

PROT@GEN[®] Protagen AG Otto-Hahn-Str. 15 44227 Dortmund	Analysis Report
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Analysis Report **Further Protein Characterization of** **Idunorate-2-sulfatase (GC1111)**

PG383-G03-01

	Name	Date	Signature
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Review and Release	Dr. Andreas Wattenberg	28.02.2011	

Prepared for :

Yo-Kyung Chung

Green Cross Corporation

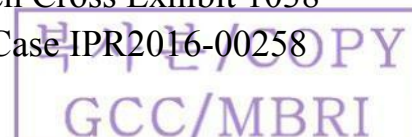
341, Bojeong-dong, Giheung-Gu, Yongin, 446-799

Korea

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Green Cross Corp., v. Shire Human Genetic Therapies, Inc. Case IPR2016-00258

Green Cross Exhibit 1038



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1 Executive Summary

1.1 Aim

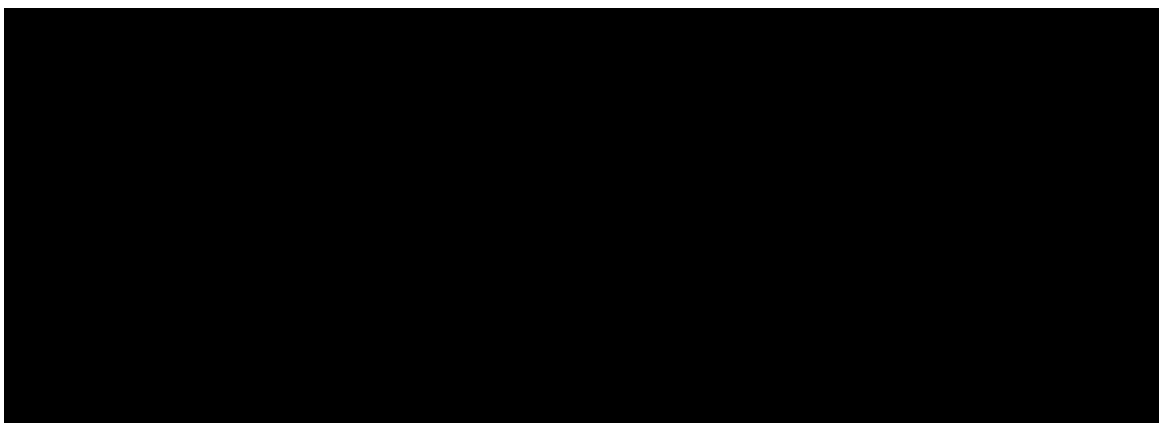
The aim of the work package is to develop and perform an assay for determination of the Formylglycine content in GC 1111, Iduronate 2-sulfatase samples.

1.2 Results

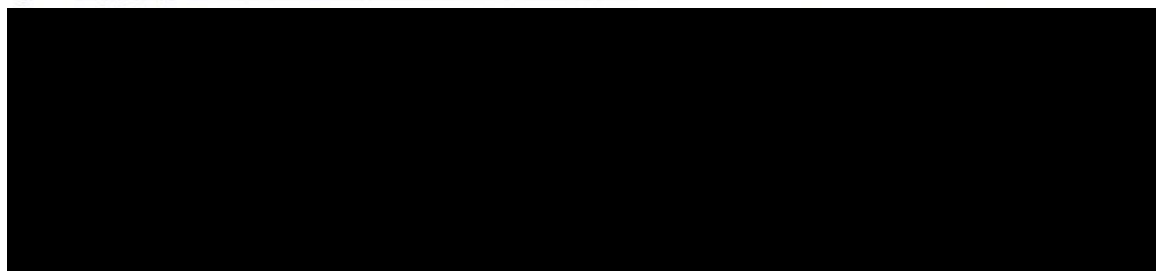
We have successfully developed and established a novel method for quantification of Formylglycine, based on the AQUA technique. With this approach, we determined that $80 \pm 15\%$ of GC1111 carries Formylglycine at position 59 (Table 2). The error of quantification was estimated to be 15% which is in the expected range for such a complex analysis.

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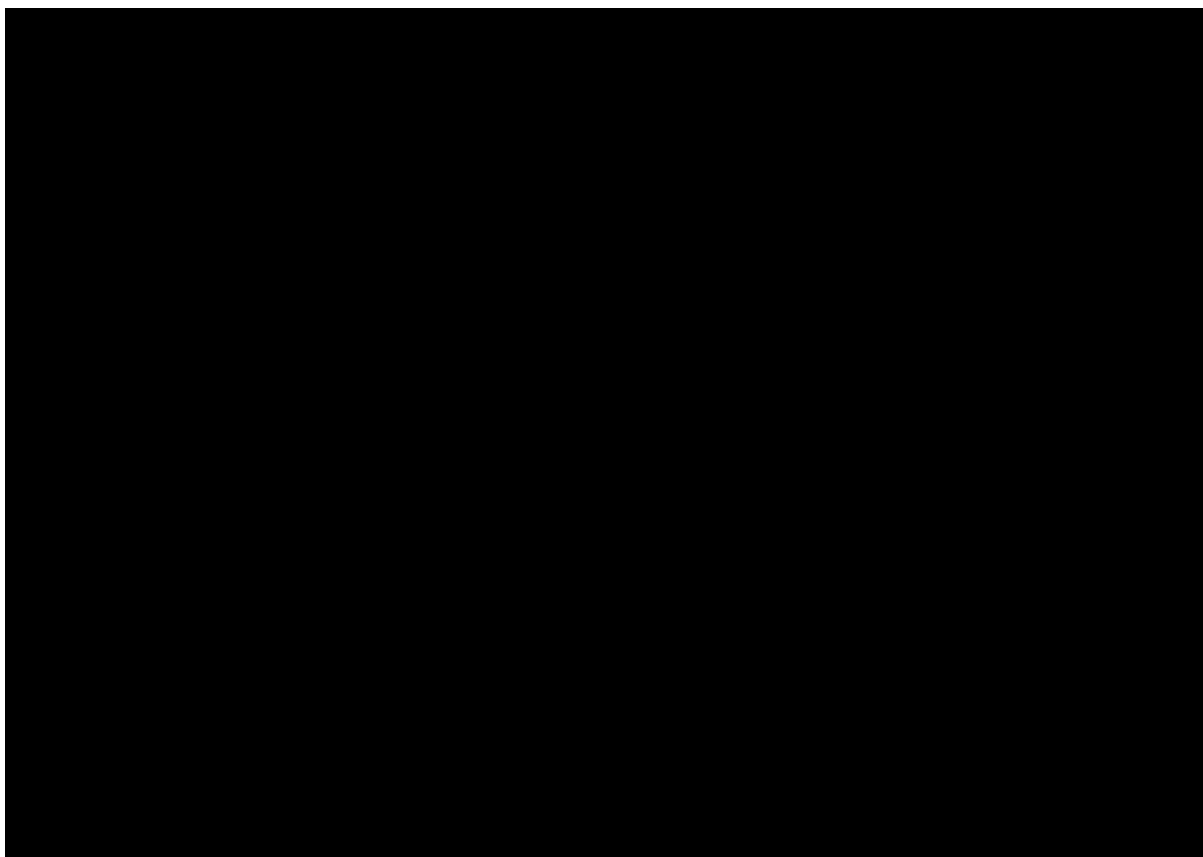
3 Terms / Abbreviations

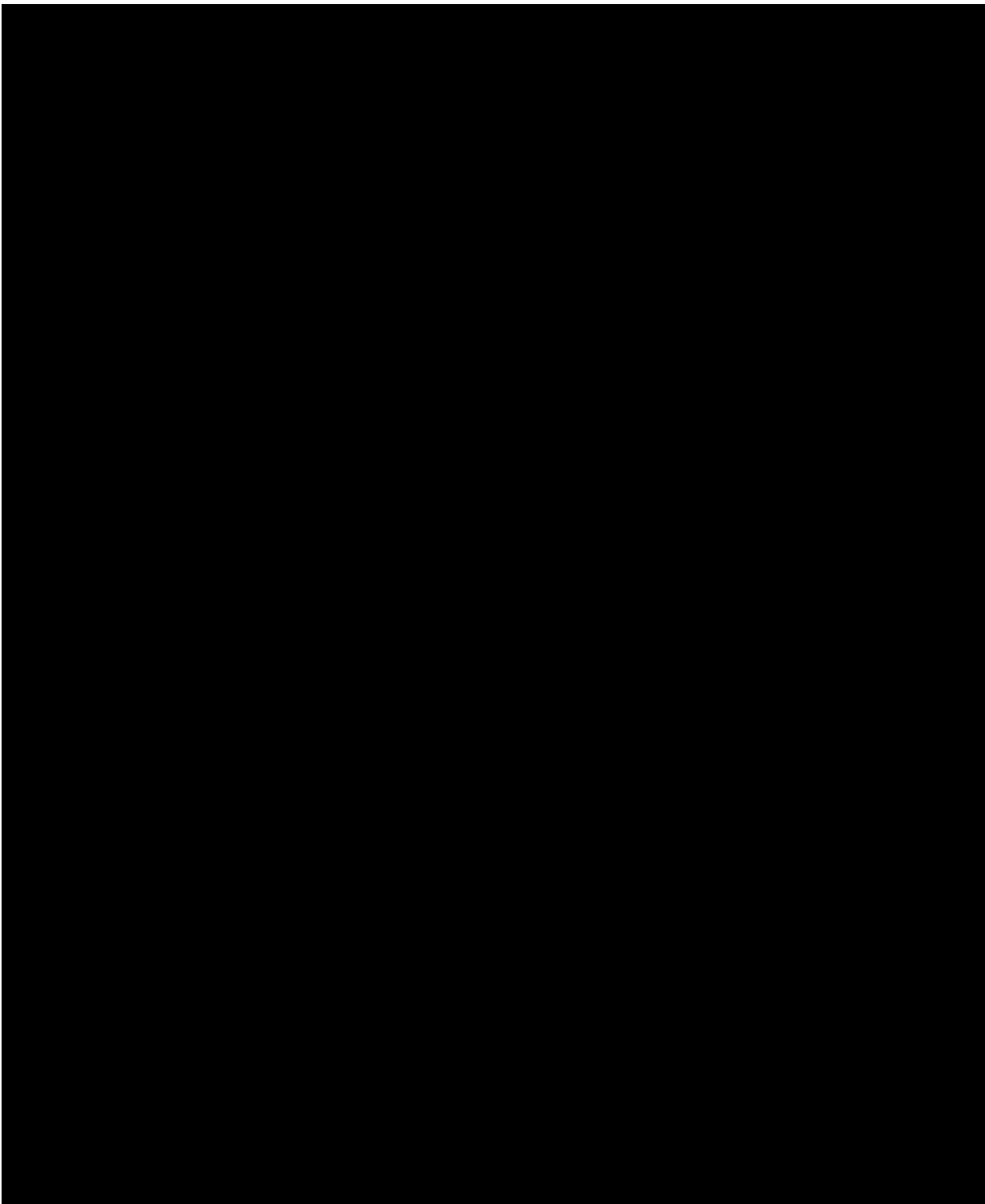
AQUA	Absolute Quantification
FGly	Formylglycine
FGE	Formylglycine-generating enzyme
Cys	Cysteine
MS	Mass spectrometry
EIC	Extracted ion chromatogram
CAM	Carbamidomethyl
MALDI-MS	Matrix-Assisted Laser Desorption-Ionization Mass Spectrometry
LC-ESI-MS/MS	Liquid-chromatography-electrospray-ionization-fragmentation mass spectrometry
Da	Dalton
rt	Retention Time

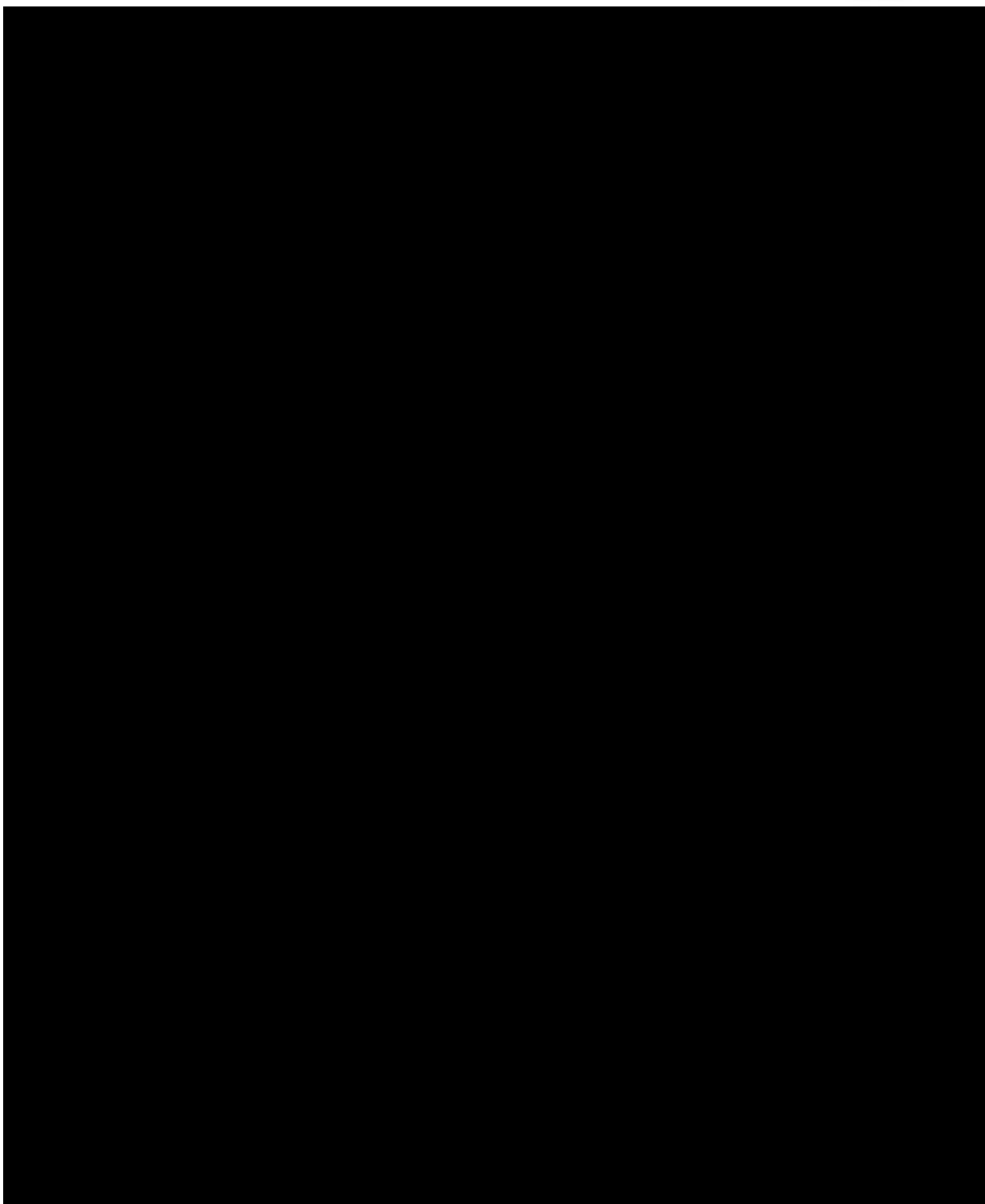
4 Facility

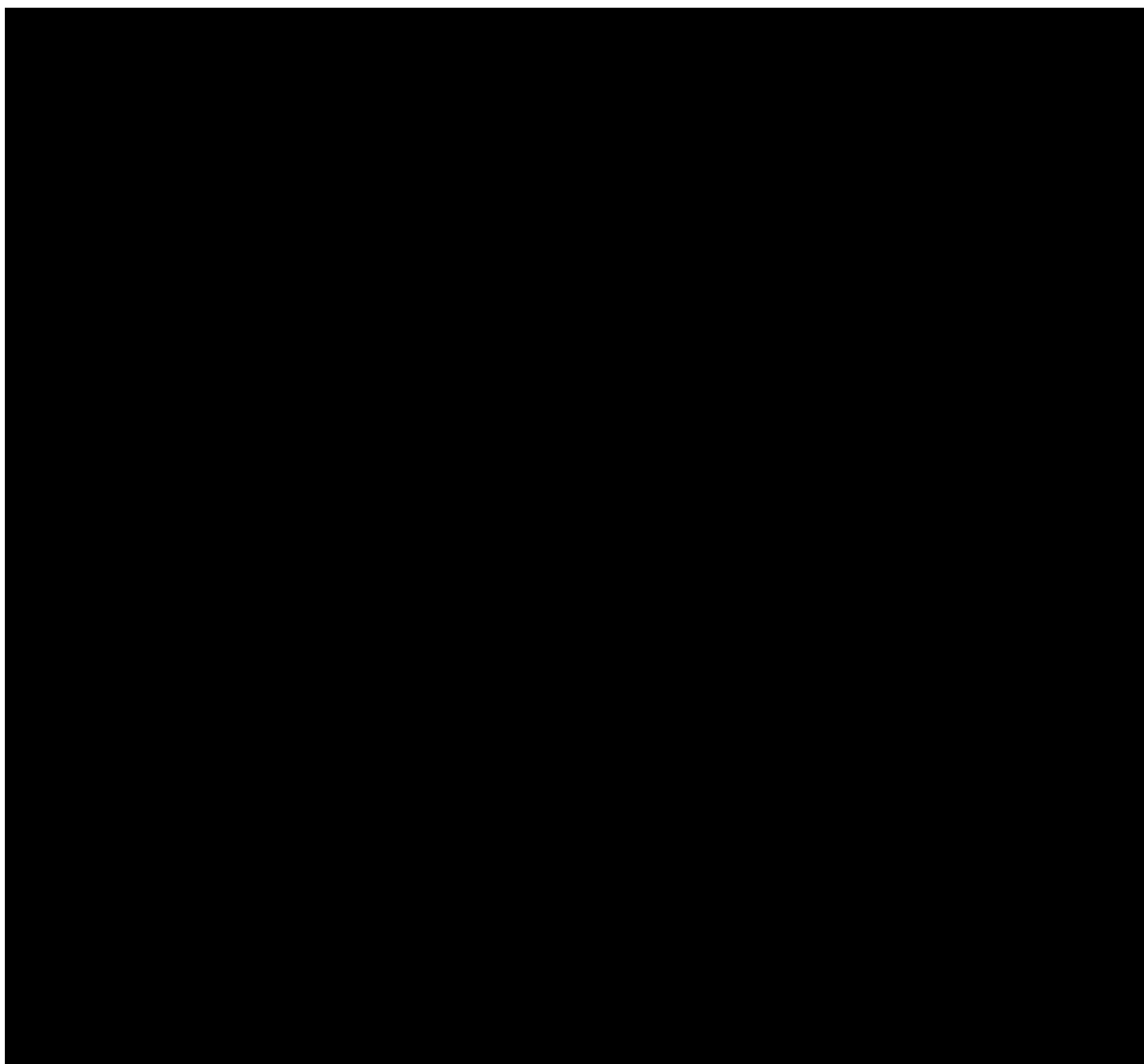
Company	Protagen AG		
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Zip	44227	City	Dortmund
Department	Protein Services		
Project Manager	Susanne Hensel		
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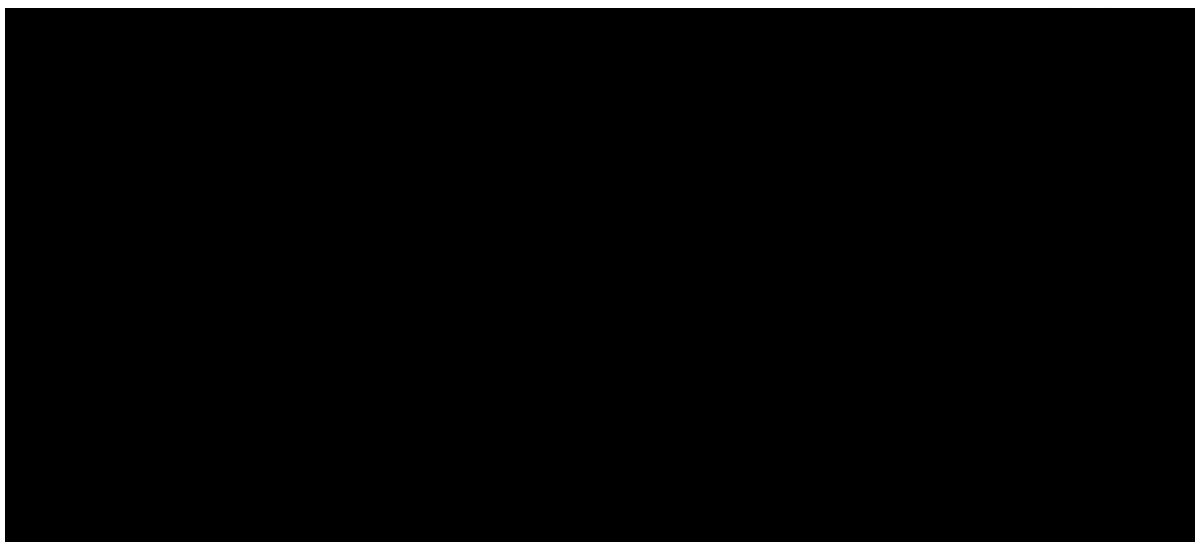
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GCC/MBRI

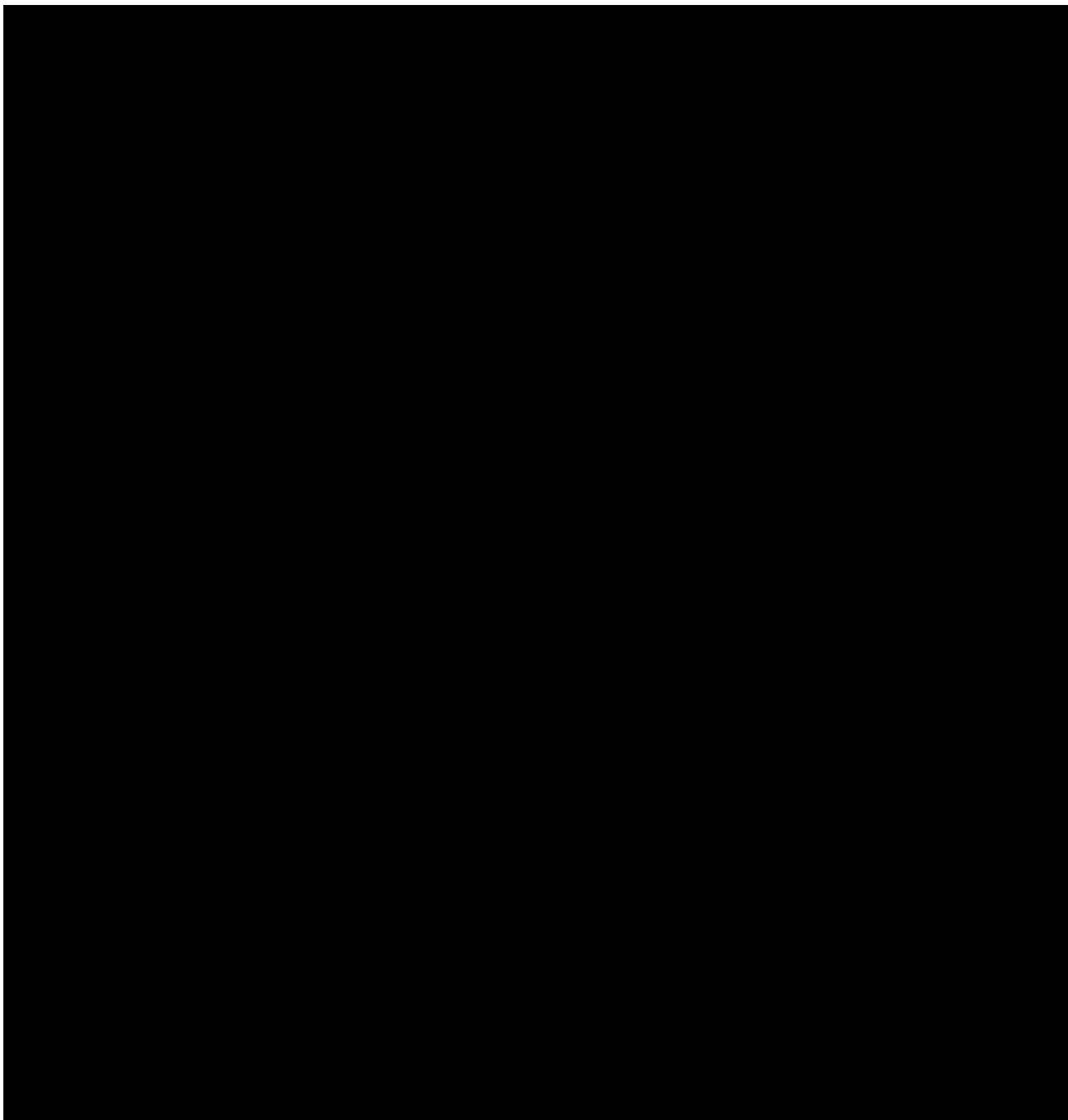












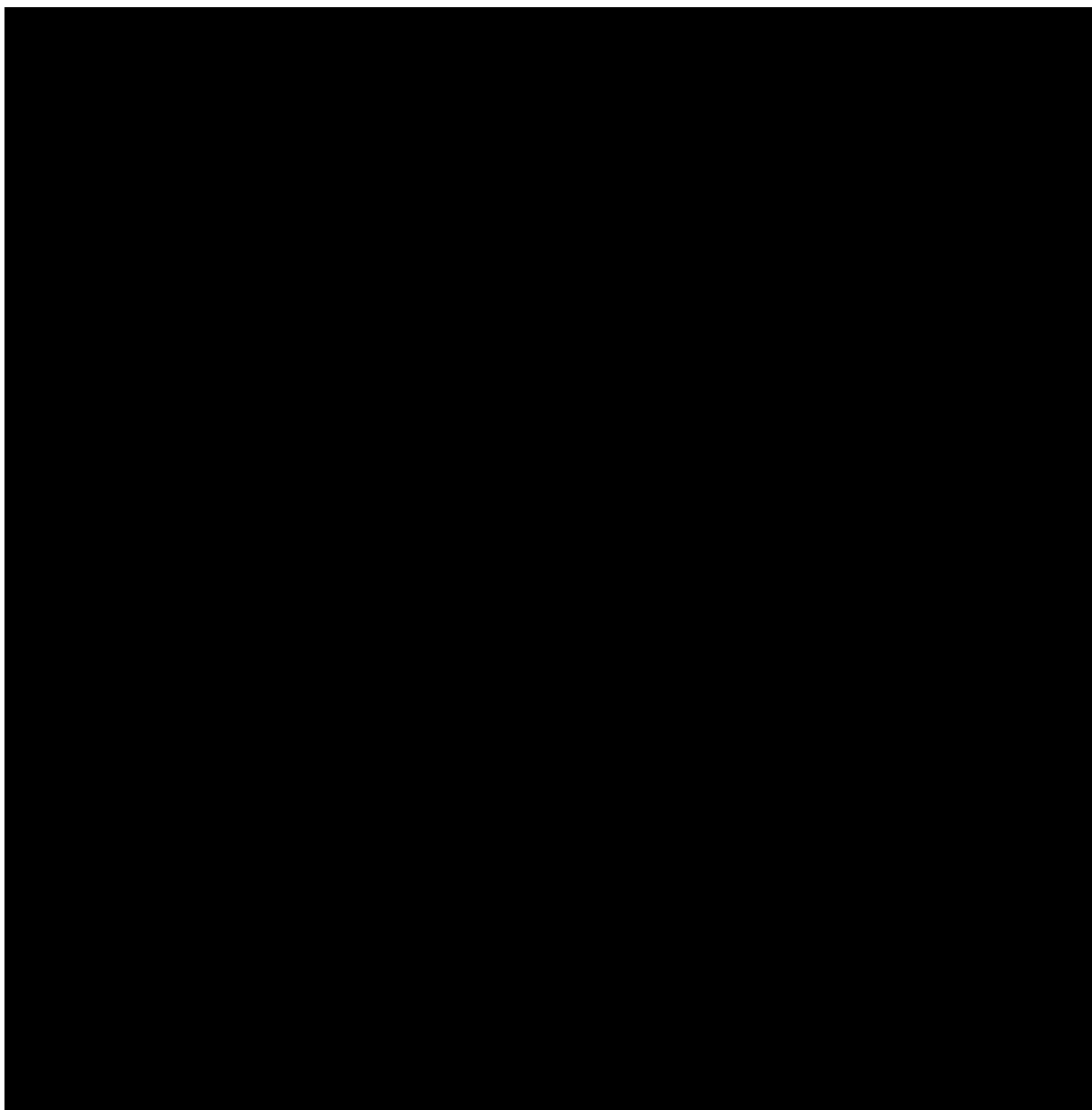
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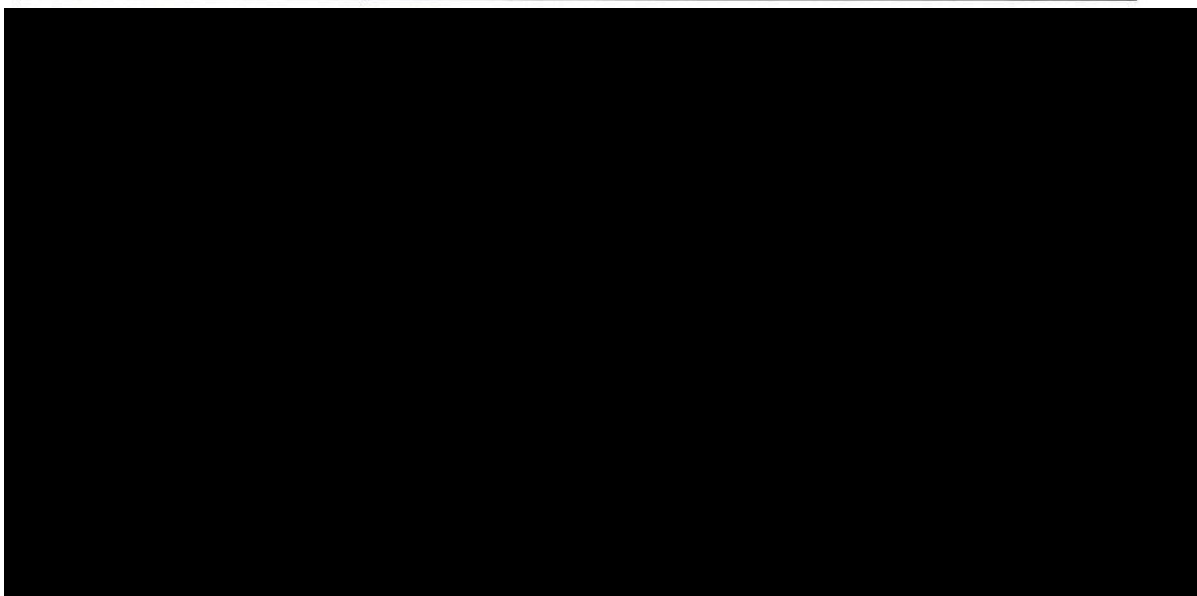
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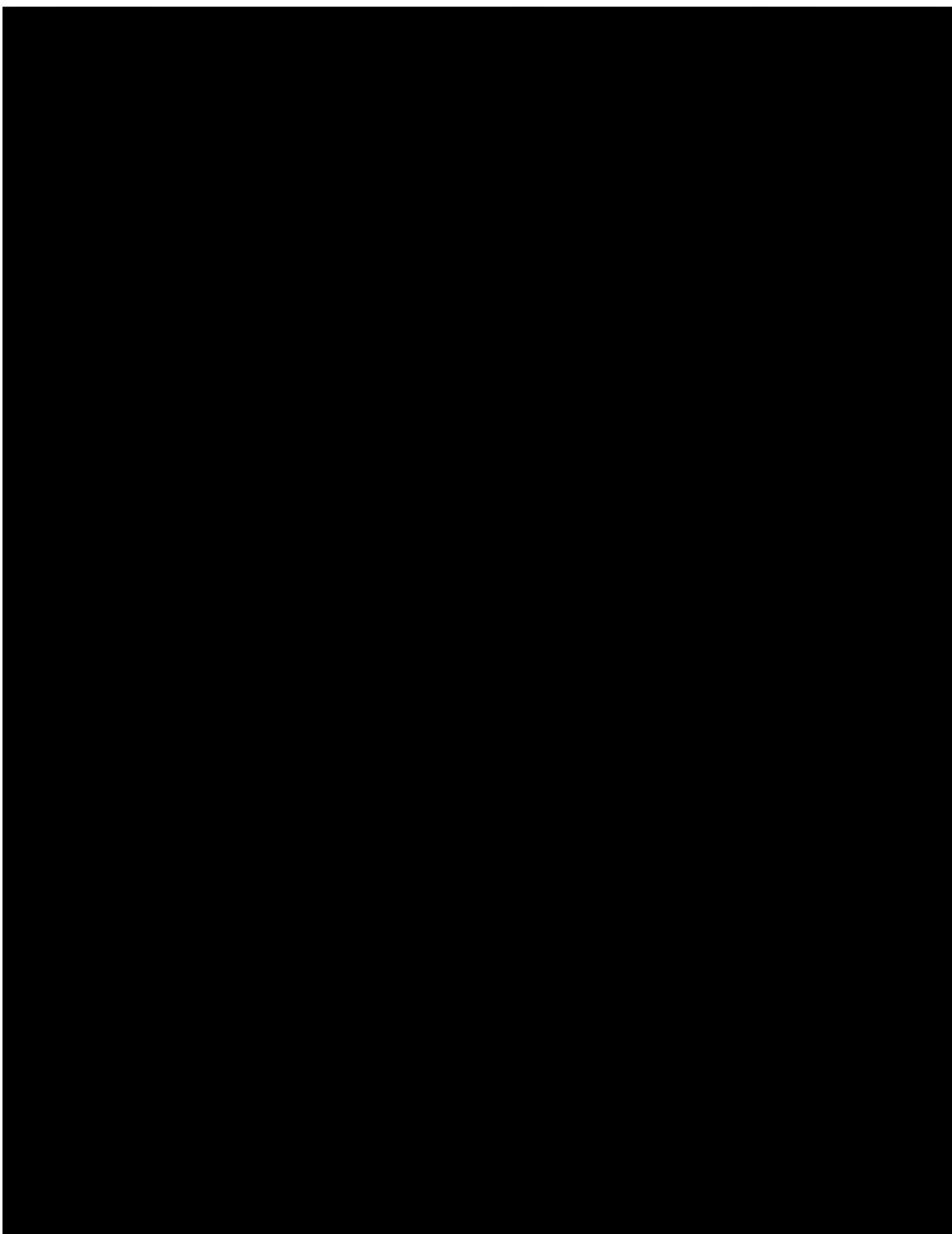
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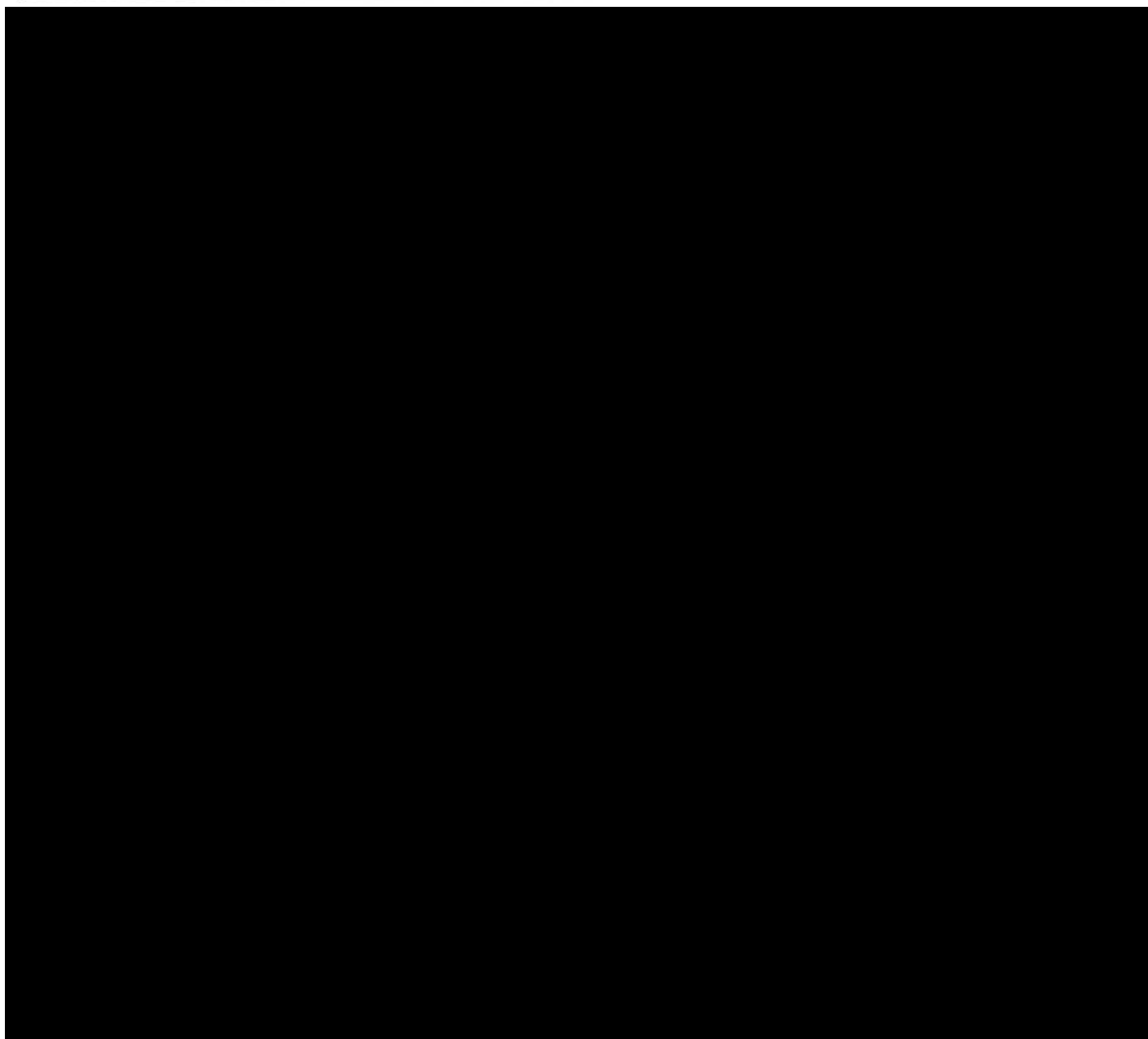
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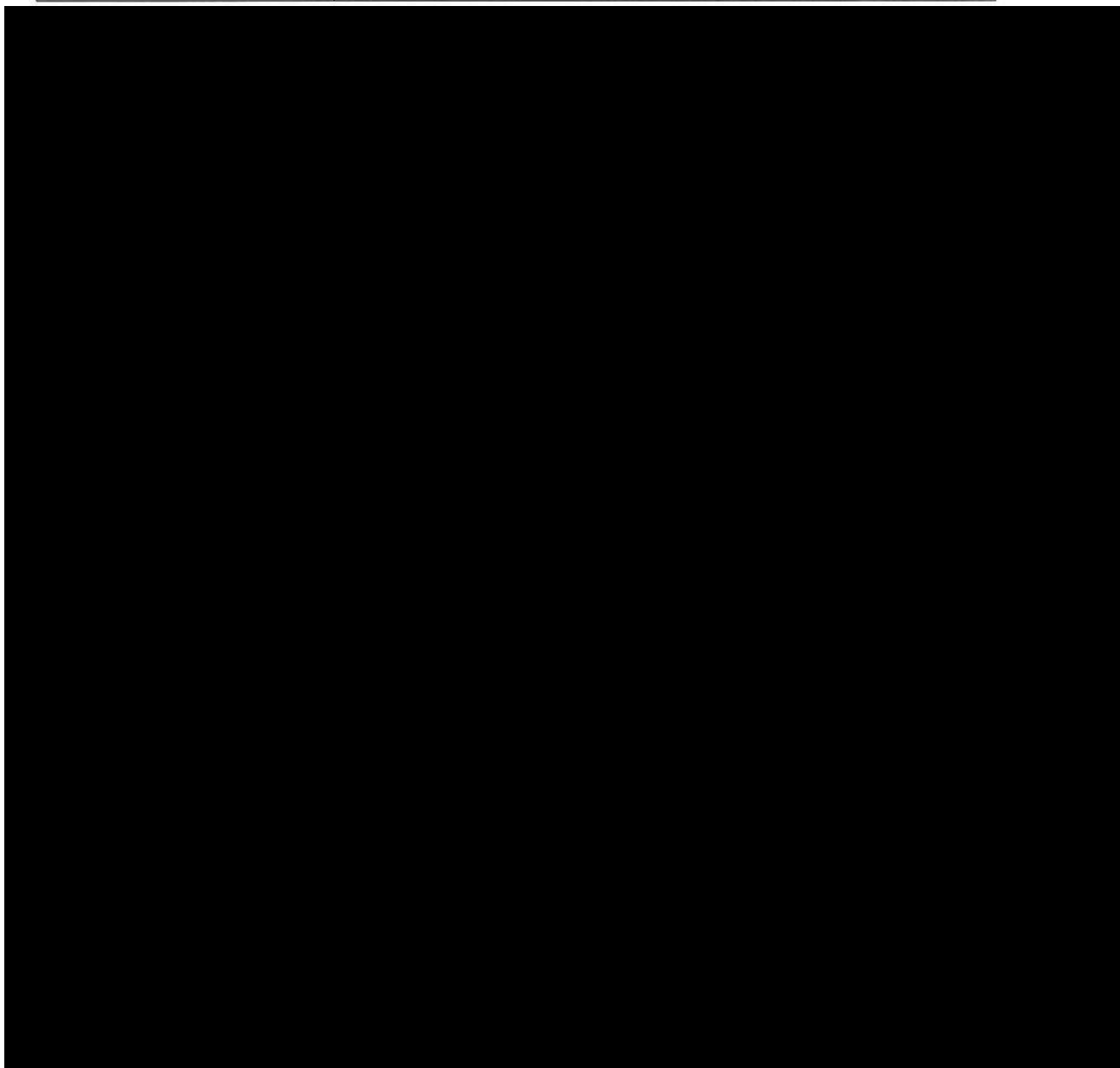
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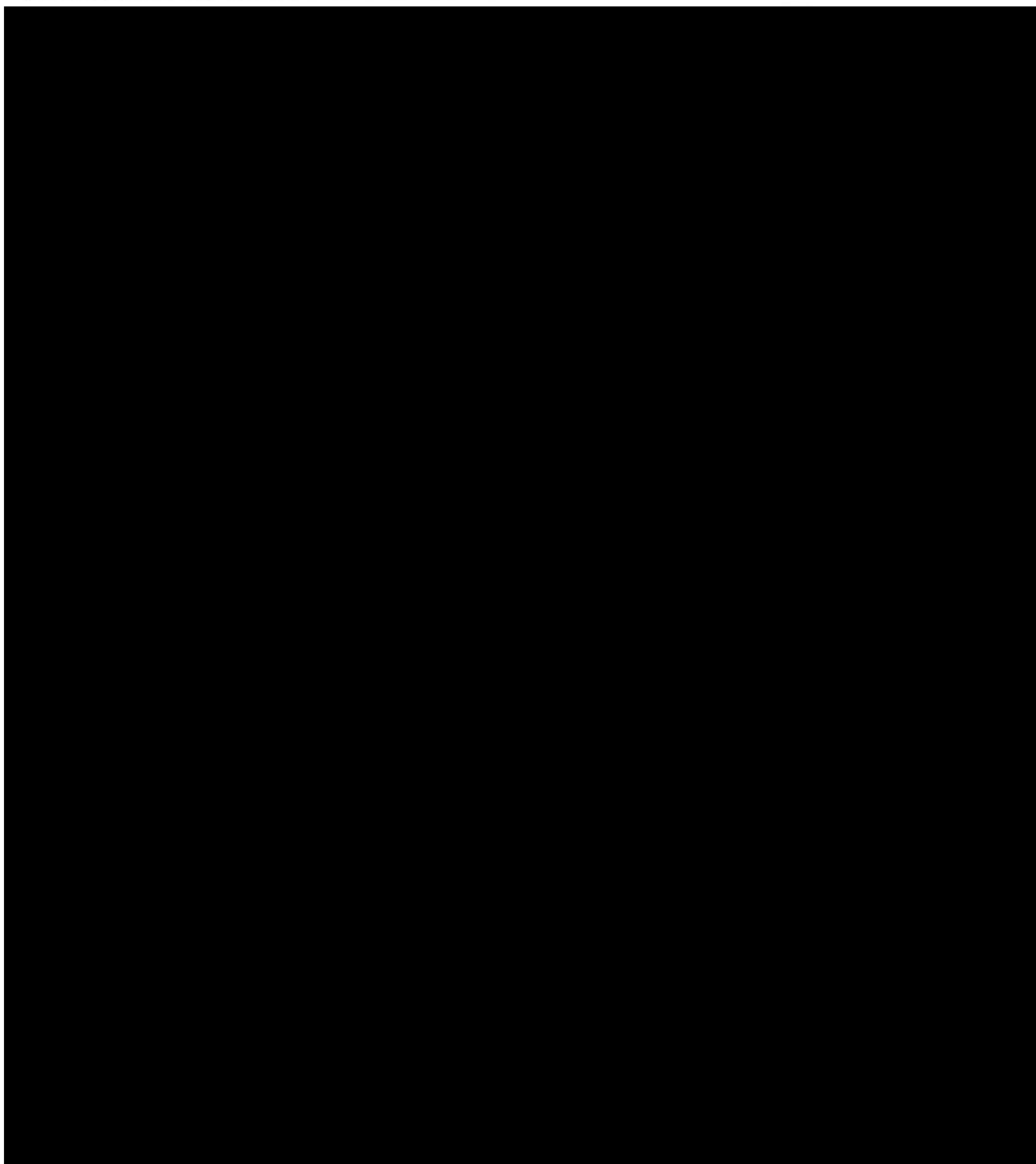
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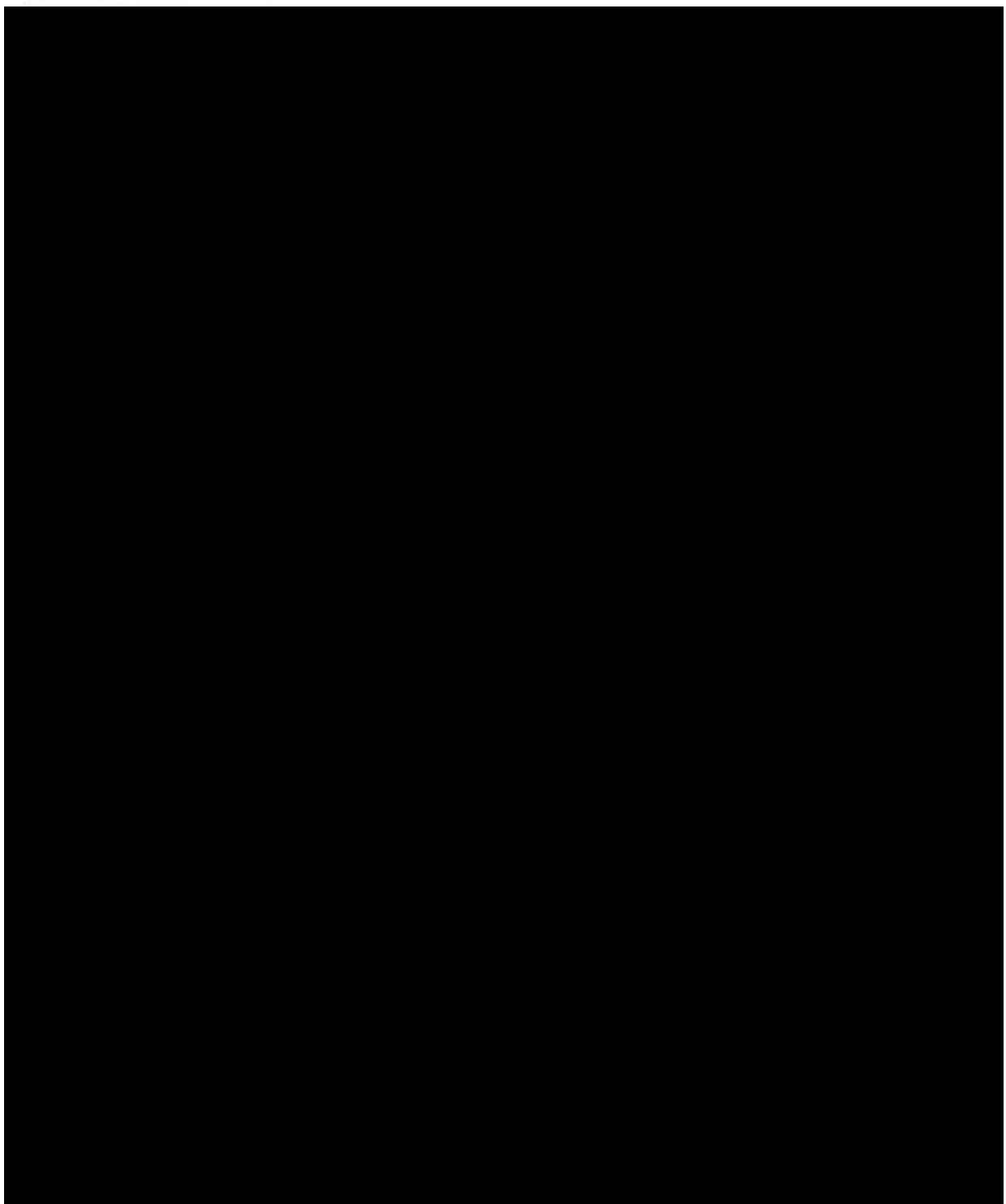
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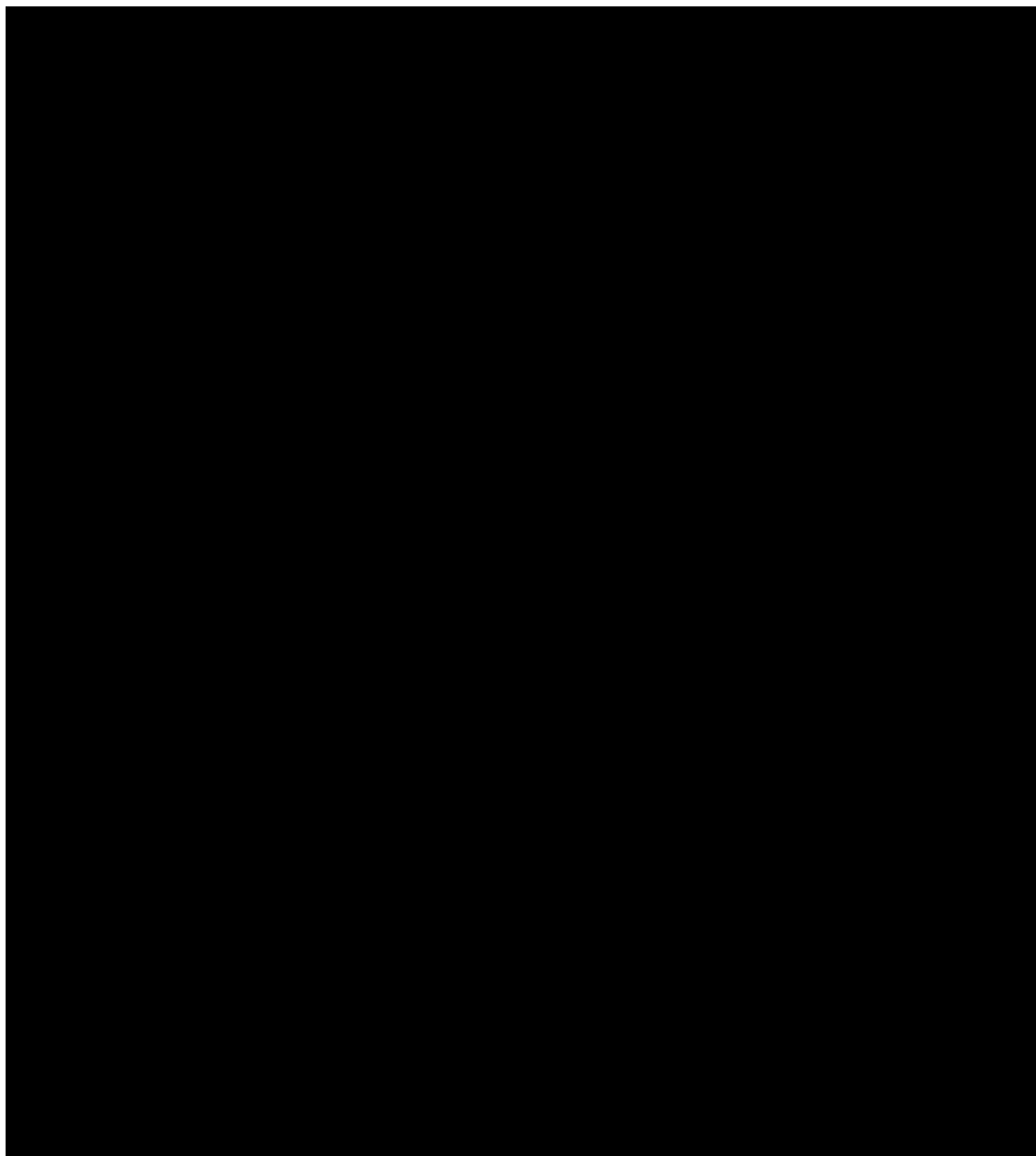
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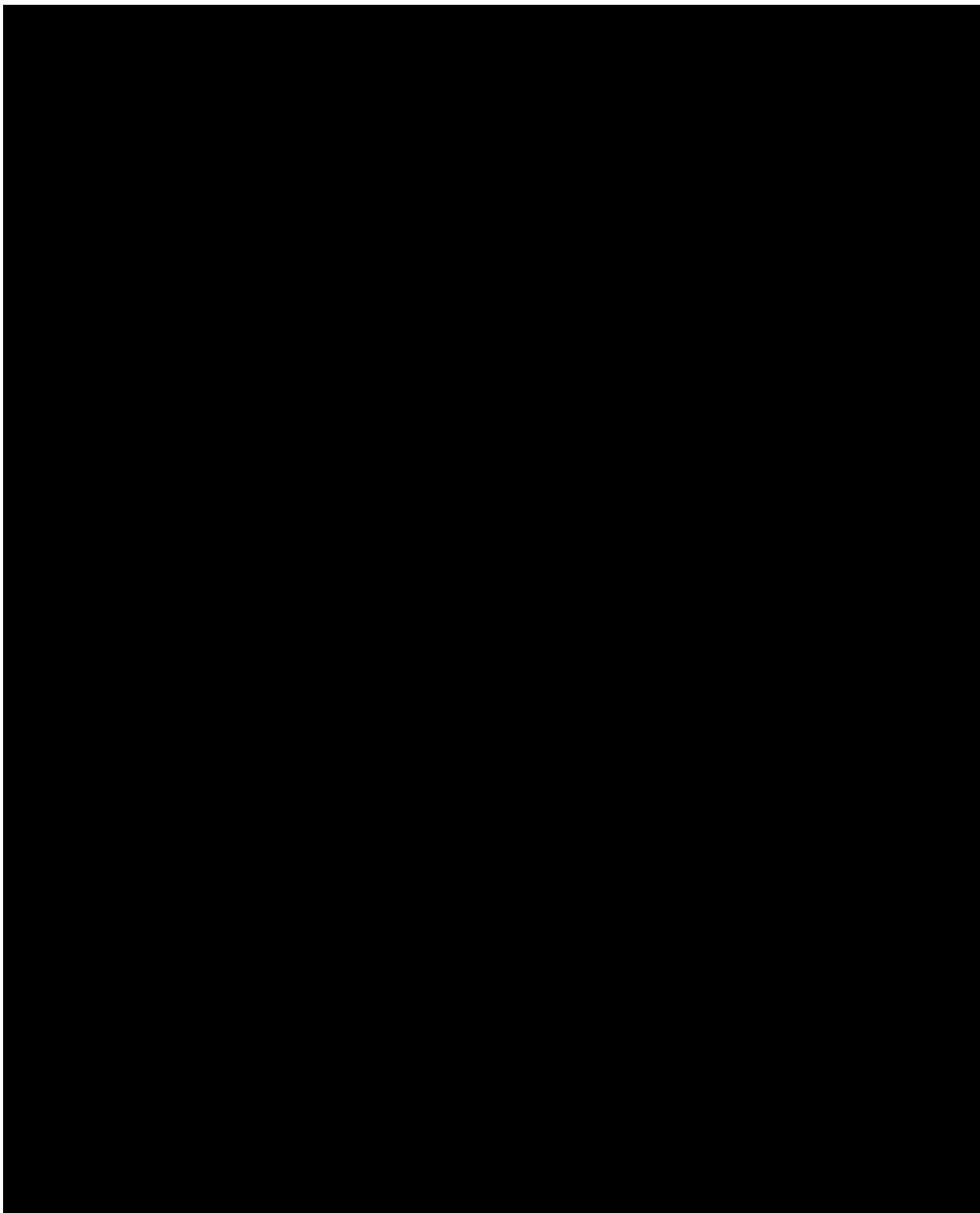
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The same approximate ratio of FGly-GC1111 to Cys-GC1111 was also found in the preliminary experiments using MALDI-MS.

Table 2: Formylglycine ratio for GC1111

Sample	Formylglycine ratio at position 59
GC1111	80±15 % FGly-GC1111 20±15 % Cys-GC1111

9.4 Conclusion/Discussion

One of the most critical steps during method development was the generation of an FGly-AQUA standard. The Cys-AQUA peptide was converted into FGly-AQUA peptide by means of an enzymatic reaction. For the Cys-AQUA peptide, the reaction was very successful. A conversion rate of approximately 90% was achieved. This conversion had never been carried out before using a synthetic peptide.

We then optimized the general workflow for AQUA quantification in several rounds. Parameters for optimization were protein concentration, spiking amounts of the standards, digestion conditions, general handling etc.

As a result of the method setup, we have successfully developed and established a novel method for quantification of Formylglycine, based on the AQUA technique. With this approach, we determined that 80±15% of GC1111 carries Formylglycine at position 59. The error of quantification was estimated to be 15% which is in the expected range for such a complex analysis.

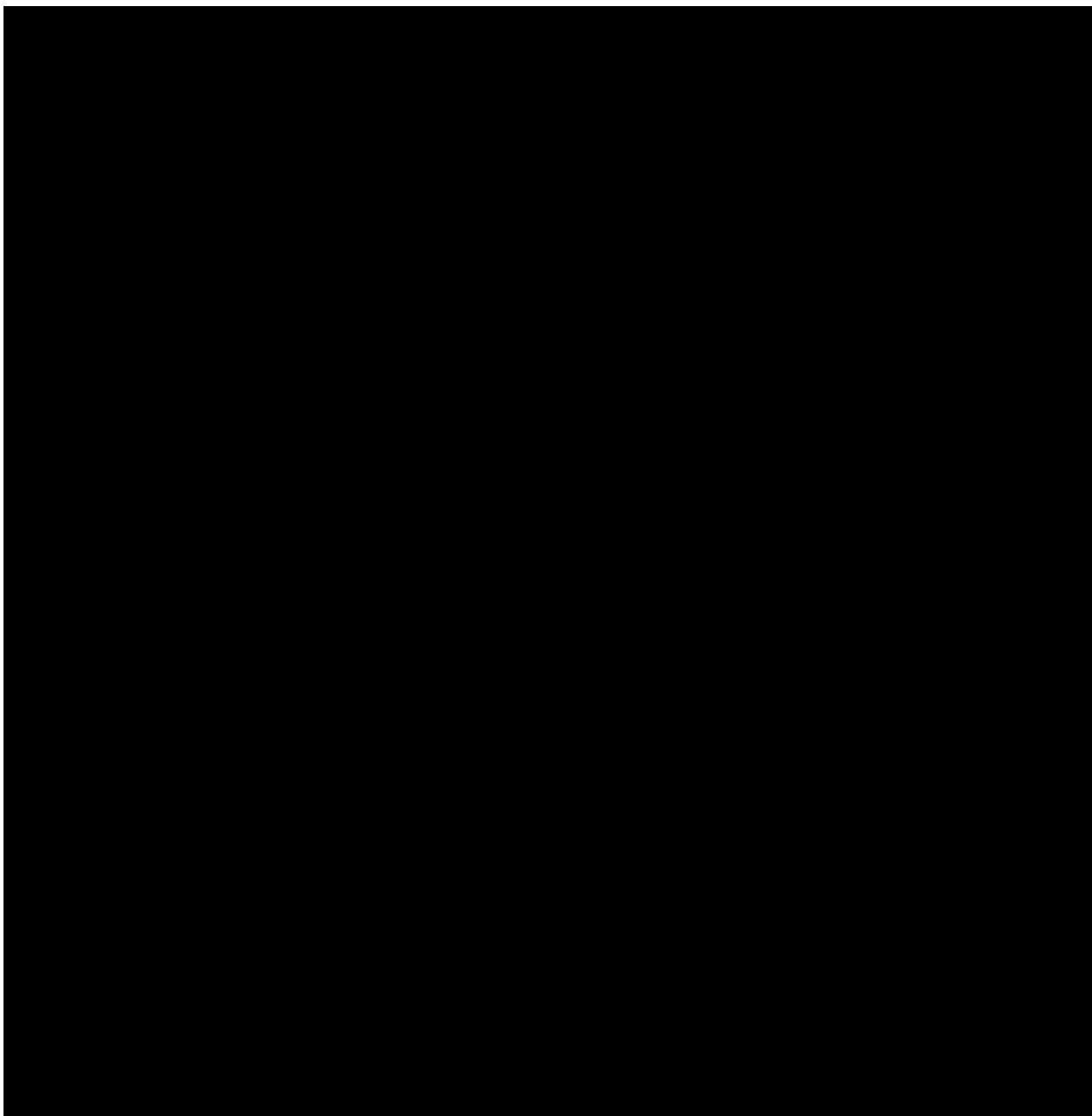
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