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Bernadette Juarez
Deputy Administrator
United States Department of Agriculture
Animal and Plant Health Inspection Service
Biotechnology Regulatory Services
4700 River Road Unit 147
Riverdale, MD 20737-1236

Re: Request for a Regulatory Status Review of Short Stature Maize MON 94804

Dear Ms. Juarez,

Bayer CropScience LP is submitting this request for an initial Regulatory Status Review (RSR) to USDA-APHIS of the enclosed information for a determination of nonregulated status for the new genetically engineered (GE) maize product, MON 94804, any progeny derived from crosses between MON 94804 and conventional maize, and any progeny derived from crosses between MON 94804 and other GE maize not subject to 7 CFR Part 340 regulations.

Bayer CropScience LP has developed short stature maize MON 94804 that can provide U.S. growers with agronomic and environmental benefits, including reduced lodging and green snap, extended in-season crop access, and potential for improved environmental sustainability. MON 94804 will be combined through traditional breeding with other deregulated traits to provide protection against maize pests, as well as herbicide tolerance. These next generation combined-trait maize products will offer broader grower choice, improved production efficiency, increased pest protection, the potential for increased yield, and promote a more sustainable agriculture system.

The enclosed information is being submitted in accordance with the Guidance for Requesting a Regulatory Status Review under 7 CFR part 340 (USDA-APHIS Document ID BRS-GD-2020-0003). We would be pleased to meet with you and other USDA officials and scientists to respond to any questions you may have, or to provide you with additional information that you may request.

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May 31, 2022

Hong S. Moon, Ph.D.
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Should you have any questions on this letter, the enclosed information or wish to set up a meeting to further discuss MON 94804, please contact James Nyangulu, Federal Engagement Lead, at (202) 304-6594, or Hong Moon at (314) 239-7524.

Thank you for your attention to this matter.

Yours sincerely,

DocuSigned by:
Hong Moon
Signer Name: Hong Moon
Signing Reason: I am the author of this document
Signing Time: 31-May-2022 | 10:09:10 PM CEST
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cc: Bayer CropScience LP Regulatory File
James M. Nyangulu, Ph.D. (202) 304-6594
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Enclosure: Request for a Regulatory Status Review of Short Stature Maize MON 94804

Request for a Regulatory Status Review of Short Stature Maize MON 94804

OECD Unique Identifier: MON-948Ø4-4

The undersigned submits this Regulatory Status Review (RSR) request under 7 CFR § 340.4 to request that the Administrator makes a determination that the article should not be regulated under 7 CFR Part 340.

May 31, 2022

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Hong Moon
Signer Name: Hong Moon
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Bayer RSR Number: CR284-22U1

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RELEASE OF INFORMATION

Bayer CropScience LP (hereafter Bayer) is submitting this request for a Regulatory Status Review (RSR) by the United States Department of Agriculture (USDA). Bayer understands that the USDA complies with the provisions of the Freedom of Information Act (FOIA). In the event the USDA receives a FOIA request, pursuant to 5 U.S.C., § 552, and 7 CFR Part 1, covering all or some of the information in this request, Bayer expects that, in advance of the release of the document(s), USDA will provide Bayer with a copy of the material proposed to be released and the opportunity to object to the release of any information based on appropriate legal grounds, *e.g.*, responsiveness, confidentiality, and/or competitive concerns. Bayer understands that a confidential business information (CBI)-deleted copy of this information may be made available to the public in a reading room and made available via the internet as part of a public comment period. Bayer also understands that if the review proceeds to the plant pest risk assessment (PPRA) step and the RSR request has been deemed complete, a copy of the RSR request may be posted to the USDA Animal and Plant Health Inspection Service (APHIS) Biotechnology Regulatory Services (BRS) website or other United States (U.S.) government websites (*e.g.*, www.regulations.gov). Except in accordance with the foregoing and required under applicable law, Bayer does not authorize the release, publication or other distribution of this information without Bayer's prior notice and consent.

ABBREVIATIONS AND DEFINITIONS

APHIS	Animal and Plant Health Inspection Service
DNA	Deoxyribonucleic Acid
GA	Gibberellic Acid/Gibberellin
GA20ox	Gibberellic Acid/Gibberellin 20 oxidase
GE	Genetically Engineered
miRNA	Micro Ribonucleic Acid
MOA	Mechanism of Action
OECD	Organization for Economic Co-operation and Development
RNAi	Ribonucleic Acid interference
RSR	Regulatory Status Review
T-DNA	Transfer Deoxyribonucleic Acid
U.S.	United States
USDA	United States Department of Agriculture
WHO	World Health Organization

I. REQUESTOR

The submitter of this initial Regulatory Status Review (RSR) request for MON 94804 maize is:

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Communications with regard to this submission should be directed to Hong S. Moon, Ph.D., Global Regulatory Manager, at the Bayer address listed above, or by email at hong.moon@bayer.com.

II. RATIONALE FOR THE DEVELOPMENT OF MON 94804

The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) has responsibility, under the Plant Protection Act (Title IV Pub. L. 106-224, 114 Stat. 438, 7 U.S.C. § 7701-7772) to prevent the introduction and dissemination of plant pests into the United States (U.S.). APHIS regulations at 7 CFR § 340.4, effective as of the date this Regulatory Status Review (RSR) request was filed, provide that an applicant may request a RSR of a plant developed using genetic engineering to evaluate whether the combination of the plant, introduced trait, and the trait's mechanism of action (MOA) pose an increased plant pest risk relative to the comparator plant. Genetically engineered (GE) plants that are unlikely to pose an increased plant pest risk relative to their conventional comparators do not fall within the scope of the 7 CFR Part 340 regulations and are no longer regulated.

II.A. Basis for the Request

Bayer is submitting this request for an initial RSR to APHIS for the agency to evaluate whether the GE maize product, MON 94804, any progeny derived from crosses between MON 94804 and conventional maize, and any progeny derived from crosses between MON 94804 and other GE maize should continue to be subject to 7 CFR Part 340 regulations.

II.B. Rationale for the Development of Short Stature Maize

Lodging (root and stalk lodging) and green snap can cause significant yield losses in maize. Annual yield losses in maize in the U.S. due to lodging and green snap range from 2 to 75%, depending upon growth stage, amount of breakage, and other factors (Nielsen and Colville, 1986, Lauer, 2011, Licht, 2020). Short stature maize MON 94804 that has approximately 1/3 reduction in plant height compared to the conventional maize comparator was developed to provide growers with agronomic and environmental benefits, including reduced lodging and green snap. Throughout product development, MON 94804 maize has consistently demonstrated reduced lodging and green snap in the fields that experienced adverse weather events such as high winds or strong storms. Severely lodged maize can lead to increased harvest losses, increased harvest time, increased drying cost, and may result in increased number of volunteer maize plants in the following year (Nielsen and Colville, 1986). Therefore, with reduced lodging and green snap, MON 94804 maize can reduce potential crop yield loss and associated impacts on costs and resources.

Another important benefit that MON 94804 maize can provide is increased flexibility through all-season access to implement more timely and precise crop input applications with standard ground equipment. The ability to access fields planted with typical height maize becomes limited when the plants grow taller than a standard ground sprayer boom height. Mid to late-season application of agrochemicals (*e.g.*, fungicide) and/or key nutrients (*e.g.*, nitrogen) for maize plants can be optimized with MON 94804 maize, enabling more precise, well-timed, and "as-needed" crop input applications with standard ground equipment. Thus, the optimized crop input application eliminates the need for aerial application or avoids early season application that may not have been needed. This optimized and timely application of crop inputs can positively

contribute to the environmental sustainability of maize farming with potential reduction of carbon or greenhouse gas emissions.

III. DESCRIPTION OF COMPARATOR PLANT

The MON 94804 transformation was conducted with maize (*Zea mays* L. subsp. *mays*) inbred line HCL301, and the molecular insert sequencing information is described in Section IV. HCL301 is a Bayer proprietary medium season yellow dent maize line of the Stiff Stalk background that is best adapted to the central regions of the U.S. corn belt. MON 94804 maize and its comparator plant, HCL301 as inbred or hybrid, share the same genetic background, with the exception of the presence of the biotechnology-derived trait.

Maize is a familiar crop that has been grown extensively throughout the world and is the largest cultivated crop in terms of total global production. In the U.S., maize is grown in almost all states and is the largest crop grown in terms of acreage planted and net value. Maize plant height is highly variable depending on genotype, environment, and other factors including agronomic practices (Zsubori et al., 2002, Liu and Wiatrak, 2011, Murányi and Pepó, 2013, Williams et al., 2021). Conventional maize germplasm varies widely in plant height, ranging from 1 ½ to 16 feet (0.5 to 5 meters) (FAO, 2003). Maize height in current U.S. hybrids typically have a plant height of around 5-12 feet (Iowa Corn Growers Association, 2022). Some shorter stature maize hybrids are commercially available in the U.S. For example, Stine brand shorter maize hybrids have been commercially sold in the U.S. since 2012 (Stine Seed, 2022). Also, sweet corn hybrids usually have shorter height (*e.g.*, approximately 4-7 feet) depending on relative maturity (Home Garden Vegetables, 2020). In addition, Bayer developed a short stature maize hybrid through conventional breeding that was commercialized in Mexico in 2020 under the brand name VITALA, and is planned for commercialization in the U.S. in the near future.

IV. GENOTYPE OF THE MODIFIED PLANT FOR MON 94804

This section describes information to understand the genetic differences between the modified and the comparator plants, including nucleotide sequence and annotation of the genetic material that has been inserted into and stably integrated in the genome of the modified plant, as described by the Guidance for Requesting a RSR under 7 CFR part 340 (USDA-APHIS Document ID BRS-GD-2020-003).

MON 94804 maize was produced by *Agrobacterium tumefaciens*-mediated transformation of maize tissue using the transfer DNA (T-DNA) transformation plasmid vector PV-ZMAP527892. This plasmid is approximately 10.1 kb and contains one T-DNA, that is delineated by Right and Left Border regions. The T-DNA contains the *GA20ox_SUP* suppression cassette that expresses a miRNA that suppresses the expression of the targeted maize endogenous gibberellin 20 oxidase (*ZmGA20ox*) genes, *ZmGA20ox3* and *ZmGA20ox5*, resulting in the reduction of gibberellic acid/gibberellin (GA) levels predominantly in the stalk, leading to a reduction of internode length and consequently reduced overall plant height compared to conventional maize comparator. The T-DNA that was inserted initially contained a *cp4 epsps* selectable marker cassette flanked by two excision targeting sequences called *loxP* sites. After MON 94804 was screened and selected as an acceptable transformant, the selectable marker cassette was excised by crossing with a Cre recombinase expressing maize line. Subsequently, segregation, selection, and screening were used to isolate those plants that contained the *GA20ox_SUP* suppression cassette and lacked the *cp4 epsps* selectable marker cassette and any sequences from the *cre* gene containing plasmid.

The nucleotide sequence of the inserted genetic material in MON 94804 maize is provided in Appendix A:, and an annotation of the different genetic elements is provided in Table IV-1.

Table IV-1. Annotation of the Inserted Genetic Material in MON 94804

Genetic Element	Location in Sequence	Function (Reference)
B[1, r1]-Left Border Region	1-40	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA (Barker et al., 1983) (GenBank accession: OK586894 positions 1 through 40)
Intervening sequence	41-107	"Synthetic"[2] sequence used in DNA cloning
loxP	108-141	Sequence from Bacteriophage P1 for the *loxP* recombination site recognized by the Cre recombinase (Russell et al., 1992) (GenBank accession: M10145 positions 24 through 57)
Intervening sequence	142-296	"Synthetic" sequence used in DNA cloning
P[3]-*RTBV-1*	297-1062	Promoter and leader from the rice tungro bacilliform virus (RTBV) (Yin and Beachy, 1995) that directs transcription in plant cells. (GenBank accession: OL473855)
Intervening sequence	1063-1082	"Synthetic" sequence used in DNA cloning
I[4]-*Hsp70*	1083-1886	Intron and flanking exon sequence of the *hsp70* gene from *Zea mays* (maize) encoding the heat shock protein 70 (HSP70) (Rochester et al., 1986) that is involved in regulating gene expression (Brown and Santino, 1997) (GenBank accession: OK586893)
GA20ox_SUP	1887-2294	Sequence composed of an inverted repeat (Plasmid location: 5742-5762 and 5797-5817) derived from coding sequence of *GA20ox3* and *GA20ox5* genes from *Zea mays* (maize) that encodes the gibberellic acid/gibbrellin 20 oxidase 3 and 5 proteins (Song et al., 2011), flanked and separated by three Osa-MIR1425 fragments (Plasmid location: 5630-5741, 5763-5796, 5818-6037) from *Oryza sativa* (rice) (Lacombe et al., 2008) that together form part of the suppression cassette. (GenBank accession: OL473856)

Table IV-1. Annotation of the Inserted Genetic Material in MON 94804 (Continued)

Genetic Element	Location in Sequence	Function (Reference)
Intervening sequence	2295-2326	"Synthetic" sequence used in DNA cloning
T[5]-*GST43*	2327-2626	A 3' UTR that has been developed from multiple 3' UTR sequences from *Zea mays* (maize) (To et al., 2021) that directs polyadenylation of the mRNA. (GenBank accession: OL473857)
Intervening sequence	2627-2733	"Synthetic" sequence used in DNA cloning

[1] B, Border

[r1] Superscript in Left Border Region indicates that the sequence in MON 94804 was truncated compared to the sequences in PV-ZMAP527892

[2] The term "synthetic" used in this table is defined and described in the USDA/APHIS-BRS Guidance Document BRS-GD-2020-0003. In the context of this table, the word "synthetic" does not indicate that the sequence was manufactured but rather that the sequence is not purposefully obtained from a known source and does not have an assigned function, although some homology may exist to known DNA sequences.

[3] P, Promoter

[4] I, Intron

[5] T, Transcription Termination Sequence

V. DESCRIPTION OF THE NEW TRAIT FOR MON 94804

This section describes the intended MON 94804 trait, intended phenotype associated with the trait, and MOA by which the intended phenotype will be conferred, as described by the Guidance for Requesting a RSR under 7 CFR part 340 (USDA-APHIS Document ID BRS-GD-2020-003).

V.A. Description of the Intended MON 94804 Trait

MON 94804 maize is intended to provide altered plant height compared to conventional maize comparator.

V.B. Intended Phenotype of MON 94804

MON 94804 maize is intended to have reduced internode length on the stalk that in turn results in the intended reduced plant height compared to conventional maize comparator.

V.C. Description of the Mechanism of Action for MON 94804

Development of short stature crops in rice and wheat had a transforming impact on world agriculture through the Green Revolution (Borlaug, 2000). Identification of the genes responsible for these traits shows that they interfere with the action or production of the plant hormone GA (Hedden, 2003). GAs are essential for many developmental processes in plants, including stem elongation, seed germination, and floral transition (Achard and Genschik, 2009). The biosynthesis of GA that has been characterized in many plant species is catalyzed by multiple enzymes (Yamaguchi, 2008). Among these enzymes, GA 20 oxidase (GA20ox) is a key enzyme in synthesizing bioactive GAs in the later steps of the GA biosynthesis pathway (Oikawa et al., 2004, Xue et al., 2013) (Figure V-1).

In maize, five *ZmGA20ox* genes were initially identified (Song et al., 2011). Four additional putative *ZmGA20ox* genes have also been identified in the Maize Genetics and Genomics database (MaizeGDB, https://www.maizegdb.org) (Portwood et al., 2019). According to the publicly available atlas of global transcription profiles for maize genes (Winter et al., 2007, Sekhon et al., 2011), *ZmGA20ox3* and *ZmGA20ox5* genes showed relatively higher expression levels in vegetative tissues and lower expression levels in reproductive tissues among the nine *ZmGA20ox* genes (Li et al, 2022). In addition, phylogenetic analysis of the *ZmGA20ox* gene family revealed that *ZmGA20ox3* and *ZmGA20ox5* shared high sequence homology with the rice semi-dwarf gene, *OsGA20ox2* known as the Green Revolution gene, that was one of the most important genes deployed in modern rice breeding (Monna et al., 2002, Sasaki et al., 2002, Spielmeyer et al., 2002). All three of these genes, *ZmGA20ox3, ZmGA20ox5, and OsGA20ox2,* are grouped together in the same clade (Song et al., 2011). With these considerations, *ZmGA20ox3* and *ZmGA20ox5* genes were selected as the target maize endogenous genes for suppression of gene expression in developing MON 94804 maize.

To precisely and effectively suppress gene expression of the above-mentioned target maize endogenous genes, a microRNA (miRNA)-mediated suppression cassette that works through an RNA interference (RNAi) process was developed. The RNAi mechanism is a natural process in

eukaryotic organisms for the regulation of endogenous gene expression (Fire et al., 1998, Jones-Rhoades et al., 2006). miRNAs can trigger the RNAi mechanism like other small non-coding RNAs such as small interfering RNA (siRNA) (Carthew and Sontheimer, 2009).

The MOA of MON 94804[1] was studied and described in detail in Paciorek et al. (2022). As described above in Table IV-1, the *GA20ox_SUP* suppression cassette is composed of the rice tungro baciliform virus (RTBV) promoter, the HSP70 intron, the miRNA encoding sequence designated *GA20ox_SUP*, and the GST43 terminator. The miRNA encoding gene, *GA20ox_SUP*, was designed with sequences from rice to provide the backbone structure of the initial transcript and sequences from maize to provide an inverted repeat sequence derived from coding sequences of *ZmGA20ox3* and *ZmGA20ox5* genes from maize. The expressed inverted repeat transcript is recognized by the endogenous RNAi machinery, resulting in down-regulation of the target endogenous GA biosynthetic genes, *ZmGA20ox3* and *ZmGA20ox5*. Expression levels of both target endogenous gene transcripts were reduced in most tissues including stalk internode (Paciorek et al., 2022).

It is important to demonstrate the target gene specificity of the *GA20ox_SUP* suppression cassette to evaluate the potential for unintended off-target gene suppression. To demonstrate the target gene specificity, expression levels of *ZmGA20ox1*, the most homologous gene to the two target genes in maize were quantified (Paciorek et al., 2022). Unlike the targeted *ZmGA20ox3* and *ZmGA20ox5* genes, no consistent trend of expression level reduction of *ZmGA20ox1* was observed in tissues evaluated. In some tissues, expression of *ZmGA20ox1* deviated from the control, which is probably a result of the well-documented compensatory mechanism of a GA-dependent feedback loop that regulates the expression level of some *GA20ox* genes (Phillips et al., 1995, Xu and Gaga, 1999, Itoh et al., 2002, Hedden, 2003, Desgagné-Penix and Sponsel, 2008). The results demonstrate suppression of gene expression by the GA20ox_SUP miRNA[2] produced from the *GA20ox_SUP* suppression cassette is specific to the targeted *ZmGA20ox3* and *ZmGA20ox5* genes (Paciorek et al., 2022).

The effect of suppression of *ZmGA20ox3* and *ZmGA20ox5* gene expression on endogenous bioactive GA levels was also assessed. Bioactive GAs, GA_1 and GA_4, were reduced significantly in the vegetative tissues of stalk internode and leaf with no significant reduction of GA levels being detected in reproductive tissues (Paciorek et al., 2022). Therefore, suppression of both targeted genes resulted in the reduction of bioactive GA levels most significantly in the stalk internode, leading to a reduction of stalk internode length, which consequently reduced overall plant height without affecting the reproductive potential when compared to the control maize (Paciorek et al., 2022).

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[1] The events, which are described as event-1 and event-2 in Paciorek et al. (2022), are MON 94804 and an experimental event containing a miRNA-mediated suppression cassette named *GA20ox_SUP* suppression cassette.

[2] GA20ox_SUP miRNA includes the original transcript, intermediate processing products and the GA20ox_SUP mature miRNA (21-nucleotide).

Additionally, GAs are known to play an important role in the regulation of seed germination (Finch-Savage and Leubner-Metzger, 2006) and flowering (Pharis and King, 1985). MON 94804 seeds showed normal germination as demonstrated by equivalent seedling stand count in the field (Paciorek et al., 2022). Further, reproductive phenology of MON 94804 maize suggested no changes in days to 50% anthesis, days to 50% silking, and normal ear development compared to the control maize. Considering all these data, the results support our conclusion that miRNA-mediated suppression of the targeted GA biosynthetic gene expression led to intended changes (*i.e.*, reduced stalk internode length leading to reduced overall plant height) with no observed unintended impacts on other metabolic, physiological or developmental processes (Paciorek et al., 2022).

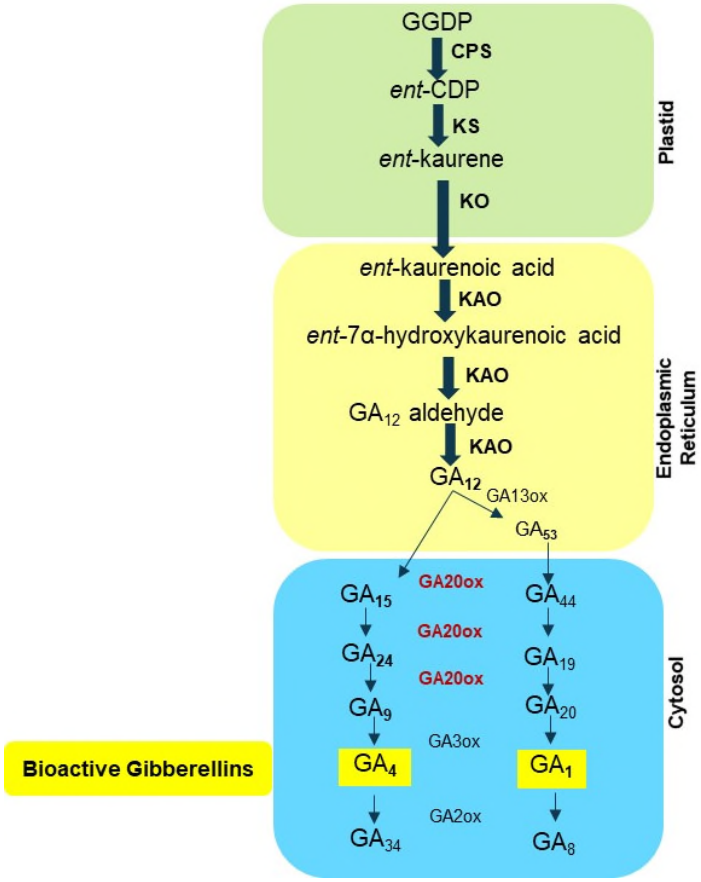

Figure V-1. Illustration of GA Biosynthesis Pathway in Plants

Figure adapted and recreated from (Binenbaum et al., 2018). GGDP: geranylgeranyl diphosphate; *ent-CDP: ent-* copalyl diphosphate; CPS: *ent*-copalyl diphosphate synthase; KS: *ent*-kaurene synthase; KO: *ent*-kaurene oxidase; KAO: *ent*-kaurenoic acid oxidase; GA20ox: Gibberellin 20-oxidase enzyme that is encoded by *ZmGA20ox* gene family in which *ZmGA20ox3* and *ZmGA20ox5* genes are targeted for suppression in MON 94804 maize.

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Appendix A: Sequence of the Insertion for MON 94804

>20210628_jdvest_MON_94804_Final_Assembly-USDA_Stage1

GACGTGTCTACATTCACGTCCAAATGGGGGCTTAGATGAGAAACTTCACGATTTGGCGCG

ACTAACTAAGCACTAGCGTACGGGACCCAGATATCGAATTCAAGCTTATAACTTCGTATA

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TGAGCCCGGGCAGGCCTACCCATAATACCCATAATAGCTGTTTGCCAATCGTTCTTCTTG

GCGCGCCAAGACGCAAACTCGGACCGACGGTACCCTCAGCGCTGTGCCTGTTGCGATCCT

ACAAAAGGGAGTAGTAATATTTAATGAGCTTGAAGGAGGATATCAACTCTCTCCAAGGTT

TATTGGAGACCTTTATGCTCATGGTTTTATTAAACAAATAAACTTCACAACCAAGGTTCC

TGAAGGGCTACCGCCAATCATAGCGGAAAAACTTCAAGACTATAAGTTCCCTGGATCAAA

TACCGTCTTAATAGAACGAGAGATTCCTCGCTGGAACTTCAATGAAATGAAAAGAGAAAC

ACAGATGAGGACCAACTTATATATCTTCAAGAATTATCGCTGTTTCTATGGCTATTCACC

ATTAAGGCCATACGAACCTATAACTCCTGAAGAATTTGGGTTTGATTACTACAGTTGGGA

AAATATGGTTGATGAAGACGAAGGAGAAGTTGTATACATCTCCAAGTATACTAAGATTAT

CAAAGTCACTAAAGAGCATGCATGGGCTTGGCCAGAACATGATGGAGACACAATGTCCTG

CACCACATCAATAGAAGATGAATGGATCCATCGTATGGACAATGCTTAAAGAAGCTTTAT

CAAAAGCAACTTTAAGTACGAATCAATAAAGAAGGACCAGAAGATATAAAGCGGGAACAT

CTTCACATGCTACCACATGGCTAGCATCTTTACTTTAGCATCTCTATTATTGTAAGAGTG

TATAATGACCAGTGTGCCCCTGGACTCCAGTATATAAGGAGCACCAGAGTAGTGTAATAG

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CCACCGTCTTCGGTACGCGCTCACTCCGCCCTCTGCCTTTGTTACTGCCACGTTTCTCTG

AATGCTCTCTTGTGTGGTGATTGCTGAGAGTGGTTTAGCTGGATCTAGAATTACACTCTG

AAATCGTGTTCTGCCTGTGCTGATTACTTGCCGTCCTTTGTAGCAGCAAAATATAGGGAC

ATGGTAGTACGAAACGAAGATAGAACCTACACAGCAATACGAGAAATGTGTAATTTGGTG

CTTAGCGGTATTTATTTAAGCACATGTTGGTGTTATAGGGCACTTGGATTCAGAAGTTTG

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CACTTGTTTTAAATATTGTTGTCTGAAGAAATAAGTACTGACAGTATTTTGATGCATTGA

TCTGCTTGTTTGTTGTAACAAAATTTAAAAATAAAGAGTTTCCTTTTTGTTGCTCTCCTT

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ATCTTGCTCGATGCCTTCTCCCTAGTGTTGACCAGTGTTACTCACATAGTCTTTGCTCAT

TTCATTGTAATGCAGATACCAAGCGGGGCAGAGCCGTGCCCGTCTCATCCCCTGCCCGTG

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