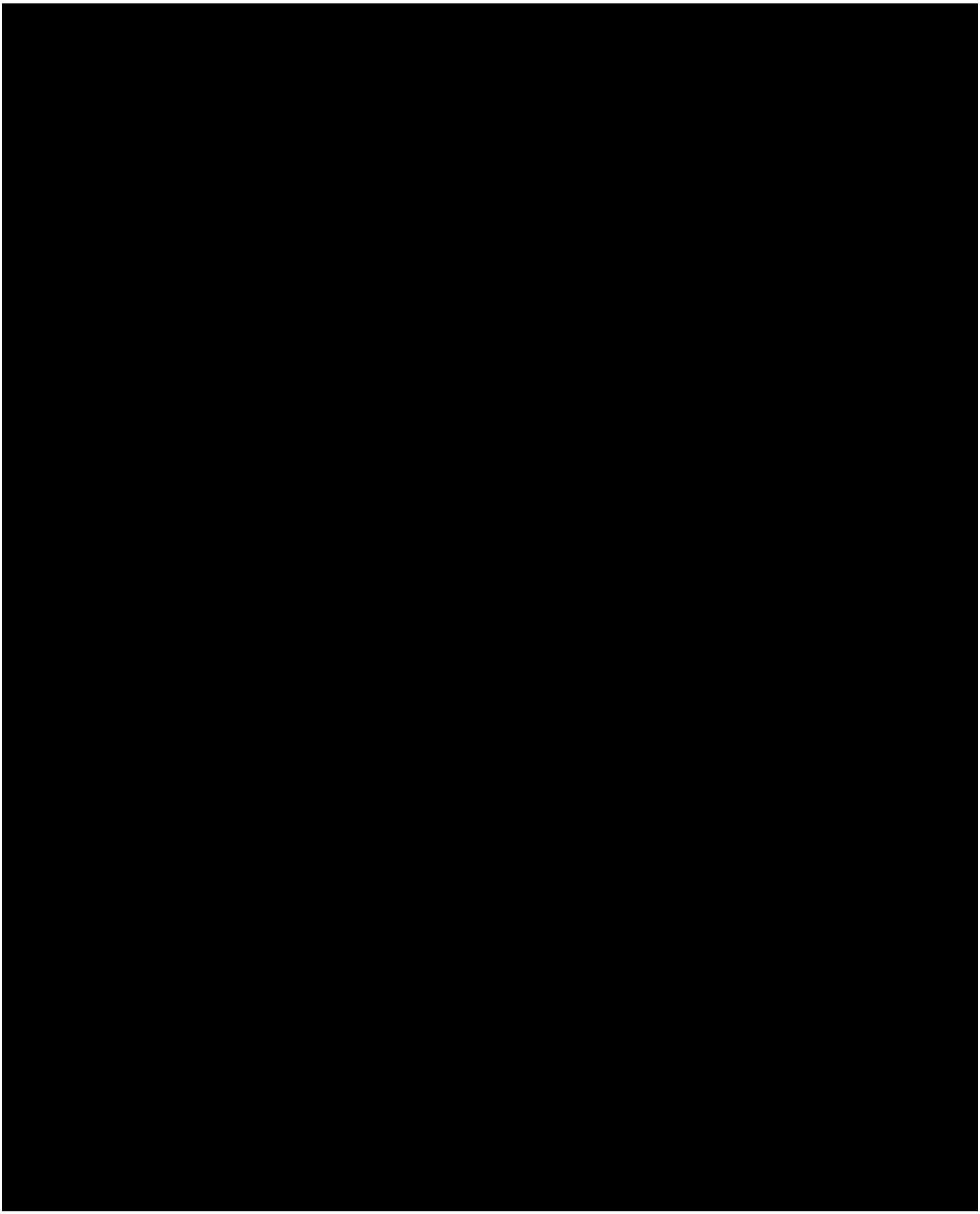
6. "… that meets the target value of the one or more of galactosylated glycans, high mannose glycans, and sialylated glycans,"

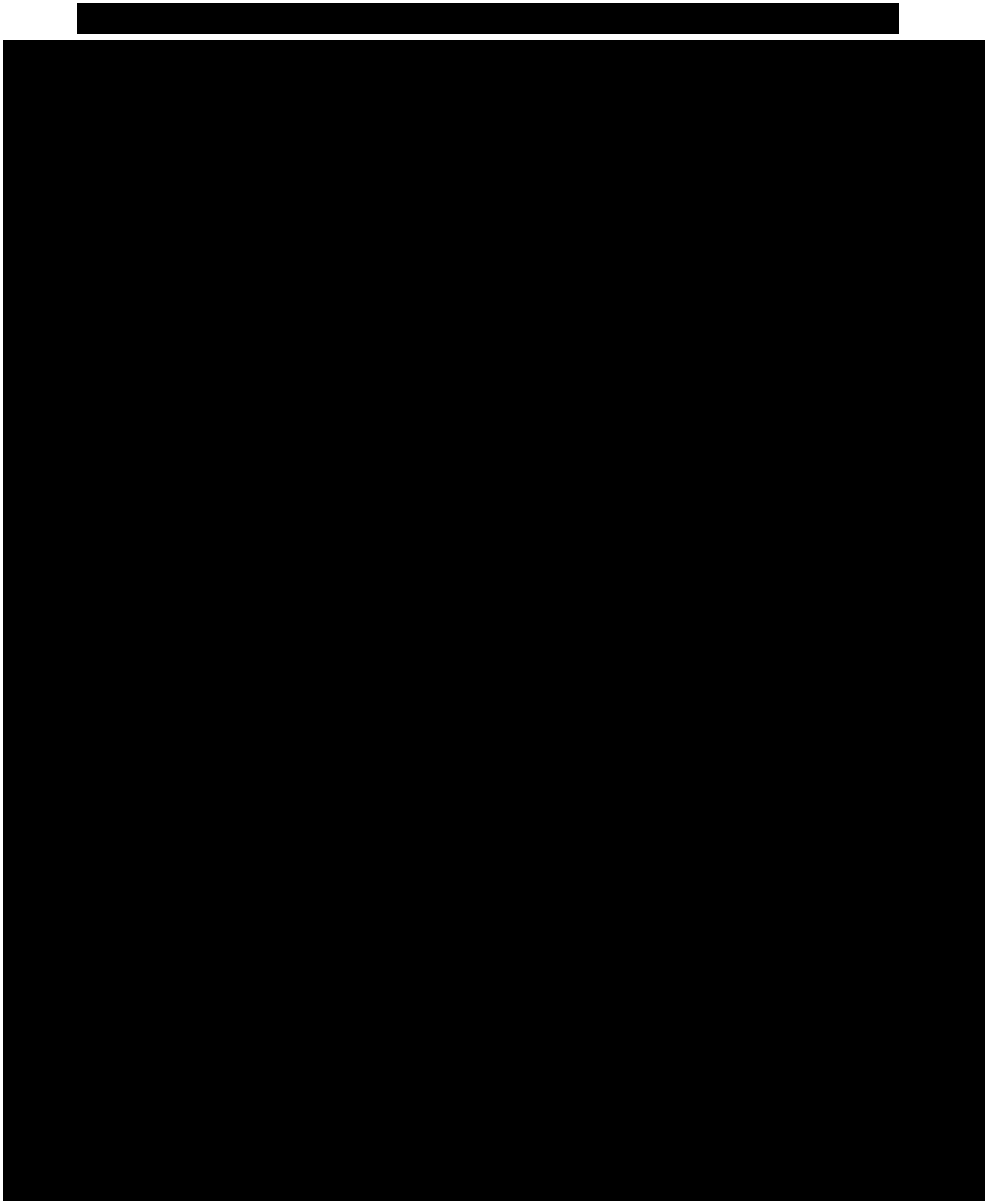
217. Amgen's manufacturing process produces ABP 654 with at least [redacted]

218. [redacted]

[redacted]

[redacted]





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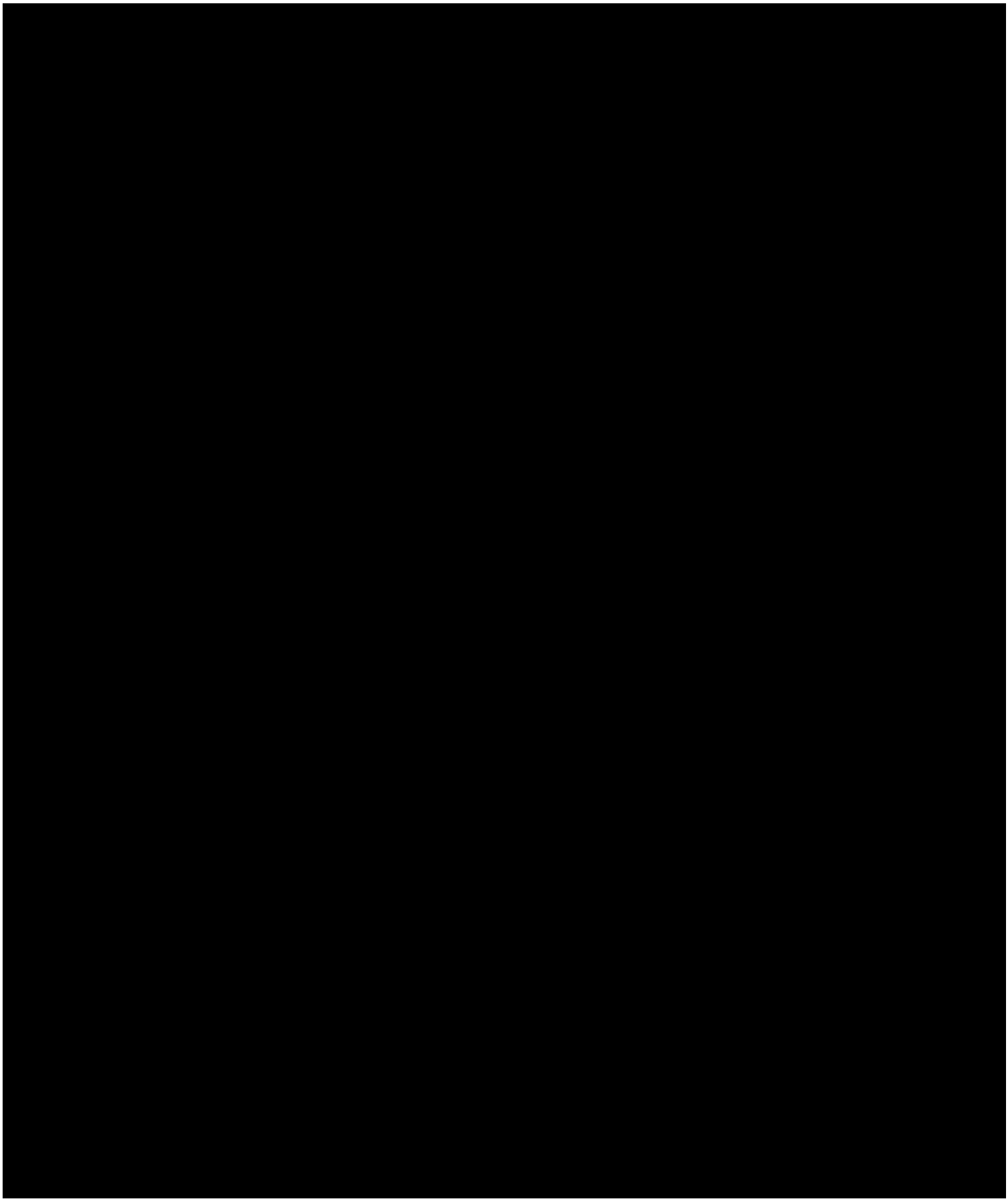
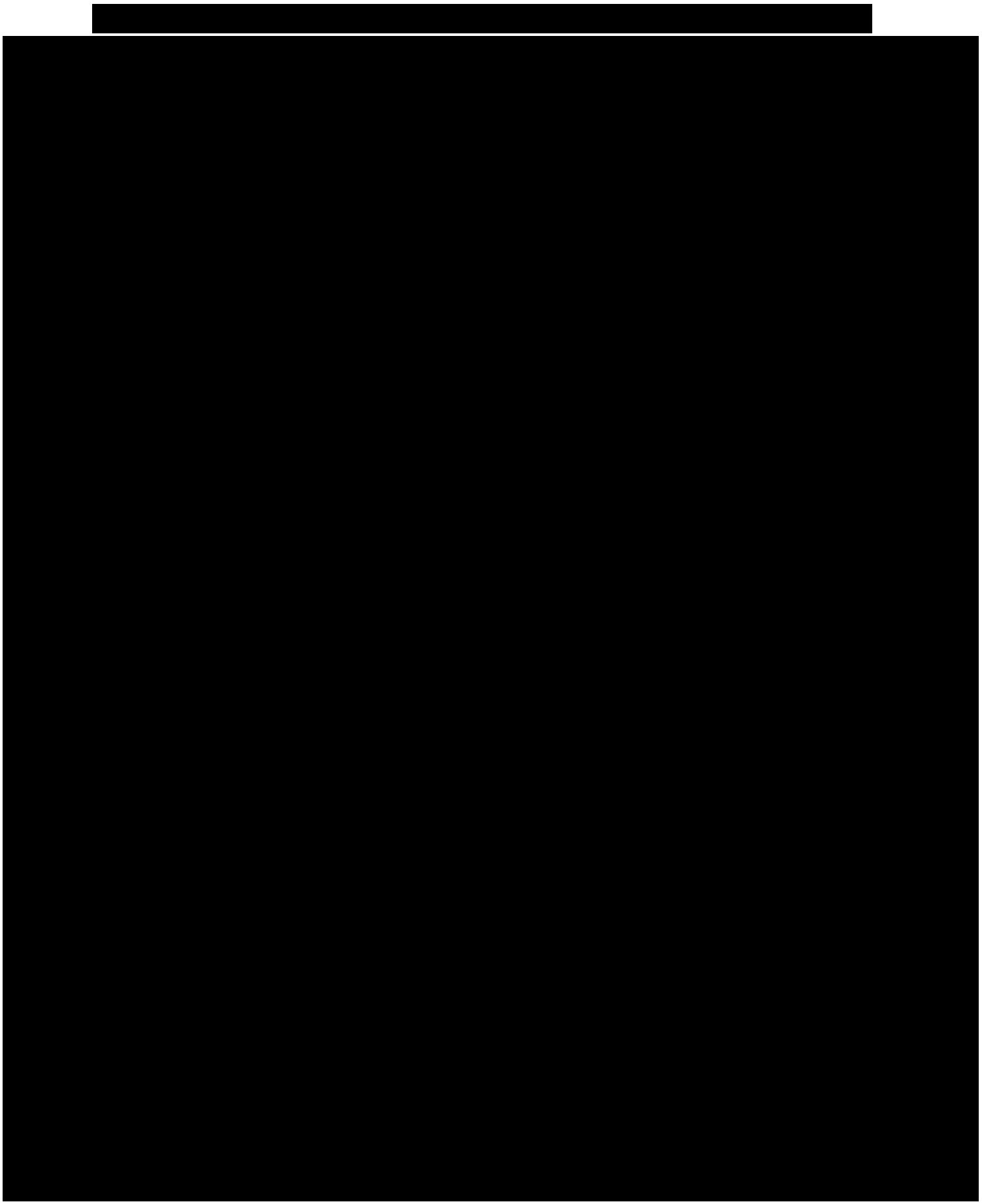
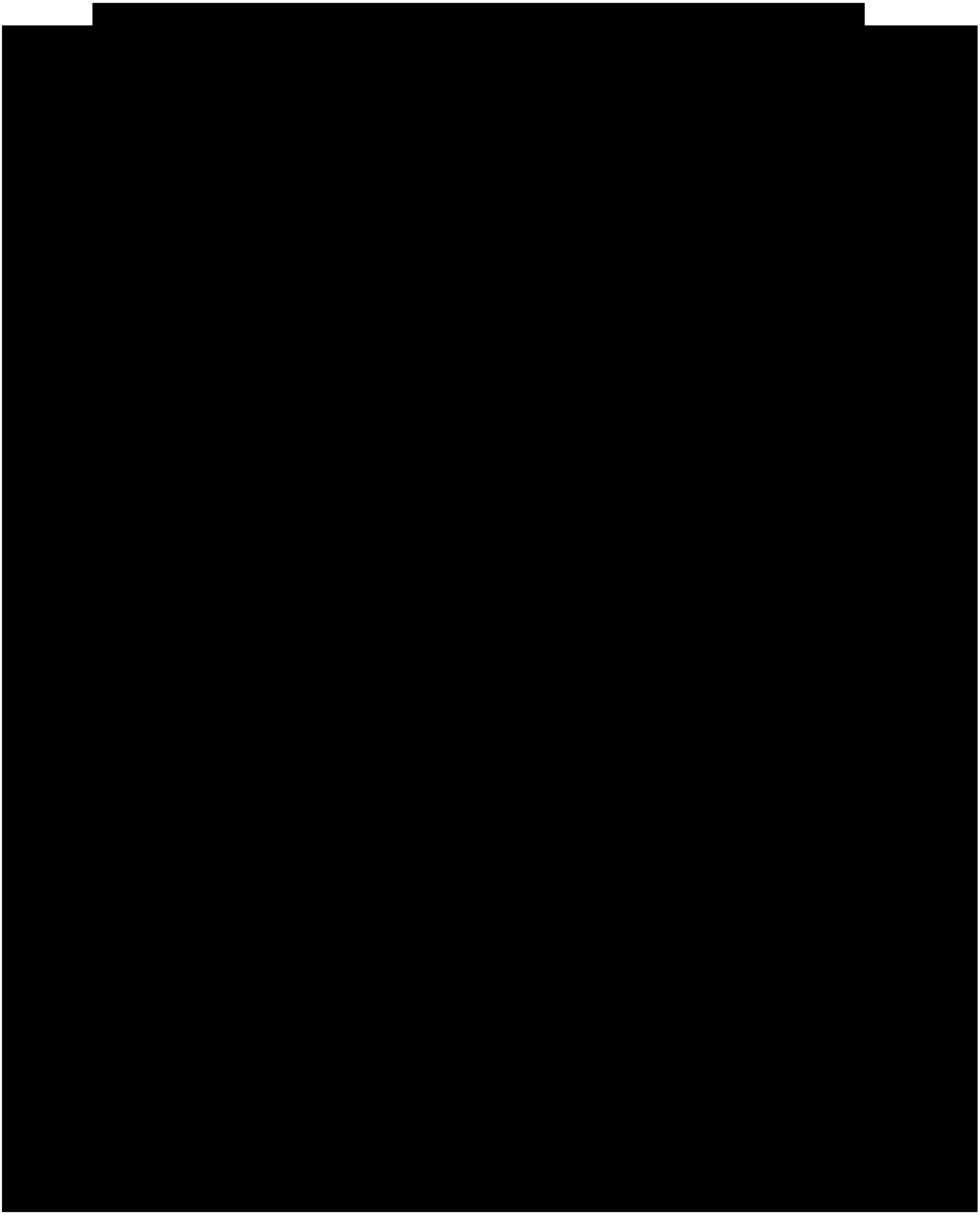
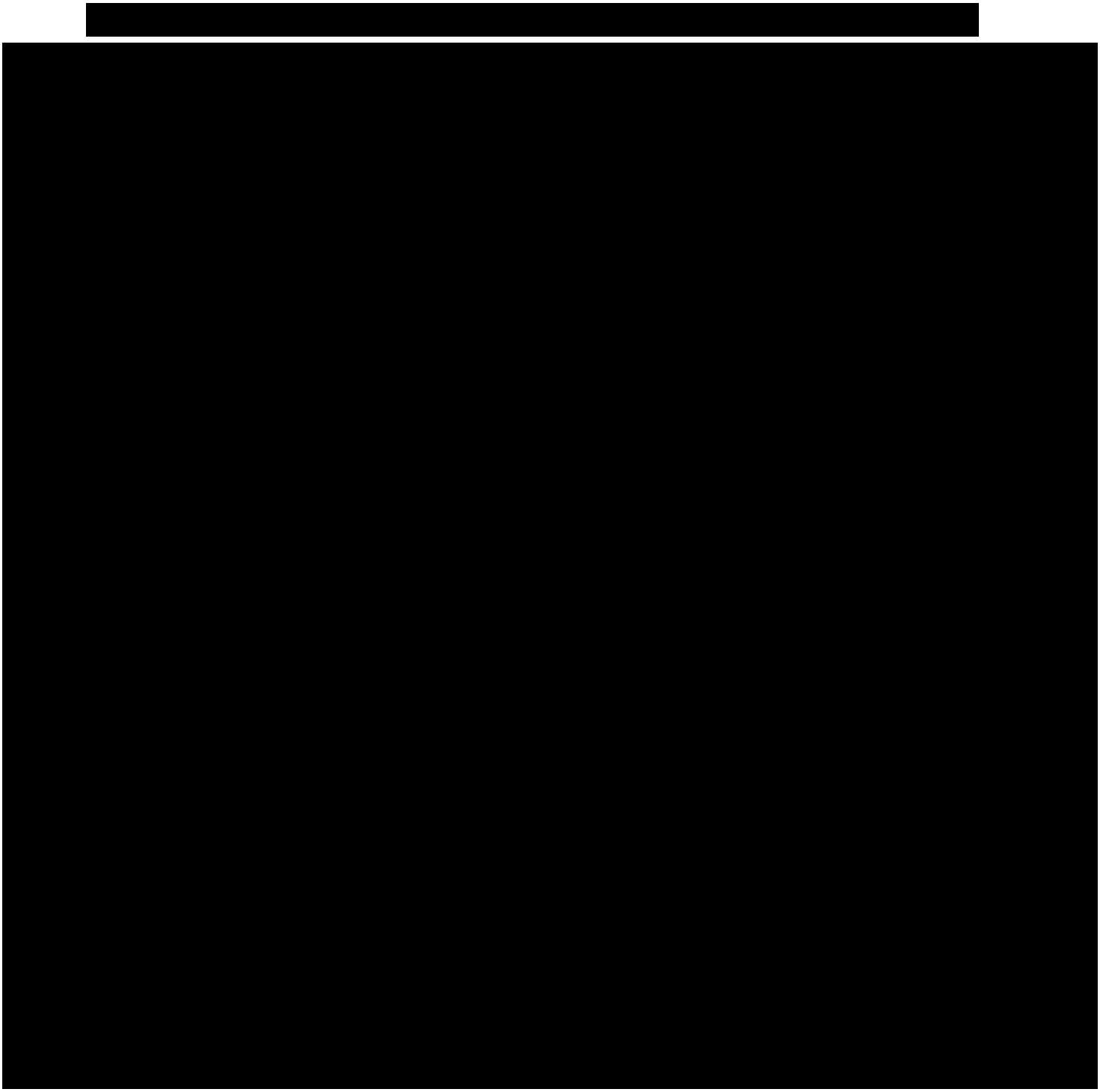






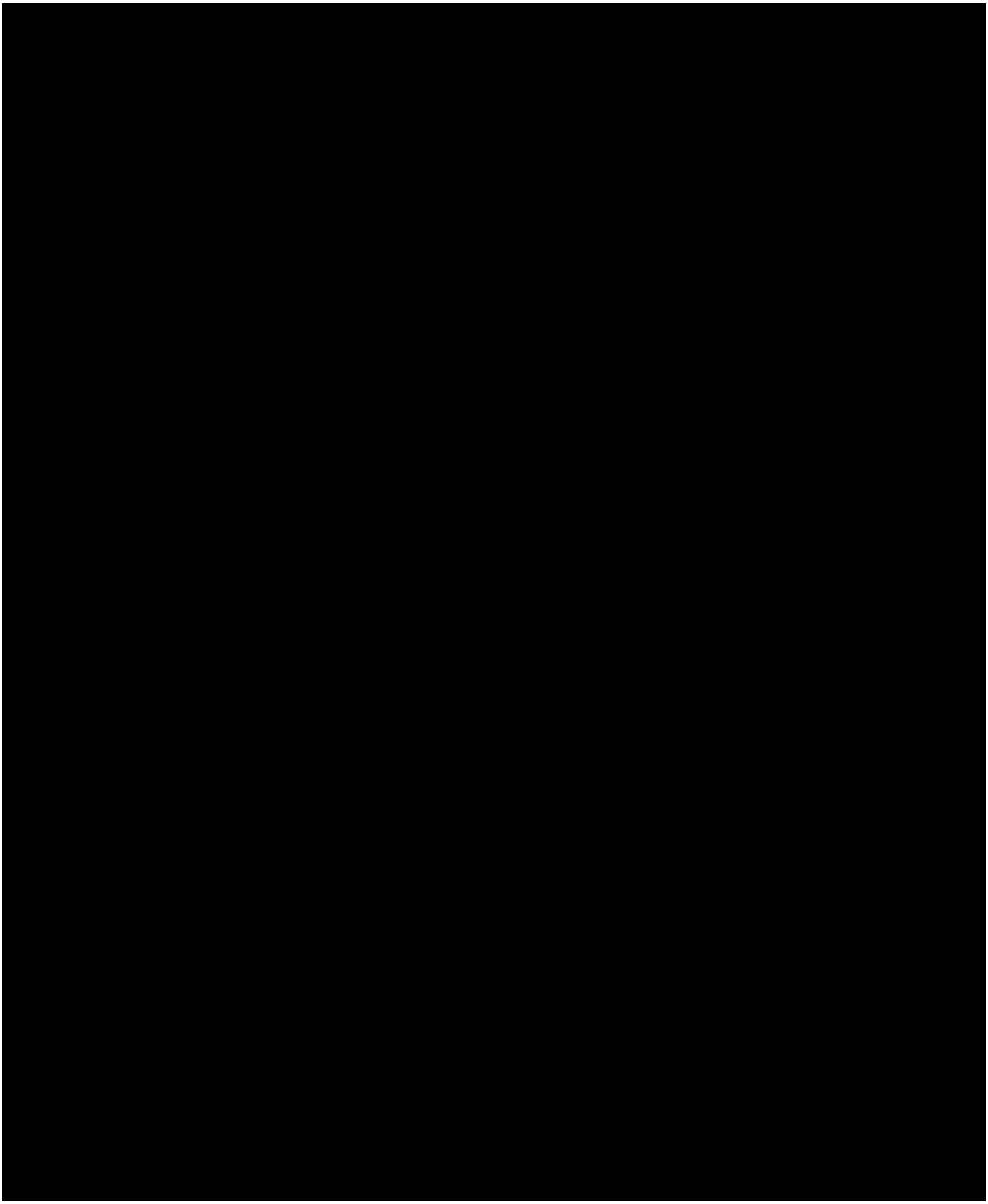


[redacted]

233. In sum, Amgen's validated commercial process meets each of the target values for the high mannose and sialylated glycans as measured by the glycan map. I summarize my above analysis of how the target values are met in the tables (one for high mannose glycans, and one for sialylated glycans) below:

[redacted]

[redacted]



[redacted]

239. Based on the '168 patent's demonstration of a cell culture medium with 0.52 mg/L putrescine versus a cell culture medium with 0.17 mg/L putrescine (about a 3-fold difference) resulting in about an 80% lower level of high mannose glycans, my conservative expectation is that use of Amgen's cell culture medium having [redacted]

240. [redacted]

241. Based on the '168 patent's demonstration of a cell culture medium with 0.52 mg/L putrescine versus a cell culture medium with 0.17 mg/L putrescine resulting in about a 58% higher

[redacted]

level of sialylated glycans, my conservative expectation is that use of Amgen's cell culture medium

[redacted]

8. "[The method of claim 1, 10, 13, or 16,] wherein the cell is a Chinese Hamster Ovary (CHO) cell."

242. [redacted]

[redacted]

B. Conclusion for '168 Patent

243. Based on the above analysis, I conclude that Amgen's manufacturing of ABP 654 performs each step of the claimed method, and thus meets each and every element of claim 23 of the '168 patent as it depends from claim 1.

244. [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

XIV. CONCLUSION

245. In sum, I conclude that the Asserted Manufacturing Patent Claims—claim 33 of the '858 patent and claim 23 of the '168 patent—are or will be infringed by Amgen's commercial manufacture of ABP 654.

[redacted]

I declare under penalty of perjury that to the best of my knowledge, information, and belief, the foregoing statements are true and correct.

Dated: March 1, 2023

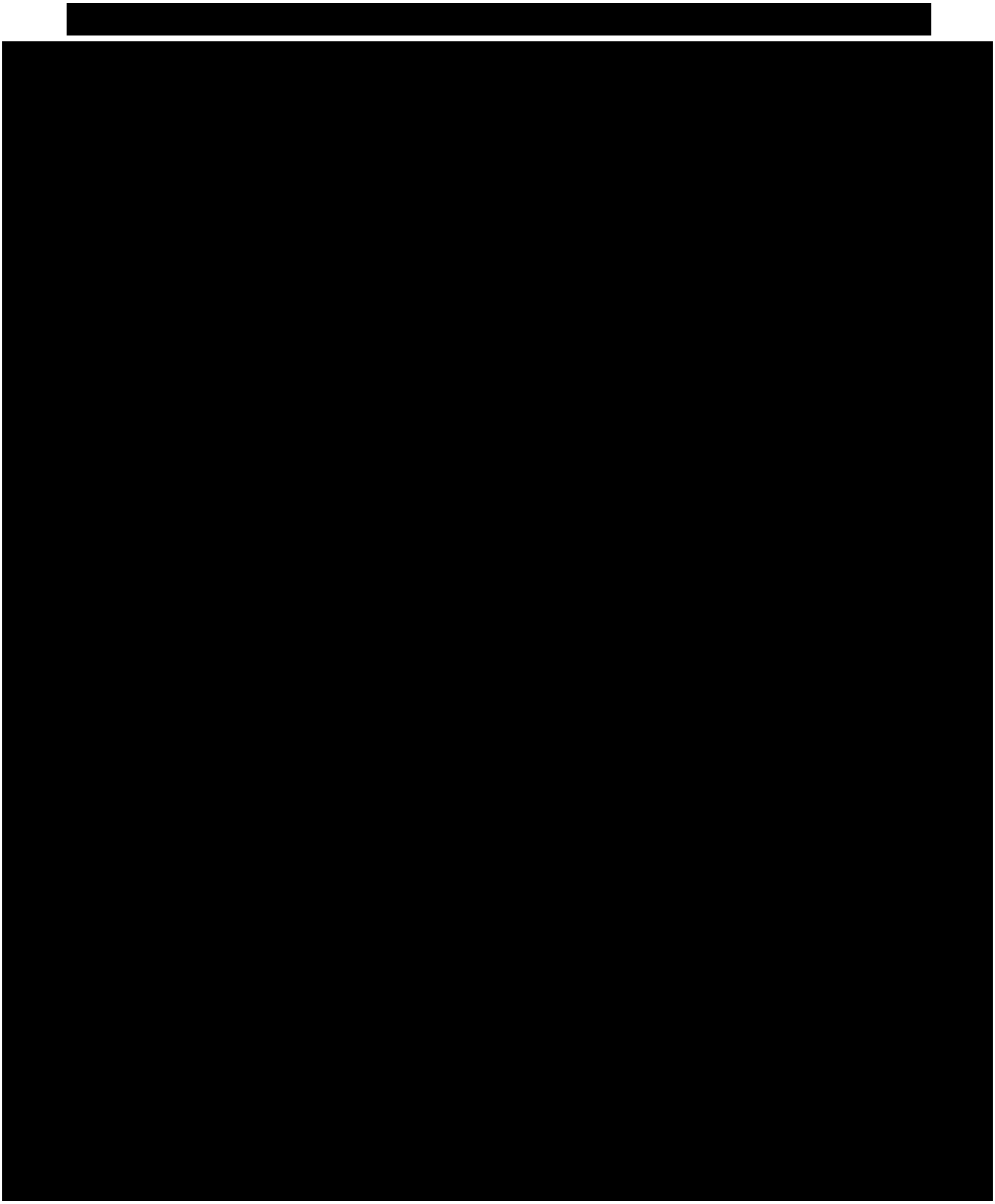
Matthew S. Croughan, Ph.D.

[redacted]

APPENDIX A – Materials Considered

Materials Considered
U.S. Patent No. 9,475,858
U.S. Patent No. 9,217,168
STELARA® (ustekinumab) prescribing information (revised Aug. 2022)

[redacted]



[redacted]

APPENDIX B – Curriculum Vitae

MATTHEW S. CROUGHAN

1044 West 1st Street, Reno, NV 89503.
Cell: 650-201-5039, E-mail: croughanmk@aol.com or shane.cruachan@gmail.com

Education

MASSACHUSETTS INSTITUTE OF TECHNOLOGY
Ph.D., Chemical Engineering, 1988. Minor in Business.

UNIVERSITY OF CALIFORNIA AT BERKELEY
B.S., High Honors, Chemical Engineering, 1983

Positions and Experience

CLAYMORE BIO LLC

Co-Founder, CEO, and Managing Partner, Part time, 12/20 - current

I co-founded Claymore Bio LLC with Professor Tim Haystead of Duke University to address shortfalls in the diagnosis and treatment of infectious disease. It is based upon technology licensed from Duke University according to three new strategies:

1. **Specific therapies for bacterial diseases,** based upon new small molecule drugs targeting species-specific sequences in bacterial proteins. This avoids killing beneficial bacteria in the gut and the generation of drug resistance across species.
2. **Non-specific therapies to treat viral diseases**, based upon inhibition of host cell factors that play a key role in the replication of many or all virus species. This game-changing technology would minimize deaths and damage to society while new specific therapies and/or vaccines are developed and distributed, and
3. **New approaches to diagnose hidden infections**, based upon imaging technologies such as PET with new radioactive agents. These new agents bind to and expose infectious organisms hiding inside tissues.

TAHOE BIOTECHNOLOGY LLC

Principal, Part time, 6/20 - current

Tahoe Biotechnology LLC holds assets that were developed over the last 20 years through the investment of over $1 million in revenues from the consulting services described below. The first product is the book: COVID-19, A Guide to Home Health Care.

MATTHEW SHANE CROUGHAN, A Consulting Services Firm, Reno, Nevada

Sole Proprietor, Part to Full time, 6/98 – 1/06 and 8/14 - current
Sole Proprietor, Part time (while professor at KGI and CTO at Sapphire), 1/06 – 8/14

Consulting on bioprocess development, scale-up, and manufacturing. Clients include major bio/pharma (Pfizer, Janssen, Merck, BMS, Regeneron, Gilead) and many other firms (>100 to date). Projects range from biopharmaceutical proteins to vaccines, biofuels, cell and gene therapies, and cellular agriculture. Service as expert witness/consultant for legal cases (20 to date). Service on the Scientific/Technical/External Advisory Board/Panel for:

Pfizer: pharmaceutical bioprocessing
AveXis: pharmaceutical bioprocessing
Pfenex: pharmaceutical bioprocessing
Anaptys Bio: pharmaceutical bioprocessing

PBS Biosystems: novel disposable bioreactors and control systems for cell therapy
Sapphire: biofuels from algae (prior to and after service as CTO)
Protein Metrics: software for proteomics analysis
NIBRT: National Institute for Bioprocessing Research and Training (Ireland)
Hampton Creek/Just: cellular agriculture
Orbillion: cellular agriculture
UC Davis: cellular agriculture

From 1998-2005, I served part-time as the Industrial Liaison Officer for the Biotechnology Process Engineering Center (BPEC) at MIT. I gave 2 plenary presentations at national NSF ERC meetings. My presentation on accomplishments of BPEC alums was shown to US Congress. BPEC alums have played a key role in Covid-19 vaccines and therapies.

SAPPHIRE ENERGY INC., San Diego, California

Chief Technology Officer, 6/13 – 8/14

Led entire R & D effort, with staff of up to 90 scientist and engineers, to invent, develop, and commercialize methods to economically produce renewable crude oil and animal feed from algae. Areas covered ranged from genetics and systems biology, through algae bioprospecting, cultivation, crop protection, pond design, harvest, drying (for feed), conversion and extraction (for oil), to product quality analysis, blending and refining, and fuel certification. Established team that doubled productivity at 100-acre outdoor pilot facility.

KECK GRADUATE INSTITUTE, Claremont, California

Industry Professor (part time), 8/13 – 2/18
George B. and Joy Rathmann Professor (full time), 1/06 – 8/13
Director of the Amgen Bioprocessing Center (full time), 1/06 – 8/13

Founding professor of the bioprocessing program at KGI, one of the largest graduate bioprocessing programs and the first one focused on educating business leaders in bioprocessing. Served as the advisor to 108 Masters in Bioscience (MBS) and Master of Science (MS) Bioprocessing Focus track graduates along with 2 doctoral graduates. As the sole full-time professor in bioprocessing, I created and taught five new courses covering both upstream and downstream topics, including two with bioprocessing labs. I also recruited adjuncts to create and teach four additional new courses.

Winner of the Student Choice Faculty Award (Top Teaching Award) for 2012 – 2013
Presented with The Godfather of Bioprocessing Cup by Bioprocessing Class of 2013

GENENTECH, Inc., South San Francisco (SSF), California

Senior Engineer, Vacaville Project, 6/95-1/98

Chief scientist on the design team for the world's largest and most automated animal cell culture facility at the time, built for production of therapeutic antibodies, with twelve 13,500-L bioreactors. Lead team to agree on consensus platform process and facility for multiple products, eliminating unnecessary variations and associated costs. The resulting process flow and plant design became the standard platform not only for Genentech but also nearly all antibody manufacturing worldwide. Helped create a new biochemical technician training program in collaboration with Professors J. DeKloe and J. Mills at Solano Community College in Fairfield, CA.

Group Leader, Manufacturing Sciences, 12/93 - 6/95
One of three group leaders in the first manufacturing sciences department in recombinant protein manufacturing. Responsible for upstream aspects of processes for 4 marketed products. Optimized tPA process within regulatory restrictions. Developed second generation tPA process; eliminated all bovine-derived raw materials (such as serum, bovine insulin, and transferrin) and increased productivity 3-fold with no change in product quality. Led viral barrier team to reduce risk of adventitious viral contamination in facilities.

ACTIVATED CELL THERAPY (later DENDREON) Inc., Mountain View, California
Director, Cell Processing and Expansion, 5/93 - 12/93
Initiated development of commercial processes to isolate, expand, and/or activate cells of the immune and hematopoietic system for adoptive immunotherapy. Set up facilities. Recruited key staff to keep program going before I left.

GENENTECH, Inc., South San Francisco, California
Group Leader, Manufacturing Sciences, 3/92 - 5/93
Described above.
Scientist, Cell Culture Research and Development (CCRD), 6/89-3/92
Developed the first licensed, high-density, fed-batch animal cell culture process, a breakthrough platform technology now used by Genentech and throughout the industry for production of high-dose therapeutic antibodies and other proteins. Successfully scaled this process, developed to produce Pulmozyme^{tm} (DNAse), from 2 to 12,500 liters.
Associate Scientist, Cell Culture Research and Development, 3/88-6/89
Established role as original cell culture scale-up and mixing expert at Genentech. Identified impact of hydrodynamics, mixing, and mass transfer, across scales from 2 liters to 12,500 liters, and developed successful scale-down models and scale-up criteria.

MASSACHUSETTS INSTITUTE OF TECHNOLOGY, Cambridge, Massachusetts
Doctoral Candidate, Chemical Engineering, 9/83-2/88
Performed experimental and theoretical research on the role of fluid mechanics and mass transfer in animal cell bioreactors. Research advisor: Institute Professor Daniel I.C. Wang
Teaching Assistant, 1/85-12/87
Gave lectures and wrote assignments for a course in Biochemical Engineering and a new course on Biotechnology of Mammalian Cells.

ENGENICS, Menlo Park, California
Consultant, 5/83-9/83
Wrote large Fortran program to model and design industrial chromatography processes, using rotating annular systems, as part of collaboration with Frances Arnold.

U.C. BERKELEY, Berkeley, California
Undergraduate Honors Researcher, 9/82-4/83
Grew 9-liter hybridoma cultures. Produced antibodies for use in affinity chromatography studies conducted by Frances Arnold (2018 Nobel laureate) under the direction of Prof. Harvey Blanch. Coauthor with Frances on her first paper in biotechnology (#1 below).

CHEVRON RESEARCH COMPANY, Richmond, California
Student Professional Engineer, 6/81-9/81
Analyzed process to reactivate alumina adsorbent in wax purification plant.

INTERNATIONAL BUSINESS MACHINES (IBM), Essex Junction, Vermont
Engineering Coop, 7/80-12/80
Optimized new ion-etch and pyrolytic deposition steps in silicon chip processing.

Honors

MATTHEW AND KATHY CROUGHAN ENDOWED SCHOLARSHIP FOR BIOPROCESSING
Keck Graduate Institute, Claremont, CA

AMONG TOP 25 PEOPLE KEY TO SUCCESS OF KGI OVER ITS FIRST 25 YEARS
Awarded by Keck Graduate Institute (KGI) as part of its 25th anniversary celebration.

HONORARY LIFETIME MEMBER, Amgen Bioprocessing Center Advisory Board
Keck Graduate Institute, Claremont, CA

COLLEGE OF FELLOWS, American Institute of Medical and Biological Engineers
College of Fellows represents the top 2% of engineers in field

40th ANNIVERSARY ISSUE, Biotechnology and Bioengineering
Paper selected as among the top 20 ever published in the journal (paper #3 below)

PETERSON AWARD, American Chemical Society
Best Student Paper in Microbial and Biotechnology Division, 1987

TAU BETA PI, Engineering Honor Society
Member, 1982-current

SIGMA XI, Scientific Research Society
Member, 1987-current

UNIVERSITY OF CALIFORNIA AT BERKELEY
Alumni Scholar, 1978-1983
President, Rush Chairman, Sports Chairman, member of Sigma Pi Fraternity, 1979 - 83

SAN MARIN HIGH SCHOOL, Novato, CA
Valedictorian, Student Body President, Varsity Track and Cross Country, 1975 -78.

Patents and Patent Applications

1. *Novel Strategy to Reduce Lactic Acid Production and Control pH in Animal Cell Culture,* Matthew S. Croughan and Nathaniel W. Freund, US Patent 8470552

2. *Methods and Compositions for Improving Protein Production,* Michael Barnett and Matthew S. Croughan, Intl. Pub. # WO 2011/091350 A2, published July 28, 2011

Peer-reviewed Publications (1238 citations, Feb 2023 numbers shown below for top 13)

1. *A Rational Approach to the Scale-up of Affinity Chromatography,* F.H. Arnold, J.J. Chalmers, M.S. Saunders, M.S. Croughan, H.W. Blanch, and C.R. Wilke, ACS Symposium Series, 271, 113-122, 1985 (F.H. Arnold is now Nobel Laureate) (17 cit.)

2. *Engineering Developments in the Homogeneous Culture of Animal Cells: Oxygenation of Reactors and Scale-up,* J.G. Aunins, J.M Goldstein, M.S. Croughan, and D.I.C. Wang, Biotech. Bioeng. Symp. #17, 699-723, 1986 (32 cit.)

3. *Hydrodynamic Effects on Animal Cells Grown in Microcarrier Cultures,* M.S. Croughan, J.-F. Hamel, and D.I.C. Wang, Biotech. Bioeng., 29, 130-141, 1987 (348 cit.)

4. *Hydrodynamic Effects on Animal Cells Grown in Microcarrier Cultures,* M.S. Croughan, Doctoral thesis, Chemical Engineering, MIT, 1988 (16 cit.)

5. *Effects of Microcarrier Concentration in Animal Cell Culture,* M.S. Croughan, J.-F. Hamel, and D.I.C. Wang, Biotech. Bioeng., 32, 975-982, 1988 (100 cit.)

6. *Growth and Death in Overagitated Microcarrier Cultures,* M.S. Croughan and D.I.C. Wang, Biotech. Bioeng., 33, 731-744, 1989 (98 cit.)

7. *Viscous Reduction of Turbulent Damage in Animal Cell Culture,* M.S. Croughan, E.S. Sayre, and D.I.C. Wang, Biotech. Bioeng., 33, 862-872, 1989 (145 cit.)

8. *Reversible Removal and Hydrodynamic Phenomena in CHO Microcarrier Cultures,* M.S. Croughan and D.I.C. Wang, Biotech. Bioeng., 36, 316-319, 1990 (14 cit.)

9. *Hydrodynamic Effects on Animal Cells in Microcarrier Bioreactors,* M.S. Croughan and D.I.C. Wang, in *Animal Cell Bioreactors,* C.S. Ho and D.I.C. Wang, eds., Butterworth-Heinemann, Stoneham, MA, 1991 (33 cit.)

10. *Immobilized Animal Cell Bioreactors,* M.S. Croughan, T-W. Chiou, and D.I.C. Wang, in *Bioreactor System Design,* J.A. Asenjo and J.C. Merchuk, eds., Marcel Dekker, N.Y., 1995

11. *Hydrodynamic Effects on Animal Cells Grown in Microcarrier Cultures,* M.S. Croughan, J.-F. Hamel, and D.I.C. Wang, Biotech. Bioeng., 67:06, 2000 (paper #3 reprinted, as one of top 20 ever published in journal, in special 40th anniversary issue) (130 cit.)

12. *From Microcarriers to Hydrodynamics: Introducing Engineering Science into Animal Cell Culture,* M.S. Croughan and W-S. Hu, Biotech. Bioeng., 95:02, 220-225, 2006

13. *Hydrodynamic Effects on Animal Cells Grown in Microcarrier Cultures,* M.S. Croughan, J.-F. Hamel, and D.I.C. Wang, Biotech. Bioeng., 95:02, 2006 (paper #3 reprinted again, as part of special 70th birthday celebration issue for Professor Daniel Wang of MIT)

14. *Accelerated Manufacturing of Large-Scale, Full-length, Human-like Glycosylated Antibodies for Biodefense,* C.M. Warner and M.S. Croughan, J. Bioterr. Biodef., 3(3), 1-7, 2012

15. *Trends Regarding Viral Barrier Implementation in Animal Cell Culture Processes,*

S. Delfosse, M. Sathavipat, N. Hsu, M. Croughan, and M. LaFond, Pharm. Bioprocess., 1(4), 351-360, 2013

16. *The Future of Industrial Bioprocessing: Batch or Continuous?*, M.S. Croughan, K.B. Konstantinov, C.L. Cooney, Biotech. Bioeng., 112(4), 648-651, 2015 (173 cit.)

17. *New Scalable Manufacturing Platform for Shear-Sensitive Cell Therapy Products*, B. Lee, D. Giroux, Y. Hashimura, N. Starkweather, F. Rosello, R. Wesselschmidt, M.S. Croughan, Cytotherapy, 18(6), S140, 2016

18. *Novel Single-Use Bioreactors for Scale-up of Anchorage-Dependent Cell Manufacturing for Cell Therapies,* M. Croughan, D. Giroux, D. Fang, B. Lee, in *Stem Cell Manufacturing*, J.M.S Cabral, C. Lobato da Silva, L.G. Chase, M.M. Diogo, eds., Elsevier, Amsterdam, 2016 (28 cit.)

19. *A Simple Method to Reduce Both Lactic Acid and Ammonium Production in Industrial Animal Cell Culture*, N.W. Freund, M. S. Croughan, Int. J. Mol. Sci., 19, 385, 2018 (40 cit.)

20. *Beyond preclinical research: production of CHO-derived biotherapeutics for toxicology and early-phase trials by transient gene expression or stable pools,* M. Stuible, F. van Lier, M.S. Croughan, Y. Durocher, Curr. Opin. Chem. Eng., 22, 145-151, 2018

21. *Computational fluid dynamic characterization of vertical-wheel bioreactors used for effective scale-up of human induced pluripotent stem cell aggregate culture*, T. Dang, B.S. Borys, S. Kanwar, J. Colter, H. Worden, A. Blatchford, M.S. Croughan, T. Hossan, D.E. Rancourt, B. Lee, M.S. Kallos, S. Jung, The Canadian J. Chem. Eng., 99, 2536-2553, 2021

22. *Initial Power Measurements for a Family of Novel Vertical-Wheel Bioreactors, M.S. Croughan, D. Giroux, O. Agbojo, E. McCain, N. Starkweather, S. Guerra, Y. Hashimura, B. Lee, S. Jung*, Canadian J. Chem. Eng., in press