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Fortsættes ...

DESCRIPTION

Field of the Invention

[0001] Embodiments of the present invention provide compounds and compositions for selectively reducing expression of an allelic variant of a gene containing a single nucleotide polymorphism (SNP). Such compounds and compositions are useful to treat, prevent, or ameliorate diseases.

Background of the Invention

[0002] Genetic diseases are caused by abnormalities in genes or chromosomes. Such abnormalities may include insertions, deletions, and expansions. Huntington's Disease (HD) is one example of a genetic disease caused by an expansion. HD is a progressive neurodegenerative disorder that is inherited in a dominant fashion and results from a mutation that expands the polymorphic trinucleotide (CAG) tract in the huntingtin gene (*HTT*). The average CAG tract size in the general population is 17-26 repeats (wild type allele), however, in HD patients the CAG tract has expanded to 36 repeats or more (mutant allele) (Huntington's Disease Collaborative Research Group 1993. Cell 72(6):971-83). The *HTT* gene encodes the HTT protein and the expanded CAG tract results in a pathological increase in the polyglutamine repeats near the N-terminal of the protein. Individuals carry two copies of the *HTT* gene and one mutant allele is sufficient to result in HD.

[0003] HTT protein appears to have a role during development of the nervous system and a protective role in cells. In mouse models, constitutive knockout of the *HTT* gene is lethal during embryonic development (Nasir et al 1995. Cell 81(5):811-23), while adult inactivation of the *HTT* gene leads to progressive cell death in the brain and the testes (Dragatsis et al 2000. Nat. Genet 26:300-306). Reduction of huntingtin expression from the wild type allele may, therefore, have negative consequences.

[0004] Like HD, there are disorders for which a strategy of selective reduction of a mutant allele would be beneficial. Antisense oligonucleotides that target mutant huntingtin alleles are mentioned in WO2009/135322. Lombardi et al., J Exp Neurol 2009; 217:312-319 describes the use of allele-specific RNA interference to treat Huntington's disease patients. Thus, there remains an unmet need to selectively reduce expression of mutant allelic variants like that of *HTT*, which are causative of disease, over the wild type variant, which appears to be necessary for normal cellular processes.

Brief Description of the Invention

[0005] Figure 1 provides the mRNA and genomic *HTT* sequence showing SNP positions.

Summary of the Invention

[0006] The invention provides a modified antisense oligonucleotide for use in treating, preventing or ameliorating a disease, wherein the disease is caused by the expression of a mutant allele containing a single nucleotide polymorphism and wherein the modified antisense oligonucleotide selectively reduces the expression of the mutant allele,
wherein the modified antisense oligonucleotide consists of 15 to 20 linked nucleosides targeted to a single nucleotide polymorphism site on the mutant allele that is associated with the disease and is capable of selectively reducing the expression of the mutant allele,
wherein the modified oligonucleotide is 100% complementary to the single nucleotide polymorphism site located on the mutant allele,
wherein the modified oligonucleotide is single-stranded and comprises a wing-gap-wing motif with a 5' wing region positioned at the 5' end of a deoxynucleoside gap, and a 3' wing region positioned at the 3' end of the deoxynucleoside gap,

wherein each of the nucleosides of each wing region comprises a modified sugar or sugar surrogate,
wherein the wing-gap-wing motif is any one of the group consisting of 5-10-5, 2-9-6, 3-9-3, 3-9-4, 3-9-5, 4-7-4, 4-9-3, 4-9-4, 4-9-5, 4-10-5, 4-11-4, 4-11-5, 5-7-5, 5-8-6, 5-9-3, 5-9-5, 5-10-4, 6-7-6, 6-8-5, and 6-9-2, and
wherein the single nucleotide polymorphism anneals to a nucleobase within the gap segment, and wherein position 4, 5, or 6 of the modified oligonucleotide, as counted from the 5' terminus of the gap, aligns with the single nucleotide polymorphism.

Summary of the Disclosure

[0007] Disclosed herein are compounds and compositions for selectively reducing expression of an allelic variant of a gene containing a single nucleotide polymorphism (SNP). Such methods, compounds, and compositions are useful to treat, prevent, or ameliorate diseases. SNPs may be associated with a mutant allele, the expression of which causes disease. In certain embodiments, the expressed gene product of a mutant allele results in aggregation of the mutant proteins causing disease. In certain embodiments, the expressed gene product of a mutant allele results in gain of function causing disease.

[0008] In certain embodiments, selective reduction of mRNA and protein expression of a mutant allele is achieved by targeting a SNP located on the mutant allele with an antisense compound. In certain embodiments, the antisense compound is an antisense oligonucleotide

[0009] In certain embodiments, antisense compounds designed to selectively reduce an allelic variant of a gene containing a SNP are created based on potency and selectivity of the antisense compound as well as population genetics.

Detailed Description

[0010] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed. Herein, the use of the singular includes the plural unless specifically stated otherwise. As used herein, the use of "or" means "and/or" unless stated otherwise. Furthermore, the use of the term "including" as well as other forms, such as "includes" and "included", is not limiting. Also, terms such as "element" or "component" encompass both elements and components comprising one unit and elements and components that comprise more than one subunit, unless specifically stated otherwise.

[0011] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Definitions

[0012] Unless specific definitions are provided, the nomenclature utilized in connection with, and the procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques may be used for chemical synthesis, and chemical analysis.

[0013] Unless otherwise indicated, the following terms have the following meanings:
"2'-O-methoxyethyl" (also 2'-MOE and 2'-$O(CH_2)_2$-OCH_3) refers to an O-methoxy-ethyl modification of the 2' position of a furosyl ring. A 2'-O-methoxyethyl modified sugar is a modified sugar.

[0014] "2'-O-methoxyethyl nucleotide" means a nucleotide comprising a 2'-O-methoxyethyl modified sugar moiety.

[0015] "5-methylcytosine" means a cytosine modified with a methyl group attached to the 5' position. A 5-methylcytosine is a modified nucleobase.

[0016] "Active pharmaceutical agent" means the substance or substances in a pharmaceutical composition that provide a therapeutic benefit when administered to an individual. For example, in certain embodiments an antisense oligonucleotide targeted to an allelic variant is an active pharmaceutical agent.

[0017] "Active target region" or "target region" means a region to which one or more active antisense compounds is targeted. "Active antisense compounds" means antisense compounds that reduce target nucleic acid levels or protein levels.

[0018] "Administered concomitantly" refers to the co-administration of two agents in any manner in which the pharmacological effects of both are manifest in the patient at the same time. Concomitant administration does not require that both agents be administered in a single pharmaceutical composition, in the same dosage form, or by the same route of administration. The effects of both agents need not manifest themselves at the same time. The effects need only be overlapping for a period of time and need not be coextensive.

[0019] "Administering" means providing a pharmaceutical agent to an individual, and includes, but is not limited to administering by a medical professional and self-administering.

[0020] "Allele" is one member of a pair of genes or one member of a series of different forms of a DNA sequences that can exist at a single locus or marker on a specific chromosome. For a diploid organism or cell or for autosomal chromosomes, each allelic pair will normally occupy corresponding positions (loci) on a pair of homologous chromosomes, one inherited from the mother and one inherited from the father. If these alleles are identical, the organism or cell is said to be 'homozygous' for that allele; if they differ, the organism or cell is said to be 'heterozygous' for that allele. "Major allele" refers to an allele containing the nucleotide present in a statistically significant proportion of individuals in the human population. "Minor allele" refers to an allele containing the nucleotide present in a relatively small proportion of individuals in the human population. "Wild type allele" refers to the genotype typically not associated with disease or dysfunction of the gene product. "Mutant allele" refers to the genotype associated with disease or dysfunction of the gene product.

[0021] "Allelic variant" refers to one of the pair of genes or DNA sequence existing at a single locus. For example, an allelic variant may refer to either the major allele or the minor allele.

[0022] "Amelioration" refers to a lessening of at least one indicator, sign, or symptom of an associated disease, disorder, or condition. The severity of indicators may be determined by subjective or objective measures, which are known to those skilled in the art.

[0023] "Animal" refers to a human or non-human animal, including, but not limited to, mice, rats, rabbits, dogs, cats, pigs, and non-human primates, including, but not limited to, monkeys and chimpanzees.

[0024] "Antibody" refers to a molecule characterized by reacting specifically with an antigen in some way, where the antibody and the antigen are each defined in terms of the other. Antibody may refer to a complete antibody molecule or any fragment or region thereof, such as the heavy chain, the light chain, Fab region, and Fc region.

[0025] "Antisense activity" means any detectable or measurable activity attributable to the hybridization of an antisense compound to its target nucleic acid. In certain embodiments, antisense activity is a decrease in the amount or expression of a target nucleic acid or protein encoded by such target nucleic acid.

[0026] "Antisense compound" means an oligomeric compound that is is capable of undergoing hybridization to a target nucleic acid through hydrogen bonding.

[0027] "Antisense inhibition" means reduction of target nucleic acid levels or target protein levels in the presence of an antisense compound complementary to a target nucleic acid compared to target nucleic acid levels or target protein levels in the absence of the antisense compound.

[0028] "Antisense oligonucleotide" means a single-stranded oligonucleotide having a nucleobase sequence that permits hybridization to a corresponding region or segment of a target nucleic acid.

[0029] "Bicyclic sugar" means a furosyl ring modified by the bridging of two ring atoms. A bicyclic sugar is a modified sugar.

[0030] "Bicyclic nucleoside" means a nucleoside having a sugar moiety comprising a bridge connecting two carbon atoms of the sugar ring, thereby forming a bicyclic ring system. In certain embodiments, the bridge connects the 4'-carbon and the 2'-carbon of the sugar ring.

[0031] "Cap structure" or "terminal cap moiety" means chemical modifications, which have been incorporated at either terminus of an antisense compound.

[0032] "cEt" or "constrained ethyl" means a bicyclic nucleoside having a sugar moiety comprising a bridge connecting the 4'-carbon and the 2'-carbon, wherein the bridge has the formula: 4'-$CH(CH_3)$-O-2'.

[0033] "Chemically distinct region" refers to a region of an antisense compound that is in some way chemically different than another region of the same antisense compound. For example, a region having 2'-O-methoxyethyl nucleotides is chemically distinct from a region having nucleotides without 2'-O-methoxyethyl modifications.

[0034] "Chimeric antisense compound" means an antisense compound that has at least two chemically distinct regions.

[0035] "Co-administration" means administration of two or more pharmaceutical agents to an individual. The two or more pharmaceutical agents may be in a single pharmaceutical composition, or may be in separate pharmaceutical compositions. Each of the two or more pharmaceutical agents may be administered through the same or different routes of administration. Co-administration encompasses parallel or sequential administration.

[0036] "Complementarity" means the capacity for pairing between nucleobases of a first nucleic acid and a second nucleic acid.

[0037] "Contiguous nucleobases" means nucleobases immediately adjacent to each other.

[0038] "Differentiating polymorphism" means a variation in a nucleotide sequence that permits differentiation between a wild type and a mutant allele of a nucleic acid sequence. Differentiating polymorphisms may include insertions or deletions of one or a few nucleotides in a sequence, or changes in one or a few nucleotides in a sequence. A differentiating polymorphism or polymorphic allele can be in linkage disequilibrium with one or more other polymorphisms or polymorphic alleles.

[0039] "Diluent" means an ingredient in a composition that lacks pharmacological activity, but is pharmaceutically necessary or desirable. For example, the diluent in an injected composition may be a liquid, e.g. saline solution.

[0040] "Dose" means a specified quantity of a pharmaceutical agent provided in a single administration, or in a specified time period. In certain embodiments, a dose may be administered in one, two, or more boluses, tablets, or injections. For example, in certain embodiments where subcutaneous administration is desired, the desired dose requires a volume not easily accommodated by a single injection, therefore, two or more injections may be used to achieve the desired dose. In certain embodiments, the pharmaceutical agent is administered by infusion over an extended period of time or continuously. Doses may be stated as the amount of pharmaceutical agent per hour, day, week, or month.

[0041] "Effective amount" means the amount of active pharmaceutical agent sufficient to effectuate a desired physiological outcome in an individual in need of the agent. The effective amount may vary among individuals depending on the health and physical condition of the individual to be treated, the taxonomic group of the individuals to be treated, the formulation of the composition, assessment of the individual's medical condition, and other relevant factors.

[0042] "Fully complementary" or "100% complementary" means each nucleobase of a first nucleic acid has a complementary nucleobase in a second nucleic acid. In certain embodiments, a first nucleic acid is an antisense compound and a target nucleic acid is a second nucleic acid.

[0043] "Gapmer" means a chimeric antisense compound in which an internal region having a plurality of nucleosides that support RNase H cleavage is positioned between external regions having one or more nucleosides, wherein the nucleosides comprising the internal region are chemically distinct from the nucleoside or nucleosides comprising the external regions. The internal region may be referred to as the "gap" and the external regions may be referred to as the "wings."

[0044] "Gap-widened" means a chimeric antisense compound having a gap segment of 12 or more contiguous 2'-deoxyribonucleosides positioned between and immediately adjacent to 5' and 3' wing segments having from one to six nucleosides.

[0045] "Gene product" refers to a biochemical material, such as RNA or protein, resulting from expression of a gene.

[0046] "Haplotype" means a set of alleles of closely linked loci on a chromosome that are generally inherited together. For example, a polymorphic allele at a first site in a nucleic acid sequence on the chromosome may be found to be associated with another polymorphic allele at a second site on the same chromosome, at a frequency other than would be expected for a random associate (e.g. "linkage equilibrium"). These two polymorphic alleles may be described as being in "linkage disequilibrium." A haplotype may comprise two, three, four, or more alleles. The set of alleles in a haplotype along a given segment of a chromosome are generally transmitted to progeny together unless there has been a recombination event.

[0047] "High-affinity sugar modification" is a modified sugar moiety which when it is included in a nucleoside and said nucleoside is incorporated into an antisense oligonucleotide, the stability (as measured by Tm) of said antisense oligonucleotide: RNA duplex is increased as compared to the stability of a DNA:RNA duplex. "High-affinity sugar-modified nucleoside" is a nucleoside comprising a modified sugar moiety that when said nucleoside is incorporated into an antisense compound, the binding affinity (as measured by Tm) of said antisense compound toward a complementary RNA molecule is increased. In certain embodiments of the invention at least one of said sugar-modified high-affinity nucleosides confers a ΔTm of at least 1 to 4 degrees per nucleoside against a complementary RNA as determined in accordance with the methodology described in Freier et al., Nucleic Acids Res., 1997, 25, 4429-4443. In another aspect, at least one of the high-affinity sugar modifications confers about 2 or more, 3 or more, or 4 or more degrees per modification. In the context of the present invention, examples of sugar-modified high affinity nucleosides include, but are not limited to, (i) certain 2'-modified nucleosides, including 2'-subtstituted and 4' to 2' bicyclic nucleosides, and (ii) certain other non-ribofuranosyl nucleosides which provide a per modification increase in binding affinity such as modified tetrahydropyran and tricycloDNA nucleosides. For other modifications that are sugar-modified high-affinity nucleosides see Freier et al., Nucleic Acids Res., 1997, 25, 4429-4443.

[0048] "Hybridization" means the annealing of complementary nucleic acid molecules. In certain embodiments, complementary nucleic acid molecules include an antisense compound and a target nucleic acid.

[0049] "Immediately adjacent" means there are no intervening elements between the immediately adjacent elements.

[0050] "Individual" means a human or non-human animal selected for treatment or therapy.

[0051] "Internucleoside linkage" refers to the chemical bond between nucleosides.

[0052] "Linked nucleosides" means adjacent nucleosides which are bonded together.

[0053] "Mismatch" or "non-complementary nucleobase" refers to the case when a nucleobase of a first nucleic acid is not capable of pairing with the corresponding nucleobase of a second or target nucleic acid.

[0054] "Modified internucleoside linkage" refers to a substitution or any change from a naturally occurring internucleoside bond (i.e. a phosphodiester internucleoside bond).

[0055] "Modified nucleobase" refers to any nucleobase other than adenine, cytosine, guanine, thymidine, or uracil. An "unmodified nucleobase" means the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T),

cytosine (C), and uracil (U).

[0056] "Modified nucleotide" means a nucleotide having, independently, a modified sugar moiety, modified internucleoside linkage, or modified nucleobase. A "modified nucleoside" means a nucleoside having, independently, a modified sugar moiety or modified nucleobase.

[0057] "Modified oligonucleotide" means an oligonucleotide comprising a modified internucleoside linkage, a modified sugar, or a modified nucleobase.

[0058] "Modified sugar" refers to a substitution or change from a natural sugar.

[0059] "Motif' means the pattern of chemically distinct regions in an antisense compound.

[0060] "Naturally occurring internucleoside linkage" means a 3' to 5' phosphodiester linkage.

[0061] "Natural sugar moiety" means a sugar found in DNA (2'-H) or RNA (2'-OH).

[0062] "Nuclease resistant modification" means a sugar modification or modified internucleoside linkage which, when incorporated into an oligonucleotide, makes said oligonucleotide more stable to degradation under cellular nucleases (e.g. exo- or endo-nucleases). Examples of nuclease resistant modifications include, but are not limited to, phosphorothioate internucleoside linkages, bicyclic sugar modifications, 2'-modified nucleotides, or neutral internucleoside linkages.

[0063] "Nucleic acid" refers to molecules composed of monomeric nucleotides. A nucleic acid includes ribonucleic acids (RNA), deoxyribonucleic acids (DNA), single-stranded nucleic acids, double-stranded nucleic acids, small interfering ribonucleic acids (siRNA), and microRNAs (miRNA).

[0064] "Nucleobase" means a heterocyclic moiety capable of pairing with a base of another nucleic acid.

[0065] "Nucleobase sequence" means the order of contiguous nucleobases independent of any sugar, linkage, or nucleobase modification.

[0066] "Nucleoside" means a nucleobase linked to a sugar.

[0067] "Nucleoside mimetic" includes those structures used to replace the sugar or the sugar and the base and not necessarily the linkage at one or more positions of an oligomeric compound such as for example nucleoside mimetics having morpholino, cyclohexenyl, cyclohexyl, tetrahydropyranyl, bicyclo or tricyclo sugar mimetics e.g. non furanose sugar units. Nucleotide mimetic includes those structures used to replace the nucleoside and the linkage at one or more positions of an oligomeric compound such as for example peptide nucleic acids or morpholinos (morpholinos linked by -N(H)-C(=O)-O- or other non-phosphodiester linkage). Sugar surrogate overlaps with the slightly broader term nucleoside mimetic but is intended to indicate replacement of the sugar unit (furanose ring) only. The tetrahydropyranyl rings provided herein are illustrative of an example of a sugar surrogate wherein the furanose sugar group has been replaced with a tetrahydropyranyl ring system.

[0068] "Nucleotide" means a nucleoside having a phosphate group covalently linked to the sugar portion of the nucleoside.

[0069] "Oligomeric compound" or "oligomer" means a polymer of linked monomeric subunits which is capable of hybridizing to at least a region of a nucleic acid molecule.

[0070] "Oligonucleotide" means a polymer of linked nucleosides each of which can be modified or unmodified, independent one from another.

[0071] "Parenteral administration" means administration through injection or infusion. Parenteral administration includes subcutaneous administration, intravenous administration, intramuscular administration, intraarterial

administration, intraperitoneal administration, or intracranial administration, e.g. intrathecal or intracerebroventricular administration.

[0072] "Peptide" means a molecule formed by linking at least two amino acids by amide bonds. Peptide refers to polypeptides and proteins.

[0073] "Pharmaceutical composition" means a mixture of substances suitable for administering to an individual. For example, a pharmaceutical composition may comprise one or more active pharmaceutical agents and a sterile aqueous solution.

[0074] "Pharmaceutically acceptable salts" means physiologically and pharmaceutically acceptable salts of antisense compounds, i.e., salts that retain the desired biological activity of the parent oligonucleotide and do not impart undesired toxicological effects thereto.

[0075] "Phosphorothioate linkage" means a linkage between nucleosides where the phosphodiester bond is modified by replacing one of the non-bridging oxygen atoms with a sulfur atom. A phosphorothioate linkage (P=S) is a modified internucleoside linkage.

[0076] "Portion" means a defined number of contiguous (i.e. linked) nucleobases of a nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of a target nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of an antisense compound.

[0077] "Prevent" refers to delaying or forestalling the onset or development of a disease, disorder, or condition for a period of time from minutes to indefinitely. Prevent also means reducing risk of developing a disease, disorder, or condition.

[0078] "Prodrug" means a therapeutic agent that is prepared in an inactive form that is converted to an active form within the body or cells thereof by the action of endogenous enzymes or other chemicals or conditions.

[0079] "Selectively reducing expression of an allelic variant" means reducing expression of one allele more than the other, differing allele among a set of alleles. For example, a mutant allele containing a single nucleotide polymorphism (SNP) may be reduced more than a wild type allele not containing the SNP.

[0080] "Side effects" means physiological responses attributable to a treatment other than the desired effects. In certain embodiments, side effects include injection site reactions, liver function test abnormalities, renal function abnormalities, liver toxicity, renal toxicity, central nervous system abnormalities, myopathies, and malaise. For example, increased aminotransferase levels in serum may indicate liver toxicity or liver function abnormality. For example, increased bilirubin may indicate liver toxicity or liver function abnormality.

[0081] "Single nucleotide polymorphism" or "SNP" means a single nucleotide variation between the genomes of individuals of the same species. In some cases, a SNP may be a single nucleotide deletion or insertion. In general, SNPs occur relatively frequently in genomes and thus contribute to genetic diversity. SNPs are thought to be mutationally more stable than other polymorphisms, lending their use in association studies in which linkage disequilibrium between markers and an unknown variant is used to map disease-causing mutations. The location of a SNP is generally flanked by highly conserved sequences. An individual may be homozygous or heterozygous for an allele at each SNP site. A heterozygous SNP allele can be a differentiating polymorphism. A SNP may be targeted with an antisense oligonucleotide, meaning that the SNP anneals to (or aligns with) position 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 of the antisense oligonucleotide. The remainder of the antisense oligonucleotide bases must have sufficient complementarity to the SNP site to facilitate hybridization.

[0082] "Single nucleotide polymorphism position" or "SNP position" refers to the nucleotide position of the SNP on a reference sequence.

[0083] "Single nucleotide polymorphism site" or "SNP site" refers to the nucleotides surrounding a SNP contained in a target nucleic acid to which an antisense compound is targeted.

[0084] "Single-stranded oligonucleotide" means an oligonucleotide which is not hybridized to a complementary strand.

[0085] "Specifically hybridizable" refers to an antisense compound having a sufficient degree of complementarity between an antisense oligonucleotide and a target nucleic acid to induce a desired effect, while exhibiting minimal or no effects on non-target nucleic acids under conditions in which specific binding is desired, i.e. under physiological conditions in the case of *in vivo* assays and therapeutic treatments.

[0086] "Targeting" or "targeted" means the process of design and selection of an antisense compound that will specifically hybridize to a target nucleic acid and induce a desired effect.

[0087] "Target nucleic acid," "target RNA," and "target RNA transcript" all refer to a nucleic acid capable of being targeted by antisense compounds.

[0088] "Target segment" means the sequence of nucleotides of a target nucleic acid to which an antisense compound is targeted. For example, for the purposes of this patent application, the target segment may be within the SNP site. "5' target site" refers to the 5'-most nucleotide of a target segment. "3' target site" refers to the 3'-most nucleotide of a target segment.

[0089] "Therapeutically effective amount" means an amount of a pharmaceutical agent that provides a therapeutic benefit to an individual.

[0090] "Treat" refers to administering a pharmaceutical composition to effect an alteration or improvement of a disease, disorder, or condition.

[0091] "Unmodified nucleotide" means a nucleotide composed of naturally occuring nucleobases, sugar moieties, and internucleoside linkages. In certain embodiments, an unmodified nucleotide is an RNA nucleotide (i.e. β-D-ribonucleosides) or a DNA nucleotide (i.e. β-D-deoxyribonucleoside).

Certain Embodiments

[0092] Disclosed herein are compounds and compositions for selectively inhibiting mRNA and protein expression of an allelic variant of a gene or DNA sequence. In certain embodiments, the allelic variant contains a single nucleotide polymorphism (SNP). In certain embodiments, the SNP is a differentiating polymorphism. In certain embodiments, a SNP is associated with a mutant allele. In certain embodiments, a SNP is in linkage disequilibrium with another polymorphism that is associated with or is causative of disease. In certain embodiments, a mutant allele is associated with disease. In certain embodiments, mRNA and protein expression of a mutant allele is associated with disease.

[0093] In certain embodiments, the expressed gene product of a mutant allele results in aggregation of the mutant proteins causing disease. In certain embodiments, the expressed gene product of a mutant allele results in gain of function causing disease. In certain embodiments, genes with an autosomal dominant mutation resulting in a toxic gain of function of the protein are the APP gene encoding amyloid precursor protein involved in Alzheimer's disease (Gene, 371: 68, 2006); the PrP gene encoding prion protein involved in Creutzfeldt-Jakob disease and in fatal familial insomnia (Nat. Med. 1997, 3: 1009); GFAP gene encoding glial fibrillary acidic protein involved in Alexander disease (J. Neurosci. 2006, 26:111623); alpha-synuclein gene encoding alpha-synuclein protein involved in Parkinson's disease (J. Clin. Invest. 2003, 111: 145); SOD-1 gene encoding the SOD-1 protein involved in amyotrophic lateral sclerosis (Science 1998, 281: 1851); atrophin-1 gene encoding atrophin-1 protein involved in dentato-rubral and pallido-luysian atrophy (DRPA) (Trends Mol. Med. 2001, 7: 479); SCA1 gene encoding ataxin-1 protein involved in spino-cerebellar ataxia-1 (SCA1) (Protein Sci. 2003, 12: 953); PLP gene encoding proteolipid protein involved in Pelizaeus-Merzbacher disease (NeuroMol Med. 2007, 4: 73); DYT1 gene encoding torsinA protein involved in Torsion dystonia (Brain Res. 2000, 877: 379); and alpha-B crystalline gene encoding alpha-B crystalline protein involved in protein aggregation diseases, including cardiomyopathy (Cell 2007, 130: 427); alpha1-antitrypsin gene encoding alpha1-antitrypsin protein involved in chronic obstructive pulmonary disease (COPD), liver disease and hepatocellular carcinoma (New Engl J Med. 2002, 346: 45); Ltk gene encoding leukocyte tyrosine kinase protein involved in systemic lupus erythematosus (Hum. Mol. Gen. 2004,13: 171); PCSK9 gene encoding PCSK9 protein involved in hypercholesterolemia (Hum Mutat. 2009, 30:

520); prolactin receptor gene encoding prolactin receptor protein involved in breast tumors (Proc. Natl. Assoc. Sci. 2008, 105: 4533); CCL5 gene encoding the chemokine CCL5 involved in COPD and asthma (Eur. Respir. J. 2008, 32: 327); PTPN22 gene encoding PTPN22 protein involved in Type 1 diabetes, Rheumatoid arthritis, Graves disease, and SLE (Proc. Natl. Assoc. Sci. 2007, 104: 19767); androgen receptor gene encoding the androgen receptor protein involved in spinal and bulbar muscular atrophy or Kennedy's disease (J Steroid Biochem. Mol. Biol. 2008, 108: 245); CHMP4B gene encoding chromatin modifying protein-4B involved in progressive childhood posterior subcapsular cataracts (Am. J. Hum. Genet 2007, 81: 596); FXR / NR1H4 gene encoding Famesoid X receptor protein involved in cholesterol gallstone disease, arthrosclerosis and diabetes (Mol. Endocrinol. 2007, 21: 1769); ABCA1 gene encoding ABCA1 protein involved in cardiovascular disease (Transl. Res. 2007, 149: 205); CaSR gene encoding the calcium sensing receptor protein involved in primary hypercalciuria (Kidney Int. 2007, 71: 1155); alpha-globin gene encoding alpha-globin protein involved in alpha-thallasemia (Science 2006, 312: 1215); httlpr gene encoding HTTLPR protein involved in obsessive compulsive disorder (Am. J. Hum. Genet. 2006, 78: 815); AVP gene encoding arginine vasopressin protein in stress-related disorders such as anxiety disorders and comorbid depression (CNS Neurol. Disord. Drug Targets 2006, 5: 167); GNAS gene encoding G proteins involved in congenital visual defects, hypertension, metabolic syndrome (Trends Pharmacol. Sci. 2006, 27: 260); APAF1 gene encoding APAF1 protein involved in a predisposition to major depression (Mol. Psychiatry 2006, 11: 76); TGF-beta1 gene encoding TGF-betal protein involved in breast cancer and prostate cancer (Cancer Epidemiol. Biomarkers Prev. 2004, 13: 759); AChR gene encoding acetylcholine receptor involved in congential myasthenic syndrome (Neurology 2004, 62: 1090); P2Y12 gene encoding adenosine diphosphate (ADP) receptor protein involved in risk of peripheral arterial disease (Circulation 2003, 108: 2971); LQT1 gene encoding LQT1 protein involved in atrial fibrillation (Cardiology 2003, 100: 109); RET protooncogene encoding RET protein involved in sporadic pheochromocytoma (J. Clin. Endocrinol. Metab. 2003, 88: 4911); filamin A gene encoding filamin A protein involved in various congenital malformations (Nat. Genet. 2003, 33: 487); TARDBP gene encoding TDP-43 protein involved in amyotrophic lateral sclerosis (Hum. Mol. Gene.t 2010, 19: 671); SCA3 gene encoding ataxin-3 protein involved in Machado-Joseph disease (PLoS One 2008, 3: e3341); SCA7 gene encoding ataxin-7 protein involved in spino-cerebellar ataxia-7 (PLoS One 2009, 4: e7232); and *HTT* gene encoding huntingtin protein involved in Huntington's disease (Neurobiol Dis. 1996, 3:183); and the CA4 gene encoding carbonic anhydrase 4 protein, CRX gene encoding cone-rod homeobox transcription factor protein, FSCN2 gene encoding retinal fascin homolog 2 protein, IMPDH1 gene encoding inosine monophosphate dehydrogenase 1 protein, NR2E3 gene encoding nuclear receptor subfamily 2 group E3 protein, NRL gene encoding neural retina leucine zipper protein, PRPF3 (RP18) gene encoding pre-mRNA splicing factor 3 protein, PRPF8 (RP13) gene encoding pre-mRNA splicing factor 8 protein, PRPF31 (RP11) gene encoding pre-mRNA splicing factor 31 protein, RDS gene encoding peripherin 2 protein, ROM1 gene encoding rod outer membrane protein 1 protein, RHO gene encoding rhodopsin protein, RP1 gene encoding RP1 protein, RPGR gene encoding retinitis pigmentosa GTPase regulator protein, all of which are involved in Autosomal Dominant Retinitis Pigmentosa disease (Adv Exp Med Biol. 2008, 613:203)

[0094] In certain embodiments, selective reduction of mRNA and protein expression of a mutant allele is achieved by targeting a SNP located on the mutant allele with an antisense compound. In certain embodiments, the antisense compound is an antisense oligonucleotide. In certain embodiments, the antisense compound is not a ribozyme, a double stranded siRNA, or an shRNA. In certain embodiments, the antisense oligonucleotide may have one or more modified sugar(s), nucleobase(s), or internucleoside linkage(s). In certain embodiments, the antisense oligonucleotide is complementary to the SNP site. The antisense oligonucleotide is 100% complementary to the SNP site. In certain embodiments, the SNP site is 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleobases in length. In certain embodiments, the SNP anneals to position 6, 7, 8, 9, 10, 11 or 12, of the antisense oligonucleotide.

[0095] In certain embodiments, antisense compounds designed to selectively reduce an allelic variant of a gene containing a SNP are created based on potency and selectivity of the antisense compound as well as population genetics.

[0096] In certain embodiments, selective reduction of mRNA and protein expression of an allelic variant of a gene containing a SNP occurs in a cell or tissue. In certain embodiments, the cell or tissue is in an animal. In certain embodiments, the animal is a human. Described herein are compounds comprising a modified antisense oligonucleotide consisting of 12 to 30 linked nucleosides targeted to a single nucleotide polymorphism site, wherein the modified oligonucleotide comprises a wing-gap-wing motif with a 5' wing region positioned at the 5' end of a deoxynucleoside gap, and a 3' wing region positioned at the 3' end of the deoxynucleoside gap, wherein position 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 of the modified oligonucleotide, as counted from the 5' terminus of the modified

oligonucleotide, or positions 1, 2, 3, 4, 5, 6, 7, 8, or 9 of the modified oligonucleotide, as counted from the 5' terminus of the gap, aligns with the single nucleotide polymorphism.

[0097] The single nucleotide polymorphism site is on a mutant allele that is associated with a disease. In certain embodiments, the single nucleotide polymorphism site contains a differentiating polymorphism.

[0098] The modified antisense oligonucleotide consists of 15 to 20 linked nucleosides. In certain embodiments, the modified antisense oligonucleotide consists of 15 to 19 linked nucleosides.

[0099] In certain embodiments, position 8, 9, or 10 of the modified oligonucleotide, as counted from the 5' terminus of the modified oligonucleotide, or positions 4, 5, or 6 of the modified oligonucleotide, as counted from the 5' terminus of the gap, aligns with the single nucleotide polymorphism.

[0100] In certain instances, the gap region is 7-11 nucleosides in length, the 5' wing region is 1-6 nucleobases in length and the 3' wing region is 1-6 nucleobases in length.

[0101] The wing-gap-wing motif is any one of the group consisting of 5-10-5, 2-9-6, 3-9-3, 3-9-4, 3-9-5, 4-7-4, 4-9-3, 4-9-4, 4-9-5, 4-10-5, 4-11-4, 4-11-5, 5-7-5, 5-8-6, 5-9-3, 5-9-5, 5-10-4, 5-10-5, 6-7-6, 6-8-5, and 6-9-2. In certain embodiments, the wing-gap-wing motif is any one of the group consisting of 2-9-6, 4-9-5, and 4-11-4.

[0102] In certain embodiments, at least one internucleoside linkage is a modified internucleoside linkage. In certain embodiments, each internucleoside linkage is a phosphorothioate internucleoside linkage.

[0103] In certain embodiments, at least one nucleoside comprises a modified nucleobase. In certain embodiments, the modified nucleobase is a 5'-methylcytosine.

[0104] In the oligonucleotides of the invention, each of the nucleosides of each wing region comprises a modified sugar or sugar surrogate. In certain embodiments, the sugar or sugar surrogate is a 2'-O-methoxyethyl modified sugar.

[0105] In certain embodiments, at least one of the wing regions comprises a 4' to 2' bicyclic nucleoside and at least one of the remaining wing nucleosides is a non-bicyclic 2'-modified nucleoside.

[0106] In certain embodiments, the non-bicyclic 2'-modified nucleoside is a 2'-O-methoxyethyl nucleoside.

[0107] In certain embodiments, the 4' to 2' bicyclic nucleoside is 4'-CH(CH3)-O-2' bicyclic nucleoside.

[0108] In certain embodiments, the modified antisense oligonucleotide consisting of 17 linked nucleosides and wherein position 9 of the modified oligonucleotide, as counted from the 5' terminus of the modified oligonucleotide, aligns with the differentiating polymorphism. In certain embodiments, the wing-gap-wing motif is 2-9-6.

[0109] In certain embodiments, described herein are compounds comprising a modified oligonucleotide consisting of 18 linked nucleosides and 100% complementary to a differentiating polymorphism, wherein the modified oligonucleotide comprises a wing-gap-wing motif, wherein position 9 of the modified oligonucleotide, as counted from the 5' terminus of the modified oligonucleotide, aligns with the differentiating polymorphism; wherein each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar; and wherein the wing-gap-wing motif is 4-9-5.

[0110] In certain embodiments, described herein are compounds comprising a modified oligonucleotide consisting of 19 linked nucleosides and 100% complementary to a differentiating polymorphism, wherein the modified oligonucleotide comprises a wing-gap-wing motif, wherein position 10 of the modified oligonucleotide, as counted from the 5' terminus of the modified oligonucleotide, aligns with the differentiating polymorphism; wherein each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar; and wherein the wing-gap-wing motif is 4-11-4.

[0111] In certain embodiments, described herein are compounds comprising a modified oligonucleotide consisting of 15 to 19 linked nucleosides and fully complementary to a differentiating polymorphism, wherein the modified oligonucleotide comprises a wing-gap-wing motif, wherein position 6, 7, 8, 9, 10, 11 or 12 of the modified

oligonucleotide, as counted from the 5' terminus of the modified oligonucleotide, aligns with the differentiating polymorphism; and at least one high-affinity sugar modification. The modified oligonucleotide is 100% complementary to the single nucleotide polymorphism site.

[0112] In certain embodiments, at least one of the wing regions comprises a high-affinity sugar modification. In certain embodiments, the high-affinity sugar modification is a bicyclic sugar. In certain embodiments, the bicyclic sugar comprises a 4'-CH(CH3)-O-2' bridge.

[0113] In certain embodiments, at least one of positions 2, 3, 6, 9, 10, 11, 13, or 14 of the modified oligonucleotide, as counted from the 5' terminus of the modified oligonucleotide, comprises the at least one high-affinity sugar modification.

[0114] In certain embodiments, at least one of positions 2, 3, 13, and 14 of the modified oligonucleotide, as counted from the 5' terminus of the modified oligonucleotide, comprises the at least one high-affinity sugar modification.

[0115] In certain embodiments, each of nucleoside positions 2, 3, 13, and 14 of the modified oligonucleotide, as counted from the 5' terminus of the modified oligonucleotide, comprise the at least one high-affinity sugar modification.

[0116] In certain embodiments, the high-affinity sugar modification is a bicyclic sugar. In certain embodiments, the bicyclic sugar comprises a 4'-CH(CH3)-O-2' bridge.

[0117] In certain embodiments, the wing-gap-wing motif is any of the group consisting of 3-9-3, 4-9-4, and 5-9-5.

[0118] In certain embodiments, described herein are compounds comprising a modified oligonucleotide consisting of 15, 17, or 19 linked nucleosides and fully complementary to a differentiating polymorphism, wherein the modified oligonucleotide comprises a wing-gap-wing motif, wherein position 6, 8, 10, or 14 of the modified oligonucleotide, as counted from the 5' terminus of the modified oligonucleotide, aligns with the differentiating polymorphism; and at least one high-affinity sugar modification.

[0119] In certain embodiments, at least one of positions 2, 3, 6, 9, 10, 11, 13, or 14 of the modified oligonucleotide, as counted from the 5' terminus of the modified oligonucleotide, comprises the at least one high-affinity sugar modification.

[0120] In certain embodiments, the high-affinity sugar modification is a bicyclic sugar. In certain embodiments, the bicyclic sugar comprises a 4'-CH(CH3)-O-2' bridge.

[0121] In certain embodiments, the wing-gap-wing motif is any of the group consisting of 3-9-3, 4-9-4, and 5-9-5.

[0122] In certain embodiments, described herein are compounds comprising a modified oligonucleotide consisting of 15 linked nucleosides and 100% complementary to a differentiating polymorphism, wherein the modified oligonucleotide comprises a wing-gap-wing motif, wherein position 8 of the modified oligonucleotide, as counted from the 5' terminus of the modified oligonucleotide, aligns with the differentiating polymorphism; and at least one high-affinity sugar modification.

[0123] In certain embodiments, each of nucleoside positions 2, 3, 13, and 14 of the modified oligonucleotide, as counted from the 5' terminus of the modified oligonucleotide, comprise the at least one high-affinity sugar modification.

[0124] In certain embodiments, the high-affinity sugar modification is a bicyclic sugar. In certain embodiments, the bicyclic sugar comprises a 4'-CH(CH3)-O-2' bridge.

[0125] In certain embodiments, the wing-gap-wing motif is 3-9-3.

[0126] In certain embodiments, described herein are methods of selectively reducing expression of an allelic variant of a gene containing a single nucleotide polymorphism in a cell, tissue, or animal, comprising administering to the cell, tissue, or animal a compound comprising a modified oligonucleotide complementary to a differentiating polymorphism, wherein the modified oligonucleotide comprises a wing-gap-wing motif and wherein position 6, 7, 8, 9, 10, 11 or 12 of the modified oligonucleotide, as counted from the 5' terminus of the modified oligonucleotide, aligns with the

differentiating polymorphism. The modified oligonucleotide is 100% complementary to the single nucleotide polymorphism site.

[0127] In certain embodiments, the single nucleotide polymorphism site is from 12 to 30 nucleobases in length. In certain embodiments, the single nucleotide polymorphism site is from 15 to 25 nucleobases in length. In certain embodiments, the single nucleotide polymorphism site is from 17 to 22 nucleobases in length. In certain embodiments, the single nucleotide polymorphism site is 17 nucleobases in length. In certain embodiments, the single nucleotide polymorphism site is 18 nucleobases in length. In certain embodiments, the single nucleotide polymorphism site is 19 nucleobases in length. In certain embodiments, the single nucleotide polymorphism site is 20 nucleobases in length.

[0128] In certain embodiments, the allelic variant is associated with disease. In certain embodiments, the disease is Huntington's Disease.

[0129] In certain embodiments, the modified oligonucleotide is a single-stranded oligonucleotide.

[0130] In certain embodiments, at least one internucleoside linkage is a modified internucleoside linkage. In certain embodiments, each internucleoside linkage is a phosphorothioate internucleoside linkage.

[0131] In certain embodiments, at least one nucleoside comprises a modified nucleobase. In certain embodiments, the at least one modified nucleobase is a 5'-methylcytosine.

[0132] In certain embodiments, at least one nucleoside comprises a modified sugar. In certain embodiments, the modified sugar is a high-affinity sugar modification. In certain embodiments, the high-affinity sugar is a bicyclic sugar. In certain embodiments, each bicyclic sugar comprises a 4'-CH(CH3)-O-2' bridge.

[0133] In certain embodiments, at least one of nucleoside positions 2, 3, 13, and 14 of the modified oligonucleotide, counting from the 5' terminus of the modified oligonucleotide, comprises a nucleoside having a bicyclic sugar wherein the bicyclic sugar comprises a 4'-CH(CH_3)-O-2' bridge.

[0134] In certain embodiments, each of nucleoside positions 2, 3, 13, and 14 of the modified oligonucleotide, counting from the 5' terminus of the modified oligonucleotide, comprises a bicyclic sugar wherein the bicyclic sugar comprises a 4'-CH(CH3)-O-2' bridge.

[0135] In certain embodiments, the at least one modified sugar comprises a 2'-O-methoxyethyl. In certain embodiments, each nucleoside positioned in a wing segment of the modified oligonucleotide comprises a 2'-O-methoxyethyl modification.

[0136] In certain embodiments, the wing-gap-wing motif is any of the group consisting of 2-9-6, 3-9-3, 3-9-4, 3-9-5, 4-7-4, 4-9-4, 4-9-5, 4-10-5, 4-11-4, 4-11-5, 5-7-5, 5-8-6, 5-9-3, 5-9-5, 5-10-4, 5-10-5, 6-7-6, 6-8-5, and 6-9-2.

[0137] In certain embodiments, the modified oligonucleotide is not a ribozyme, a double stranded siRNA, or an shRNA.

[0138] In certain embodiments, the single nucleotide polymorphism site is on a mutant allele that is associated with disease. In certain embodiments, the single nucleotide polymorphism site contains a differentiating polymorphism.

[0139] In certain embodiments, the modified antisense oligonucleotide consists of 15 to 19 linked nucleosides.

[0140] In certain instances, the gap region is 7 to 11 nucleosides in length, the 5' wing region is 1 to 6 nucleobases in length and 3' wing region is 1 to 6 nucleobases in length.

[0141] In certain embodiments, wherein at least one nucleoside of at least one of the wing regions comprises a modified sugar or sugar surrogate.

[0142] In certain embodiments, each of the nucleosides of each wing region comprises a modified sugar or sugar surrogate. In certain embodiments, the sugar or sugar surrogate is a 2'-O-methoxyethyl modified sugar.

[0143] In certain embodiments, at least one of the wing regions comprises a 4' to 2' bicyclic nucleoside and at least one of the remaining wing nucleosides is a non-bicyclic 2'-modified nucleoside.

[0144] In certain embodiments, the non-bicyclic 2'-modified nucleoside is a 2'-O-methoxyethyl nucleoside.

[0145] In certain embodiments, 4' to 2' bicyclic nucleoside is a 4'-CH(CH3)-O-2' bicyclic nucleoside. Described herein are methods of selectively reducing expression of an allelic variant of a gene containing a single nucleotide polymorphism in a cell, tissue, or animal, comprising administering to the cell, tissue, or animal a compound comprising a modified oligonucleotide complementary to a differentiating polymorphism, wherein the modified oligonucleotide comprises a wing-gap-wing motif and wherein position 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 of the modified oligonucleotide, as counted from the 5' terminus of the modified oligonucleotide, aligns with the differentiating polymorphism. Described herein are methods of selectively reducing expression of an allelic variant of a gene containing a single nucleotide polymorphism in a cell, tissue, or animal, comprising administering to the cell, tissue, or animal a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and complementary to a differentiating polymorphism, wherein the modified oligonucleotide comprises a wing-gap-wing motif and wherein position 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 of the modified oligonucleotide, as counted from the 5' terminus of the modified oligonucleotide aligns with the differentiating polymorphism; and wherein the allelic variant is a mutant allele.

[0146] In certain embodiments, the mutant allele is associated with any disease from the group consisting of Alzheimer's disease, Creutzfeldt-Jakob disease, fatal familial insomnia, Alexander disease, Parkinson's disease, amyotrophic lateral sclerosis, dentato-rubral and pallido-luysian atrophy DRPA, spino-cerebellar ataxia, Torsion dystonia, cardiomyopathy, chronic obstructive pulmonary disease (COPD), liver disease, hepatocellular carcinoma, systemic lupus erythematosus, hypercholesterolemia, breast cancer, asthma, Type 1 diabetes, Rheumatoid arthritis, Graves disease, SLE, spinal and bulbar muscular atrophy, Kennedy's disease, progressive childhood posterior subcapsular cataracts, cholesterol gallstone disease, arthrosclerosis, cardiovascular disease, primary hypercalciuria, alpha-thallasemia, obsessive compulsive disorder, Anxiety, comorbid depression, congenital visual defects, hypertension, metabolic syndrome, prostate cancer, congential myasthenic syndrome, peripheral arterial disease, atrial fibrillation, sporadic pheochromocytoma, congenital malformations, Machado-Joseph disease, Huntington's disease, and Autosomal Dominant Retinitis Pigmentosa disease. Described herein are oligonucleotides of the invention for use in methods of treating Huntington's Disease, comprising selectively reducing expression of an allelic variant of a gene containing a single nucleotide polymorphism in a cell, tissue, or animal, comprising administering to the cell, tissue, or animal a compound comprising a modified oligonucleotide consisting of 15 to 20 linked nucleosides and complementary to differentiating polymorphism, wherein the modified oligonucleotide comprises a wing-gap-wing motif and wherein position 6, 7, 8, 9, 10, 11 or 12 of the modified oligonucleotide, as counted from the 5' terminus of the modified oligonucleotide, aligns with differentiating polymorphism; and wherein the allelic variant is associated with Huntington's Disease.

[0147] In certain embodiments, position 8, 9, or 10 of the modified oligonucleotide, as counted from the 5' terminus of the modified oligonucleotide, or positions 4, 5, or 6 of the modified oligonucleotide, as counted from the 5' terminus of the gap, aligns with the single nucleotide polymorphism.

Single Nucleotide Polymorphisms (SNPs)

[0148] Single-nucleotide polymorphisms (SNPs) are single base-pair alterations in the DNA sequence that represent a major source of genetic heterogeneity (Gene. 1999, 234:177). SNP genotyping is an important tool with which to investigate these genetic variants (Genome Res. 2000, 10:895; Trends Biotechnol. 2000, 18:77). In certain embodiments, antisense compounds designed to selectively reduce an allelic variant of a gene containing an SNP were selected based on potency, selectivity and population genetics coverage.

Potency

[0149] In certain embodiments, antisense compounds designed to selectively reduce an allelic variant of a gene

containing a SNP are created based on potency of the antisense compound. Potency generally refers to how amenable the targeted sequence area is to antisense inhibition. In certain embodiments, specific SNP sites may be particularly amenable to antisense inhibition. Certain such highly amenable SNP sites may be targeted by antisense compounds for selectively reducing an allelic variant of a gene. Potency is demonstrated by the percent inhibition of mutant mRNA achieved by the antisense oligonucleotides targeting a SNP compared to the percent inhibition of mutant mRNA achieved by the benchmark oligonucleotide.

Selectivity

[0150] In certain embodiments, antisense compounds designed to selectively reduce an allelic variant of a gene containing a SNP are created based on selectivity of the antisense compound. Selectivity generally refers to antisense compounds comprising a particular sequence, motif, and chemical modification(s) that preferentially target the one or more differentiating polymorphisms (SNPs) in the RNA encoding a mutant HTT protein compared to the RNA encoding a wild type HTT protein. In certain embodiments, specific sequences, motifs, and chemical modification(s) are particularly selective in reducing an allelic variant of a gene containing a SNP. Certain such sequences, motifs, and chemical modification(s) are utilized to selectively reduce an allelic variant of a gene. Selectivity is demonstrated by the ability of the antisense oligonucleotide targeting a SNP to inhibit expression of the major allele or mutant allele preferentially compared to the minor allele or wild type allele.

Population Genetics

[0151] In certain embodiments, antisense compounds designed to selectively reduce an allelic variant of a gene containing an SNP are created based on the population genetics of a population afflicted with disease. Population genetics means the frequency at which the SNP appears in the disease chromosome of patients afflicted with a particular disease. In certain embodiments, the disease is Huntington disease. Where potency and selectivity amongst antisense compounds is equal, SNP targets that have higher population genetics coverage are favored over SNPs that have a weaker association with disease chromosomes.

Antisense Compounds

[0152] Oligomeric compounds may include, but are not limited to, oligonucleotides, oligonucleosides, oligonucleotide analogs, oligonucleotide mimetics, antisense compounds, antisense oligonucleotides, and siRNAs. An oligomeric compound may be "antisense" to a target nucleic acid, meaning that is is capable of undergoing hybridization to a target nucleic acid through hydrogen bonding.

[0153] In certain embodiments, an antisense compound is an antisense oligonucleotide. In certain embodiments, the antisense compound is not a ribozyme, a double stranded siRNA, or an shRNA.

[0154] In certain embodiments, an antisense compound has a nucleobase sequence that, when written in the 5' to 3' direction, comprises the reverse complement of the target segment of a target nucleic acid to which it is targeted. In certain such embodiments, an antisense oligonucleotide has a nucleobase sequence that, when written in the 5' to 3' direction, comprises the reverse complement of the target segment of a target nucleic acid to which it is targeted.

[0155] In certain parts of the disclosure, antisense compounds are 12 to 30 subunits in length. In other words, such antisense compounds are from 12 to 30 linked subunits. In other embodiments, the antisense compound is 8 to 80, 12 to 50, 15 to 30, 18 to 24, 19 to 22, or 20 linked subunits. In certain parts of the disclosure, the antisense compounds are 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 linked subunits in length, or a range defined by any two of the above values. In some embodiments the antisense compound is an antisense oligonucleotide, and the linked subunits are nucleosides.

[0156] In certain embodiments antisense oligonucleotides targeted to a nucleic acid may be shortened or truncated. For example, a single subunit may be deleted from the 5' end (5' truncation), or alternatively from the 3' end (3' truncation). A shortened or truncated antisense compound targeted to a nucleic acid may have two subunits deleted from the 5' end, or alternatively may have two subunits deleted from the 3' end, of the antisense compound. Alternatively, the deleted nucleosides may be dispersed throughout the antisense compound, for example, in an antisense compound having one nucleoside deleted from the 5' end and one nucleoside deleted from the 3' end.

[0157] When a single additional subunit is present in a lengthened antisense compound, the additional subunit may be located at the 5' or 3' end of the antisense compound. When two or more additional subunits are present, the added subunits may be adjacent to each other, for example, in an antisense compound having two subunits added to the 5' end (5' addition), or alternatively to the 3' end (3' addition), of the antisense compound. Alternatively, the added subunits may be dispersed throughout the antisense compound, for example, in an antisense compound having one subunit added to the 5' end and one subunit added to the 3' end.

[0158] It is possible to increase or decrease the length of an antisense compound, such as an antisense oligonucleotide, and/or introduce mismatch bases without eliminating activity. For example, in Woolf et al. (Proc. Natl. Acad. Sci. USA 89:7305-7309, 1992), a series of antisense oligonucleotides 13-25 nucleobases in length were tested for their ability to induce cleavage of a target RNA in an oocyte injection model. Antisense oligonucleotides 25 nucleobases in length with 8 or 11 mismatch bases near the ends of the antisense oligonucleotides were able to direct specific cleavage of the target mRNA, albeit to a lesser extent than the antisense oligonucleotides that contained no mismatches. Similarly, target specific cleavage was achieved using 13 nucleobase antisense oligonucleotides, including those with 1 or 3 mismatches.

[0159] Gautschi et al (J. Natl. Cancer Inst. 93:463-471, March 2001) demonstrated the ability of an oligonucleotide having 100% complementarity to the bcl-2 mRNA and having 3 mismatches to the bcl-xL mRNA to reduce the expression of both bcl-2 and bcl-xL *in vitro* and *in vivo*. Furthermore, this oligonucleotide demonstrated potent anti-tumor activity *in vivo*.

[0160] Maher and Dolnick (Nuc. Acid. Res. 16:3341-3358,1988) tested a series of tandem 14 nucleobase antisense oligonucleotides, and a 28 and 42 nucleobase antisense oligonucleotides comprised of the sequence of two or three of the tandem antisense oligonucleotides, respectively, for their ability to arrest translation of human DHFR in a rabbit reticulocyte assay. Each of the three 14 nucleobase antisense oligonucleotides alone was able to inhibit translation, albeit at a more modest level than the 28 or 42 nucleobase antisense oligonucleotides.

[0161] However, selective reduction of expression of an allelic variant is optimized when the SNP contained in the target nucleic anneals to a complementary base in the antisense compound and not a mismatched base. Moreover, selectivity in general is increased when there are fewer mismatches between the SNP site and the antisense compound. However, a certain number of mismatches may be tolerated.

Antisense Compound Motifs

[0162] In certain embodiments, antisense compounds targeted to a nucleic acid have chemically modified subunits arranged in patterns, or motifs, to confer to the antisense compounds properties such as enhanced the inhibitory activity, increased binding affinity for a target nucleic acid, or resistance to degradation by *in vivo* nucleases.

[0163] Chimeric antisense compounds typically contain at least one region modified so as to confer increased resistance to nuclease degradation, increased cellular uptake, increased binding affinity for the target nucleic acid, and/or increased inhibitory activity. A second region of a chimeric antisense compound may optionally serve as a substrate for the cellular endonuclease RNase H, which cleaves the RNA strand of an RNA:DNA duplex.

[0164] Antisense compounds having a gapmer motif are considered chimeric antisense compounds. In a gapmer an internal region having a plurality of nucleotides that supports RNaseH cleavage is positioned between external regions having a plurality of nucleotides that are chemically distinct from the nucleosides of the internal region. In the case of an antisense oligonucleotide having a gapmer motif, the gap segment generally serves as the substrate for endonuclease

cleavage, while the wing segments comprise modified nucleosides. In the case of an antisense oligonucleotide for selectively reducing expression of an allelic variant of a gene containing a SNP, the SNP anneals to a nucleobase within the gap segment.

[0165] In certain embodiments, the SNP anneals or is complementary to a nucleobase at position 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 of the antisense oligonucleotide, wherein position refers to the orientation of a nucleobase within the antisense oligonucleotide counting from the 5' terminus of the antisense oligonucleotide. For example, the 5' most nucleobase within the antisense oligonucleotide is in the first position of the antisense oligonucleotide. In certain embodiments, the SNP anneals or is complementary to a nucleobase at position 6, 7, 8, 9, or 10 of the antisense oligonucleotide (counting from the 5' terminus). In certain embodiments, the SNP anneals or is complementary to a nucleobase at position 9 or 10 of the antisense oligonucleotide (counting from the 5' terminus).

[0166] In certain embodiments, the SNP anneals to a nucleobase at position 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 of the gap segment, wherein position refers to the orientation of a nucleobase within the gap segment counting from the 5' terminus of the gap segment. For example, the 5' most nucleobase within the gap segment is in the first position of the gap segment. In certain embodiments, the SNP anneals to a nucleobase at position 4, 5, 6, or 7 counting from the 5' terminus of the gap segment. In certain embodiments, the SNP anneals to a nucleobase at position 4 or 5 beginning from the 5' terminus of the gap segment.

[0167] In certain embodiments, the regions of a gapmer are differentiated by the types of sugar moieties comprising each distinct region. The types of sugar moieties that are used to differentiate the regions of a gapmer may in some embodiments include β-D-ribonucleosides, β-D-deoxyribonucleosides, 2'-modified nucleosides (such 2'-modified nucleosides may include 2'-MOE, and 2'-O-CH_3, among others), and bicyclic sugar modified nucleosides (such bicyclic sugar modified nucleosides may include those having a 4'-(CH_2)n-O-2' bridge, where n=1 or n=2). The bicyclic moiety may be a cEt having the formula 4'-$CH(CH_3)$-O-2.'

[0168] The wing-gap-wing motif is frequently described as "X-Y-Z", where "X" represents the length of the 5' wing region, "Y" represents the length of the gap region, and "Z" represents the length of the 3' wing region. As used herein, a gapmer described as "X-Y-Z" has a configuration such that the gap segment is positioned immediately adjacent to each of the 5' wing segment and the 3' wing segment. Thus, no intervening nucleotides exist between the 5' wing segment and gap segment, or the gap segment and the 3' wing segment. Any of the antisense compounds described herein can have a gapmer motif. In some embodiments, X and Z are the same, in other embodiments they are different. In certain embodiments, Y is between 8 and 15 nucleotides. In certain embodiments, Y is comprised of deoxynucleotides. X, Y or Z can be any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more nucleotides. Thus, gapmers disclosed herein include, but are not limited to, for example 1-10-1, 1-18-1, 2-8-2, 2-9-6, 2-10-2, 2-13-5, 2-16-2, 3-9-3, 3-9-5, 3-10-3, 3-14-3, 4-8-4, 4-9-5, 4-10-5, 4-11-4, 4-12-3, 4-12-4, 5-8-5, 5-9-5, 5-10-4, 5-10-5, or 6-8-6.

[0169] In certain embodiments, the antisense compound has a "wingmer" motif, having a wing-gap or gap-wing configuration, i.e. an X-Y or Y-Z configuration as described above for the gapmer configuration. Thus, wingmer configurations disclosed herein include, but are not limited to, for example 5-10, 8-4, 4-12, 12-4, 3-14, 16-2, 18-1, 10-3, 2-10, 1-10, 8-2, 2-13, 5-13, 5-8, or 6-8.

[0170] In certain embodiments, antisense compounds targeted to a nucleic acid possess a 2-9-6 gapmer motif or a 6-9-2 gapmer motif.

[0171] In certain embodiments, antisense compounds targeted to a nucleic acid possess a 3-9-3 gapmer motif.

[0172] In certain embodiments, antisense compounds targeted to a nucleic acid possess a 3-9-5 gapmer motif or 5-9-3 gapmer motif.

[0173] In certain embodiments, antisense compounds targeted to a nucleic acid possess a 4-9-5 gapmer motif. In certain instances, antisense compounds targeted to a nucleic acid possess a 5-9-4 gapmer motif.

[0174] In certain embodiments, antisense compounds targeted to a nucleic acid possess a 4-10-5 gapmer motif or 5-

10-4 gapmer motif.

[0175] In certain embodiments, antisense compounds targeted to a nucleic acid possess a 4-11-4 gapmer motif.

[0176] In certain embodiments, antisense compounds targeted to a nucleic acid possess a 5-9-5 gapmer motif.

[0177] In certain embodiments, antisense compounds targeted to a nucleic acid possess a 5-8-6 gapmer motif or a 6-8-5 gapmer motif.

[0178] In certain embodiments, antisense compounds targeted to a nucleic acid possess a 6-7-6 gapmer motif.

[0179] In certain embodiments, antisense compounds targeted to a nucleic acid possess a 6-8-5 gapmer motif or a 5-8-6 gapmer motif.

[0180] In certain embodiments, antisense compounds targeted to a nucleic acid possess a 3-9-4 gapmer motif or a 4-9-3 gapmer motif.

[0181] In certain embodiments, antisense compounds targeted to a nucleic acid possess a 5-7-5 gapmer motif.

[0182] In certain embodiments, antisense compounds targeted to a nucleic acid possess a 4-7-4 gapmer motif.

[0183] In certain embodiments, antisense compounds targeted to a nucleic acid possess a 5-10-5 gapmer motif.

[0184] In certain embodiments, an antisense compound targeted to a nucleic acid has a gap-widened motif.

Certain Mixed Wings

[0185] In certain embodiments, the invention provides gapmer compounds wherein at least one nucleoside of one wing is differently modified compared to at least one other nucleoside of the same wing. Such antisense compounds are referred to as mixed wing antisense compounds (see WO 2008/049085). In certain embodiments, the modifications (or no modification) of one or more nucleosides of the 3' wing are different from those of one or more other nucleosides of the 3' wing. Such antisense compounds may be referred to as 3' mixed wing gapmers. In certain embodiments, the modifications (or no modification) of one or more nucleosides of the 5' wing are different from those of one or more other nucleosides of the 5' wing. Such antisense compounds may be referred to as 5' mixed wing gapmers. In certain embodiments, the modifications (or no modification) of one or more nucleosides of the 3' wing are different from those of one or more other nucleosides of the 3' wing and the modifications (or no modification) of one or more nucleosides of the 5' wing are different from those of one or more other nucleosides of the 5' wing. Such antisense compounds may be referred to as 3', 5' mixed wing gapmers. In such embodiment, the modifications and combination of modifications at the 3' wing and at the 5' wing may be the same or they may be different.

[0186] In certain embodiments, mixed wing compounds have desirable properties. Certain nucleoside modifications confer on the antisense compound a desirable property, for example increased affinity for a target or nuclease resistance, but also confer an undesirable property, for example increased toxicity. Incorporation of certain other nucleoside modifications results in antisense compounds with different profiles of properties. In certain embodiments, one may combine modifications in one or both wings to optimize desirable characteristics and/or minimize undesirable characteristics. In certain embodiments, the wings of a mixed wing antisense compound comprise one or more nucleoside comprising a first modification that increases affinity of the antisense compound for a target nucleic acid compared to an antisense compound comprising unmodified nucleosides; and one or more nucleoside comprising a second modification that results in reduced toxicity compared to an antisense compound with wings comprising nucleosides that all comprise the first modification.

[0187] In certain embodiments, an antisense compound comprises at least one wing comprising at least one MOE substituted nucleoside and at least one high affinity modification. In certain such embodiments, the at least one MOE substituted nucleoside and the at least one high affinity are in the 3' wing. In certain such embodiments, the at least one

MOE substituted nucleoside and the at least one high affinity are in the 5' wing.

[0188] In certain embodiments, an antisense compound comprises 1, 2 or 3 high affinity modifications in the 5' and/or 3' wings.

Target Nucleic Acids, Target Regions and Nucleotide Sequences

[0189] In certain embodiments, an allelic variant of huntingtin is selectively reduced. Nucleotide sequences that encode huntingtin include, without limitation, the following: GENBANK Accession No. NT_006081.18, truncated from nucleotides 1566000 to 1768000 (replaced by GENBANK Accession No. NT_006051), incorporated herein as SEQ ID NO: 1, and NM_002111.6, incorporated herein as SEQ ID NO: 2.

[0190] It is understood that the sequence set forth in each SEQ ID NO in the Examples contained herein is independent of any modification to a sugar moiety, an internucleoside linkage, or a nucleobase. As such, antisense compounds defined by a SEQ ID NO may comprise, independently, one or more modifications to a sugar moiety, an internucleoside linkage, or a nucleobase. Antisense compounds described by Isis Number (Isis No) indicate a combination of nucleobase sequence and motif.

[0191] In certain embodiments, a target region is a structurally defined region of the target nucleic acid. For example, a target region may encompass a 3' UTR, a 5' UTR, an exon, an intron, an exon/intron junction, a coding region, a translation initiation region, translation termination region, or other defined nucleic acid region. The structurally defined regions for huntingtin can be obtained by accession number from sequence databases such as NCBI. In certain embodiments, a target region may encompass the sequence from a 5' target site of one target segment within the target region to a 3' target site of another target segment within the same target region.

[0192] Targeting includes determination of at least one target segment to which an antisense compound hybridizes, such that a desired effect occurs. In certain embodiments, the desired effect is a reduction in mRNA target nucleic acid levels of a particular allelic variant. In certain embodiments, the desired effect is reduction of levels of the protein encoded by the target nucleic acid or a phenotypic change associated with a particular alleleic variant.

[0193] A target region may contain one or more target segments. Multiple target segments within a target region may be overlapping. Alternatively, they may be non-overlapping. In certain embodiments, target segments within a target region are separated by no more than about 300 nucleotides. In certain emodiments, target segments within a target region are separated by a number of nucleotides that is, is about, is no more than, is no more than about, 250, 200, 150, 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10 nucleotides on the target nucleic acid, or is a range defined by any two of the preceeding values. In certain embodiments, target segments within a target region are separated by no more than, or no more than about, 5 nucleotides on the target nucleic acid. In certain embodiments, target segments are contiguous. Contemplated are target regions defined by a range having a starting nucleic acid that is any of the 5' target sites or 3' target sites listed herein.

[0194] Suitable target segments may be found within a 5' UTR, a coding region, a 3' UTR, an intron, an exon, or an exon/intron junction. Target segments containing a start codon or a stop codon are also suitable target segments. A suitable target segment may specifcally exclude a certain structurally defined region such as the start codon or stop codon.

[0195] The determination of suitable target segments may include a comparison of the sequence of a target nucleic acid to other sequences throughout the genome. For example, the BLAST algorithm may be used to identify regions of similarity amongst different nucleic acids. This comparison can prevent the selection of antisense compound sequences that may hybridize in a non-specific manner to sequences other than a selected target nucleic acid (i.e., non-target or off-target sequences).

Cell Lines

[0196] In certain embodiments, the GM04281, GM02171, and GM02173B cell lines are used in experiments described herein below. The GM04281 cell line has a wild-type *HTT* allele that contains 17 repeats and a mutant *HTT* allele that contains 69 repeats. The cell line was derived from a patient both of whose parents were also affected by the disease. The GM02171 cell line was chosen as a counter screen control to the GM04281. This cell line was derived from the daughter of parents, only one of whom had the disease. The daughter had not developed HD but was considered to be at risk. The GM02173B cell line was also patient-derived and was used as a haplotype test control.

[0197] Table 1 provides SNPs found in the GM04281, GM02171, and GM02173B cell lines. Also provided are the allelic variants found at each SNP position, the genotype for each of the cell lines, and the percentage of HD patients having a particular allelic variant. For example, the two allelic variants for SNP rs6446723 are T and C. The GM02171 cell line is homozygous CC, the GM02173 cell line is heterozygous TC, and the GM04281 cell line is homozygous TT. Fifty percent of HD patients have a T at SNP position rs6446723.

Table 1

Allelic Variations for SNPs Associated with HD						
SNP	Variation	GM02171	GM02173	GM04281	TargetPOP	allele
rs6446723	T/C	CC	TC	TT	0.50	T
rs3856973	A/G	AA	AG	GG	0.50	G
rs2285086	A/G	GG	AG	AA	0.50	A
rs363092	A/C	AA	AC	CC	0.49	C
rs916171	C/G	GG	GC	CC	0.49	C
rs6844859	T/C	CC	TC	TT	0.49	T
rs7691627	A/G	AA	AG	GG	0.49	G
rs4690073	A/G	AA	AG	GG	0.49	G
rs2024115	A/G	GG	AG	AA	0.48	A
rs11731237	T/C	CC	TC	TT	0.43	T
rs362296	A/C	AC	AC	AC	0.42	C
rs10015979	A/G	AA	AG	GG	0.42	G
rs7659144	C/G	CG	CG	CC	0.41	C
rs363096	T/C	CC	TC	TT	0.40	T
rs362273	A/G	AG	AG	AA	0.39	A
rs16843804	T/C	TC	TC	CC	0.38	C
rs362271	A/G	AG	AG	GG	0.38	G
rs362275	T/C	TC	TC	CC	0.38	C
rs3121419	T/C	TC	TC	CC	0.38	C
rs362272	A/G	--	AG	GG	0.38	G
rs3775061	A/G	AG	AG	AA	0.38	A
rs34315806	T/C	TC	TC	CC	0.38	C
rs363099	T/C	TC	TC	CC	0.38	C
rs2298967	T/C	TC	TC	TT	0.38	T
rs363088	A/T	TA	TA	AA	0.38	A
rs363064	T/C	TC	TC	CC	0.35	C
rs363102	A/G	AA	AA	AA	0.23	G
rs2798235	A/G	GG	GG	GG	0.21	A
rs363080	T/C	CC	CC	CC	0.21	T
rs363072	A/T	TA	AA	AA	0.13	A
rs363125	A/C	AC	CC	CC	0.12	C
rs362303	T/C	TC	CC	CC	0.12	C

Allelic Variations for SNPs Associated with HD						
SNP	Variation	GM02171	GM02173	GM04281	TargetPOP	allele
rs362310	T/C	TC	CC	CC	0.12	C
rs10488840	A/G	AG	GG	GG	0.12	G
rs362325	T/C	TC	TT	TT	0.11	T
rs35892913	A/G	GG	GG	GG	0.10	A
rs363102	A/G	AA	AA	AA	0.09	A
rs363096	T/C	CC	TC	TT	0.09	C
rs11731237	T/C	CC	TC	TT	0.09	C
rs10015979	A/G	AA	AG	GG	0.08	A
rs363080	T/C	CC	CC	CC	0.07	c
rs2798235	A/G	GG	GG	GG	0.07	G
rs1936032	C/G	CC	CC	CC	0.06	C
rs2276881	A/G	GG	GG	GG	0.06	G
rs363070	A/G	AA	AA	AA	0.06	A
rs35892913	A/G	GG	GG	GG	0.04	G
rs12502045	T/C	CC	CC	CC	0.04	C
rs6446723	T/C	CC	TC	TT	0.04	C
rs7685686	A/G	GG	AG	AA	0.04	G
rs3733217	T/C	CC	CC	CC	0.03	C
rs6844859	T/C	CC	TC	TT	0.03	C
rs362331	T/C	CC	TC	TT	0.03	C

Hybridization

[0198] In some embodiments, hybridization occurs between an antisense compound disclosed herein and a SNP site. The most common mechanism of hybridization involves hydrogen bonding (e.g., Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding) between complementary nucleobases of the nucleic acid molecules.

[0199] Hybridization can occur under varying conditions. Stringent conditions are sequence-dependent and are determined by the nature and composition of the nucleic acid molecules to be hybridized.

[0200] In certain embodiments, the antisense compounds provided herein are specifically hybridizable with the nucleic acid of a particular allelic variant.

Complementarity

[0201] An antisense compound and a target nucleic acid are complementary to each other when a sufficient number of nucleobases of the antisense compound can hydrogen bond with the corresponding nucleobases of the target nucleic acid, such that a desired effect will occur (e.g., selective reduction of a gene product of an allelic variant).

[0202] Non-complementary nucleobases between an antisense compound and a target nucleic acid may be tolerated provided that the antisense compound remains able to specifically hybridize to a target nucleic acid. Moreover, an antisense compound may hybridize over one or more segments of a target nucleic acid such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure, mismatch or hairpin structure).

[0203] The antisense compounds disclosed herein, or a specified portion thereof, are, or are at least, 70%, 80%, 85%,

86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% complementary to a target nucleic acid, a target region, target segment, SNP site, or specified portion thereof. Percent complementarity of an antisense compound with a target nucleic acid can be determined using routine methods.

[0204] For example, an antisense compound in which 18 of 20 nucleobases of the antisense compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining noncomplementary nucleobases may be clustered or interspersed with complementary nucleobases and need not be contiguous to each other or to complementary nucleobases. As such, an antisense compound which is 18 nucleobases in length having 4 (four) noncomplementary nucleobases which are flanked by two regions of complete complementarity with the target nucleic acid would have 77.8% overall complementarity with the target nucleic acid.. Percent complementarity of an antisense compound with a region of a target nucleic acid can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art (Altschul et al., J. Mol. Biol., 1990, 215, 403 410; Zhang and Madden, Genome Res., 1997, 7, 649 656). Percent homology, sequence identity or complementarity, can be determined by, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wis.), using default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2, 482 489).

[0205] In certain embodiments, the antisense compounds provided herein, or specified portions thereof, are fully complementary (i.e. 100% complementary) to a target nucleic acid, a SNP site, target region, target segment, or specified portion thereof. As used herein, "fully complementary" means each nucleobase of an antisense compound is capable of precise base pairing with the corresponding nucleobases of a target nucleic acid. For example, a 20 nucleobase antisense compound is fully complementary to a target sequence that is 400 nucleobases long, so long as there is a corresponding 20 nucleobase portion of the target nucleic acid that is fully complementary to the antisense compound. Fully complementary can also be used in reference to a specified portion of the first and /or the second nucleic acid. For example, a 20 nucleobase portion of a 30 nucleobase antisense compound can be "fully complementary" to a target sequence that is 400 nucleobases long. The 20 nucleobase portion of the 30 nucleobase oligonucleotide is fully complementary to the target sequence if the target sequence has a corresponding 20 nucleobase portion wherein each nucleobase is complementary to the 20 nucleobase portion of the antisense compound. At the same time, the entire 30 nucleobase antisense compound may or may not be fully complementary to the target sequence, depending on whether the remaining 10 nucleobases of the antisense compound are also complementary to the target sequence.

[0206] The location of a non-complementary nucleobase may be at the 5' end or 3' end of the antisense compound. Alternatively, the non-complementary nucleobase or nucleobases may be at an internal position of the antisense compound. When two or more non-complementary nucleobases are present, they may be contiguous (i.e. linked) or non-contiguous. In one embodiment, a non-complementary nucleobase is located in the wing segment of a gapmer antisense oligonucleotide.

[0207] In certain embodiments, antisense compounds that are, or are up to 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleobases in length comprise no more than 6, no more than 5, no more than 4, no more than 3, no more than 2, or no more than 1 non-complementary nucleobase(s) relative to a target nucleic acid, SNP site, or specified portion thereof.

[0208] In certain embodiments, antisense oligonucleotides that are, or are up to 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleobases in length comprise no more than 6, no more than 5, no more than 4, no more than 3, no more than 2, or no more than 1 non-complementary nucleobase(s) relative to a target nucleic acid, SNP site, or specified portion thereof.

[0209] The antisense compounds provided herein also include those which are complementary to a portion of a target nucleic acid. As used herein, "portion" refers to a defined number of contiguous (i.e. linked) nucleobases within a region or segment of a target nucleic acid. A "portion" can also refer to a defined number of contiguous nucleobases of an antisense compound. In certain embodiments, the antisense compounds, are complementary to at least an 8 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 12 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are

complementary to at least a 15 nucleobase portion of a target segment. Also contemplated are antisense compounds that are complementary to at least a 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more nucleobase portion of a target segment, or a range defined by any two of these values.

Identity

[0210] The antisense compounds provided herein may also have a defined percent identity to a particular nucleotide sequence, SEQ ID NO, or compound represented by a specific Isis number, or portion thereof. As used herein, an antisense compound is identical to the sequence disclosed herein if it has the same nucleobase pairing ability. For example, a RNA which contains uracil in place of thymidine in a disclosed DNA sequence would be considered identical to the DNA sequence since both uracil and thymidine pair with adenine. Shortened and lengthened versions of the antisense compounds described herein as well as compounds having non-identical bases relative to the antisense compounds provided herein also are contemplated. The non-identical bases may be adjacent to each other or dispersed throughout the antisense compound. Percent identity of an antisense compound is calculated according to the number of bases that have identical base pairing relative to the sequence to which it is being compared.

[0211] In certain embodiments, the antisense compounds, or portions thereof, are at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to one or more of the antisense compounds or SEQ ID NOs, or a portion thereof, disclosed herein.

[0212] In certain embodiments, a portion of the antisense compound is compared to an equal length portion of the target nucleic acid. In certain embodiments, an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleobase portion is compared to an equal length portion of the target nucleic acid.

[0213] In certain embodiments, a portion of the antisense oligonucleotide is compared to an equal length portion of the target nucleic acid. In certain embodiments, an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleobase portion is compared to an equal length portion of the target nucleic acid.

Modifications

[0214] A nucleoside is a base-sugar combination. The nucleobase (also known as base) portion of the nucleoside is normally a heterocyclic base moiety. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to the 2', 3' or 5' hydroxyl moiety of the sugar. Oligonucleotides are formed through the covalent linkage of adjacent nucleosides to one another, to form a linear polymeric oligonucleotide. Within the oligonucleotide structure, the phosphate groups are commonly referred to as forming the internucleoside linkages of the oligonucleotide.

[0215] Modifications to antisense compounds encompass substitutions or changes to internucleoside linkages, sugar moieties, or nucleobases. Modified antisense compounds are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target, increased stability in the presence of nucleases, or increased inhibitory activity.

[0216] Chemically modified nucleosides may also be employed to increase the binding affinity of a shortened or truncated antisense oligonucleotide for its target nucleic acid. Consequently, comparable results can often be obtained with shorter antisense compounds that have such chemically modified nucleosides.

[0217] Chemically modified nucleosides may also be employed to increase selectivity in reducing expression the gene product of an allelic variant.

Modified Internucleoside Linkages

[0218] The naturally occuring internucleoside linkage of RNA and DNA is a 3' to 5' phosphodiester linkage. Antisense compounds having one or more modified, i.e. non-naturally occurring, internucleoside linkages are often selected over antisense compounds having naturally occurring internucleoside linkages because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for target nucleic acids, and increased stability in the presence of nucleases.

[0219] Oligonucleotides having modified internucleoside linkages include internucleoside linkages that retain a phosphorus atom as well as internucleoside linkages that do not have a phosphorus atom. Representative phosphorus containing internucleoside linkages include, but are not limited to, phosphodiesters, phosphotriesters, methylphosphonates, phosphoramidate, and phosphorothioate. Methods of preparation of phosphorous-containing and non-phosphorous-containing linkages are well known.

[0220] In certain embodiments, antisense compounds comprise one or more modified internucleoside linkages. In certain embodiments, the modified internucleoside linkages are phosphorothioate linkages. In certain embodiments, each internucleoside linkage of an antisense compound is a phosphorothioate internucleoside linkage.

Modified Sugar Moieties

[0221] Antisense compounds of the invention contain one or more nucleosides wherein the sugar group has been modified. Such sugar modified nucleosides may impart enhanced nuclease stability, increased binding affinity, increased selectivity for an allelic variant, or some other beneficial biological property to the antisense compounds. In certain embodiments, nucleosides comprise a chemically modified ribofuranose ring moieties. Examples of chemically modified ribofuranose rings include without limitation, addition of substitutent groups (including 5' and 2' substituent groups, bridging of non-geminal ring atoms to form bicyclic nucleic acids (BNA), replacement of the ribosyl ring oxygen atom with S, N(R), or C(R1)(R)2 (R = H, C1-C12 alkyl or a protecting group) and combinations thereof. Examples of chemically modified sugars include 2'-F-5'-methyl substituted nucleoside (see PCT International Application WO 2008/101157 Published on 8/21/08 for other disclosed 5',2'-bis substituted nucleosides) or replacement of the ribosyl ring oxygen atom with S with further substitution at the 2'-position (see published U.S. Patent Application US2005-0130923, published on June 16, 2005) or alternatively 5'-substitution of a BNA (see PCT International Application WO 2007/134181 Published on 11/22/07 wherein LNA is substituted with for example a 5'-methyl or a 5'-vinyl group).

[0222] Examples of nucleosides having modified sugar moieties include without limitation nucleosides comprising 5'-vinyl, 5'-methyl (R or S), 4'-S, 2'-F, 2'-OCH3 and 2'-O(CH2)2OCH3 substituent groups. The substituent at the 2' position can also be selected from allyl, amino, azido, thio, O-allyl, O-C1-C10 alkyl, OCF3, O(CH2)2SCH3, O(CH2)2-O-N(Rm)(Rn), and O-CH2-C(=O)-N(Rm)(Rn), where each Rm and Rn is, independently, H or substituted or unsubstituted C1-C10 alkyl.

[0223] As used herein, "bicyclic nucleosides" refer to modified nucleosides comprising a bicyclic sugar moiety. Examples of bicyclic nucleosides include without limitation nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. In certain embodiments, antisense compounds provided herein include one or more bicyclic nucleosides wherein the bridge comprises a 4' to 2' bicyclic nucleoside. Examples of such 4' to 2' bicyclic nucleosides, include but are not limited to one of the formulae: 4'-(CH_2)-O-2' (LNA); 4'-(CH_2)-S-2'; 4'-$(CH_2)_2$-O-2' (ENA); 4'-$CH(CH_3)$-O-2' and 4'-$CH(CH_2OCH_3)$-O-2' (and analogs thereof see U.S. Patent 7,399,845, issued on July 15, 2008); 4'-$C(CH_3)(CH_3)$-O-2' (and analogs thereof see published International Application WO/2009/006478, published January 8, 2009); 4'-CH_2-$N(OCH_3)$-2' (and analogs thereof see published International Application WO/2008/150729, published December 11, 2008); 4'-CH_2-$ON(CH_3)$-2' (see published U.S. Patent Application US2004-0171570, published September 2, 2004); 4'-CH_2-N(R)-O-2', wherein R is H, C_1-C_{12} alkyl, or a protecting group (see U.S. Patent 7,427,672, issued on September 23, 2008); 4'-CH_2-$C(H)(CH_3)$-2' (see Chattopadhyaya, et al., J. Org. Chem., 2009, 74, 118-134); and 4'-CH_2-$C(=CH_2)$-2' (and analogs thereof see published International Application WO 2008/154401, published on December 8, 2008). See, for example: Singh et al., Chem. Commun., 1998, 4, 455-456; Koshkin et al., Tetrahedron, 1998, 54, 3607-3630; Wahlestedt et al., Proc. Natl. Acad. Sci. U. S. A., 2000, 97, 5633-5638; Kumar et al., Bioorg. Med. Chem. Lett., 1998, 8, 2219-2222; Singh et al., J. Org. Chem., 1998, 63, 10035-10039; Srivastava et al., J. Am. Chem. Soc., 129(26) 8362-8379 (Jul. 4, 2007); U.S. Patent Nos. 7,053,207; 6,268,490; 6,770,748; 6,794,499; 7,034,133; and

6,525,191; Elayadi et al., Curr. Opinion Invens. Drugs, 2001, 2, 558-561; Braasch et al., Chem. Biol., 2001, 8, 1-7; and Orum et al., Curr. Opinion Mol. Ther., 2001, 3, 239-243; and U.S. 6,670,461; International applications WO 2004/106356; WO 94/14226; WO 2005/021570; U.S. Patent Publication Nos. US2004-0171570; US2007-0287831; US2008-0039618; U.S. Patent Nos. 7,399,845; U.S. Patent Serial Nos. 12/129,154; 60/989,574; 61/026,995; 61/026,998; 61/056,564; 61/086,231; 61/097,787; 61/099,844; PCT International Applications Nos. PCT/US2008/064591; PCT/US2008/066154; PCT/US2008/068922; and Published PCT International Applications WO 2007/134181. Each of the foregoing bicyclic nucleosides can be prepared having one or more stereochemical sugar configurations including for example α-L-ribofuranose and β-D-ribofuranose (see PCT international application PCT/DK98/00393, published on March 25, 1999 as WO 99/14226).

[0224] In certain embodiments, bicyclic sugar moieties of BNA nucleosides include, but are not limited to, compounds having at least one bridge between the 4' and the 2' position of the pentofuranosyl sugar moiety wherein such bridges independently comprises 1 or from 2 to 4 linked groups independently selected from $-[C(R_a)(R_b)]_n-$, $-C(R_a)=C(R_b)-$, $-C(R_a)=N-$, $-C(=NR_a)-$, $-C(=O)-$, $-C(=S)-$, $-O-$, $-Si(R_a)_2-$, $-S(=O)_x-$, and $-N(R_a)-$;
wherein:

x is 0, 1, or 2;

n is 1, 2, 3, or 4;

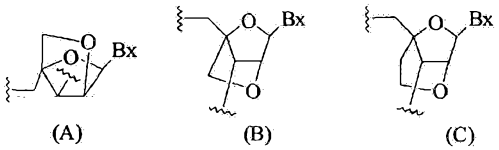
each R_a and R_b is, independently, H, a protecting group, hydroxyl, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, heterocycle radical, substituted heterocycle radical, heteroaryl, substituted heteroaryl, C_5-C_7 alicyclic radical, substituted C_5-C_7 alicyclic radical, halogen, OJ_1, NJ_1J_2, SJ_1, N_3, $COOJ_1$, acyl (C(=O)-H), substituted acyl, CN, sulfonyl ($S(=O)_2$-J_1), or sulfoxyl ($S(=O)$-J_1); and

each J_1 and J_2 is, independently, H, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, acyl (C(=O)-H), substituted acyl, a heterocycle radical, a substituted heterocycle radical, C_1-C_{12} aminoalkyl, substituted C_1-C_{12} aminoalkyl or a protecting group.

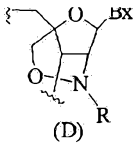
[0225] In certain embodiments, the bridge of a bicyclic sugar moiety is, $-[C(R_a)(R_b)]_n-$, $-[C(R_a)(R_b)]_n-O-$, $-C(R_aR_b)-N(R)-O-$ or $-C(R_aR_b)-O-N(R)-$. In certain embodiments, the bridge is 4'-CH_2-2', 4'-$(CH_2)_2$-2', 4'-$(CH_2)_3$-2', 4'-CH_2-O-2', 4'-$(CH_2)_2$-O-2', 4'-CH_2-O-N(R)-2' and 4'-CH_2-N(R)-O-2'- wherein each R is, independently, H, a protecting group or C_1-C_{12} alkyl.

[0226] In certain embodiments, bicyclic nucleosides are further defined by isomeric configuration. For example, a nucleoside comprising a 4'-2' methylene-oxy bridge, may be in the α-L configuration or in the β-D configuration. Previously, α-L-methyleneoxy (4'-CH_2-O-2') BNA's have been incorporated into antisense oligonucleotides that showed antisense activity (Frieden et al., Nucleic Acids Research, 2003, 21, 6365-6372).

[0227] In certain embodiments, bicyclic nucleosides include, but are not limited to, (A) α-L-Methyleneoxy (4'-CH_2-O-2') BNA, (B) β-D-Methyleneoxy (4'-CH_2-O-2') BNA, (C) Ethyleneoxy (4'-$(CH_2)_2$-O-2') BNA, (D) Aminooxy (4'-CH_2-O-N(R)-2') BNA, (E) Oxyamino (4'-CH_2-N(R)-O-2') BNA, and (F) Methyl(methyleneoxy) (4'-$CH(CH_3)$-O-2') BNA, (G) methylene-thio (4'-CH_2-S-2') BNA, (H) methylene-amino (4'-CH2-N(R)-2') BNA, (I) methyl carbocyclic (4'-CH_2-$CH(CH_3)$-2') BNA, (J) propylene carbocyclic (4'-$(CH_2)_3$-2') BNA, and (K) ethylene carbocyclic (4'-CH_2-CH_2-2') (carba LNA or "cLNA") as depicted below.



(A) (B) (C)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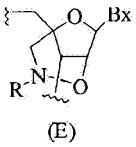
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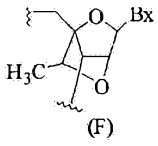
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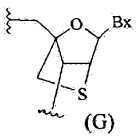
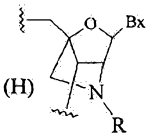

(G)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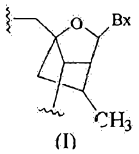
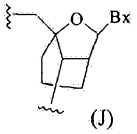
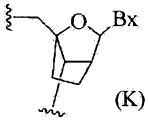
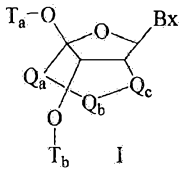

(I)


(J)


(K)

wherein Bx is the base moiety and R is independently H, a protecting group or C_1-C_{12} alkyl.

[0228] In certain embodiments, bicyclic nucleoside having Formula I:


I

wherein:

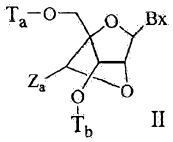
Bx is a heterocyclic base moiety;

$-Q_a-Q_b-Q_c-$ is $-CH_2-N(R_c)-CH_2-$, $-C(=O)-N(R_c)-CH_2-$, $-CH_2-O-N(R_c)-$, $-CH_2-N(R_c)-O-$ or $-N(R_c)-O-CH_2$;

R_c is C_1-C_{12} alkyl or an amino protecting group; and

T_a and T_b are each, independently H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety or a covalent attachment to a support medium.

[0229] In certain embodiments, bicyclic nucleoside having Formula II:


II

wherein:

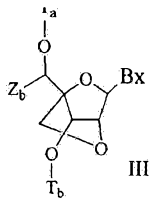
Bx is a heterocyclic base moiety;

T_a and T_b are each, independently H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety or a covalent attachment to a support medium;

Z_a is C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, substituted C_1-C_6 alkyl, substituted C_2-C_6 alkenyl, substituted C_2-C_6 alkynyl, acyl, substituted acyl, substituted amide, thiol or substituted thio.

[0230] In one embodiment, each of the substituted groups, is, independently, mono or poly substituted with substituent groups independently selected from halogen, oxo, hydroxyl, OJ_c, NJ_cJ_d, SJ_c, N_3, $OC(=X)J_c$, and $NJ_eC(=X)NJ_cJ_d$, wherein each J_c, J_d and J_e is, independently, H, C_1-C_6 alkyl, or substituted C_1-C_6 alkyl and X is O or NJ_c.

[0231] In certain embodiments, bicyclic nucleoside having Formula III:



III

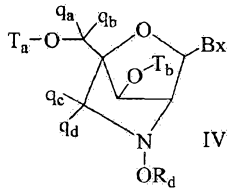
wherein:

Bx is a heterocyclic base moiety;

T_a and T_b are each, independently H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety or a covalent attachment to a support medium;

Z_b is C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, substituted C_1-C_6 alkyl, substituted C_2-C_6 alkenyl, substituted C_2-C_6 alkynyl or substituted acyl (C(=O)-).

[0232] In certain embodiments, bicyclic nucleoside having Formula IV:



IV

wherein:

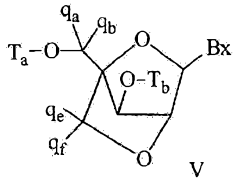
Bx is a heterocyclic base moiety;

T_a and T_b are each, independently H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety or a covalent attachment to a support medium;

R_d is C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl or substituted C_2-C_6 alkynyl;

each q_a, q_b, q_c and q_d is, independently, H, halogen, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl or substituted C_2-C_6 alkynyl, C_1-C_6 alkoxyl, substituted C_1-C_6 alkoxyl, acyl, substituted acyl, C_1-C_6 aminoalkyl or substituted C_1-C_6 aminoalkyl;

[0233] In certain embodiments, bicyclic nucleoside having Formula V:



V

wherein:

Bx is a heterocyclic base moiety;

T_a and T_b are each, independently H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety or a covalent attachment to a support medium;

q_a, q_b, q_e and q_f are each, independently, hydrogen, halogen, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_1-C_{12} alkoxy, substituted C_1-C_{12} alkoxy, OJ_j, SJ_j, SOJ_j, SO_2J_j, NJ_jJ_k, N_3, CN, $C(=O)OJ_j$, $C(=O)NJ_jJ_k$, $C(=O)J_j$, $O\text{-}C(=O)NJ_jJ_k$, $N(H)C(=NH)NJ_jJ_k$, $N(H)C(=O)NJ_jJ_k$ or $N(H)C(=S)NJ_jJ_k$;

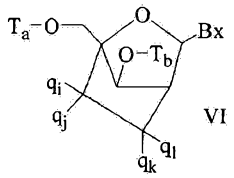
or q_e and q_f together are $=C(q_g)(q_h)$;

q_g and q_h are each, independently, H, halogen, C_1-C_{12} alkyl or substituted C_1-C_{12} alkyl.

[0234] The synthesis and preparation of the methyleneoxy (4'-CH_2-O-2') BNA monomers adenine, cytosine, guanine, 5-methyl-cytosine, thymine and uracil, along with their oligomerization, and nucleic acid recognition properties have been described (Koshkin et al., Tetrahedron, 1998, 54, 3607-3630). BNAs and preparation thereof are also described in WO 98/39352 and WO 99/14226.

[0235] Analogs of methyleneoxy (4'-CH_2-O-2') BNA, methyleneoxy (4'-CH_2-O-2') BNA and 2'-thio-BNAs, have also been prepared (Kumar et al., Bioorg. Med. Chem. Lett., 1998, 8, 2219-2222). Preparation of locked nucleoside analogs comprising oligodeoxyribonucleotide duplexes as substrates for nucleic acid polymerases has also been described (Wengel et al., WO 99/14226). Furthermore, synthesis of 2'-amino-BNA, a novel comformationally restricted high-affinity oligonucleotide analog has been described in the art (Singh et al., J. Org. Chem., 1998, 63, 10035-10039). In addition, 2'-Amino- and 2'-methylamino-BNA's have been prepared and the thermal stability of their duplexes with complementary RNA and DNA strands has been previously reported.

[0236] In certain embodiments, bicyclic nucleoside having Formula VI:



VI

wherein:

Bx is a heterocyclic base moiety;

T_a and T_b are each, independently H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety or a covalent attachment to a support medium;

each q_i, q_j, q_k and q_l is, independently, H, halogen, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_1-C_{12} alkoxyl, substituted C_1-C_{12} alkoxyl, OJ_j, SJ_j, SOJ_j, SO_2J_j, NJ_jJ_k, N_3, CN, $C(=O)OJ_j$, $C(=O)NJ_jJ_k$, $C(=O)J_j$, $O\text{-}C(=O)NJ_jJ_k$, $N(H)C(=NH)NJ_jJ_k$, $N(H)C(=O)NJ_jJ_k$ or $N(H)C(=S)NJ_jJ_k$; and

q_i and q_j or q_i and q_k together are $=C(q_g)(q_h)$, wherein q_g and q_h are each, independently, H, halogen, C_1-C_{12} alkyl or substituted C_1-C_{12} alkyl.

[0237] One carbocyclic bicyclic nucleoside having a 4'-$(CH_2)_3$-2' bridge and the alkenyl analog bridge 4'-CH=CH-CH_2-2' have been described (Frier et al., Nucleic Acids Research, 1997, 25(22), 4429-4443 and Albaek et al., J. Org. Chem., 2006, 71, 7731-7740). The synthesis and preparation of carbocyclic bicyclic nucleosides along with their oligomerization and biochemical studies have also been described (Srivastava et al., J. Am. Chem. Soc. 2007, 129(26), 8362-8379).

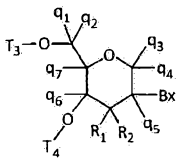
[0238] As used herein, "4'-2' bicyclic nucleoside" or "4' to 2' bicyclic nucleoside" refers to a bicyclic nucleoside comprising a furanose ring comprising a bridge connecting two carbon atoms of the furanose ring connects the 2' carbon atom and the 4' carbon atom of the sugar ring.

[0239] As used herein, "monocylic nucleosides" refer to nucleosides comprising modified sugar moieties that are not bicyclic sugar moieties. In certain embodiments, the sugar moiety, or sugar moiety analogue, of a nucleoside may be modified or substituted at any position.

[0240] As used herein, "2'-modified sugar" means a furanosyl sugar modified at the 2' position. In certain embodiments, such modifications include substituents selected from: a halide, including, but not limited to substituted and unsubstituted alkoxy, substituted and unsubstituted thioalkyl, substituted and unsubstituted amino alkyl, substituted and unsubstituted alkyl, substituted and unsubstituted allyl, and substituted and unsubstituted alkynyl. In certain embodiments, 2' modifications are selected from substituents including, but not limited to: $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, $OCH_2C(=O)N(H)CH_3$, and $O(CH_2)_nON[(CH_2)_nCH_3]_2$, where n and m are from 1 to about 10. Other 2'- substituent groups can also be selected from: C_1-C_{12} alkyl, substituted alkyl, alkenyl, alkynyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving pharmacokinetic properties, or a group for improving the pharmacodynamic properties of an antisense compound, and other substituents having similar properties. In certain embodiments, modifed nucleosides comprise a 2'-MOE side chain (Baker et al., J. Biol. Chem., 1997, 272, 11944-12000). Such 2'-MOE substitution have been described as having improved binding affinity compared to unmodified nucleosides and to other modified nucleosides, such as 2'-*O*-methyl, *O*-propyl, and *O*-aminopropyl. Oligonucleotides having the 2'-MOE substituent also have been shown to be antisense inhibitors of gene expression with promising features for *in vivo* use (Martin, P., Helv. Chim. Acta, 1995, 78, 486-504; Altmann et al., Chimia, 1996, 50, 168-176; Altmann et al., Biochem. Soc. Trans., 1996, 24, 630-637; and Altmann et al., Nucleosides Nucleotides, 1997, 16, 917-926).

[0241] As used herein, a "modified tetrahydropyran nucleoside" or "modified THP nucleoside" means a nucleoside having a six-membered tetrahydropyran "sugar" substituted in for the pentofuranosyl residue in normal nucleosides (a sugar surrogate). Modified THP nucleosides include, but are not limited to, what is referred to in the art as hexitol nucleic acid (HNA), anitol nucleic acid (ANA), manitol nucleic acid (MNA) (see Leumann, CJ. Bioorg. & Med. Chem. (2002) 10:841-854), fluoro HNA (F-HNA) or those compounds having Formula X:

Formula X:



X

wherein independently for each of said at least one tetrahydropyran nucleoside analog of Formula X:

Bx is a heterocyclic base moiety;

T_3 and T_4 are each, independently, an internucleoside linking group linking the tetrahydropyran nucleoside analog to the antisense compound or one of T_3 and T_4 is an internucleoside linking group linking the tetrahydropyran nucleoside analog to the antisense compound and the other of T_3 and T_4 is H, a hydroxyl protecting group, a linked conjugate group or a 5' or 3'-terminal group;

q_1, q_2, q_3, q_4, q_5, q_6 and q_7 are each independently, H, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl or substituted C_2-C_6 alkynyl; and

one of R_1 and R_2 is hydrogen and the other is selected from halogen, substituted or unsubstituted alkoxy, NJ_1J_2, SJ_1, N_3, $OC(=X)J_1$, $OC(=X)NJ_1J_2$, $NJ_3C(=X)NJ_1J_2$ and CN, wherein X is O, S or NJ_1 and each J_1, J_2 and J_3 is, independently, H or C_1-C_6 alkyl.

[0242] In certain embodiments, the modified THP nucleosides of Formula X are provided wherein q_m, q_n, q_p, q_r, q_s, q_t and q_u are each H. In certain embodiments, at least one of q_m, q_n, q_p, q_r, q_s, q_t and q_u is other than H. In certain embodiments, at least one of q_m, q_n, q_p, q_r, q_s, q_t and q_u is methyl. In certain embodiments, THP nucleosides of Formula X are provided wherein one of R_1 and R_2 is F. In certain embodiments, R_1 is fluoro and R_2 is H; R_1 is methoxy and R_2 is H, and R_1 is methoxyethoxy and R_2 is H.

[0243] As used herein, "2'-modified" or "2'-substituted" refers to a nucleoside comprising a sugar comprising a substituent at the 2' position other than H or OH. 2'-modified nucleosides, include, but are not limited to, bicyclic nucleosides wherein the bridge connecting two carbon atoms of the sugar ring connects the 2' carbon and another carbon of the sugar ring; and nucleosides with non-bridging 2'substituents, such as allyl, amino, azido, thio, O-allyl, O-C_1-C_{10} alkyl, -OCF_3, O-$(CH_2)_2$-O-CH_3, 2'-O$(CH_2)_2SCH_3$, O-$(CH_2)_2$-O-N$(R_m)(R_n)$, or O-CH_2-C(=O)-N$(R_m)(R_n)$, where each R_m and R_n is, independently, H or substituted or unsubstituted C_1-C_{10} alkyl. 2'-modifed nucleosides may further comprise other modifications, for example at other positions of the sugar and/or at the nucleobase.

[0244] As used herein, "2'-F" refers to a nucleoside comprising a sugar comprising a fluoro group at the 2' position.

[0245] As used herein, "2'-OMe" or "2'-OCH_3" or "2'-O-methyl" each refers to a nucleoside comprising a sugar comprising an -OCH_3 group at the 2' position of the sugar ring.

[0246] As used herein, "MOE" or "2'-MOE" or "2'-$OCH_2CH_2OCH_3$" or "2'-O-methoxyethyl" each refers to a nucleoside comprising a sugar comprising a -$OCH_2CH_2OCH_3$ group at the 2' position of the sugar ring.

[0247] As used herein, "oligonucleotide" refers to a compound comprising a plurality of linked nucleosides. In certain embodiments, one or more of the plurality of nucleosides is modified. In certain embodiments, an oligonucleotide comprises one or more ribonucleosides (RNA) and/or deoxyribonucleosides (DNA).

[0248] Many other bicyclo and tricyclo sugar surrogate ring systems are also know in the art that can be used to modify nucleosides for incorporation into antisense compounds (see for example review article: Leumann, J. C, Bioorganic & Medicinal Chemistry, 2002, 10, 841-854). Such ring systems can undergo various additional substitutions to enhance activity.

[0249] Methods for the preparations of modified sugars are well known to those skilled in the art.

[0250] In nucleotides having modified sugar moieties, the nucleobase moieties (natural, modified or a combination thereof) are maintained for hybridization with an appropriate nucleic acid target.

[0251] In certain embodiments, antisense compounds comprise one or more nucleotides having modified sugar moieties. In certain embodiments, the modified sugar moiety is 2'-MOE. In certain embodiments, the 2'-MOE modified nucleotides are arranged in a gapmer motif. In certain embodiments, the modified sugar moiety is a cEt. In certain embodiments, the cEt modified nucleotides are arranged throughout the wings of a gapmer motif.

Modified Nucleobases

[0252] Nucleobase (or base) modifications or substitutions are structurally distinguishable from, yet functionally interchangeable with, naturally occurring or synthetic unmodified nucleobases. Both natural and modified nucleobases are capable of participating in hydrogen bonding. Such nucleobase modifications may impart nuclease stability, binding affinity, increased selectivity for an allelic variant, or some other beneficial biological property to antisense compounds. Modified nucleobases include synthetic and natural nucleobases such as, for example, 5-methylcytosine (5-me-C). Certain nucleobase substitutions, including 5-methylcytosine substitutions, are particularly useful for increasing the binding affinity of an antisense compound for a target nucleic acid. For example, 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C (Sanghvi, Y.S., Crooke, S.T. and Lebleu, B., eds., Antisense Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278).

[0253] Additional modified nucleobases include 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl (-C≡C-CH_3) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 2-F-

adenine, 2-amino-adenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine.

[0254] Heterocyclic base moieties may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone. Nucleobases that are particularly useful for increasing the binding affinity of antisense compounds include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2 aminopropyladenine, 5-propynyluracil and 5-propynylcytosine.

[0255] In certain embodiments, antisense compounds comprise one or more modified nucleobases. In certain embodiments, gap-widened antisense oligonucleotides comprise one or more modified nucleobases. In certain embodiments, the modified nucleobase is 5-methylcytosine. In certain embodiments, each cytosine is a 5-methylcytosine.

Compositions and Methods for Formulating Pharmaceutical Compositions

[0256] Antisense oligonucleotides may be admixed with pharmaceutically acceptable active or inert substances for the preparation of pharmaceutical compositions or formulations. Compositions and methods for the formulation of pharmaceutical compositions are dependent upon a number of criteria, including, but not limited to, route of administration, extent of disease, or dose to be administered.

[0257] An antisense compound can be utilized in pharmaceutical compositions by combining the antisense compound with a suitable pharmaceutically acceptable diluent or carrier. A pharmaceutically acceptable diluent includes phosphate-buffered saline (PBS). PBS is a diluent suitable for use in compositions to be delivered parenterally. Accordingly, in one embodiment, employed in the methods described herein is a pharmaceutical composition comprising an antisense compound and a pharmaceutically acceptable diluent. In certain embodiments, the pharmaceutically acceptable diluent is PBS. In certain embodiments, the antisense compound is an antisense oligonucleotide.

[0258] Pharmaceutical compositions comprising antisense compounds encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other oligonucleotide which, upon administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to pharmaceutically acceptable salts of antisense compounds, prodrugs, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents. Suitable pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts.

[0259] A prodrug can include the incorporation of additional nucleosides at one or both ends of an antisense compound which are cleaved by endogenous nucleases within the body, to form the active antisense compound.

Conjugated Antisense Compounds

[0260] Antisense compounds may be covalently linked to one or more moieties or conjugates which enhance the activity, cellular distribution, increased selectivity for an allelic variant, or cellular uptake of the resulting antisense oligonucleotides. Typical conjugate groups include cholesterol moieties and lipid moieties. Additional conjugate groups include carbohydrates, phospholipids, biotin, phenazine, folate, phenanthridine, anthraquinone, acridine, fluoresceins, rhodamines, coumarins, and dyes.

[0261] Antisense compounds can also be modified to have one or more stabilizing groups that are generally attached to one or both termini of antisense compounds to enhance properties such as, for example, nuclease stability. Included in stabilizing groups are cap structures. These terminal modifications protect the antisense compound having terminal nucleic acid from exonuclease degradation, and can help in delivery and/or localization within a cell. The cap can be present at the 5'-terminus (5'-cap), or at the 3'-terminus (3'-cap), or can be present on both termini. Cap structures are well known in the art and include, for example, inverted deoxy abasic caps. Further 3' and 5'-stabilizing groups that can

be used to cap one or both ends of an antisense compound to impart nuclease stability include those disclosed in WO 03/004602 published on January 16, 2003.

Cell culture and antisense compounds treatment

[0262] The effects of antisense compounds on the level, activity or expression target nucleic acids can be tested *in vitro* in a variety of cell types. Cell types used for such analyses are available from commerical vendors (e.g. American Type Culture Collection, Manassus, VA; Zen-Bio, Inc., Research Triangle Park, NC; Clonetics Corporation, Walkersville, MD) and are cultured according to the vendor's instructions using commercially available reagents (e.g. Invitrogen Life Technologies, Carlsbad, CA). Illustrative cell types include, but are not limited to, HepG2 cells, Hep3B cells, and primary hepatocytes. Illustrative cell lines include GM04281, GM02171, and GM02173B cells.

In vitro testing of antisense oligonucleotides

[0263] Described herein are methods for treatment of cells with antisense oligonucleotides, which can be modified appropriately for treatment with other antisense compounds.

[0264] In general, cells are treated with antisense oligonucleotides when the cells reach approximately 60-80% confluency in culture.

[0265] One reagent commonly used to introduce antisense oligonucleotides into cultured cells includes the cationic lipid transfection reagent LIPOFECTIN (Invitrogen, Carlsbad, CA). Antisense oligonucleotides are mixed with LIPOFECTIN in OPTI-MEM 1 (Invitrogen, Carlsbad, CA) to achieve the desired final concentration of antisense oligonucleotide and a LIPOFECTIN concentration that typically ranges 2 to 12 ug/mL per 100 nM antisense oligonucleotide.

[0266] Another reagent used to introduce antisense oligonucleotides into cultured cells includes LIPOFECTAMINE (Invitrogen, Carlsbad, CA). Antisense oligonucleotide is mixed with LIPOFECTAMINE in OPTI-MEM 1 reduced serum medium (Invitrogen, Carlsbad, CA) to achieve the desired concentration of antisense oligonucleotide and a LIPOFECTAMINE concentration that typically ranges 2 to 12 ug/mL per 100 nM antisense oligonucleotide.

[0267] Another technique used to introduce antisense oligonucleotides into cultured cells includes electroporation.

[0268] Cells are treated with antisense oligonucleotides by routine methods. Cells are typically harvested 16-24 hours after antisense oligonucleotide treatment, at which time RNA or protein levels of target nucleic acids are measured by methods known in the art and described herein. In general, when treatments are performed in multiple replicates, the data are presented as the average of the replicate treatments.

[0269] The concentration of antisense oligonucleotide used varies from cell line to cell line. Methods to determine the optimal antisense oligonucleotide concentration for a particular cell line are well known in the art. Antisense oligonucleotides are typically used at concentrations ranging from 1 nM to 300 nM when transfected with LIPOFECTAMINE. Antisense oligonucleotides are used at higher concentrations ranging from 625 to 20,000 nM when transfected using electroporation.

RNA Isolation

[0270] RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. Methods of RNA isolation are well known in the art. RNA is prepared using methods well known in the art, for example, using the TRIZOL Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's recommended protocols.

Analysis of inhibition of target levels or expression

[0271] Reduction, inhibition, or expression of a target nucleic acid can be assayed in a variety of ways known in the art. For example, target nucleic acid levels can be quantitated by, e.g., Northern blot analysis, competitive polymerase chain reaction (PCR), or quantitaive real-time PCR. RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. Methods of RNA isolation are well known in the art. Northern blot analysis is also routine in the art. Quantitative real-time PCR can be conveniently accomplished using the commercially available ABI PRISM 7600, 7700, or 7900 Sequence Detection System, available from PE-Applied Biosystems, Foster City, CA and used according to manufacturer's instructions.

Quantitative Real-Time PCR Analysis of Target RNA Levels

[0272] Quantitation of target RNA levels may be accomplished by quantitative real-time PCR using the ABI PRISM 7600, 7700, or 7900 Sequence Detection System (PE-Applied Biosystems, Foster City, CA) according to manufacturer's instructions. Methods of quantitative real-time PCR are well known in the art.

[0273] Prior to real-time PCR, the isolated RNA is subjected to a reverse transcriptase (RT) reaction, which produces complementary DNA (cDNA) that is then used as the substrate for the real-time PCR amplification. The RT and real-time PCR reactions are performed sequentially in the same sample well. RT and real-time PCR reagents are obtained from Invitrogen (Carlsbad, CA). RT real-time-PCR reactions are carried out by methods well known to those skilled in the art.

[0274] Gene (or RNA) target quantities obtained by real time PCR are normalized using either the expression level of a gene whose expression is constant, such as cyclophilin A, or by quantifying total RNA using RIBOGREEN (Invitrogen, Inc. Carlsbad, CA). Cyclophilin A expression is quantified by real time PCR, by being run simultaneously with the target, multiplexing, or separately. Total RNA is quantified using RIBOGREEN RNA quantification reagent (Invetrogen, Inc. Eugene, OR). Methods of RNA quantification by RIBOGREEN are taught in Jones, L.J., et al, (Analytical Biochemistry, 1998, 265, 368-374). A CYTOFLUOR 4000 instrument (PE Applied Biosystems) is used to measure RIBOGREEN fluorescence.

[0275] Probes and primers are designed to hybridize to target nucleic acids. Methods for designing real-time PCR probes and primers are well known in the art, and may include the use of software such as PRIMER EXPRESS Software (Applied Biosystems, Foster City, CA).

Analysis of Protein Levels

[0276] Reduction, inhibition, or expression of target nucleic acids can be assessed by measuring target protein levels. Target protein levels can be evaluated or quantitated in a variety of ways well known in the art, such as immunoprecipitation, Western blot analysis (immunoblotting), enzyme-linked immunosorbent assay (ELISA), quantitative protein assays, protein activity assays (for example, caspase activity assays), immunohistochemistry, immunocytochemistry or fluorescence-activated cell sorting (FACS). Antibodies directed to a target can be identified and obtained from a variety of sources, such as the MSRS catalog of antibodies (Aerie Corporation, Birmingham, MI), or can be prepared via conventional monoclonal or polyclonal antibody generation methods well known in the art. Antibodies useful for the detection of mouse, rat, monkey, and human proteins are commercially available.

In vivo testing of antisense compounds

[0277] Antisense compounds, for example, antisense oligonucleotides, are tested in animals to assess their ability to selectively reduce or inhibit expression of target gene product and produce phenotypic changes, such as, amelioration of a disease symptom. Testing may be performed in normal animals, or in experimental disease models. For administration to animals, antisense oligonucleotides are formulated in a pharmaceutically acceptable diluent, such as phosphate-buffered saline. Administration includes parenteral routes of administration, such as intraperitoneal, intravenous, and subcutaneous. Calculation of antisense oligonucleotide dosage and dosing frequency is within the

abilities of those skilled in the art, and depends upon factors such as route of administration and animal body weight. Following a period of treatment with antisense oligonucleotides, RNA or protein is isolated from tissue and changes in target nucleic acid or protein expression are measured.

Administration

[0278] In certain embodiments, the compounds and compositions described herein may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic, vaginal, rectal, intranasal), oral, pulmonary (including by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal) or parenteral, for example, by intravenous drip, intravenous injection or subcutaneous, intraperitoneal, intraocular, intravitreal, or intramuscular injection.

[0279] In certain embodiments, the compounds and compositions as described herein are administered parenterally.

[0280] In certain embodiments, parenteral administration is by infusion. Infusion can be chronic or continuous or short or intermittent. In certain embodiments, infused pharmaceutical agents are delivered with a pump. In certain embodiments, parenteral administration is by injection.

[0281] In certain embodiments, compounds and compositions are delivered to the CNS. In certain embodiments, compounds and compositions are delivered to the cerebrospinal fluid. In certain embodiments, compounds and compositions are administered to the brain parenchyma. In certain embodiments, compounds and compositions are delivered to an animal by intrathecal administration, or intracerebroventricular administration. Broad distribution of compounds and compositions, described herein, within the central nervous system may be achieved with intraparenchymal administration, intrathecal administration, or intracerebroventricular administration.

[0282] In certain embodiments, parenteral administration is by injection. The injection may be delivered with a syringe or a pump. In certain embodiments, the injection is a bolus injection. In certain embodiments, the injection is administered directly to a tissue, such as striatum, caudate, cortex, hippocampus and cerebellum.

[0283] In certain embodiments, methods of specifically localizing a pharmaceutical agent, such as by bolus injection, decreases median effective concentration (EC50) by a factor of 20, 25, 30, 35, 40, 45 or 50. In certain embodiments, the pharmaceutical agent in an antisense compound as further described herein. In certain embodiments, the targeted tissue is brain tissue. In certain embodiments, the targeted tissue is brain tissue. In certain embodiments the targeted tissue is striatal tissue. In certain embodiments, decreasing EC50 is desirable because it reduces the dose required to achieve a pharmacological result in a patient in need thereof.

[0284] In certain embodiments, an antisense oligonucleotide is delivered by injection or infusion once every month, every two months, every 90 days, every 3 months, every 6 months, twice a year or once a year.

Certain Compounds and Indications

[0285] Provided herein are compounds that provide potent inhibition and increased selectivity for a mutant allele. Potency is demonstrated by the percent inhibition of mutant mRNA achieved by the antisense oligonucleotides targeting a SNP compared to the percent inhibition of mutant mRNA achieved by the benchmark oligonucleotide. Selectivity is demonstrated by the ability of the antisense oligonucleotide targeting a SNP to inhibit expression of the major allele or mutant allele preferentially compared to the minor allele or wild type allele. The usage of three cell lines with different genotypes at each SNP position have facilitated the determination of design rules that provide for potent and selective SNP targeting antisense oligonucleotides.

[0286] In certain embodiments, the compounds are antisense oligonucleotides as further described herein. The antisense oligonucleotides preferentially target a SNP or differentiating polymorphism. Oligonucleotides of various lengths were tested and certain lengths were determined to be beneficial for the targeting of SNPs.

[0287] The antisense oligonucleotides have a sequence that is 15-20 nucleobases in length. In certain embodiments, the antisense oligonucleotides have a sequence that is 15-19, nuceobases in length. In certain embodiments, the antisense oligonucleotides have a sequence that is 16-19 nucleobases in lenth. In certain embodiments, the antisense oligonucleotides have a sequence that is 17-19 nucleobases in length. In certain embodiments, the antisense oligonucleotides have a sequence that is 15, 16, 17, 18 or 19 nucleobases in length.

[0288] For oligonucleotides of various lengths, the position of the nucleoside complementary to the SNP position was shifted within the gap and the wings and the effect was tested. Certain positions within the antisense oligonucleotide are shown to be beneficial for targeting SNPs.

[0289] In certain embodiments, the antisense oligonucleotide is at least 12, at least 13, at least 14, at least 15, at least 16, at least 17 at least 18 or at least 19 nucleobases in length and the SNP is complementary to positions 8-10 counting from the 5' terminus of the antisense oligonuceotide and/or positions 4-6 counting from the 5' end of the gap.

[0290] In certain embodiments, the SNP is complementary to position 8, 9, or 10 counting from the 5' terminus of the oligonucleotide or position 4, 5, or 6, counting from the 5' end of the gap. For oligonucleotides of various lengths, the effect of the length of the gap, 5' wing, and 3' wing was tested.

[0291] Certain wing-gap-wing combinations were shown to be beneficial for a SNP targeting antisense oligonucleotide. In certain embodiments the gap is 7-11 nucleobases in length and each wing is independently 2-6 nucleobases in length. In certain embodiments the gap is 8-11 nucleobases in length and each wing is independently 2-6 nucleobases in length. In certain embodiments the gap is 9 nucleobases in length and each wing is independently 2-6 nucleobases in length. In certain embodiments the gap is 10 nucleobases in length and each wing is independently 2-6 or 4-5 nucleobases in length. In certain embodiments the gap is 11 nucleobases in length and each wing is independently 2-6, or 4-5 nucleobases in length. In certain embodiments, the wing-gap-wing configuration is one of 4-7-4, 5-8-6, 6-8-5, 6-7-6, 5-7-5. 6-8-5, 5-8-6, 3-9-4, 4-9-3, 2-9-6, 6,9,2,3-9-3, 3-9-5, 5-9-3, 5-9-4, 4-9-5, 5-9-5, 4-11-4, 4-10-5 and 5-10-4.

[0292] For oligonucleotides of various lengths, the effect of certain chemistries was tested. Certain chemistry modifications were shown to be beneficial for a SNP targeting antisense oligonucleotide. In certain embodiments, each nucleoside of each wing of the modified antisense oligonucleotide has a 2'-MOE modification. In certain embodiments, each nucleoside of each wing of the modified antisense oligonucleotide has a high affinity modification. In certain embodiments, the antisense oligonucleotide is a mixed wing gapmer. In such embodiment, the modifications and combination of modifications at the 3' wing and at the 5' wing may be the same or they may be different. In certain embodiments, the antisense oligonucleotide has one or more 2'-MOE modifications in the wings and/or one or more high affinity modifications in the wings. In certain embodiments, the high affinity modification is a cEt modification. In certain embodiments, the antisense oligonucleotide has a high affinity modification at positions 2, 3, 13, and 14 of the antisense oligonucleotide (counting from the 5' terminus). In certain embodiments, the antisense oligonuceotide has one, two, three, or four high affinity modifications in at least one of the wings. In certain embodiments, the antisense oligonuceotide has one, two, three, or four high affinity modifications in each of the 5' and 3' wings independently. In certain embodiments, the antisense oligonucleotide has a high affinity modification at positions 2 and 3 in one or both of the 5' and 3' wings (counting from the 5' terminus of the 5' wing and the 3' terminus of the 3'wing). In certain embodiments, the antisense oligonuceotide has a high affinity modification at positions 2, 3 and 4 in one or both of the 5' and 3' wings (counting from the 5' terminus of the 5' wing and the 3' terminus of the 3'wing,). In certain embodiments, the antisense oligonuceotide has a high affinity modification at positions 1 of the 5' and/or 3' wings (counting from the 5' terminus of the 5' wing and the 3' terminus of the 3'wing,). In certain embodiments, the antisense oligonuceotide has a high affinity modification at positions 1 of the 5' and 3' wings (counting from the 5' terminus of the 5' wing and the 3' terminus of the 3'wing,) and at least one other position in the wing. In certain embodiments, the antisense oligonuceotide has alternating 2'-MOE and high affinity modification in at least one of the 5' and 3'wings.

[0293] In certain embodiments, the compound comprises an antisense oligonucleotide incorporating one or more of the design rules provided above.

[0294] In certain instances, the compound comprises a modified antisense oligonucleotide consisting of 12 to 30 linked nucleosides, fully complementary to a single nucleotide polymorphism site, wherein the modified antisense oligonucleotide comprises a wing-gap-wing motif, wherein the single nucleotide polymorphism aligns with any one of

positions 6-15 begining from the 5' terminus of the antisense oligonuceotide or positions 1-9 begining from the 5' end of the gap of the modified antisense oligonucleotide; and wherein each nucleoside of each wing has a modified sugar or sugar surrogate. In certain embodiments the single nucleotide polymorphism site contains a differentiating polymorphism. The single nucleotide polymorphism site is on a mutant allele. The mutant allele is associated with disease. In certain instances, the wing-gap-wing motif is any one of the group consisting of 4-7-4, 5-8-6, 6-8-5, 6-7-6, 5-7-5. 6-8-5, 5-8-6, 3-9-4, 4-9-3, 2-9-6, 6,9,2,3-9-3, 3-9-5,5-9-3, 5-9-4, 4-9-5, 5-9-5, 4-11-4,4-10-5 and 5-10-4.

[0295] In certain instances, the compound comprises a modified antisense oligonucleotide consisting of 12 to 20 linked nucleosides, fully complementary to a single nucleotide polymorphism site, wherein the modified antisense oligonucleotide comprises a wing-gap-wing motif, wherein the single nucleotide polymorphism aligns with any one of positions 6-15 begining from the 5' terminus of the antisense oligonuceotide or positions 1-9 begining from the 5' end of the gap of the modified antisense oligonucleotide; and wherein each nucleoside of each wing has a modified sugar or sugar surrogate. In certain instances, the wing-gap-wing motif is any one of the group consisting of 4-7-4, 5-8-6, 6-8-5, 6-7-6, 5-7-5. 6-8-5, 5-8-6, 3-9-4, 4-9-3, 2-9-6, 6-9-2,3-9-3, 3-9-5,5-9-3, 5-9-4, 4-9-5, 5-9-5, 4-11-4,4-10-5 and 5-10-4.

[0296] In certain instances, the compound comprises a modified antisense oligonucleotide consisting of 12 to 20 linked nucleosides, fully complementary to a single nucleotide polymorphism site, wherein the modified antisense oligonucleotide comprises a wing-gap-wing motif, wherein the single nucleotide polymorphism aligns with any one of positions 8-14 begining from the 5' terminus of the antisense oligonuceotide or positions 1-9 begining from the 5' end of the gap of the modified antisense oligonucleotide; and wherein each nucleoside of each wing has a modified sugar or sugar surrogate. In certain instances, the wing-gap-wing motif is any one of the group consisting of 4-7-4, 5-8-6, 6-8-5, 6-7-6, 5-7-5. 6-8-5, 5-8-6, 3-9-4, 4-9-3, 2-9-6, 6,9,2,3-9-3, 3-9-5,5-9-3, 5-9-4, 4-9-5, 5-9-5, 4-11-4,4-10-5 and 5-10-4.

[0297] In certain instances, the compound comprises a modified antisense oligonucleotide consisting of 12 to 20 linked nucleosides, fully complementary to a single nucleotide polymorphism site, wherein the modified antisense oligonucleotide comprises a wing-gap-wing motif, wherein the single nucleotide polymorphism aligns with any one of positions 8-14 beginning from the 5' terminus of the antisense oligonuceotide or positions 4-7 begining from the 5' end of the gap of the modified antisense oligonucleotide; and wherein each nucleoside of each wing has a modified sugar or sugar surrogate. In certain instances, the wing-gap-wing motif is any one of the group consisting of 4-7-4, 5-8-6, 6-8-5, 6-7-6, 5-7-5. 6-8-5, 5-8-6, 3-9-4, 4-9-3, 2-9-6, 6,9,2,3-9-3, 3-9-5,5-9-3, 5-9-4, 4-9-5, 5-9-5, 4-11-4,4-10-5 and 5-10-4.

[0298] In certain instances, the compound comprises a modified antisense oligonucleotide consisting of 12 to 20 linked nucleosides, fully complementary to a single nucleotide polymorphism site, wherein the modified antisense oligonucleotide comprises a wing-gap-wing motif, wherein the single nucleotide polymorphism aligns with any one of positions 8-10 begining from the 5' terminus of the antisense oligonuceotide or positions 4-6 begining from the 5' end of the gap of the modified antisense oligonucleotide; and wherein each nucleoside of each wing has a modified sugar or sugar surrogate. In certain instances, the wing-gap-wing motif is any one of the group consisting of 4-7-4, 5-8-6, 6-8-5, 6-7-6, 5-7-5. 6-8-5, 5-8-6, 3-9-4, 4-9-3, 2-9-6, 6,9,2,3-9-3, 3-9-5,5-9-3, 5-9-4, 4-9-5, 5-9-5, 4-11-4,4-10-5 and 5-10-4.

[0299] In certain instances, the compound comprises a modified antisense oligonucleotide consisting of 12 to 19 linked nucleosides, fully complementary to a single nucleotide polymorphism site, wherein the modified antisense oligonucleotide comprises a wing-gap-wing motif, wherein the single nucleotide polymorphism aligns with any one of positions 8-10 begining from the 5' terminus of the antisense oligonuceotide or positions 4-6 begining from the 5' end of the gap of the modified antisense oligonucleotide; and wherein each nucleoside of each wing has a modified sugar or sugar surrogate. In certain instances, the wing-gap-wing motif is any one of the group consisting of 4-7-4, 5-8-6, 6-8-5, 6-7-6, 5-7-5. 6-8-5, 5-8-6, 3-9-4, 4-9-3, 2-9-6, 6,9,2,3-9-3, 3-9-5,5-9-3, 5-9-4, 4-9-5, 5-9-5, 4-11-4,4-10-5 and 5-10-4.

[0300] In certain instances, the compound comprises a modified antisense oligonucleotide consisting of 13 to 19 linked nucleosides, fully complementary to a single nucleotide polymorphism site, wherein the modified antisense oligonucleotide comprises a wing-gap-wing motif, wherein the single nucleotide polymorphism aligns with any one of positions 8-10 begining from the 5' terminus of the antisense oligonuceotide or positions 4-6 begining from the 5' end of the gap of the modified antisense oligonucleotide; and wherein each nucleoside of each wing has a modified sugar or sugar surrogate. In certain instances, the wing-gap-wing motif is any one of the group consisting of 4-7-4, 5-8-6, 6-8-5, 6-7-6, 5-7-5. 6-8-5, 5-8-6, 3-9-4, 4-9-3, 2-9-6, 6,9,2,3-9-3, 3-9-5,5-9-3, 5-9-4, 4-9-5, 5-9-5, 4-11-4,4-10-5 and 5-10-4.

[0301] In certain instances, the compound comprises a modified antisense oligonucleotide consisting of 14 to 19 linked nucleosides, fully complementary to a single nucleotide polymorphism site, wherein the modified antisense oligonucleotide comprises a wing-gap-wing motif, wherein the single nucleotide polymorphism aligns with any one of positions 8-10 beginning from the 5' terminus of the antisense oligonuceotide or positions 4-6 begining from the 5' end of the gap of the modified antisense oligonucleotide; and wherein each nucleoside of each wing has a modified sugar or sugar surrogate. In certain instances, the wing-gap-wing motif is any one of the group consisting of 4-7-4, 5-8-6, 6-8-5, 6-7-6, 5-7-5. 6-8-5, 5-8-6, 3-9-4, 4-9-3, 2-9-6, 6,9,2,3-9-3, 3-9-5,5-9-3, 5-9-4, 4-9-5, 5-9-5, 4-11-4,4-10-5 and 5-10-4.

[0302] In certain instances, the compound comprises a modified antisense oligonucleotide consisting of 15 to 19 linked nucleosides, fully complementary to a single nucleotide polymorphism site, wherein the modified antisense oligonucleotide comprises a wing-gap-wing motif, wherein the single nucleotide polymorphism aligns with any one of positions 6-15 beginning from the 5' terminus of the antisense oligonuceotide or positions 1-9 begining from the 5' end of the gap of the modified antisense oligonucleotide; and wherein each nucleoside of each wing has a modified sugar or sugar surrogate. In certain instances, the wing-gap-wing motif is any one of the group consisting of 4-7-4, 5-8-6, 6-8-5, 6-7-6, 5-7-5. 6-8-5, 5-8-6, 3-9-4, 4-9-3, 2-9-6, 6,9,2,3-9-3, 3-9-5,5-9-3, 5-9-4, 4-9-5, 5-9-5, 4-11-4,4-10-5 and 5-10-4.

[0303] In certain embodiments, the compound comprises a modified antisense oligonucleotide consisting of 15 to 19 linked nucleosides, fully complementary to a single nucleotide polymorphism site, wherein the modified antisense oligonucleotide comprises a wing-gap-wing motif, wherein the single nucleotide polymorphism aligns with any one of positions 8-10 beginning from the 5' terminus of the antisense oligonuceotide or positions 4-6 begining from the 5' end of the gap of the modified antisense oligonucleotide; and wherein each nucleoside of each wing has a modified sugar or sugar surrogate. In certain embodiments, the wing-gap-wing motif is any one of the group consisting of 4-7-4, 5-8-6, 6-8-5, 6-7-6, 5-7-5. 6-8-5, 5-8-6, 3-9-4, 4-9-3, 2-9-6, 6,9,2,3-9-3, 3-9-5,5-9-3, 4-9-5, 5-9-5, 4-11-4,4-10-5 and 5-10-4.

[0304] In certain embodiments, the compound comprises a modified antisense oligonucleotide consisting of 15 to 19 linked nucleosides, fully complementary to a single nucleotide polymorphism site, wherein the modified antisnese oligonucleotide comprises a wing-gap-wing motif, wherein position 8-10 beginning from the 5' terminus of the modified antisense oligonucleotide aligns with the single nucleotide polymorphism; and wherein each nucleoside of each wing segment modified sugar or sugar surrogate. In certain embodiments, the wing-gap-wing motif is any one of the group consisting of 2-9-6, 3-9-3, 3-9-5, 4-9-5, 4-11-4, and 5-10-4.

[0305] In certain embodiments, the compound comprises a modified antisense oligonucleotide consisting of 15 to 19 linked nucleosides, fully complementary to a single nucleotide polymorphism site, wherein the modified antisense oligonucleotide comprises a wing-gap-wing motif, wherein position 4, 5 or 6 of the gap segment aligns with the single nucleotide polymorphism; and wherein each nucleoside of each wing segment has a modified sugar or sugar surrogate. In certain embodiments, the wing-gap-wing motif is any one of the group consisting of 2-9-6, 3-9-3, 3-9-5, 4-9-5, 4-11-4, and 5-10-4.

[0306] In certain embodiments, the compound comprises a modified antisense oligonucleotide consisting of 15 to 19 linked nucleosides, fully complementary to a single nucleotide polymorphism site, wherein the modified antisense oligonucleotide comprises a wing-gap-wing motif, wherein position 8-10 of the modified antisense oligonucleotide aligns with the single nucleotide polymorphism; and positions 2 and 3 of the 5' and 3' wing segments comprise a 4'-CH(CH_3)-O-2' bridge. In certain embodiments, the wing-gap-wing motif is any one of the group consisting of 2-9-6, 3-9-3, 3-9-5, 4-9-5, 4-11-4, and 5-10-4.

[0307] In certain embodiments, the compound comprises a modified antisense oligonucleotide consisting of 15 to 19 linked nucleosides and fully complementary to a single nucleotide polymorphism site, wherein the modified antisense oligonucleotide comprises a wing-gap-wing motif, wherein position 4, 5 or 6 of the gap segment aligns with the single nucleotide polymorphism; and positions 2 and 3 of the 5' and 3' wing segments comprise a 4'-CH(CH_3)-O-2' bridge. In certain embodiments, the wing-gap-wing motif is any one of the group consisting of 2-9-6, 3-9-3, 3-9-5, 4-9-5, 4-11-4, and 5-10-4.

[0308] A compound comprising a modified antisense oligonucleotide consisting of 15 to 19 linked nucleosides and fully

complementary to a single nucleotide polymorphism site, wherein the modified antisense oligonucleotide comprises a wing-gap-wing motif, wherein position 8-10 of the modified antisense oligonucleotide aligns with the single nucleotide polymorphism; and positions 2, 3, 13, and 14 of the antisense oligonucleotide comprise a 4'-CH(CH_3)-O-2' bridge.. In certain embodiments, the wing-gap-wing motif is any one of the group consisting of 2-9-6, 3-9-3, 3-9-5, 4-9-5, 4-11-4, and 5-10-4.

[0309] A compound comprising a modified antisense oligonucleotide consisting of 15 to 19 linked nucleosides and fully complementary to a single nucleotide polymorphism site, wherein the modified antisense oligonucleotide comprises a wing-gap-wing motif, wherein position 4, 5 or 6 of the gap segment aligns with the single nucleotide polymorphism; and positions 2, 3, 13, and 14 of the antisense antisense oligonucleotide comprise a 4'-CH(CH_3)-O-2' bridge. In certain embodiments, the wing-gap-wing motif is any one of the group consisting of 2-9-6, 3-9-3, 3-9-5, 4-9-5, 4-11-4, and 5-10-4.

[0310] In certain embodiments, the compound comprise a modified antisense oligonucleotide consisting of 17 to 19 linked nucleosides, fully complementary to a single nucleotide polymorphism site, wherein the modified antisense oligonucleotide comprises a wing-gap-wing motif, wherein position 8, 9, or 10 of the modified antisense oligonucleotide aligns with the single nucleotide polymorphism; and wherein each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar. In certain embodiments, the wing-gap-wing motif is any one of the group consisting of 2-9-6, 3-9-3, 3-9-5, 4-9-5, 4-11-4, and 5-10-4.

[0311] In certain embodiments, the compound comprises a modified antisense oligonucleotide consisting of 17 to 19 linked nucleosides, fully complementary to a single nucleotide polymorphism site, wherein the modified antisense oligonucleotide comprises a wing-gap-wing motif, wherein position 5 or 6, of the gap segment aligns with the single nucleotide polymorphism; and wherein each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar. In certain embodiments, the wing-gap-wing motif is any one of the group consisting of 2-9-6, 3-9-3, 3-9-5, 4-9-5, 4-11-4, and 5-10-4.

[0312] In certain embodiments, the compound comprises a modified antisense oligonucleotide consisting of 17 to 19 linked nucleosides, fully complementary to a single nucleotide polymorphism site, wherein the modified antisense oligonucleotide comprises a wing-gap-wing motif, wherein position 8, 9, or 10 of the modified antisense oligonucleotide aligns with the single nucleotide polymorphism; and positions 2 and 3 of the 5' and 3' wing segments comprise a 4'-CH(CH_3)-O-2' bridge. In certain embodiments, the wing-gap-wing motif is any one of the group consisting of 2-9-6, 3-9-3, 3-9-5, 4-9-5, 4-11-4, and 5-10-4.

[0313] In certain embodiments, the compound comprises a modified antisense oligonucleotide consisting of 17 to 19 linked nucleosides and fully complementary to a single nucleotide polymorphism site, wherein the modified antisense oligonucleotide comprises a wing-gap-wing motif, wherein position 5 or 6 of the gap segment aligns with the single nucleotide polymorphism; and positions 2 and 3 of the 5' and 3' wing segments comprise a 4'-CH(CH_3)-O-2' bridge. In certain embodiments, the wing-gap-wing motif is any one of the group consisting of 2-9-6, 3-9-3, 3-9-5, 4-9-5, 4-11-4, and 5-10-4.

[0314] A compound comprising a modified antisense oligonucleotide consisting of 17 to 19 linked nucleosides and fully complementary to a single nucleotide polymorphism site, wherein the modified antisense oligonucleotide comprises a wing-gap-wing motif, wherein position 8, 9, or 10 of the modified oligonucleotide aligns with the single nucleotide polymorphism; and positions 2, 3, 13, and 14 of the antisense antisense oligonucleotide comprise a 4'-CH(CH_3)-O-2' bridge.. In certain embodiments, the wing-gap-wing motif is any one of the group consisting of 2-9-6, 3-9-3, 3-9-5, 4-9-5, 4-11-4, and 5-10-4.

[0315] A compound comprising a modified antisense oligonucleotide consisting of 17 to 19 linked nucleosides and fully complementary to a single nucleotide polymorphism site, wherein the modified antisense oligonucleotide comprises a wing-gap-wing motif, wherein position 5,or 6 of the gap segment aligns with the single nucleotide polymorphism; and positions 2, 3, 13, and 14 of the antisense oligonucleotide comprise a 4'-CH(CH_3)-O-2' bridge. In certain embodiments, the wing-gap-wing motif is any one of the group consisting of 2-9-6, 3-9-3, 3-9-5, 4-9-5, 4-11-4, and 5-10-4.

[0316] In a certain embodiment, the antisense oligonucleotide is 11 to 20 linked nucleosides in length and has,

independently, 2 to 5 linked nucleosides in the 5' and 3' wings and 7 to 11 linked nucleosides in the gap. The SNP is complementary to position 8-10 of the antisense oligonucleotide (counting from the 5' terminus of the antisense oligonucleotide) or position 4, 5 or 6 counting from the 5' terminus of the gap segment.

[0317] In a certain embodiment, the antisense oligonucleotide is 15 to 19 linked nucleosides in length and has, independently, 2 to 5 linked nucleosides in the 5' and 3' wings and 7 to 11 linked nucleosides in the gap. The SNP is complementary to position 8-10 of the antisense oligonucleotide (counting from the 5' terminus of the antisense oligonucleotide) or position 4, 5 or 6, counting from the 5' terminus of the gap segment.

[0318] In a certain embodiment, the antisense oligonucleotide is 17 linked nucleosides in length and has, independently, 2 to 5 linked nucleosides in the 5' and 3' wing segments and 9 to 11 linked nucleosides in the gap segment. The SNP is complementary to position 8, 9, or 10 of the antisense oligonucleotide (counting from the 5' terminus of the antisense oligonucleotide) or position 5 or 6(counting from the 5' terminus of the gap segment).

[0319] In a certain embodiment, the antisense oligonucleotide is 18 linked nucleosides in length and has, independently, 2 to 5 linked nucleosides in the 5' and 3' wing segments and 9 to 11 linked nucleosides in the gap segment. The SNP is complementary to position 8, 9, or 10 of the antisense oligonucleotide (counting from the 5' terminus of the antisense oligonucleotide) or position 5 or 6 (counting from the 5' terminus of the gap segment).

[0320] In a certain embodiment, the antisense oligonucleotide is 19 linked nucleosides in length and has, independently, 2 to 5 linked nucleosides in the 5' and 3' wing segments and 9 to 11 linked nucleosides in the gap segment. The SNP is complementary to position 8, 9, or 10 of the antisense oligonucleotide (counting from the 5' terminus of the antisense oligonucleotide) or position 5 or 6(counting from the 5' terminus of the gap segment).

[0321] In certain embodiments, the oligonucleotides of the invention are for use in methods of treating an individual, the method comprising administering one or more pharmaceutical compositions described herein. In certain embodiments, the individual has an allelic variant associated with a disease or disorder. The pharmaceutical compositions provided herein preferentially target a SNP. In certain embodiments, the SNP is a differentiating polymorphism.

[0322] Methods have been described for determining whether a SNP is specific to a disease associated allele and more specifically whether a SNP variant of an allele of a heterozygous patient is on the same allele as a disease-causing mutation that is at a remote region of the gene's mRNA (WO 2008/147930 and WO 2008/143774).

[0323] Diseases associated with SNPs have been described for certain genes. In certain embodiments, the gene and associated disease are any of the following: APP gene encoding amyloid precursor protein involved in Alzheimer's disease (Gene, 371: 68, 2006); the PrP gene encoding prion protein involved in Creutzfeldt-Jakob disease and in fatal familial insomnia (Nat. Med. 1997, 3: 1009); GFAP gene encoding glial fibrillary acidic protein involved in Alexander disease (J. Neurosci. 2006, 26:111623); alpha-synuclein gene encoding alpha-synuclein protein involved in Parkinson's disease (J. Clin. Invest. 2003, 111: 145); SOD-1 gene encoding the SOD-1 protein involved in amyotrophic lateral sclerosis (Science 1998, 281: 1851); atrophin-1 gene encoding atrophin-1 protein involved in dentato-rubral and pallido-luysian atrophy (DRPA) (Trends Mol. Med. 2001, 7: 479); SCA1 gene encoding ataxin-1 protein involved in spino-cerebellar ataxia-1 (SCA1) (Protein Sci. 2003, 12: 953); PLP gene encoding proteolipid protein involved in Pelizaeus-Merzbacher disease (NeuroMol Med. 2007, 4: 73); DYT1 gene encoding torsinA protein involved in Torsion dystonia (Brain Res. 2000, 877: 379); and alpha-B crystalline gene encoding alpha-B crystalline protein involved in protein aggregation diseases, including cardiomyopathy (Cell 2007, 130: 427); alpha 1-antitrypsin gene encoding alpha1-antitrypsin protein involved in chronic obstructive pulmonary disease (COPD), liver disease and hepatocellular carcinoma (New Engl J Med. 2002, 346: 45); Ltk gene encoding leukocyte tyrosine kinase protein involved in systemic lupus erythematosus (Hum. Mol. Gen. 2004, 13: 171); PCSK9 gene encoding PCSK9 protein involved in hypercholesterolemia (Hum Mutat. 2009, 30: 520); prolactin receptor gene encoding prolactin receptor protein involved in breast tumors (Proc. Natl. Assoc. Sci. 2008, 105: 4533); CCL5 gene encoding the chemokine CCL5 involved in COPD and asthma (Eur. Respir. J. 2008, 32: 327); PTPN22 gene encoding PTPN22 protein involved in Type 1 diabetes, Rheumatoid arthritis, Graves disease, and SLE (Proc. Natl. Assoc. Sci. 2007, 104: 19767); androgen receptor gene encoding the androgen receptor protein involved in spinal and bulbar muscular atrophy or Kennedy's disease (J Steroid Biochem. Mol. Biol. 2008, 108: 245); CHMP4B gene encoding chromatin modifying protein-4B involved in progressive childhood posterior subcapsular cataracts (Am. J. Hum. Genet 2007, 81: 596); FXR / NR1H4 gene

encoding Farnesoid X receptor protein involved in cholesterol gallstone disease, arthrosclerosis and diabetes (Mol. Endocrinol. 2007, 21: 1769); ABCA1 gene encoding ABCA1 protein involved in cardiovascular disease (Transl. Res. 2007, 149: 205); CaSR gene encoding the calcium sensing receptor protein involved in primary hypercalciuria (Kidney Int. 2007, 71: 1155); alpha-globin gene encoding alpha-globin protein involved in alpha-thallasemia (Science 2006, 312: 1215); httlpr gene encoding HTTLPR protein involved in obsessive compulsive disorder (Am. J. Hum. Genet. 2006, 78: 815); AVP gene encoding arginine vasopressin protein in stress-related disorders such as anxiety disorders and comorbid depression (CNS Neurol. Disord. Drug Targets 2006, 5: 167); GNAS gene encoding G proteins involved in congenital visual defects, hypertension, metabolic syndrome (Trends Pharmacol. Sci. 2006, 27: 260); APAF1 gene encoding APAF1 protein involved in a predisposition to major depression (Mol. Psychiatry 2006, 11: 76); TGF-beta1 gene encoding TGF-betal protein involved in breast cancer and prostate cancer (Cancer Epidemiol. Biomarkers Prev. 2004, 13: 759); AChR gene encoding acetylcholine receptor involved in congential myasthenic syndrome (Neurology 2004, 62: 1090); P2Y12 gene encoding adenosine diphosphate (ADP) receptor protein involved in risk of peripheral arterial disease (Circulation 2003, 108: 2971); LQT1 gene encoding LQT1 protein involved in atrial fibrillation (Cardiology 2003, 100: 109); RET protooncogene encoding RET protein involved in sporadic pheochromocytoma (J. Clin. Endocrinol. Metab. 2003, 88: 4911); filamin A gene encoding filamin A protein involved in various congenital malformations (Nat. Genet. 2003, 33: 487); TARDBP gene encoding TDP-43 protein involved in amyotrophic lateral sclerosis (Hum. Mol. Gene.t 2010, 19: 671); SCA3 gene encoding ataxin-3 protein involved in Machado-Joseph disease (PLoS One 2008, 3: e3341); SCA7 gene encoding ataxin-7 protein involved in spino-cerebellar ataxia-7 (PLoS One 2009, 4: e7232); *HTT* gene encoding huntingtin protein involved in Huntington's disease (Neurobiol Dis. 1996, 3:183); and the CA4 gene encoding carbonic anhydrase 4 protein, CRX gene encoding cone-rod homeobox transcription factor protein, FSCN2 gene encoding retinal fascin homolog 2 protein, IMPDH1 gene encoding inosine monophosphate dehydrogenase 1 protein, NR2E3 gene encoding nuclear receptor subfamily 2 group E3 protein, NRL gene encoding neural retina leucine zipper protein, PRPF3 (RP18) gene encoding pre-mRNA splicing factor 3 protein, PRPF8 (RP13) gene encoding pre-mRNA splicing factor 8 protein, PRPF31 (RP11) gene encoding pre-mRNA splicing factor 31 protein, RDS gene encoding peripherin 2 protein, ROM1 gene encoding rod outer membrane protein 1 protein, RHO gene encoding rhodopsin protein, RP1 gene encoding RP1 protein, RPGR gene encoding retinitis pigmentosa GTPase regulator protein, all of which are involved in Autosomal Dominant Retinitis Pigmentosa disease (Adv Exp Med Biol. 2008, 613:203)

[0324] In certain embodiments, the disease is a neurodegenerative disorder. In certain embodiments, the neurodegenerative disorder is Huntington's Disease. In certain embodiments, the targeted SNP is one or more of: rs6446723, rs3856973, rs2285086, rs363092, rs916171, rs6844859, rs7691627, rs4690073, rs2024115, rs11731237, rs362296, rs10015979, rs7659144, rs363096, rs362273, rs16843804, rs362271, rs362275, rs3121419, rs362272, rs3775061, rs34315806, rs363099, rs2298967, rs363088, rs363064, rs363102, rs2798235, rs363080, rs363072, rs363125, rs362303, rs362310, rs10488840, rs362325, rs35892913, rs363102, rs363096, rs11731237, rs10015979, rs363080, rs2798235, rs1936032, rs2276881, rs363070, rs35892913, rs12502045, rs6446723, rs7685686, rs3733217, rs6844859, rs362331, rs1143646, rs2285086, rs2298969, rs4690072, rs916171, rs3025849, rs7691627, rs4690073, rs3856973, rs363092, rs362310, rs362325, rs363144, rs362303, rs34315806, rs363099, rs363081, rs3775061, rs2024115, rs10488840, rs363125, rs362296, rs2298967, rs363088, rs363064, rs362275, rs3121419, rs3025849, rs363070, rs362273, rs362272, rs362306, rs362271, rs363072, rs16843804, rs7659144, rs363120, and rs12502045. In certain embodiments the compounds areISIS460065, ISIS 459978, ISIS 460028, ISIS 460209, ISIS 460208, and ISIS 460206.

Therapeutically Effective Dosages

[0325] In certain embodiments, administration of a therapeutically effective amount of an antisense compound targeted to the mutant huntingtin allele is accompanied by monitoring of expression of a gene product in an individual, to determine an individual's response to administration of the antisense compound. In certain embodiments, the gene product is huntingtin mRNA or protein. An individual's response to administration of the antisense compound is used by a physician to determine the amount and duration of therapeutic intervention.

[0326] In certain embodiments, administration of an antisense compound targeted to a mutant nucleic acid results in reduction of mRNA or protein expression by at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99%, or a range defined by any two of these values. In certain embodiments, the mutant nucleic acid is huntingtin

nucleic acid, the mRNA is huntingtin mRNA, and the protein is huntingtin protein.

[0327] In certain embodiments, pharmaceutical compositions comprising an antisense compound targeted to a mutant allele are used for the preparation of a medicament for treating a patient suffering or susceptible to any of Huntington's Disease, Alzheimer's Disease, Crutzfeldt-Jakob Disease, Fatal Familial Insomnia, Huntington's Disease, Alexander Disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis (ALS), Dentato-Rubral and Pallido-Luysian Atrophy, Spino-Cerebellar Ataxia 1, Pelizaeus-Merzbacher Disease, Torsion Dystonia, Cardiomyopathy, Chronic Obstructive Pulmonary Disease (COPD), liver disease and hepatocellular carcinoma, SLE, Hypercholesterolemia, breast tumors, Asthma, Type 1 Diabetes, Rheumatoid Arthritis, Graves Disease, Spinal and Bulbar Muscular Atrophy, Kennedy's Disease, progressive childhood posterior subcapsular cataracts, Cholesterol Gallstone Disease, Arthrosclerosis, cardiovascular disease, primary hypercalciuria, alpha-thallasemia, OCD, stress-related disorders (including anxiety disorders and comorbid depression), congential visual defects, hypertension, metabolic syndrome, major depression, breast cancer, prostate cancer, congenital myasthenic syndrome, peripheral arterial syndrome, atrial fibrillation, sporadic pheochromocytoma, congenital malformations, NJD, SCA7, and autosomal dominant retinitis pigmentosa adRP.

Certain Combination Therapies

[0328] In certain embodiments, one or more pharmaceutical compositions of the present invention are co-administered with one or more other pharmaceutical agents. In certain embodiments, such one or more other pharmaceutical agents are designed to treat the same disease, disorder, or condition as the one or more pharmaceutical compositions of the present invention. In certain embodiments, such one or more other pharmaceutical agents are designed to treat a different disease, disorder, or condition as the one or more pharmaceutical compositions of the present invention. In certain embodiments, such one or more other pharmaceutical agents are designed to treat an undesired side effect of one or more pharmaceutical compositions of the present invention. In certain embodiments, one or more pharmaceutical compositions of the present invention are co-administered with another pharmaceutical agent to treat an undesired effect of that other pharmaceutical agent. In certain embodiments, one or more pharmaceutical compositions of the present invention are co-administered with another pharmaceutical agent to produce a combinational effect. In certain embodiments, one or more pharmaceutical compositions of the present invention are co-administered with another pharmaceutical agent to produce a synergistic effect.

[0329] In certain embodiments, one or more pharmaceutical compositions of the present invention and one or more other pharmaceutical agents are administered at the same time. In certain embodiments, one or more pharmaceutical compositions of the present invention and one or more other pharmaceutical agents are administered at different times. In certain embodiments, one or more pharmaceutical compositions of the present invention and one or more other pharmaceutical agents are prepared together in a single formulation. In certain embodiments, one or more pharmaceutical compositions of the present invention and one or more other pharmaceutical agents are prepared separately.

EXAMPLES

Non-limiting disclosure

[0330] While certain compounds, compositions and methods described herein have been described with specificity in accordance with certain embodiments, the following examples serve only to illustrate the compounds described herein and are not intended to limit the same.

Example 1: Single nucleotide polymorphisms (SNPs) in the huntingtin (*HTT*) gene sequence

[0331] The *HTT* genomic sequence, designated herein as SEQ ID NO: 1 (NT_006081.18 truncated from nucleotides

1566000 to 1768000) was aligned with the *HTT* mRNA, designated herein as SEQ ID NO: 2 (NM_002111.6), using the EMBL-EBI sequence database (ClustalW2, http://www.ebi.ac.uk/Tools/clustalw2/index.html), and the output is presented in Figure 1. SNP positions (identified by Hayden et al, WO/2009/135322) associated with the *HTT* gene were mapped to the two sequences and have been demarcated in Figure 1 by their reference SNP ID number from the Entrez SNP database at the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp),. Table 2 furnishes further details on each SNP. The 'Reference SNP ID number' or 'RS number' is the number designated to each SNP from the Entrez SNP database at NCBI. 'SNP position' refers to the nucleotide position of the SNP on SEQ ID NO: 1. 'Polymorphism' indicates the nucleotide variants at that SNP position. 'Major allele' indicates the nucleotide associated with the major allele, or the nucleotide present in a statistically significant proportion of individuals in the human population. 'Minor allele' indicates the nucleotide associated with the minor allele, or the nucleotide present in a relatively small proportion of individuals in the human population.

Table 2

Single Nuclear Polymorphisms (SNPs) and their positions on SEQ ID NO: 1				
RS No.	**SNP position**	**Polymorphism**	**Major allele**	**Minor allele**
rs2857936	1963	C/T	C	T
rs12506200	3707	A/G	G	A
rs762855	14449	A/G	G	A
rs3856973	19826	G/A	G	A
rs2285086	28912	G/A	A	G
rs7659144	37974	C/G	C	G
rs16843804	44043	C/T	C	T
rs2024115	44221	G/A	A	G
rs10015979	49095	A/G	A	G
rs7691627	51063	A/G	G	A
rs2798235	54485	G/A	G	A
rs4690072	62160	G/T	T	G
rs6446723	66466	C/T	T	C
rs363081	73280	G/A	G	A
rs363080	73564	T/C	C	T
rs363075	77327	G/A	G	A
rs363064	81063	T/C	C	T
rs3025849	83420	A/G	A	G
rs6855981	87929	A/G	G	A
rs363102	88669	G/A	A	G
rs11731237	91466	C/T	C	T
rs4690073	99803	A/G	G	A
rs363144	100948	T/G	T	G
rs3025838	101099	C/T	C	T
rs34315806	101687	A/G	G	A
rs363099	101709	T/C	C	T
rs363096	119674	T/C	T	C
rs2298967	125400	C/T	T	C
rs2298969	125897	A/G	G	A
rs6844859	130139	C/T	T	C
rs3 63 092	135682	C/A	C	A
rs7685686	146795	A/G	A	G

Single Nuclear Polymorphisms (SNPs) and their positions on SEQ ID NO: 1				
RS No.	**SNP position**	**Polymorphism**	**Major allele**	**Minor allele**
rs363088	149983	A/T	A	T
rs362331	155488	C/T	T	C
rs916171	156468	G/C	c	G
rs362322	161018	A/G	A	G
rs362275	164255	T/C	C	T
rs362273	167080	A/G	A	G
rs2276881	171314	G/A	G	A
rs3121419	171910	T/C	C	T
rs362272	174633	G/A	G	A
rs362271	175171	G/A	G	A
rs3775061	178407	C/T	C	T
rs362310	179429	A/G	G	A
rs362307	181498	T/C	C	T
rs362306	181753	G/A	G	A
rs362303	181960	T/C	C	T
rs362296	186660	C/A	C	A
rs1006798	198026	A/G	A	G

Example 2: Design of antisense oligonucleotides targeting huntingtin gene SNPs and inhibition of *HTT* mRNA in Coriell fibroblast cell lines (GM04281, GM02171, and GM02173B)

[0332] Antisense oligonucleotides targeting nucleotides overlapping SNP positions presented in Table 1 were designed and tested for potency in three huntingtin patient-derived Coriell fibroblast cell lines, GM04281, GM02171, and GM02173B (from the Coriell Institute for Medical Research). Cultured GM04281 cells or GM02171 cells or GM02173B cells at a density of 20,000 cells per well were transfected using electroporation with 10,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and *HTT* mRNA levels were measured by quantitative real time PCR using primer probe set RTS2617 (forward sequence CTCCGTCCGGTAGACATGCT, designated herein as SEQ ID NO: 3; reverse sequence GGAAATCAGAACCCTCAAAATGG, designated herein as SEQ ID NO: 4; probe sequence TGAGCACTGTTCAACTGTGGATATCGGGAX, designated herein as SEQ ID NO: 5). *HTT* mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented as percent inhibition of *HTT* mRNA, relative to untreated control cells.

[0333] ISIS 387916 (TCTCTATTGCACATTCCAAG, 5-10-5 MOE (SEQ ID NO: 6)) and ISIS 388816 (GCCGTAGCCTGGGACCCGCC, 5-10-5 MOE (SEQ ID NO: 7)) were included in each study as benchmark oligonucleotides against which the potency of the antisense oligonucleotides targeting nucleotides overlapping each SNP position could be compared.

[0334] The chimeric antisense oligonucleotides in Tables 3 and 4 were designed as 5-9-5 MOE gapmers. The gapmers are 19 nucleotides in length, wherein the central gap segment is comprised of nine 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising five nucleotides each. Each nucleotide in the 5' wing segment and each nucleotide in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine nucleobases throughout each gapmer are 5-methylcytosines.

[0335] The oligonucleotides are further described in Table 3. The percent inhibition of *HTT* mRNA by the antisense oligonucleotides in each cell line is shown in Table 4. 'Target allele' indicates whether the gapmer is targeted to the

major or the minor allele at the SNP position. The number in parentheses indicates the nucleotide position in the gapmer opposite to the SNP position, starting from the 5'-terminus of the oligonucleotide. 'Start site' indicates the 5'-most nucleotide to which the gapmer is targeted. "Stop site" indicates the 3'-most nucleotide to which the gapmer is targeted. Each gapmer listed in Tables 3 and 4 is targeted to human *HTT* pre-mRNA, which is SEQ ID NO: 1.

Table 3

Chimeric oligonucleotides targeting SNP positions on the *HTT* gene						
ISIS No	SNP RS No.	Target allele	Sequence	Start Site	Stop Site	SEQ ID NO
387916	n/a	n/a	TCTCTATTGCACATTCCAAG	145466	145485	6
388816	n/a	n/a	GCCGTAGCCTGGGACCCGCC	16501	16520	7
435330	rs3856973	Major (8)	TAACACTCGATTAACCCTG	19815	19833	8
435348	rs3856973	Minor (8)	TAACACTTGATTAACCCTG	19815	19833	9
435294	rs3856973	Major (10)	GTTAACACTCGATTAACCC	19817	19835	10
435312	rs3856973	Minor (10)	GTTAACACTTGATTAACCC	19817	19835	11
435864	rs2285086	Major (10)	GCTAGTTCATCCCAGTGAG	28903	28921	12
435889	rs2285086	Minor (10)	GCTAGTTCACCCCAGTGAG	28903	28921	13
435878	rs7659144	Major (10)	TGGAAATGGGTTTTTCCAC	37965	37983	14
435903	rs7659144	Minor (10)	TGGAAATGGCTTTTTCCAC	37965	37983	15
435863	rs16843804	Major (10)	TTTAACCGTGGCATGGGCA	44034	44052	16
435888	rs16843804	Minor (10)	TTTAACCGTAGCATGGGCA	44034	44052	17
435331	rs2024115	Major (8)	TTCAAGCTAGTAACGATGC	44210	44228	18
435349	rs2024115	Minor (8)	TTCAAGCCAGTAACGATGC	44210	44228	19
435295	rs2024115	Major (10)	ACTTCAAGCTAGTAACGAT	44212	44230	20
435313	rs2024115	Minor (10)	ACTTCAAGCCAGTAACGAT	44212	44230	21
435862	rs10015979	Major (10)	GCAGCTAGGTTAAAGAGTC	49086	49104	22
435887	rs10015979	Minor (10)	GCAGCTAGGCTAAAGAGTC	49086	49104	23
435880	rs7691627	Major (10)	AATAAGAAACACAATCAAA	51054	51072	24
435905	rs7691627	Minor (10)	AATAAGAAATACAATCAAA	51054	51072	25
435885	rs2798235	Major (10)	CAGAGGAGGCATACTGTAT	54476	54494	26
435910	rs2798235	Minor (10)	CAGAGGAGGTATACTGTAT	54476	54494	27
435874	rs4690072	Major (10)	CACAGTGCTACCCAACCTT	62151	62169	28
435899	rs4690072	Minor (10)	CACAGTGCTCCCCAACCTT	62151	62169	29
435875	rs6446723	Major (10)	TAATTTTCTAGACTTTATG	66457	66475	30
435900	rs6446723	Minor (10)	TAATTTTCTGGACTTTATG	66457	66475	31
435332	rs363081	Major (8)	GCTACAACGCAGGTCAAAT	73269	73287	32
435350	rs363081	Minor (8)	GCTACAATGCAGGTCAAAT	73269	73287	33
435296	rs363081	Major (10)	GAGCTACAACGCAGGTCAA	73271	73289	34
435314	rs363081	Minor (10)	GAGCTACAATGCAGGTCAA	73271	73289	35
435886	rs363080	Major (10)	AGAGAGAACGAGAAGGCTC	73555	73573	36
435911	rs363080	Minor (10)	AGAGAGAACAAGAAGGCTC	73555	73573	37
435914	rs363075	Major (6)	AGCCCCTCTGTGTAAGTTT	77314	77332	38
435926	rs363075	Minor (6)	AGCCCTTCTGTGTAAGTTT	77314	77332	39
435916	rs363075	Major (7)	GAGCCCCTCTGTGTAAGTT	77315	77333	40
435928	rs363075	Minor (7)	GAGCCCTTCTGTGTAAGTT	77315	77333	41
435333	rs363075	Major (8)	TGAGCCCCTCTGTGTAAGT	77316	77334	42
435351	rs363075	Minor (8)	TGAGCCCTTCTGTGTAAGT	77316	77334	43
435918	rs363075	Major (9)	ATGAGCCCCTCTGTGTAAG	77317	77335	44

Chimeric oligonucleotides targeting SNP positions on the *HTT* gene						
ISIS No	SNP RS No.	Target allele	Sequence	Start Site	Stop Site	SEQ ID NO
435930	rs363075	Minor (9)	ATGAGCCCTTCTGTGTAAG	77317	77335	45
435297	rs363075	Major (10)	GATGAGCCCCTCTGTGTAA	77318	77336	46
435315	rs363075	Minor (10)	GATGAGCCCTTCTGTGTAA	77318	77336	47
435920	rs363075	Major (11)	TGATGAGCCCCTCTGTGTA	77319	77337	48
435932	rs363075	Minor (11)	TGATGAGCCCTTCTGTGTA	77319	77337	49
435366	rs363075	Major (12)	ATGATGAGCCCCTCTGTGT	77320	77338	50
435924	rs363075	Minor (12)	ATGATGAGCCCTTCTGTGT	77320	77338	51
435922	rs363075	Major (14)	TAATGATGAGCCCCTCTGT	77322	77340	52
435934	rs363075	Minor (14)	TAATGATGAGCCCTTCTGT	77322	77340	53
435334	rs363064	Major (8)	AGAATACGGGTAACATTTT	81052	81070	54
435352	rs363064	Minor (8)	AGAATACAGGTAACATTTT	81052	81070	55
435298	rs363064	Major (10)	GGAGAATACGGGTAACATT	81054	81072	56
435316	rs363064	Minor (10)	GGAGAATACAGGTAACATT	81054	81072	57
435335	rs3025849	Major (8)	TTAGTAATCAATTTTAATG	83409	83427	58
435353	rs3025849	Minor (8)	TTAGTAACCAATTTTAATG	83409	83427	59
435299	rs3025849	Major (10)	AGTTAGTAATCAATTTTAA	83411	83429	60
435317	rs3025849	Minor (10)	AGTTAGTAACCAATTTTAA	83411	83429	61
435877	rs6855981	Major (10)	GAAGGAATGCTTTTACTAG	87920	87938	62
435902	rs6855981	Minor (10)	GAAGGAATGTTTTTACTAG	87920	87938	63
435336	rs363102	Major (8)	CTAAAACTAACTTGAGAAT	88658	88676	64
435354	rs363102	Minor (8)	CTAAAACCAACTTGAGAAT	88658	88676	65
435300	rs363102	Major (10)	ATCTAAAACTAACTTGAGA	88660	88678	66
435318	rs363102	Minor (10)	ATCTAAAACCAACTTGAGA	88660	88678	67
435884	rs11731237	Major (10)	GGTGGGCAGGAAGGACTGA	91457	91475	68
435909	rs11731237	Minor (10)	GGTGGGCAGAAAGGACTGA	91457	91475	69
435337	rs4690073	Major (8)	CCTAAATCAATCTACAAGT	99792	99810	70
435355	rs4690073	Minor (8)	CCTAAATTAATCTACAAGT	99792	99810	71
435301	rs4690073	Major (10)	TCCCTAAATCAATCTACAA	99794	99812	72
435319	rs4690073	Minor (10)	TCCCTAAATTAATCTACAA	99794	99812	73
435883	rs363144	Major (10)	GAAAATGTGAGTGGATCTA	100939	100957	74
435908	rs363144	Minor (10)	GAAAATGTGCGTGGATCTA	100939	100957	75
435338	rs3025838	Major (8)	GTAAGGCGAGACTGACTAG	101088	101106	76
435356	rs3025838	Minor (8)	GTAAGGCAAGACTGACTAG	101088	101106	77
435302	rs3025838	Major (10)	AGGTAAGGCGAGACTGACT	101090	101108	78
435320	rs3025838	Minor (10)	AGGTAAGGCAAGACTGACT	101090	101108	79
435339	rs363099	Major (8)	CTGAGCGGAGAAACCCTCC	101698	101716	80
435357	rs363099	Minor (8)	CTGAGCGAAGAAACCCTCC	101698	101716	81
435303	rs363099	Major (10)	GGCTGAGCGGAGAAACCCT	101700	101718	82
435321	rs363099	Minor (10)	GGCTGAGCGAAGAAACCCT	101700	101718	83
435367	rs363099	Major(12)	AAGGCTGAGCGGAGAAACC	101702	101720	84
435340	rs363096	Major (8)	TTCCCTAAAAACAAAAACA	119663	119681	85
435358	rs363096	Minor (8)	TTCCCTAGAAACAAAAACA	119663	119681	86
435304	rs363096	Major (10)	GATTCCCTAAAAACAAAAA	119665	119683	87

Chimeric oligonucleotides targeting SNP positions on the *HTT* gene						
ISIS No	SNP RS No.	Target allele	Sequence	Start Site	Stop Site	SEQ ID NO
435322	rs363096	Minor (10)	GATTCCCTAGAAACAAAAA	119665	119683	88
435341	rs2298967	Major (8)	CTTTTCTATTGTCTGTCCC	125389	125407	89
435359	rs2298967	Minor (8)	CTTTTCTGTTGTCTGTCCC	125389	125407	90
435305	rs2298967	Major (10)	TGCTTTTCTATTGTCTGTC	125391	125409	91
435323	rs2298967	Minor (10)	TGCTTTTCTGTTGTCTGTC	125391	125409	92
435865	rs2298969	Major (10)	AAGGGATGCCGACTTGGGC	125888	125906	93
435890	rs2298969	Minor (10)	AAGGGATGCTGACTTGGGC	125888	125906	94
435876	rs6844859	Major (10)	ACCTTCCTCACTGAGGATG	130130	130148	95
435901	rs6844859	Minor (10)	ACCTTCCTCGCTGAGGATG	130130	130148	96
435872	rs363092	Major (10)	CAAACCACTGTGGGATGAA	135673	135691	97
435897	rs363092	Minor (10)	CAAACCACTTTGGGATGAA	135673	135691	98
435879	rs7685686	Major (10)	AATAAATTGTCATCACCAG	146786	146804	99
435904	rs7685686	Minor (10)	AATAAATTGCCATCACCAG	146786	146804	100
435871	rs363088	Major (10)	TCACAGCTATCTTCTCATC	149974	149992	101
435896	rs363088	Minor (10)	TCACAGCTAACTTCTCATC	149974	149992	102
435870	rs362331	Major (10)	GCACACAGTAGATGAGGGA	155479	155497	103
435895	rs362331	Minor (10)	GCACACAGTGGATGAGGGA	155479	155497	104
435881	rs916171	Major (10)	CAGAACAAAGAGAAGAATT	156459	156477	105
435906	rs916171	Minor (10)	CAGAACAAACAGAAGAATT	156459	156477	106
435342	rs362322	Major (8)	GCTTACATGCCTTCAGTGA	161007	161025	107
435360	rs362322	Minor (8)	GCTTACACGCCTTCAGTGA	161007	161025	108
435306	rs362322	Major (10)	CAGCTTACATGCCTTCAGT	161009	161027	109
435324	rs362322	Minor (10)	CAGCTTACACGCCTTCAGT	161009	161027	110
435868	rs362275	Major (10)	AAGAAGCCTGATAAAATCT	164246	164264	111
435893	rs362275	Minor (10)	AAGAAGCCTAATAAAATCT	164246	164264	112
435343	rs2276881	Major (8)	CATACATCAGCTCAAACTG	171303	171321	113
435361	rs2276881	Minor (8)	CATACATTAGCTCAAACTG	171303	171321	114
435307	rs2276881	Major (10)	CACATACATCAGCTCAAAC	171305	171323	115
435325	rs2276881	Minor (10)	CACATACATTAGCTCAAAC	171305	171323	116
435368	rs2276881	Major (12)	GTCACATACATCAGCTCAA	171307	171325	117
435866	rs3121419	Major (10)	GAGACTATAGCACCCAGAT	171901	171919	118
435891	rs3121419	Minor (10)	GAGACTATAACACCCAGAT	171901	171919	119
435344	rs362272	Major (8)	TAGAGGACGCCGTGCAGGG	174622	174640	120
435362	rs362272	Minor (8)	TAGAGGATGCCGTGCAGGG	174622	174640	121
435308	rs362272	Major (10)	CATAGAGGACGCCGTGCAG	174624	174642	122
435326	rs362272	Minor (10)	CATAGAGGATGCCGTGCAG	174624	174642	123
435369	rs362272	Major (12)	CACATAGAGGACGCCGTGC	174626	174644	124
435867	rs362271	Major (10)	ACGTGTGTACAGAACCTGC	175162	175180	125
435892	rs362271	Minor (10)	ACGTGTGTATAGAACCTGC	175162	175180	126
435873	rs3775061	Major (10)	TGTTCAGAATGCCTCATCT	178398	178416	127
435898	rs3775061	Minor (10)	TGTTCAGAACGCCTCATCT	178398	178416	128
435345	rs362310	Major (8)	AAACGGCGCAGCGGGAAGG	179418	179436	129
435363	rs362310	Minor (8)	AAACGGCACAGCGGGAAGG	179418	179436	130

Chimeric oligonucleotides targeting SNP positions on the *HTT* gene						
ISIS No	SNP RS No.	Target allele	Sequence	Start Site	Stop Site	SEQ ID NO
435309	rs362310	Major (10)	AGAAACGGCGCAGCGGGAA	179420	179438	131
435327	rs362310	Minor (10)	AGAAACGGCACAGCGGGAA	179420	179438	132
435915	rs362307	Major (6)	AGGGCGCAGACTTCCAAAG	181485	181503	133
435927	rs362307	Minor (6)	AGGGCACAGACTTCCAAAG	181485	181503	134
435917	rs362307	Major (7)	AAGGGCGCAGACTTCCAAA	181486	181504	135
435929	rs362307	Minor (7)	AAGGGCACAGACTTCCAAA	181486	181504	136
435346	rs362307	Major (8)	CAAGGGCGCAGACTTCCAA	181487	181505	137
435364	rs362307	Minor (8)	CAAGGGCACAGACTTCCAA	181487	181505	138
435919	rs362307	Major (9)	ACAAGGGCGCAGACTTCCA	181488	181506	139
435931	rs362307	Minor (9)	ACAAGGGCACAGACTTCCA	181488	181506	140
435310	rs362307	Major (10)	CACAAGGGCGCAGACTTCC	181489	181507	141
435328	rs362307	Minor (10)	CACAAGGGCACAGACTTCC	181489	181507	142
435921	rs362307	Major (11)	GCACAAGGGCGCAGACTTC	181490	181508	143
435933	rs362307	Minor (11)	GCACAAGGGCACAGACTTC	181490	181508	144
435370	rs362307	Major (12)	GGCACAAGGGCGCAGACTT	181491	181509	145
435925	rs362307	Minor (12)	GGCACAAGGGCACAGACTT	181491	181509	146
435923	rs362307	Major (14)	AGGGCACAAGGGCGCAGAC	181493	181511	147
435935	rs362307	Minor (14)	AGGGCACAAGGGCACAGAC	181493	181511	148
435869	rs362306	Major (10)	GAGCAGCTGCAACCTGGCA	181744	181762	149
435894	rs362306	Minor (10)	GAGCAGCTGTAACCTGGCA	181744	181762	150
435347	rs362303	Major (8)	TGGTGCCGGGTGTCTAGCA	181949	181967	151
435365	rs362303	Minor (8)	TGGTGCCAGGTGTCTAGCA	181949	181967	152
435311	rs362303	Major (10)	AATGGTGCCGGGTGTCTAG	181951	181969	153
435329	rs362303	Minor (10)	AATGGTGCCAGGTGTCTAG	181951	181969	154
435882	rs362296	Major (10)	GGGGACAGGGTGTGCTCTC	186651	186669	155
435907	rs362296	Minor (10)	GGGGACAGGTTGTGCTCTC	186651	186669	156

Table 4

Comparison of inhibition of *HTT* mRNA levels by ISIS 387916 and ISIS 388816 with that by chimeric oligonucleotides targeting SNP positions on the *HTT* gene (SEQ ED NO: 1)						
ISIS No	SNP RS No.	Target allele	% inhibition			SEQ ID NO
			GM04281	GM02171	GM02173B	
387916	n/a	n/a	96	96	98	6
388816	n/a	n/a	76	88	85	7
435330	rs3856973	Major (8)	64	51	36	8
435348	rs3856973	Minor (8)	50	88	80	9
435294	rs3856973	Major (10)	54	46	54	10
435312	rs3856973	Minor (10)	20	82	58	11
435864	rs2285086	Major (10)	54	28	26	12
435889	rs2285086	Minor (10)	17	43	41	13
435878	rs7659144	Major (10)	43	32	39	14
435903	rs7659144	Minor (10)	16	37	29	15
435863	rs16843804	Major (10)	63	78	81	16
435888	rs16843804	Minor (10)	58	75	77	17

Comparison of inhibition of *HTT* mRNA levels by ISIS 387916 and ISIS 388816 with that by chimeric oligonucleotides targeting SNP positions on the *HTT* gene (SEQ ED NO: 1)						
ISIS No	SNP RS No.	Target allele	% inhibition			SEQ ID NO
			GM04281	GM02171	GM02173B	
435331	rs2024115	Major (8)	56	27	56	18
435349	rs2024115	Minor (8)	26	91	66	19
435295	rs2024115	Major (10)	53	57	62	20
435313	rs2024115	Minor (10)	25	87	53	21
435862	rs10015979	Major (10)	8	51	40	22
435887	rs10015979	Minor (10)	40	22	28	23
435880	rs7691627	Major (10)	43	17	21	24
435905	rs7691627	Minor (10)	13	27	15	25
435885	rs2798235	Major (10)	38	39	30	26
435910	rs2798235	Minor (10)	17	30	16	27
435874	rs4690072	Major (10)	61	34	48	28
435899	rs4690072	Minor (10)	50	41	45	29
435875	rs6446723	Major (10)	28	13	35	30
435900	rs6446723	Minor (10)	24	56	37	31
435332	rs363081	Major (8)	76	95	88	32
435350	rs363081	Minor (8)	27	61	43	33
435296	rs363081	Major (10)	59	77	66	34
435314	rs363081	Minor (10)	38	66	40	35
435886	rs363080	Major (10)	74	72	79	36
435911	rs363080	Minor (10)	57	58	54	37
435914	rs363075	Major (6)	95	92	95	38
435926	rs363075	Minor (6)	88	81	79	39
435916	rs363075	Major (7)	90	92	94	40
435928	rs363075	Minor (7)	83	79	85	41
435333	rs363075	Major (8)	86	97	91	42
435351	rs363075	Minor (8)	59	80	58	43
435918	rs363075	Major (9)	83	90	91	44
435930	rs363075	Minor (9)	29	49	49	45
435297	rs363075	Major (10)	74	84	83	46
435315	rs363075	Minor (10)	47	63	45	47
435920	rs363075	Major (11)	78	66	83	48
435932	rs363075	Minor (11)	39	20	19	49
435366	rs363075	Major (12)	80	91	85	50
435924	rs363075	Minor (12)	37	49	58	51
435922	rs363075	Major (14)	80	90	91	52
435934	rs363075	Minor (14)	63	70	80	53
435334	rs363064	Major (8)	50	59	44	54
435352	rs363064	Minor (8)	12	37	48	55
435298	rs363064	Major (10)	81	92	87	56
435316	rs363064	Minor (10)	69	90	80	57
435335	rs3025849	Major (8)	0	40	37	58

Comparison of inhibition of *HTT* mRNA levels by ISIS 387916 and ISIS 388816 with that by chimeric oligonucleotides targeting SNP positions on the *HTT* gene (SEQ ED NO: 1)						
ISIS No	SNP RS No.	Target allele	% inhibition			SEQ ID NO
			GM04281	GM02171	GM02173B	
435353	rs3025849	Minor (8)	0	29	18	59
435299	rs3025849	Major (10)	0	34	67	60
435317	rs3025849	Minor (10)	0	38	34	61
435877	rs6855981	Major (10)	31	59	58	62
435902	rs6855981	Minor (10)	0	43	27	63
435336	rs363102	Major (8)	0	21	19	64
435354	rs363102	Minor (8)	0	36	33	65
435300	rs363102	Major (10)	0	34	24	66
435318	rs363102	Minor (10)	0	30	20	67
435884	rs11731237	Major (10)	7	46	51	68
435909	rs11731237	Minor (10)	30	47	41	69
435337	rs4690073	Major (8)	12	0	12	70
435355	rs4690073	Minor (8)	0	26	33	71
435301	rs4690073	Major (10)	23	0	10	72
435319	rs4690073	Minor (10)	0	45	53	73
435883	rs363144	Major (10)	24	23	39	74
435908	rs363144	Minor (10)	27	20	22	75
435338	rs3025838	Major (8)	31	46	69	76
435356	rs3025838	Minor (8)	3	25	17	77
435302	rs3025838	Major (10)	39	73	67	78
435320	rs3025838	Minor (10)	21	49	32	79
435339	rs363099	Major (8)	84	87	76	80
435357	rs363099	Minor (8)	71	91	90	81
435303	rs363099	Major (10)	83	92	85	82
435321	rs363099	Minor (10)	84	95	89	83
435367	rs363099	Major (12)	76	82	72	84
435340	rs363096	Major (8)	0	47	52	85
435358	rs363096	Minor (8)	0	25	35	86
435304	rs363096	Major (10)	5	33	36	87
435322	rs363096	Minor (10)	2	30	32	88
435341	rs2298967	Major (8)	54	72	56	89
435359	rs2298967	Minor (8)	25	59	63	90
435305	rs2298967	Major (10)	66	80	78	91
435323	rs2298967	Minor (10)	36	79	66	92
435865	rs2298969	Major (10)	53	72	79	93
435890	rs2298969	Minor (10)	65	46	54	94
435876	rs6844859	Major (10)	70	67	77	95
435901	rs6844859	Minor (10)	39	83	80	96
435872	rs363092	Major (10)	46	41	54	97
435897	rs363092	Minor (10)	37	69	57	98
435879	rs7685686	Major (10)	83	31	70	99

Comparison of inhibition of *HTT* mRNA levels by ISIS 387916 and ISIS 388816 with that by chimeric oligonucleotides targeting SNP positions on the *HTT* gene (SEQ ED NO: 1)						
ISIS No	SNP RS No.	Target allele	% inhibition			SEQ ID NO
			GM04281	GM02171	GM02173B	
435904	rs7685686	Minor (10)	30	92	72	100
435871	rs363088	Major (10)	70	55	70	101
435896	rs363088	Minor (10)	66	74	80	102
435870	rs362331	Major (10)	88	74	88	103
435895	rs362331	Minor (10)	78	92	86	104
435881	rs916171	Major (10)	0	57	51	105
435906	rs916171	Minor (10)	14	26	17	106
435342	rs362322	Major (8)	47	74	67	107
435360	rs362322	Minor (8)	17	58	52	108
435306	rs362322	Major (10)	50	77	65	109
435324	rs362322	Minor (10)	42	61	64	110
435868	rs362275	Major (10)	54	35	43	111
435893	rs362275	Minor (10)	3	27	33	112
435343	rs2276881	Major (8)	59	76	65	113
435361	rs2276881	Minor (8)	58	44	20	114
435307	rs2276881	Major (10)	69	82	81	115
435325	rs2276881	Minor (10)	17	47	43	116
435368	rs2276881	Major (12)	84	96	92	117
435866	rs3121419	Major (10)	67	61	64	118
435891	rs3121419	Minor (10)	53	76	73	119
435344	rs362272	Major (8)	35	46	36	120
435362	rs362272	Minor (8)	34	68	57	121
435308	rs362272	Major (10)	26	30	35	122
435326	rs362272	Minor (10)	29	50	39	123
435369	rs362272	Major (12)	66	74	65	124
435867	rs362271	Major (10)	73	74	75	125
435892	rs362271	Minor (10)	52	74	79	126
435873	rs3775061	Major (10)	40	32	47	127
435898	rs3775061	Minor (10)	13	20	24	128
435345	rs362310	Major (8)	38	55	52	129
435363	rs362310	Minor (8)	45	67	60	130
435309	rs362310	Major (10)	33	44	56	131
435327	rs362310	Minor (10)	33	71	61	132
435915	rs362307	Major (6)	61	54	58	133
435927	rs362307	Minor (6)	31	35	44	134
435917	rs362307	Major (7)	67	76	66	135
435929	rs362307	Minor (7)	33	34	55	136
435346	rs362307	Major (8)	67	89	66	137
435364	rs362307	Minor (8)	46	72	66	138
435919	rs362307	Major (9)	84	79	70	139
435931	rs362307	Minor (9)	74	74	86	140

Comparison of inhibition of *HTT* mRNA levels by ISIS 387916 and ISIS 388816 with that by chimeric oligonucleotides targeting SNP positions on the *HTT* gene (SEQ ED NO: 1)						
ISIS No	SNP RS No.	Target allele	% inhibition			SEQ ID NO
			GM04281	GM02171	GM02173B	
435310	rs362307	Major (10)	74	81	71	141
435328	rs362307	Minor (10)	47	69	75	142
435921	rs362307	Major (11)	74	77	69	143
435933	rs362307	Minor (11)	38	47	74	144
435370	rs362307	Major (12)	64	74	38	145
435925	rs362307	Minor (12)	60	66	80	146
435923	rs362307	Major (14)	73	66	71	147
435935	rs362307	Minor (14)	68	75	87	148
435869	rs362306	Major (10)	82	77	81	149
435894	rs362306	Minor (10)	28	79	72	150
435347	rs362303	Major (8)	68	74	71	151
435365	rs362303	Minor (8)	69	83	76	152
435311	rs362303	Major (10)	46	56	72	153
435329	rs362303	Minor (10)	49	62	39	154
435882	rs362296	Major (10)	29	48	56	155
435907	rs362296	Minor (10)	42	56	52	156

Example 3: Dose-dependent antisense inhibition of human huntingtin mRNA levels in Coriell fibroblast cell lines

[0336] Gapmers from the study described in Example 2 were selected and tested at various doses in GM04281, GM02171, and GM02173B cell lines. Each cell line was plated at a density of 25,000 cells per well and transfected using electroporation with 750 nM, 1,500 nM, 3,000 nM, 6,000 nM, and 12,000 nM concentrations of antisense oligonucleotide, as specified in Table 5, 6, and 7. After a treatment period of approximately 16 hours, RNA was isolated from the cells and *HTT* mRNA levels were measured by quantitative real-time PCR. Human *HTT* primer probe set RTS2617 was used to measure mRNA levels. *HTT* mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented as percent inhibition of *HTT* mRNA, relative to untreated control cells. IC_{50} values are also provided in Tables 5, 6, and 7.

Table 5

Dose-dependent antisense inhibition of human *HTT* in GM04281 cells						
ISIS No.	750 nM	1,500 nM	3,000 nM	6,000 nM	12,000 nM	IC_{50} (μM)
387916	51	81	80	91	97	0.6
435330	24	49	50	73	85	2.5
435331	23	38	64	72	74	2.4
435868	3	17	7	29	63	6.7
435870	53	73	77	86	93	0.6
435871	28	51	52	78	89	1.7
435874	14	21	28	64	82	3.3
435879	42	57	57	81	91	1.1
435890	48	56	62	76	91	0.9
435929	10	0	5	12	48	13.8

Dose-dependent antisense inhibition of human *HTT* in GM04281 cells						
ISIS No.	750 nM	1,500 nM	3,000 nM	6,000 nM	12,000 nM	IC_{50} (μM)
435931	20	17	53	62	81	2.9
435933	0	7	24	43	49	10.7
435935	0	38	38	62	29	4.2

Table 6

Dose-dependent antisense inhibition of human *HTT* in GM02171 cells						
ISIS No.	750 nM	1,500 nM	3,000 nM	6,000 nM	12,000 nM	IC_{50} (μM)
387916	57	73	81	93	98	0.4
435330	27	37	0	44	63	4.4
435331	35	34	19	41	63	3.5
435868	21	21	39	24	12	>12.0
435870	50	53	57	70	79	0.9
435871	32	46	45	58	62	3.9
435874	1	0	4	11	6	>12.0
435879	32	14	17	45	38	>12.0
435890	34	33	40	51	62	5.4
435929	25	22	31	5	29	>12.0
435931	15	28	27	60	79	3.7
435933	13	36	21	43	48	12.2
435935	25	42	27	61	68	3.2

Table 7

Dose-dependent antisense inhibition of human *HTT* in GM02173B cells						
ISIS No.	750 nM	1,500 nM	3,000 nM	6,000 nM	12,000 nM	IC_{50} (μM)
387916	43	67	80	86	97	1.1
435330	22	21	0	52	62	5.3
435331	19	17	32	50	55	9.4
435868	17	25	41	13	26	>12.0
435870	24	57	70	78	75	1.8
435871	8	30	42	50	48	5.0
435874	31	35	28	35	42	>12.0
435879	39	44	42	60	64	2.5
435890	38	36	50	65	73	3.1
435929	19	17	19	42	35	7.7
435931	40	19	31	48	71	5.8
435933	35	24	47	52	59	4.4
435935	25	23	40	73	77	3.7

Example 4: Dose-dependent antisense inhibition of human huntingtin mRNA levels in Coriell fibroblast cell lines

[0337] Gapmers from the study described in Example 2 were selected and tested at various doses in GM04281, GM02171, and GM02173B cell lines. Each cell line was plated at a density of 25,000 cells per well and transfected using electroporation with 750 nM, 1,500 nM, 3,000 nM, 6,000 nM, and 12,000 nM concentrations of antisense

oligonucleotide, as specified in Table 8, 9, and 10. After a treatment period of approximately 16 hours, RNA was isolated from the cells and *HTT* mRNA levels were measured by quantitative real-time PCR. Human *HTT* primer probe set RTS2617 was used to measure mRNA levels. *HTT* mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented as percent inhibition of *HTT mRNA* relative to untreated control cells. IC_{50} values are also provided in Tables 8, 9, and 10.

Table 8

Dose-dependent antisense inhibition of human *HTT* in GM04281 cells						
ISIS No.	750 nM	1,500 nM	3,000 nM	6,000 nM	12,000 nM	IC_{50} (μM)
387916	61	78	90	94	97	**<0.8**
435303	33	39	69	79	91	1.5
435328	0	12	16	51	75	5.3
435331	27	48	48	70	82	2.1
435339	46	37	61	73	89	2.3
435869	17	35	44	66	80	3.3
435870	44	60	64	84	84	1.1
435871	41	50	71	78	87	1.2
435874	24	36	35	65	73	3.1
435879	46	52	78	81	92	0.9
435890	41	53	63	80	86	1.3
435925	0	14	39	60	87	4.2
435926	20	28	67	81	89	2.0
435928	32	49	73	86	86	1.8
435931	22	24	40	59	90	3.8

Table 9

Dose-dependent antisense inhibition of human *HTT* in GM02171 cells						
ISIS No.	750 nM	1,500 nM	3,000 nM	6,000 nM	□□□□ nM	IC_{50} (μM)
387916	50	64	90	95	96	0.7
435303	14	32	68	79	85	2.8
435328	0	12	20	38	55	10.3
435331	0	13	5	30	36	>12.0
435339	30	40	58	63	49	2.5
435869	13	25	31	47	87	4.0
435870	18	31	44	66	74	3.5
435871	1	20	29	49	64	6.5
435874	3	6	12	17	31	>12.0
435879	0	2	12	35	44	>12.0
435890	15	16	30	48	72	5.8
435925	0	0	22	48	29	6.3
435926	25	28	58	74	85	2.3
435928	18	53	61	86	83	2.5
435931	0	4	25	46	68	6.7

Table 10

Dose-dependent antisense inhibition of human *HTT* in GM02173B cells						
ISIS No.	750 nM	1,500 nM	3,000 nM	6,000 nM	12,000 nM	IC_{50} (μM)
387916	27	65	84	81	96	1.9
435303	23	48	52	76	76	2.9

Dose-dependent antisense inhibition of human *HTT* in GM02173B cells						
ISIS No.	750 nM	1,500 nM	3,000 nM	6,000 nM	12,000 nM	IC_{50} (μM)
435328	8	14	19	34	50	15.7
435331	10	17	16	27	32	>12.0
435339	28	26	38	67	82	3.8
435869	12	24	37	45	79	4.2
435870	20	26	58	53	78	2.7
435871	15	16	32	45	71	6.0
435874	13	8	28	36	31	>12.0
435879	22	20	36	53	60	6.0
435890	21	28	34	54	71	4.3
435925	2	10	28	43	78	5.9
435926	7	25	37	73	79	3.5
435928	15	39	60	73	87	2.5
435931	13	13	32	61	62	6.7

Example 5: Antisense inhibition of human *HTT* in GM04281 cells

[0338] Additional antisense oligonucleotides were designed based on the gapmers selected from studies described in Example 4. These oligonucleotides were designed by creating gapmers shifted slightly upstream and downstream (i.e. "microwalk") of the original gapmers from Tables 8, 9, and 10. Antisense oligonucleotides were also created with uniform MOE, as well as with various motifs, 2-9-6 MOE, 3-9-3 MOE, 3-9-4 MOE, 3-9-5 MOE, 4-10-5 MOE, 4-11-4 MOE, 4-7-4 MOE, 4-9-4 MOE, 4-9-5 MOE, 5-10-4 MOE, 5-7-5 MOE, 5-8-6 MOE, 5-9-3 MOE, 5-9-5 MOE, 6-7-6 MOE, 6-9-2 MOE, and 6-8-5 MOE.

[0339] In addition, antisense oligonucleotides were designed targeting SNP RS Nos. rs2857936, rs12506200, rs762855, and rs1006798 (refer to Table 2). The oligonucleotides were designed targeting either the major allele or the minor allele, and with the SNP position opposite either position 8 or position 10 of the gapmer.

[0340] These gapmers were tested *in vitro.* Cultured GM04281 cells at a density of 25,000 cells per well were transfected using electroporation with 10,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and *HTT* mRNA levels were measured by quantitative real-time PCR. *HTT* mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented in Tables 11-19 as percent inhibition of *HTT* mRNA, relative to untreated control cells.

[0341] The gapmers, ISIS 435869, ISIS 435870, ISIS 435874, ISIS 435879, and ISIS 435890, from which some of the newly designed gapmers were derived are marked with an asterisk (*) in the table. ISIS 387916 was included in the study as a benchmark oligonucleotide against which the potency of the antisense oligonucleotides targeting nucleotides overlapping each SNP position could be compared.

[0342] The uniform MOE oligonucleotides are 15 nucleotides in length.

[0343] The 2-9-6 gapmers are 17 nucleotides in length, wherein the central gap segment is comprised of nine 2'-deoxynucleotides and is flanked on the 5' direction by a wing comprising 2 nucleotides and on the 3' direction by a wing comprising 6 nucleotides.

[0344] The 3-9-3 gapmers are 15 nucleotides in length, wherein the central gap segment is comprised of nine 2'-deoxynucleotides and is flanked on both 5' and 3' directions by wings comprising 3 nucleotides each.

[0345] The 3-9-4 gapmers are 16 nucleotides in length, wherein the central gap segment is comprised of nine 2'-deoxynucleotides and is flanked on the 5' direction by a wing comprising 3 nucleotides and on the 3' direction by a wing comprising 4 nucleotides.

[0346] The 3-9-5 gapmers are 17 nucleotides in length, wherein the central gap segment is comprised of nine 2'-deoxynucleotides and is flanked on the 5' direction by a wing comprising 3 nucleotides and on the 3' direction by a wing comprising 5 nucleotides.

[0347] The 4-10-5 gapmers are 19 nucleotides in length, wherein the central gap segment is comprised of ten 2'-deoxynucleotides and is flanked on the 5' direction by a wing comprising 4 nucleotides and on the 3' direction by a wing comprising 5 nucleotides.

[0348] The 4-11-4 gapmers are 19 nucleotides in length, wherein the central gap segment is comprised of eleven 2'-deoxynucleotides and is flanked on both 5' and 3' directions by wings comprising 4 nucleotides each.

[0349] The 4-7-4 gapmers are 15 nucleotides in length, wherein the central gap segment is comprised of seven 2'-deoxynucleotides and is flanked on both 5' and 3' directions by wings comprising 4 nucleotides each.

[0350] The 4-9-4 gapmers are 17 nucleotides in length, wherein the central gap segment is comprised of nine 2'-deoxynucleotides and is flanked on both 5' and 3' directions by wings comprising 4 nucleotides each.

[0351] The 4-9-5 gapmers are 18 nucleotides in length, wherein the central gap segment is comprised of nine 2'-deoxynucleotides and is flanked on the 5' direction by a wing comprising 4 nucleotides and on the 3' direction by a wing comprising 5 nucleotides.

[0352] The 5-10-4 gapmers are 19 nucleotides in length, wherein the central gap segment is comprised of ten 2'-deoxynucleotides and is flanked on the 5' direction by a wing comprising 5 nucleotides and on the 3' direction by a wing comprising 4 nucleotides.

[0353] The 5-7-5 gapmers are 17 nucleotides in length, wherein the central gap segment is comprised of seven 2'-deoxynucleotides and is flanked on both 5' and 3' directions by wings comprising 5 nucleotides each.

[0354] The 5-8-6 gapmers are 19 nucleotides in length, wherein the central gap segment is comprised of eight 2'-deoxynucleotides and is flanked on the 5' direction by a wing comprising 5 nucleotides and on the 3' direction by a wing comprising 6 nucleotides.

[0355] The 5-9-3 gapmers are 17 nucleotides in length, wherein the central gap segment is comprised of nine 2'-deoxynucleotides and is flanked on the 5' direction by a wing comprising 5 nucleotides and on the 3' direction by a wing comprising 3 nucleotides.

[0356] The 5-9-5 gapmers are 19 nucleotides in length, wherein the central gap segment is comprised of nine 2'-deoxynucleotides and is flanked on both 5' and 3' directions by wings comprising 5 nucleotides each.

[0357] The 6-7-6 gapmers are 19 nucleotides in length, wherein the central gap segment is comprised of seven 2'-deoxynucleotides and is flanked on both 5' and 3' directions by wings comprising 6 nucleotides each.

[0358] The 6-9-2 gapmers are 17 nucleotides in length, wherein the central gap segment is comprised of nine 2'-deoxynucleotides and is flanked on the 5' direction by a wing comprising 6 nucleotides and on the 3' direction by a wing comprising 2 nucleotides.

[0359] The 6-8-5 gapmers are 19 nucleotides in length, wherein the central gap segment is comprised of eight 2'-deoxynucleotides and is flanked on the 5' direction by a wing comprising 6 nucleotides and on the 3' direction by a wing comprising 5 nucleotides.

[0360] For each of the motifs, each nucleotide in the 5' wing segment and each nucleotide in the 3' wing segment has a

2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine nucleobases throughout each gapmer are 5-methylcytosines.

[0361] The oligonucleotides are organized in tables according to the SNP they target. "Start site" indicates the 5'-most nucleotide to which the gapmer is targeted. "Stop site" indicates the 3'-most nucleotide to which the gapmer is targeted. 'Target allele' indicates whether the gapmer is targeted to the major or the minor allele. The number in parentheses indicates the position on the oligonucleotide opposite to the SNP position.

Table 11

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs2857936 (nucleobases 1952 to 1972 of SEQ ID NO: 1)							
Start Site	Stop Site	Target allele	ISIS No.	Sequence	Motif	% inhibition	SEQ ID NO
145466	145485	n/a	387916	TCTCTATTGCACATTCCAAG	5-10-5	98	6
1952	1970	Minor (8)	459908	GCTTTTCATTGAAAAGAAA	5-9-5	26	157
1952	1970	Major (8)	459916	GCTTTTCGTTGAAAAGAAA	5-9-5	8	158
1954	1972	Minor (10)	459904	CTGCTTTTCATTGAAAAGA	5-9-5	23	159
1954	1972	Major (10)	459912	CTGCTTTTCGTTGAAAAGA	5-9-5	8	160

Table 12

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs12506200 (nucleobases 3695 to 3715 of SEQ ID NO: 1)							
Start Site	Stop Site	Target allele	ISIS No.	Sequence	Motif	% inhibition	SEQ ID NO
145466	145485	n/a	387916	TCTCTATTGCACATTCCAAG	5-10-5	98	6
3695	3713	Major (8)	459909	ACTAGGCCGGGCATGCTGG	5-9-5	48	161
3695	3713	Minor (8)	459917	ACTAGGCTGGGCATGCTGG	5-9-5	35	162
3697	3715	Major (10)	459905	AGACTAGGCCGGGCATGCT	5-9-5	33	163
3697	3715	Minor (10)	459913	AGACTAGGCTGGGCATGCT	5-9-5	45	164

Table 13

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs762855 (nucleobases 14437 to 14457 of SEQ ID NO: 1)							
Start Site	Stop Site	Target allele	ISIS No.	Sequence	Motif	% inhibition	SEQ ID NO
145466	145485	n/a	387916	TCTCTATTGCACATTCCAAG	5-10-5	98	6
14437	14455	Minor (8)	459910	AAACAGCTGTTAGTTCCCA	5-9-5	27	165
14437	14455	Major (8)	459918	AAACAGCCGTTAGTTCCCA	5-9-5	39	166
14439	14457	Minor (10)	459906	AGAAACAGCTGTTAGTTCC	5-9-5	24	167
14439	14457	Major (10)	459914	AGAAACAGCCGTTAGTTCC	5-9-5	28	168

Table 14

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs4690072 (nucleobases 62147 to 62173 of SEQ ID NO: 1)							
Start Site	Stop Site	Target allele	ISIS No.	Sequence	Motif	% inhibition	SEQ ID NO
145466	145485	n/a	387916	TCTCTATTGCACATTCCAAG	5-10-5	98	6
62147	62165	Major (6)	460145	GTGCTACCCAACCTTTCTG	5-9-5	62	169
62148	62166	Major (7)	460144	AGTGCTACCCAACCTTTCT	5-9-5	61	170

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs4690072 (nucleobases 62147 to 62173 of SEQ ID NO: 1)							
Start Site	Stop Site	Target allele	ISIS No.	Sequence	Motif	% inhibition	SEQ ID NO
62149	62167	Major (8)	460143	CAGTGCTACCCAACCTTTC	5-9-5	65	171
62150	62168	Major (9)	460142	ACAGTGCTACCCAACCTTT	5-9-5	83	172
62151	62169	Major (10)	*435874	CACAGTGCTACCCAACCTT	5-9-5	76	28
62151	62169	Major (10)	460022	CACAGTGCTACCCAACCTT	4-10-5	75	28
62151	62169	Major (10)	460033	CACAGTGCTACCCAACCTT	4-11-4	89	28
62151	62168	Major (9)	460063	ACAGTGCTACCCAACCTT	4-9-5	77	173
62151	62169	Major (10)	460073	CACAGTGCTACCCAACCTT	5-10-4	86	28
62151	62169	Major (10)	460093	CACAGTGCTACCCAACCTT	5-8-6	61	28
62151	62169	Major (10)	460169	CACAGTGCTACCCAACCTT	6-7-6	16	28
62151	62169	Major (10)	460188	CACAGTGCTACCCAACCTT	6-8-5	53	28
62152	62168	Major (9)	459978	ACAGTGCTACCCAACCT	2-9-6	87	174
62152	62167	Major (8)	459999	CAGTGCTACCCAACCT	3-9-4	48	175
62152	62168	Major (9)	460012	ACAGTGCTACCCAACCT	3-9-5	84	174
62152	62168	Major (9)	460052	ACAGTGCTACCCAACCT	4-9-4	51	174
62152	62168	Major (9)	460083	ACAGTGCTACCCAACCT	5-7-5	37	174
62152	62168	Major (9)	460103	ACAGTGCTACCCAACCT	5-9-3	80	174
62152	62170	Major (11)	460137	TCACAGTGCTACCCAACCT	5-9-5	65	176
62152	62168	Major (9)	460179	ACAGTGCTACCCAACCT	6-9-2	67	174
62153	62167	Major (8)	459989	CAGTGCTACCCAACC	3-9-3	60	177
62153	62167	Major (8)	460043	CAGTGCTACCCAACC	4-7-4	24	177
62153	62171	Major (12)	460138	ATCACAGTGCTACCCAACC	5-9-5	76	178
62154	62172	Major (13)	460139	TATCACAGTGCTACCCAAC	5-9-5	68	179
62155	62173	Major (14)	460140	ATATCACAGTGCTACCCAA	5-9-5	79	180

Table 15

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs2298969 (nucleobases 125883 to 125911 of SEQ ID NO: 1)							
Start Site	Stop Site	Target allele	ISIS No.	Sequence	Motif	% inhibition	SEQ ID NO
145466	145485	n/a	387916	TCTCTATTGCACATTCCAAG	5-10-5	98	6
125883	125901	Minor (5)	460166	ATGCTGACTTGGGCCATTC	5-9-5	83	181
125884	125902	Minor (6)	460165	GATGCTGACTTGGGCCATT	5-9-5	88	182
125885	125903	Minor (7)	460164	GGATGCTGACTTGGGCCAT	5-9-5	68	183

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs2298969 (nucleobases 125883 to 125911 of SEQ ID NO: 1)							
Start Site	Stop Site	Target allele	ISIS No.	Sequence	Motif	% inhibition	SEQ ID NO
125886	125904	Minor (8)	460163	GGGATGCTGACTTGGGCCA	5-9-5	73	184
125887	125905	Minor (9)	460162	AGGGATGCTGACTTGGGCC	5-9-5	88	185
125888	125906	Minor (10)	*435890	AAGGGATGCTGACTTGGGC	5-9-5	83	94
125888	125906	Minor (10)	460026	AAGGGATGCTGACTTGGGC	4-10-5	90	94
125888	125906	Minor (10)	460037	AAGGGATGCTGACTTGGGC	4-11-4	86	94
125888	125905	Minor (9)	460068	AGGGATGCTGACTTGGGC	4-9-5	90	186
125888	125906	Minor (10)	460076	AAGGGATGCTGACTTGGGC	5-10-4	90	94
125888	125906	Minor (10)	460096	AAGGGATGCTGACTTGGGC	5-8-6	88	94
125888	125906	Minor (10)	460171	AAGGGATGCTGACTTGGGC	6-7-6	87	94
125888	125906	Minor (10)	460190	AAGGGATGCTGACTTGGGC	6-8-5	69	94
125889	125905	Minor (9)	459983	AGGGATGCTGACTTGGG	2-9-6	80	187
125889	125904	Minor (8)	460005	GGGATGCTGACTTGGG	3-9-4	80	284
125889	125905	Minor (9)	460016	AGGGATGCTGACTTGGG	3-9-5	90	187
125889	125905	Minor (9)	460057	AGGGATGCTGACTTGGG	4-9-4	86	187
125889	125905	Minor (9)	460087	AGGGATGCTGACTTGGG	5-7-5	86	187
125889	125905	Minor (9)	460107	AGGGATGCTGACTTGGG	5-9-3	79	187
125889	125907	Major (11)	460157	CAAGGGATGCTGACTTGGG	5-9-5	88	188
125889	125905	Minor (9)	460181	AGGGATGCTGACTTGGG	6-9-2	62	187
125890	125904	Minor (8)	459972	GGGATGCTGACTTGG	Uniform	18	189
125890	125904	Minor (8)	459992	GGGATGCTGACTTGG	3-9-3	90	189
125890	125904	Minor (8)	460046	GGGATGCTGACTTGG	4-7-4	59	189
125890	125908	Major (12)	460158	CCAAGGGATGCTGACTTGG	5-9-5	79	190
125891	125909	Major (13)	460159	GCCAAGGGATGCTGACTTG	5-9-5	82	191
125892	125910	Major (14)	460160	TGCCAAGGGATGCTGACTT	5-9-5	87	192

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs2298969 (nucleobases 125883 to 125911 of SEQ ID NO: 1)							
Start Site	Stop Site	Target allele	ISIS No.	Sequence	Motif	% inhibition	SEQ ID NO
125893	125911	Major (15)	460161	CTGCCAAGGGATGCTGACT	5-9-5	78	193

Table 16

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs7685686 (nucleobases 146781 to 146809 of SEQ ID NO: 1)							
Start Site	Stop Site	Target allele	ISIS No.	Sequence	Motif	% inhibition	SEQ ID NO
145466	145485	n/a	387916	TCTCTATTGCACATTCCAAG	5-10-5	98	6
146781	146799	Major (5)	460156	ATTGTCATCACCAGAAAAA	5-9-5	88	194
146782	146800	Major (6)	460155	AATTGTCATCACCAGAAAA	5-9-5	89	195
146783	146801	Major (7)	460154	AAATTGTCATCACCAGAAA	5-9-5	89	196
146784	146802	Major (8)	460153	TAAATTGTCATCACCAGAA	5-9-5	93	197
146785	146803	Major (9)	460152	ATAAATTGTCATCACCAGA	5-9-5	95	198
146786	146804	Major (10)	*435879	AATAAATTGTCATCACCAG	5-9-5	94	99
146786	146804	Major (10)	460024	AATAAATTGTCATCACCAG	4-10-5	88	99
146786	146804	Major (10)	460035	AATAAATTGTCATCACCAG	4-11-4	91	99
146786	146803	Major (9)	460065	ATAAATTGTCATCACCAG	4-9-5	96	199
146786	146804	Major (10)	460074	AATAAATTGTCATCACCAG	5-10-4	94	99
146786	146804	Major (10)	460095	AATAAATTGTCATCACCAG	5-8-6	92	99
146786	146804	Major (10)	460170	AATAAATTGTCATCACCAG	6-7-6	91	99
146786	146804	Major (10)	460189	AATAAATTGTCATCACCAG	6-8-5	94	99
146787	146803	Major (9)	459981	ATAAATTGTCATCACCA	2-9-6	85	200
146787	146802	Major (8)	460002	TAAATTGTCATCACCA	3-9-4	86	201
146787	146803	Major (9)	460014	ATAAATTGTCATCACCA	3-9-5	91	200
146787	146803	Major (9)	460055	ATAAATTGTCATCACCA	4-9-4	90	200
146787	146803	Major (9)	460085	ATAAATTGTCATCACCA	5-7-5	94	200
146787	146803	Major (9)	460104	ATAAATTGTCATCACCA	5-9-3	93	200
146787	146805	Major (11)	460147	TAATAAATTGTCATCACCA	5-9-5	91	202
146787	146803	Major (9)	460180	ATAAATTGTCATCACCA	6-9-2	91	200
146788	146802	Major (8)	459970	TAAATTGTCATCACC	Uniform	9	203
146788	146802	Major (8)	459990	TAAATTGTCATCACC	3-9-3	67	203
146788	146802	Major (8)	460045	TAAATTGTCATCACC	4-7-4	84	203
146788	146806	Major (12)	460148	TTAATAAATTGTCATCACC	5-9-5	88	204
146789	146807	Major (13)	460149	ATTAATAAATTGTCATCAC	5-9-5	32	205
146790	146808	Major (14)	460150	TATTAATAAATTGTCATCA	5-9-5	29	206

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs7685686 (nucleobases 146781 to 146809 of SEQ ID NO: 1)							
Start Site	Stop Site	Target allele	ISIS No.	Sequence	Motif	% inhibition	SEQ ID NO
146791	146809	Major (15)	460151	CTATTAATAAATTGTCATC	5-9-5	33	207

Table 17

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs362331 (nucleobases 155474 to 155502 of SEQ ID NO: 1)							
Start Site	Stop Site	Target allele	ISIS No.	Sequence	Motif	% inhibition	SEQ ID NO
145466	145485	n/a	387916	TCTCTATTGCACATTCCAAG	5-10-5	98	6
155474	155492	Major (5)	460136	CAGTAGATGAGGGAGCAGG	5-9-5	81	208
155475	155493	Major (6)	460135	ACAGTAGATGAGGGAGCAG	5-9-5	84	209
155476	155494	Major (7)	460134	CACAGTAGATGAGGGAGCA	5-9-5	87	210
155477	155495	Major (8)	460133	ACACAGTAGATGAGGGAGC	5-9-5	85	211
155478	155496	Major (9)	460132	CACACAGTAGATGAGGGAG	5-9-5	86	212
155479	155497	Major (10)	*435870	GCACACAGTAGATGAGGGA	5-9-5	91	103
155479	155497	Major (10)	460019	GCACACAGTAGATGAGGGA	4-10-5	92	103
155479	155497	Major (10)	460031	GCACACAGTAGATGAGGGA	4-11-4	95	103
155479	155496	Major (9)	460061	CACACAGTAGATGAGGGA	4-9-5	87	213
155479	155497	Major (10)	460071	GCACACAGTAGATGAGGGA	5-10-4	94	103
155479	155497	Major (10)	460090	GCACACAGTAGATGAGGGA	5-8-6	86	103
155479	155497	Major (10)	460168	GCACACAGTAGATGAGGGA	6-7-6	84	103
155479	155497	Major (10)	460187	GCACACAGTAGATGAGGGA	6-8-5	89	103
155480	155496	Major (9)	459977	CACACAGTAGATGAGGG	2-9-6	90	214
155480	155495	Major (8)	459996	ACACAGTAGATGAGGG	3-9-4	37	215
155480	155496	Major (9)	460009	CACACAGTAGATGAGGG	3-9-5	90	214
155480	155496	Major (9)	460051	CACACAGTAGATGAGGG	4-9-4	73	214
155480	155496	Major (9)	460081	CACACAGTAGATGAGGG	5-7-5	77	214
155480	155496	Major (9)	460101	CACACAGTAGATGAGGG	5-9-3	84	214
155480	155498	Major (11)	460127	TGCACACAGTAGATGAGGG	5-9-5	89	216
155480	155496	Major (9)	460178	CACACAGTAGATGAGGG	6-9-2	92	214
155481	155495	Major (8)	459967	ACACAGTAGATGAGG	Uniform	81	217
155481	155495	Major (8)	459987	ACACAGTAGATGAGG	3-9-3	18	217
155481	155495	Major (8)	460041	ACACAGTAGATGAGG	4-7-4	54	217
155481	155499	Major (12)	460128	GTGCACACAGTAGATGAGG	5-9-5	73	218
155482	155500	Major (13)	460129	AGTGCACACAGTAGATGAG	5-9-5	86	219
155483	155501	Major (14)	460130	AAGTGCACACAGTAGATGA	5-9-5	60	220

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs362331 (nucleobases 155474 to 155502 of SEQ ID NO: 1)							
Start Site	Stop Site	Target allele	ISIS No.	Sequence	Motif	% inhibition	SEQ ID NO
155484	155502	Major (15)	460131	GAAGTGCACACAGTAGATG	5-9-5	73	221

Table 18

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs362306 (nucleobases 181739 to 181767 of SEQ ID NO: 1)							
Start Site	Stop Site	Target allele	ISIS No.	Sequence	Motif	% inhibition	SEQ ID NO
145466	145485	n/a	387916	TCTCTATTGCACATTCCAAG	5-10-5	98	6
181739	181757	Major (5)	460126	GCTGCAACCTGGCAACAAC	5-9-5	87	222
181740	181758	Major (6)	460125	AGCTGCAACCTGGCAACAA	5-9-5	70	223
181741	181759	Major (7)	460123	CAGCTGCAACCTGGCAACA	5-9-5	83	224
181742	181760	Major (8)	460121	GCAGCTGCAACCTGGCAAC	5-9-5	47	225
181743	181761	Major (9)	460118	AGCAGCTGCAACCTGGCAA	5-9-5	75	226
181744	181762	Major (10)	*435869	GAGCAGCTGCAACCTGGCA	5-9-5	91	149
181744	181762	Major (10)	460018	GAGCAGCTGCAACCTGGCA	4-10-5	86	149
181744	181762	Major (10)	460028	GAGCAGCTGCAACCTGGCA	4-11-4	89	149
181744	181761	Major (9)	460058	AGCAGCTGCAACCTGGCA	4-9-5	85	227
181744	181762	Major (10)	460069	GAGCAGCTGCAACCTGGCA	5-10-4	91	149
181744	181762	Major (10)	460089	GAGCAGCTGCAACCTGGCA	5-8-6	54	149
181744	181762	Major (10)	460167	GAGCAGCTGCAACCTGGCA	6-7-6	85	149
181744	181762	Major (10)	460186	GAGCAGCTGCAACCTGGCA	6-8-5	84	149
181745	181761	Major (9)	459975	AGCAGCTGCAACCTGGC	2-9-6	86	228
181745	181760	Major (8)	459995	GCAGCTGCAACCTGGC	3-9-4	87	229
181745	181761	Major (9)	460008	AGCAGCTGCAACCTGGC	3-9-5	83	228
181745	181761	Major (9)	460049	AGCAGCTGCAACCTGGC	4-9-4	88	228
181745	181761	Major (9)	460079	AGCAGCTGCAACCTGGC	5-7-5	46	228
181745	181761	Major (9)	460099	AGCAGCTGCAACCTGGC	5-9-3	44	228
181745	181763	Major (11)	460108	AGAGCAGCTGCAACCTGGC	5-9-5	50	230
181745	181761	Major (9)	460177	AGCAGCTGCAACCTGGC	6-9-2	67	228
181746	181760	Major (8)	459966	GCAGCTGCAACCTGG	Uniform	26	231
181746	181760	Major (8)	459985	GCAGCTGCAACCTGG	3-9-3	69	231
181746	181760	Major (8)	460039	GCAGCTGCAACCTGG	4-7-4	56	231
181746	181764	Major (12)	460110	AAGAGCAGCTGCAACCTGG	5-9-5	75	232
181747	181765	Major (13)	460113	CAAGAGCAGCTGCAACCTG	5-9-5	36	233
181748	181766	Major (14)	460115	GCAAGAGCAGCTGCAACCT	5-9-5	78	234

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs362306 (nucleobases 181739 to 181767 of SEQ ID NO: 1)							
Start Site	Stop Site	Target allele	ISIS No.	Sequence	Motif	% inhibition	SEQ ID NO
181749	181767	Major (15)	460117	TGCAAGAGCAGCTGCAACC	5-9-5	73	235

Table 19

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs1006798 (nucleobases 198015 to 198035 of SEQ ID NO: 1)							
Start Site	Stop Site	Target allele	ISIS No.	Sequence	Motif	% inhibition	SEQ ID NO
145466	145485	n/a	387916	TCTCTATTGCACATTCCAAG	5-10-5	98	6
198015	198033	Minor (8)	459911	ACCATGATATCTCCAGCAC	5-9-5	33	236
198015	198033	Minor (8)	459919	ACCATGACATCTCCAGCAC	5-9-5	26	237
198017	198035	Major (10)	459907	CCACCATGATATCTCCAGC	5-9-5	32	238
198017	198035	Minor (10)	459915	CCACCATGACATCTCCAGC	5-9-5	51	239

Example 6: Dose-dependent antisense inhibition of human huntingtin mRNA levels in Coriell fibroblast cell lines

[0362] Gapmers from the studies described in Example 5 were selected and tested at various doses in GM04281, GM02171, and GM02173B cell lines. Each cell line was plated at a density of 25,000 cells per well and transfected using electroporation with 750 nM, 1,500 nM, 3,000 nM, 6,000 nM, and 12,000 nM concentrations of antisense oligonucleotide, as specified in Tables 20, 21, and 22. After a treatment period of approximately 16 hours, RNA was isolated from the cells and *HTT* mRNA levels were measured by quantitative real-time PCR. Human *HTT* primer probe set RTS2617 was used to measure mRNA levels. *HTT* mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented as percent inhibition of *HTT* mRNA, relative to untreated control cells. IC_{50} values are also provided in Tables 20, 21, and 22.

Table 20

Dose-dependent antisense inhibition of human *HTT* in GM04281 cells						
ISIS No.	750 nM	1,500 nM	3,000 nM	6,000 nM	12,000 nM	IC_{50} (μM)
387916	56	81	89	96	98	0.6
435869	38	49	66	86	91	1.4
435874	33	27	37	49	62	8.4
435879	42	55	73	86	96	1.1
435890	39	51	74	83	89	1.3
459978	29	33	51	69	86	2.5
459992	14	27	51	54	84	3.2
460012	15	24	54	70	81	3.1
460016	3	36	48	71	77	3.3
460019	54	59	74	87	94	0.7
460026	48	47	71	79	88	0.8
460028	39	38	73	77	87	1.4
460031	44	62	72	87	92	0.9
460033	11	38	52	64	87	3.0

Dose-dependent antisense inhibition of human *HTT* in GM04281 cells						
ISIS No.	750 nM	1,500 nM	3,000 nM	6,000 nM	12,000 nM	IC_{50} (μM)
460065	43	54	74	89	96	1.1
460068	47	28	63	76	90	2.6
460069	38	50	65	77	91	1.4
460071	53	61	80	89	93	0.6
460073	16	39	42	58	75	4.0
460076	26	47	54	70	86	2.1
460085	48	60	79	89	94	0.8
460140	6	24	44	44	64	6.6
460142	2	38	46	46	68	4.8
460152	35	61	76	92	94	1.2
460157	51	36	53	74	89	2.6
460162	64	41	71	76	85	2.1
460165	41	50	56	76	84	1.5

Table 21

Dose-dependent antisense inhibition of human *HTT* in GM02171 cells						
ISIS No.	750 nM	1,500 nM	3,000 nM	6,000 nM	12,000 nM	IC_{50} (μM)
387916	53	66	88	96	98	0.7
435869	4	20	36	63	86	3.9
435870	25	39	48	62	83	2.8
435874	12	20	18	27	37	>12.0
435879	10	7	11	42	51	10.6
435890	10	23	29	29	55	9.2
459978	15	7	6	29	52	12.7
459992	11	19	26	39	62	8.7
460012	3	3	10	19	41	>12.0
460016	0	14	12	22	48	>12.0
460019	27	21	41	60	73	4.4
460026	9	25	30	46	58	7.8
460028	24	8	32	54	77	5.3
460031	8	25	42	60	83	3.8
460033	11	25	30	40	75	4.1
460065	11	16	11	31	53	10.3
460068	15	13	39	44	53	8.8
460069	17	28	37	60	79	3.9
460071	16	36	58	70	88	2.6
460073	5	19	24	33	56	8.7
460076	19	29	44	54	83	3.3
460085	10	15	17	28	31	>12.0
460140	8	22	22	28	47	>12.0
460142	11	24	28	36	38	>12.0
460152	14	21	8	25	44	22
460157	22	21	29	44	66	6.7
460162	24	55	52	62	82	2.8

Dose-dependent antisense inhibition of human *HTT* in GM02171 cells						
ISIS No.	750 nM	1,500 nM	3,000 nM	6,000 nM	12,000 nM	IC_{50} (μM)
460165	14	34	50	69	81	3.1

Table 22

Dose-dependent antisense inhibition of human *HTT* in GM02173B cells						
ISIS No.	750 nM	1,500 nM	3,000 nM	6,000 nM	12,000 nM	IC_{50} (μM)
387916	37	63	86	88	98	1.0
435869	10	20	43	70	85	3.5
435870	24	24	56	72	87	2.3
435874	0	11	12	30	44	>12.0
435879	4	17	43	64	74	4.3
435890	31	29	54	57	69	4.4
459978	7	13	17	35	64	8.4
459992	18	15	30	51	71	5.7
460012	0	10	24	37	72	7.1
460016	15	5	30	38	59	9.5
460019	10	32	51	65	87	3.1
460026	0	34	21	55	65	6.4
460028	0	14	31	51	77	5.2
460031	0	31	53	71	88	3.2
460033	11	8	6	52	84	5.0
460065	19	37	53	58	74	3.6
460068	17	11	31	59	69	5.5
460069	11	21	37	55	75	4.6
460071	6	42	61	83	88	2.6
460073	7	13	19	49	66	6.3
460076	27	31	49	43	81	2.9
460085	17	34	51	54	68	4.4
460140	0	2	28	18	46	>12.0
460142	2	32	37	42	59	7.6
460152	17	32	35	51	66	5.5
460157	9	34	38	52	74	4.5
460162	22	45	57	65	79	2.5
460165	5	45	52	72	84	3.2

Example 7: Antisense inhibition of human *HTT* in GM04281 cells and GM02171 cells

[0363] Additional antisense oligonucleotides were designed based on the gapmers selected from studies described in Example 2. These oligonucleotides were designed by creating gapmers shifted slightly upstream and downstream (i.e. "microwalk") of the original gapmers from Table 4.

[0364] The gapmers were tested in the GM04281 and the GM02171 cell lines. Cultured GM04281 or GM02171 cells at a density of 25,000 cells per well were transfected using electroporation with 10,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and *HTT* mRNA levels were measured by quantitative real-time PCR using primer probe set RTS2617. *HTT* mRNA levels were adjusted according to total RNA

content, as measured by RIBOGREEN. Results are presented as percent inhibition of *HTT* mRNA, relative to untreated control cells.

[0365] The gapmers, from which the newly designed oligonucleotides were derived, were also included in the assay. These parent gapmers, ISIS 435294, ISIS 435295, ISIS 435301, ISIS 435303, ISIS 435304, ISIS 435305, ISIS 435308, ISIS 435330, ISIS 435331, ISIS 435337, ISIS 435339, ISIS 435340, ISIS 435341, ISIS 435344, ISIS 435862, ISIS 435863, ISIS 435864, ISIS 435866, ISIS 435867, ISIS 435868, ISIS 435871, ISIS 435873, ISIS 435875, ISIS 435876, ISIS 435878, ISIS 435880, ISIS 435881, ISIS 435882, ISIS 435884, ISIS 435890, and ISIS 435897 are marked with an asterisk (*) in the table. ISIS 387916 was included in the study as a benchmark oligonucleotide against which the potency of the antisense oligonucleotides targeting nucleotides overlapping each SNP position could be compared.

[0366] The chimeric antisense oligonucleotides in Tables 23-48 were designed as 5-9-5 MOE gapmers. The 5-9-5 gapmers are 19 nucleotides in length, wherein the central gap segment is comprised of nine 2'-deoxynucleotides and is flanked on both 5' and 3' directions by wings comprising 5 nucleotides each. Each nucleotide in the 5' wing segment and each nucleotide in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine nucleobases throughout each gapmer are 5-methylcytosines.

[0367] The gapmers are organized in Tables 23-48, according to the SNP site they target. "Start site" indicates the 5'-most nucleotide to which the gapmer is targeted. "Stop site" indicates the 3'-most nucleotide to which the gapmer is targeted. 'Target allele' indicates whether the gapmer is targeted to the major or the minor allele. The number in parentheses indicates the position on the oligonucleotide opposite to the SNP position.

Table 23

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs3856973 (nucleobases 19815 to 19835 of SEQ ID NO: 1)							
Start Site	Stop Site	ISIS No	Target allele	Sequence	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	n/a	TCTCTATTGCACATTCCAAG	100	99	6
19815	19833	*435330	Major (8)	TAACACTCGATTAACCCTG	88	31	8
19816	19834	476441	Major (9)	TTAACACTCGATTAACCCT	88	0	240
19817	19835	*435294	Major (10)	GTTAACACTCGATTAACCC	72	30	10

Table 24

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs2285086 (nucleobases 28901 to 28921 of SEQ ID NO: 1)							
Start Site	Stop Site	ISIS No	Target allele	Sequence	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	n/a	TCTCTATTGCACATTCCAAG	100	99	6
28901	28919	463570	Major (8)	TAGTTCATCCCAGTGAGAA	66	12	241
28902	28920	463573	Major (9)	CTAGTTCATCCCAGTGAGA	66	36	242
28903	28921	*435864	Major (10)	GCTAGTTCATCCCAGTGAG	40	18	12

Table 25

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs7659144 (nucleobases 37963 to 37983 of SEQ ID NO: 1)							
Start Site	Stop Site	ISIS No	Target allele	Sequence	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	n/a	TCTCTATTGCACATTCCAAG	100	99	6

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs7659144 (nucleobases 37963 to 37983 of SEQ ID NO: 1)							
Start Site	Stop Site	ISIS No	Target allele	Sequence	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
37963	37981	476462	Major (8)	GAAATGGGTTTTTCCACAT	38	0	243
37964	37982	476439	Major (9)	GGAAATGGGTTTTTCCACA	80	45	244
37965	37983	*435878	Major (10)	TGGAAATGGGTTTTTCCAC	76	3	14

Table 26

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs16843804 (nucleobases 44032 to 44052 of SEQ ID NO: 1)							
Start Site	Stop Site	ISIS No	Target allele	Sequence	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	n/a	TCTCTATTGCACATTCCAAG	100	99	6
44032	44050	476471	Major (8)	TAACCGTGGCATGGGCAGT	82	53	245
44033	44051	476452	Major (9)	TTAACCGTGGCATGGGCAG	84	44	246
44034	44052	*435863	Major (10)	TTTAACCGTGGCATGGGCA	89	89	16

Table 27

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs2024115 (nucleobases 44210 to 44230 of SEQ ID NO: 1)							
Start Site	Stop Site	ISIS No	Target allele	Sequence	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	n/a	TCTCTATTGCACATTCCAAG	100	99	6
44210	44228	*435331	Major (8)	TTCAAGCTAGTAACGATGC	84	20	18
44211	44229	476447	Major (9)	CTTCAAGCTAGTAACGATG	87	57	247
44212	44230	*435295	Major (10)	ACTTCAAGCTAGTAACGAT	85	67	20

Table 28

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs10015979 (nucleobases 49084 to 49104 of SEQ ID NO: 1)							
Start Site	Stop Site	ISIS No	Target allele	Sequence	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	n/a	TCTCTATTGCACATTCCAAG	100	99	6
49084	49102	476470	Major (8)	AGCTAGGTTAAAGAGTCAC	55	74	248
49085	49103	476450	Major (9)	CAGCTAGGTTAAAGAGTCA	44	5	249
49086	49104	*435862	Major (10)	GCAGCTAGGTTAAAGAGTC	56	49	22

Table 29

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs7691627 (nucleobases 51052 to 51072 of SEQ ID NO: 1)							
Start Site	Stop Site	ISIS No	Target allele	Sequence	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	n/a	TCTCTATTGCACATTCCAAG	100	99	6
51052	51070	476467	Major (8)	TAAGAAACACAATCAAAGA	45	21	250
51053	51071	476445	Major (9)	ATAAGAAACACAATCAAAG	34	1	251
51054	51072	*435880	Major (10)	AATAAGAAACACAATCAAA	68	7	24

Table 30

Comparison of inhibition of human *HTT mRNA* levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs6446723 (nucleobases 66455 to 66475 of SEQ ID NO: 1)							
Start Site	Stop Site	ISIS No	Target allele	Sequence	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	n/a	TCTCTATTGCACATTCCAAG	100	99	6
66455	66473	476463	Major (8)	ATTTTCTAGACTTTATGAT	37	7	252
66456	66474	476440	Major (9)	AATTTTCTAGACTTTATGA	57	0	253
66457	66475	*435875	Major (10)	TAATTTTCTAGACTTTATG	42	0	30

Table 31

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and a chimeric antisense oligonucleotide targeted to SNP rs363064 (nucleobases 81053 to 81071 of SEQ ID NO: 1)							
Start Site	Stop Site	ISIS No	Target allele	Sequence	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	n/a	TCTCTATTGCACATTCCAAG	100	99	6
81053	81071	476461	Major (9)	GAGAATACGGGTAACATTT	87	62	254

Table 32

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs11731237 (nucleobases 91455 to 91475 of SEQ ID NO: 1)							
Start Site	Stop Site	ISIS No	Target allele	Sequence	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	n/a	TCTCTATTGCACATTCCAAG	100	99	6
91455	91473	476468	Major (8)	TGGGCAGGAAGGACTGAAC	58	56	255
91456	91474	476448	Major (9)	GTGGGCAGGAAGGACTGAA	61	69	256
91457	91475	*435884	Major (10)	GGTGGGCAGGAAGGACTGA	59	49	68

Table 33

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs4690073 (nucleobases 99792 to 99812 of SEQ ID NO: 1)							
Start Site	Stop Site	ISIS No	Target allele	Sequence	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	n/a	TCTCTATTGCACATTCCAAG	100	99	6
99792	99810	*435337	Major (8)	CCTAAATCAATCTACAAGT	69	7	70
99793	99811	476446	Major (9)	CCCTAAATCAATCTACAAG	61	0	257
99794	99812	*435301	Major (10)	TCCCTAAATCAATCTACAA	63	1	72

Table 34

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs34315806 (nucleobases 101676 to 101696 of SEQ ID NO: 1)							
Start Site	Stop Site	ISIS No	Target allele	Sequence	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	n/a	TCTCTATTGCACATTCCAAG	100	99	6
101676	101694	463569	Major (8)	CTTTTCCGTGCTGTTCTGA	96	95	258
101677	101695	463572	Major (9)	ACTTTTCCGTGCTGTTCTG	93	91	259
101678	101696	463567	Major (10)	AACTTTTCCGTGCTGTTCT	98	97	260

Table 35

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs363099 (nucleobases 101698 to 101718 of SEQ ID NO: 1)							
Start Site	Stop Site	ISIS No	Target allele	Sequence	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	n/a	TCTCTATTGCACATTCCAAG	100	99	6
101698	101716	*435339	Major (8)	CTGAGCGGAGAAACCCTCC	94	85	80
101699	101717	476458	Major (9)	GCTGAGCGGAGAAACCCTC	92	79	261
101700	101718	*435303	Major (10)	GGCTGAGCGGAGAAACCCT	96	93	82

Table 36

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs363096 (nucleobases 119663 to 119683 of SEQ ID NO: 1)							
Start Site	Stop Site	ISIS No	Target allele	Sequence	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	n/a	TCTCTATTGCACATTCCAAG	100	99	6
119663	119681	*435340	Major (8)	TTCCCTAAAAACAAAAACA	42	21	85
119664	119682	476451	Major (9)	ATTCCCTAAAAACAAAAAC	0	0	262
119665	119683	*435304	Major (10)	GATTCCCTAAAAACAAAAA	41	27	87

Table 37

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs2298967 (nucleobases 125389 to 125409 of SEQ ID NO: 1)							
Start Site	Stop Site	ISIS No	Target allele	Sequence	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	n/a	TCTCTATTGCACATTCCAAG	100	99	6
125389	125407	*435341	Major (8)	CTTTTCTATTGTCTGTCCC	83	65	89
125390	125408	476459	Major (9)	GCTTTTCTATTGTCTGTCC	89	82	263
125391	125409	*435305	Major (10)	TGCTTTTCTATTGTCTGTC	92	85	91

Table 38

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and a chimeric antisense oligonucleotide targeted to SNP rs2298969 (nucleobases 125888 to 125906 of SEQ ID NO: 1)							
Start Site	Stop Site	ISIS No	Target allele	Sequence	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	n/a	TCTCTATTGCACATTCCAAG	100	99	6
125888	125906	*435890	Minor (10)	AAGGGATGCTGACTTGGGC	91	64	94

Table 39

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs6844859 (nucleobases 130128 to 130148 of SEQ ID NO: 1)							
Start Site	Stop Site	ISIS No	Target allele	Sequence	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	n/a	TCTCTATTGCACATTCCAAG	100	99	6
130128	130146	476466	Major (8)	CTTCCTCACTGAGGATGAA	87	64	264
130129	130147	476444	Major (9)	CCTTCCTCACTGAGGATGA	92	77	265
130130	130148	*435876	Major (10)	ACCTTCCTCACTGAGGATG	94	87	95

Table 40

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs363092 (nucleobases 135671 to 135691 of SEQ ID NO: 1)							
Start Site	Stop Site	ISIS No	Target allele	Sequence	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	n/a	TCTCTATTGCACATTCCAAG	100	99	6
135671	135689	476464	Major (8)	AACCACTTTGGGATGAATA	51	71	266
135672	135690	476442	Major (9)	AAACCACTTTGGGATGAAT	58	59	267
135673	135691	*435897	Minor (10)	CAAACCACTTTGGGATGAA	48	78	98

Table 41

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs363088 (nucleobases 149972 to 149992 of SEQ ID NO: 1)							
Start Site	Stop Site	ISIS No	Target allele	Sequence	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	n/a	TCTCTATTGCACATTCCAAG	100	99	6
149972	149990	476476	Major (8)	ACAGCTATCTTCTCATCAA	90	65	268
149973	149991	476460	Major (9)	CACAGCTATCTTCTCATCA	86	39	269
149974	149992	*435871	Major (10)	TCACAGCTATCTTCTCATC	91	54	101

Table 42

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs916171 (nucleobases 156457 to 156477 of SEQ ID NO: 1)							
Start Site	Stop Site	ISIS No	Target allele	Sequence	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	n/a	TCTCTATTGCACATTCCAAG	100	99	6
156457	156475	476465	Major (8)	GAACAAAGAGAAGAATTTC	38	0	270
156458	156476	476443	Major (9)	AGAACAAAGAGAAGAATTT	58	0	271
156459	156477	*435881	Major (10)	CAGAACAAAGAGAAGAATT	59	16	105

Table 43

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs362275 (nucleobases 164244 to 164264 of SEQ ID NO: 1)							
Start Site	Stop Site	ISIS No	Target allele	Sequence	% inhibition in GM04281	% inhibition in GM0217 1	SEQ ID NO
145466	145485	387916	n/a	TCTCTATTGCACATTCCAAG	100	99	6
164244	164262	476473	Major (8)	GAAGCCTGATAAAATCTCT	83	51	272
164245	164263	476454	Major (9)	AGAAGCCTGATAAAATCTC	79	61	273
164246	164264	*435868	Major (10)	AAGAAGCCTGATAAAATCT	69	56	111

Table 44

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs362273 (nucleobases 167061 to 167081 of SEQ ID NO: 1)							
Start Site	Stop Site	ISIS No	Target allele	Sequence	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	n/a	TCTCTATTGCACATTCCAAG	100	99	6
167061	167079	463568	Major (8)	TGATCTGTAGCAGCAGCTT	96	78	274
167062	167080	463571	Major (9)	TTGATCTGTAGCAGCAGCT	95	86	275
167063	167081	463566	Major (10)	GTTGATCTGTAGCAGCAGC	94	78	276

Table 45

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs362272 (nucleobases 174622 to 174642 of SEQ ID NO: 1)							
Start Site	Stop Site	ISIS No	Target allele	Sequence	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	n/a	TCTCTATTGCACATTCCAAG	100	99	6
174622	174640	*435344	Major (8)	TAGAGGACGCCGTGCAGGG	78	63	120
174623	174641	476456	Major (9)	ATAGAGGACGCCGTGCAGG	87	60	277
174624	174642	*435308	Major (10)	CATAGAGGACGCCGTGCAG	76	48	122

Table 46

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs362271 (nucleobases 175160 to 175180 of SEQ ID NO: 1)							
Start Site	Stop Site	ISIS No	Target allele	Sequence	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	n/a	TCTCTATTGCACATTCCAAG	100	99	6
175160	175178	476472	Major (8)	GTGTGTACAGAACCTGCCG	85	52	278
175161	175179	476453	Major (9)	CGTGTGTACAGAACCTGCC	88	69	279
175162	175180	*435867	Major (10)	ACGTGTGTACAGAACCTGC	91	80	125

Table 47

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs3775061 (nucleobases 178396 to 178416 of SEQ ID NO: 1)							
Start Site	Stop Site	ISIS No	Target allele	Sequence	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	n/a	TCTCTATTGCACATTCCAAG	100	99	6
178396	178414	476475	Major (8)	TTCAGAATGCCTCATCTGG	61	1	280
178397	178415	476457	Major (9)	GTTCAGAATGCCTCATCTG	80	50	281
178398	178416	*435873	Major (10)	TGTTCAGAATGCCTCATCT	80	43	127

Table 48

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs362296 (nucleobases 186649 to 1786669 of SEQ ID NO: 1)							
Start Site	Stop Site	ISIS No	Target allele	Sequence	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	n/a	TCTCTATTGCACATTCCAAG	100	99	6
186649	186667	476469	Major (8)	GGACAGGGTGTGCTCTCCG	80	58	282
186650	186668	476449	Major (9)	GGGACAGGGTGTGCTCTCC	80	64	283
186651	186669	*435882	Major (10)	GGGGACAGGGTGTGCTCTC	61	61	155

Example 8: Dose-dependent antisense inhibition of human huntingtin mRNA levels in Coriell fibroblast cell lines

[0368] Gapmers from the studies described in Example 7 were selected and tested at various doses in GM04281, GM02171, and GM02173B cell lines. Each cell line was plated at a density of 25,000 cells per well and transfected using electroporation with 750 nM, 1,500 nM, 3,000 nM, 6,000 nM, and 12,000 nM concentrations of antisense oligonucleotide, as specified in Tables 49, 50, and 51. After a treatment period of approximately 16 hours, RNA was isolated from the cells and *HTT* mRNA levels were measured by quantitative real-time PCR. Human *HTT* primer probe set RTS2617 was used to measure mRNA levels. *HTT* mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented as percent inhibition of *HTT* mRNA, relative to untreated control cells. IC_{50} values are also provided in Tables 49, 50, and 51.

Table 49

Dose-dependent antisense inhibition of human *HTT* in GM04281 cells						
ISIS No.	750 nM	1500 nM	3000 nM	6000 nM	12000 nM	IC_{50} (μM)
387916	67	88	95	97	99	<0.8
463566	25	65	79	88	95	1.5
463567	34	73	90	93	98	1.1
463568	33	56	75	87	92	1.3
463571	32	21	70	90	93	1.4
476441	11	27	50	70	87	3.1
476444	20	31	68	49	93	2.3
476449	4	28	34	47	77	4.9
476453	21	21	48	73	85	2.7
476455	5	19	34	56	80	4.6
476458	36	72	83	93	96	1.1
476459	23	59	75	85	91	1.5
476469	17	27	47	47	67	5.5
476473	0	6	32	50	68	6.2
476476	3	7	32	53	86	4.9

Table 50

Dose-dependent antisense inhibition of human *HTT* in GM02171 cells						
ISIS No.	750 nM	1500 nM	3000 nM	6000 nM	12000 nM	IC_{50} (μM)
387916	59	79	93	98	98	<0.8
463566	4	33	42	62	79	3.8
463567	38	41	69	85	94	1.5
463568	21	26	41	58	64	4.8
463571	8	23	56	63	75	3.7
476441	0	13	7	0	12	>12.0
476444	11	0	0	67	59	8.8
476449	4	27	37	51	63	5.8
476453	6	40	40	51	73	4.9
476455	32	15	18	47	61	7.8
476458	42	54	71	86	84	1.2
476459	22	38	70	44	73	4.3
476469	7	24	30	56	58	7.8

Dose-dependent antisense inhibition of human *HTT* in GM02171 cells						
ISIS No.	750 nM	1500 nM	3000 nM	6000 nM	12000 nM	IC_{50} (μM)
476473	4	10	15	33	43	>12.0
476476	5	16	18	23	41	>12.0

Table 51

Dose-dependent antisense inhibition of human *HTT* in GM02171 cells						
ISIS No.	750 nM	1500 nM	3000 nM	6000 nM	12000 nM	IC_{50} (μM)
387916	66	89	95	97	99	<0.8
463566	32	55	76	77	93	1.3
463567	51	61	87	94	97	0.7
463568	26	23	72	87	94	1.6
463571	32	34	60	86	94	1.9
476441	18	18	27	47	44	>12.0
476444	15	0	31	51	58	7.1
476449	27	33	56	80	81	2.6
476453	24	28	55	75	83	2.7
476455	24	26	52	55	73	3.7
476458	63	77	87	89	94	0.2
476459	37	55	56	62	86	1.5
476469	22	41	40	63	76	2.9
476473	7	28	33	51	73	5.0
476476	11	29	26	55	69	4.6

Example 9: Antisense inhibition of human *HTT* in GM04281 cells by oligonucleotides designed by microwalk

[0369] Additional gapmers were designed based on the gapmers selected from studies described in Example 4. These gapmers were designed by creating gapmers shifted slightly upstream and downstream (i.e. "microwalk") of the original gapmers from Tables 8, 9, and 10. Gapmers were also created with 3-9-3 or 5-9-5 motifs, and with constrained 6(S)-CH_3-bicyclic nucleic acid (BNA) molecules at various nucleoside positions.

[0370] These gapmers were tested *in vitro*. Cultured GM04281 cells at a density of 25,000 cells per well were transfected using electroporation with 5,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and *HTT* mRNA levels were measured by quantitative real-time PCR. *HTT* mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented as percent inhibition of *HTT* mRNA, relative to untreated control cells.

[0371] The chimeric antisense oligonucleotides in Tables 52-56 were designed as 3-9-3 or 5-9-5 gapmers. The parent gapmers, ISIS 435869, ISIS 435870, ISIS 435874, ISIS 435879, and ISIS 435890, from which the newly designed gapmers were derived are marked with an asterisk (*) in the table. ISIS 387916 was included in the study as a benchmark oligonucleotide against which the potency of the antisense oligonucleotides targeting nucleotides overlapping each SNP position could be compared.

[0372] The 3-9-3 gapmers are 15 nucleotides in length, wherein the central gap segment is comprised of nine 2'-deoxynucleosides and is flanked on both 5' and 3' directions by wings comprising 3 sugar modified nucleosides each.

[0373] The 5-9-5 gapmers are 19 nucleotides in length, wherein the central gap segment is comprised of nine 2'-deoxynucleosides and is flanked on both 5' and 3' directions by wings comprising 5 sugar modified nucleosides each.

[0374] The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine nucleobases throughout each gapmer are 5-methylcytosines. Bolded and underlined nucleotides in Tables 52-56 indicate the positions of the 6(S)-CH_3-BNA molecules (e.g. cEt molecules) in each gapmer. Italicized nucleotides are MOE subunits.

[0375] "Start site" indicates the 5'-most nucleotide to which the gapmer is targeted. "Stop site" indicates the 3'-most nucleotide to which the gapmer is targeted. 'Target allele' indicates whether the gapmer is targeted to the major or the minor allele. The number in parentheses indicates the position on the oligonucleotide opposite to the SNP position.

Table 52

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs4690072 (nucleobases 62147 to 62173 of SEQ ID NO: 1)							
Start Site	Stop Site	Target allele	ISIS No.	Sequence	Motif	% inhibition	SEQ ID NO
145466	145485	n/a	387916	*TCTCT*ATTGCACATT*CCAAG*	5-10-5	97	6
62147	62165	Major (6)	460266	*GTGCT***A**CCCAACCT*TTCTG*	5-9-5	63	169
62151	62169	Major (10)	*435874	*CACAG*TGCTACCCA*ACCTT*	5-9-5	50	28
62151	62169	Major (10)	460213	*CACAG*TGCT**A**CCCA*ACCTT*	5-9-5	22	28
62151	62169	Major (10)	460220	*CACAG*TGC**T**ACCCA*ACCTT*	5-9-5	24	28
62151	62169	Major (10)	460221	*CACAG*TGCTA**C**CCA*ACCTT*	5-9-5	28	28
62153	62167	Major (8)	460208	C**AG**TGCTACCCA**AC***C*	3-9-3	81	177
62155	62173	Major (14)	460267	*ATATC*ACAGTGCT**A***CCCAA*	5-9-5	37	180

Table 53

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs2298969 (nucleobases 125884 to 125910 of SEQ ID NO: 1)							
Start Site	Stop Site	Target allele	ISIS No.	Sequence	Motif	% inhibition	SEQ ID NO
145466	145485	n/a	387916	*TCTCT*ATTGCACATT*CCAAG*	5-10-5	97	6
125884	125902	Minor (6)	460233	*GATGC***T**GACTTGGG*CCATT*	5-9-5	76	182
125888	125906	Minor (10)	*435890	*AAGGG*ATGCTGACT*TGGGC*	5-9-5	75	94
125888	125906	Minor (10)	460215	*AAGGG*ATGC**T**GACT*TGGGC*	5-9-5	26	94
125888	125906	Minor (10)	460224	*AAGGG*ATG**C**TGACT*TGGGC*	5-9-5	38	94
125888	125906	Minor (10)	460225	*AAGGG*ATGCT**G**ACT*TGGGC*	5-9-5	49	94
125890	125904	Minor (8)	460210	*G***GG**ATGCTGACT**TG***G*	3-9-3	97	189
125892	125910	Minor (14)	460229	*TGCCA*AGGGATGC**T***GACTT*	5-9-5	60	192

Table 54

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs7685686 (nucleobases 146782 to 146808 of SEQ ID NO: 1)							
Start Site	Stop Site	Target allele	ISIS No.	Sequence	Motif	% inhibition	SEQ ID NO
145466	145485	n/a	387916	*TCTCT*ATTGCACATT*CCAAG*	5-10-5	97	6
146782	146800	Major (6)	460232	AATT**G**TCATCACCAGAAAA	5-9-5	82	195

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs7685686 (nucleobases 146782 to 146808 of SEQ ID NO: 1)							
Start Site	Stop Site	Target allele	ISIS No.	Sequence	Motif	% inhibition	SEQ ID NO
146786	146804	Major (10)	*435879	*AATAA*ATTGTCATC*ACCAG*	5-9-5	84	99
146786	146804	Major (10)	460214	*AATAA*ATTG**T**CATC*ACCAG*	5-9-5	33	99
146786	146804	Major (10)	460222	*AATAA*ATT**G**TCATC*ACCAG*	5-9-5	87	99
146786	146804	Major (10)	460223	*AATAA*ATTGT**C**ATC*ACCAG*	5-9-5	75	99
146788	146802	Major (8)	460209	*T***AA**ATTGTCATC**AC***C*	3-9-3	96	203
146790	146808	Major (14)	460228	*TATTA*ATAAATTG**T***CATCA*	5-9-5	0	206

Table 55

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs362331 (nucleobases 155475 to 155501 of SEQ ID NO: 1)							
Start Site	Stop Site	Target allele	ISIS No.	Sequence	Motif	% inhibition	SEQ ID NO
145466	145485	n/a	387916	*TCTCT*ATTGCACATT*CCAAG*	5-10-5	97	6
155475	155493	Major (6)	460231	*ACAGT***A**GATGAGGG*AGCAG*	5-9-5	88	209
155479	155497	Major (10)	*435870	*GCACA*CAGTAGATG*AGGGA*	5-9-5	86	103
155479	155497	Major (10)	460212	*GCACA*CAGT**A**GATG*AGGGA*	5-9-5	89	103
155479	155497	Major (10)	460218	*GCACA*CAG**T**AGATG*AGGGA*	5-9-5	90	103
155479	155497	Major (10)	460219	*GCACA*CAGTA**G**ATG*AGGGA*	5-9-5	88	103
155481	155495	Major (8)	460207	*A***CA**CAGTAGATG**AG***G*	3-9-3	89	217
155483	155501	Major (14)	460227	*AAGTG*CACACAGT**A***GATGA*	5-9-5	45	220

Table 56

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs362306 (nucleobases 181740 to 181766 of SEQ ID NO: 1)							
Start Site	Stop Site	Target allele	ISIS No.	Sequence	Motif	% inhibition	SEQ ID NO
145466	145485	n/a	387916	*TCTCT*ATTGCACATTC*CAAG*	5-10-5	97	6
181740	181758	Major (6)	460230	*AGCTG***C**AACCTGGC*AACAA*	5-9-5	66	223
181744	181762	Major (10)	*435869	*GAGCA*GCTGCAACC*TGGCA*	5-9-5	69	149
181744	181762	Major (10)	460211	*GAGCA*GCTG**C**AACC*TGGCA*	5-9-5	22	149
181744	181762	Major (10)	460216	*GAGCA*GCT**G**CAACC*TGGCA*	5-9-5	18	149
181744	181762	Major (10)	460217	*GAGCA*GCTGC**A**ACC*TGGCA*	5-9-5	56	149
181746	181760	Major (8)	460206	*G***CA**GCTGCAACC**TG***G*	3-9-3	83	231
181748	181766	Major (14)	460226	*GCAAG*AGCAGCTG**C***AACCT*	5-9-5	51	234

Example 10: Dose-dependent antisense inhibition of human huntingtin mRNA levels in Coriell fibroblast cell lines

[0376] Gapmers from studies described in Example 9 were selected and tested at various doses in GM04281, GM02171 and GM02173B cell lines. Each cell line was plated at a density of 25,000 cells per well and transfected using electroporation with 312.5 nM, 625 nM, 1,250 nM, 2,500 nM, 5,000 nM and 10,000 nM concentrations of antisense oligonucleotide, as specified in Tables 75, 58, and 59. After a treatment period of approximately 16 hours, RNA was isolated from the cells and *HTT* mRNA levels were measured by quantitative real-time PCR. Human *HTT* primer probe set RTS2617 was used to measure mRNA levels. *HTT* mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented as percent inhibition of *HTT* mRNA, relative to untreated control cells. IC_{50} values are also provided in Tables 57, 58, and 59.

Table 57

Dose-dependent antisense inhibition of human *HTT* in GM04281 cells							
ISIS No.	312.5 nM	625 nM	1,250 nM	2,500 nM	5,000 nM	10,000 nM	IC_{50} (μM)
387916	26	49	68	86	94	97	0.7
435869	0	0	23	48	62	82	3.2
435870	15	38	50	65	85	88	1.3
435874	14	22	32	49	65	73	2.7
435879	0	17	40	61	83	94	1.8
435890	5	13	37	56	70	82	2.3
460206	10	18	37	52	66	85	2.3
460207	20	27	50	65	80	91	1.4
460208	21	34	51	63	70	79	1.5
460209	52	74	89	94	94	95	0.2
460210	34	61	84	91	97	98	0.5
460212	13	31	50	62	75	82	1.6
460218	14	27	50	63	78	86	1.8
460219	9	32	42	64	77	87	1.6
460222	19	21	42	57	73	78	1.7
460231	12	24	41	57	71	84	1.9
460233	16	28	59	66	72	74	1.8
460266	4	17	32	48	60	75	2.9

Table 58

Dose-dependent antisense inhibition of human *HTT* in GM02171 cells							
ISIS No.	312.5 nM	625 nM	1,250 nM	2,500 nM	5,000 nM	10,000 nM	IC_{50} (μM)
387916	32	56	77	89	95	97	0.7
435869	0	6	22	40	69	84	2.9
435870	15	19	32	51	68	77	2.4
435874	0	5	1	17	17	30	>10.0
435879	0	8	0	16	36	47	15.3
435890	14	16	19	19	39	57	9.3
460206	5	13	33	41	68	80	2.7
460207	13	10	22	22	33	39	45.6
460208	13	15	11	11	15	53	10.8
460209	8	27	46	70	80	86	1.6

Dose-dependent antisense inhibition of human *HTT* in GM02171 cells							
ISIS No.	312.5 nM	625 nM	1,250 nM	2,500 nM	5,000 nM	10,000 nM	IC_{50} (μM)
460210	19	37	55	75	88	96	1.1
460212	8	23	30	43	57	74	2.2
460218	15	26	27	36	52	78	3.2
460219	16	17	32	44	69	76	2.5
460222	14	3	0	0	13	0	>10.0
460231	6	8	13	16	33	56	10.4
460233	27	30	39	46	61	73	2.4
460266	0	15	20	15	18	34	>10.0

Table 59

Dose-dependent antisense inhibition of human *HTT* in GM02173B cells							
ISIS No.	312.5 nM	625 nM	1,250 nM	2,500 nM	5,000 nM	10,000 nM	IC_{50} (μM)
387916	22	47	76	88	96	98	0.7
435869	10	0	16	38	59	76	3.9
435870	22	36	44	58	69	81	2.0
435874	11	6	25	23	32	42	>10.0
435879	0	9	21	30	52	68	4.8
435890	12	16	30	31	48	66	4.5
460206	11	13	18	35	59	74	3.5
460207	15	25	30	37	42	66	4.3
460208	5	14	27	32	52	51	9.0
460209	27	49	61	79	81	74	0.8
460210	19	40	61	77	89	95	1.0
460212	0	19	32	32	61	78	2.9
460218	4	17	26	38	64	82	3.0
460219	5	6	26	47	68	84	2.9
460222	13	19	23	30	35	50	16.1
460231	7	33	25	35	54	77	3.7
460233	11	20	37	52	68	69	2.3
460266	12	6	10	21	25	47	>10.0

Example 11: Dose-dependent antisense inhibition of human *HTT* in GM04281 and GM02171 cells by oligonucleotides designed by microwalk

[0377] Additional gapmers were designed based on the gapmers selected from studies described in Example 10. These gapmers were designed by creating gapmers shifted slightly upstream and downstream (i.e. "microwalk") of the original gapmers from Tables 57, 58, and 59. Gapmers were also created with 4-9-4 MOE or 5-9-5 MOE motifs, and with constrained 6(S)-CH_3-bicyclic nucleic acid (BNA) molecules at various nucleotide positions.

[0378] These gapmers were tested in the GM04281 and GM02171 cell lines. Cultured GM04281 or GM02171 cells at a density of 25,000 cells per well were transfected using electroporation with 2,500 nM or 5,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and *HTT* mRNA levels were measured by quantitative real-time PCR. *HTT* mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented as percent inhibition of *HTT* mRNA, relative to untreated control

cells.

[0379] The chimeric antisense oligonucleotides in Tables 60, 61, and 62 were designed as 3-9-3, 4-9-4, or 5-9-5 MOE gapmers. The parent gapmers, ISIS 435890, ISIS 460210, ISIS 435879, ISIS 460209, ISIS 435870, and ISIS 460207, from which the newly designed gapmers were derived are marked with an asterisk (*) in the table. ISIS 387916 was included in the study as a benchmark oligonucleotide against which the potency of the antisense oligonucleotides targeting nucleotides overlapping each SNP position could be compared.

[0380] The 3-9-3 gapmers are 15 nucleotides in length, wherein the central gap segment is comprised of nine 2'-deoxynucleotides and is flanked on both 5' and 3' directions by wings comprising 3 nucleotides each.

[0381] The 4-9-4 gapmers are 17 nucleotides in length, wherein the central gap segment is comprised of nine 2'-deoxynucleotides and is flanked on both 5' and 3' directions by wings comprising 4 nucleotides each.

[0382] The 5-9-5 gapmers are 19 nucleotides in length, wherein the central gap segment is comprised of nine 2'-deoxynucleotides and is flanked on both 5' and 3' directions by wings comprising 5 nucleotides each.

[0383] The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine nucleobases throughout each gapmer are 5-methylcytosines. Bolded and underlined nucleotides in Tables 60, 61, and 62 indicate the positions of the 6(S)-CH_3-BNA (e.g. cEt molecules)molecules in each gapmer. Italicized nucleotides are MOE subunits.

[0384] The gapmers are organized in Tables 60, 61, and 62, according to the SNP site they target. "Start site" indicates the 5'-most nucleotide to which the gapmer is targeted. "Stop site" indicates the 3'-most nucleotide to which the gapmer is targeted. 'Target allele' indicates whether the gapmer is targeted to the major or the minor allele. The number in parentheses indicates the position on the oligonucleotide opposite to the SNP position.

Table 60

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs2298969 (nucleobases 125888 to 125907 of SEQ ID NO: 1)								
Start position	Stop position	ISIS No.	Sequence	Motif	Concentration (nM)	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	TCTCTATTGCA CATTCCAAG	5-10-5	5000	57	24	6
125888	125907	*435890	*AAGGG*ATGCTG ACT*TGGGC*	5-9-5	2500	22	0	94
					5000	41	23	
125890	125904	*460210	G**GG**ATGCTGAC T**TG**G	3-9-3	2500	59	24	189
					5000	81	33	
125889	125905	474870	A**GGG**ATGCTG ACT**TGG**G	4-9-4	2500	23	3	187
					5000	44	34	
125889	125905	474890	A**GG**GATGCTG ACT**TG**G**G**	4-9-4	2500	38	6	187
					5000	49	25	
125889	125905	474910	A**G**G**G**ATGCTGA CT**T**GG**G**	4-9-4	2500	34	8	187
					5000	49	41	
125889	125905	474914	A**G**G**G**ATGCTGA CTTGGG	4-9-4	2500	44	14	187

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs2298969 (nucleobases 125888 to 125907 of SEQ ID NO: 1)								
Start position	Stop position	ISIS No.	Sequence	Motif	Concentration (nM)	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
					5000	44	21	
125888	125907	474918	AAGGGATGCT GACTTGGGC	5-9-5	2500	31	0	94
					5000	26	25	
125888	125907	474922	AAGGGATGCT GACTTGGGC	5-9-5	2500	33	14	94
					5000	65	24	
125889	125905	476332	AGGGATGCTG	4-9-4	2500	23	13	187
			ACTTGGG		5000	51	42	
125888	125907	476336	AAGGGATGCTG ACTTGGGC	5-9-5	2500	5	0	94
					5000	43	9	

Table 61

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs7685686 (nucleobases 146786 to 146805 of SEQ ID NO: 1)								
Start position	Stop position	ISIS No.	Sequence	Motif	Concentration (nM)	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	TCTCTATTGCA CATTCCAAG	5-10-5	5000	57	24	6
146786	146805	*435879	AATAAATTGTC ATCACCAG	5-9-5	2500	39	0	99
					5000	59	19	
146788	146802	*460209	TAAATTGTCAT CACC	3-9-3	2500	3	0	203
					5000	13	5	
146787	146803	474871	ATAAATTGTCA TCACCA	4-9-4	2500	82	32	200
					5000	83	58	
146787	146803	474891	ATAAATTGTCA TCACCA	4-9-4	2500	84	29	200
					5000	89	56	
146787	146803	474911	ATAAATTGTCA TCACCA	4-9-4	2500	70	18	200
					5000	83	40	
146787	146803	474915	ATAAATTGTCA TCACCA	4-9-4	2500	38	9	200
					5000	74	14	
146786	146805	474919	AATAAATTGTC ATCACCAG	5-9-5	2500	80	7	99

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs7685686 (nucleobases 146786 to 146805 of SEQ ID NO: 1)								
Start position	Stop position	ISIS No.	Sequence	Motif	Concentration (nM)	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
					5000	84	37	
146786	146805	474923	AATAAATTGTC ATCACCAG	5-9-5	2500	74	32	99
					5000	83	51	
146787	146803	476333	ATAAATTGTCA TCACCA	4-9-4	2500	75	28	200
					5000	86	21	
146786	146805	476337	AATAAATTGTC ATCACCAG	5-9-5	2500	71	6	99
					5000	83	31	

Table 62

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs362331 (nucleobases 155478 to 155498 of SEQ ID NO: 1)								
Start position	Stop position	ISIS No.	Sequence	Motif	Concentration (nM)	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
145466	145485	387916	TCTCTATTGCAC ATTCCAAG	5-10-5	5000	57	24	6
155479	155498	*435870	TCTCTATTGCAC ATTCCAAG	5-9-5	2500 5000	19 49	1 34	103
155481	155495	*460207	ACACAGTAGAT GAGG	3-9-3	2500	0	0	217
					5000	7	8	
155480	155496	474872	CACACAGTAGA TGAGGG	4-9-4	2500	35	9	214
					5000	63	37	
155480	155496	474892	CACACAGTAGA TGAGGG	4-9-4	2500	43	16	214
					5000	69	31	
155480	155496	474912	CACACAGTAGA TGAGGG	4-9-4	2500	16	9	214
					5000	36	6	
155480	155496	474916	CACACAGTAGA TGAGGG	4-9-4	2500	22	5	214
					5000	47	7	
155479	155498	474920	GCACACAGTAG ATGAGGGA	5-9-5	2500	19	0	103
					5000	43	23	
155479	155498	474924	GCACACAGTAG ATGAGGGA	5-9-5	2500	29	8	103
					5000	48	22	
					2500	35	7	

Comparison of inhibition of human *HTT* mRNA levels by ISIS 387916 and chimeric antisense oligonucleotides targeted to SNP rs362331 (nucleobases 155478 to 155498 of SEQ ID NO: 1)								
Start position	Stop position	ISIS No.	Sequence	Motif	Concentration (nM)	% inhibition in GM04281	% inhibition in GM02171	SEQ ID NO
155480	155496	476334	CACACAGTAGA TGAGGG	4-9-4	5000	62	32	214
155479	155498	476338	GCACACAGTAG ATGAGGGA	5-9-5	2500	26	9	103
					5000	40	4	
155479	155495	474873	ACACAGTAGAT GAGGGA	4-9-4	2500	53	9	285
					5000	61	29	
155479	155495	474893	ACACAGTAGAT GAGGGA	4-9-4	2500	47	5	285
					5000	59	30	
155479	155495	474913	ACACAGTAGAT GAGGGA	4-9-4	2500	30	16	285
					5000	29	17	
155479	155495	474917	ACACAGTAGAT GAGGGA	4-9-4	2500	23	12	285
					5000	40	5	
155478	155497	474921	CACACAGTAGA TGAGGGAG	5-9-5	2500	28	0	212
					5000	43	23	
155478	155497	474925	CACACAGTAGA TGAGGGAG	5-9-5	2500	30	9	212
					5000	61	34	
155479	155495	476335	ACACAGTAGAT GAGGGA	4-9-4	2500	35	2	285
					5000	53	31	
155478	155497	476339	CACACAGTAGA TGAGGGAG	5-9-5	2500	15	0	212
					5000	34	13	

Example 12: Dose-dependent antisense inhibition of human huntingtin mRNA levels in Coriell fibroblast cell lines

[0385] Gapmers from the studies described in Example 11 were selected and tested at various doses in GM04281, GM02171 and GM02173B cell lines. Each cell line was plated at a density of 25,000 cells per well and transfected using electroporation with 625 nM, 1,250 nM, 2,500 nM, 5,000 nM and 10,000 nM concentrations of antisense oligonucleotide, as specified in Tables 63, 64, and 65. After a treatment period of approximately 16 hours, RNA was isolated from the cells and *HTT* mRNA levels were measured by quantitative real-time PCR. Human *HTT* primer probe set RTS2617 was used to measure mRNA levels. *HTT* mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented as percent inhibition of *HTT* mRNA, relative to untreated control cells. IC_{50} values are also provided in Tables 63, 64, and 65.

Table 63

Dose-dependent antisense inhibition of human *HTT* in GM04281 cells						
ISIS No	625 nM	1250 nM	2500 nM	5000 nM	10000 nM	IC_{50} (μM)
387916	70	83	94	96	98	<0.6
460207	51	63	83	91	93	0.5
460209	83	93	96	97	97	<0.6
460210	70	89	94	97	98	0.6
474871	94	97	96	96	95	<0.6
474873	51	73	89	94	95	0.5
474891	93	95	97	96	95	<0.6
474892	48	72	89	93	95	0.6
474911	85	92	96	95	94	<0.6
474919	89	94	95	94	96	<0.6
474922	21	47	73	86	96	1.5
474923	86	94	96	95	94	<0.6
476333	92	94	95	95	96	<0.6
476334	45	70	87	92	95	0.6
476337	83	92	95	96	96	<0.6

Table 64

Dose-dependent antisense inhibition of human *HTT* in GM02171 cells						
ISIS No	625 nM	1250 nM	2500 nM	5000 nM	10000 nM	IC_{50} (μM)
387916	28	38	63	82	99	1.6
460207	16	0	20	22	55	10.0
460209	27	50	61	87	94	9.9
460210	34	60	80	86	97	0.9
474871	62	74	84	87	90	0.1
474873	13	29	61	77	89	2.2
474891	57	72	80	83	88	0.2
474892	23	26	51	68	81	2.5
474911	47	58	68	72	82	0.7
474919	44	48	65	71	83	1.1
474922	15	27	49	74	79	2.6
474923	27	53	74	79	84	1.5
476333	42	53	75	76	84	1.0
476334	20	23	58	71	87	2.3
476337	23	34	60	62	75	2.7

Table 65

Dose-dependent antisense inhibition of human *HTT* in GM02173B cells						
ISIS No	625 nM	1250 nM	2500 nM	5000 nM	10000 nM	IC_{50} (μM)
387916	38	75	89	95	99	0.9
460207	13	27	52	46	63	6.5
460209	79	68	84	90	92	<0.6
460210	37	62	79	92	97	0.9
474871	74	83	87	92	89	<0.6
474873	22	32	67	72	92	1.9
474891	69	78	84	89	89	<0.6

Dose-dependent antisense inhibition of human *HTT* in GM02173B cells						
ISIS No	625 nM	1250 nM	2500 nM	5000 nM	10000 nM	IC_{50} (μM)
474892	26	50	75	83	91	1.3
474911	50	66	76	86	86	0.6
474919	57	67	74	87	82	<0.6
474922	15	32	61	71	90	2.2
474923	49	67	78	83	85	0.5
476333	58	71	78	87	89	<0.6
476334	20	42	63	76	91	1.8
476337	48	63	71	79	80	0.6

Example 13: Strategy for selection of antisense oligonucleotides based on potency and selectivity

[0386] Gapmers from each of the studies described above were selected for further analysis based on potency and selectivity.

[0387] Potency was based on the percent inhibition of *HTT mRNA* achieved by the antisense oligonucleotides targeting a SNP compared to the percent inhibition of *HTT mRNA* achieved by the benchmark oligonucleotide, ISIS 387916.

[0388] Selectivity was based on the ability of the antisense oligonucleotides targeting a SNP to inhibit expression of the major allele and not of the minor allele. The usage of the three cell lines with different genotypes at each SNP position facilitated this process.

[0389] ISIS 460065 (5'-ATAAATTGTCATCACCAG-3' (SEQ ID NO: 199)) is a 4-9-5 MOE gapmer targeted to SNP rs7685686 (major allele A, minor allele G) at position 9 of the oligonucleotide. The GM04281 cell line is homozygous AA at SNP position rs7685686. The GM02173B cell line is heterozygous AG at SNP position rs7685686. The GM02171 cell line is homozygous GG at SNP position rs7685686. Therefore, selectivity is shown if ISIS 460065 causes potent inhibition of *HTT* mRNA in GM04281, less potent inhibition of *HTT* mRNA in GM02173, and little to no significant inhibition of *HTT* mRNA in GM02171. IC50 values taken from Table 20, 21, and 22, and presented below in Table 66, confirm varying degrees of inhibition in the three cell lines, wherein expression was most reduced in the homozygous AA cell line, moderately reduced in the heterozygous AG cell line, and less reduced in the homozygous GG cell line. IC50 is the concentration of antisense oligonucleotide required for 50 percent inhibition *HTT* mRNA. IC50 values are in μM.

Table 66

Genotype of the Coriell cell lines for SNP rs7685686 and comparison of inhibition of *HTT* mRNA by ISIS 460065 in each cell line			
	GM04281	GM02173B	GM02171
Genotype	AA	AG	GG
IC_{50} with ISIS 460065	1.1	3.6	10.3

[0390] ISIS 459978 (5'-ACAGTGCTACCCAACCT-3' (SEQ ID NO: 174)) is a 2-9-6 MOE gapmer targeted to SNP rs4690072 (major allele T, minor allele G) at position 9 of the oligonucleotide. The GM04281 cell line is homozygous TT at SNP position rs4690072. The GM02173B cell line is heterozygous TG at SNP position rs4690072. The GM02171 cell line is homozygous GG at SNP position rs4690072. Therefore, selectivity is shown if ISIS 459978 causes potent inhibition of *HTT* mRNA in GM04281, less potent inhibition of *HTT mRNA* in GM02173, and little to no significant inhibition of *HTT* mRNA in GM02171. IC50 values taken from Table 20, 21, and 22, and presented below in Table 67, confirm varying degrees of inhibition in the three cell lines, wherein expression was most reduced in the homozygous TT cell line, moderately reduced in the heterozygous TG cell line, and less reduced in the homozygous GG cell line.

IC50 is the concentration of antisense oligonucleotide required for 50 percent inhibition *HTT* mRNA. IC50 values are in μM.

Table 67

Genotype of the Coriell cell lines for SNP rs4690072 and comparison of inhibition of *HTT* mRNA by ISIS 459978 in each cell line			
	GM04281	GM02173B	GM02171
Genotype	TT	TG	GG
IC_{50} with ISIS 459978	2.5	8.4	12.7

[0391] ISIS 460028 (5'-GAGCAGCTGCAACCTGGCA -3' (SEQ ID NO: 149)) is a 4-11-4 MOE gapmer targeted to SNP rs362306 (major allele G, minor allele A) at position 10 of the oligonucleotide. The GM04281 cell line is homozygous GG at SNP position rs362306. The GM02173B and GM02171 cell lines are heterozygous GA at SNP position rs362306. Therefore, selectivity is shown if ISIS 460028 causes potent inhibition of *HTT* mRNA in GM04281 and less potent inhibition of *HTT* mRNA in GM02173 and GM02171. IC50 values taken from Table 20, 21, and 22, and presented below in Table 68, confirm varying degrees of inhibition between the GM04281 cell line and the GM02173B and GM02171 cell lines, wherein expression was most reduced in the homozygous GG cell line and less reduced in the heterozygous AG cell line. IC50 is the concentration of antisense oligonucleotide required for 50 percent inhibition *HTT* mRNA. IC50 values are in μM.

Table 68

Genotype of the Coriell cell lines for SNP rs362306 and comparison of inhibition of *HTT* mRNA by ISIS 460028 in each cell line			
	GM04281	GM02173B	GM02171
Genotype	GG	AG	AG
IC_{50} with ISIS 460028	1.4	5.2	5.3

Example 14: Strategy for selection of antisense oligonucleotides with cEt motifs based on potency and selectivity

[0392] Gapmers from each of the studies described above were selected for further analysis based on potency and selectivity.

[0393] Potency was based on the percent inhibition o*f HTT mRNA* achieved by the antisense oligonucleotides targeting a SNP compared to the percent inhibition of *HTT* mRNA achieved by the benchmark oligonucleotide, ISIS 387916.

[0394] Selectivity was based on the ability of the antisense oligonucleotides targeting a SNP to inhibit expression of the major allele and not of the minor allele. The usage of the three cell lines with different genotypes at each SNP position facilitated this process.

[0395] ISIS 460209 (5'- TAAATTGTCATCACC -3' (SEQ ID NO: 203)) is a 3-9-3 gapmer with cEt subunits at positions 2, 3, 13, and 14, targeted to SNP rs7685686 (major allele A, minor allele G) at position 8 of the oligonucleotide. The GM04281 cell line is homozygous AA at SNP position rs7685686. The GM02173B cell line is heterozygous AG at SNP position rs7685686. The GM02171 cell line is homozygous GG at SNP position rs7685686. Therefore, selectivity is shown if ISIS 460209 causes potent inhibition of HTT mRNA in GM04281, less potent inhibition of HTT mRNA in GM02173, and little to no significant inhibition of HTT mRNA in GM02171. IC_{50} values taken from Table 57, 58, and 59, and presented below in Table 69, confirm varying degrees of inhibition in the three cell lines, wherein expression was most reduced in the homozygous AA cell line, moderately reduced in the heterozygous AG cell line, and less reduced in the homozygous GG cell line. IC_{50} is the concentration of antisense oligonucleotide required for 50 percent inhibition HTT mRNA. IC_{50} values are in μM.

Table 69

Genotype of the Coriell cell lines for SNP rs7685686 and comparison of inhibition of HTT mRNA by ISIS 460209 in each cell line			
	GM04281	GM02173B	GM02171
Genotype	AA	AG	GG
IC_{50} with ISIS 460209	0.2	0.8	1.6

[0396] ISIS 460208 (5'- CAGTGCTACCCAACC -3' (SEQ ID NO: 177)) is a 3-9-3 gapmer with cEt subunits at positions 2, 3, 13, and 14, targeted to SNP rs4690072 (major allele T, minor allele G) at position 8 of the oligonucleotide. The GM04281 cell line is homozygous TT at SNP position rs4690072. The GM02173B cell line is heterozygous TG at SNP position rs4690072. The GM02171 cell line is homozygous GG at SNP position rs4690072. Therefore, selectivity is shown if ISIS 460208 causes potent inhibition of HTT mRNA in GM04281, less potent inhibition of HTT mRNA in GM02173, and little to no significant inhibition of HTT mRNA in GM02171. IC_{50} values taken from Table 57, 58, and 59, and presented below in Table 70, confirm varying degrees of inhibition in the three cell lines, wherein expression was most reduced in the homozygous TT cell line, moderately reduced in the heterozygous TG cell line, and less reduced in the homozygous GG cell line. IC_{50} is the concentration of antisense oligonucleotide required for 50 percent inhibition HTT mRNA. IC_{50} values are in μM.

Table 70

Genotype of the Coriell cell lines for SNP rs4690072 and comparison of inhibition of HTT mRNA by ISIS 460208 in each cell line			
	GM04281	GM02173B	GM02171
Genotype	TT	TG	GG
IC_{50} with ISIS 460208	1.5	9.0	10.8

[0397] ISIS 460206 (5'- GCAGCTGCAACCTGG -3' (SEQ ID NO: 231)) is a 3-9-3 gapmer with cEt subunits at positions 2, 3, 13, and 14, targeted to SNP rs362306 (major allele G, minor allele A) at position 8 of the oligonucleotide. The GM04281 cell line is homozygous GG at SNP position rs362306. The GM02173B and GM02171 cell lines are heterozygous GA at SNP position rs362306. Therefore, selectivity is shown if ISIS 460206 causes potent inhibition of HTT mRNA in GM04281 and less potent inhibition of HTT mRNA in GM02173 and GM02171. IC_{50} values taken from Table 57, 58, and 59, and presented below in Table 71, confirm varying degrees of inhibition between the GM04281 cell line and the GM02173B and GM02171 cell lines, wherein expression was most reduced in the homozygous GG cell line and less reduced in the heterozygous AG cell line. IC_{50} is the concentration of antisense oligonucleotide required for 50 percent inhibition HTT mRNA. IC_{50} values are in μM.

Table 71

Genotype of the Coriell cell lines for SNP rs362306 and comparison of inhibition of HTT mRNA by ISIS 460206 in each cell line			
	GM04281	GM02173B	GM02171
Genotype	GG	AG	AG
IC_{50} with ISIS 460206	2.3	2.7	2.7

Example 15: Comparison of SNPs in various cell lines and mouse models associated with Huntington's disease

[0398] The genotype at various SNP positions associated with Huntington's disease was compared amongst the three Corriell cell lines, used in the above Examples, as well as with the GM04022 fibroblast, the BACHD mouse model and the YAC18 mouse model.

[0399] The donor patient of the GM04022 fibroblast cell line was heterozygous at SNP position rs363125 (NCBI Entrez

SNP database), harboring an A allele (adenine) and a C allele (cytosine) at nucleotide 5310 of SEQ ID NO: 2 (van Bilsen, P.H.J. et al., Human Gene Therapy.19: 710-718, 2008). YAC18 mice were developed with a YAC transgene containing human huntingtin gene (Hodgson, et al. Hum. Mol. Genet. 5: 1875-85, 1996). BACHD mice were developed expressing a full-length mutant huntingtin gene with 97 glutamine repeats under the control of a bacterial artificial chromosome (Gray, M. et al., J. Neurosc. 28: 6182-95, 2008). The comparative genotype at the indicated SNP positions in all four cell lines and mouse models is presented in Table 72.

Table 72

Genotypes of the Coriell cell lines and Huntington mouse models						
SNP	GM02171	GM02173	GM04281	GM04022	BACHD	YAC18
rs3856973	AA	AG	GG	AG	GG	AA
rs2285086	GG	AG	AA	AG	AA	GG
rs7659144	CG	CG	CC	CG	CC	GG
rs16843804	TC	TC	CC	CC	CC	TT
rs2024115	GG	AG	AA	AG	AA	GG
rs3733217	CC	CC	CC	CC	CC	CC
rs10015979	AA	AG	GG	AA	AA	AA
rs7691627	AA	AG	GG	AG	GG	AA
rs2798235	GG	GG	GG	AG	GG	GG
rs4690072	GG	TG	TT	TG	TT	GG
rs6446723	CC	TC	TT	TC	TT	CC
rs363081	GG	GG	GG	GG	GG	GG
rs363080	CC	CC	CC	TC	CC	CC
rs363075	GG	GG	GG	GG	GG	GG
rs363064	TC	TC	CC	CC	CC	TT
rs3025849	AA	AA	AA	AA	AA	AA
rs363102	AA	AA	AA	AG	AA	AA
rs11731237	CC	TC	TT	CC	CC	CC
rs4690073	AA	AG	GG	AG	GG	AA
rs363144	TT	TT	TT	TT	TT	TT
rs3025838	CC	CC	CC	CC	CC	CC
rs34315806	TC	TC	CC	CC	CC	TT
rs363099	TC	TC	CC	CC	CC	TT
rs363096	CC	TC	TT	CC	TT	CC
rs2298967	TC	TC	TT	TT	TT	CC
rs2298969	GG	AG	AA	AG	AA	GG
rs6844859	CC	TC	TT	TC	TT	CC
rs363092	AA	AC	CC	AC	AA	AA
rs7685686	GG	AG	AA	AG	AA	GG
rs363088	TA	TA	AA	AA	AA	TT
rs362331	CC	TC	TT	TC	TT	CC
rs916171	GG	GC	CC	GC	CC	GG
rs362322	AA	AA	AA	AA	AA	AA
rs362275	TC	TC	CC	CC	CC	TT
rs362273	AG	AG	AA	AA	AA	GG
rs2276881	GG	GG	GG	GG	GG	GG
rs3121419	TC	TC	CC	CC	CC	TT

Genotypes of the Coriell cell lines and Huntington mouse models						
SNP	GM02171	GM02173	GM04281	GM04022	BACHD	YAC18
rs362272	-	AG	GG	GG	GG	AA
rs362271	AG	AG	GG	GG	GG	AA
rs3775061	AG	AG	AA	AA	AA	GG
rs362310	TC	CC	CC	TC	CC	CC
rs362307	CC	TC	CC	CC	CC	CC
rs362306	AG	AG	GG	GG	GG	AA
rs362303	TC	CC	CC	TC	CC	CC
rs362296	AC	AC	AC	CC	CC	AA

Example 16: Allele-specific inhibition measured in BacHD cortical neurons

[0400] Antisense oligonucleotides, ISIS 460209 (5'-TAAATTGTCATCACC-3' (SEQ ID NO: 203)), targeting SNP rs7685686 of human *HTT*, and ISIS 387916 (TCTCTATTGCACATTCCAAG (SEQ ID NO: 6)), and with no human or murine SNP target site, were tested for their effect on Htt protein levels in *vitro*. ISIS 387916 is cross-reactive with murine Htt mRNA (GENBANK Accession No. NM_010414.1, designated herein as SEQ ID NO: 286) at target start site 5763 with one mismatch. ISIS 460209 is cross-reactive with murine Htt mRNA at target start site 6866 with three mismatches.

[0401] Primary BacHD cortical neurons, which express human Htt and murine Htt, were isolated in the following way: Embryos were dissected from E15.5-E17.5 pregnant females. Cortices were dissected into ice-cold divalent-free Hank's Balanced Salt Solution (Invitrogen, 14025-134). The cortices were chopped into pieces and digested with 0.05% Trypsin-EDTA (Invitrogen, 25300-120) at 37°C for 8 minutes. The digestion was halted by addition of complete neurobasal media (Invitrogen, 10888-022). Cells were resuspended in media and treated with DNAse I (Invitrogen, 18047-019). After titration through a 100ul pipette tip, cells are resuspended in neurobasal media with B27 supplement (Invitrogen, 17504-044), and counted. 1.7 x 10^5 cells/well were plated in 24-well plates precoated with poly-D-lysine (BD Biosciences, 354210). Neurons were fed with 200 µl neurobasal media with B27 on the second day in vitro.

[0402] ISIS 460209 or ISIS 387916 was added to the supplementary media fed to neurons on division 2 at 0.7 µM, 1.4 µM or 1.5 µM final concentrations. Cells were harvested after 8 days with into 1 mL of media using a cell scraper. Cells were centrifuged at 2,500 rpm for 5 min at 4°C and the pellets were resuspended in a buffer of 50 mM Tris, pH = 8.0, 150 mM NaCl, 1% Igepal, 40 mM β-glycerophosphate, 10mM NaF, 1 x Roche complete protease inhibitor, 1mM Sodium Orthovanadate and 800 µM PMSF. The lysates were centrifuged after 15 min incubation and protein concentration was measured with the DC assay (BioRad).

[0403] Protein lysates were run on low-bis gels to separate huntingtin alleles (resolving gel - 2001:Acrylamide:BIS (10% acrylamide, 0.5% BIS, 375mMTris pH 8.8; stacking gel - 4%Acrylamide-BIS(29:1), 156 mM Tris pH6.8; Running buffer - 25mM Tris, 190mM Glycine, 0.1% SDS + 10µM beta-mercaptoethanol added fresh). After electrophoresis, proteins in the gel were transferred to a nitrocellulose membrane (Hybond-C Extra; GE Healthcare Bio-Sciences) at 90V for 40' to allow samples to penetrate the stacking gel and then at 190V for 2.5h to resolve proteins.

[0404] Primary antibodies specific for human Htt and murine calnexin protein were used at 1:10,000 dilutions. HRP-conjugated anti-mouse secondary antibody (1:10,000, Jackson ImmunoResearch Laboratories) was used for visualizing proteins using SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific). Protein bands were quantified using ImageJ software and normalized to calnexin levels. Protein bands were quantified using ImageJ software. Table 73 provides an estimate of the percentage inhibition relative to the negative control sample. The comparative percent inhibitions of the human Htt protein and the murine Htt protein are presented.

Table 73

Effect of antisense inhibition on mutant human and wild-type murine Htt protein (percent inhibition normalized to PBS control)			
	Dose (μM)	Human	Murine
ISIS 387916	0.7	54	38
	1.4	75	58
	1.5	92	88
ISIS 460209	0.2	71	35
	0.4	82	41
	1.5	94	56

Example 17: Dose-dependent antisense inhibition of human huntingtin mRNA levels in Coriell fibroblast cell lines

[0405] Gapmers from the studies described in Examples, 3, 4, 10, and 12 were selected and tested at various doses in GM04281, GM02171 and GM02173B cell lines. Each cell line was plated at a density of 25,000 cells per well and transfected using electroporation with 0.4747 nM, 1.5011 nM, 4.7463 nM, 15.0079 nM 45.455 nM, 150.0527 nM, 474.4673 nM, 1,500.27 nM, 4,743.833 nM, and 15,000 nM concentrations of antisense oligonucleotide, as specified in Tables 72, 73, and 74. After a treatment period of approximately 16 hours, RNA was isolated from the cells and *HTT* mRNA levels were measured by quantitative real-time PCR. Human *HTT* primer probe set RTS2617 was used to measure mRNA levels. *HTT* mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN. Results are presented as percent inhibition of *HTT* mRNA, relative to untreated control cells. IC_{50} values are also provided in Tables 72, 73, and 74.

Table 74

Dose-dependent antisense inhibition of human *HTT* in GM04281 cells											
ISIS No	0.4747 nM	1.5011 nM	4.7463 nM	15.0079 nM	47.455 nM	150.0527 nM	474.4673 nM	1500.27 nM	4743.833 nM	15000.0 nM	IC_{50} (μM)
387916	15	12	4	5	7	26	70	89	98	99	0.33
435879	0	8	19	13	24	23	45	53	84	93	0.25
435890	16	1	8	12	25	23	32	52	61	91	0.82
460209	2	9	21	17	36	46	80	89	94	93	0.09
460210	4	7	5	19	20	35	69	85	98	98	0.21
476333	7	10	8	11	42	65	86	93	93	95	0.05

Table 75

Dose-dependent antisense inhibition of human *HTT* in GM02171 cells											
ISIS No	0.4747 nM	1.5011 nM	4.7463 nM	15.0079 nM	47.455 nM	150.0527 nM	474.4673 nM	1500.27 nM	4743.833 nM	15000.0 nM	IC_{50} (μM)
387916	22	8	0	9	0	32	60	90	96	97	0.27
435879	0	1	6	2	0	0	8	9	46	57	7.62
435890	0	0	0	6	0	0	0	31	27	71	4.37
460209	11	5	15	0	0	7	30	69	82	88	0.96
460210	0	0	0	2	17	18	38	70	93	95	0.56
476333	0	0	0	0	13	18	44	69	72	91	0.75

Table 76

Dose-dependent antisense inhibition of human *HTT* in GM02173B cells											
ISIS No	0.4747 nM	1.5011 nM	4.7463 nM	15.0079 nM	47.455 nM	150.0527 nM	474.4673 nM	1500.27 nM	4743.833 nM	15000.0 nM	IC_{50} (μM)
387916	3	17	7	25	27	33	65	88	98	99	0.19
435879	0	6	0	8	3	10	16	24	50	68	3.72
435890	0	13	0	1	2	12	16	23	49	82	4.60
460209	0	7	29	2	9	32	52	71	82	86	0.27
460210	0	13	0	5	16	18	49	74	93	97	0.27
476333	11	13	20	7	23	36	63	75	83	90	0.13

Example 18: Validation of the specificity of ISIS oligonucleotides targeting SNPs of human huntingtin by the Molecular Beacon assay

[0406] Some of the gapmers from the study described in Example 17 were tested in GM04022 fibroblasts (from the Coriell Institute for Medical Research).

[0407] To verify allele-specific suppression of *HTT* mRNA in GM04022 fibroblasts by ISIS 435879, ISIS 460209, and ISIS 476333, the Molecular Beacon assay, as described in the van Bilsen at el publication (van Bilsen, P.H.J. et al., Human Gene Therapy.19: 710-718, 2008), was conducted using 'molecular beacon' synthetic oligonucleotides linked with a fluorophore and quencher. GM04022 fibroblasts were transfected by electroporation with ISIS 435879, ISIS 460209, or ISIS 476333 at 0.06 μM, 0.19 μM, 0.56 μM, 1.67 μM, 5 μM and 15 μM concentrations of antisense oligonucleotide, as specified in Tables 75-77. ISIS 387916 was included in the assay as a benchmark oligonucleotide. The qRT-PCR assay for molecular beacon for the A allele was conducted with the annealing temperature at 56.5°C. The qRT-PCR assay for molecular beacon for the C allele was conducted with the annealing temperature at 62.0°C. Primer probe set RTS2617 was used to measure the total *HTT* mRNA reduction. The results of the assay are presented in Tables 77-79 as percent inhibition over the PBS control. The results demonstrate that the SNP-specific ISIS oligonucleotides specifically target the C allele of rs7685686 compared to the A allele (Table 80).

Table 77

Dose-dependent antisense inhibition of the A allele of rs7685686 in GM04022 fibroblasts							
ISIS No	0.06 μM	0.19 μM	0.56 μM	1.67 μM	5.00 μM	15.00 μM	IC_{50} (μM)
387916	33	40	53	90	99	98	0.56
435879	0	0	50	29	38	47	10.8
460209	14	4	54	73	81	95	0.53
476333	2	44	41	77	91	86	0.64

Table 78

Dose-dependent antisense inhibition of the C allele of rs7685686 in GM04022 fibroblasts							
ISIS No	0.06 μM	0.19 μM	0.56 μM	1.67 μM	5.00 μM	15.00 μM	IC_{50} (μM)
387916	41	42	46	86	95	92	0.54
435879	0	0	75	60	68	81	2.9
460209	35	48	76	84	88	92	0.19
476333	22	60	75	84	90	93	0.15

Table 79

Dose-dependent antisense inhibition of total HTT mRNA in GM04022 fibroblasts						
ISIS No	0.06 μM	0.19 μM	0.56 μM	1.67 μM	5.00 μM	15.00 μM
387916	32	59	49	89	98	99
435879	0	0	42	25	41	62
460209	26	27	54	75	84	96

Dose-dependent antisense inhibition of total HTT mRNA in GM04022 fibroblasts						
ISIS No	0.06 μM	0.19 μM	0.56 μM	1.67 μM	5.00 μM	15.00 μM
476333	25	51	58	82	92	90

Table 80

IC_{50} ratio (A/C) in GM04022 fibroblasts	
ISIS No	Ratio
387916	1.0
435879	4.2
460209	2.8
476333	4.3

Example 19: Allele-specific inhibition measured in cortical neurons from BACHD and YAC18 mice.

[0408] In order to identify potential SNPs for screening of human allele-specific ISIS oligonucleotides, the *HTT* mRNA of YAC18 and BACHD mice were sequenced by the Goldengate 96SNP assay. It was determined that the BAC and YAC mice carried different alleles at several key SNP positions (Table 72) and could therefore be used as a screening tool for allele-specific knockdown. Each of the SNP positions chosen for targeting in the mouse strains were also compared to human HD chromosomes. For each target, approximately 50% of the human HD population is heterozygous for the target expressed in the BACHD mice, but not the YAC18 mice.

[0409] In order to verify the allele-specificity of the ISIS oligonucleotides (described in Examples 2, 9, 17 and 18), the antisense oligonucleotides, ISIS 460207, targeting SNP rs362331; ISIS 460209, targeting SNP rs7685686; ISIS 435879, targeting SNP rs7685686; ISIS 476333, targeting SNP rs7685686; ISIS 460210, targeting SNP rs2298969; ISIS 435874, targeting SNP rs4690072; ISIS 460208, targeting SNP rs4690072; ISIS 435331, targeting SNP rs2024115; and ISIS 435871, targeting SNP rs363088, were tested for their effect on HTT protein levels in BACHD and YAC18 cortical neurons. ISIS 387916, which has no human or murine SNP target site, was used as the benchmark. ISIS 387916 is cross-reactive with murine *HTT* mRNA (GENBANK Accession No. NM_010414.1, designated herein as SEQ ID NO: 286) at target start site 5763 with one mismatch. It was expected that treatment with the allele-specific antisense oligonucleotides would cause significant inhibition of *HTT mRNA* in the BACHD neurons and not in the YAC18 neurons. It was also expected that treatment with ISIS 387916 would cause inhibition of *HTT* mRNA in both sets of neurons.

[0410] YAC18 cultures were prepared from E16.5 pregnant female YAC18 (line 60, +/+) mice who had been bred with YAC18 (line 60, +/+) males. All progeny are thus homozygous YAC18 (line 60), facilitating pooled cortical cultures. BACHD E16.5 embryos were isolated from pregnant BACHD (+/-) mice who had been bred with pregnant BACHD (+/-) male mice, necessitating single pup cultures and genotyping. Single cortices were isolated, using caution to prevent cross-contamination of samples. Each dissociated cortex was used to seed 5 wells of a 6-well plate. After genotyping, only BACHD (+/-) cultures were used for ASO treatment. The antisense oligonucleotides were added to the supplementary media fed to the neurons on division 2. Cells were harvested after 8 days with into 1 mL of media using a cell scraper. Cells were centrifuged at 2,500 rpm for 5 min at 4°C and the pellets were resuspended in a buffer of 50 mM Tris, pH = 8.0, 150 mM NaCl, 1% Igepal, 40 mM β-glycerophosphate, 10mM NaF, 1 x Roche complete protease inhibitor, 1mM Sodium Orthovanadate and 800 μM PMSF. The lysates were centrifuged after 15 min incubation and protein concentration was measured with the DC assay (BioRad).

[0411] Protein lysates were run on low-bis gels to separate huntingtin alleles (resolving gel - 2001:Acrylamide:BIS (10% acrylamide, 0.5% BIS, 375mMTris pH 8.8; stacking gel - 4%Acrylamide-BIS(29:1), 156 mM Tris pH6.8; Running buffer-25mM Tris, 190mM Glycine, 0.1% SDS + 10μM beta-mercaptoethanol added fresh). After electrophoresis, proteins in the gel were transferred to a nitrocellulose membrane (Hybond-C Extra; GE Healthcare Bio-Sciences) at 90V for 40' to allow samples to penetrate the stacking gel and then at 190V for 2.5h to resolve proteins.

[0412] Primary antibodies specific for human HTT and murine calnexin protein were used at 1:10,000 dilutions. HRP-conjugated anti-mouse secondary antibody (1:10,000, Jackson ImmunoResearch Laboratories) was used for visualizing proteins using SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific). Protein bands were quantified using ImageJ software and normalized to calnexin levels. Tables 81-91 provide the percentage inhibition relative to the untreated control sample. The percentage inhibition of human HTT protein levels in BACHD and YAC18 neurons are presented.

Table 81

HTT SNPs in BACHD and YAC18 mice and correlation with human HTT SNPs				
SNP	Allele present in YAC18 Mice	Allele present in BACHD Mice	Allele present in human patients with high CAG repeats	% of human patients heterozgous at the SNP position
rs2024115	G	A	A	48
rs2298969	G	A	A	52
rs362331	C	T	T	49
rs363088	G	T	T	38
rs4690072	T	A	A	49
rs7685686	G	A	A	49

Table 82

Effect of antisense inhibition by ISIS 387916 in BACHD and YAC18 neurons		
	500 nM	1500 nM
YAC18	69	81
BACHD	84	90

Table 83

Effect of antisense inhibition by ISIS 435331, targeting rs2024115 in BACHD and YAC18 neurons		
	500 nM	1500 nM
YAC18	0	0
BACHD	39	43

Table 84

Effect of antisense inhibition by ISIS 460210, targeting rs2298969 in BACHD and YAC18 neurons		
	500 nM	1500 nM
YAC18	31	51
BACHD	79	89

Table 85

Effect of antisense inhibition by ISIS 460207, targeting rs362331 in BACHD and YAC18 neurons		
	500 nM	1500 nM
YAC18	0	0
BACHD	29	44

Table 86

Effect of antisense inhibition by ISIS 435871, targeting rs363088 in BACHD and YAC18 neurons		
	500 nM	1500 nM
YAC18	0	0
BACHD	51	68

Table 87

Effect of antisense inhibition by ISIS 435874, targeting rs4690072 in BACHD and YAC18 neurons		
	500 nM	1500 nM
YAC18	9	5
BACHD	30	44

Table 88

Effect of antisense inhibition by ISIS 460208, targeting rs4690072 in BACHD and YAC18 neurons		
	500 nM	1500 nM
YAC18	1	8
BACHD	54	68

Table 89

Effect of antisense inhibition by ISIS 460209, targeting rs7685686 in BACHD and YAC18 neurons		
	500 nM	1500 nM
YAC18	12	32
BACHD	72	83

Table 90

Effect of antisense inhibition by ISIS 435879, targeting rs7685686 in BACHD and YAC18 neurons		
	500 nM	1500 nM
YAC18	0	7
BACHD	36	58

Table 91

Effect of antisense inhibition by ISIS 476333, targeting rs7685686 in BACHD and YAC18 neurons		
	500 nM	1500 nM
YAC18	46	61
BACHD	89	91

SEQUENCE LISTING

[0413]

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<120> SELECTIVE REDUCTION OF ALLELIC VARIANTS

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Patentkrav

1. Modificeret antisense-oligonukleotid til anvendelse i behandling, forebyggelse eller lindring af en sygdom, hvor sygdommen er forårsaget af ekspressionen af en mutant-allel indeholdende en enkeltnukleotidpolymorfisme og hvor det modificerede antisense-oligonukleotid selektivt reducerer ekspressionen af mutantallelen,
hvor det modificerede antisense-oligonukleotid består af 15 til 20 bundne nukleosider målrettet til et enkeltnukleotidpolymorfisme-sted på mutantallelen der er associeret med sygdommen og i stand til selektivt at reducere ekspressionen af mutantallelen,
hvor det modificerede oligonukleotid er 100% komplementært til enkeltnukleotidpolymorfisme-stedet lokaliseret på den mutantallelen,
hvor det modificerede oligonukleotid er enkelt-strenget og omfatter et wing-gap-wing-motiv med en 5' wing region positioneret ved 5'-enden af et deoxynukleosid-gap, og en 3' wing region positioneret ved 3'-enden af deoxynukleosid-gapet,
hvor hver af nukleosiderne af hver wing-region omfatter et modificeret sukker eller sukkersurrogat,
hvor wing-gap-wing-motivet er en hvilket som helst af gruppen bestående af 5-10-5, 2-9-6, 3-9-3, 3-9-4, 3-9-5, 4-7-4, 4-9-3, 4-9-4, 4-9-5, 4-10-5, 4-11-4, 4-11-5, 5-7-5, 5-8-6, 5-9-3, 5-9-5, 5-10-4, 6-7-6, 6-8-5, og 6-9-2, og
hvor enkeltnukleosidpolymorfismen annealerer til en nukleobase inde i gap-segmentet, og hvor position 4, 5, eller 6 af det modificerede oligonukleotid, som talt fra 5'-terminus af gap'et, annealerer med enkeltnukleotidpolymorfismen.

2. Oligonukleotidet til anvendelse ifølge krav 1, hvor sygdommen er valgt fra gruppen bestående af Alzheimer's sygdom, Creutzfeldt-Jakob sygdom, fatal familiær søvnløshed, Alexander sygdom, Parkinson's sygdom, amyotrof lateral sclerose, dentato-rubral og pallido-luysian atrofi DRPA, spino-cerebellar ataxi, Torsion dystoni, myokardiopati, kronisk obstruktiv pulmonær sygdom (COPD), leversygdom, hepatocellulær carcinom, systemisk lupus erythematosus, hypercholesterolæmi, brystcancer, astma, Type 1 diabetes, Rheumatoid arthritis, Graves sygdom, SLE, spinal- og bulbær muskulær atrofi, Kennedy's sygdom, progressiv childhood posterior subkapsulært katarakt, cholesterolgaldestensygdom, arthrosclerose, kardiovaskulær sygdom, primær

hypercalciuria, alfa-thallasæmi, obsessiv compulsiv sygdom, angst, comorbid depression, kongenitale visuelle defekter, hypertension, metabolisk syndrom, prostatacancer, kongenital myasthenisk syndrom, perifer arteriel sygdom, atrial fibrillation, sporadisk pheochromocytoma, kongenital misdannelser, Machado-Joseph sygdom, Huntington's sygdom, og Autosomal Dominant Retinitis Pigmentosa sygdom.

3. Oligonukleotidet til anvendelse ifølge krav 1, hvor sygdommen er Huntington's sygdom og enkeltnukleotidpolymorfisme-stedet er på en mutant allel der er associeret med Huntington's sygdom.

4. Oligonukleotidet til anvendelse ifølge et hvilket som helst af de foregående krav, hvor enkeltnukleotidpolymorfisme-stedet indeholder en differentierings-polymorfisme.

5. Oligonukleotidet til anvendelse ifølge et hvilket som helst af de foregående krav, hvor det modificerede antisense-oligonukleotid består af 15 til 19 bundne nukleosider.

6. Oligonukleotidet til anvendelse ifølge et hvilket som helst af de foregående krav, hvor wing-gap-wing-motivet er et hvilket som helst fra gruppen bestående af 2-9-6, 4-9-5, og 4-11-4.

7. Oligonukleotidet til anvendelse ifølge et hvilket som helst af de foregående krav, hvor det modificerede sukker eller sukkersurrogat er en 2'-O-methoxyethyl-modificeret sukker.

8. Oligonukleotidet til anvendelse ifølge et hvilket som helst af kravene 1-6, hvor mindst én af wing-regionerne omfatter et 4'- til 2'-bicyklisk nukleosid og mindst ét af de tilbageværende wing-nukleosider er et ikke-bicyklisk 2'-modificeret nukleosid, fx et 2'-O-methoxyethylnucleosid.

9. Oligonukleotidet til anvendelse ifølge krav 8, hvor det 4'- til 2'-bicykliske nukleosid er et 4'-$CH(CH_3)$-O-2'-bicyclisk nukleosid.

10. Oligonukleotidet til anvendelse ifølge et hvilket som helst af de foregående krav, hvor mindst én internukleosidbinding er en modificeret internukleosid-binding, eller hver internukleosidbinding er en phosphorthioat-internukleosid-binding.

11. Oligonukleotidet til anvendelse ifølge et hvilket som helst af de foregående krav, hvor mindst ét nukleosid omfatter en modificeret nukleobase, fx 5'-methylcytosin.

12. Oligonukleotidet til anvendelse ifølge et hvilket som helst af de foregående krav, hvor oligonukleotidet anvendes i en farmaceutisk sammensætning ved at kombinere oligonukleotidet og et farmaceutisk acceptabelt fortyndingsmiddel eller bærer.

DRAWINGS

Figure 1

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CLUSTAL 2.0.12 multiple sequence alignment

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Figure 1

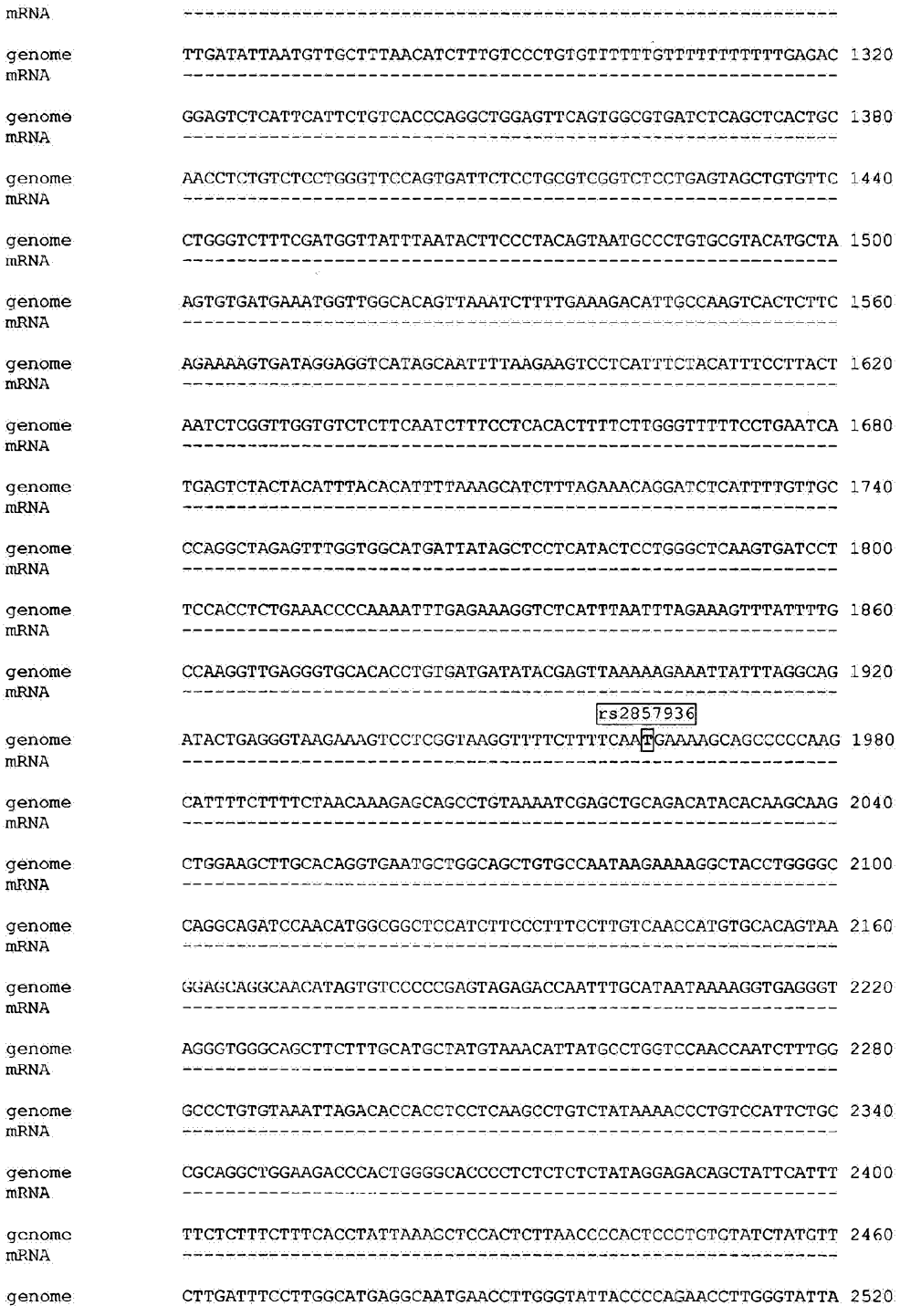
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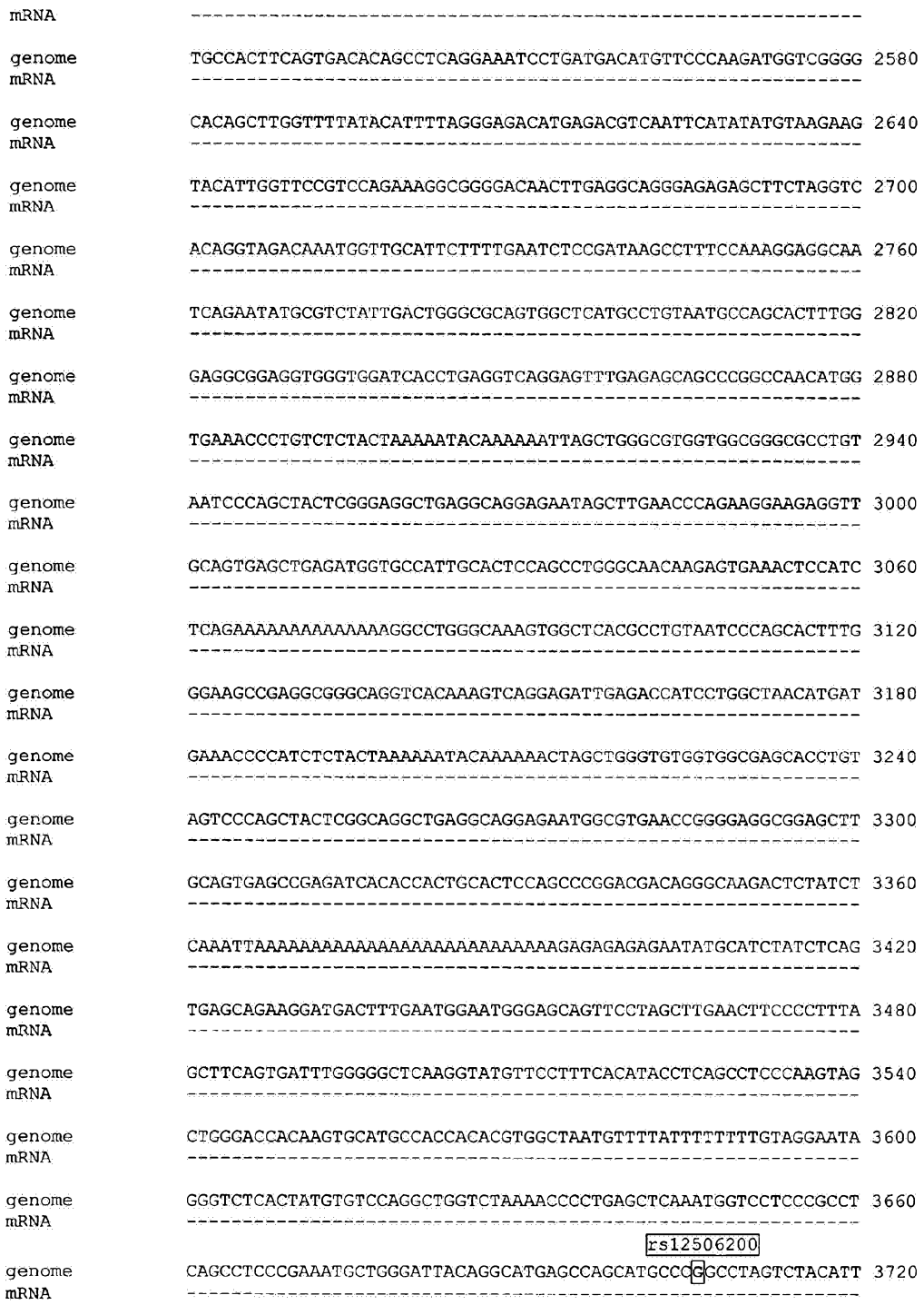
Figure 1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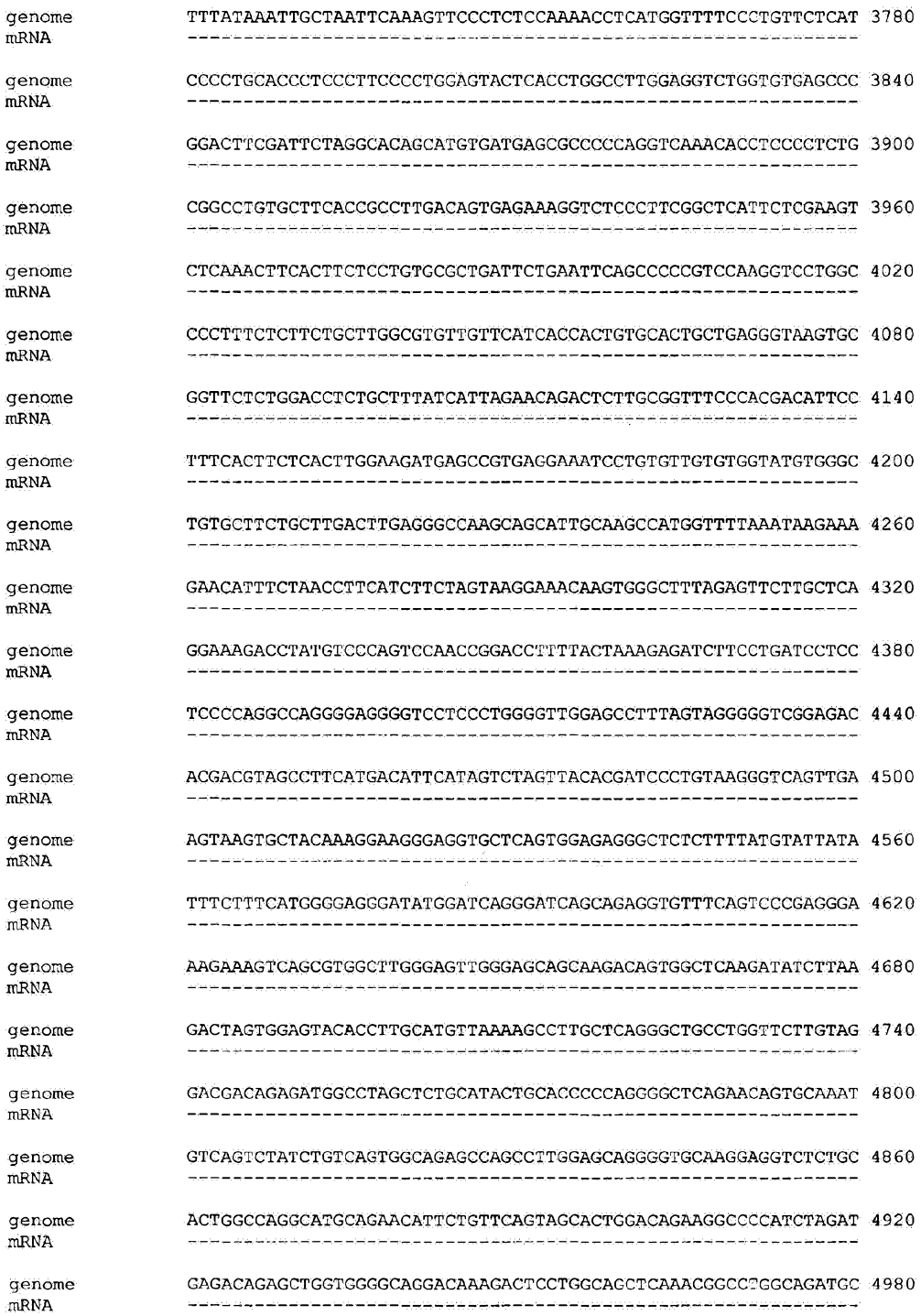
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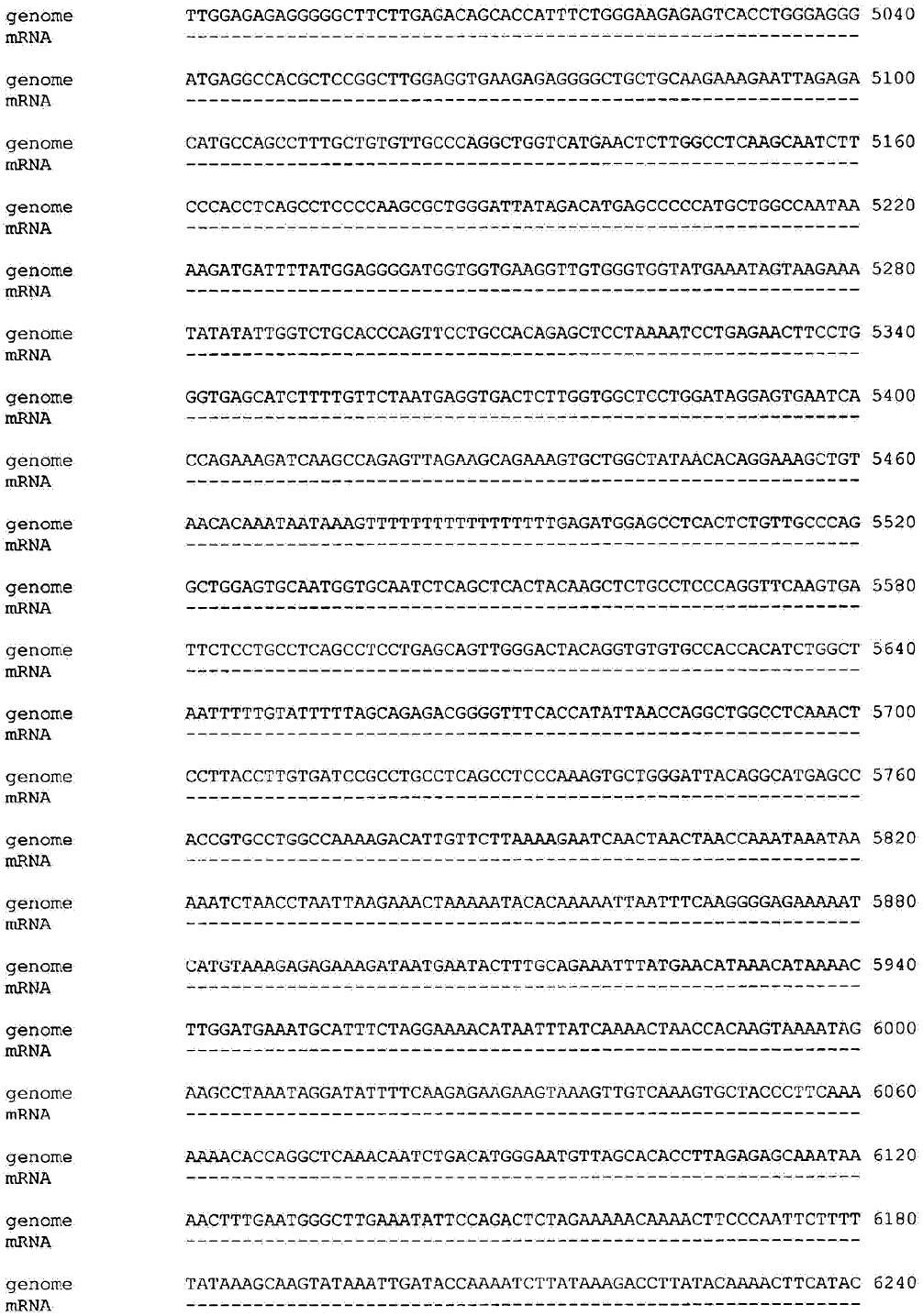
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Figure 1

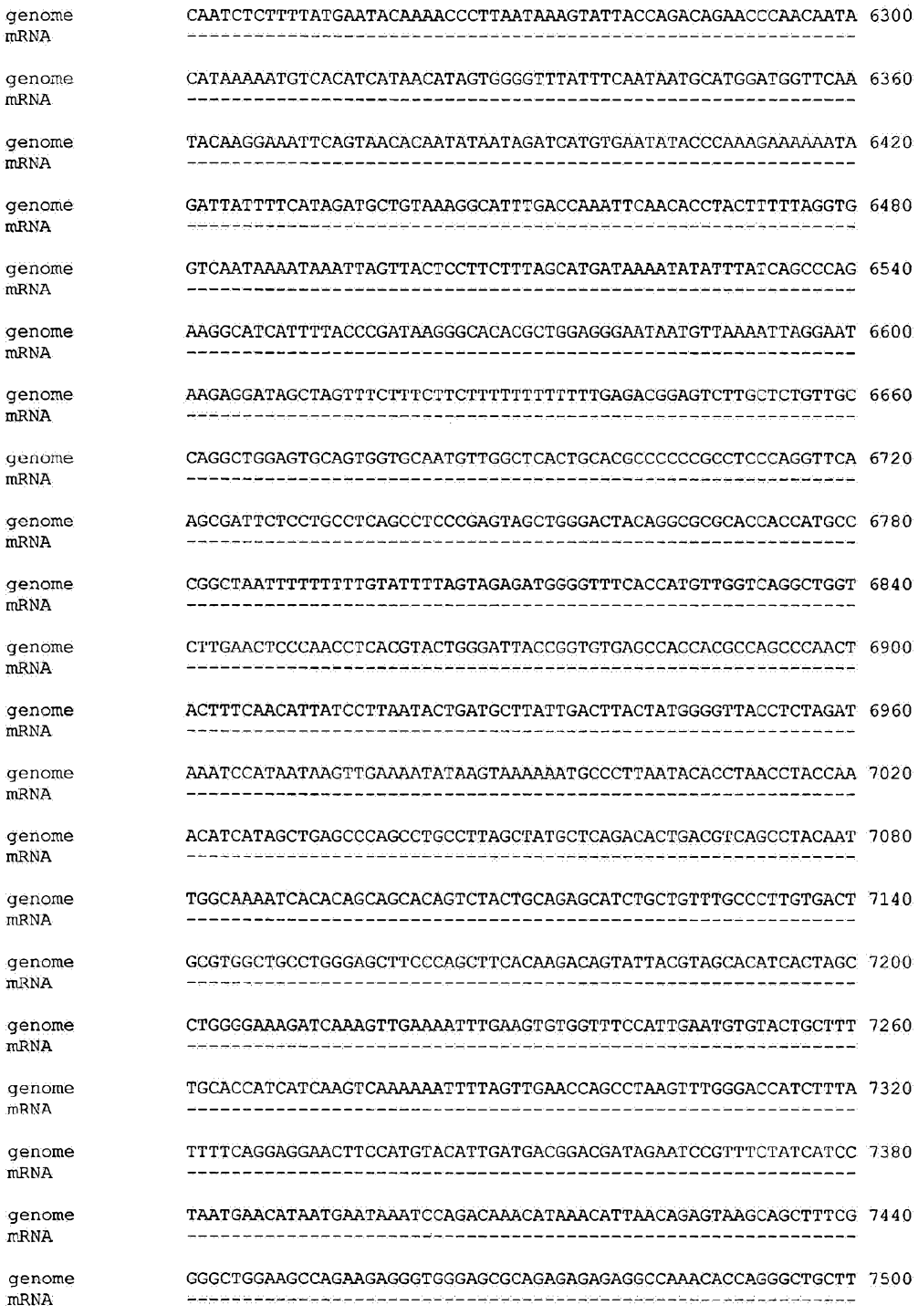
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Figure 1

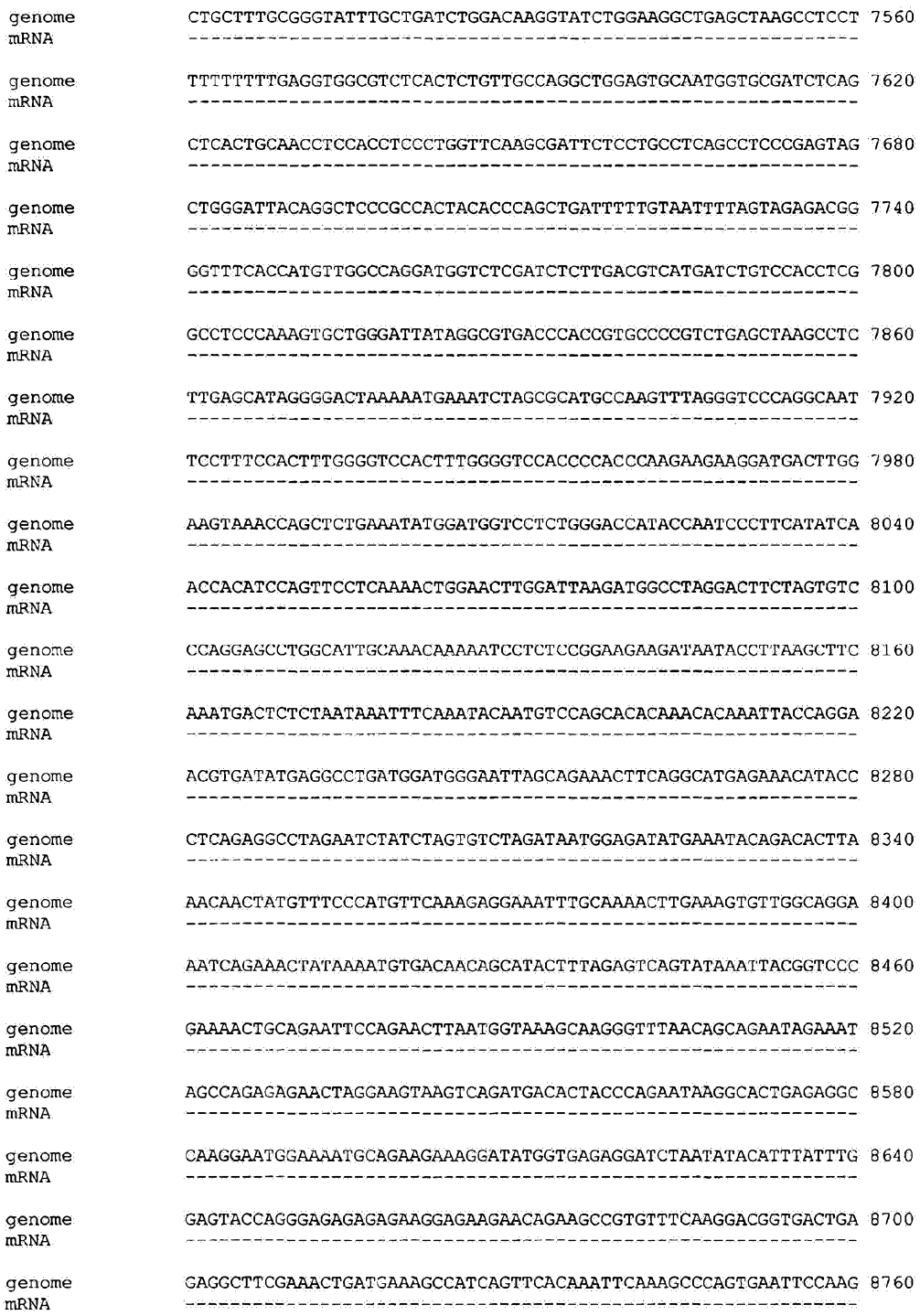
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Figure 1

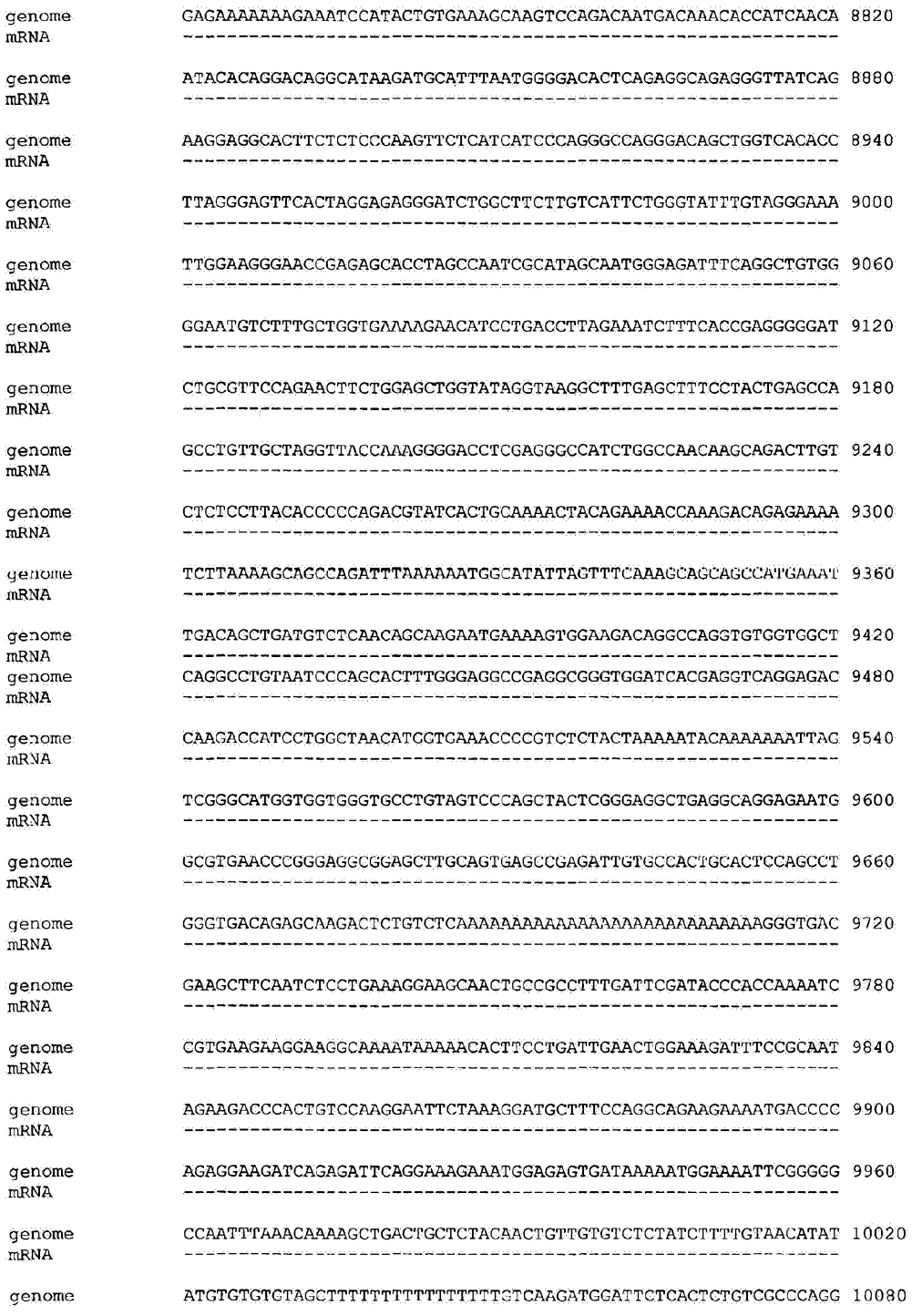
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Figure 1

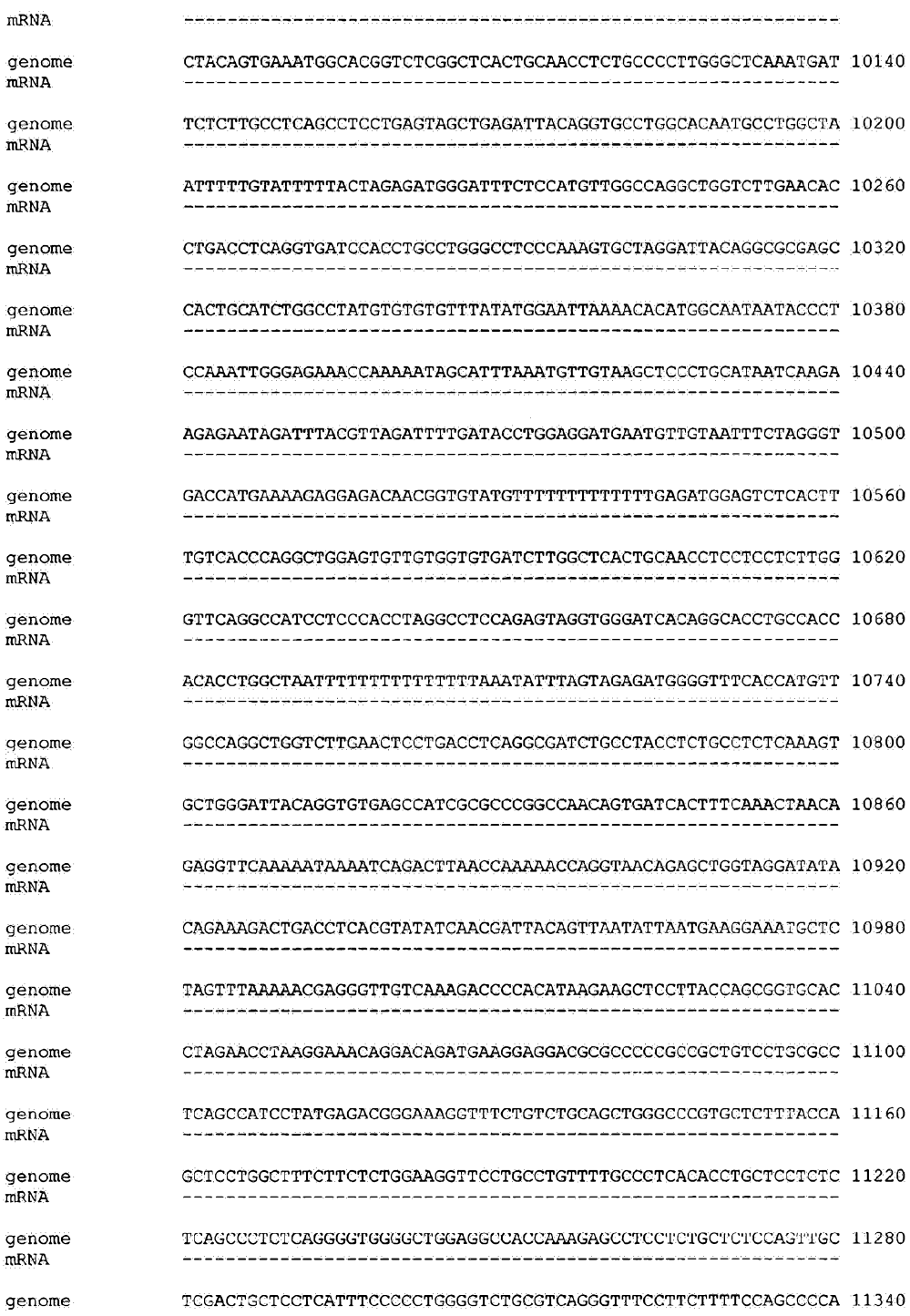
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Figure 1

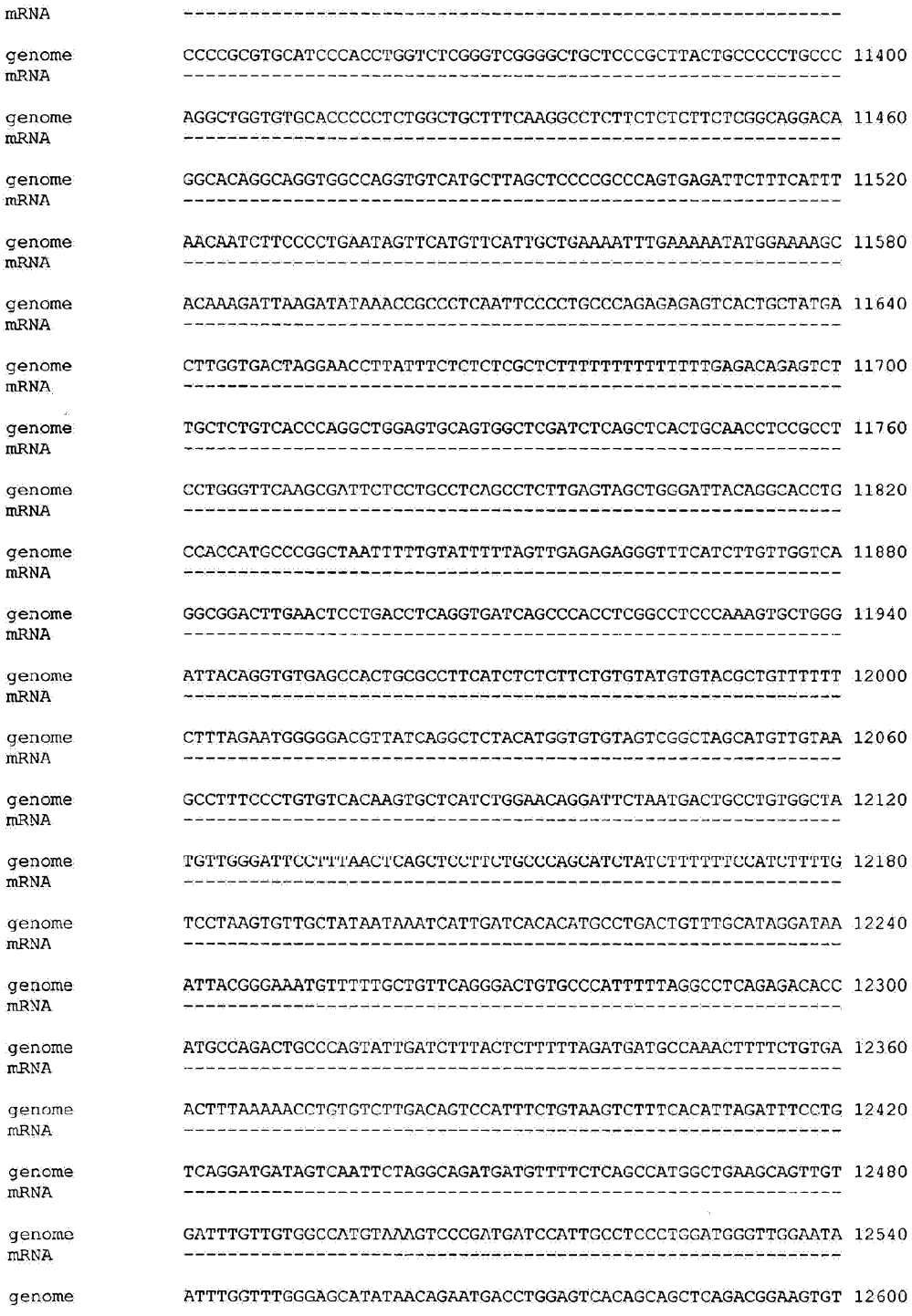
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Figure 1

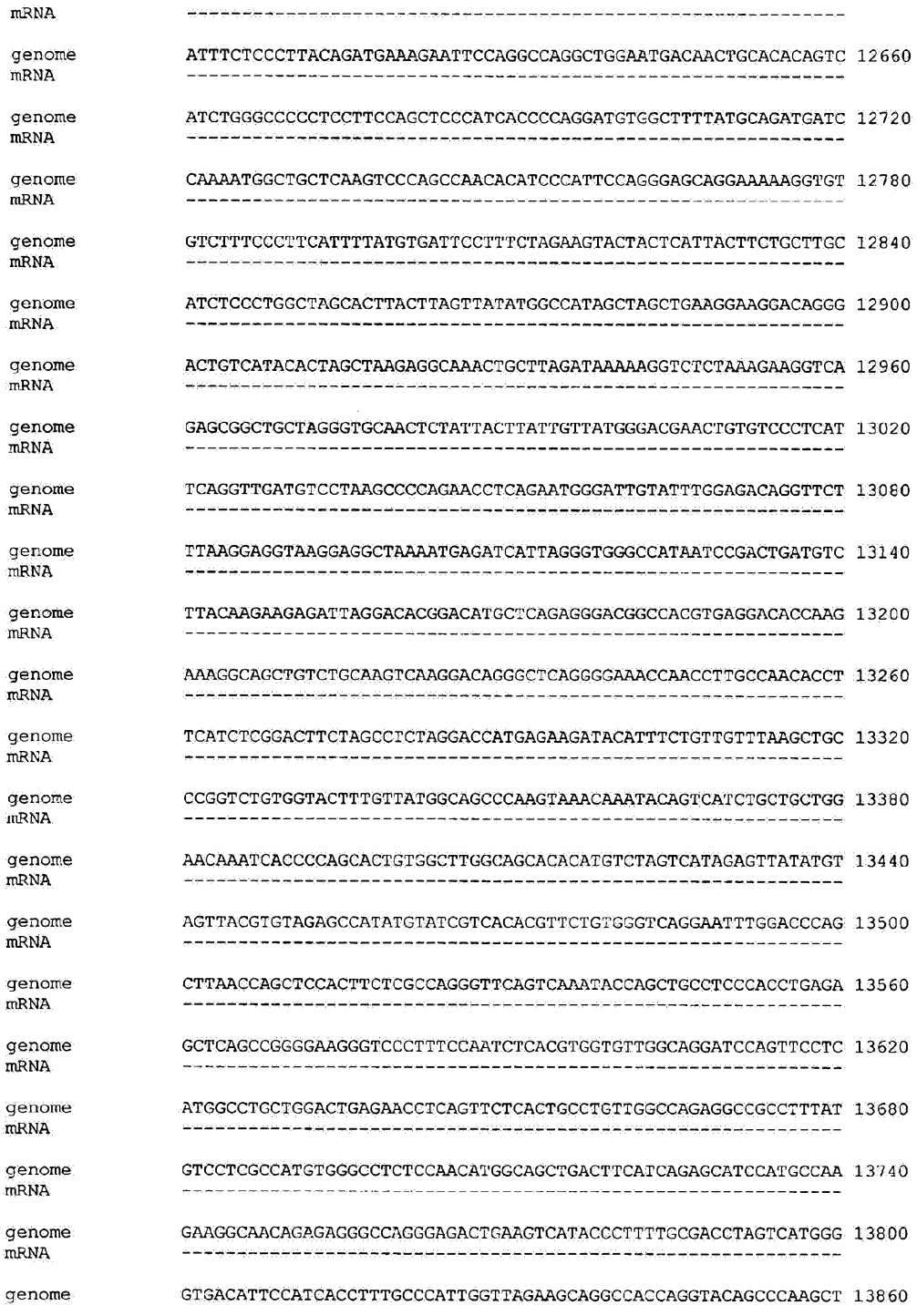
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Figure 1

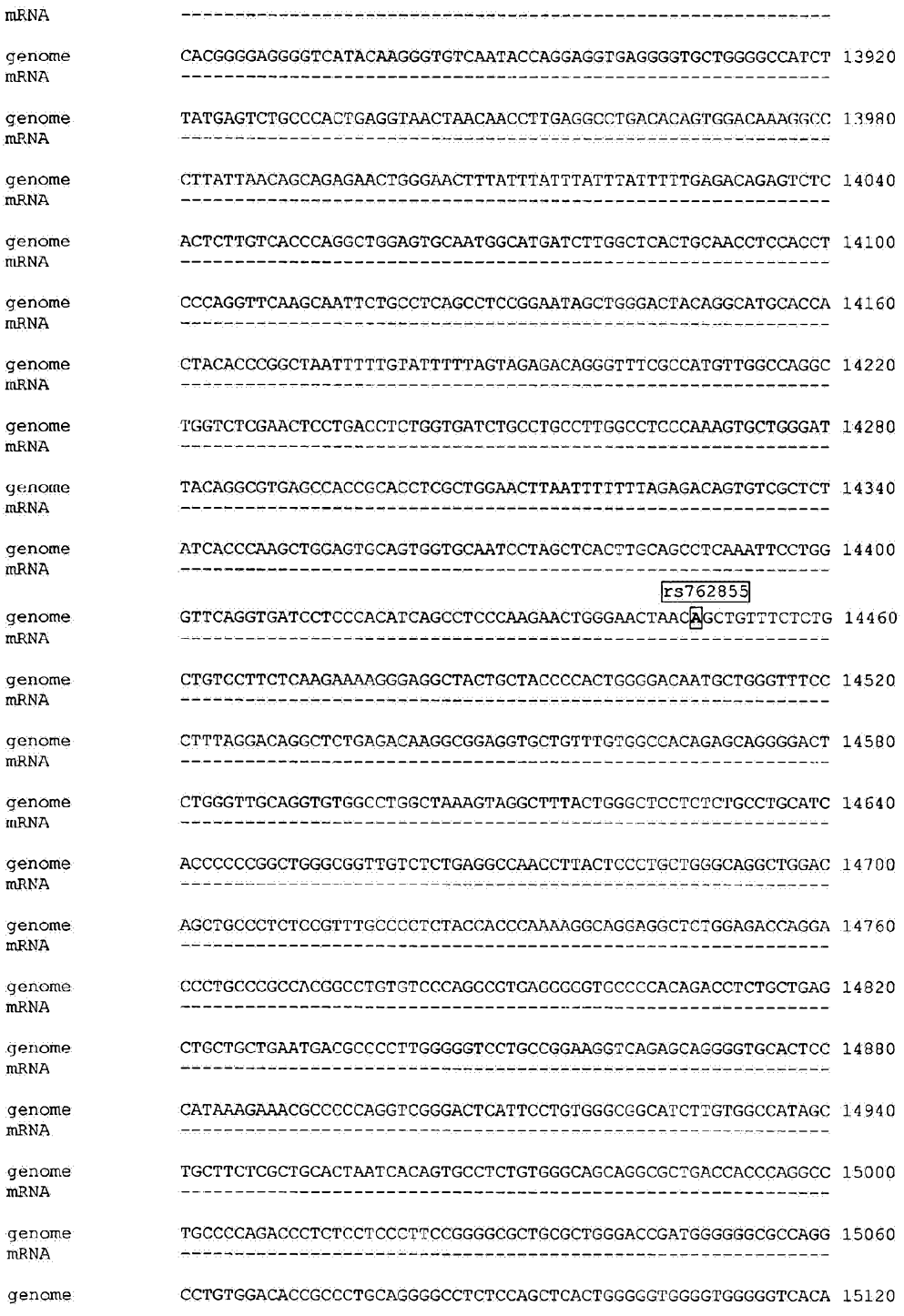
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Figure 1

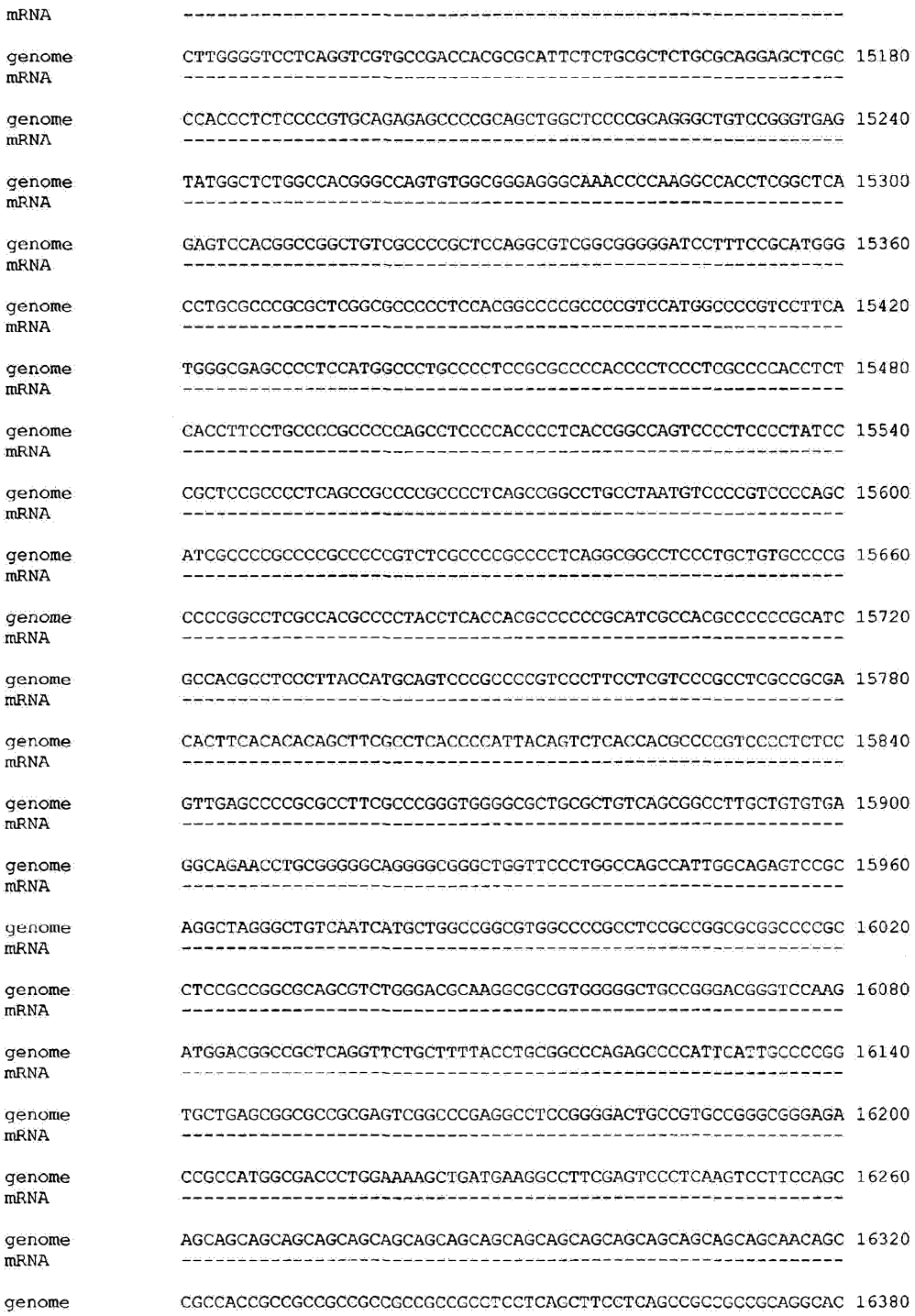
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Figure 1

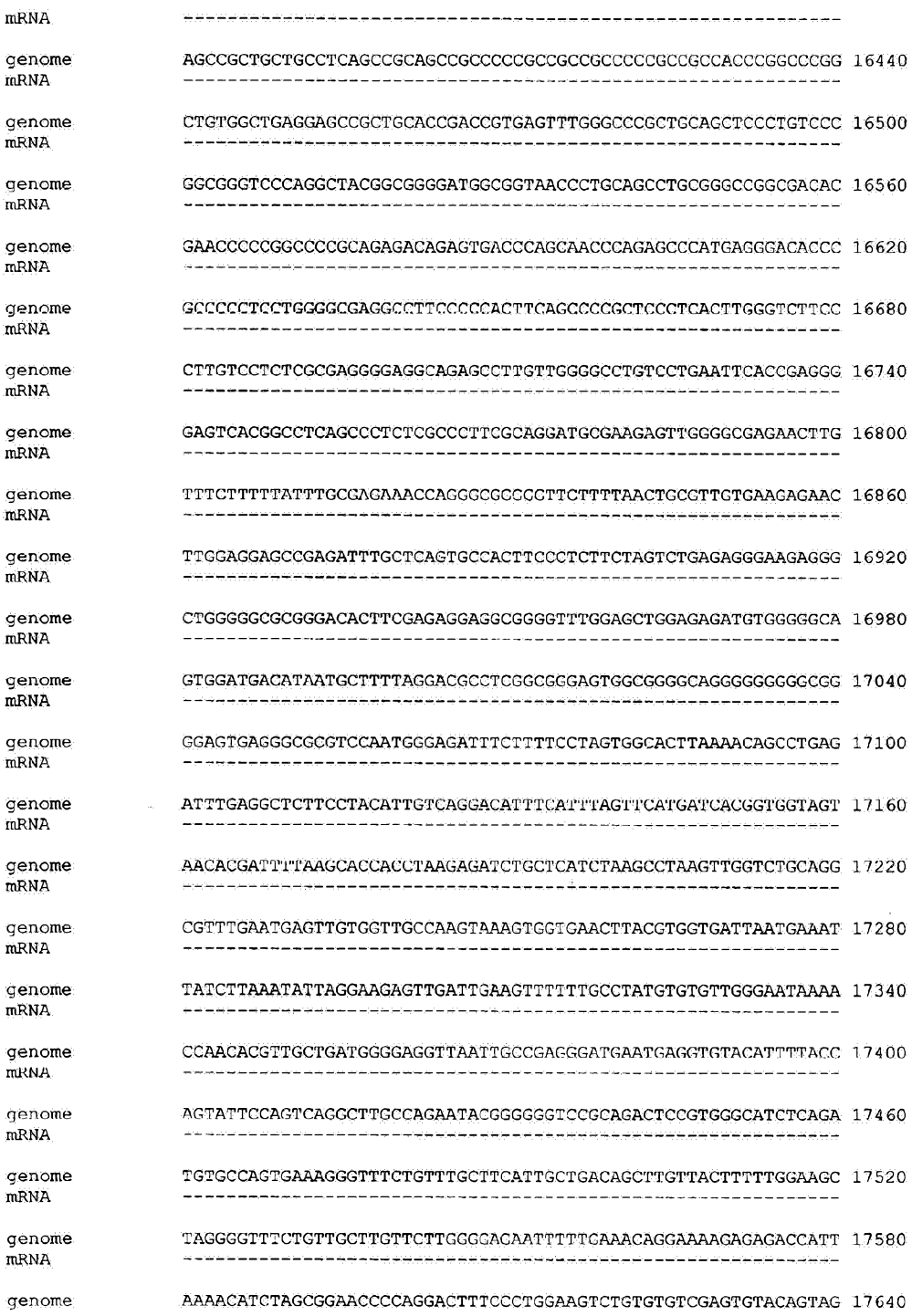
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Figure 1

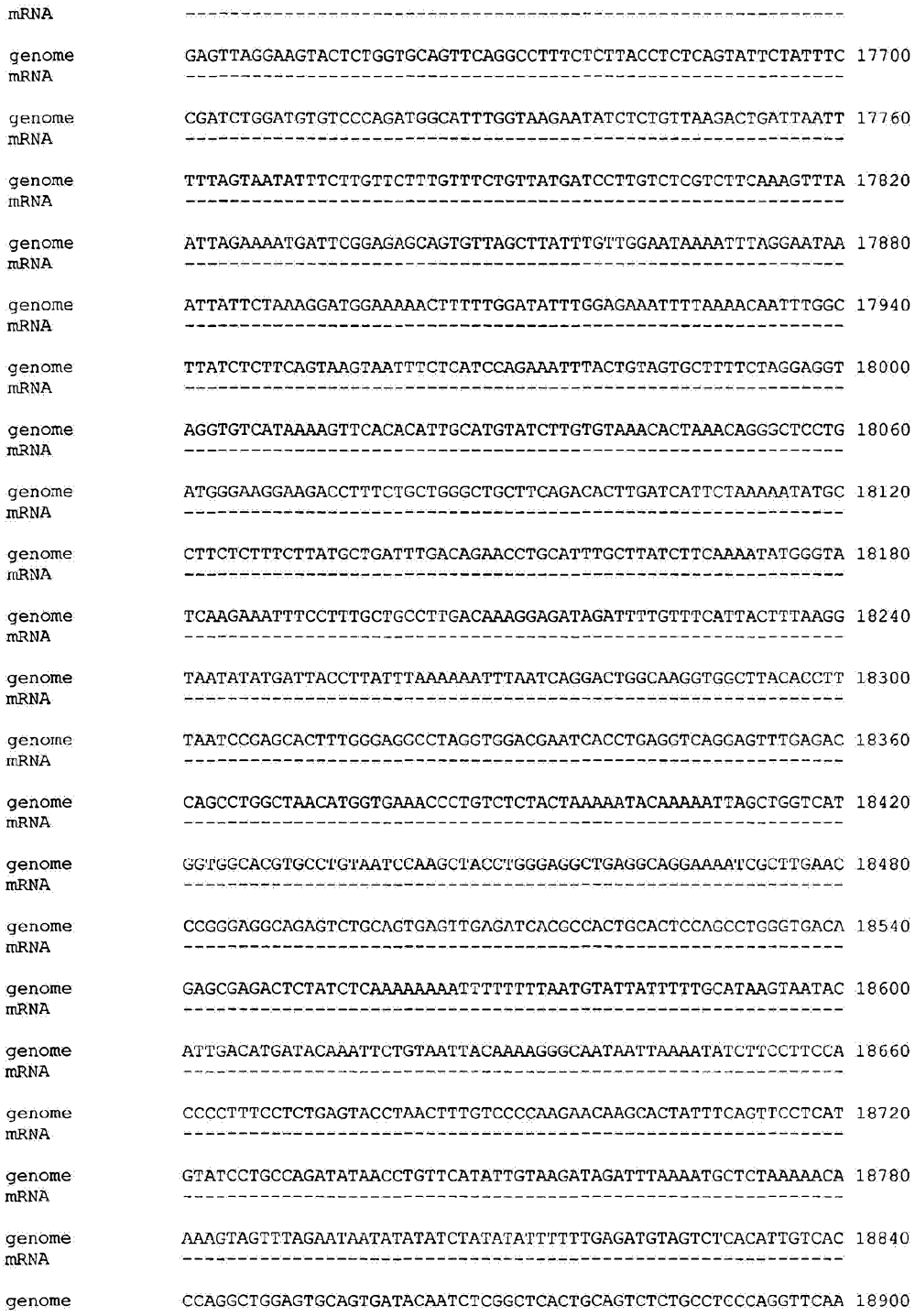
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Figure 1

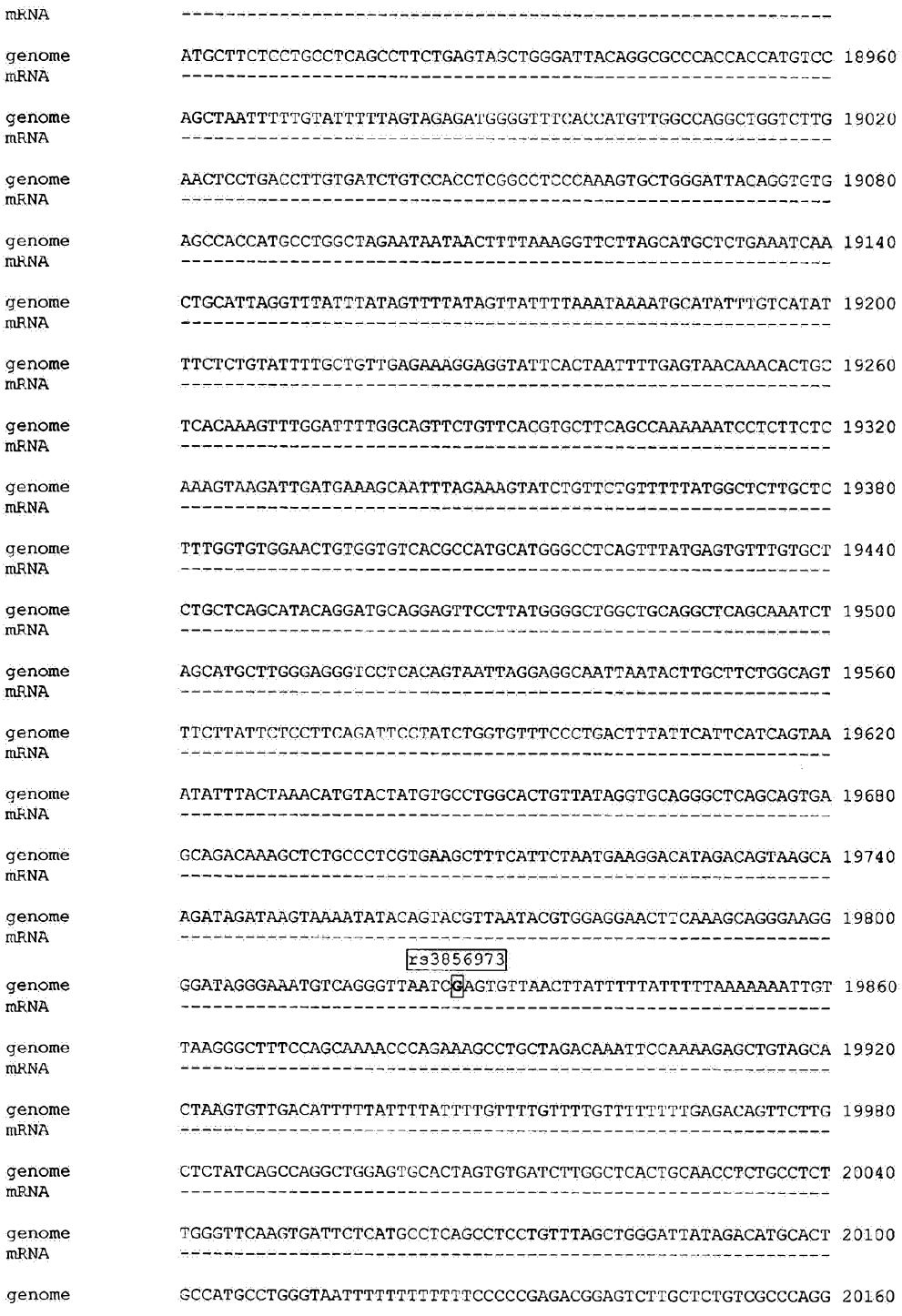
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Figure 1

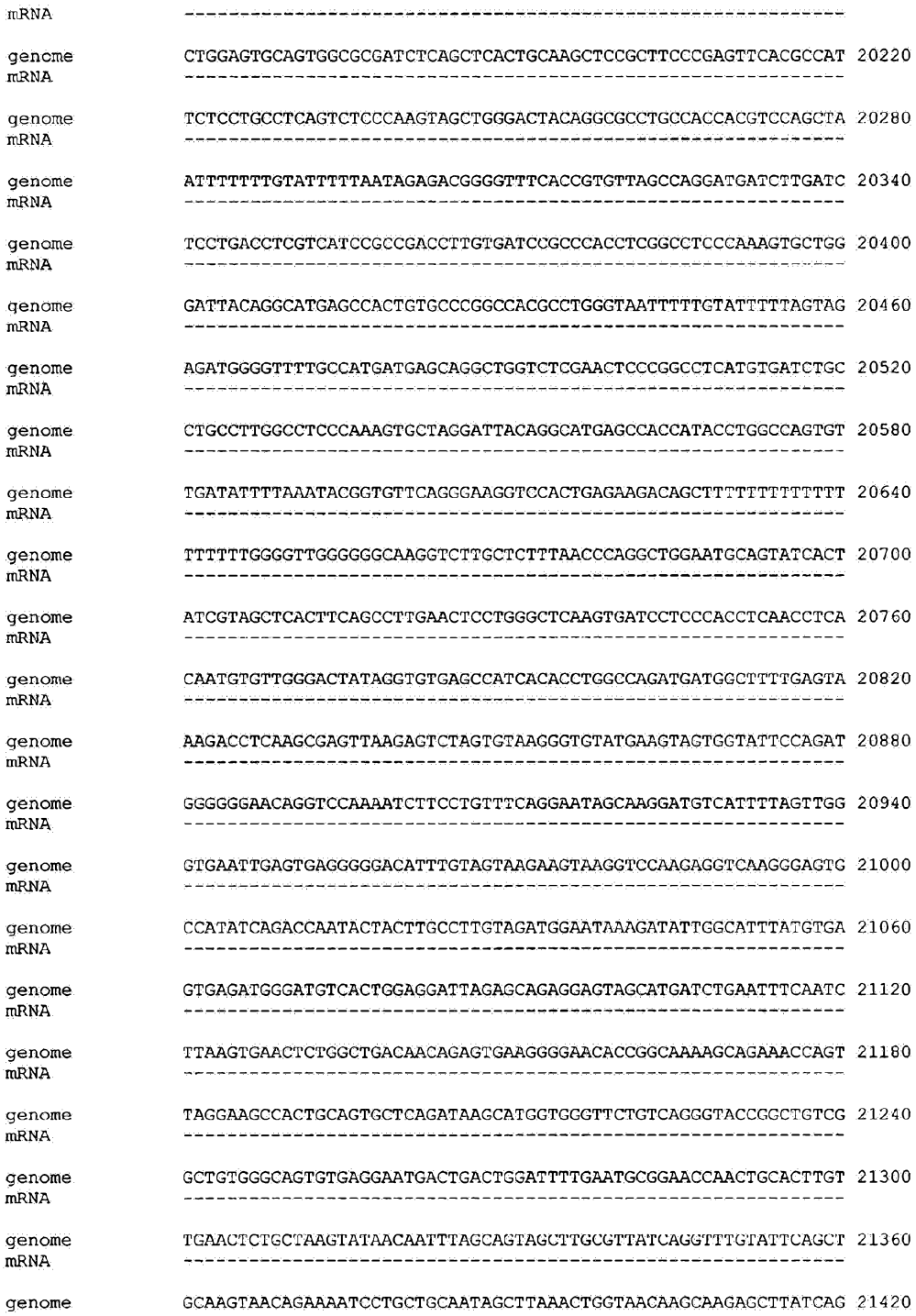
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Figure 1

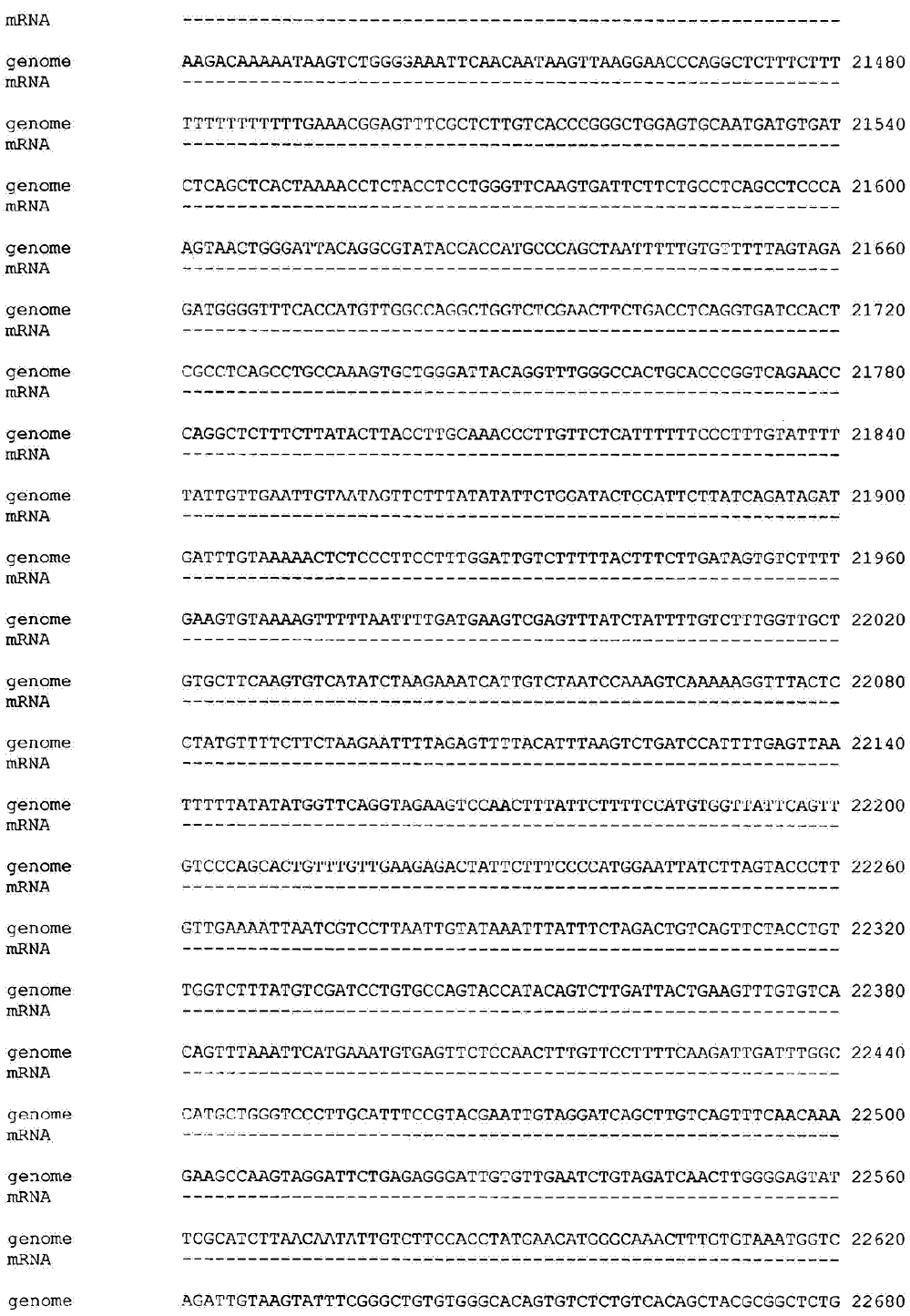
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Figure 1

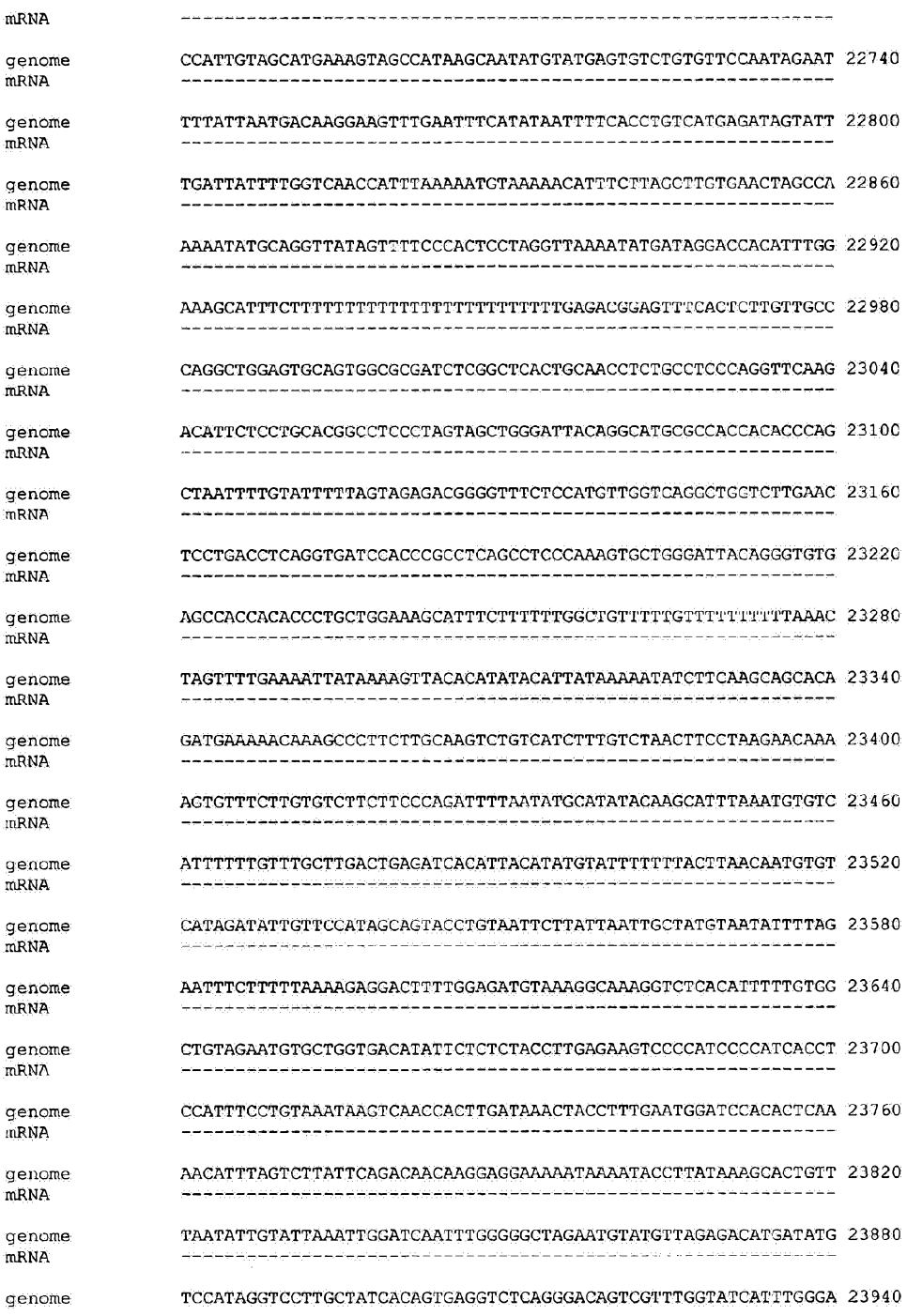
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Figure 1

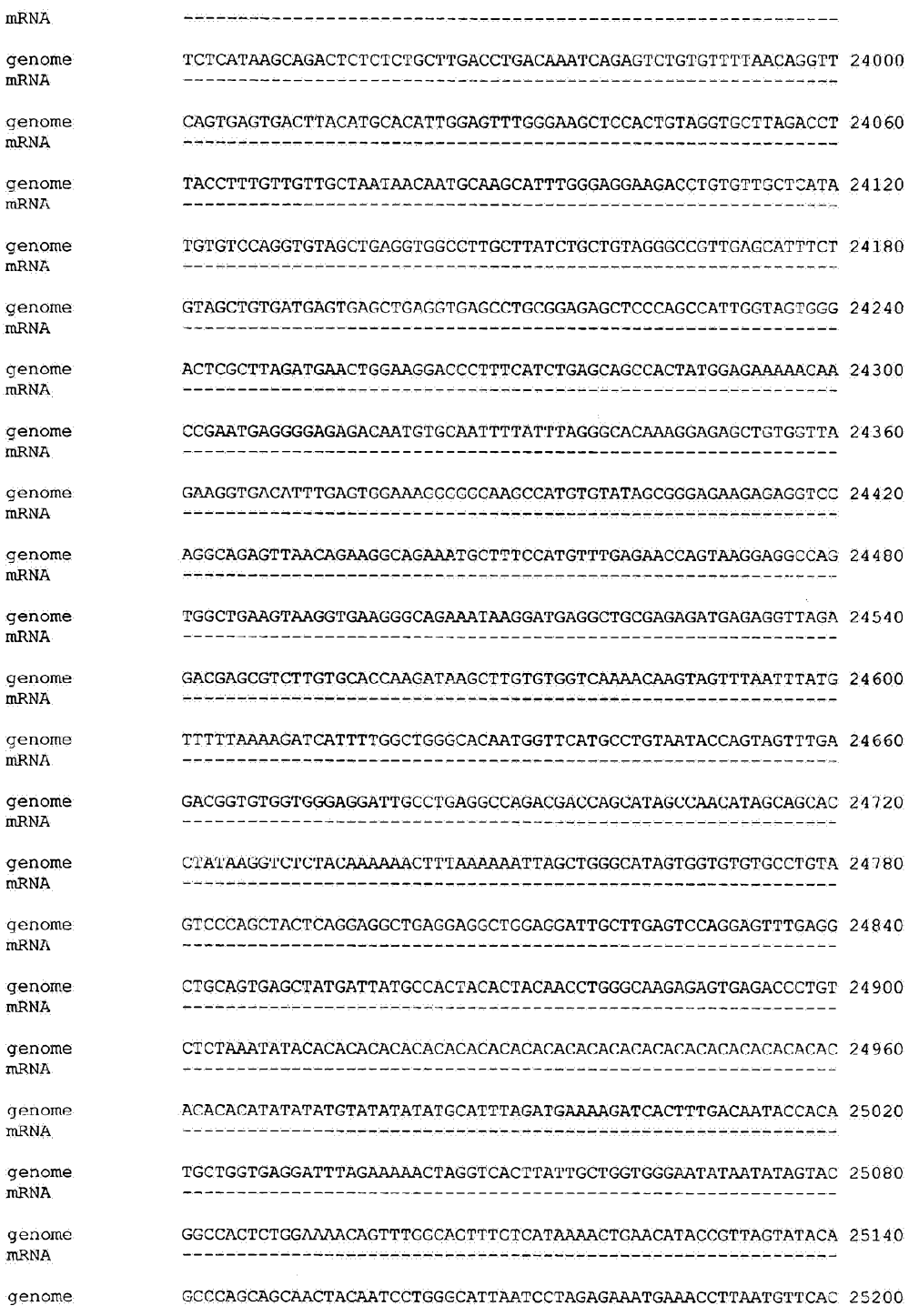
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Figure 1

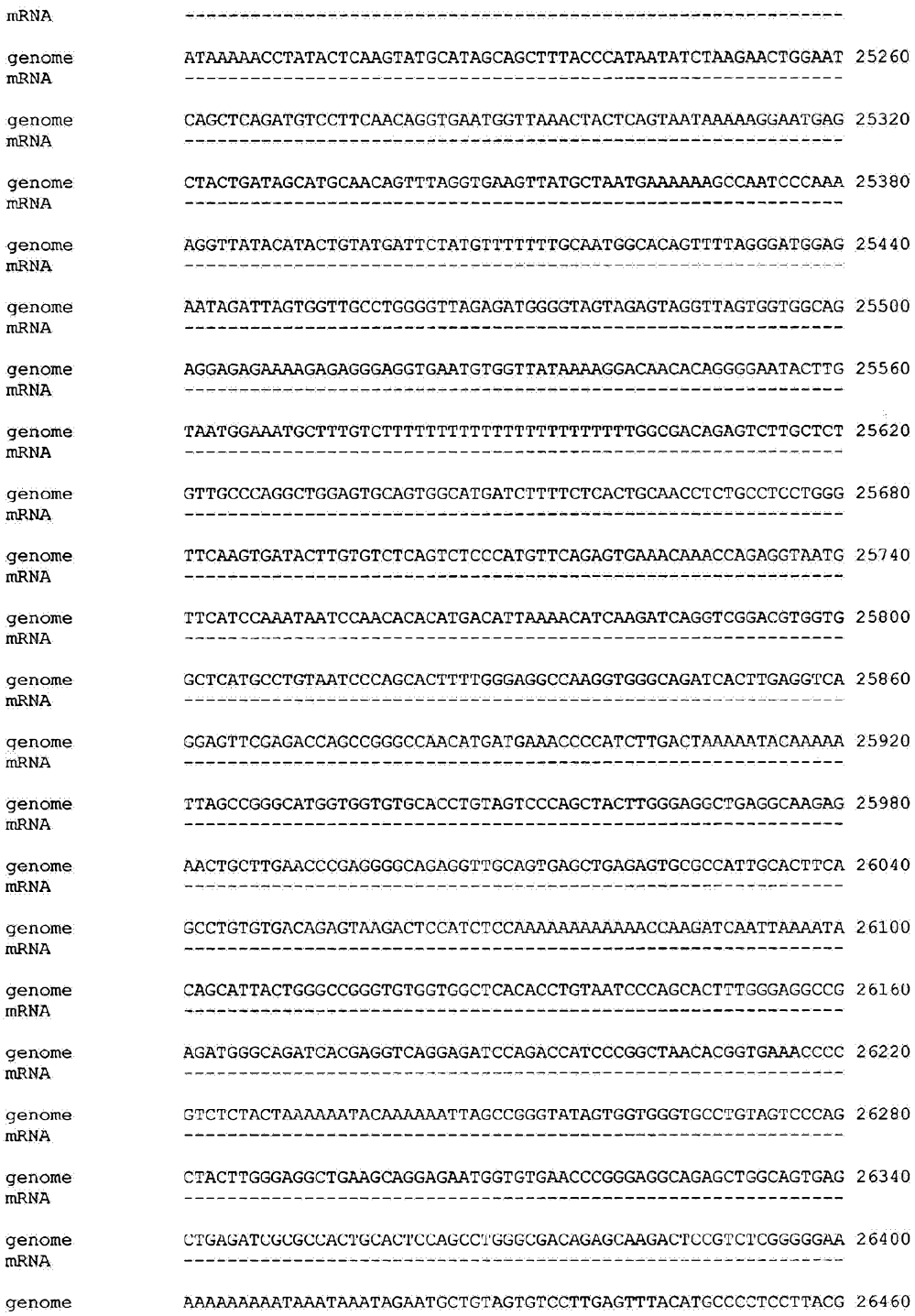
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Figure 1

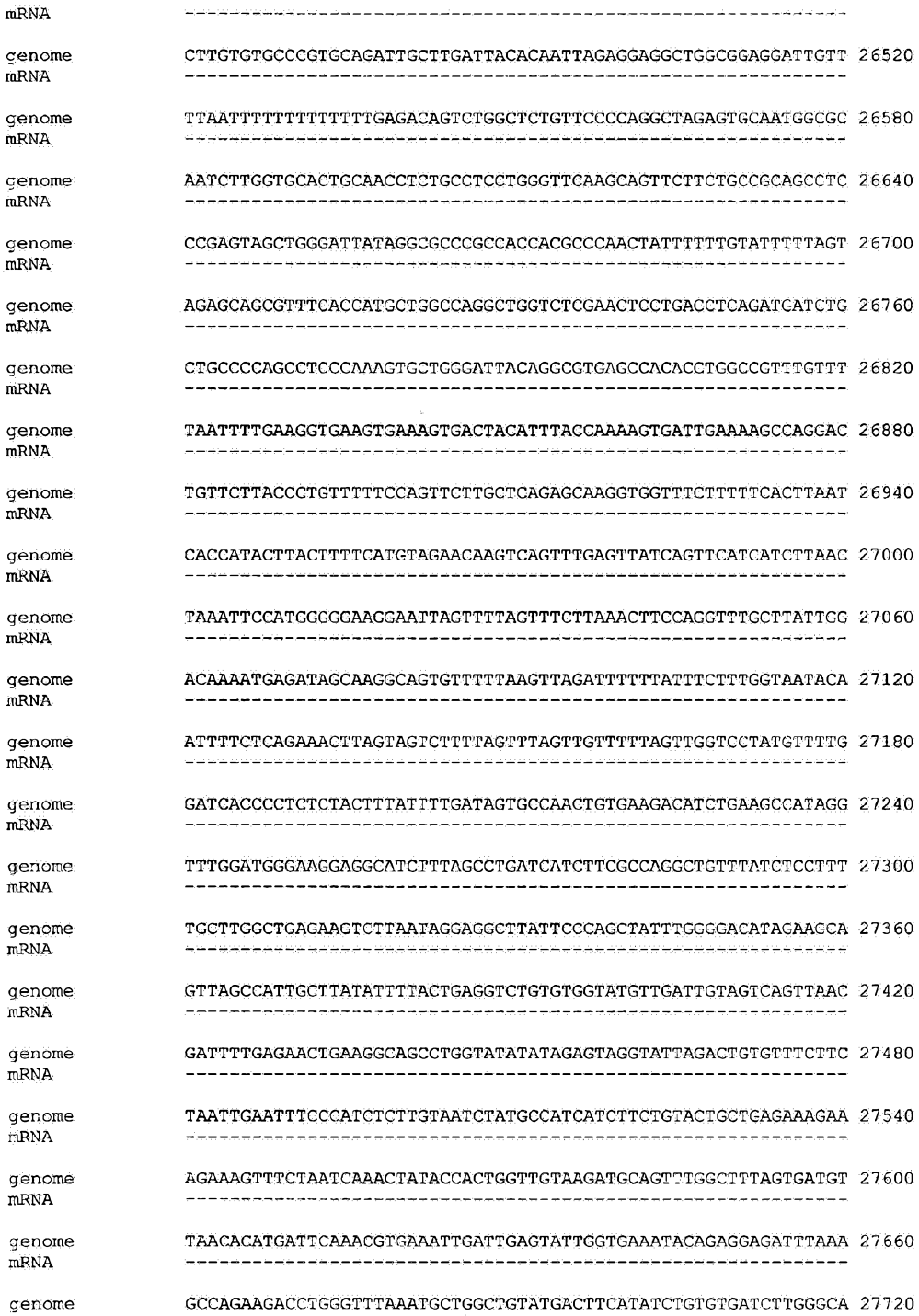
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Figure 1

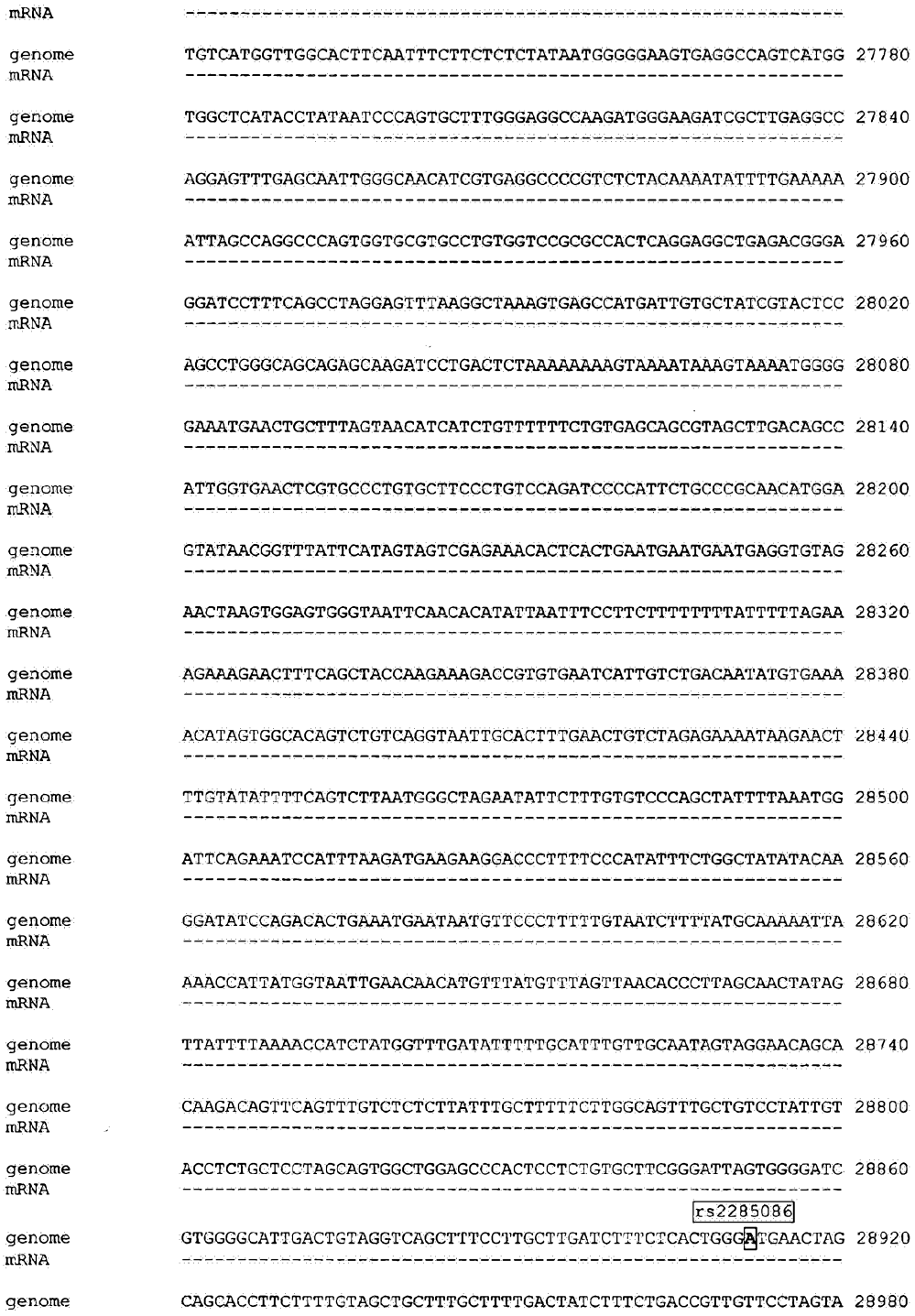
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Figure 1

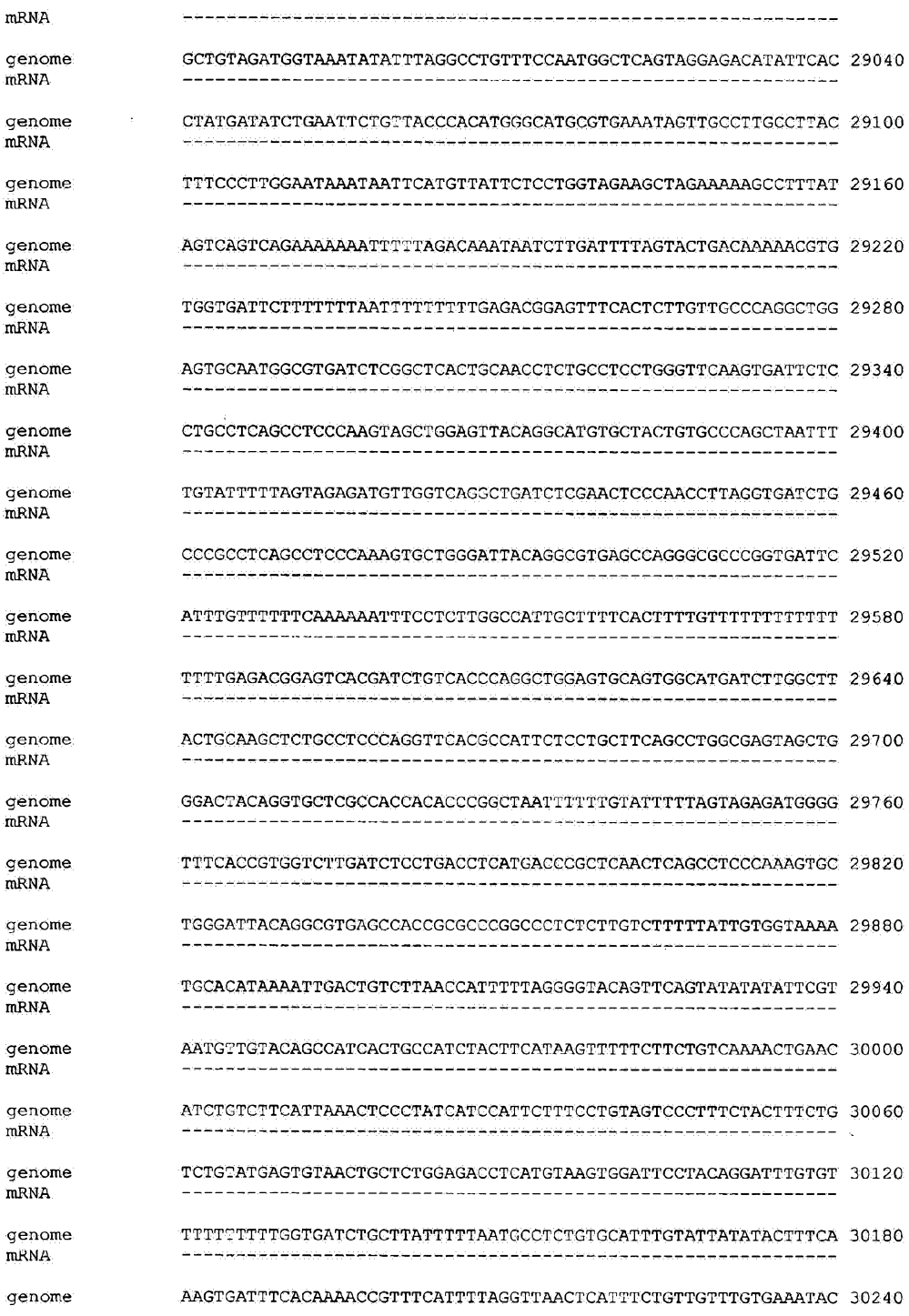
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Figure 1

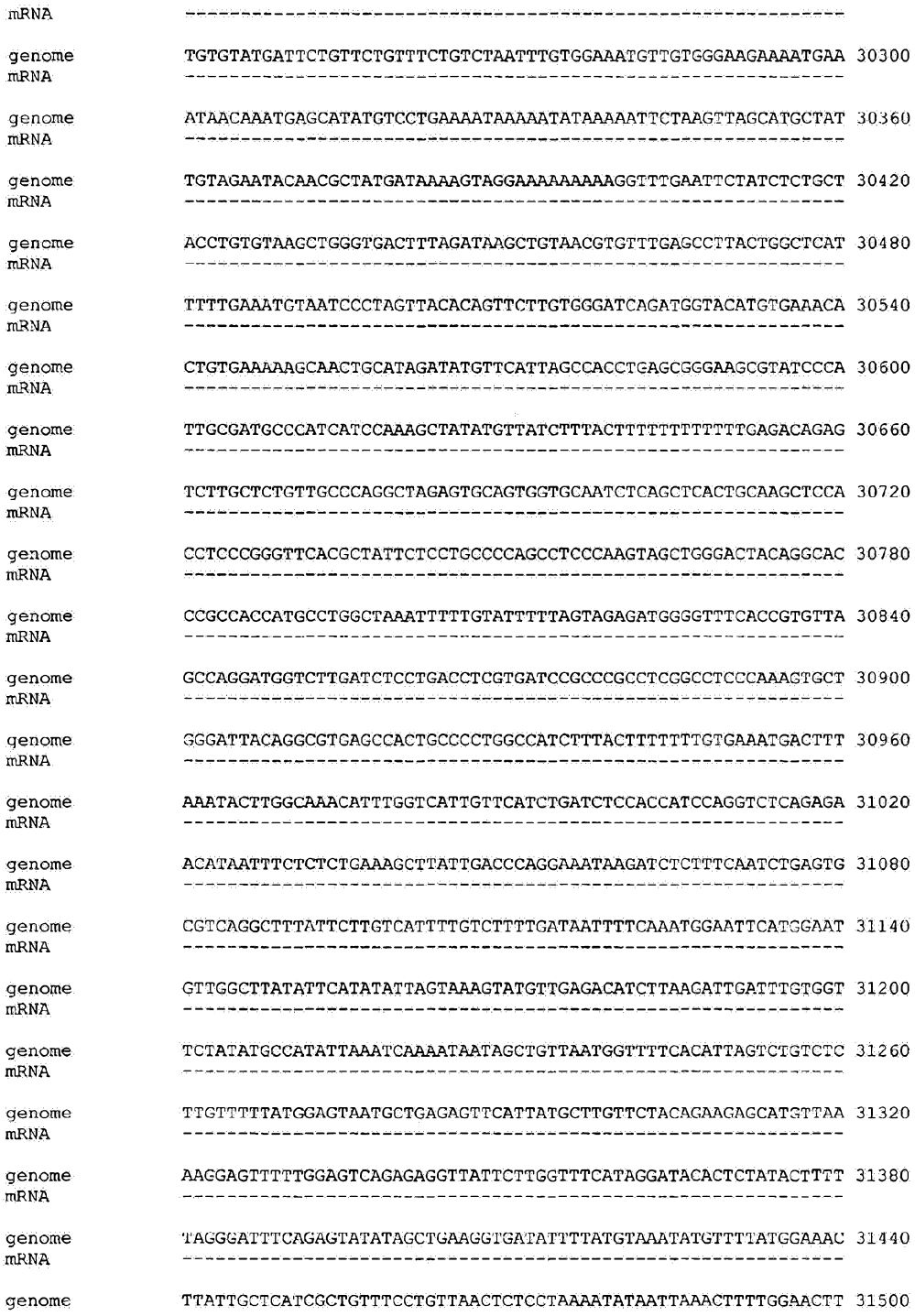
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Figure 1

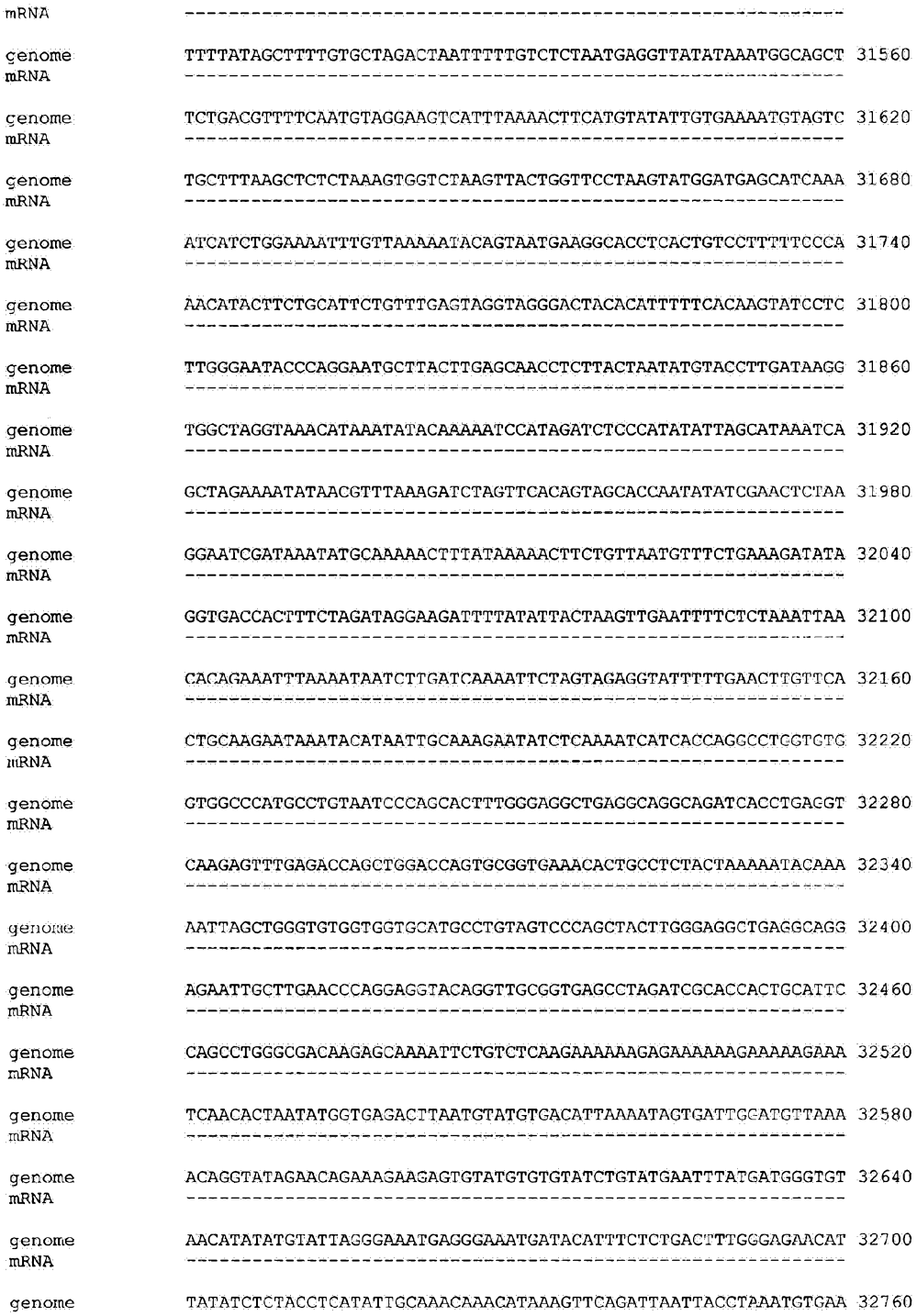
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Figure 1

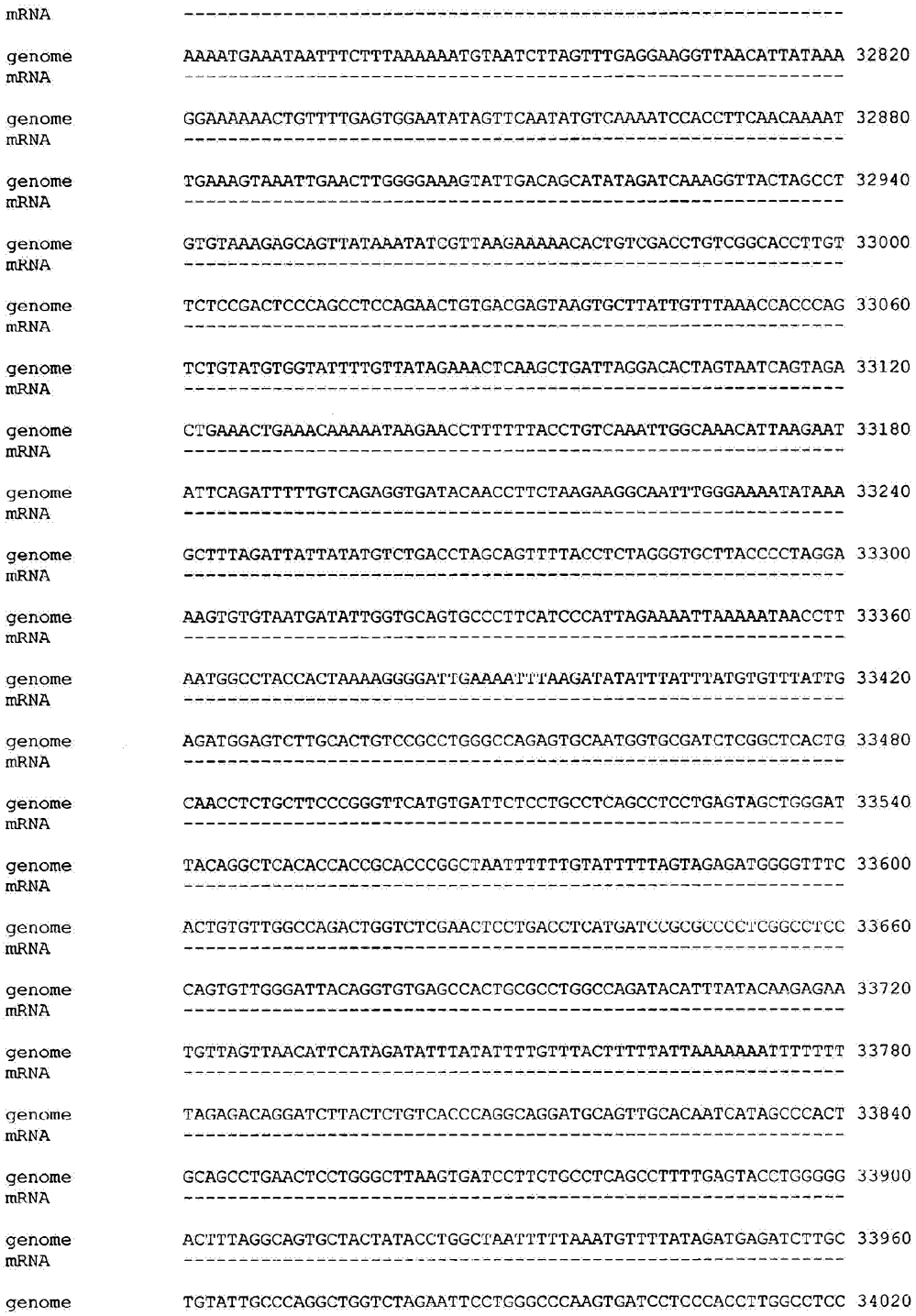
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Figure 1

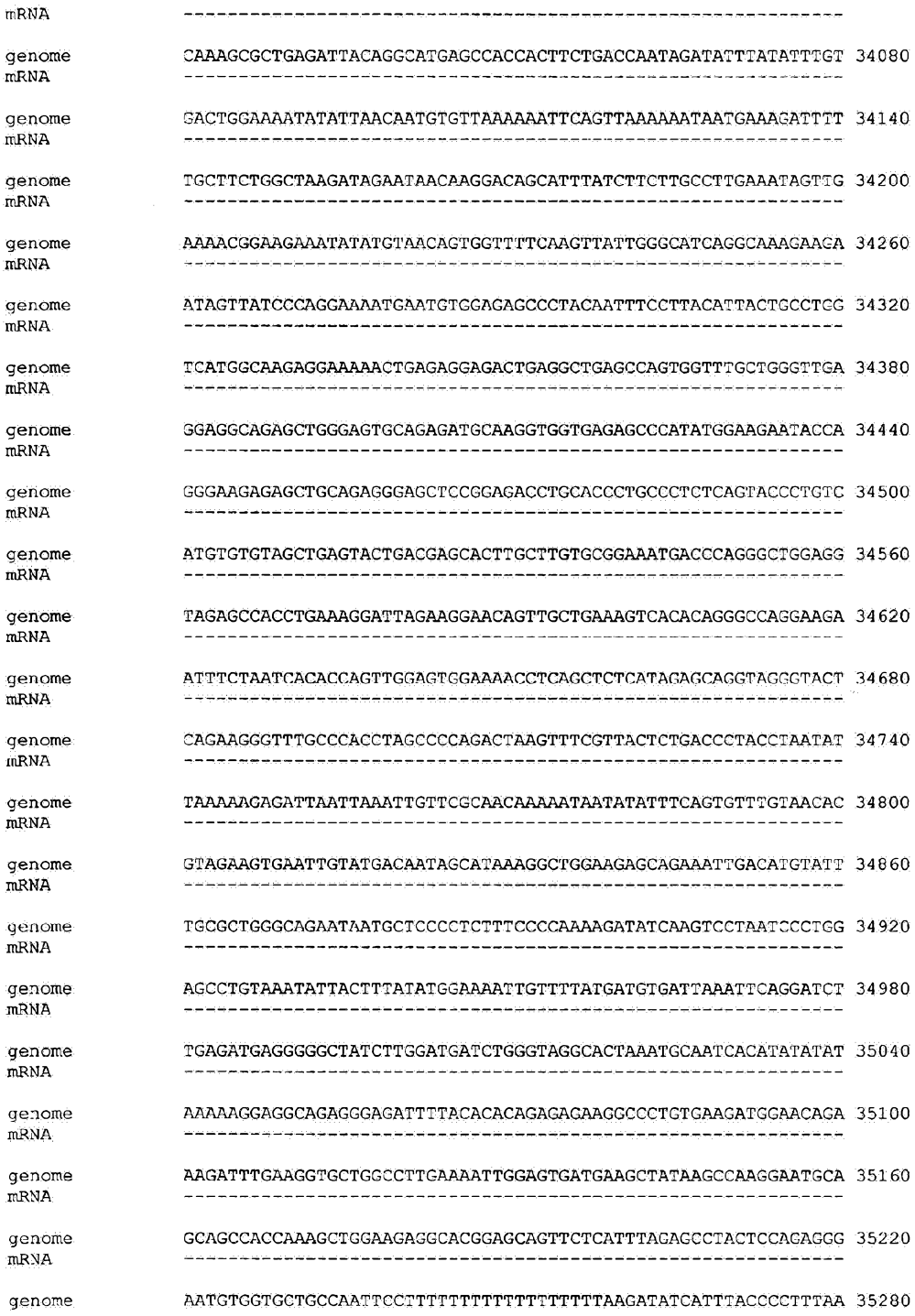
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Figure 1

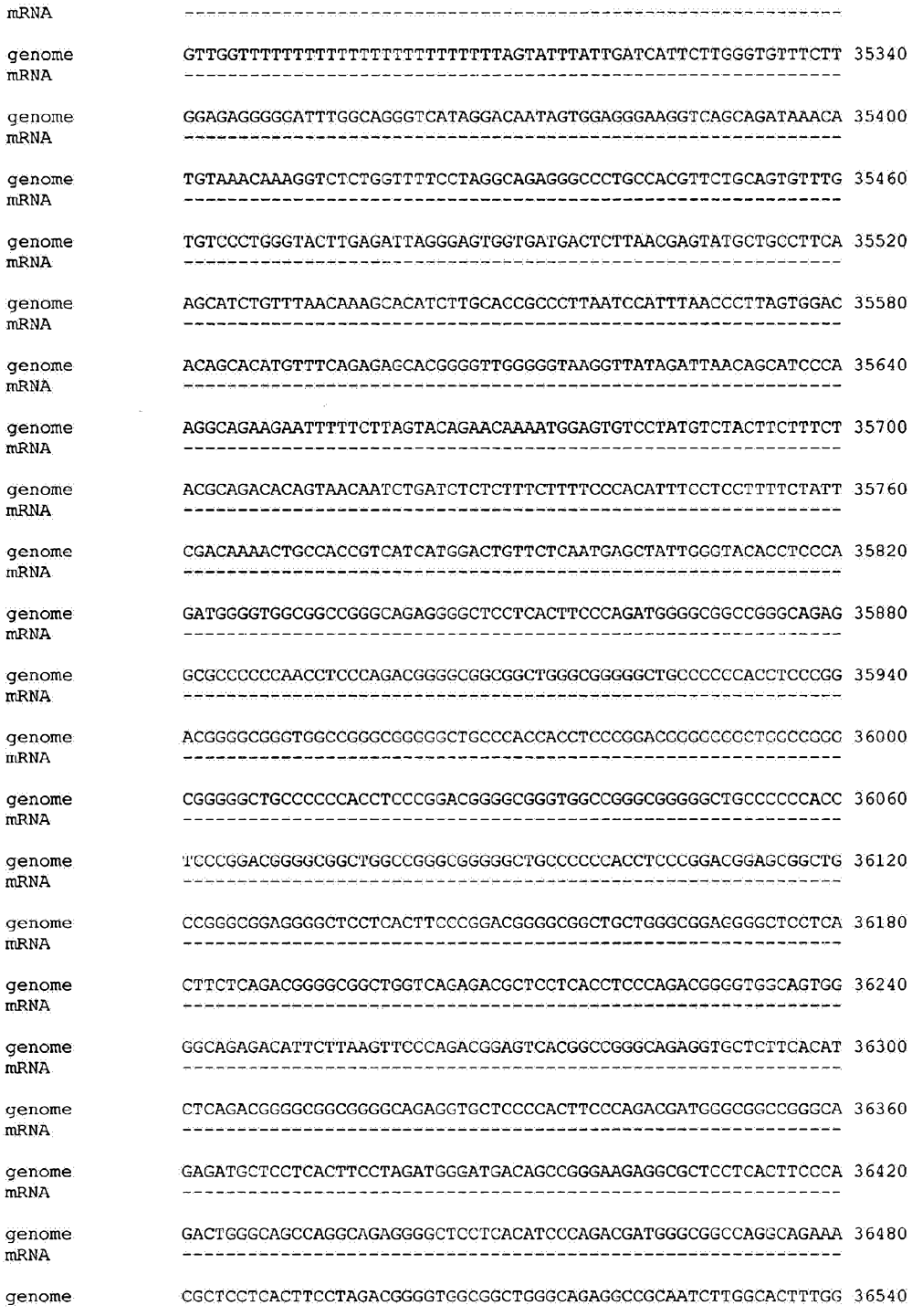
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Figure 1

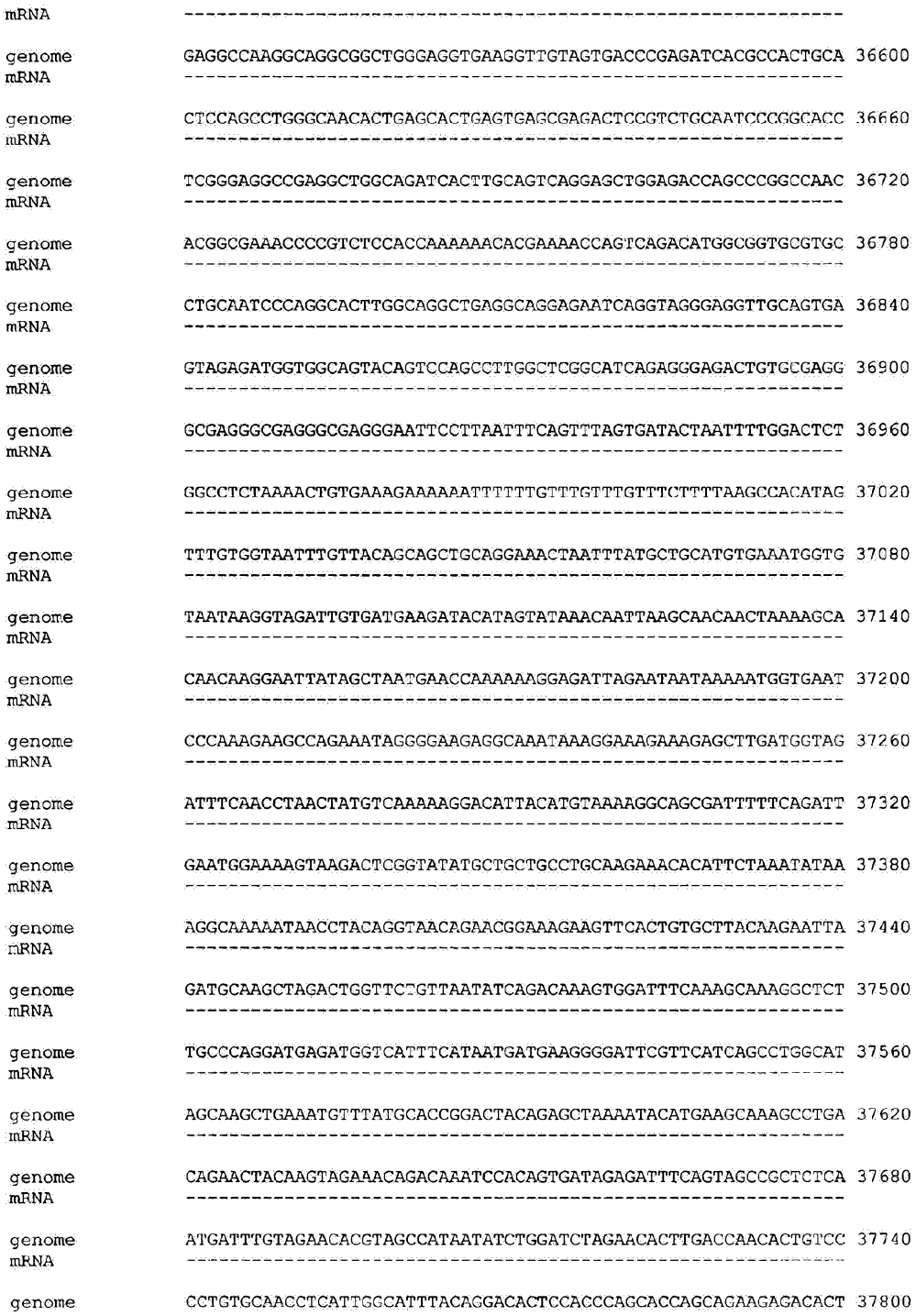
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Figure 1

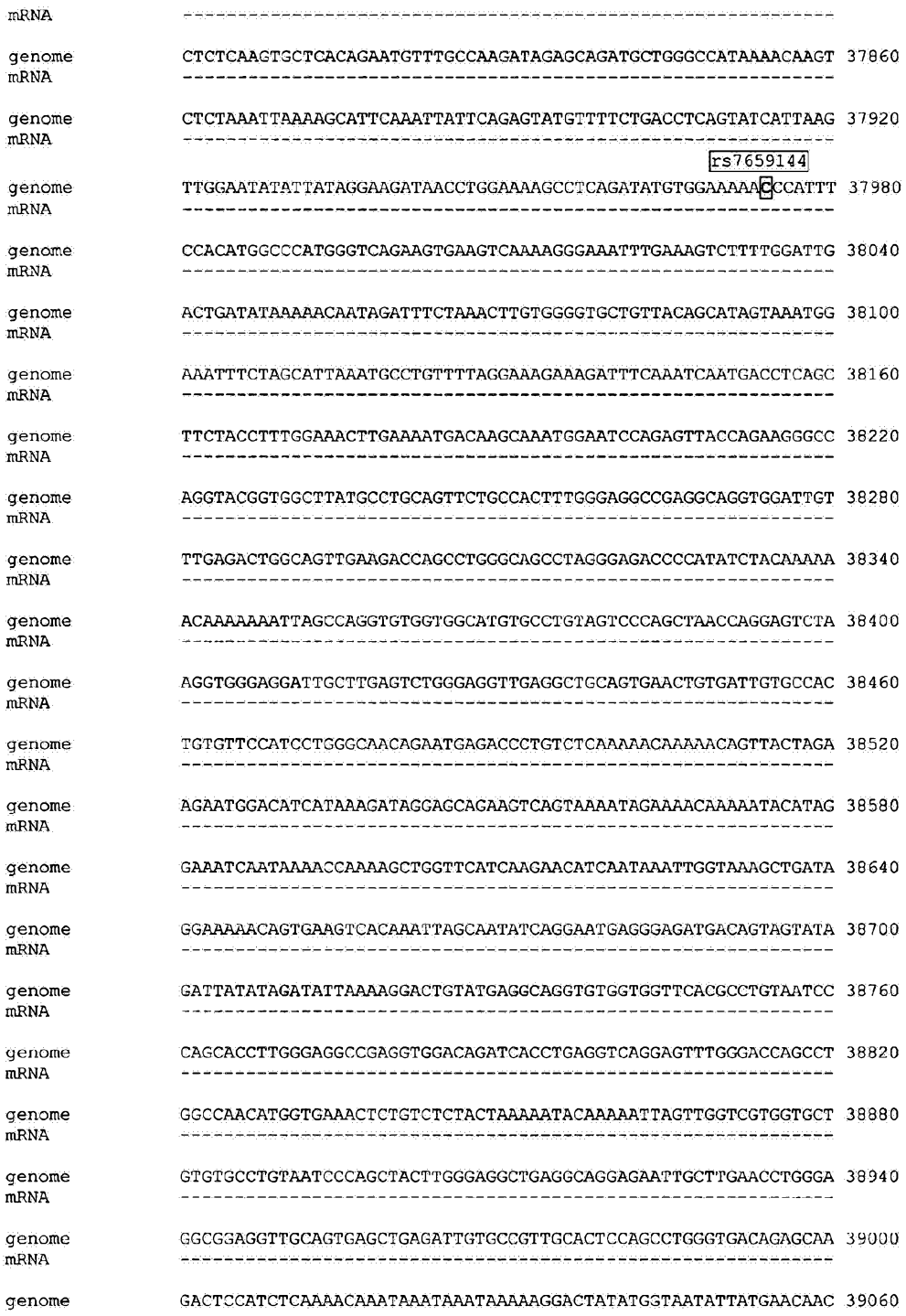
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Figure 1

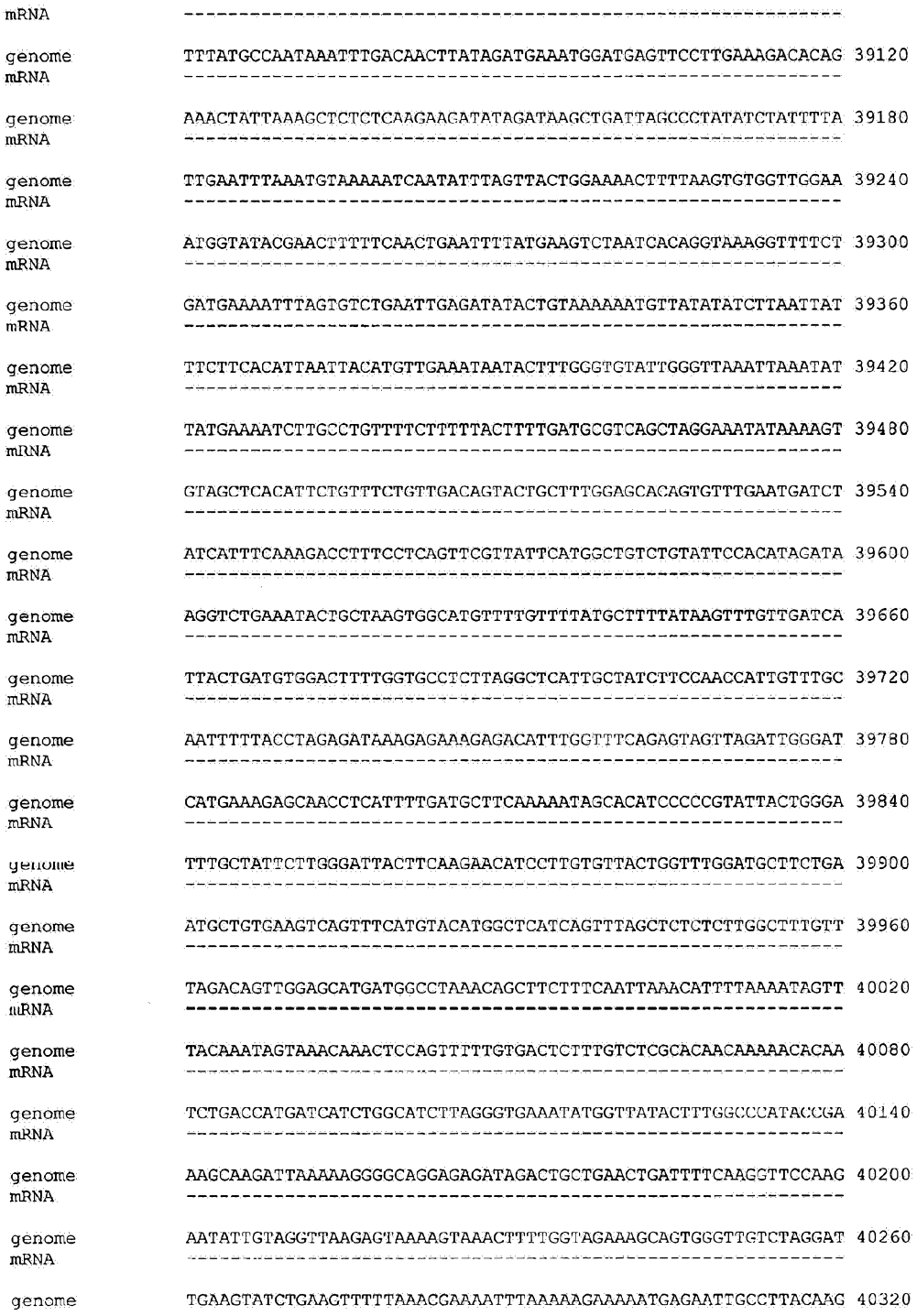
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Figure 1

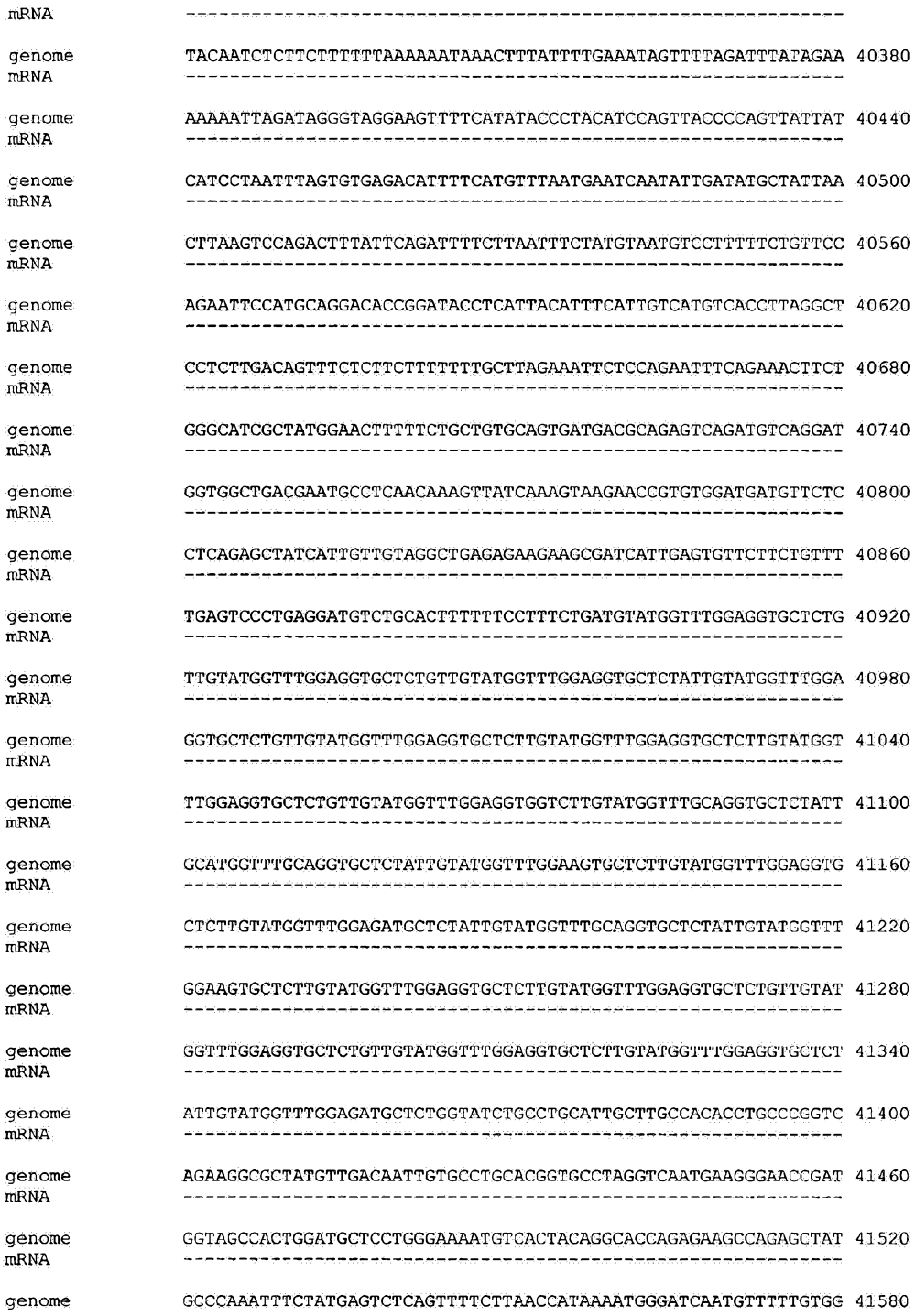
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Figure 1

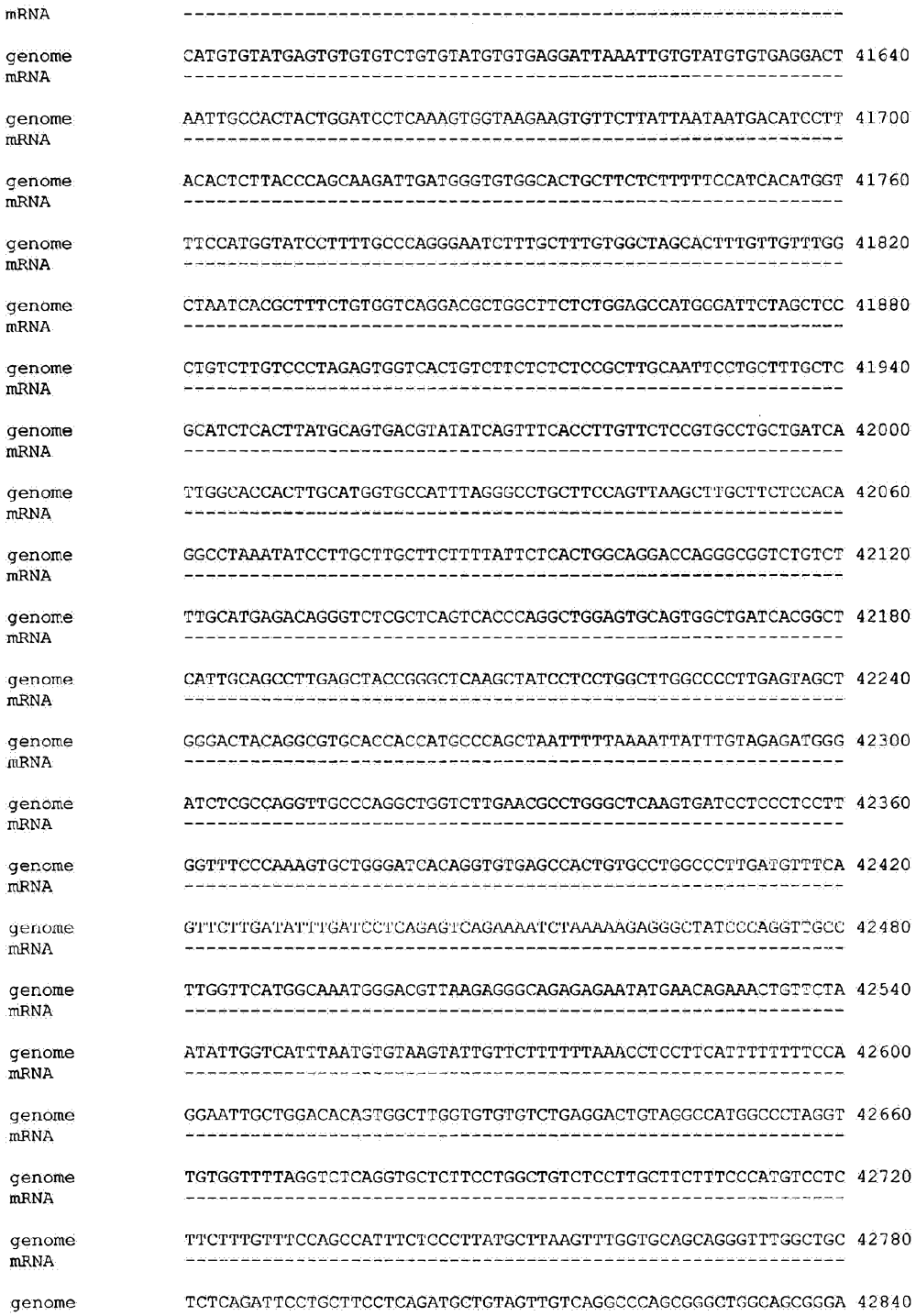
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Figure 1

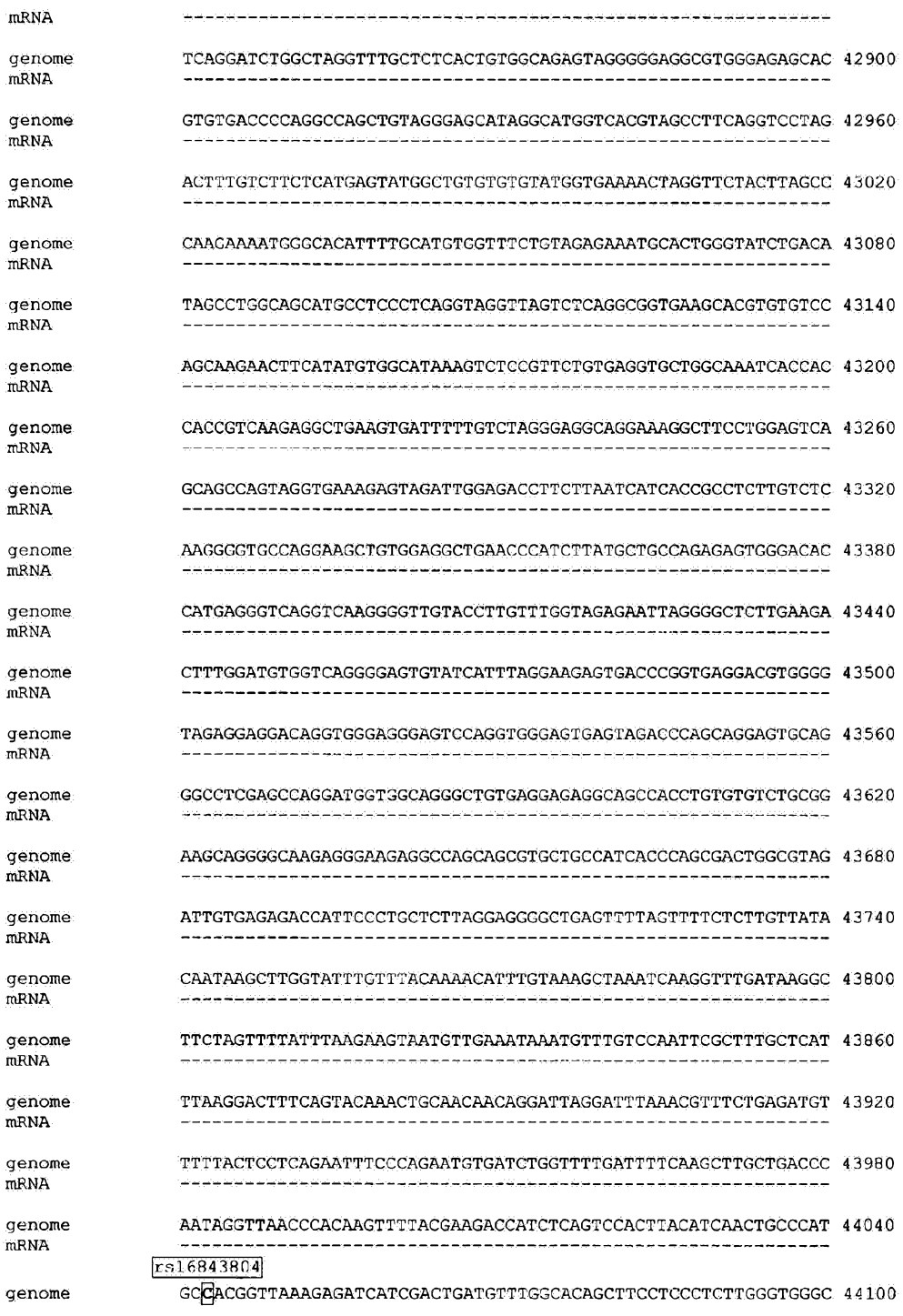
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Figure 1

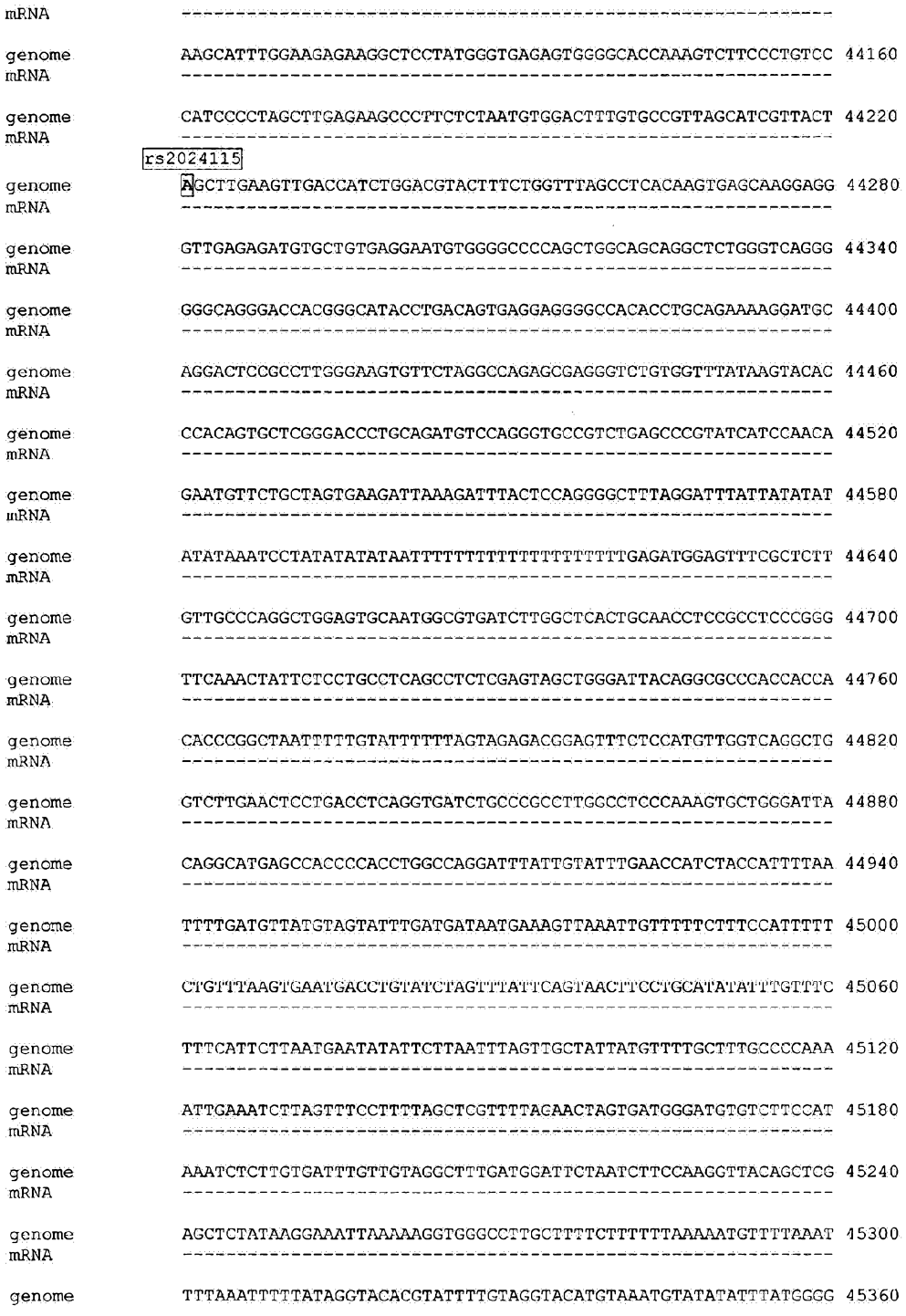
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Figure 1

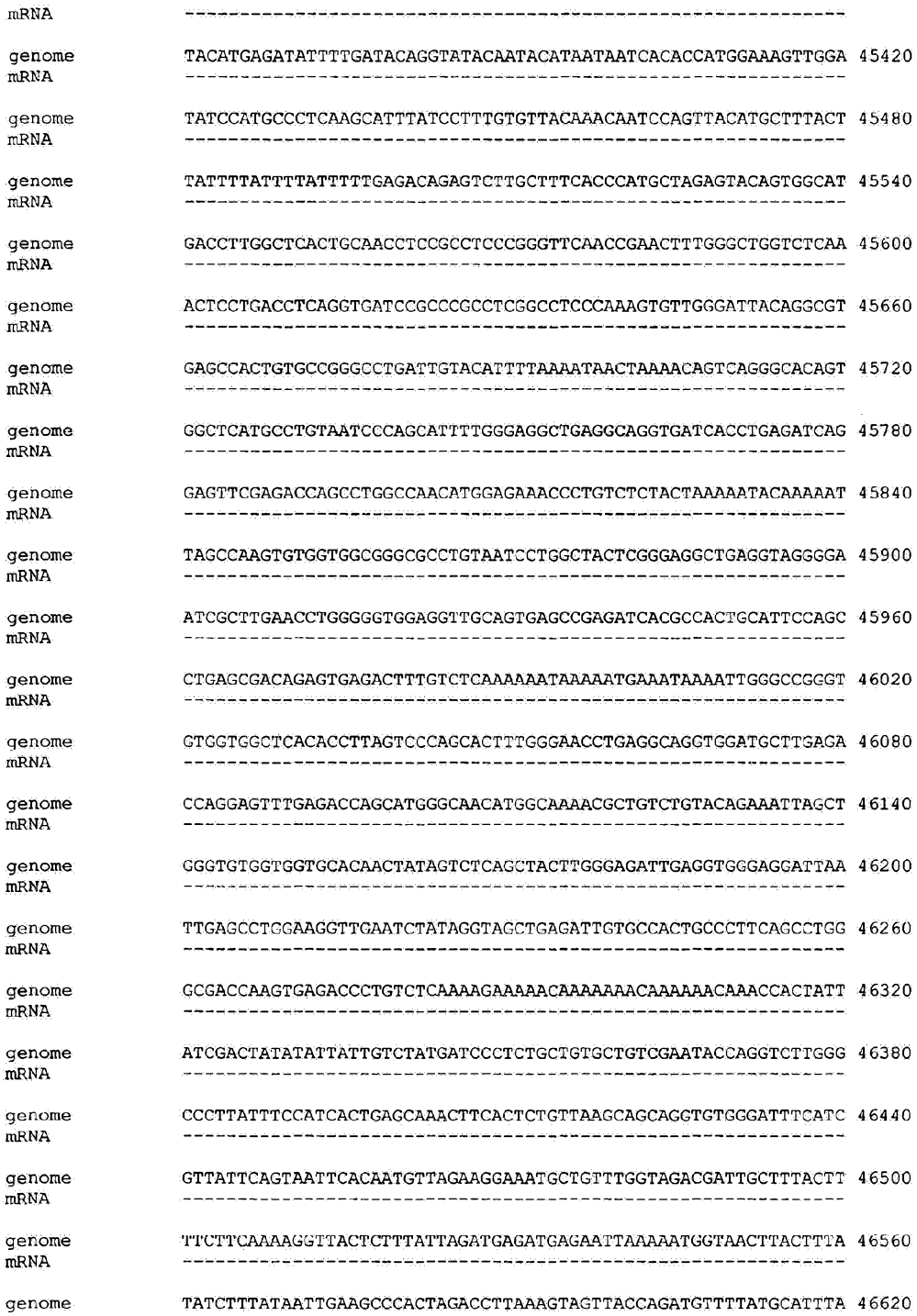
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Figure 1

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Figure 1

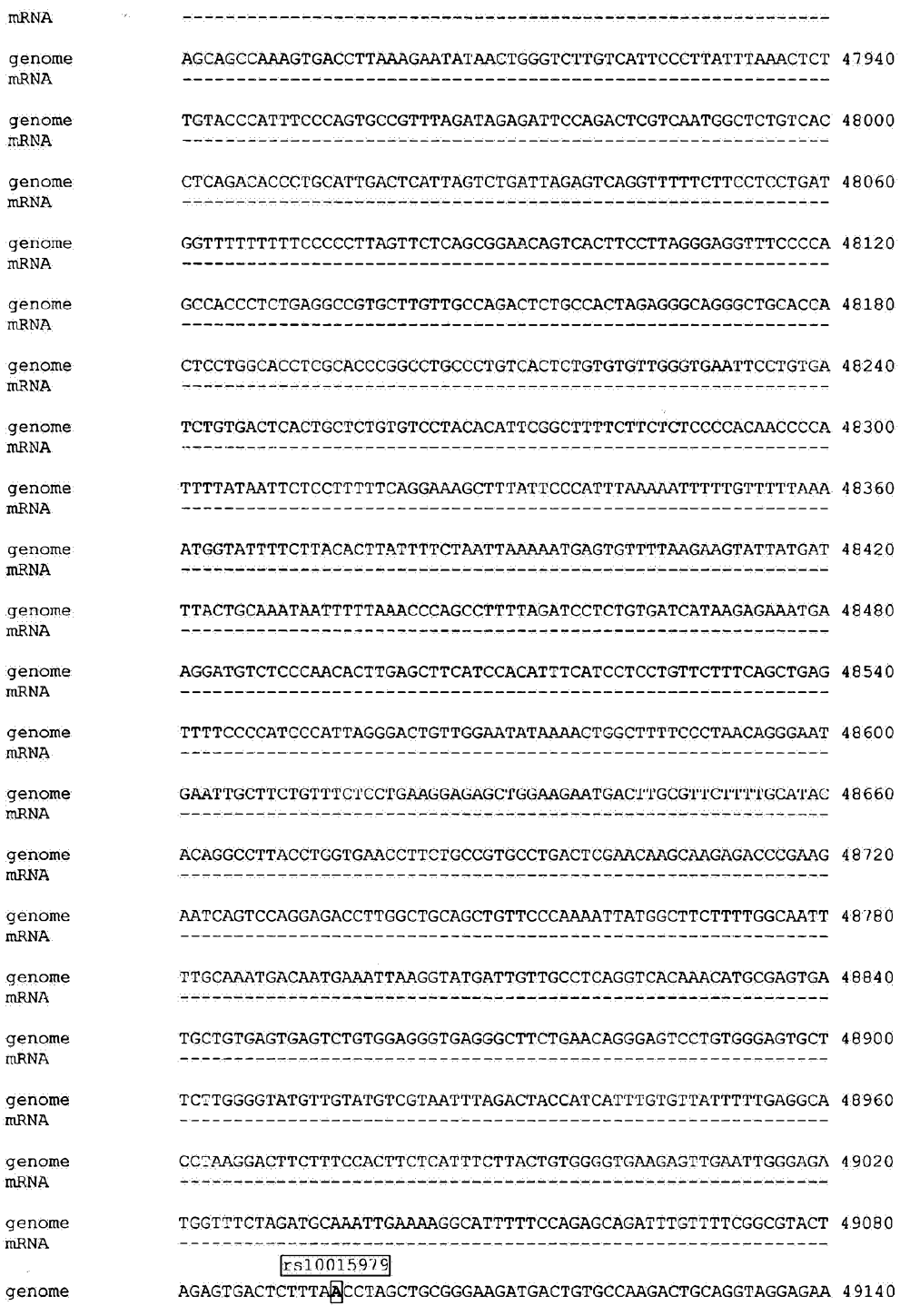
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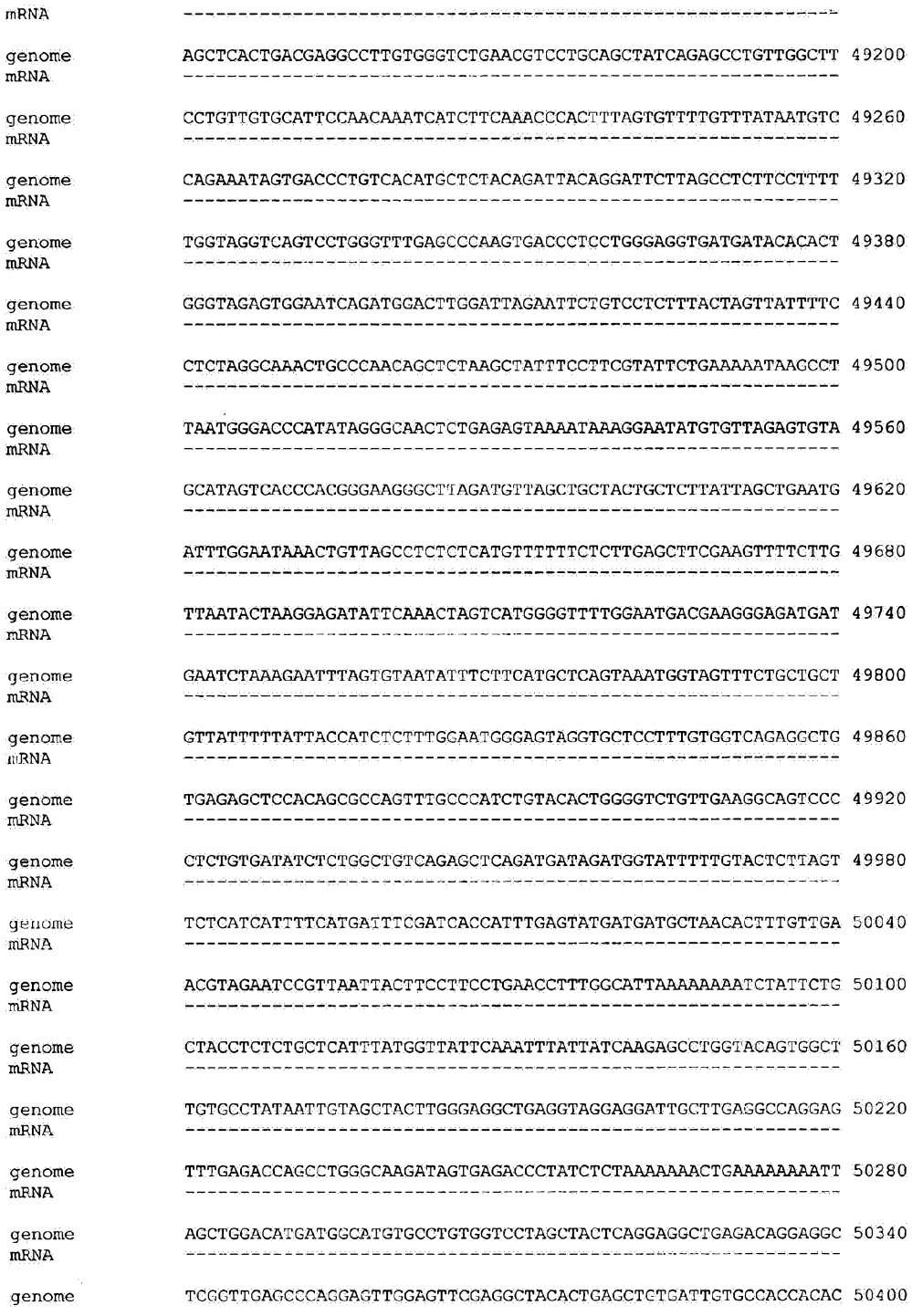
Figure 1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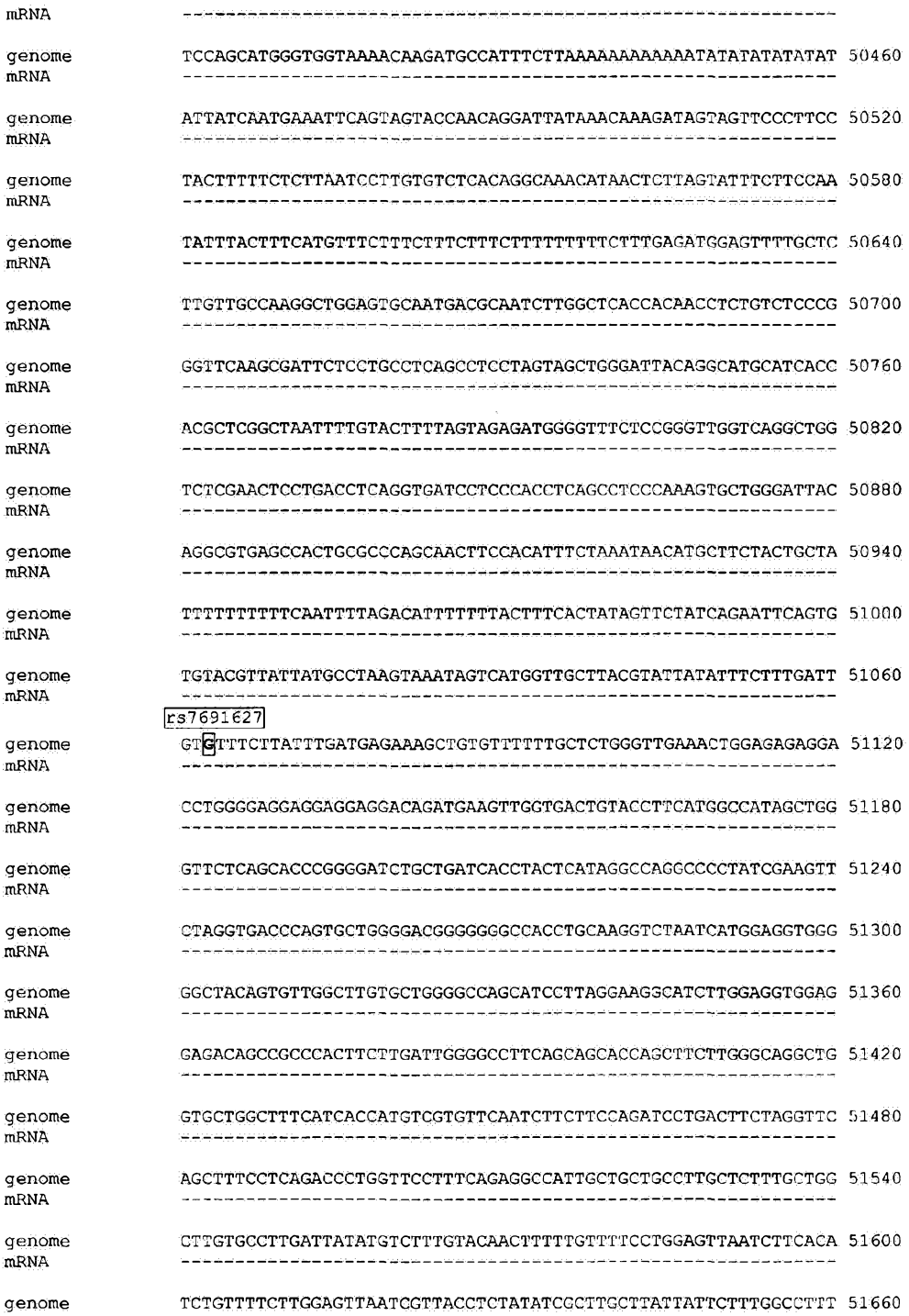
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Figure 1

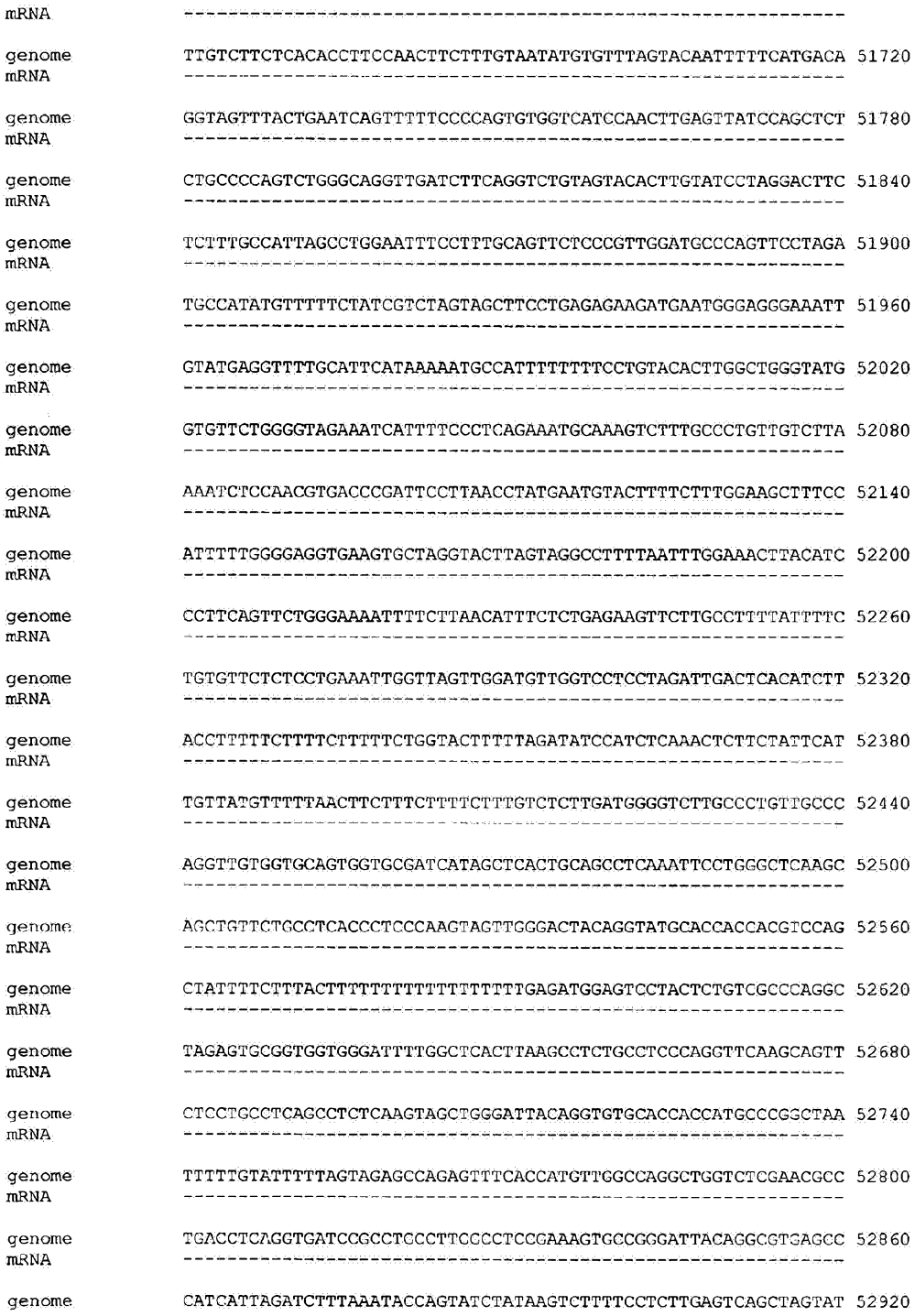
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Figure 1

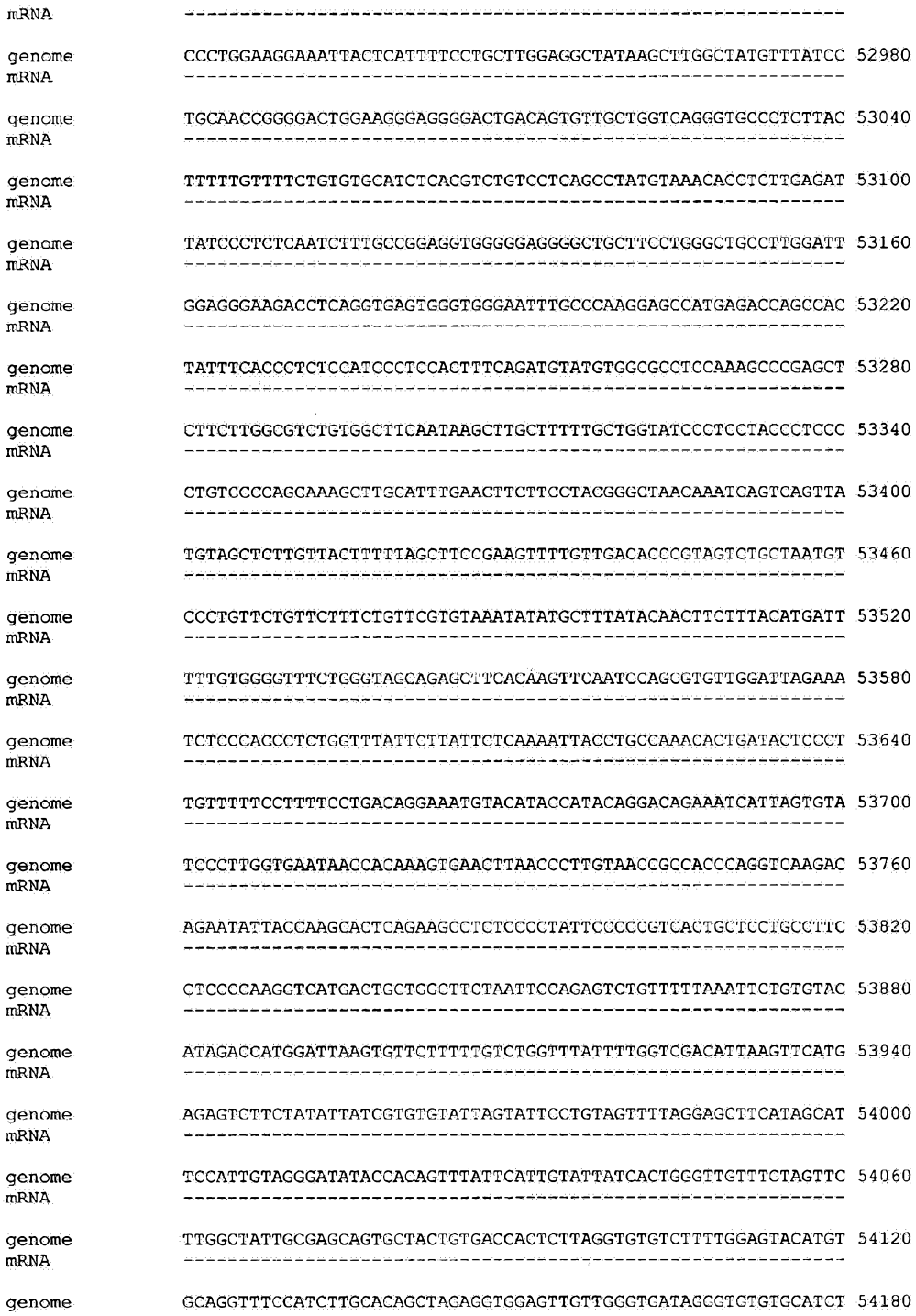
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Figure 1

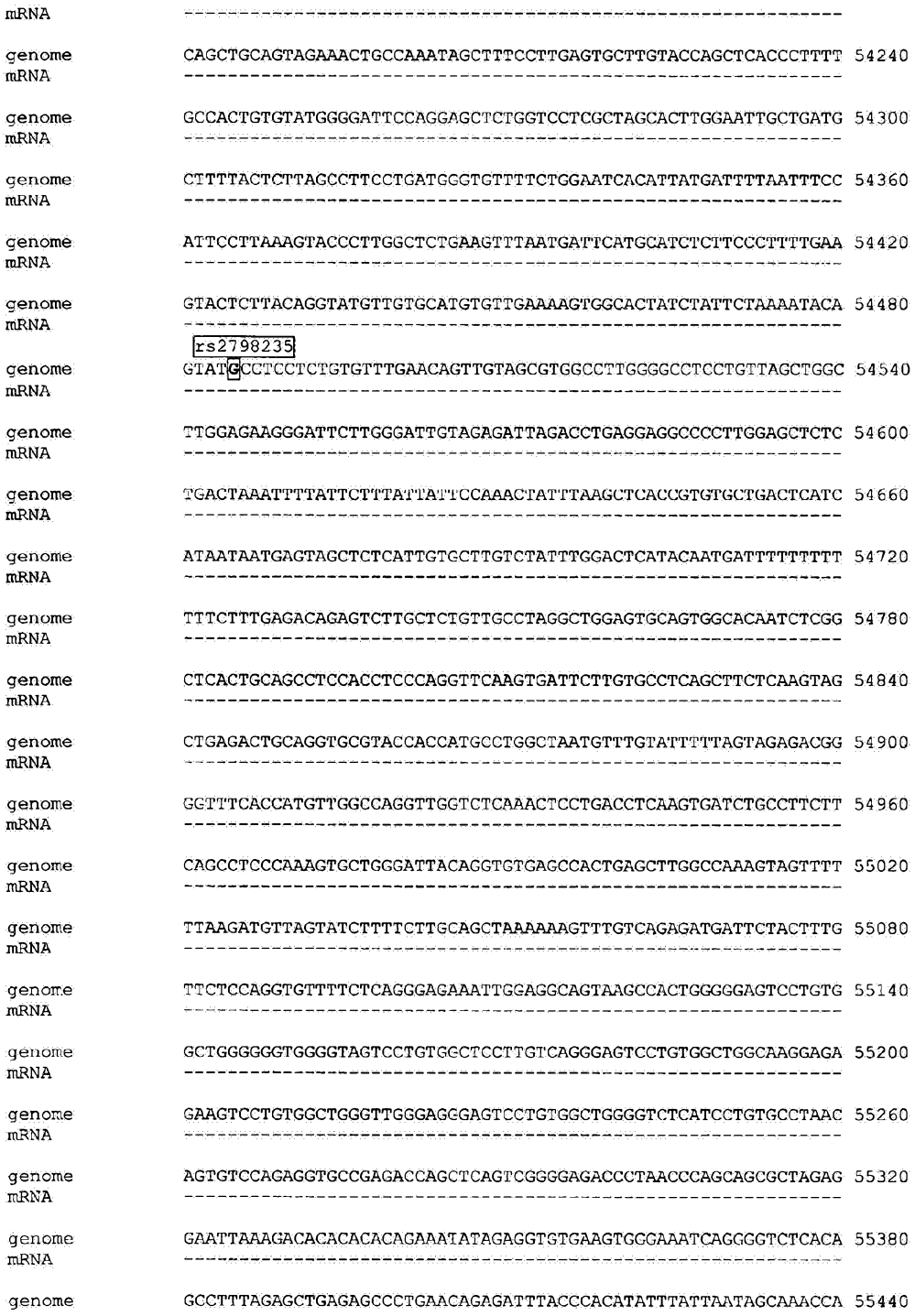
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Figure 1

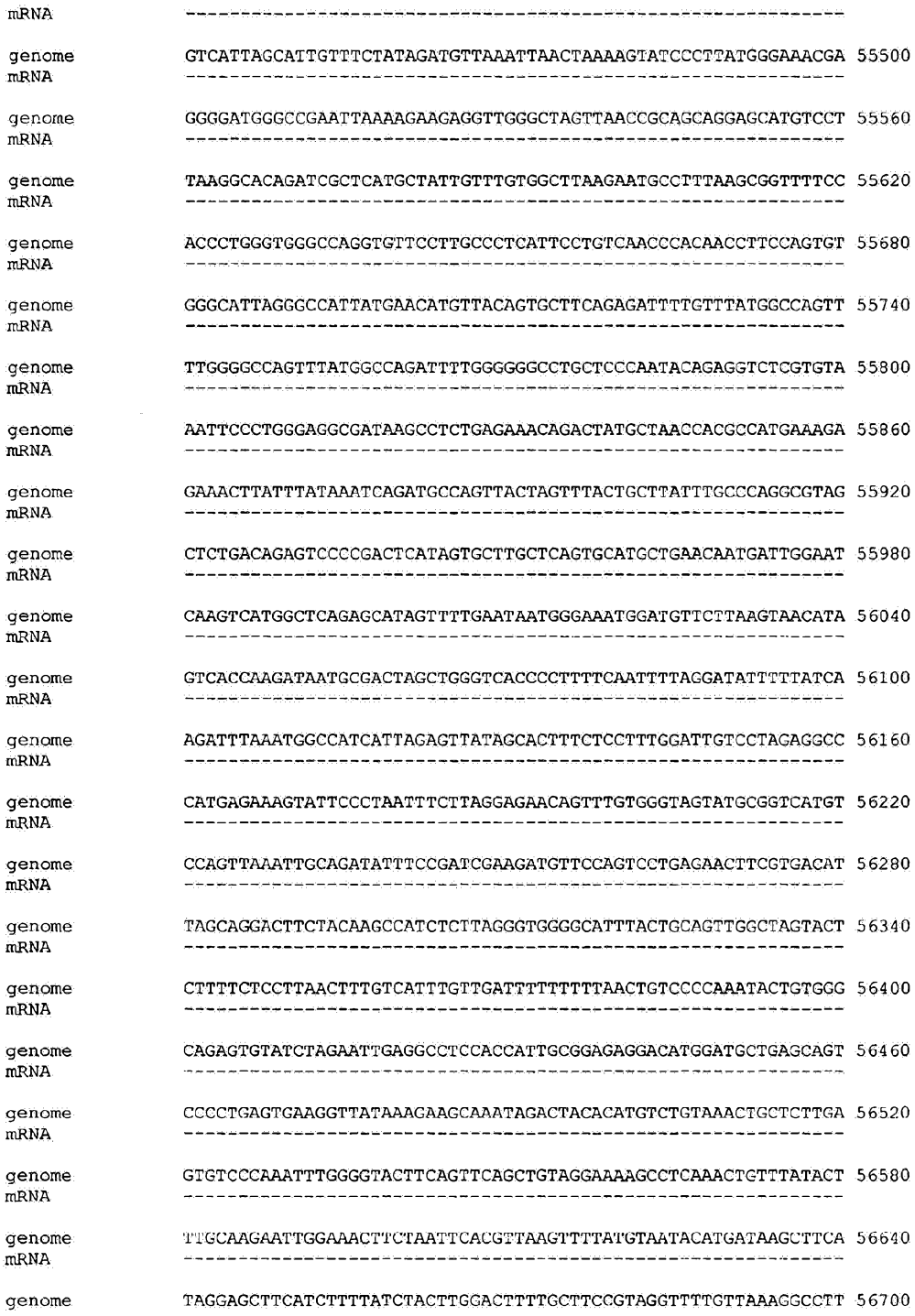
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Figure 1

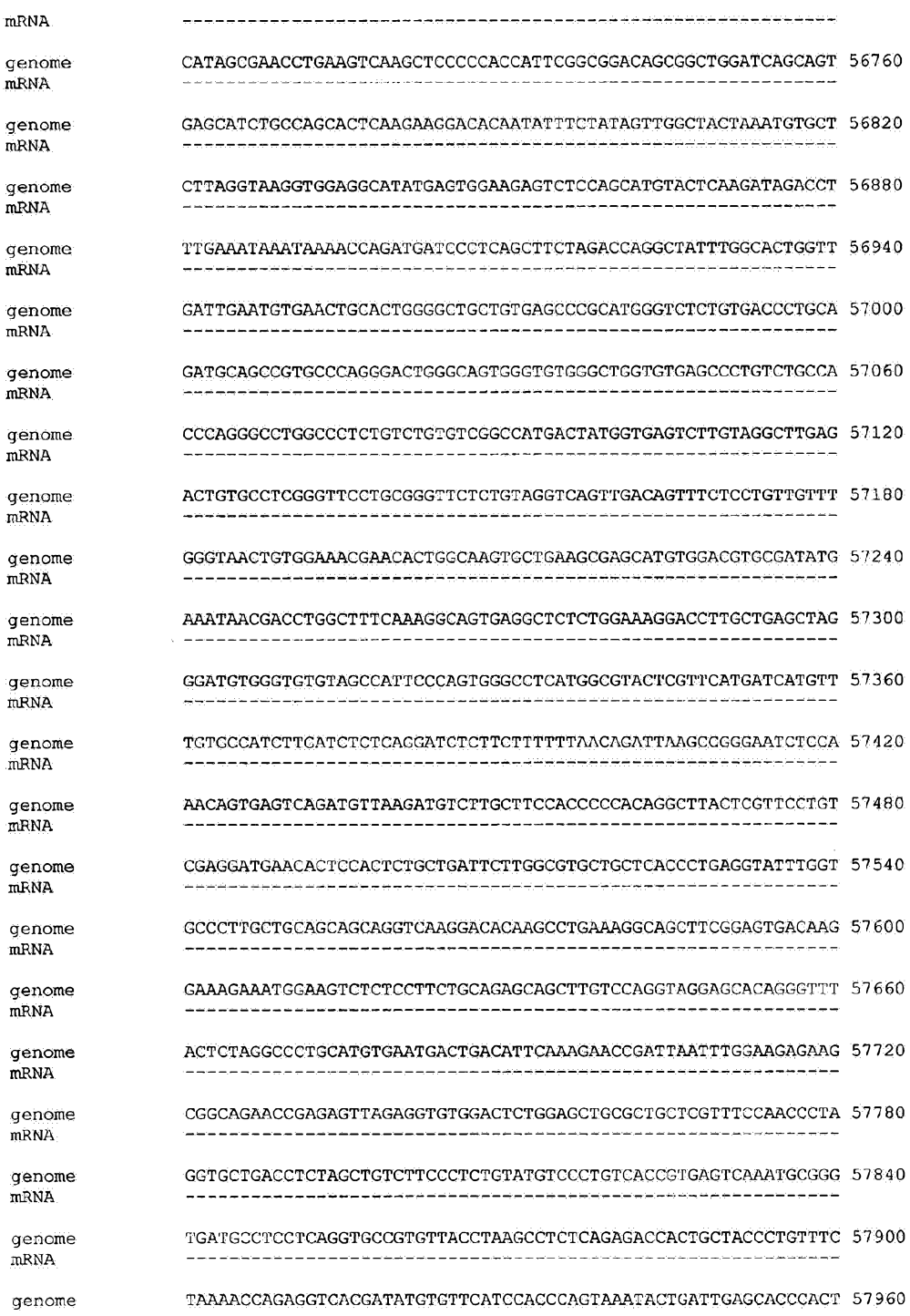
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Figure 1

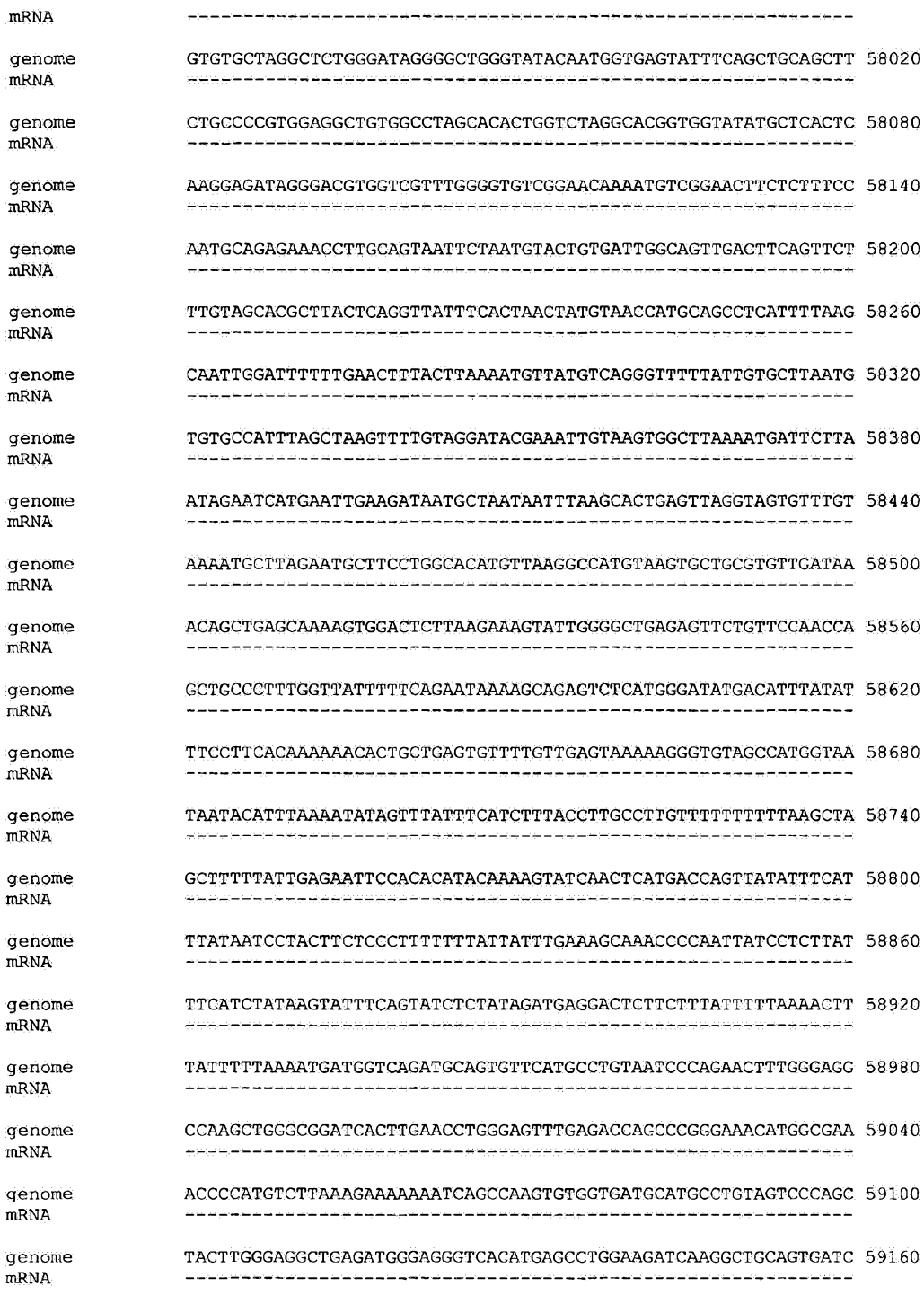
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Figure 1

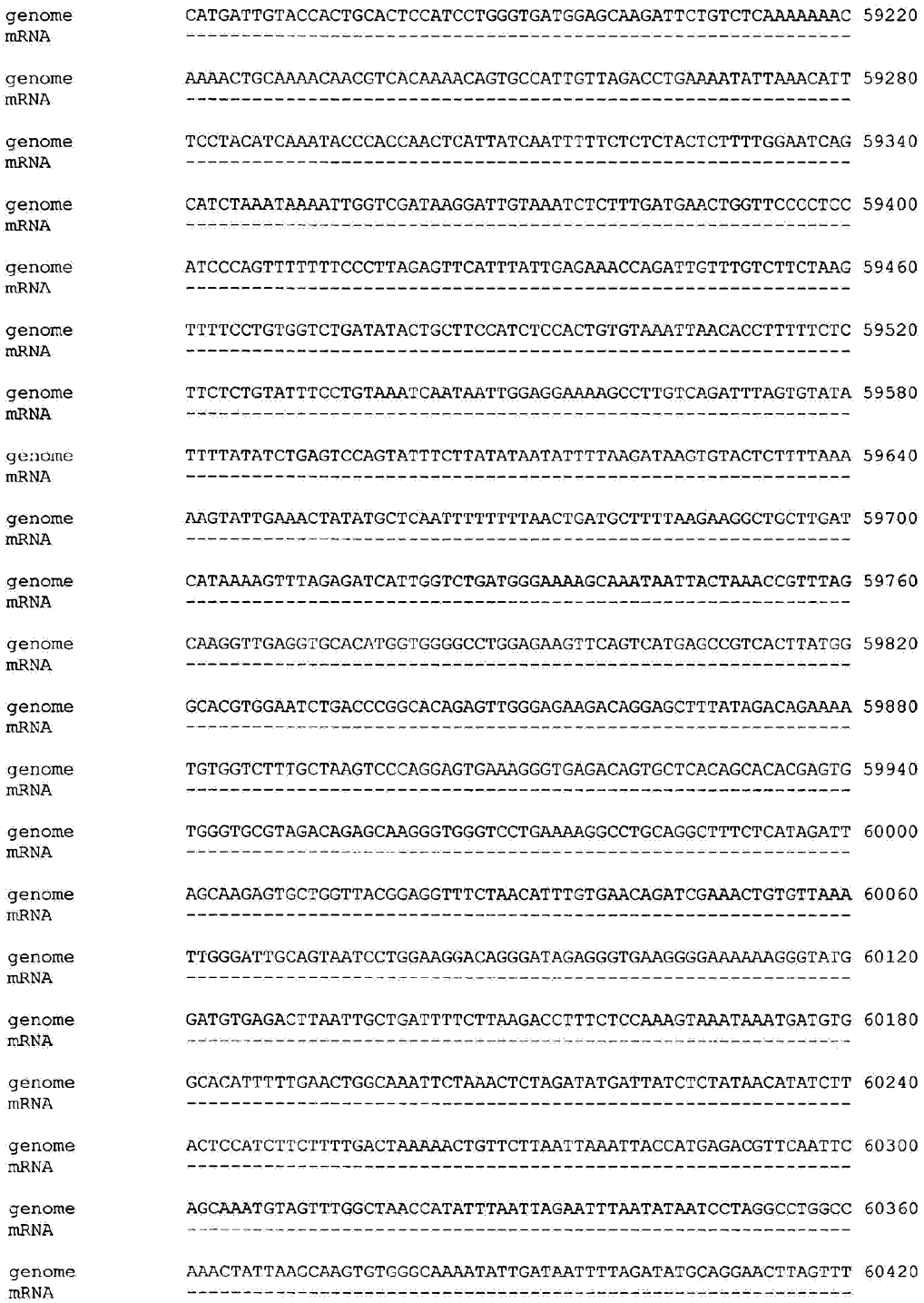
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Figure 1

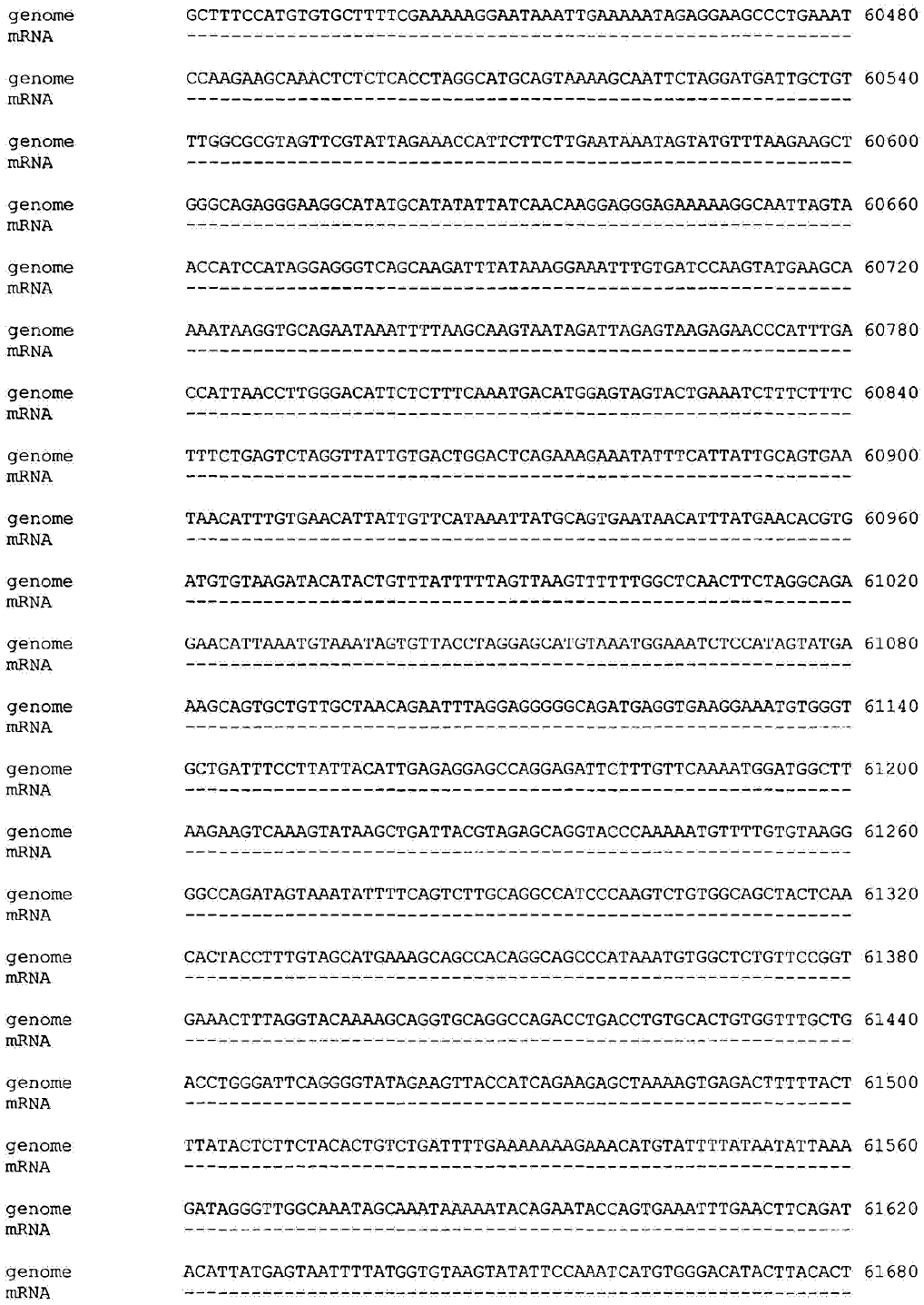
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Figure 1

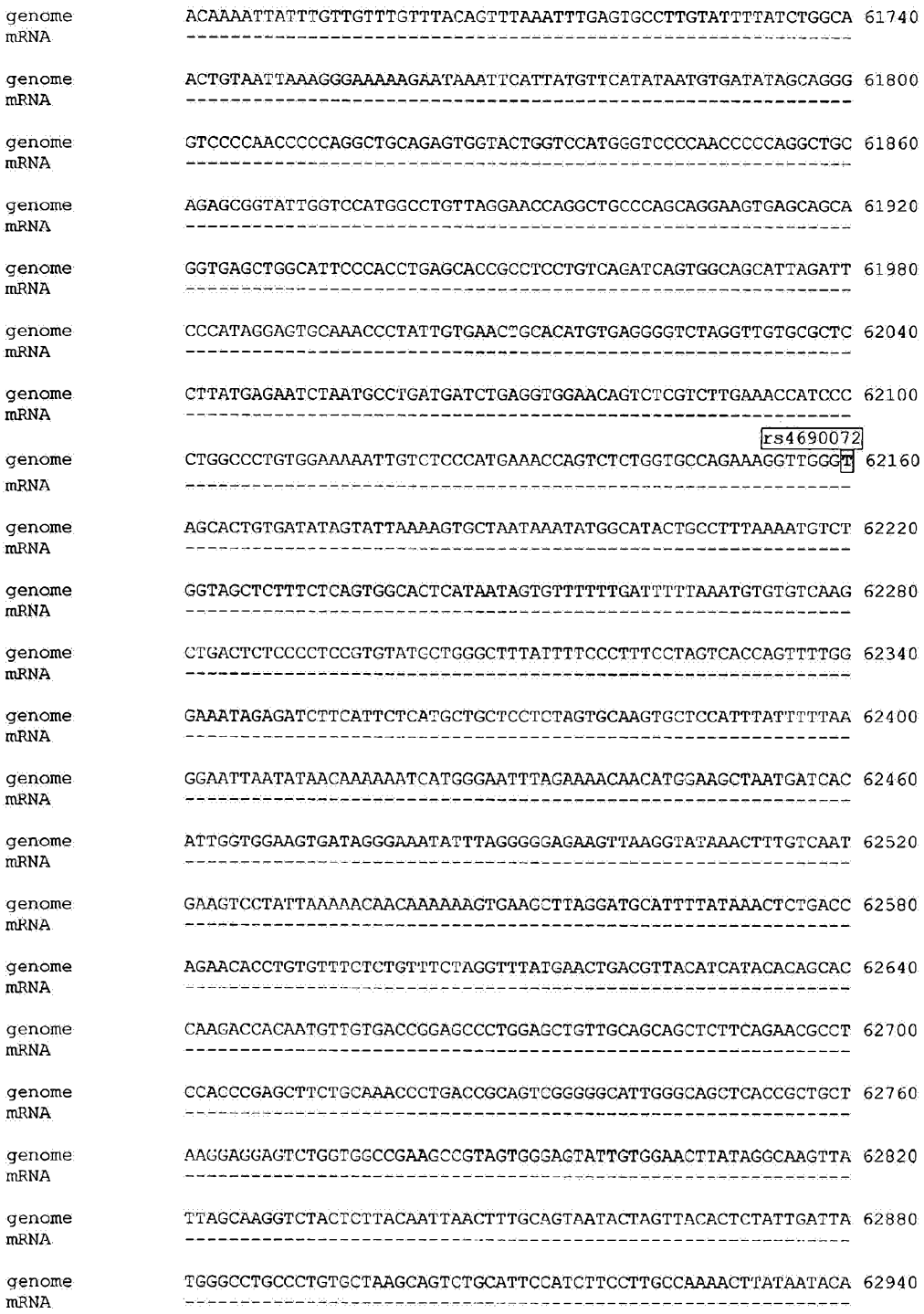
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Figure 1

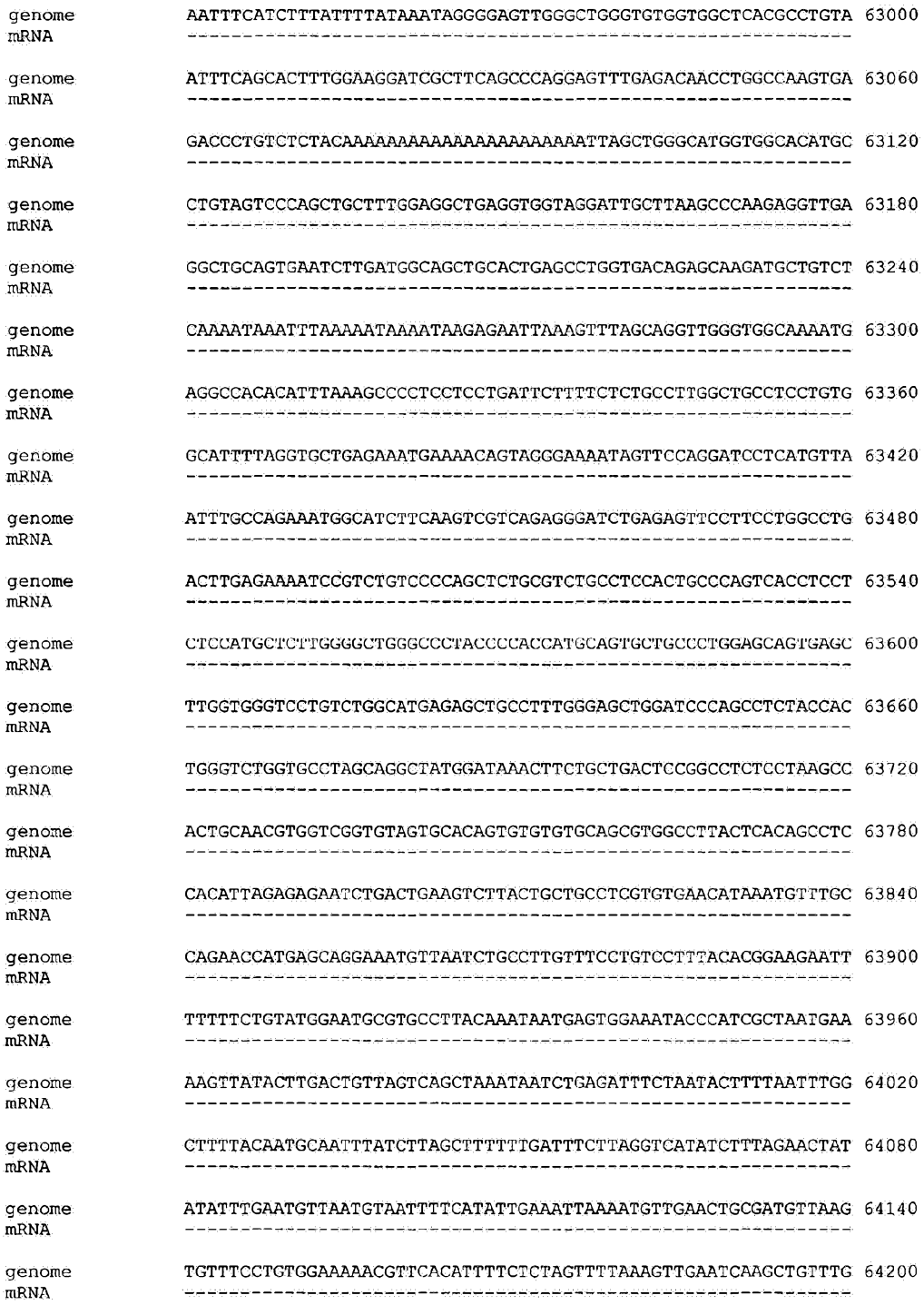
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Figure 1

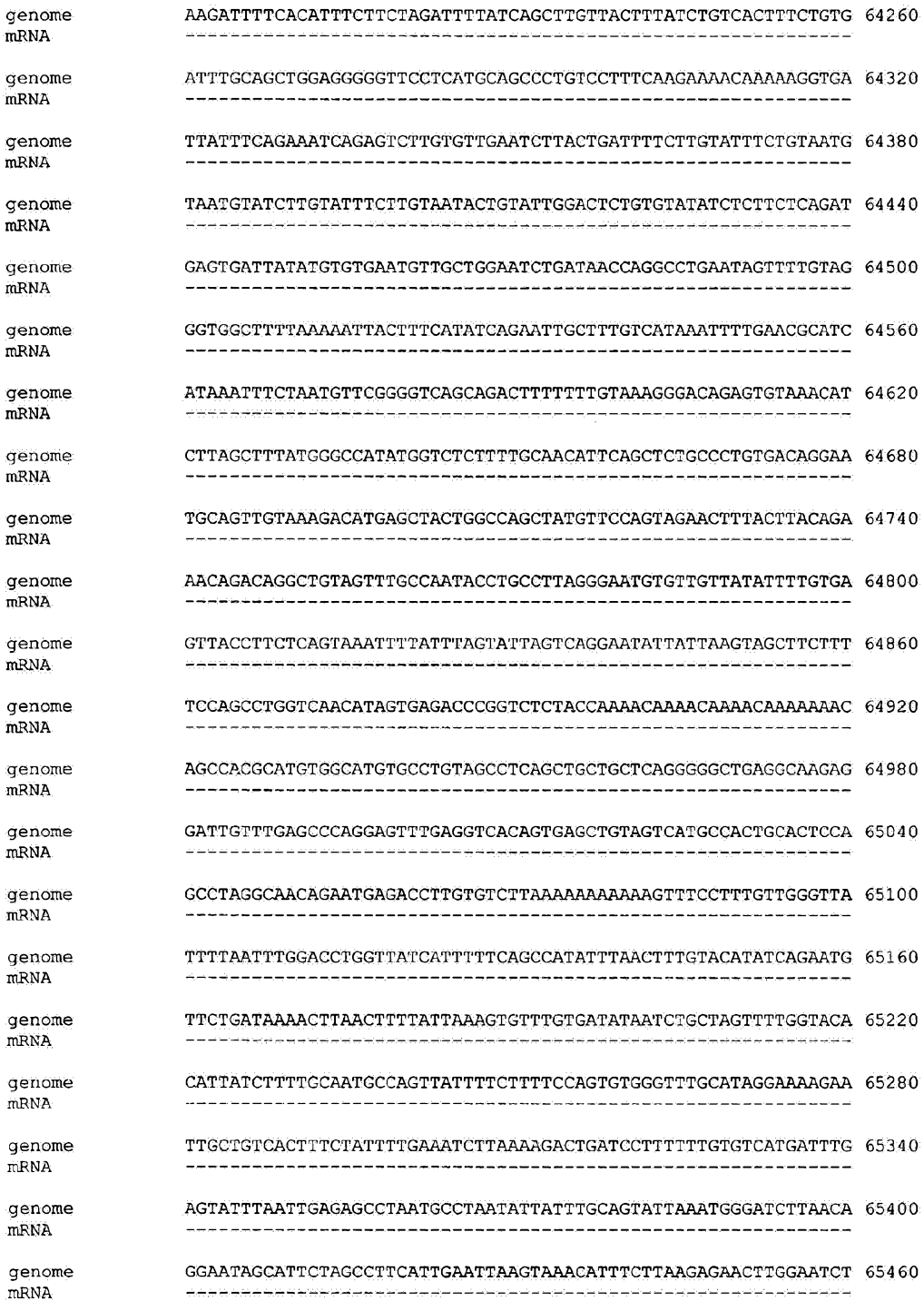
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Figure 1

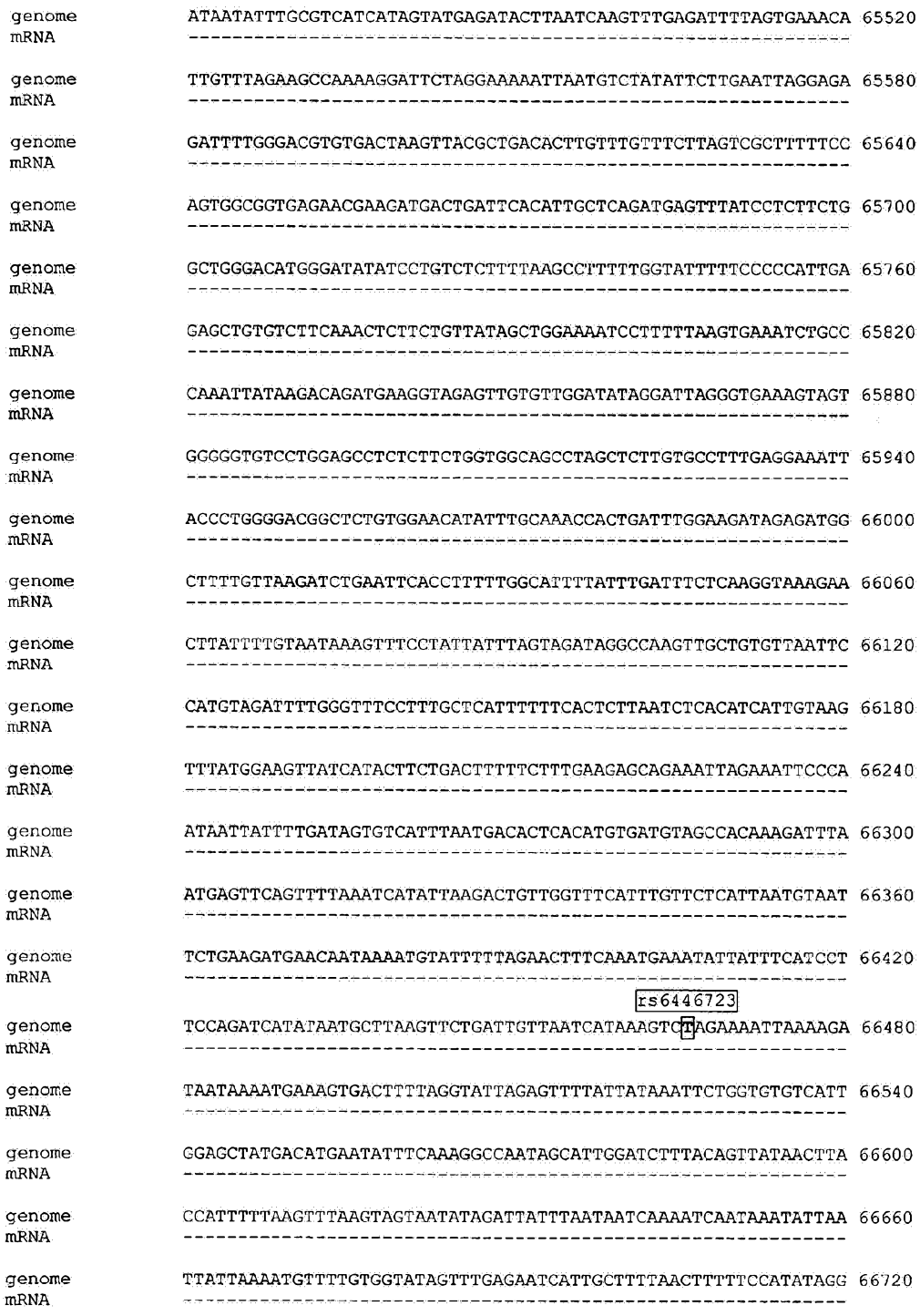
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Figure 1

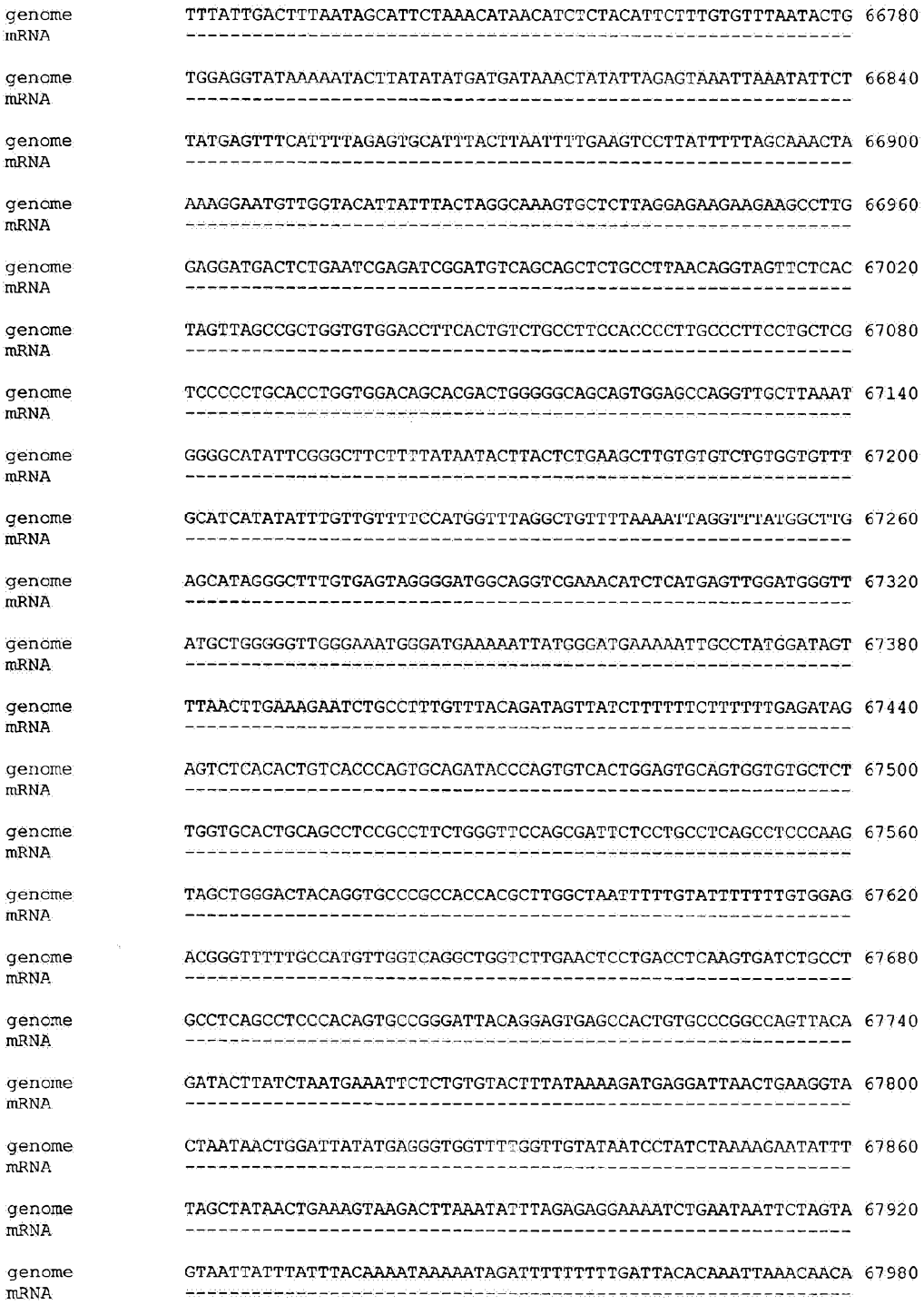
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Figure 1

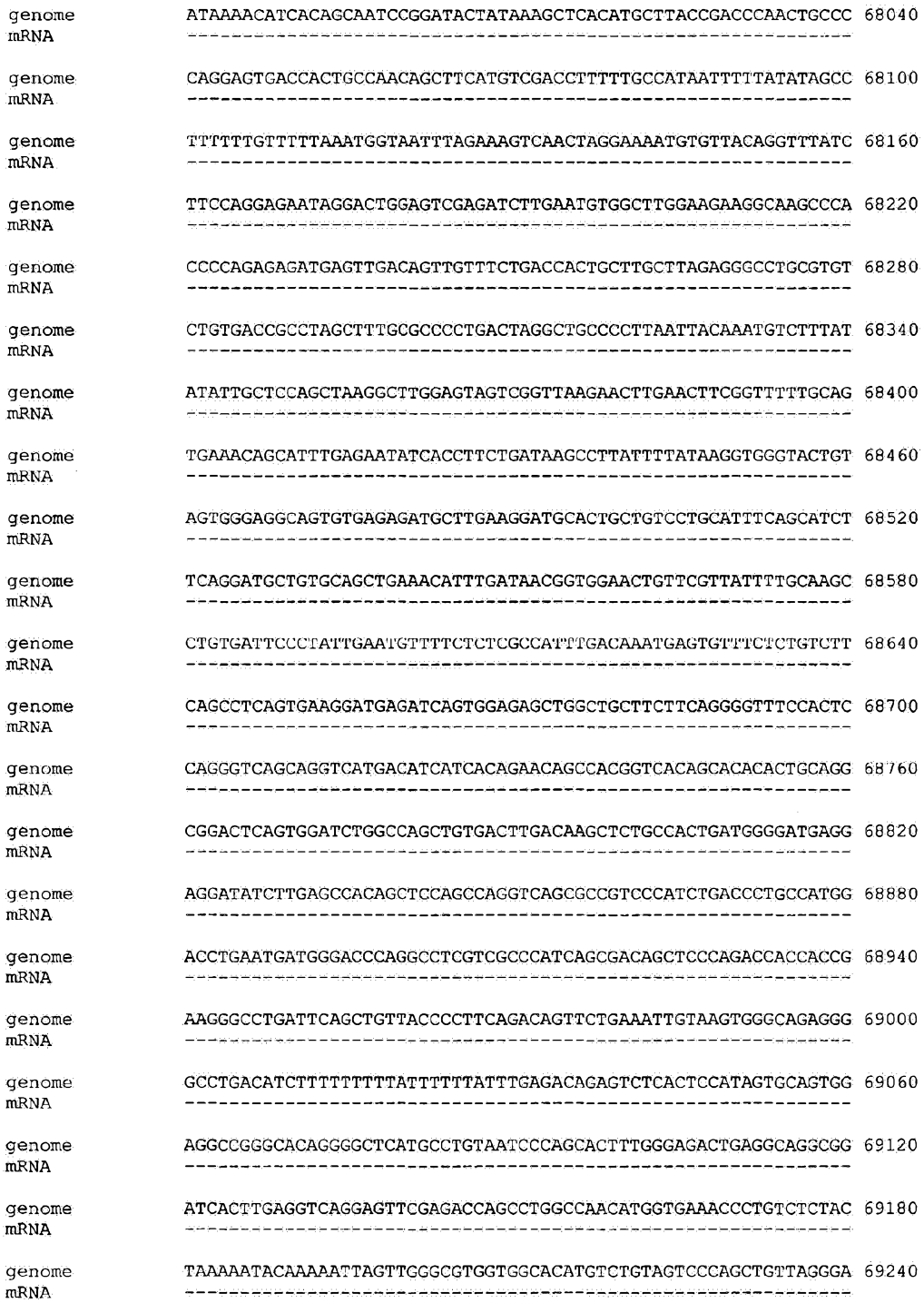
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Figure 1

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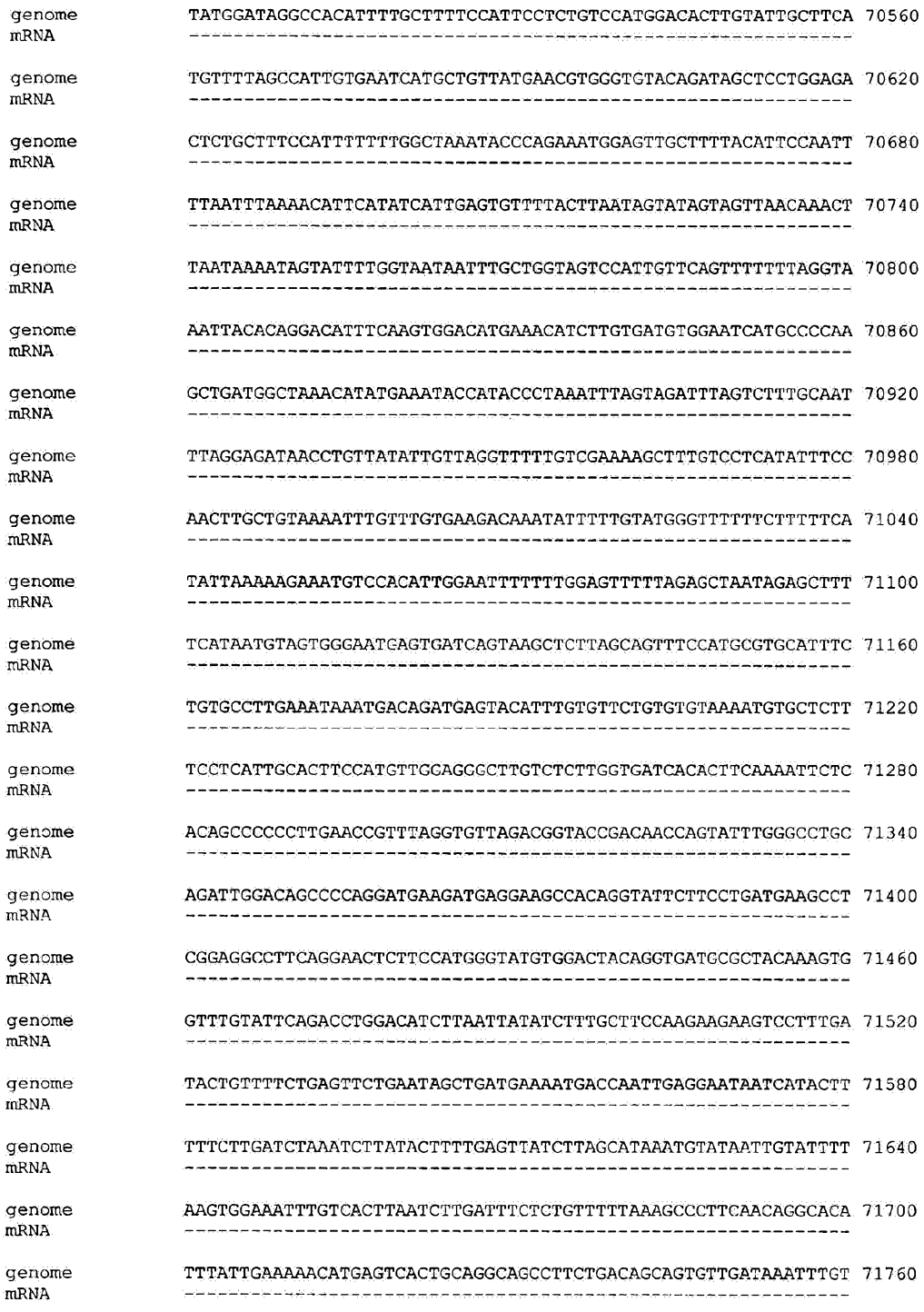
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Figure 1

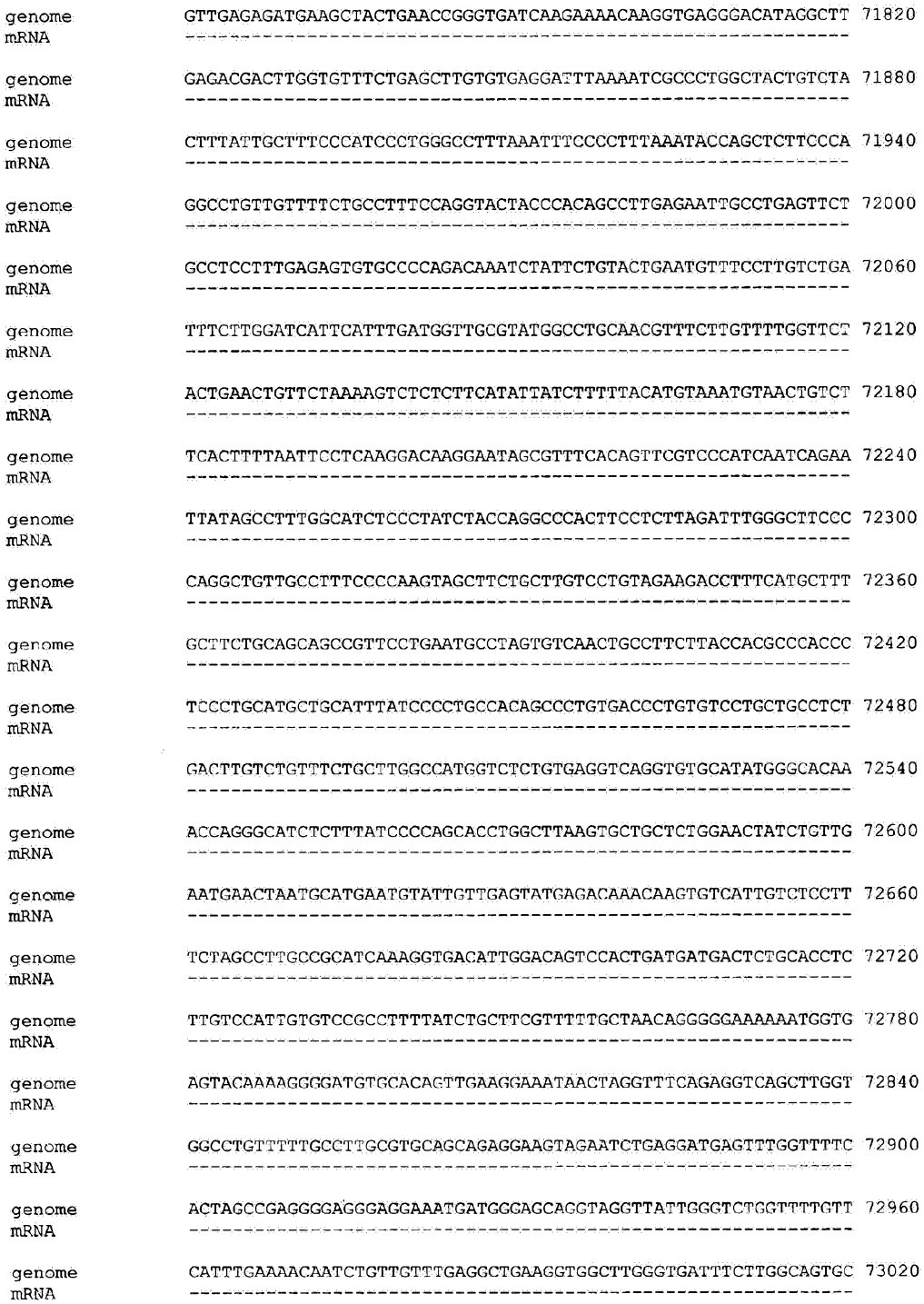
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Figure 1

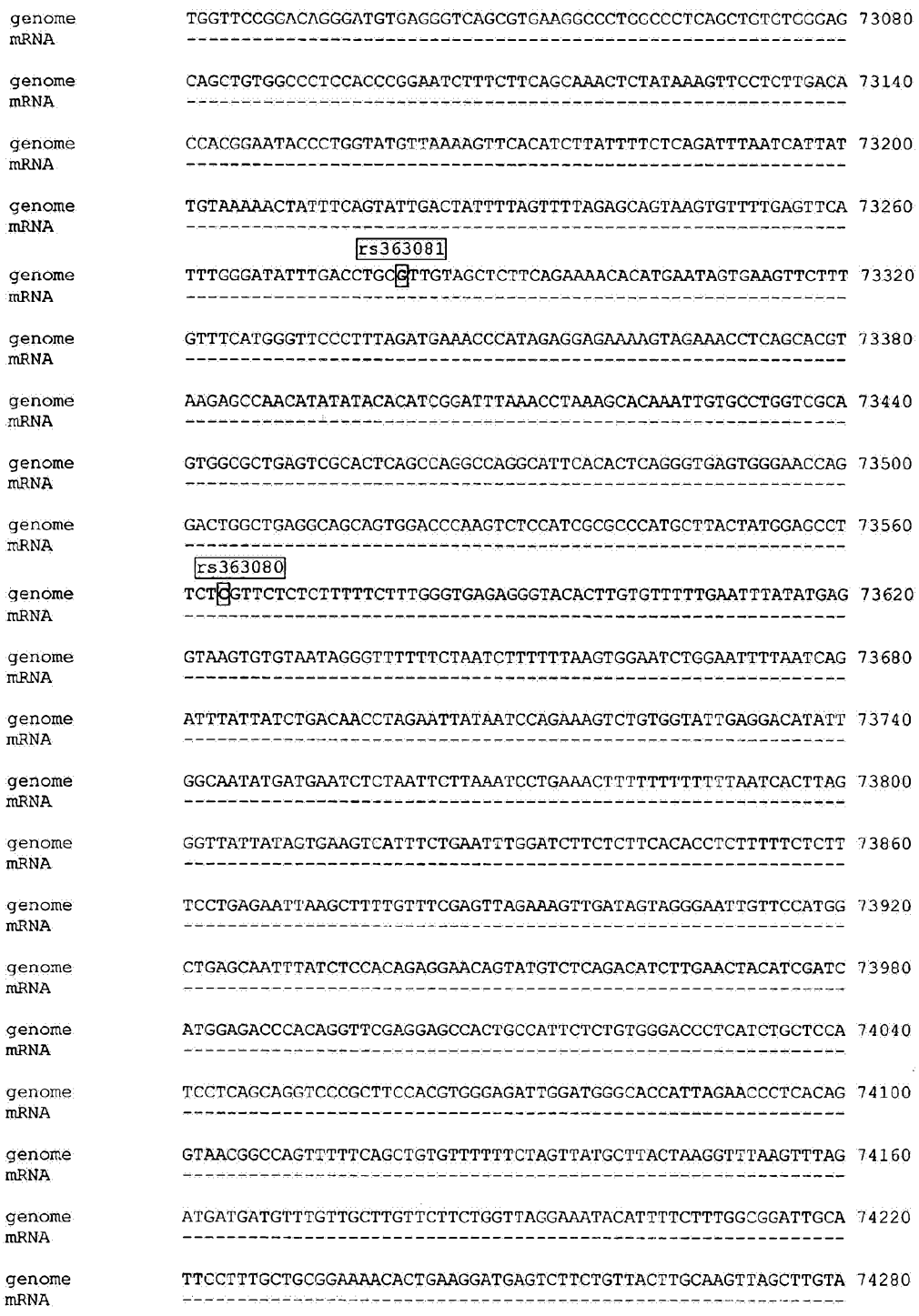
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Figure 1

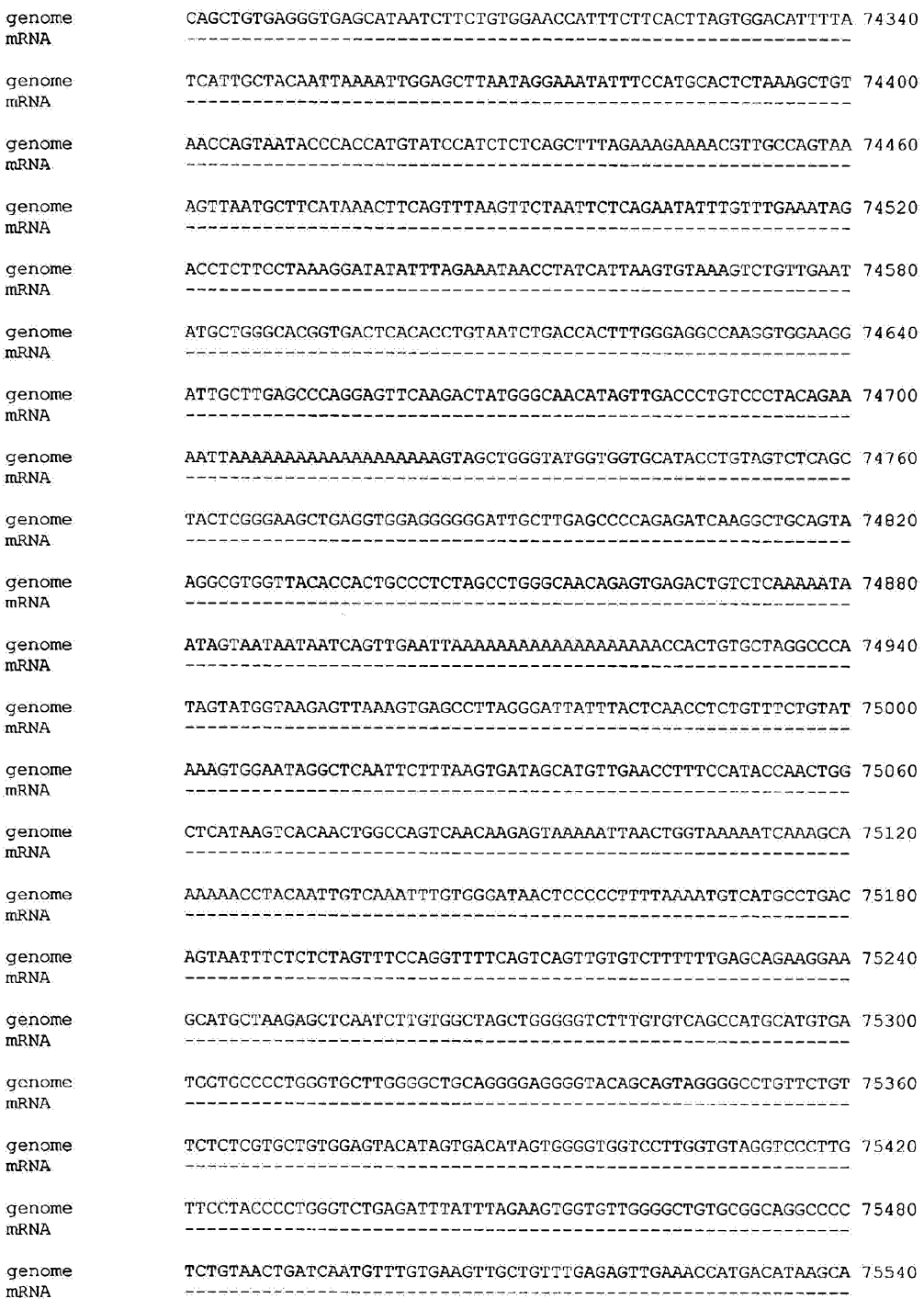
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Figure 1

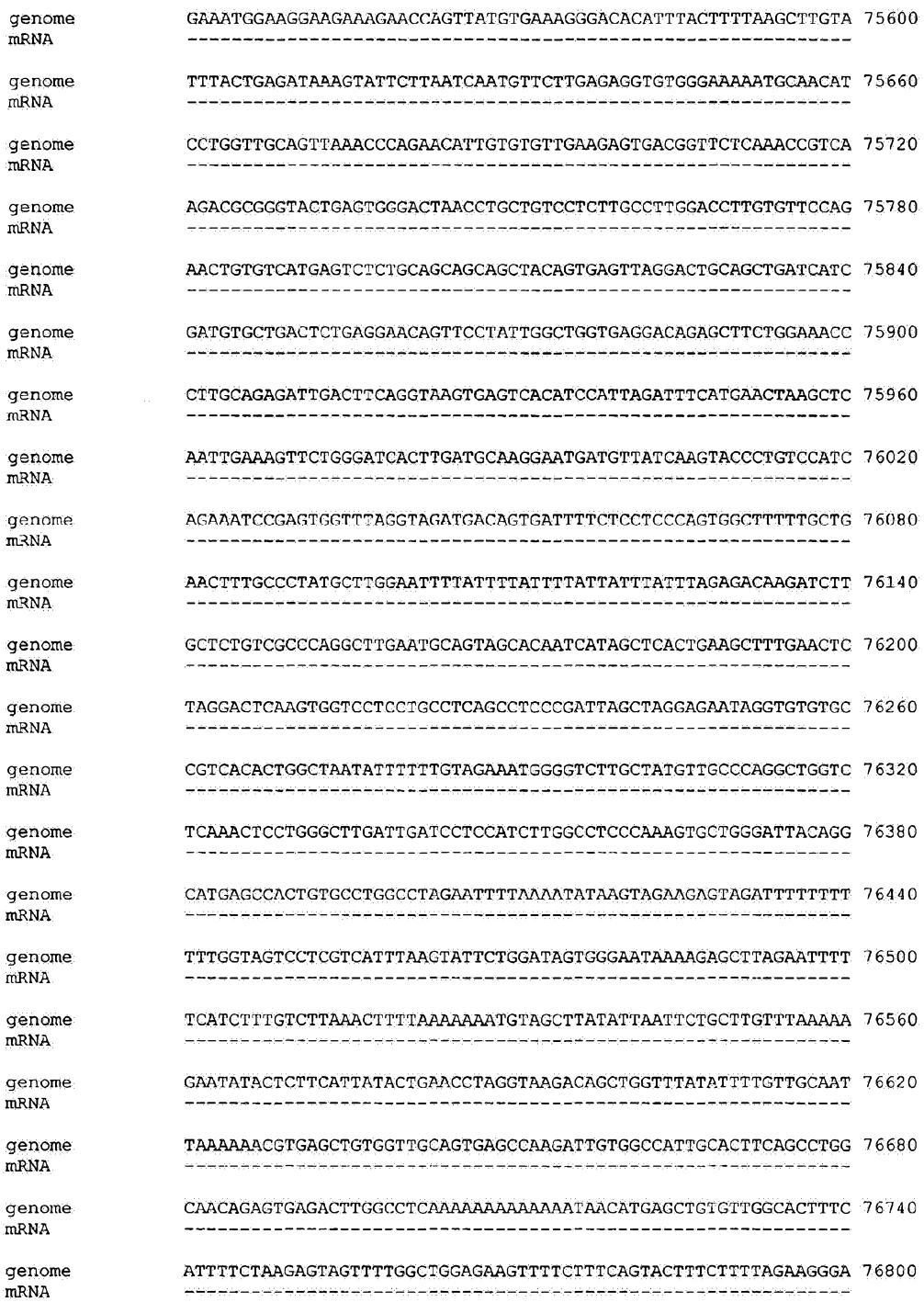
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Figure 1

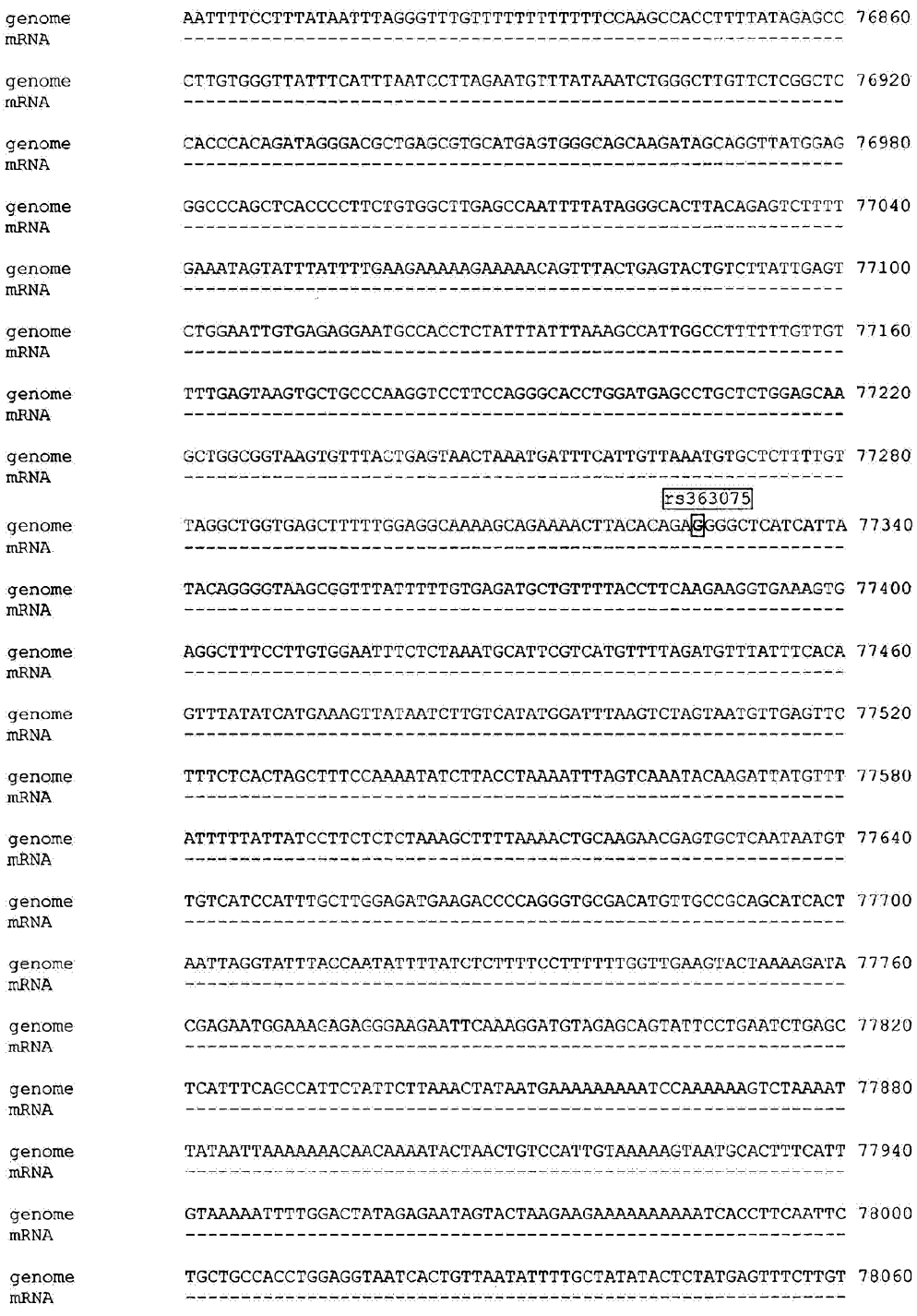
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Figure 1

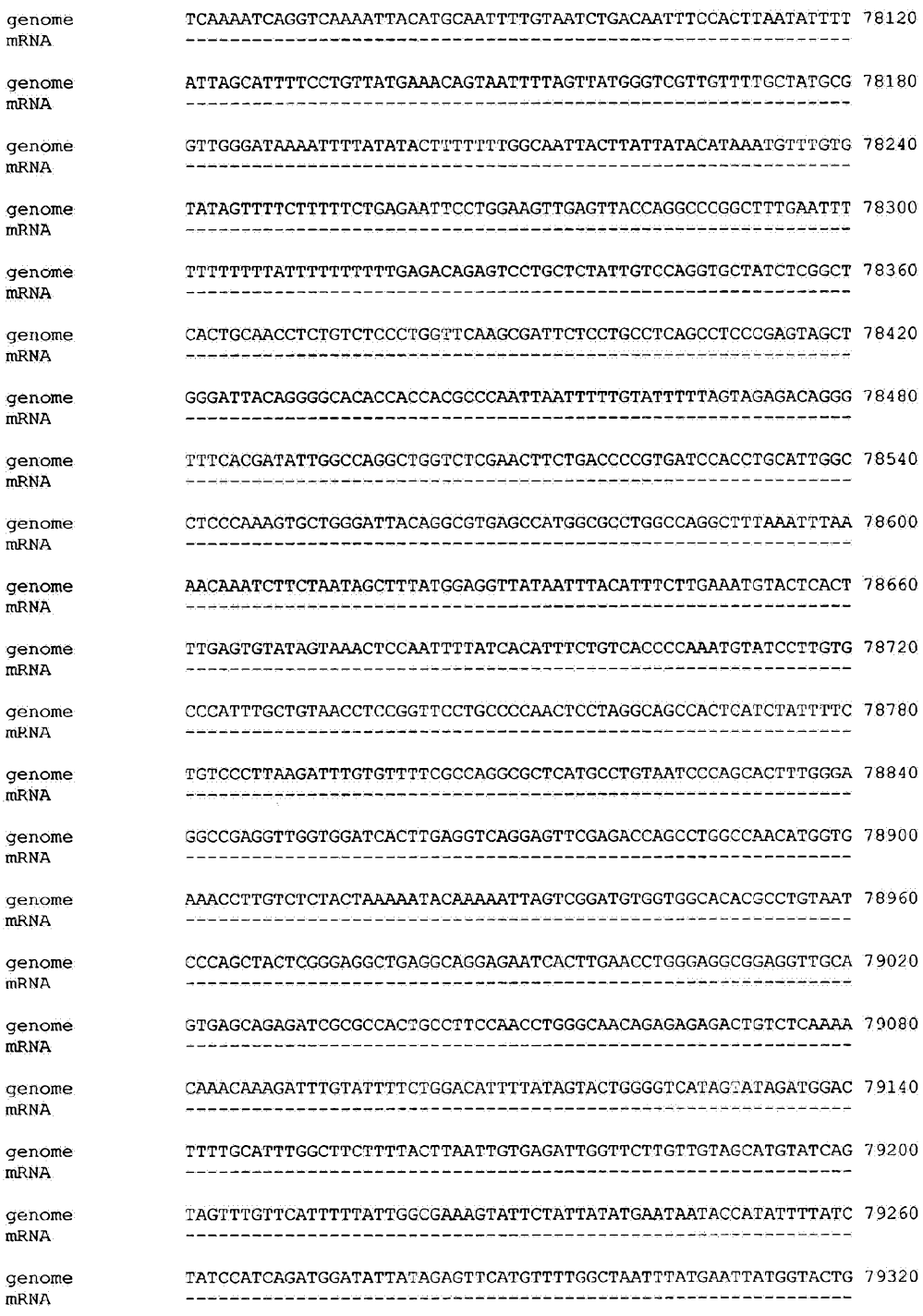
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Figure 1

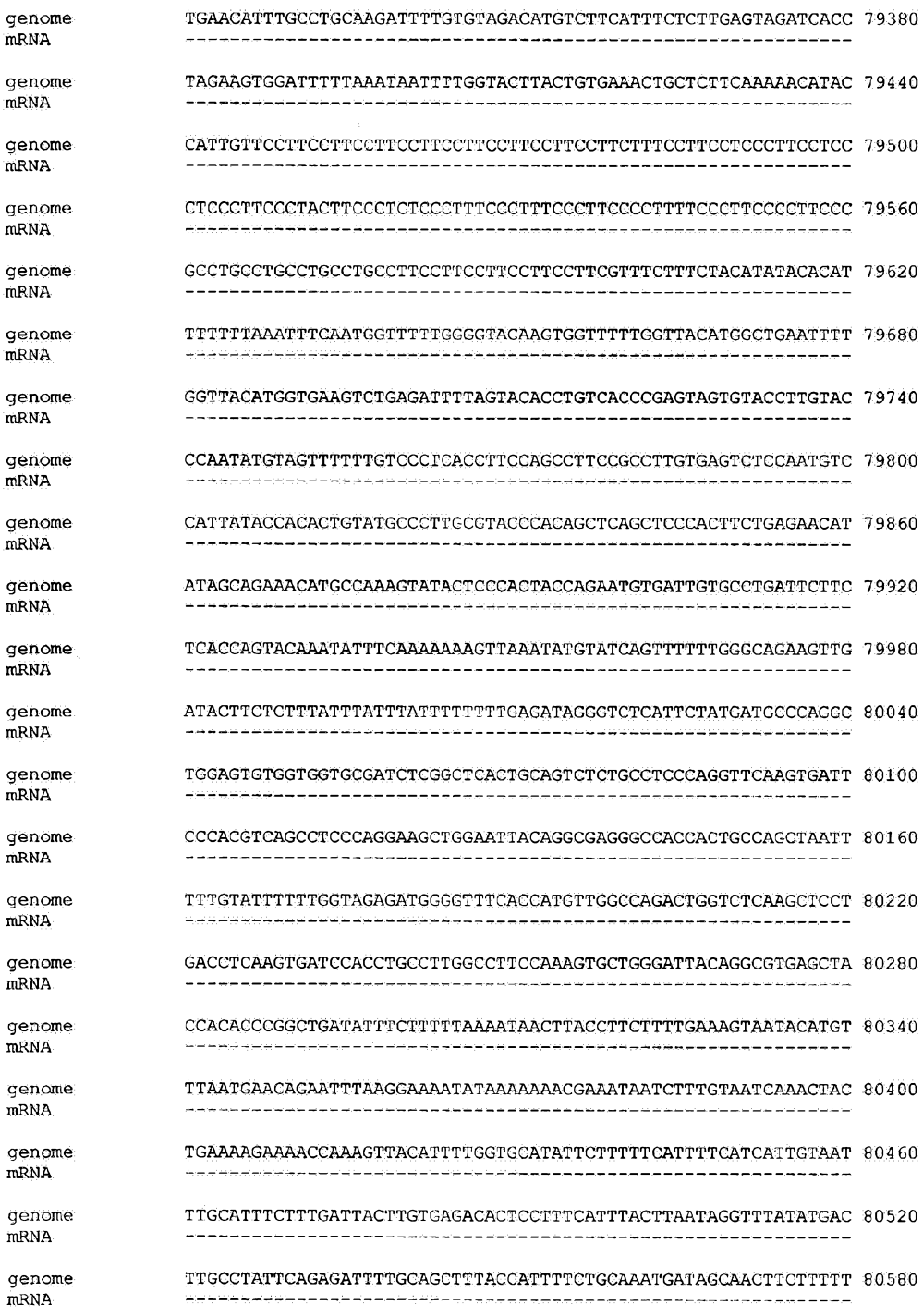
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Figure 1

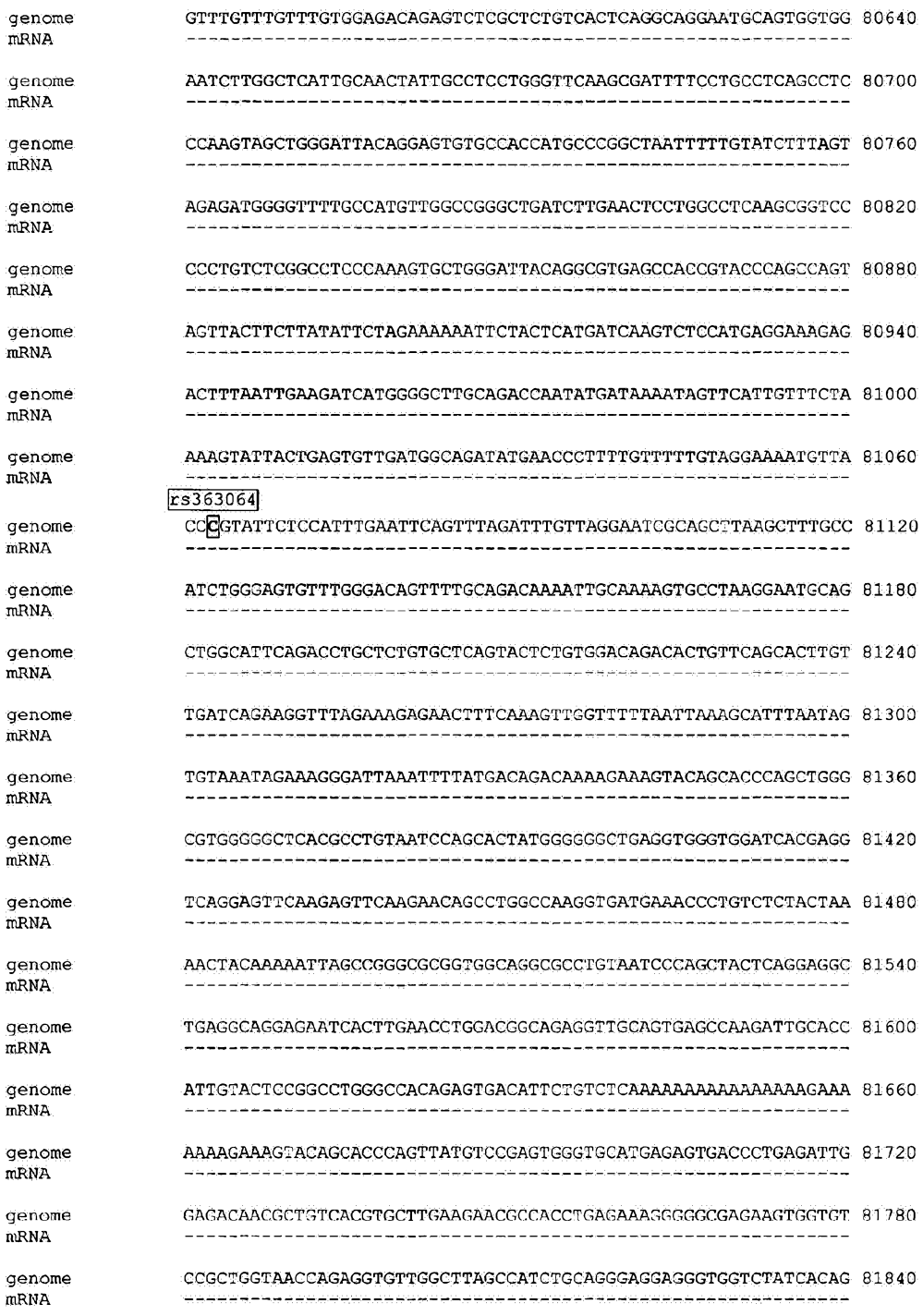
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Figure 1

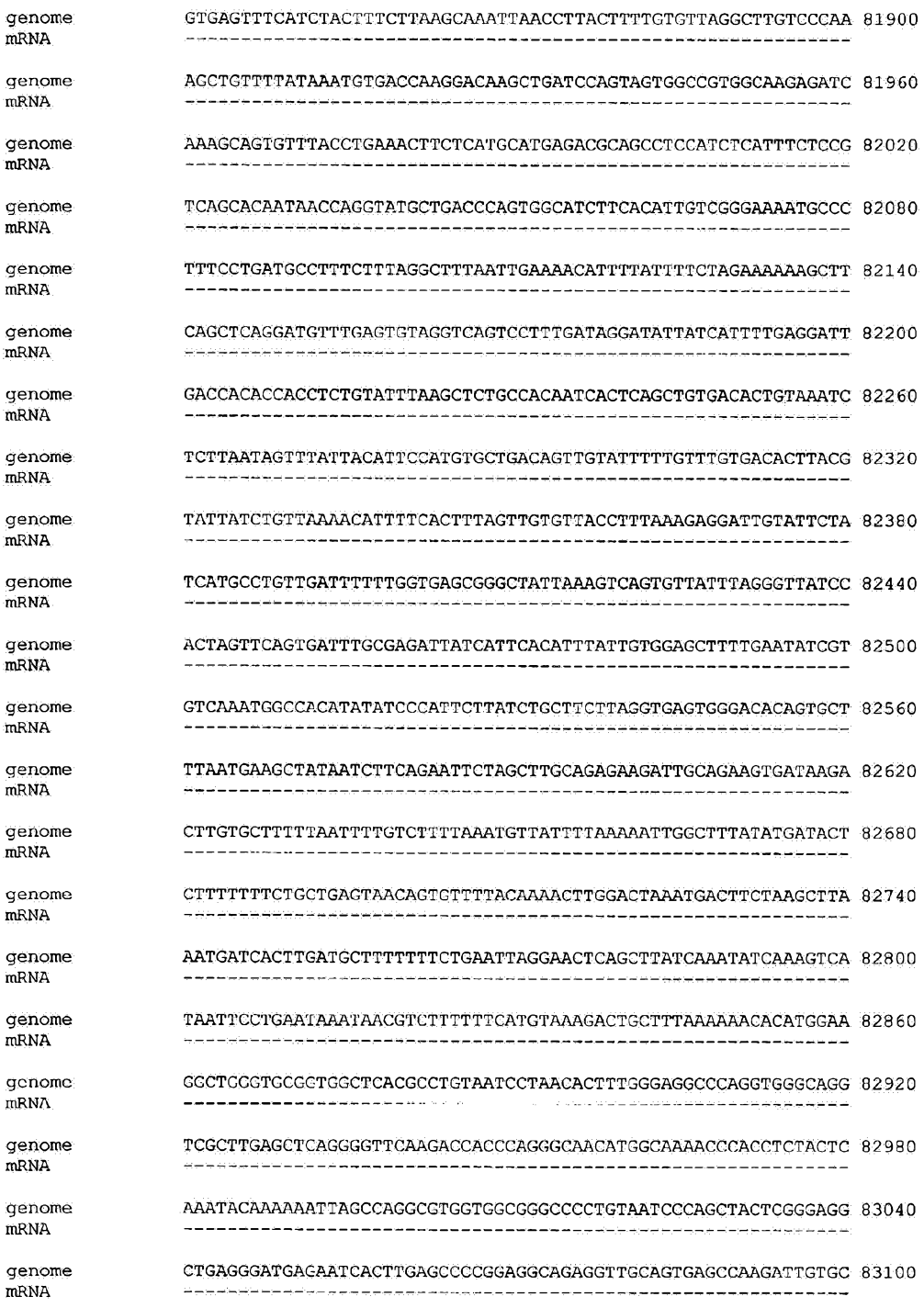
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Figure 1

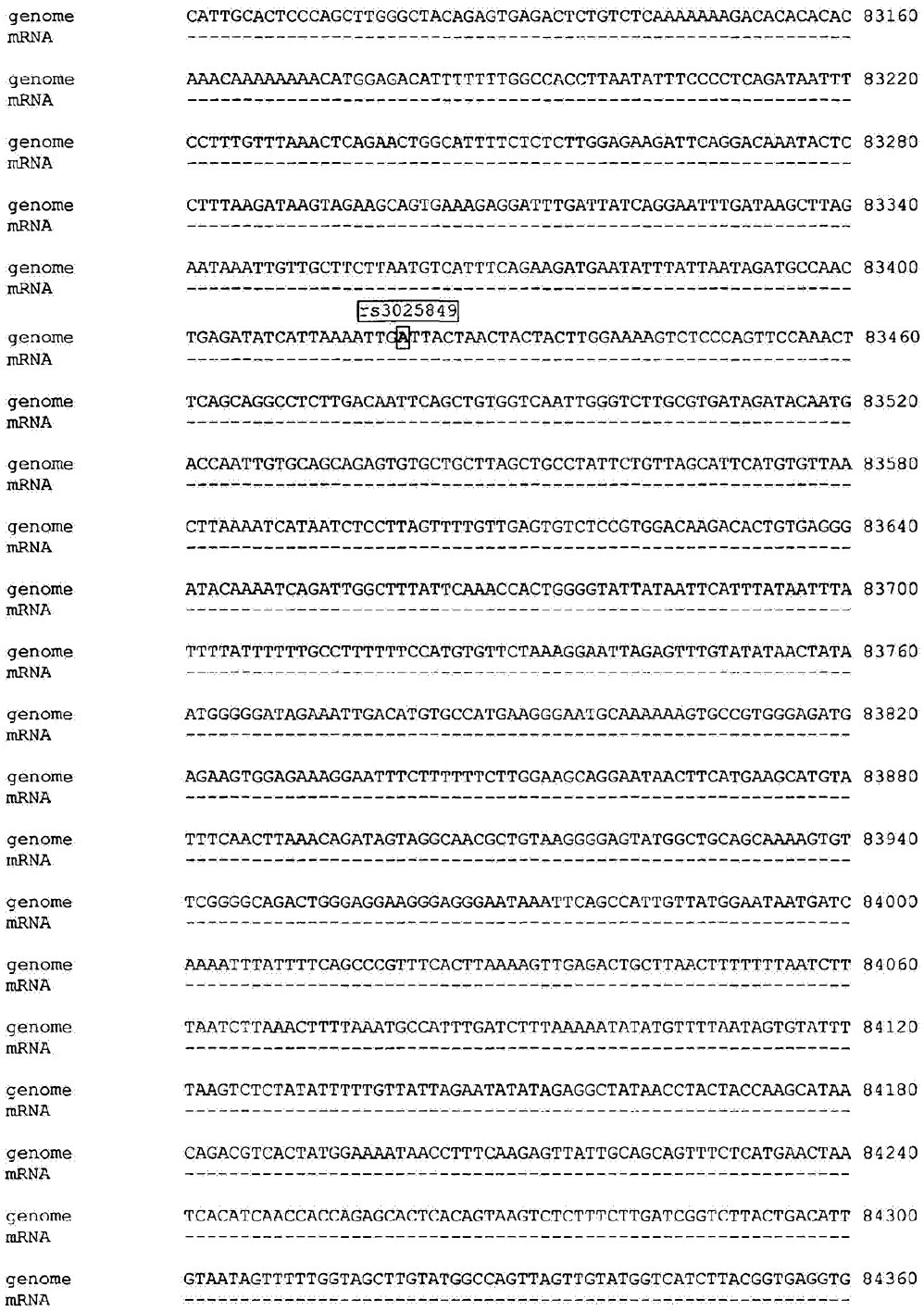
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Figure 1

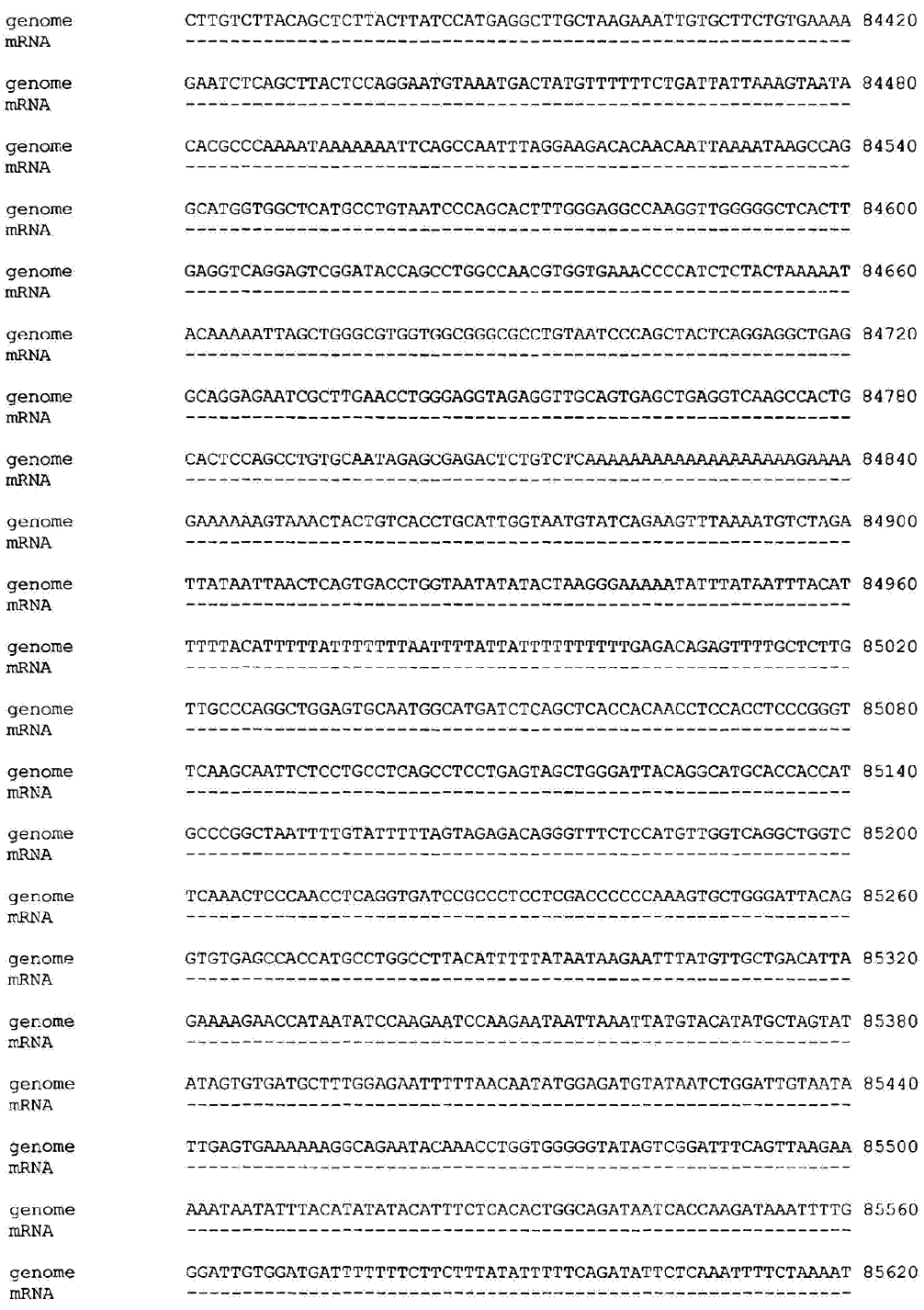
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Figure 1

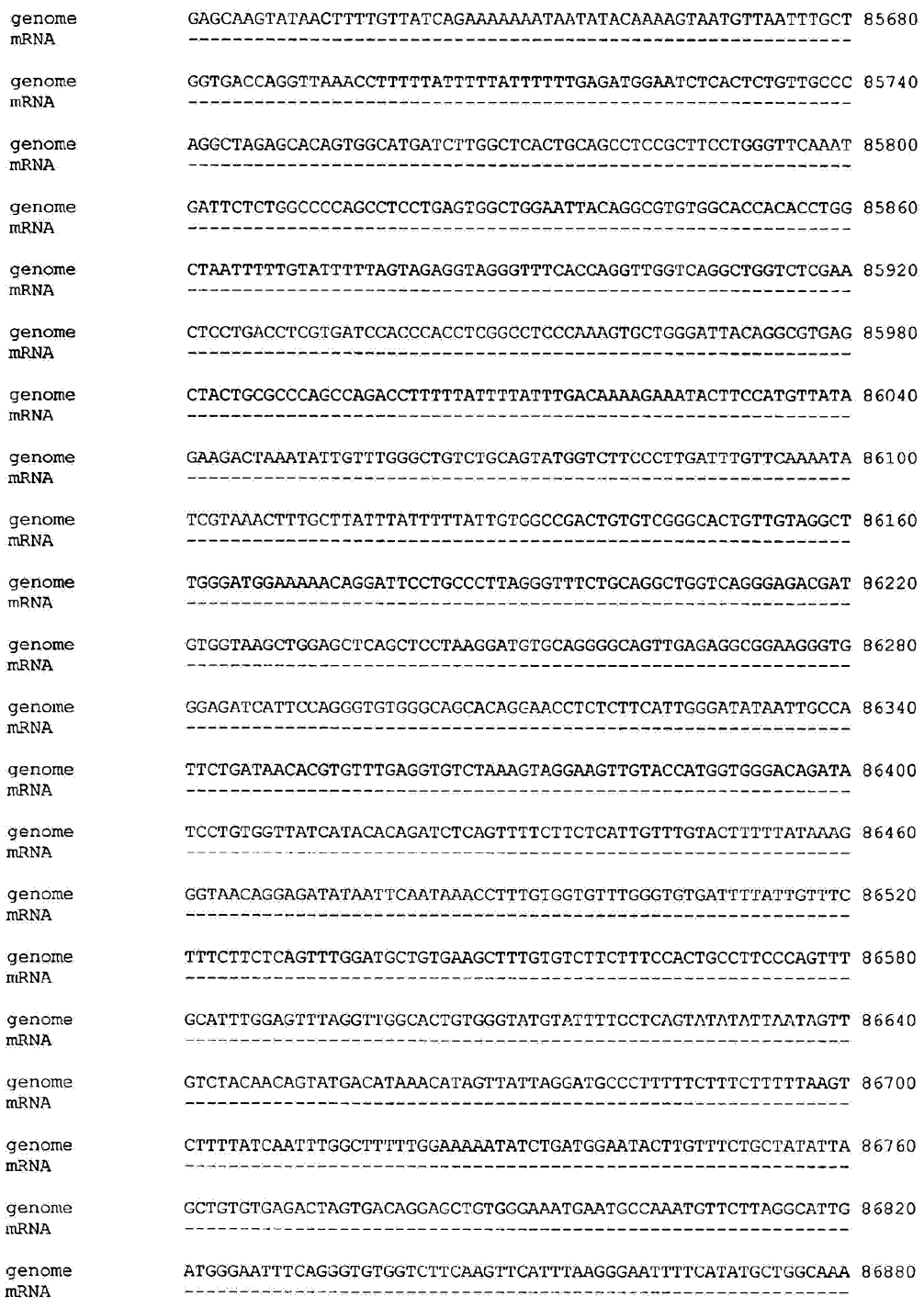
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Figure 1

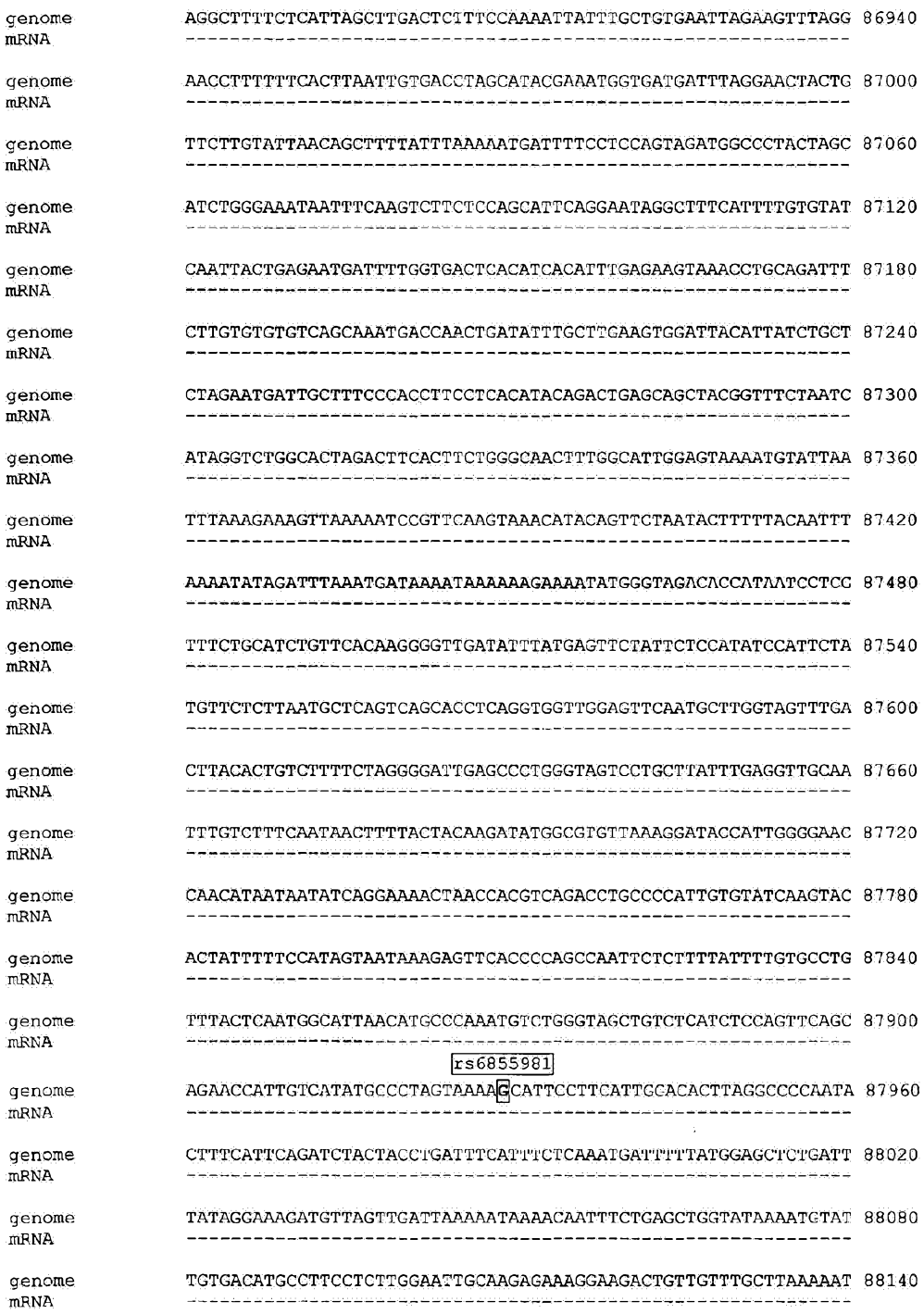
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Figure 1

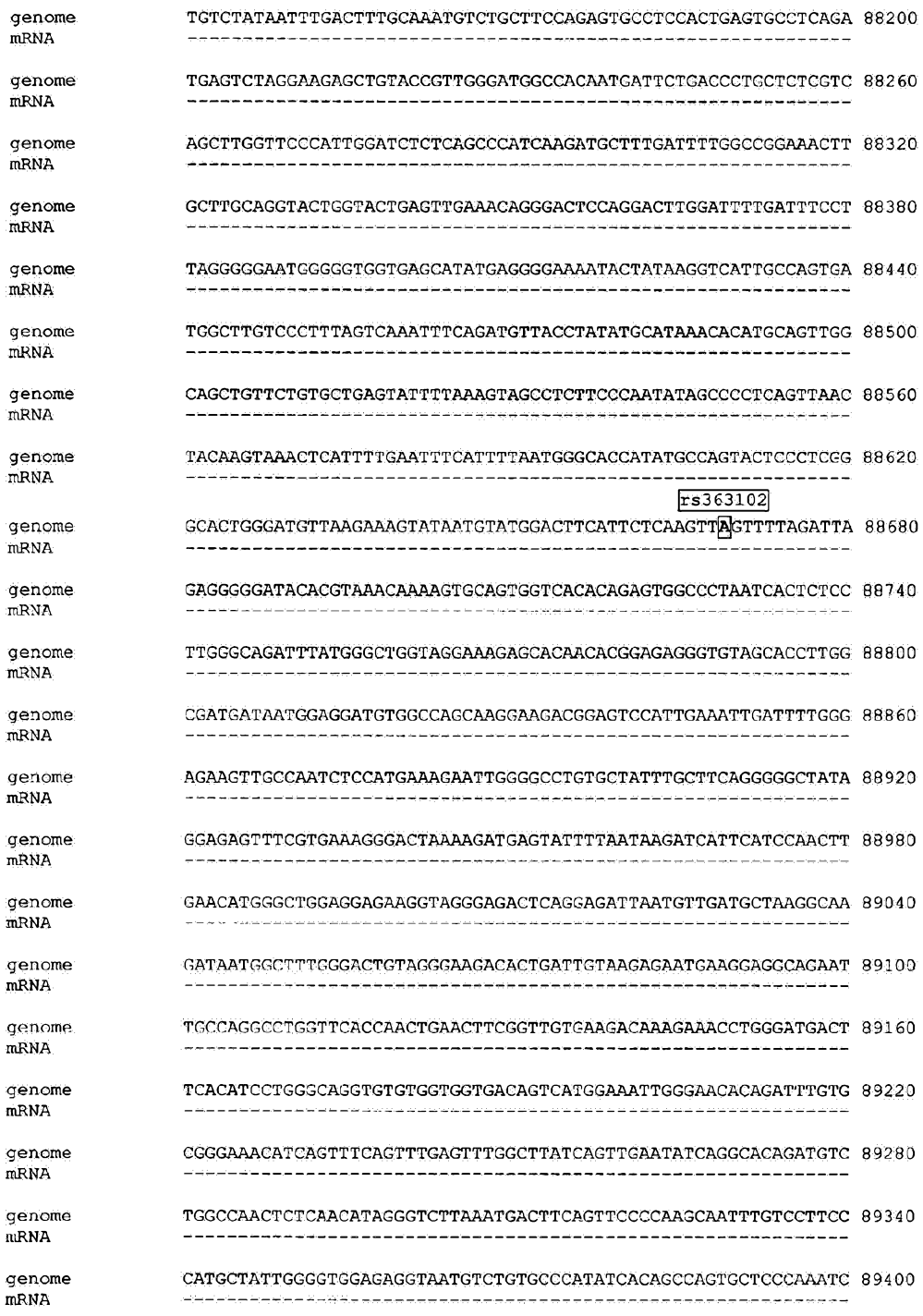
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Figure 1

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Figure 1

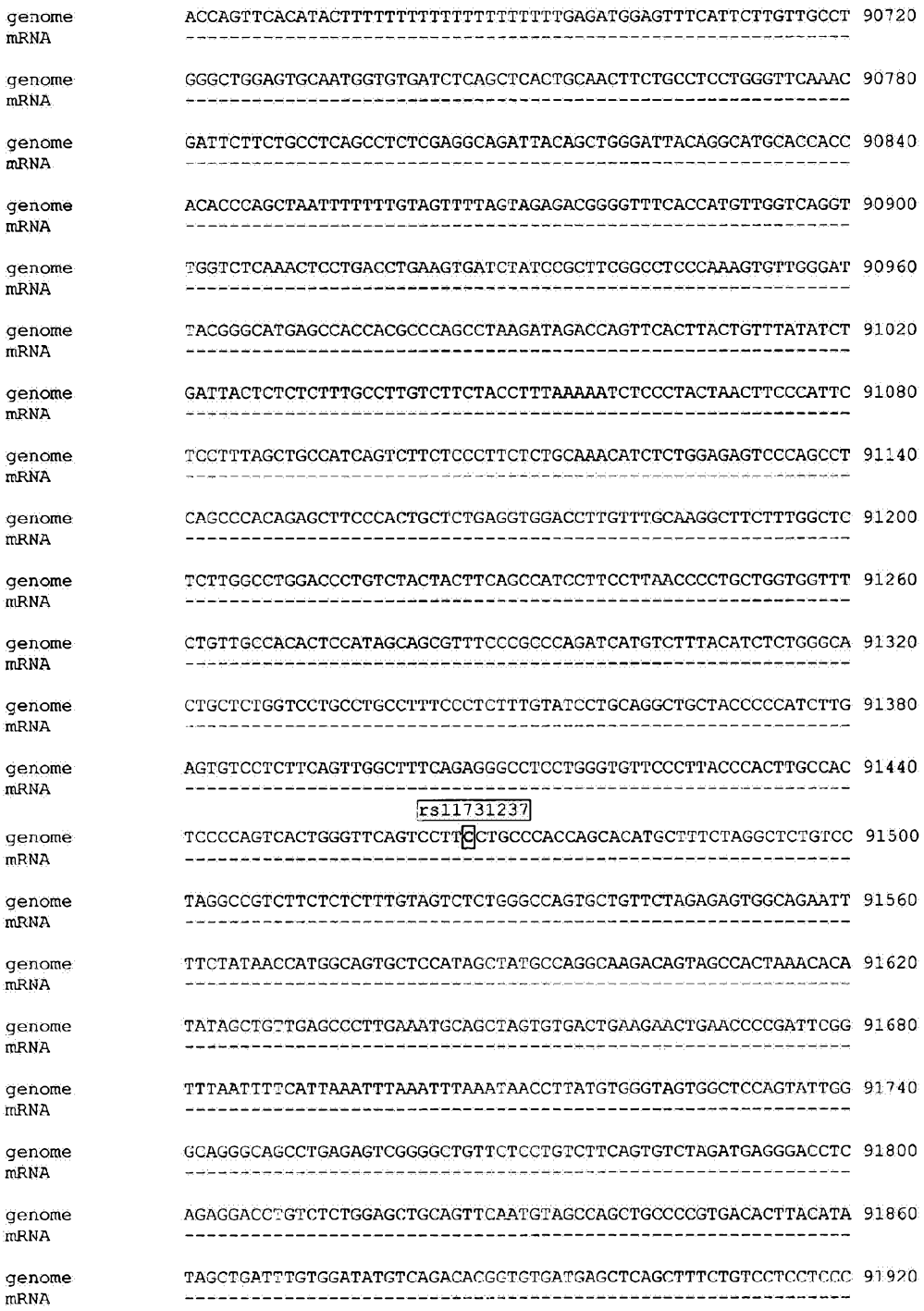
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Figure 1

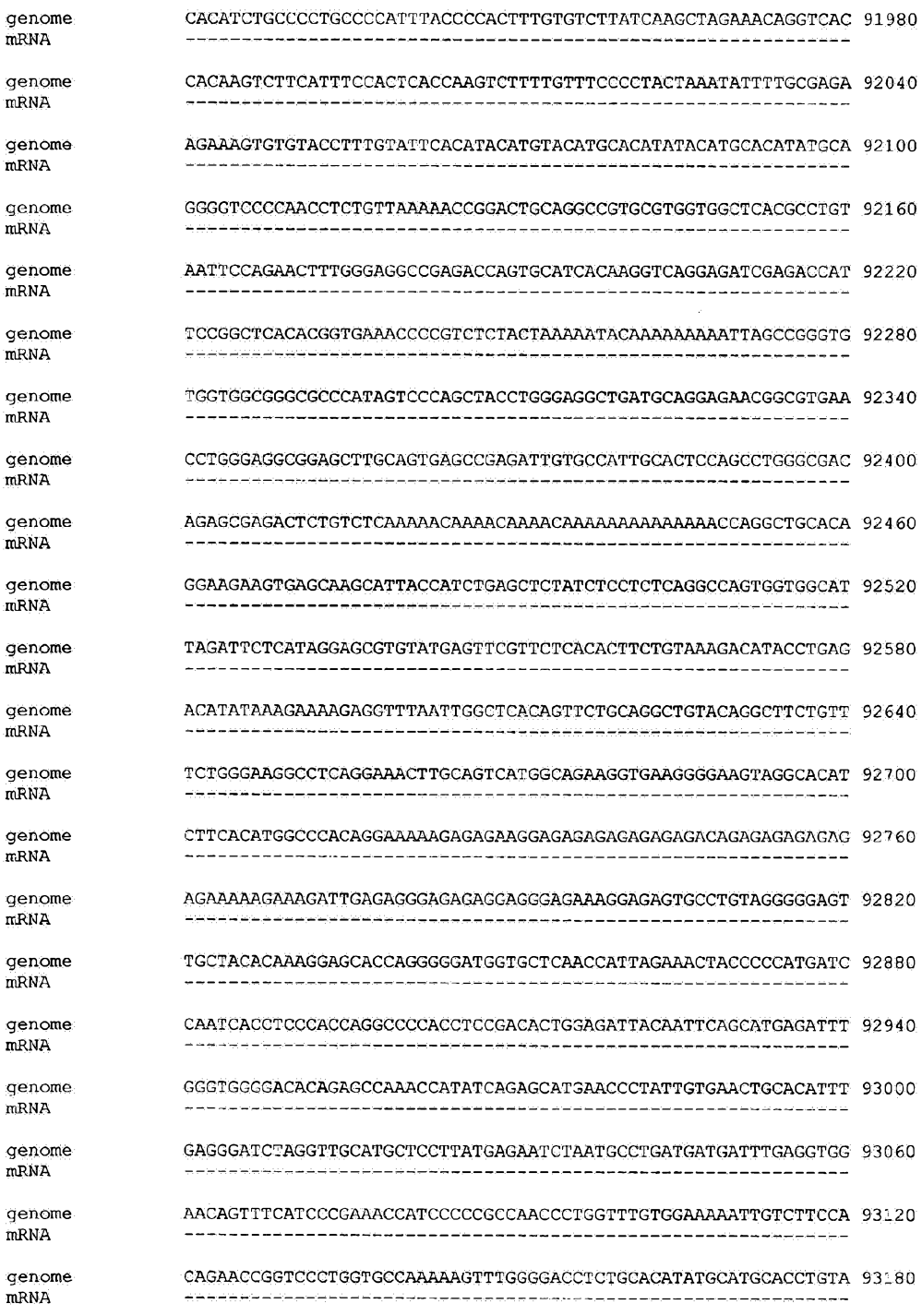
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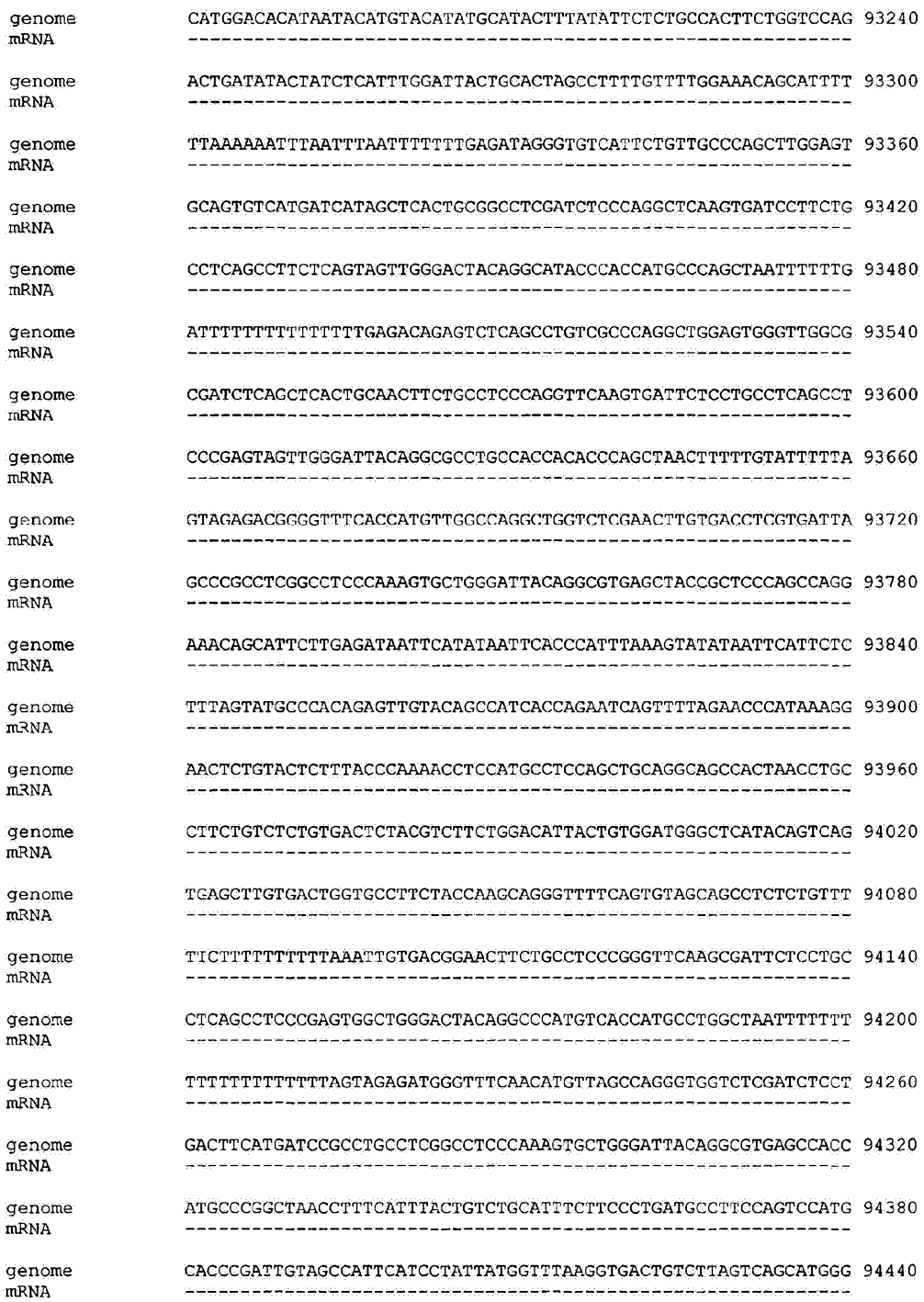
Figure 1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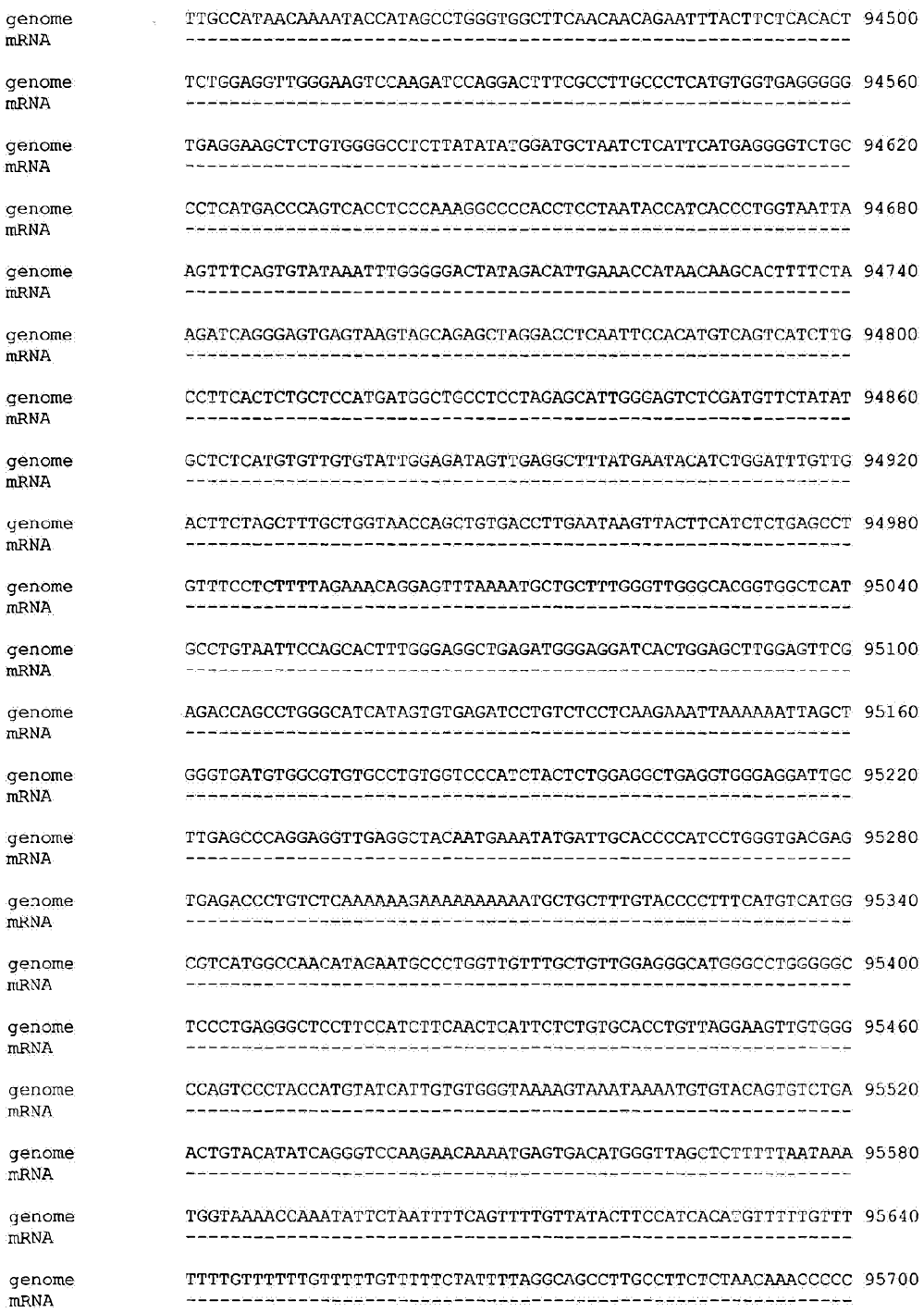
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Figure 1

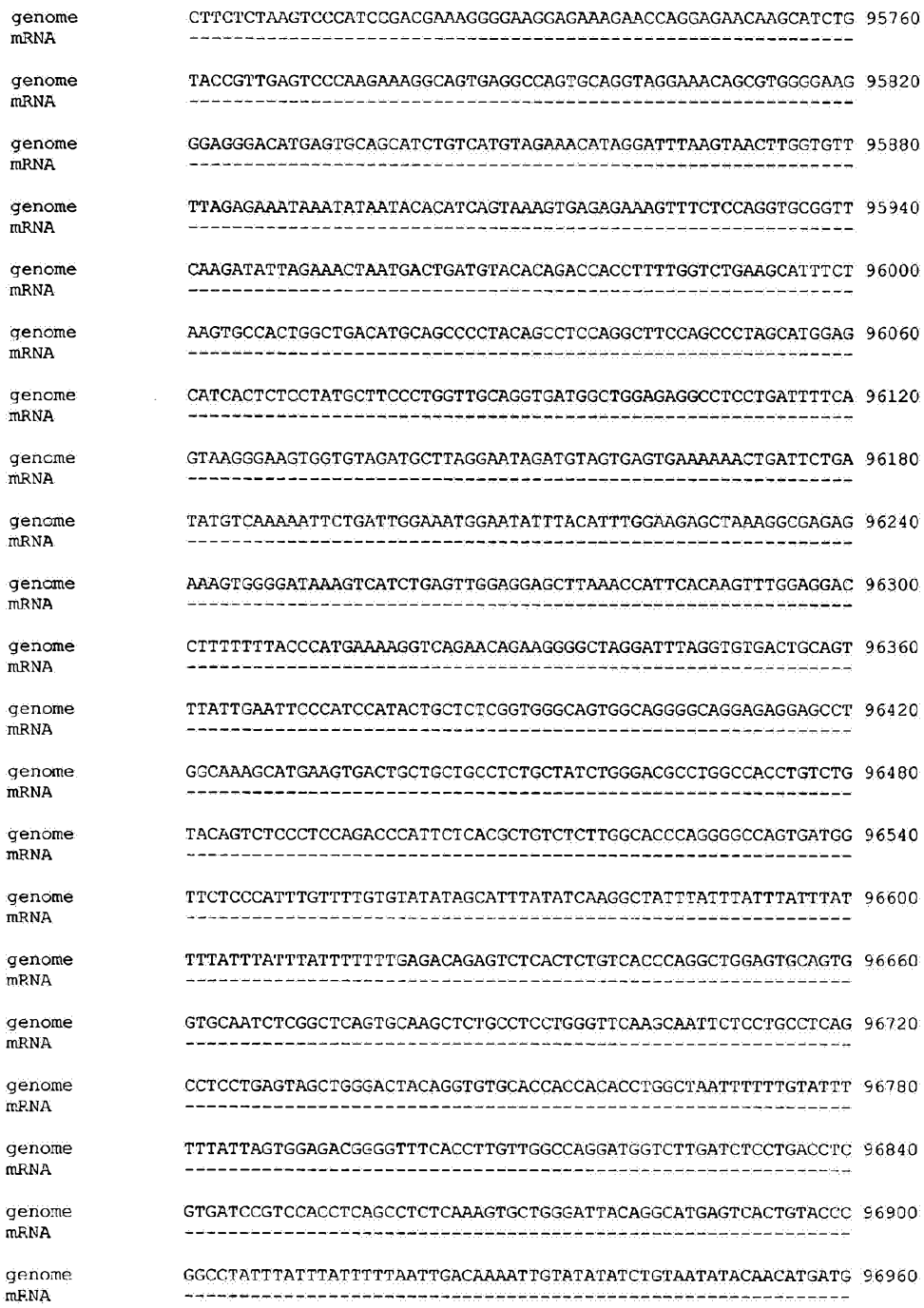
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Figure 1

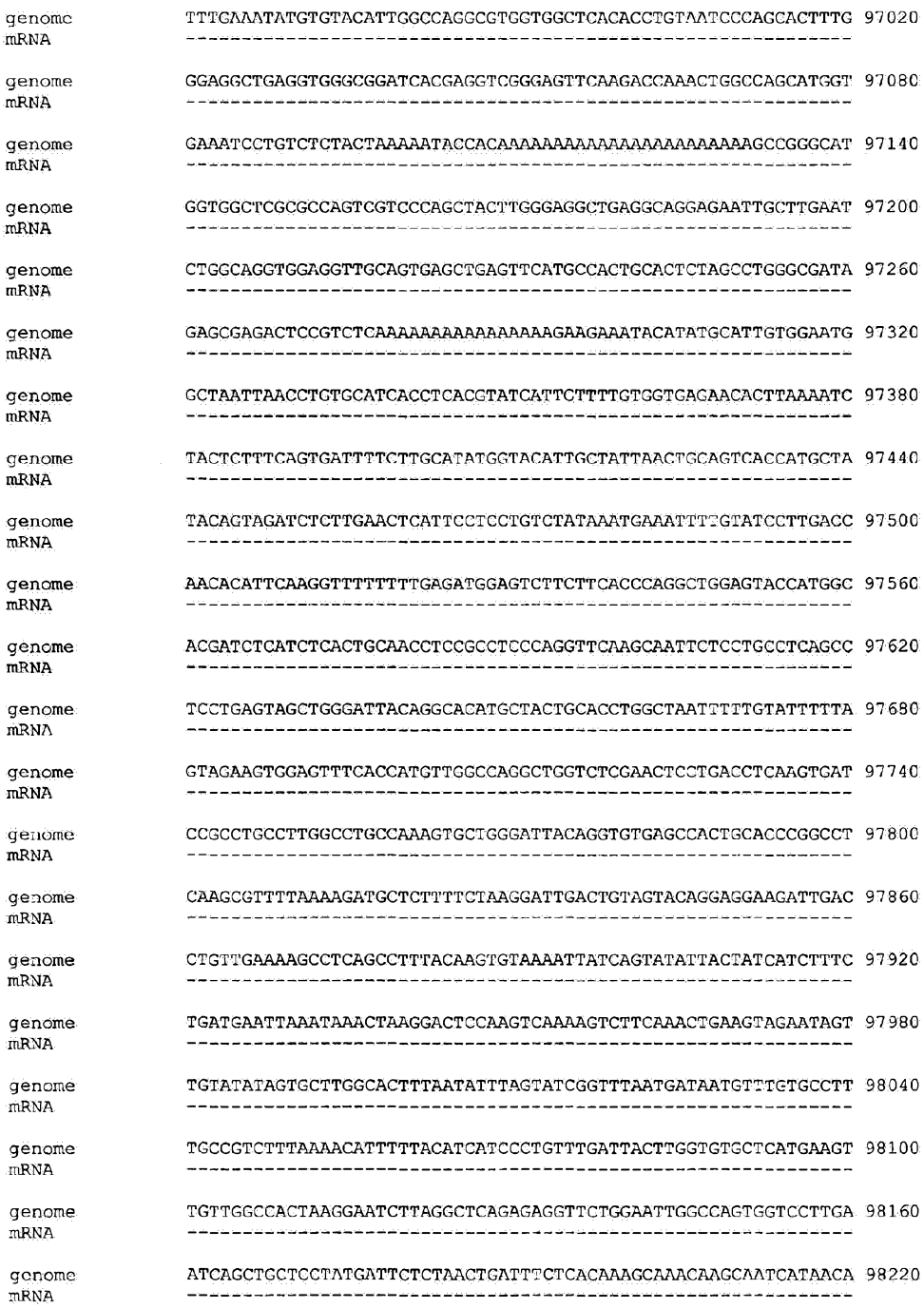
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Figure 1

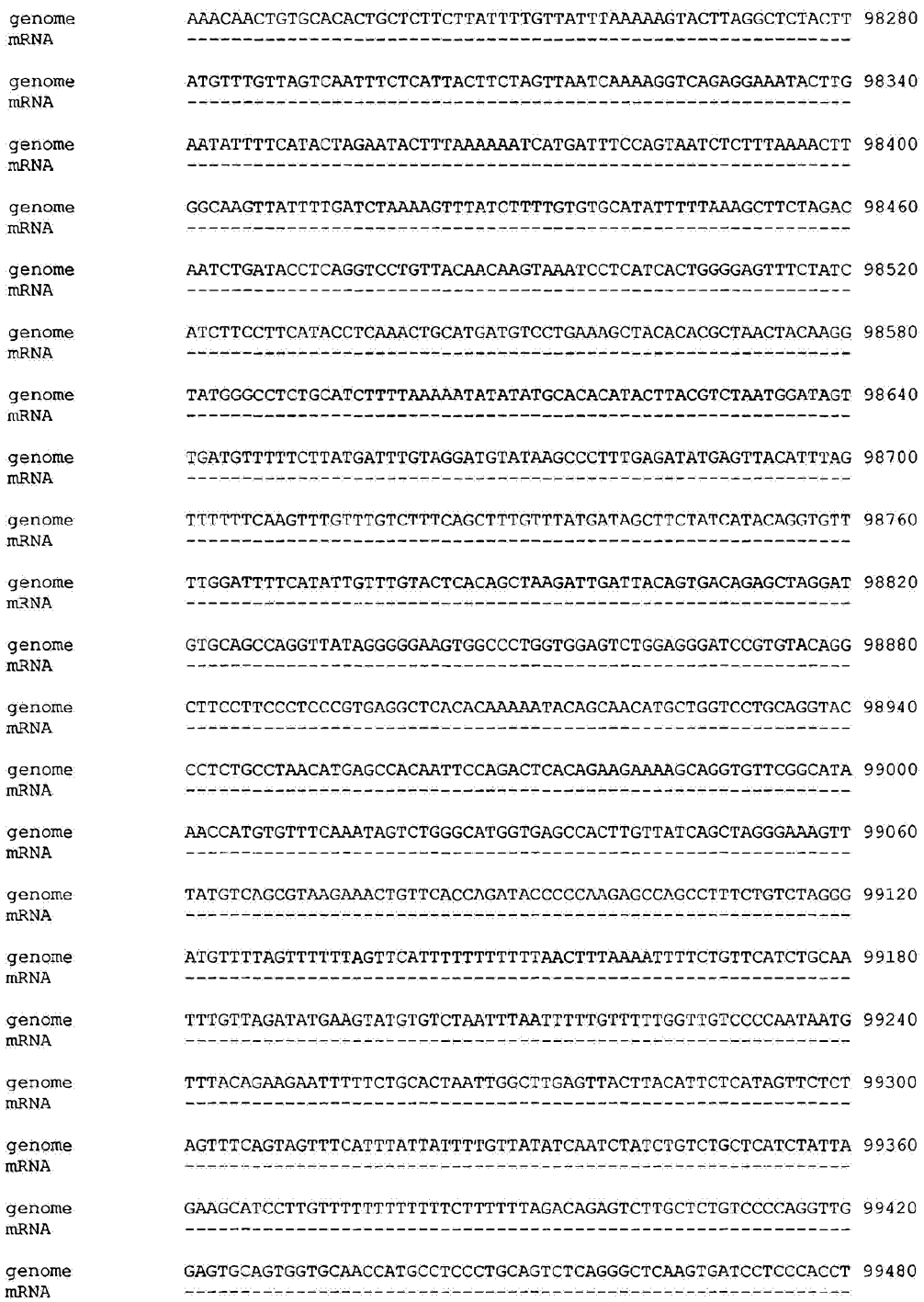
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Figure 1

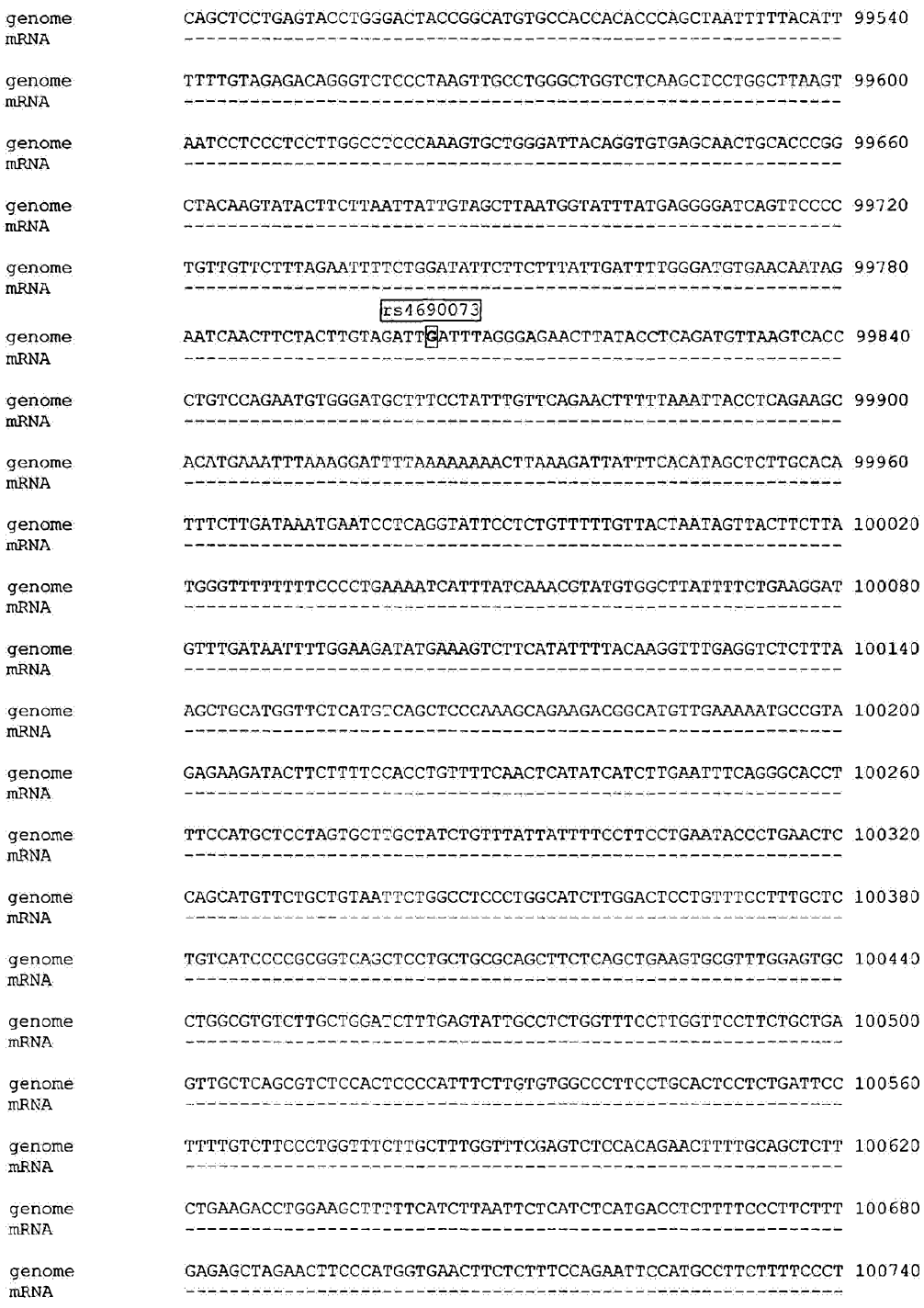
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Figure 1

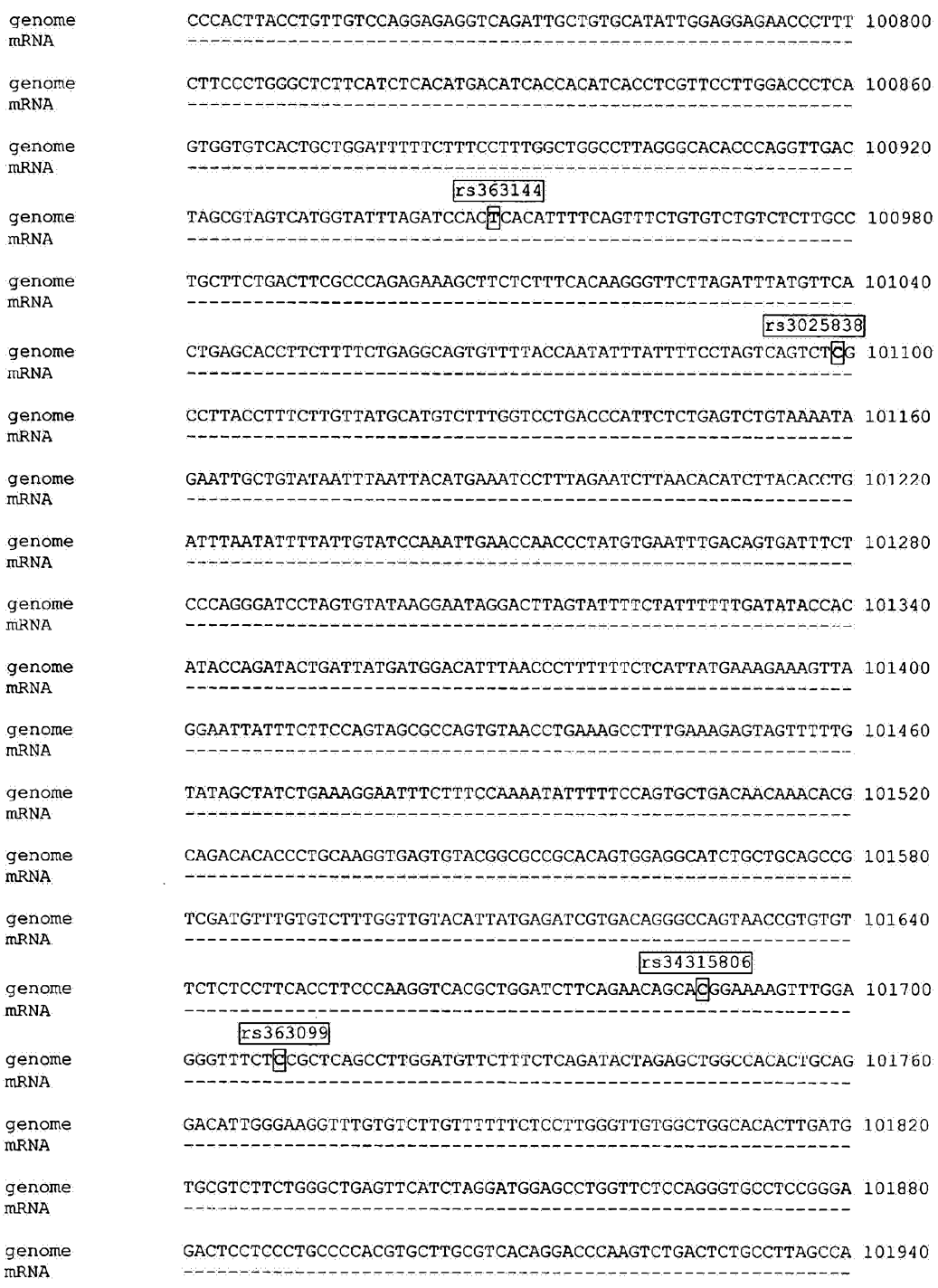
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Figure 1

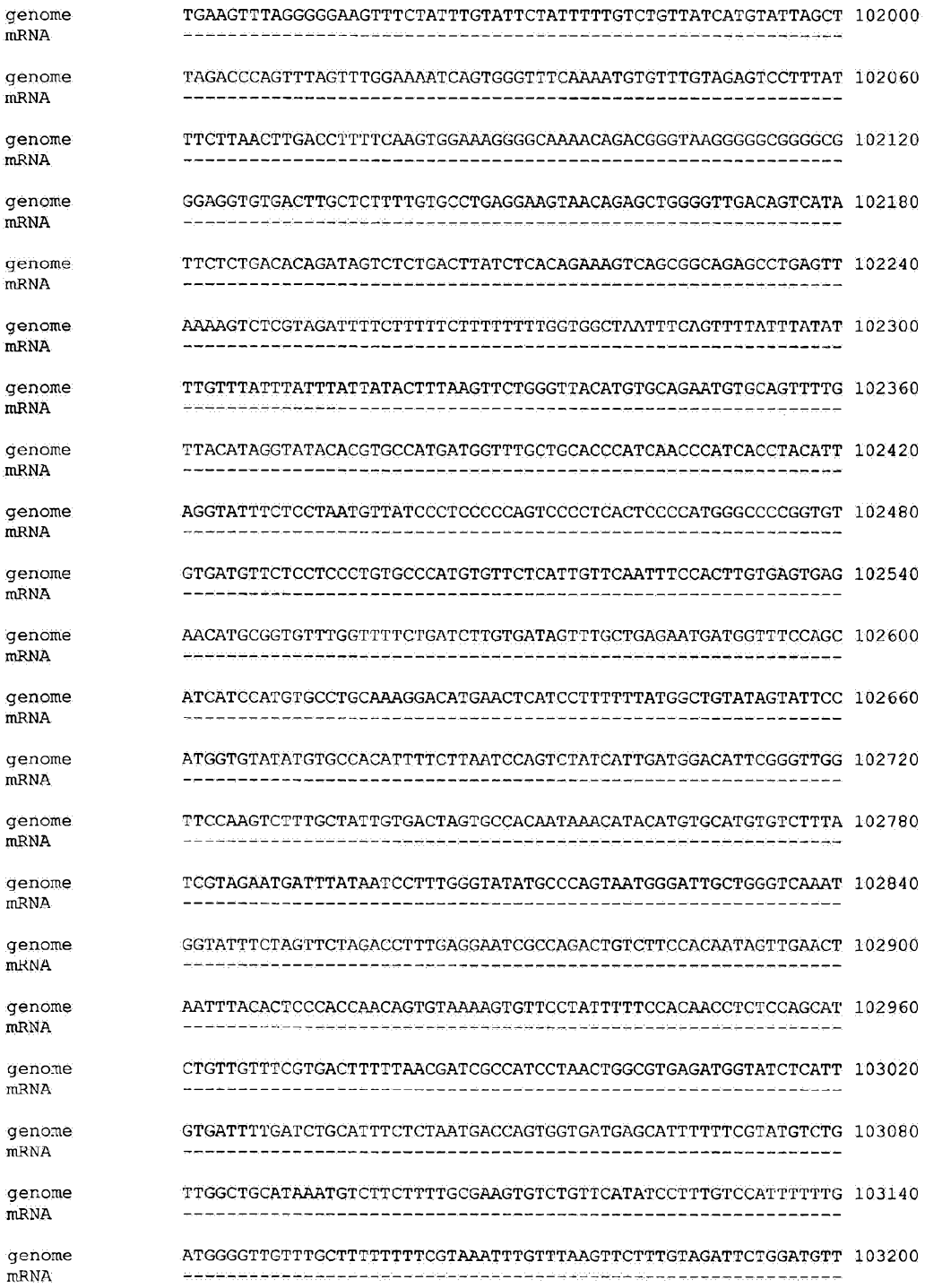
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Figure 1

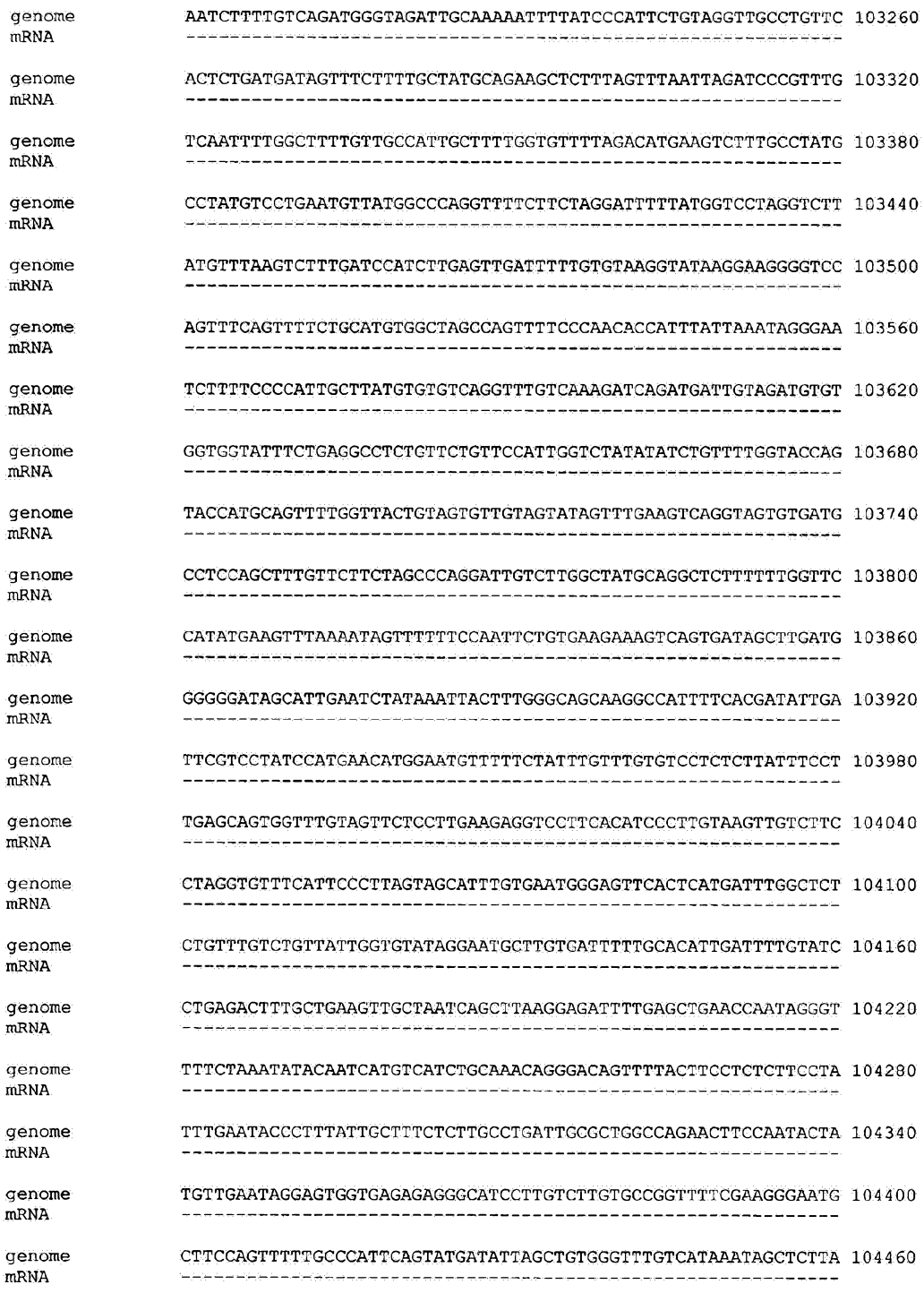
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Figure 1

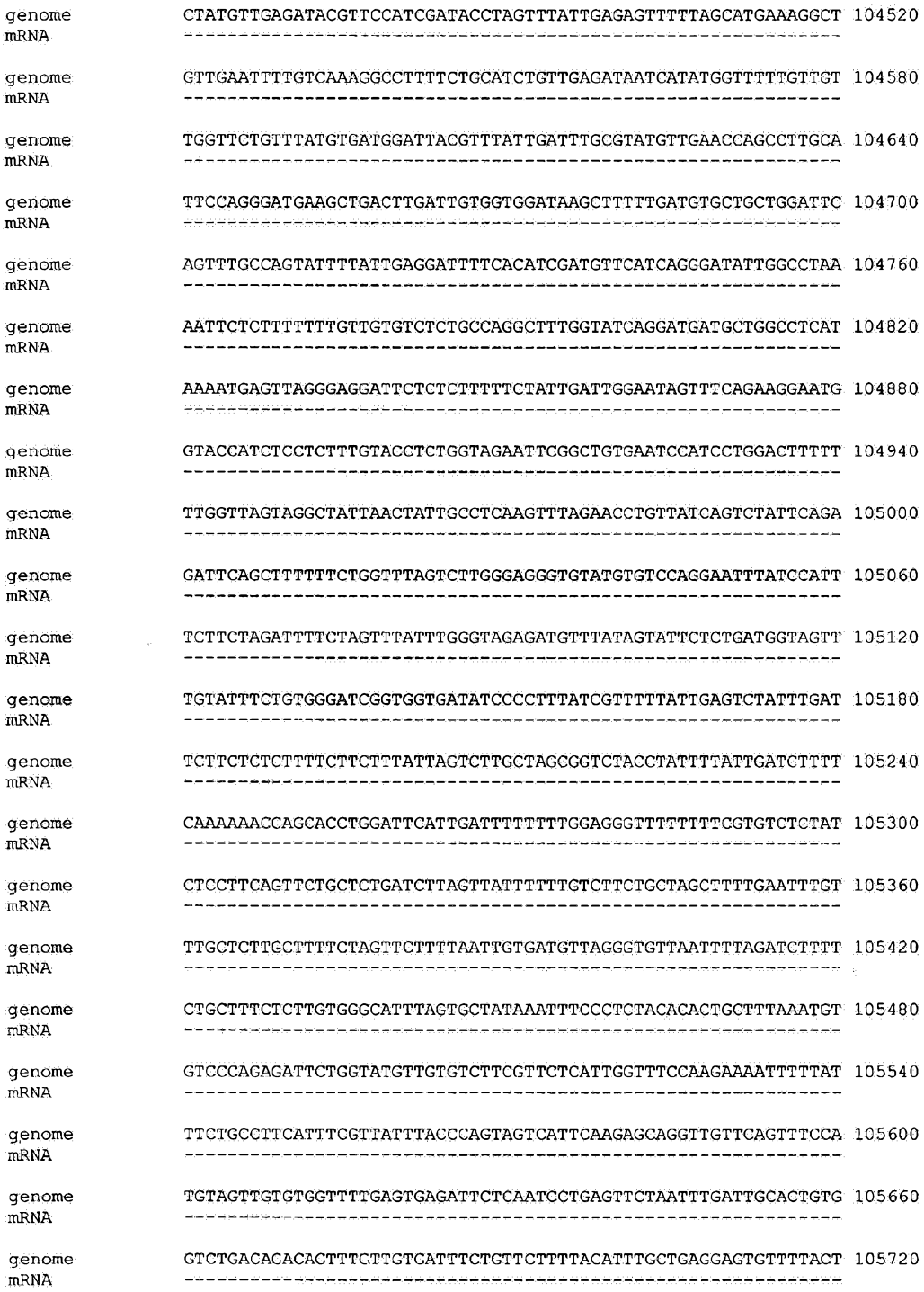
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Figure 1

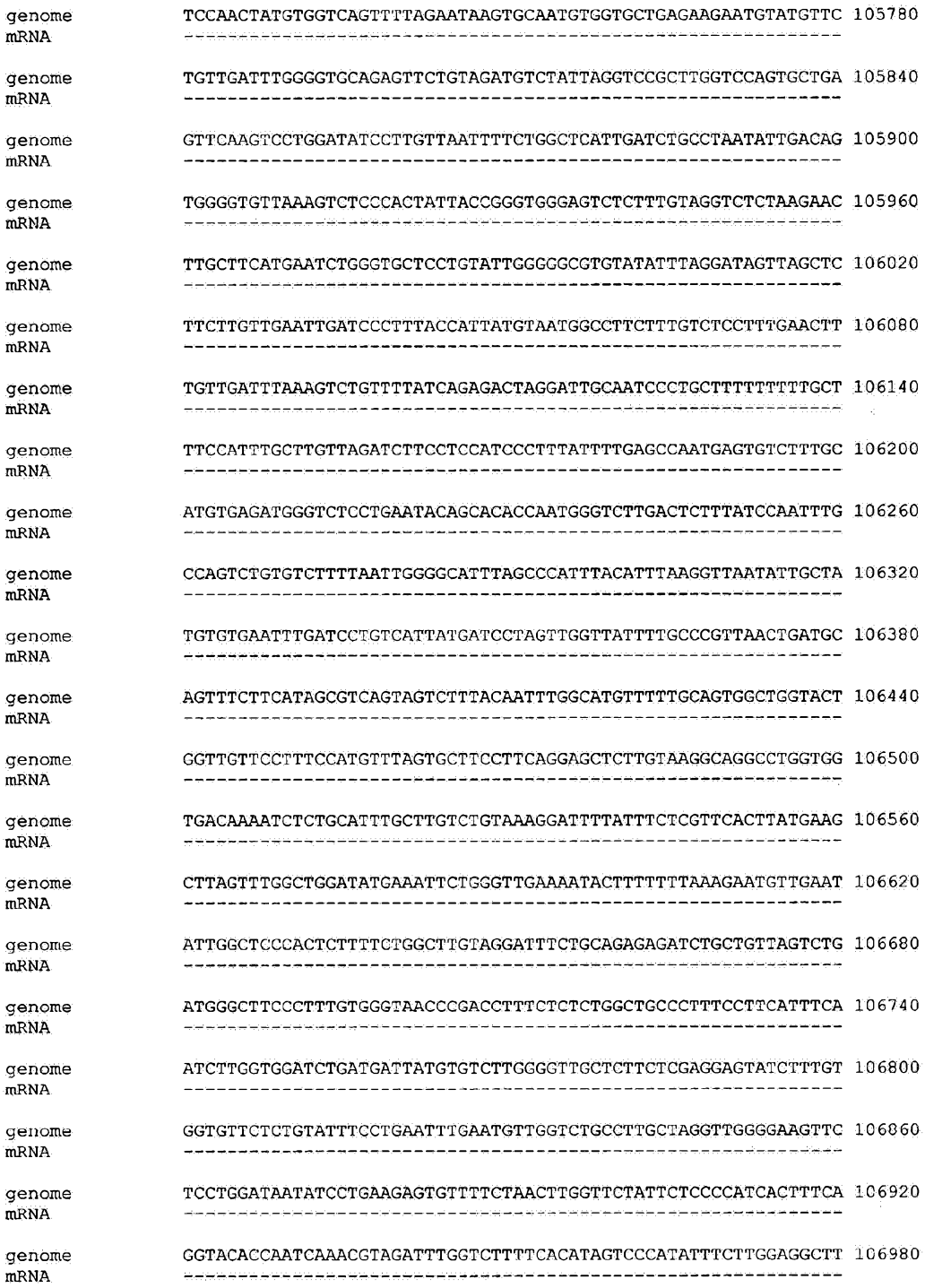
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Figure 1

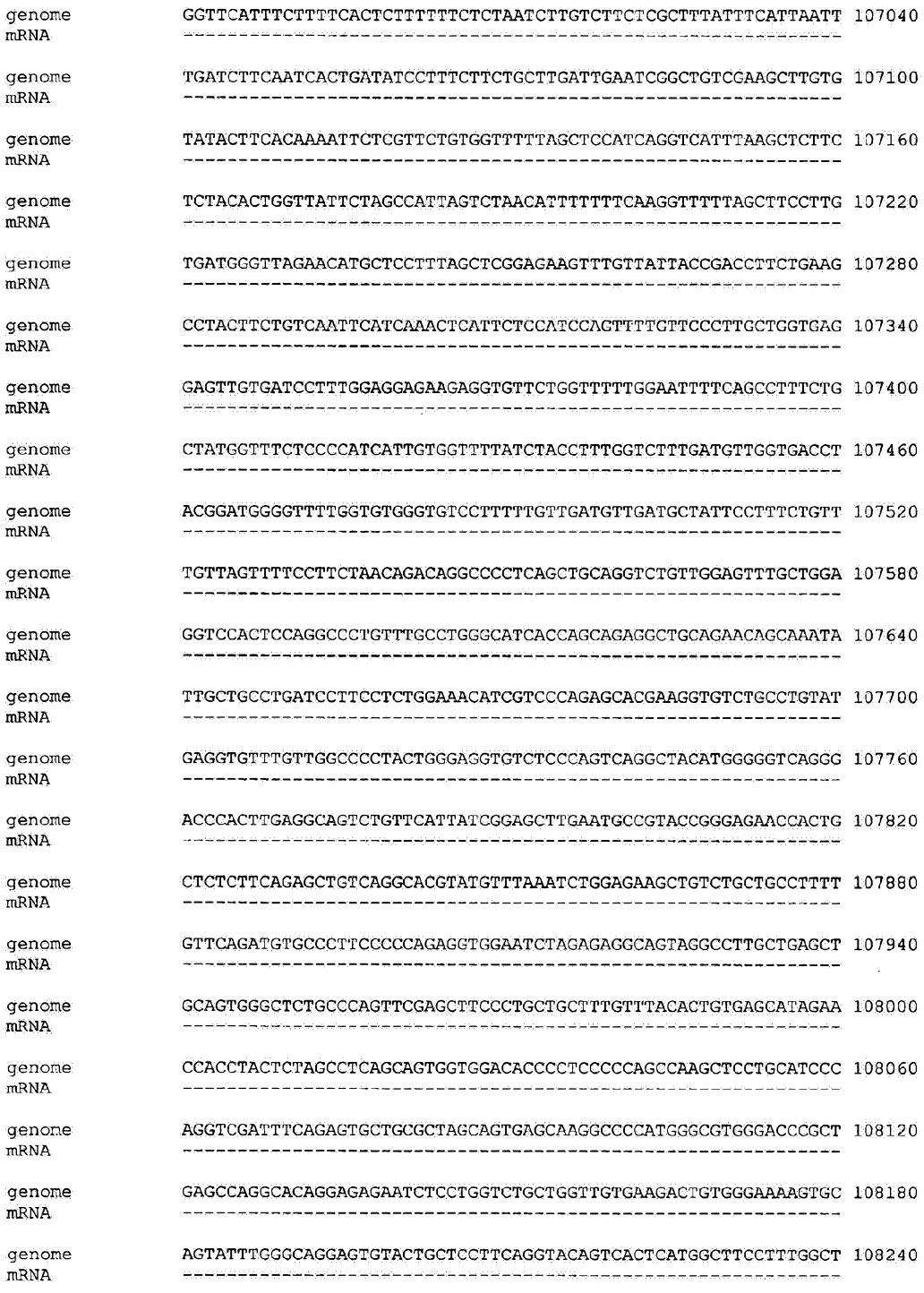
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Figure 1

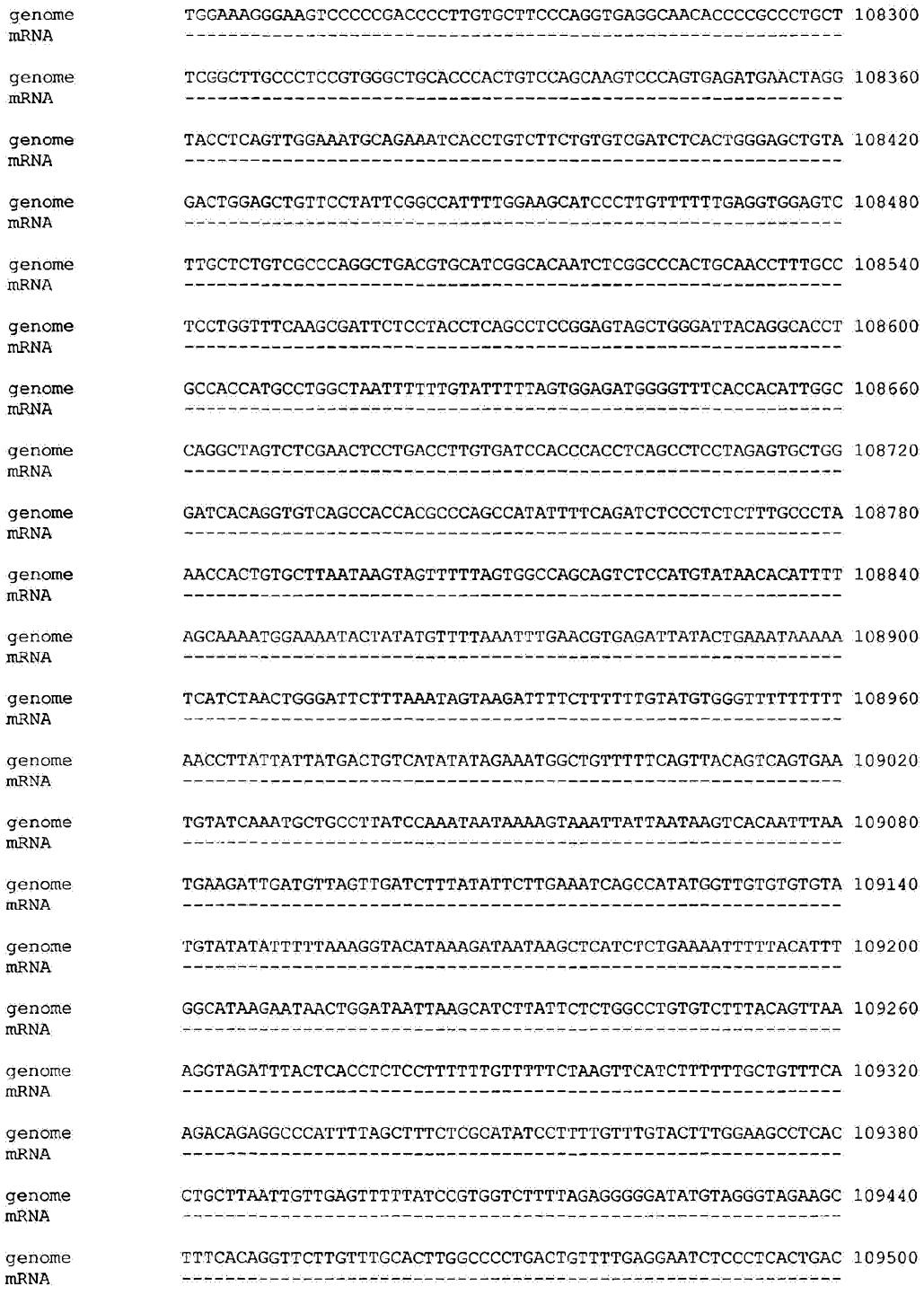
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Figure 1

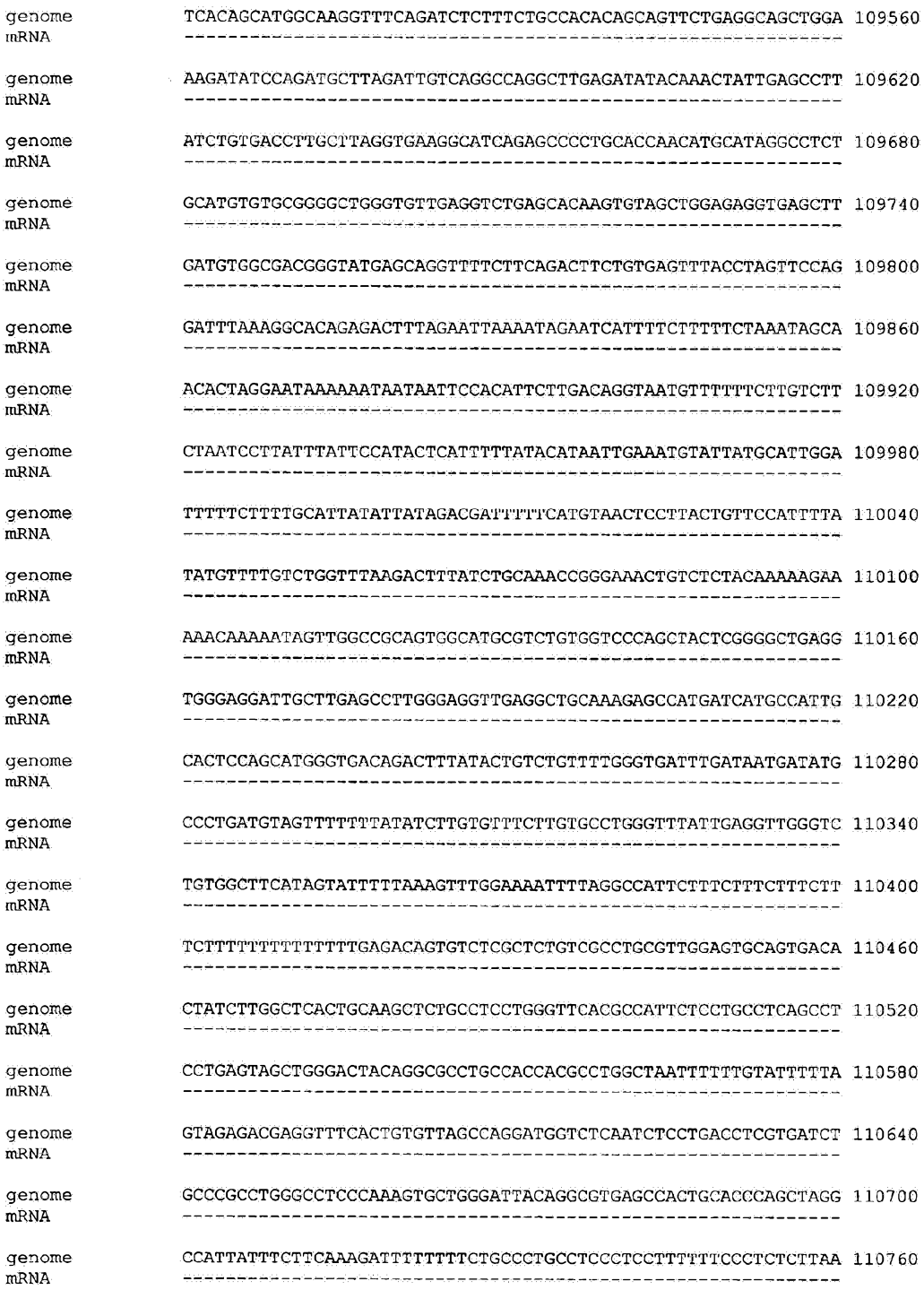
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Figure 1

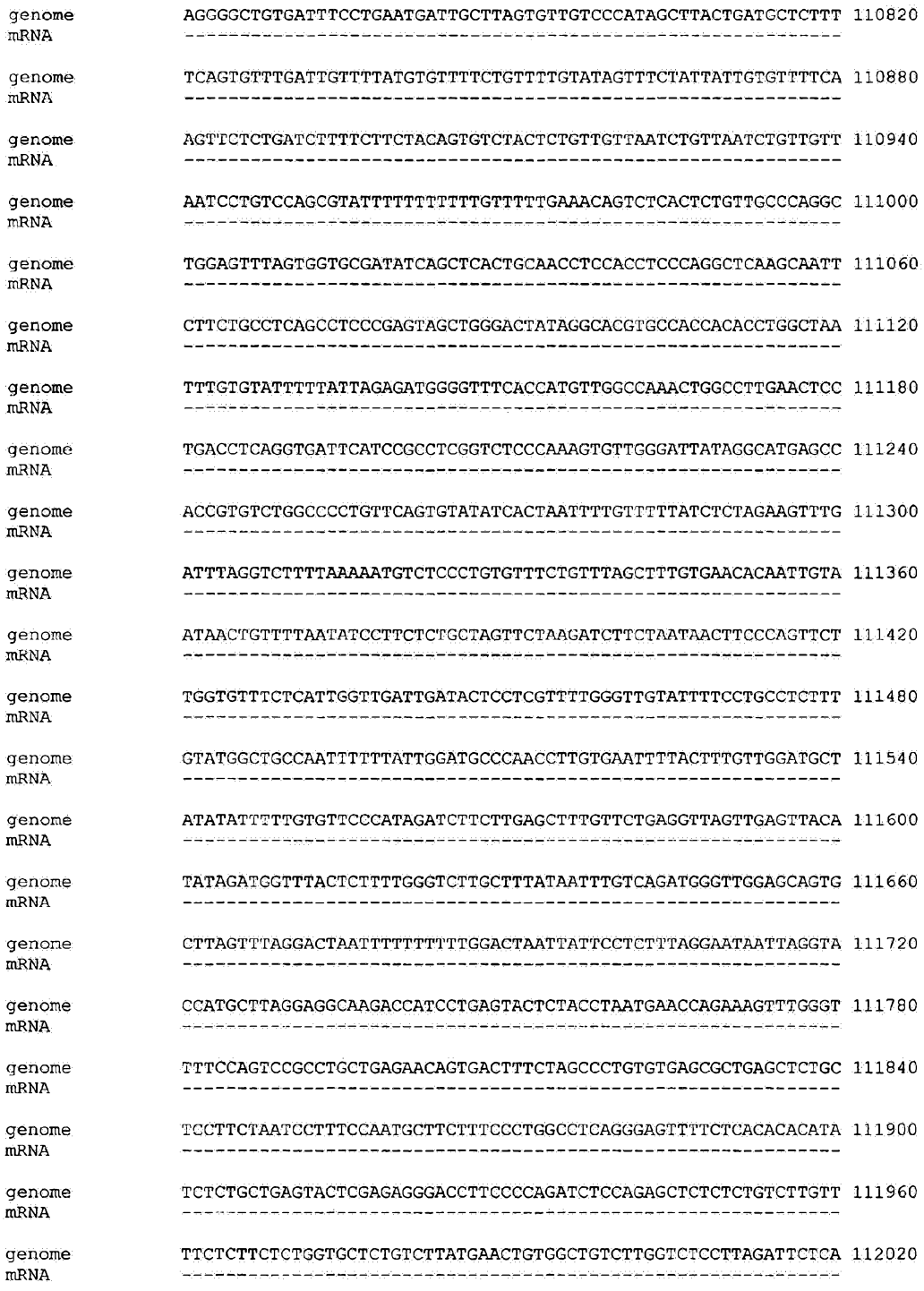
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Figure 1

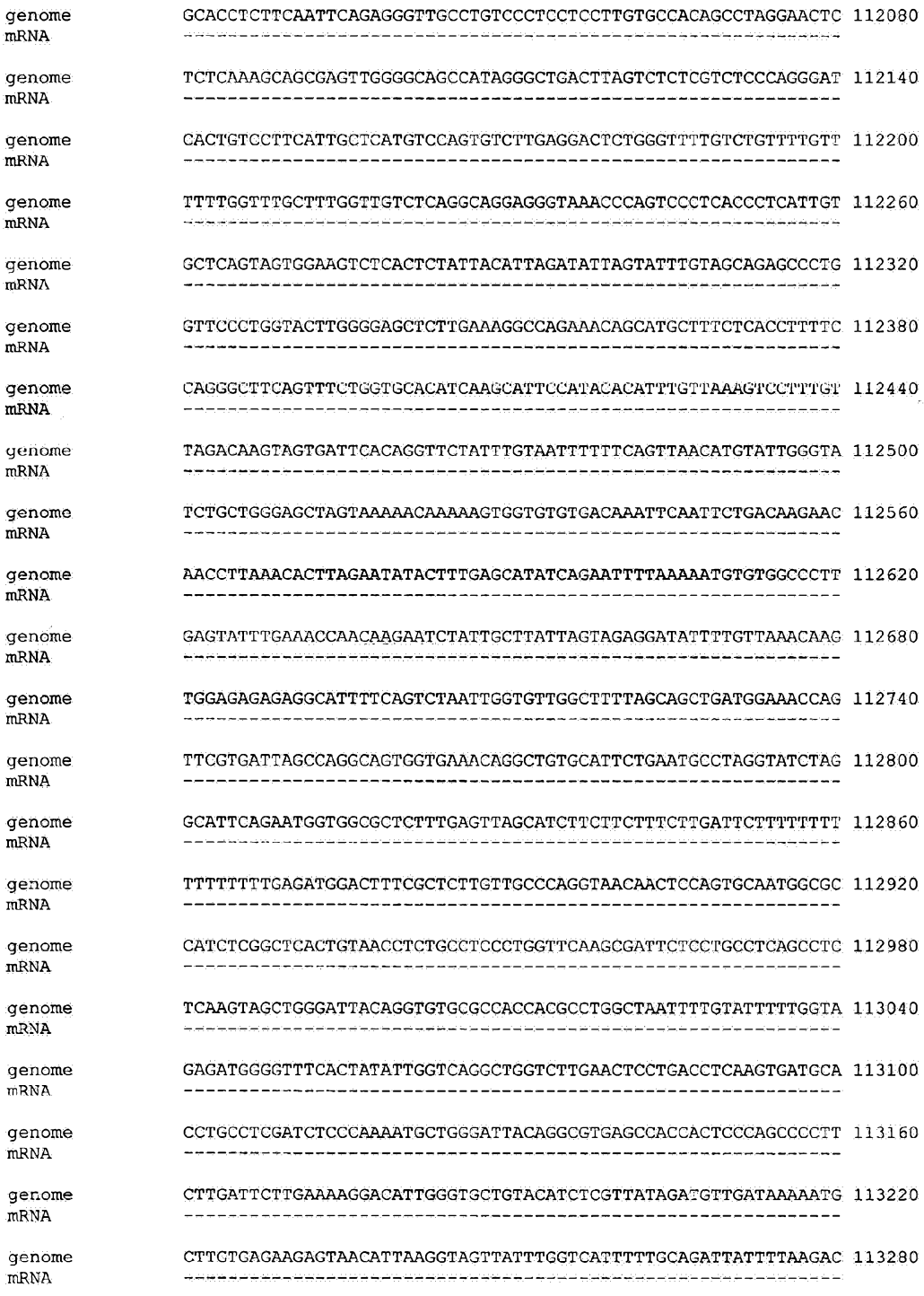
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Figure 1

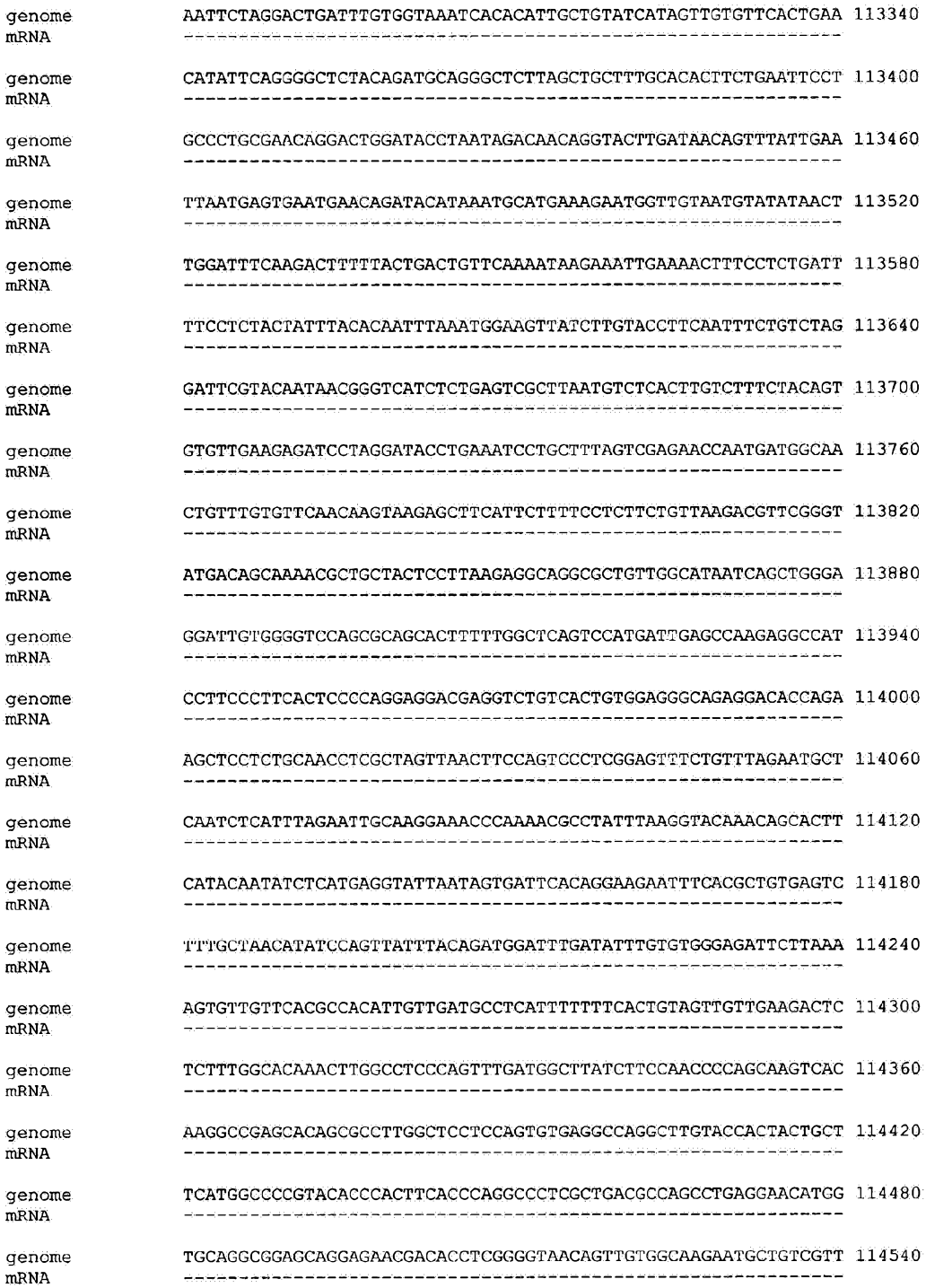
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Figure 1

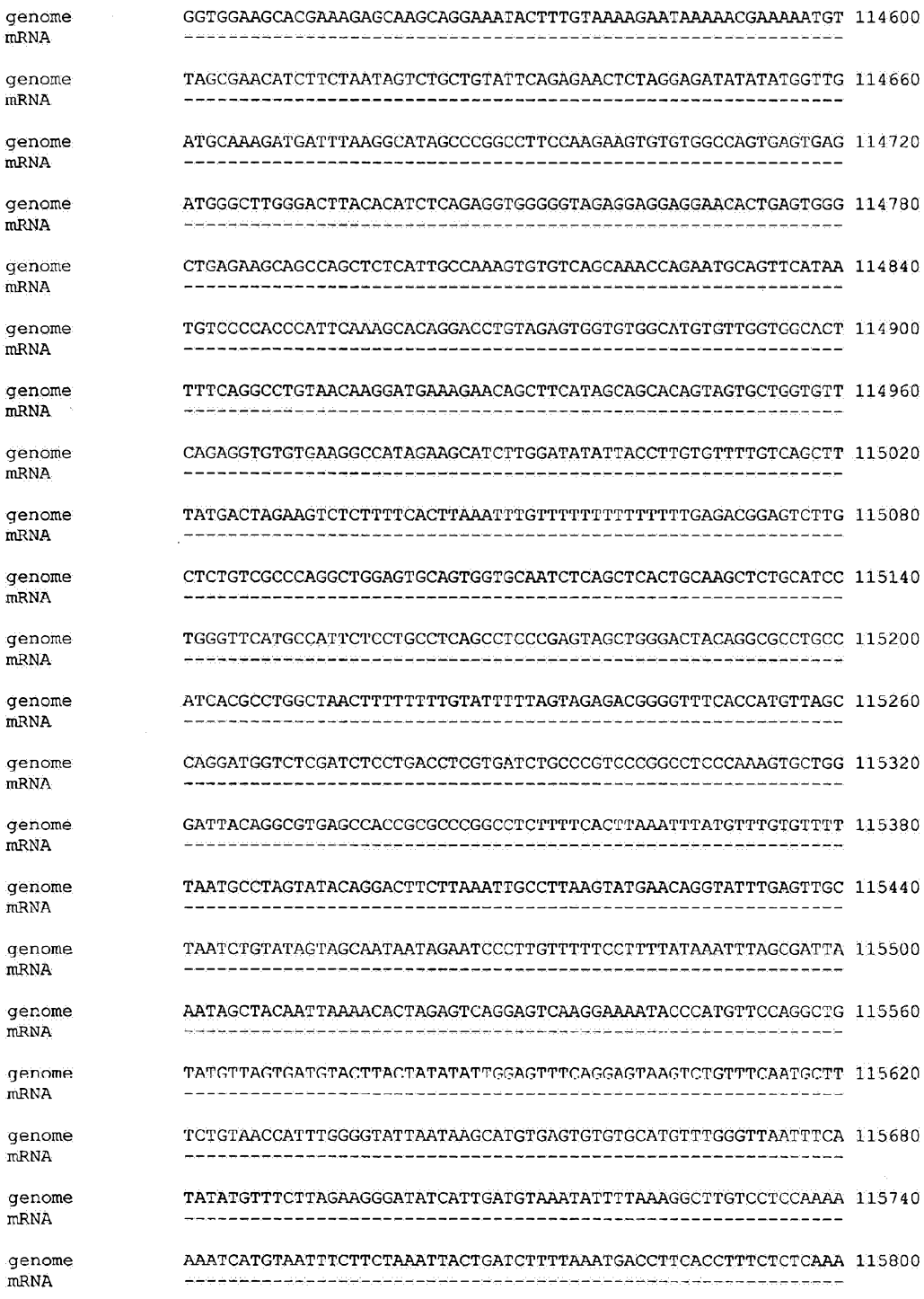
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Figure 1

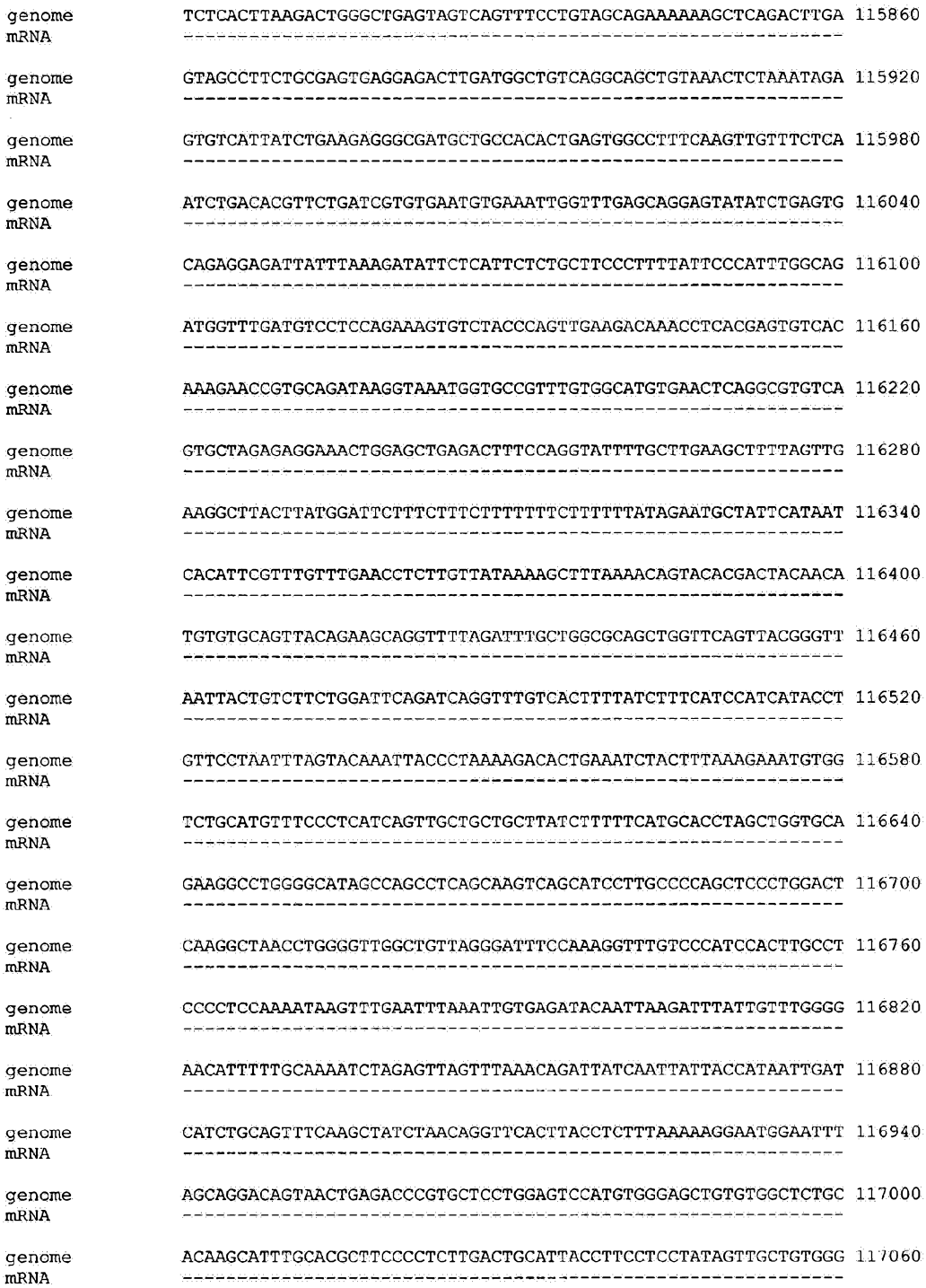
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Figure 1

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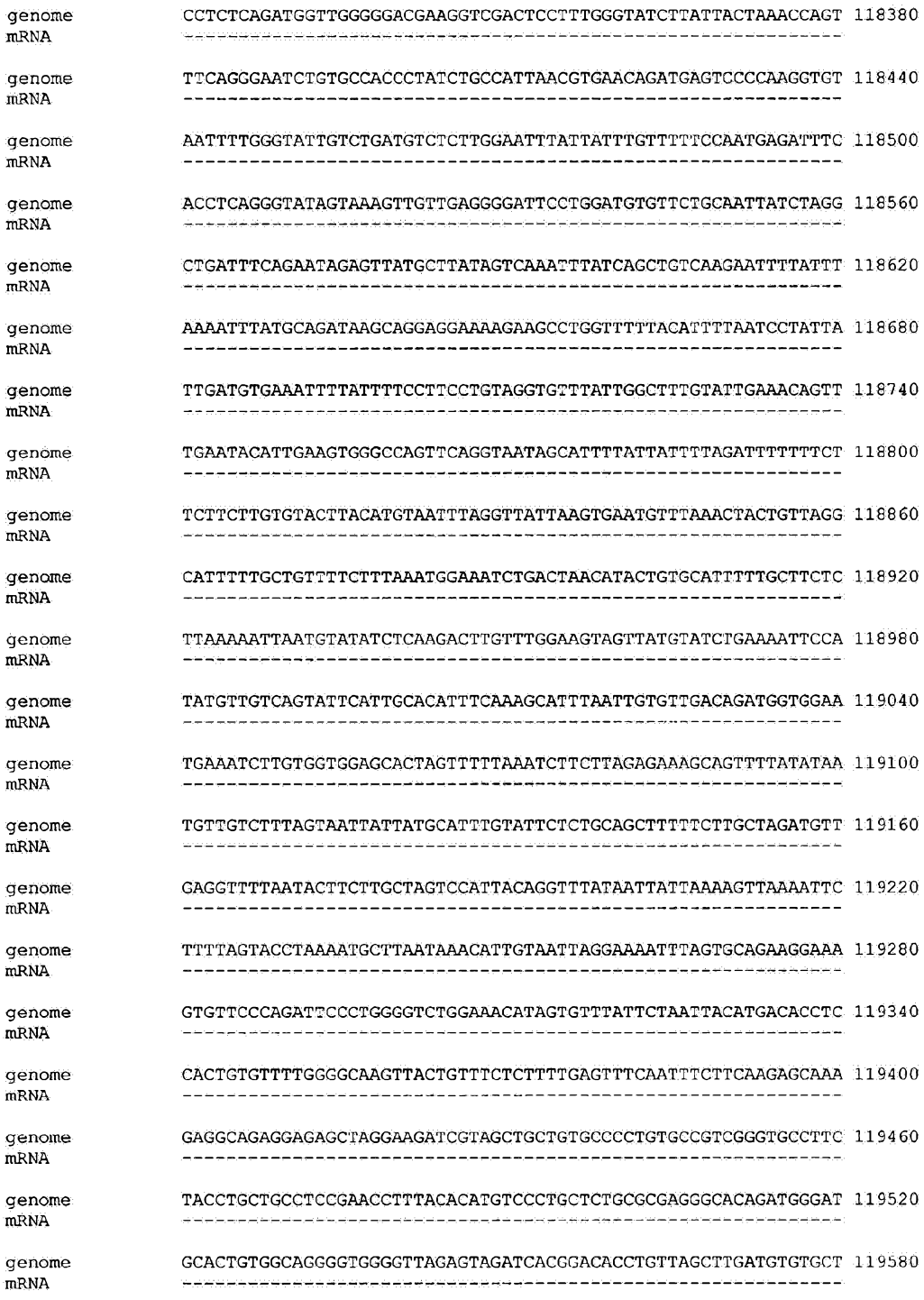
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Figure 1

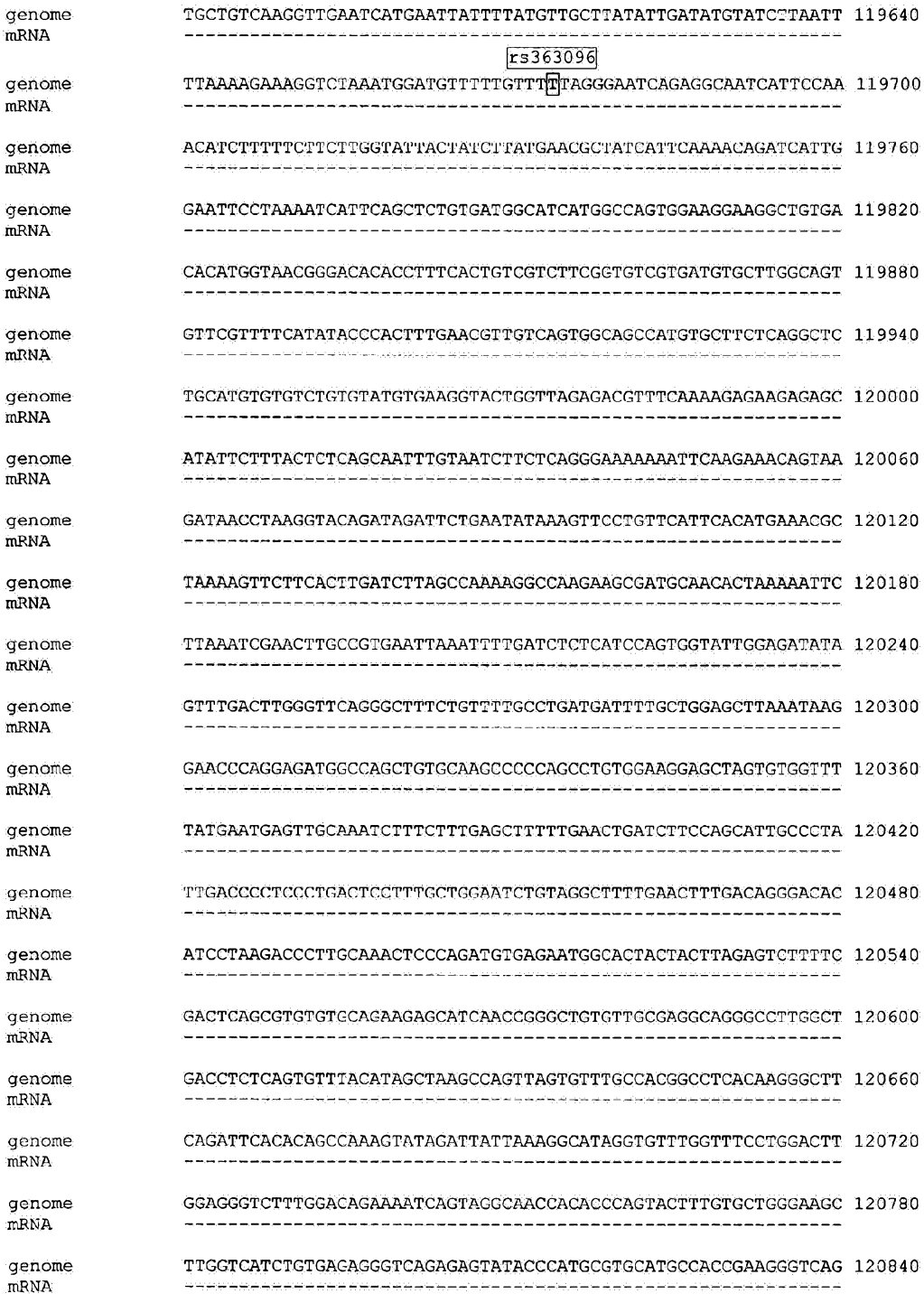
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Figure 1

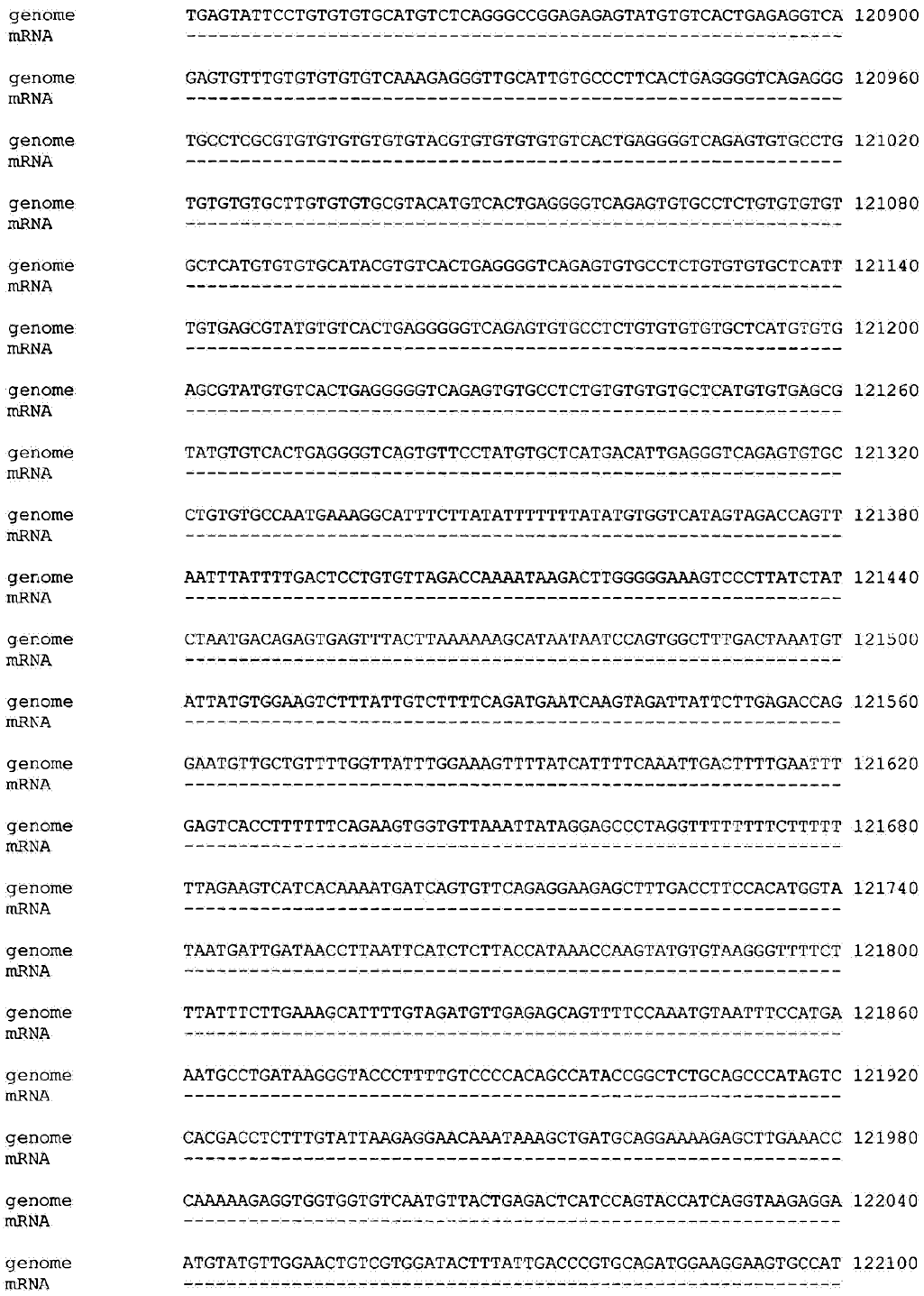
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Figure 1

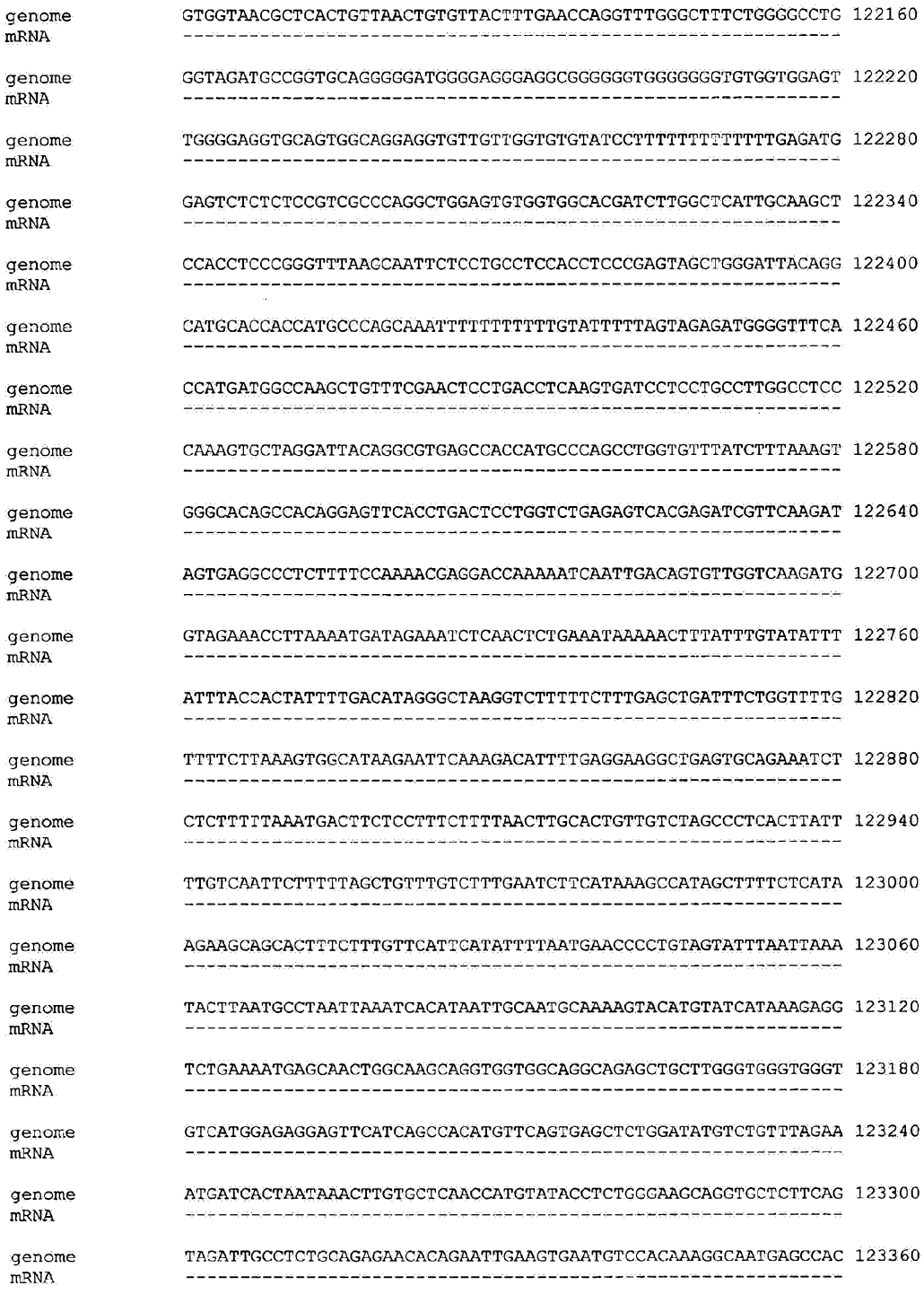
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Figure 1

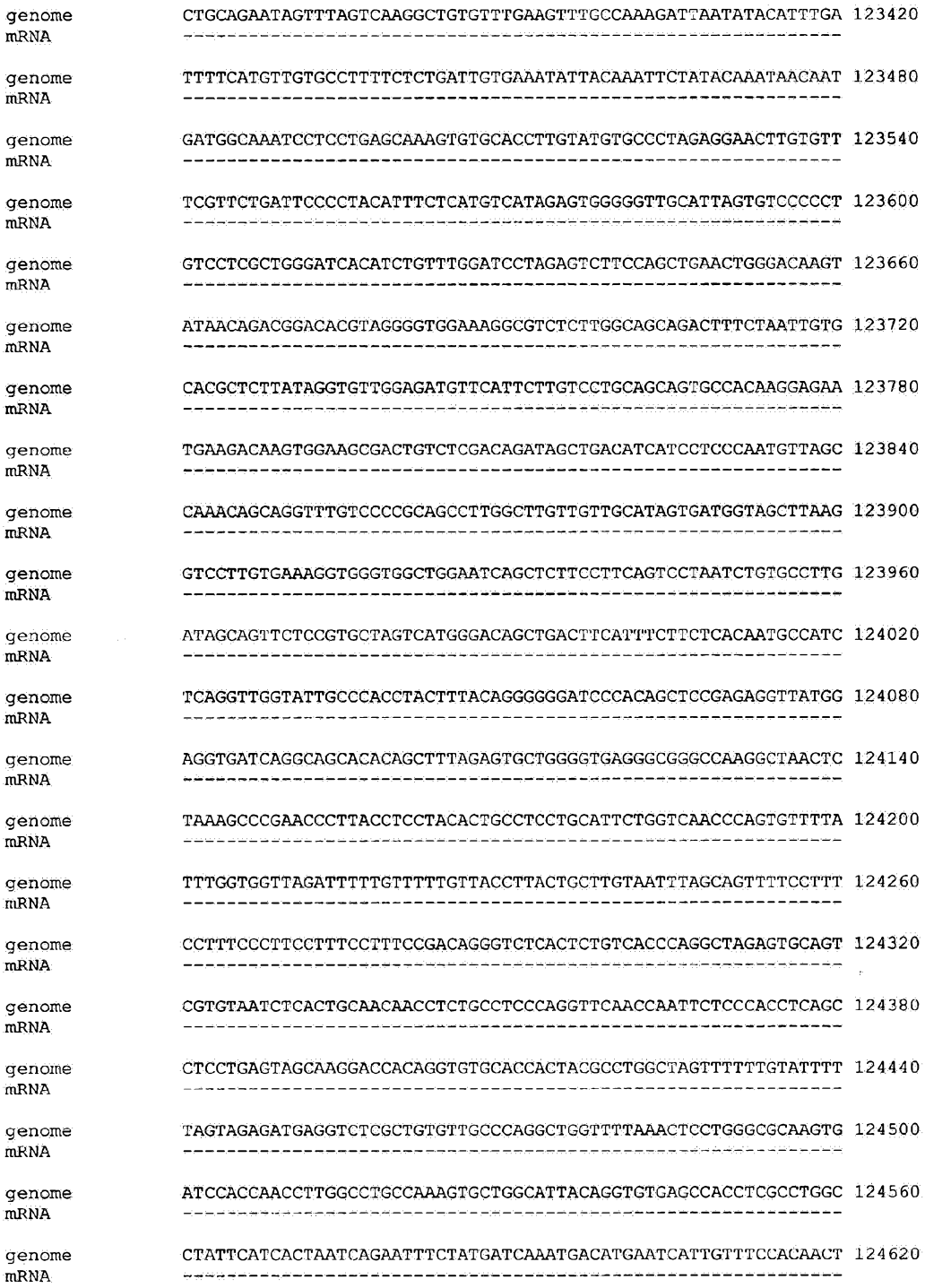
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Figure 1

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Figure 1

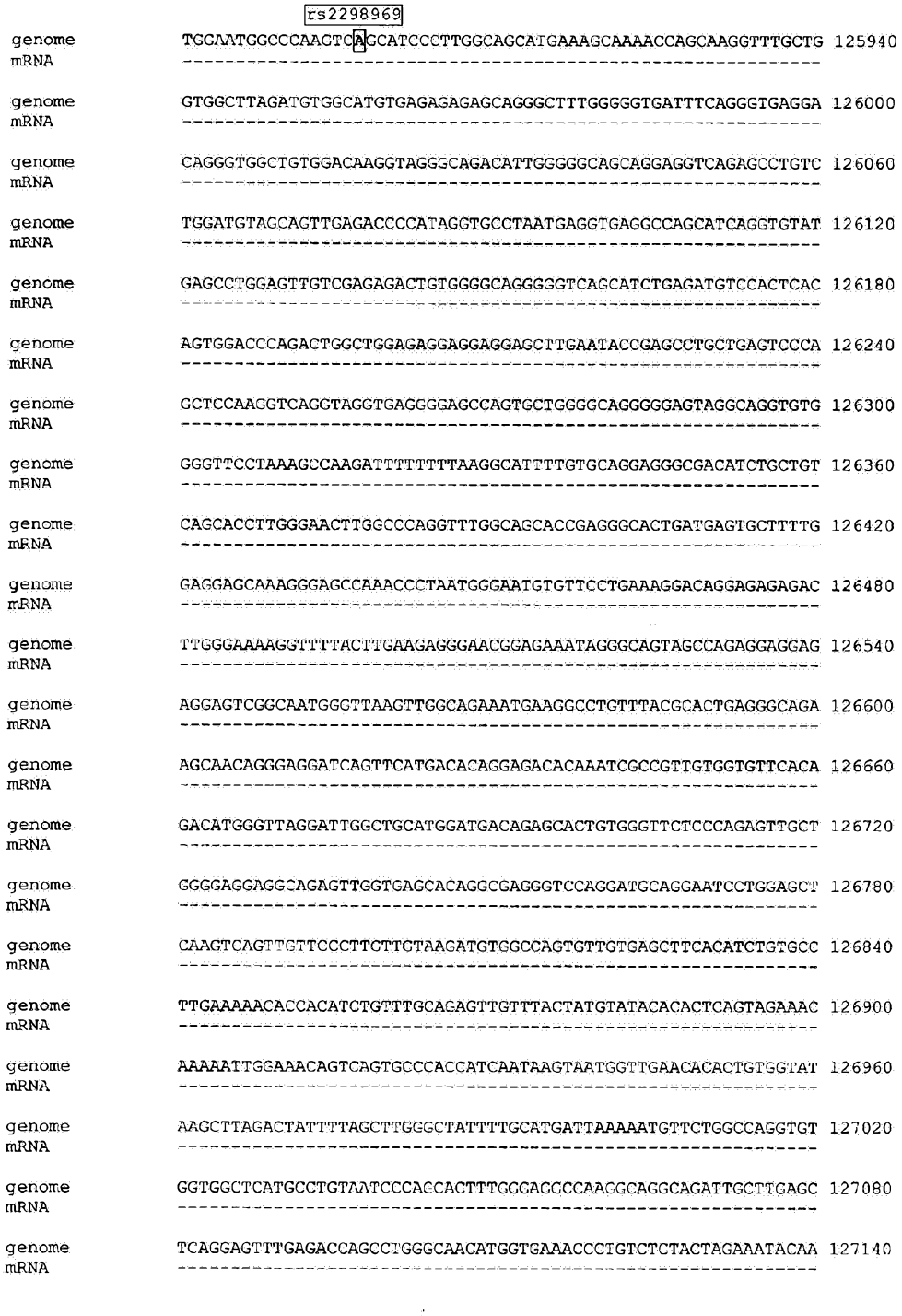
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Figure 1

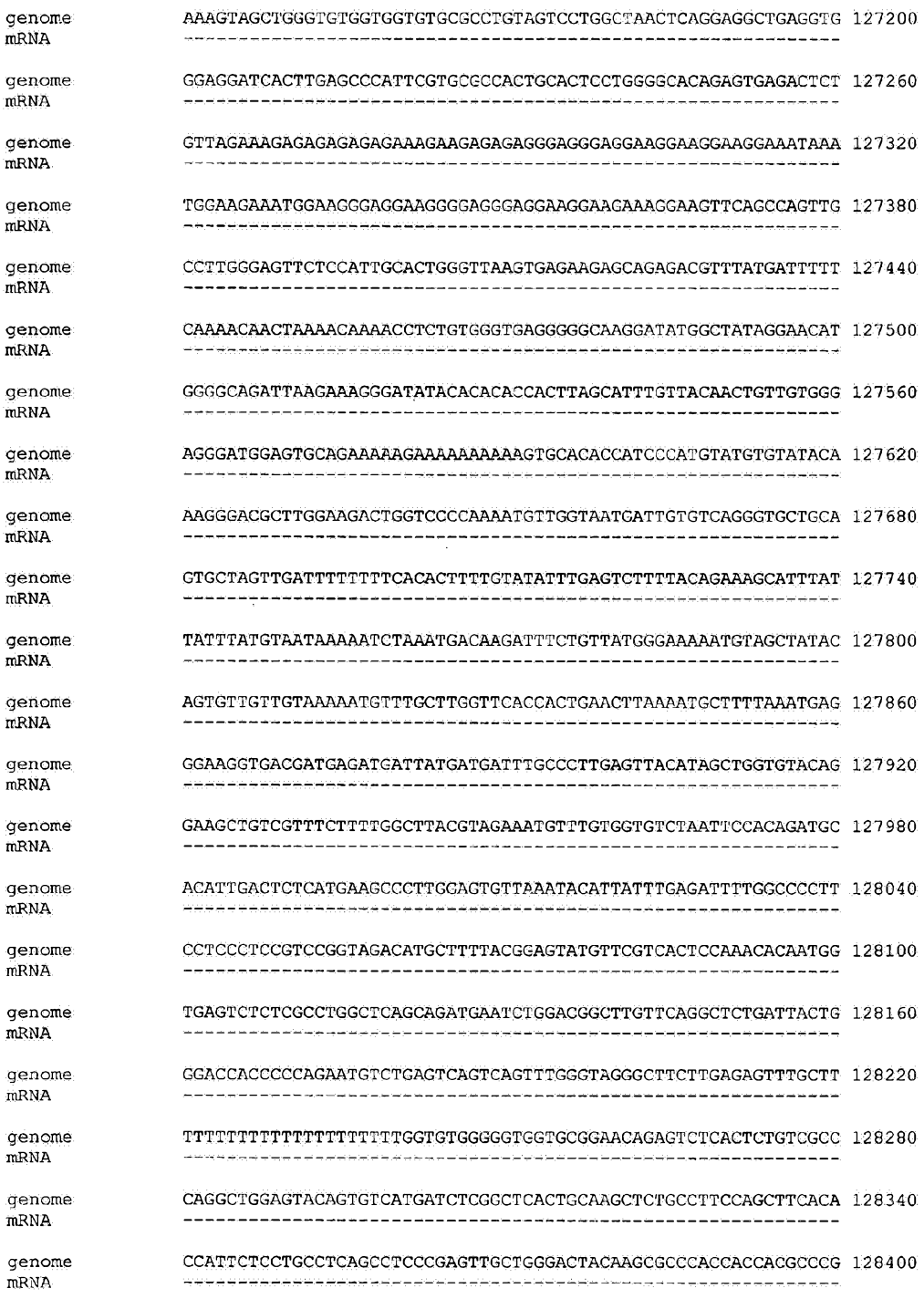
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Figure 1

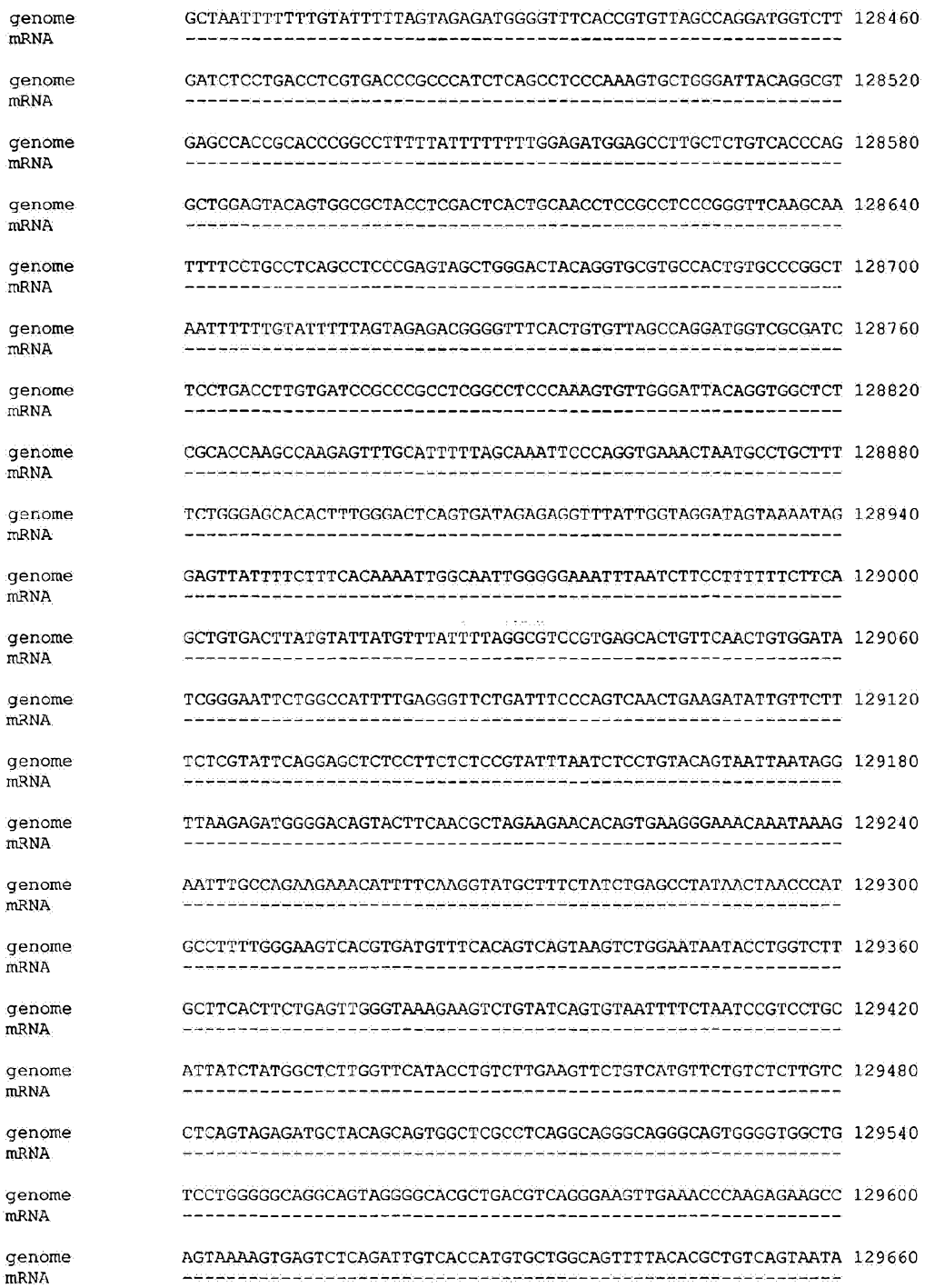
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Figure 1

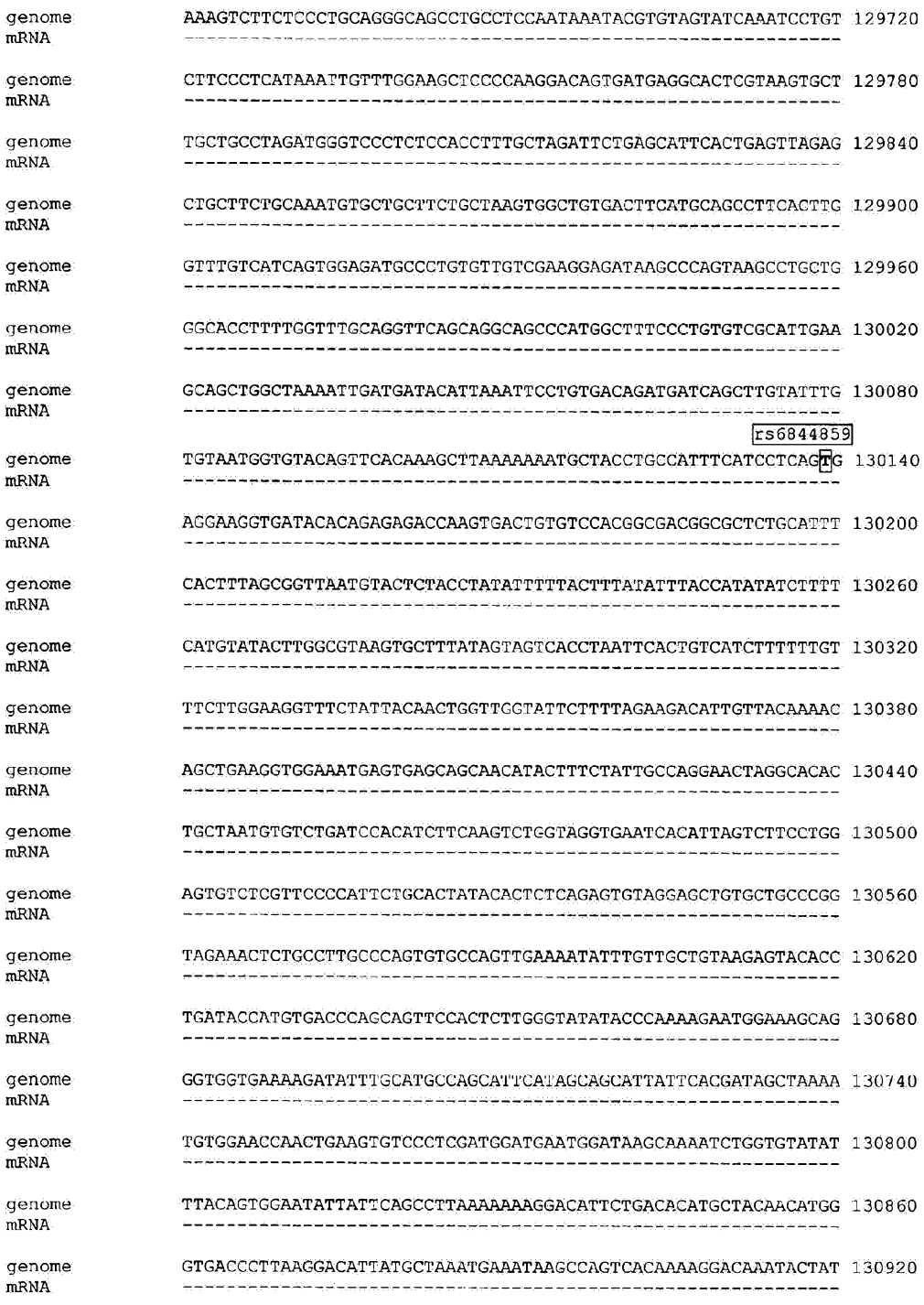
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Figure 1

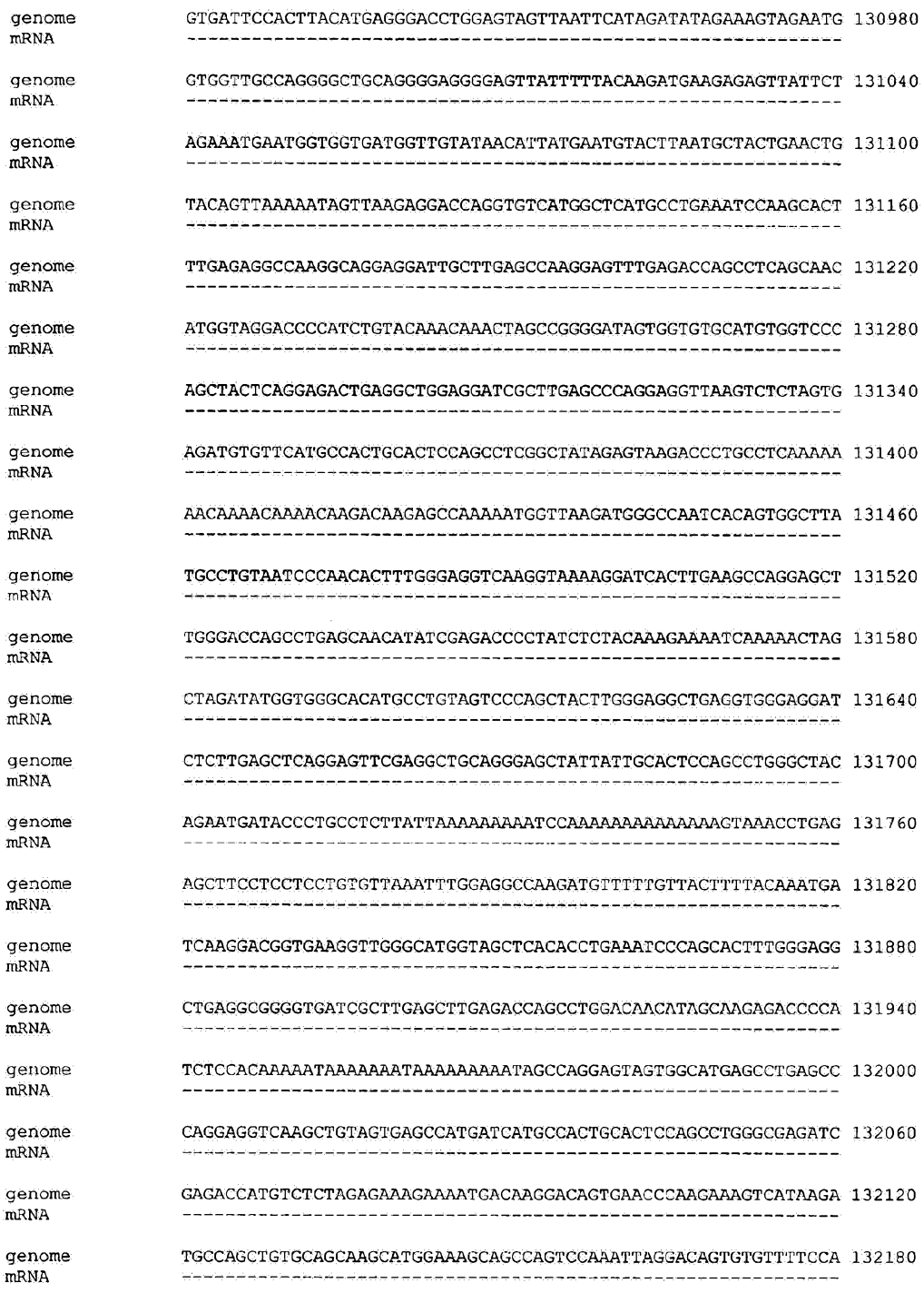
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Figure 1

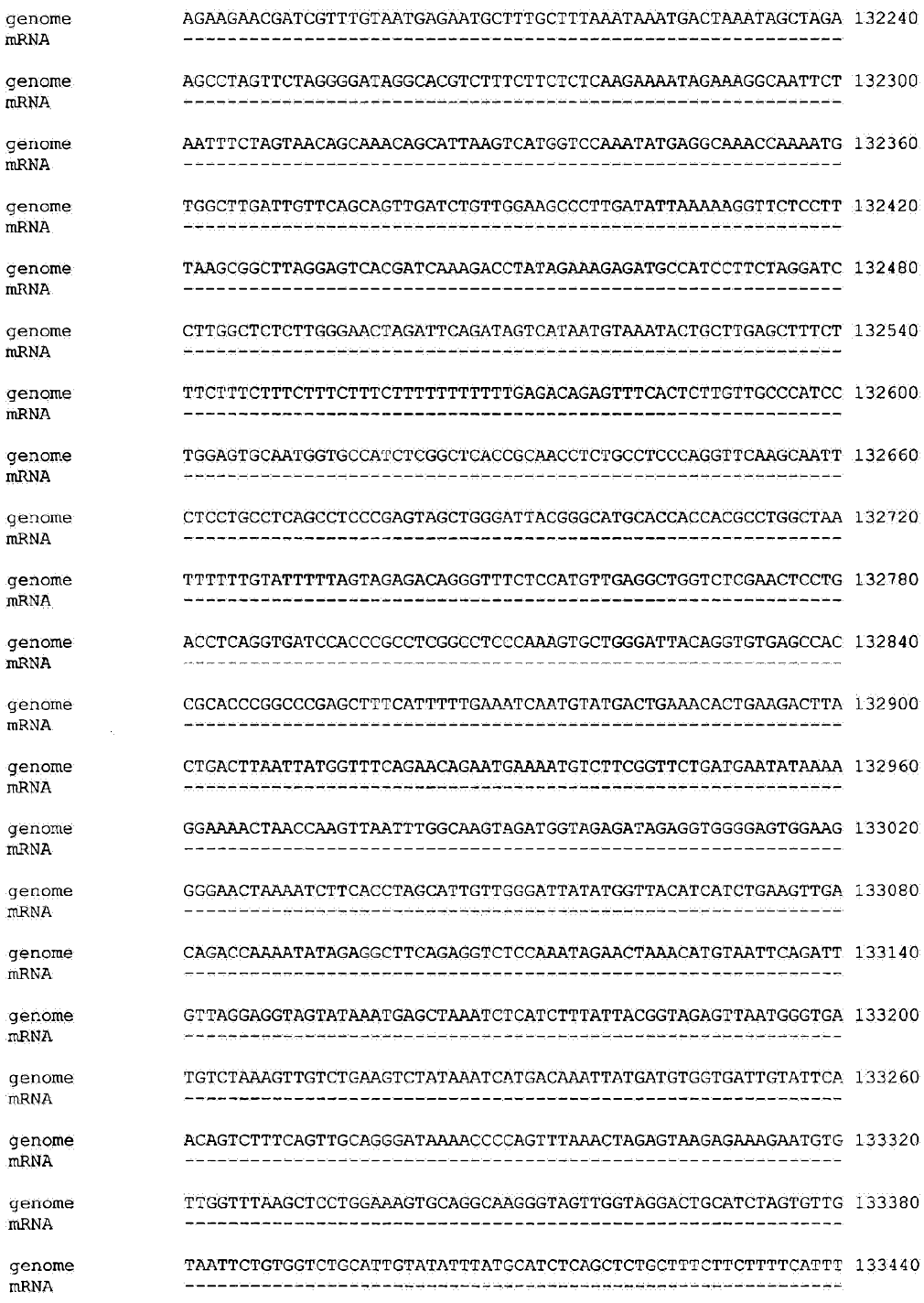
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Figure 1

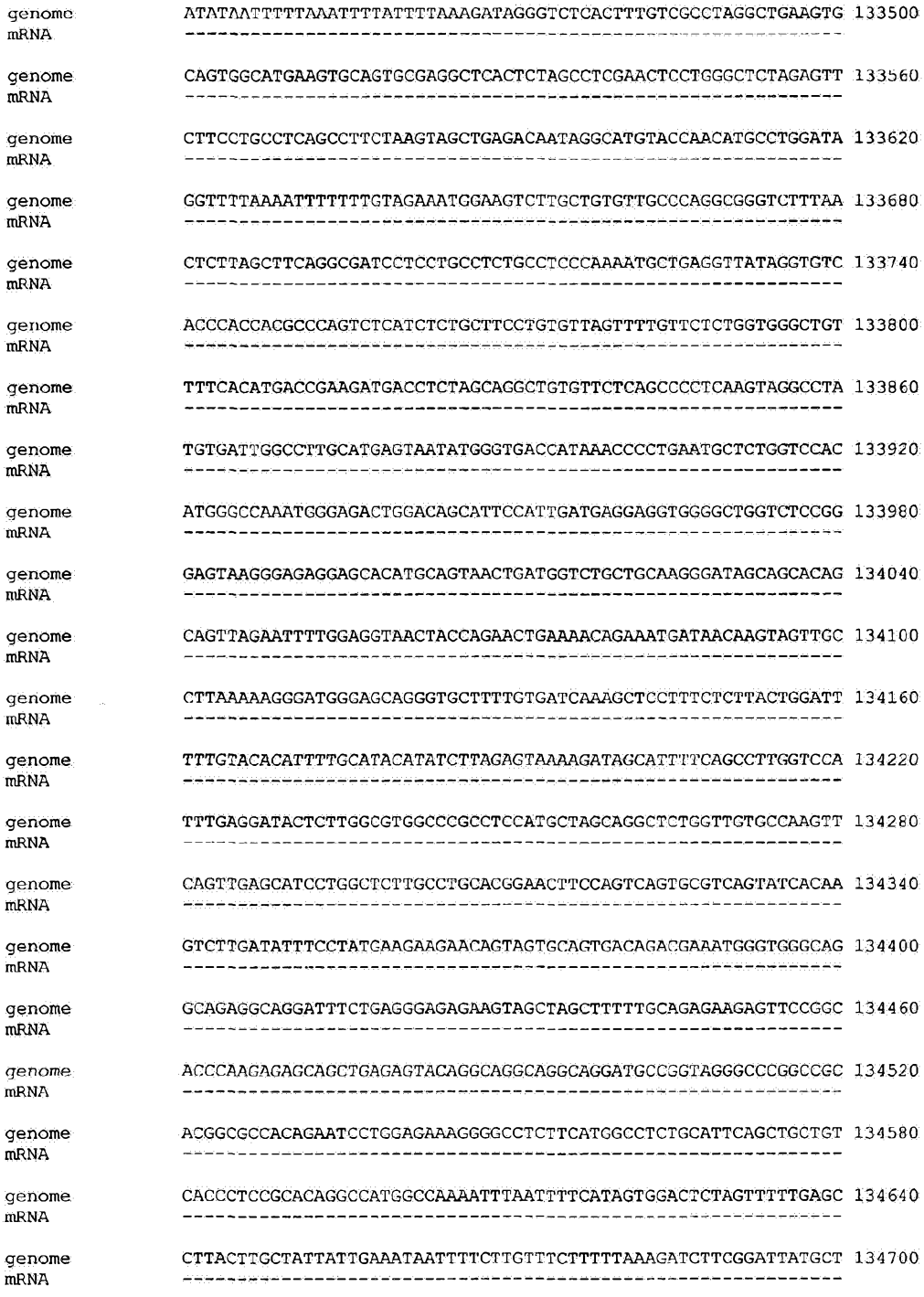
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Figure 1

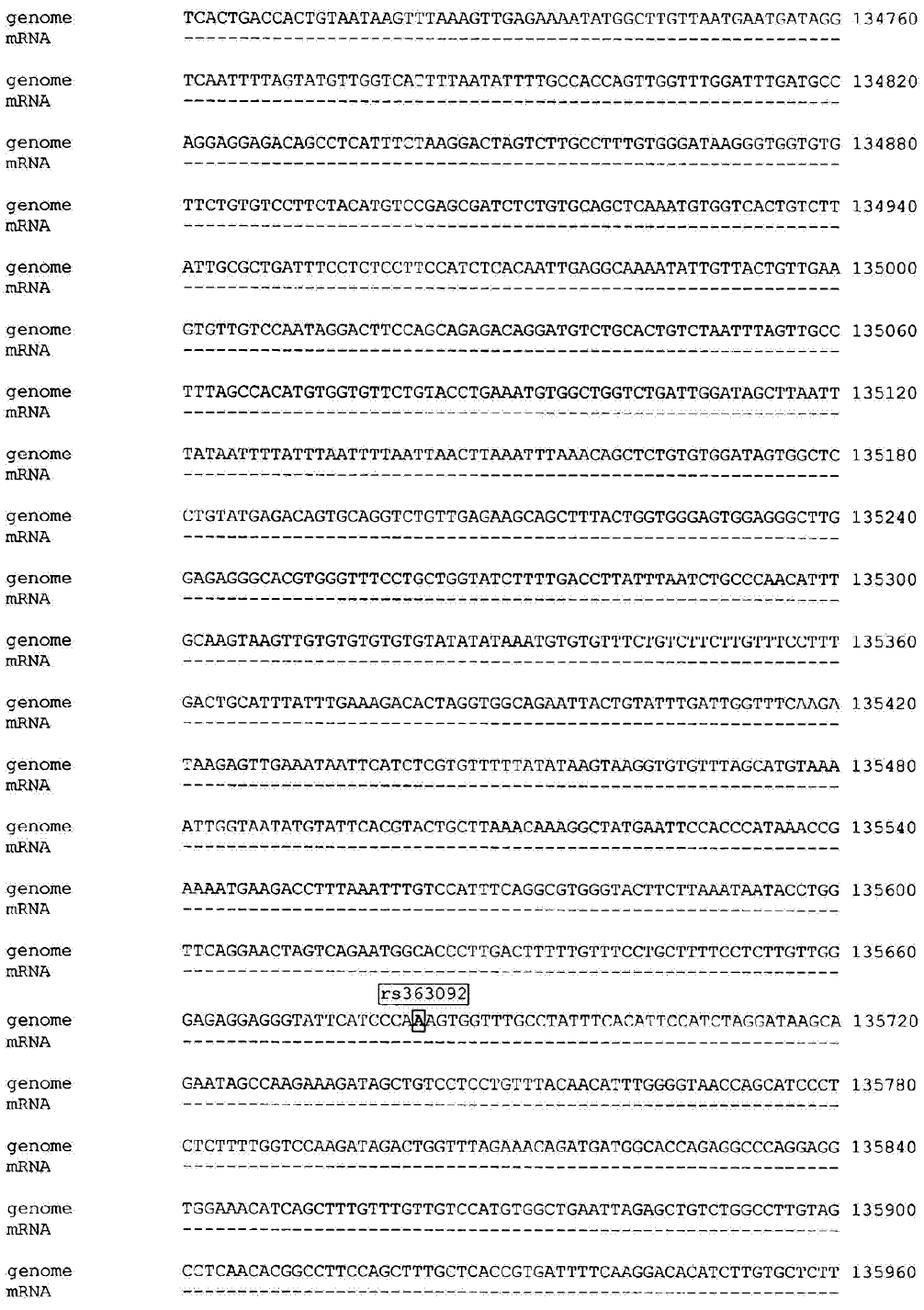
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Figure 1

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Figure 1

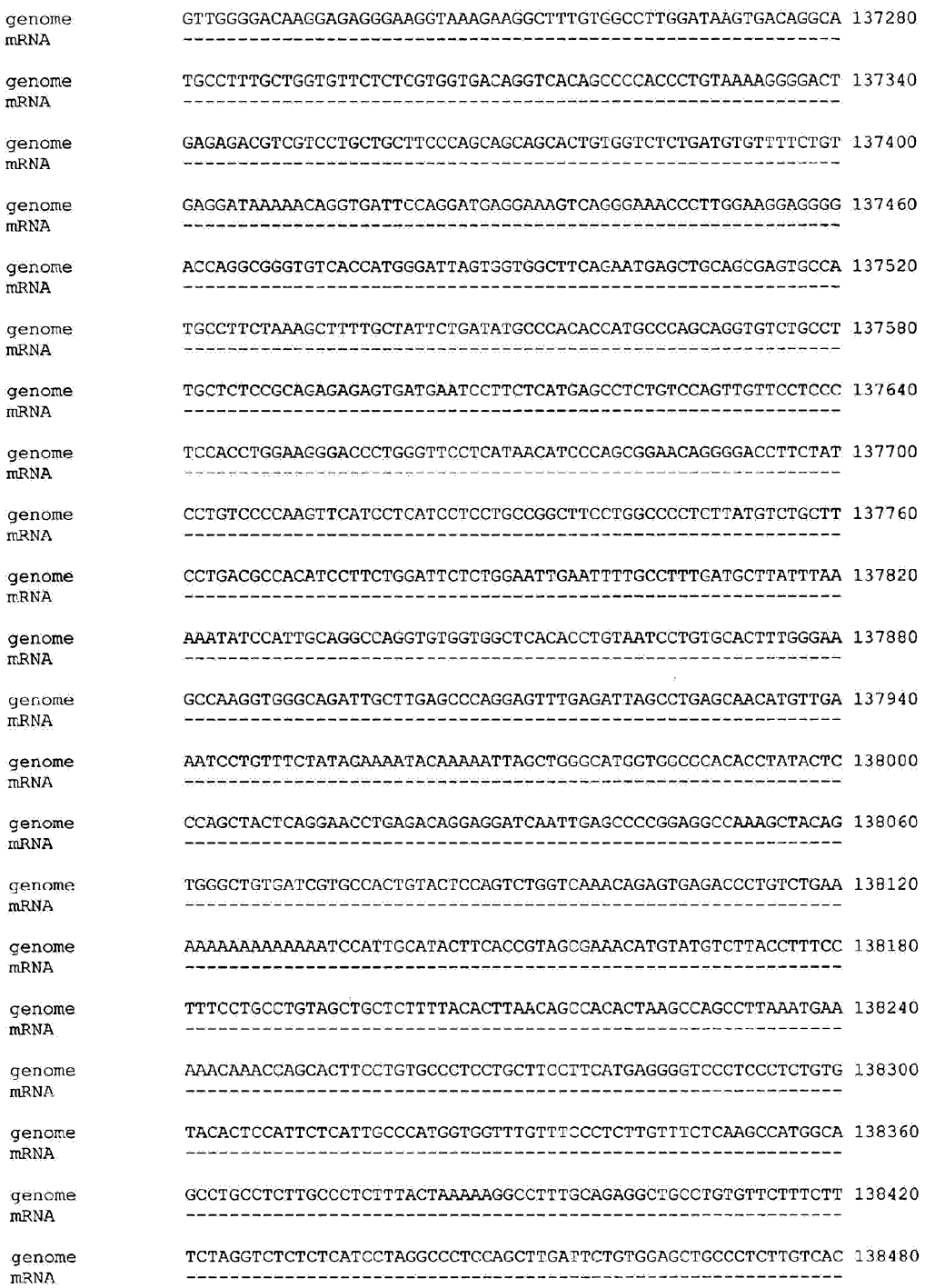
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Figure 1

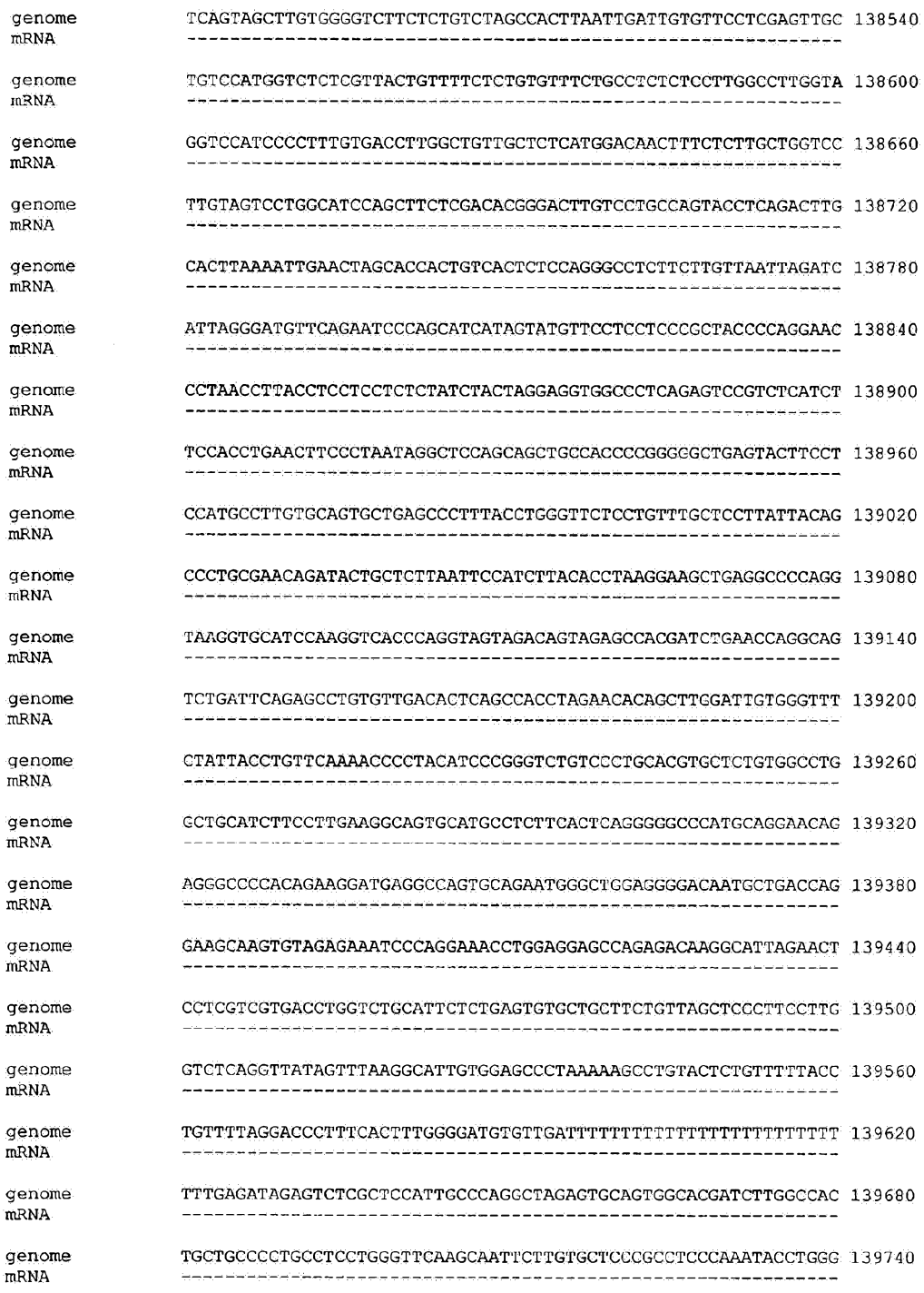
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Figure 1

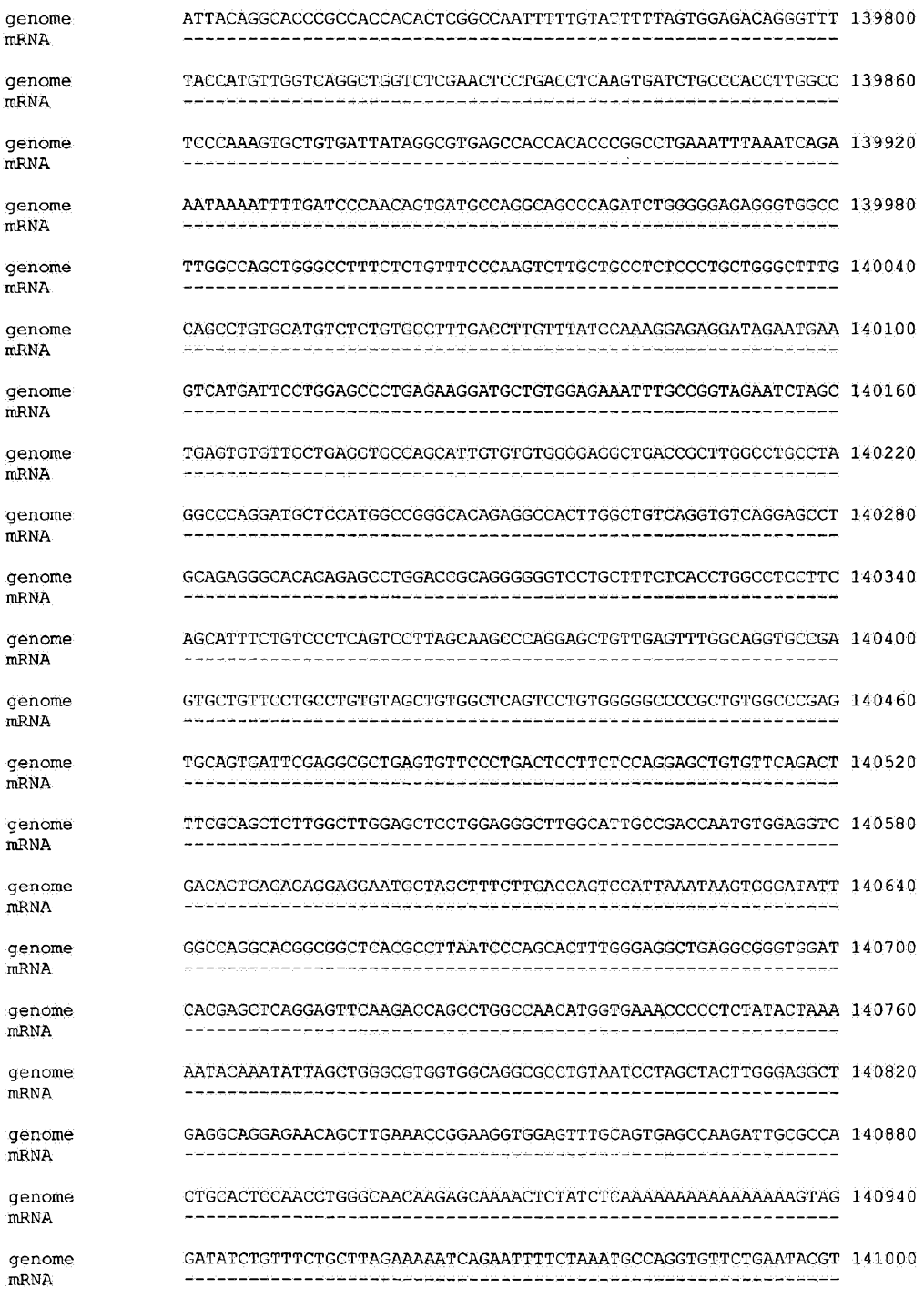
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Figure 1

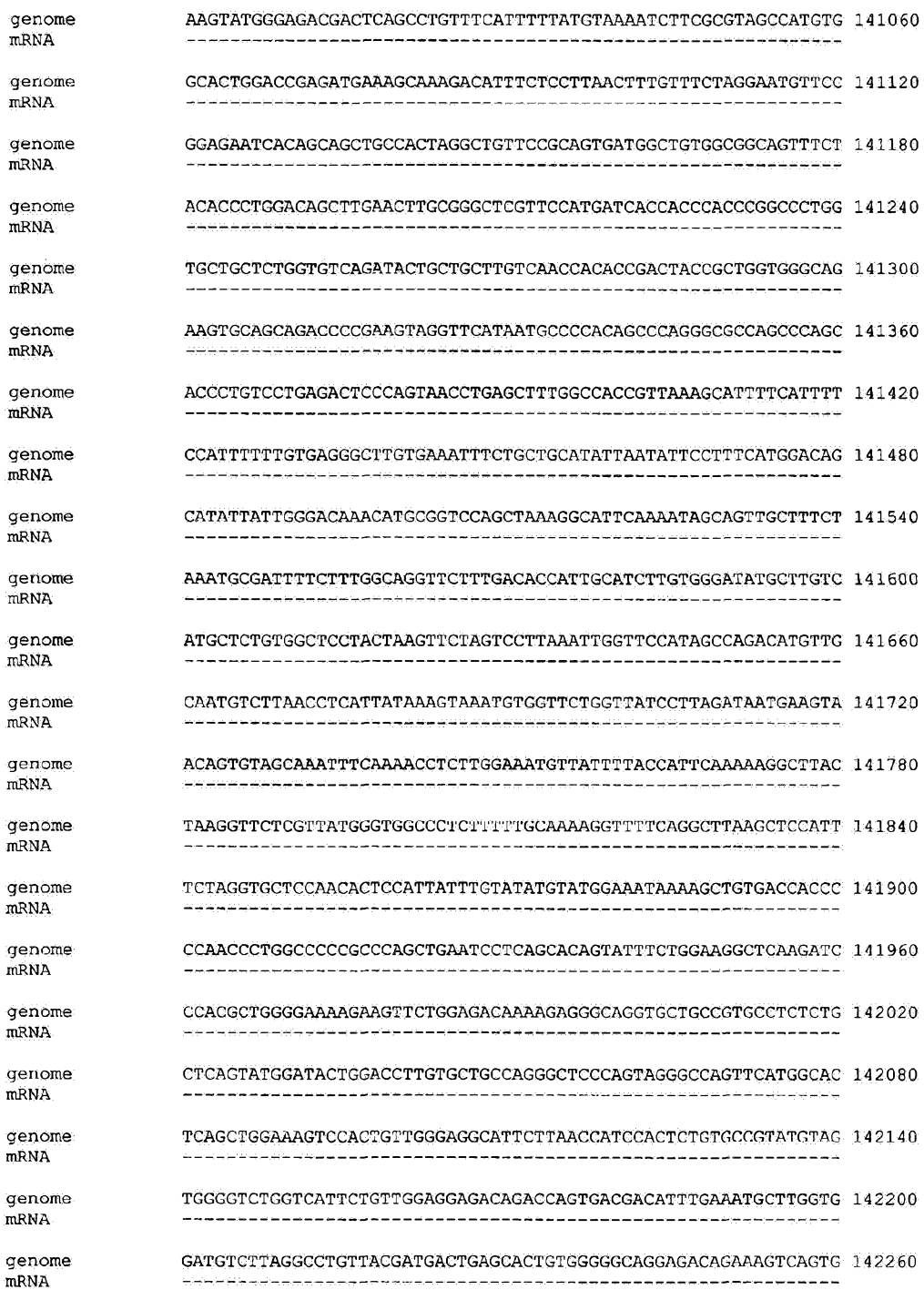
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Figure 1

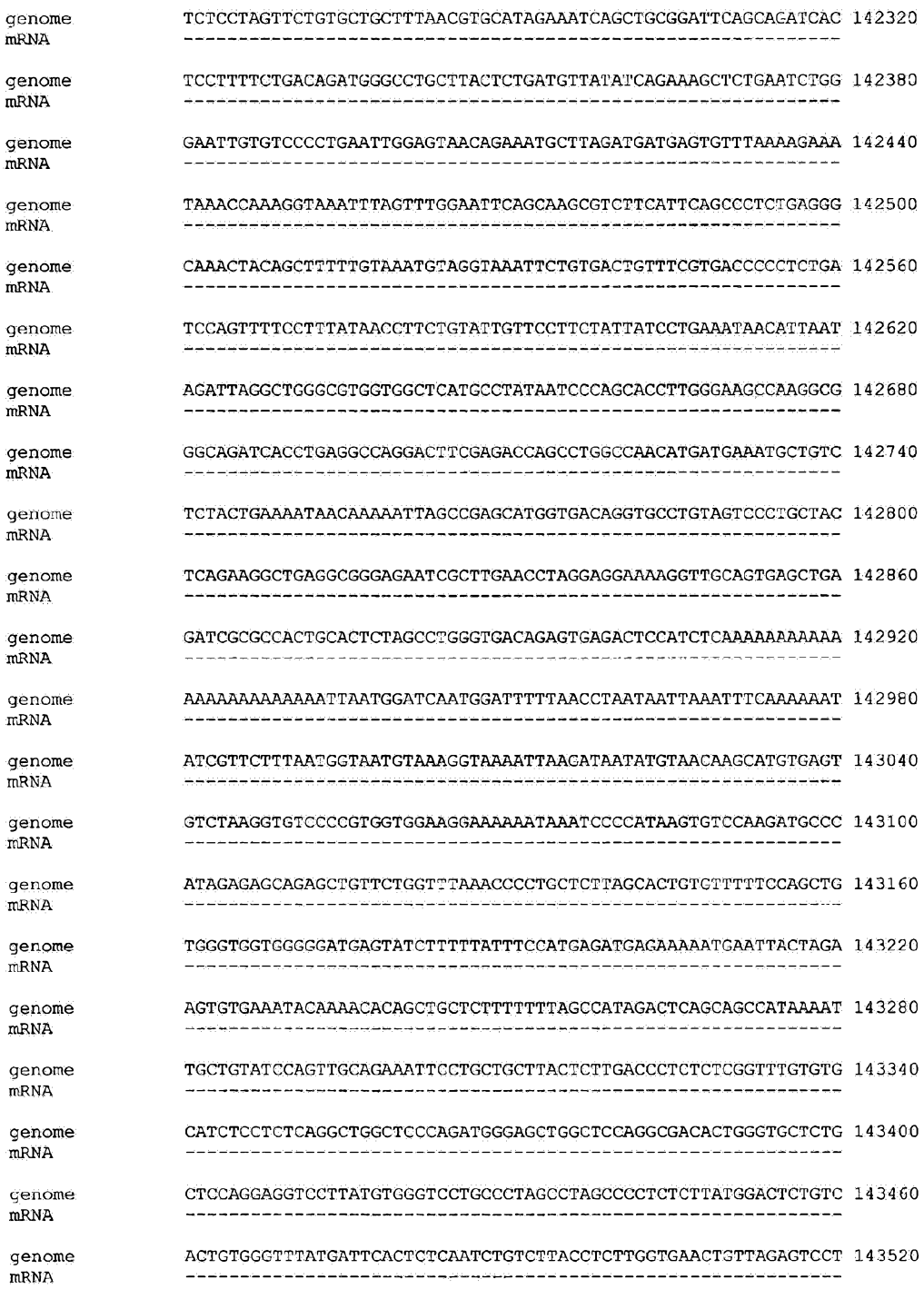
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Figure 1

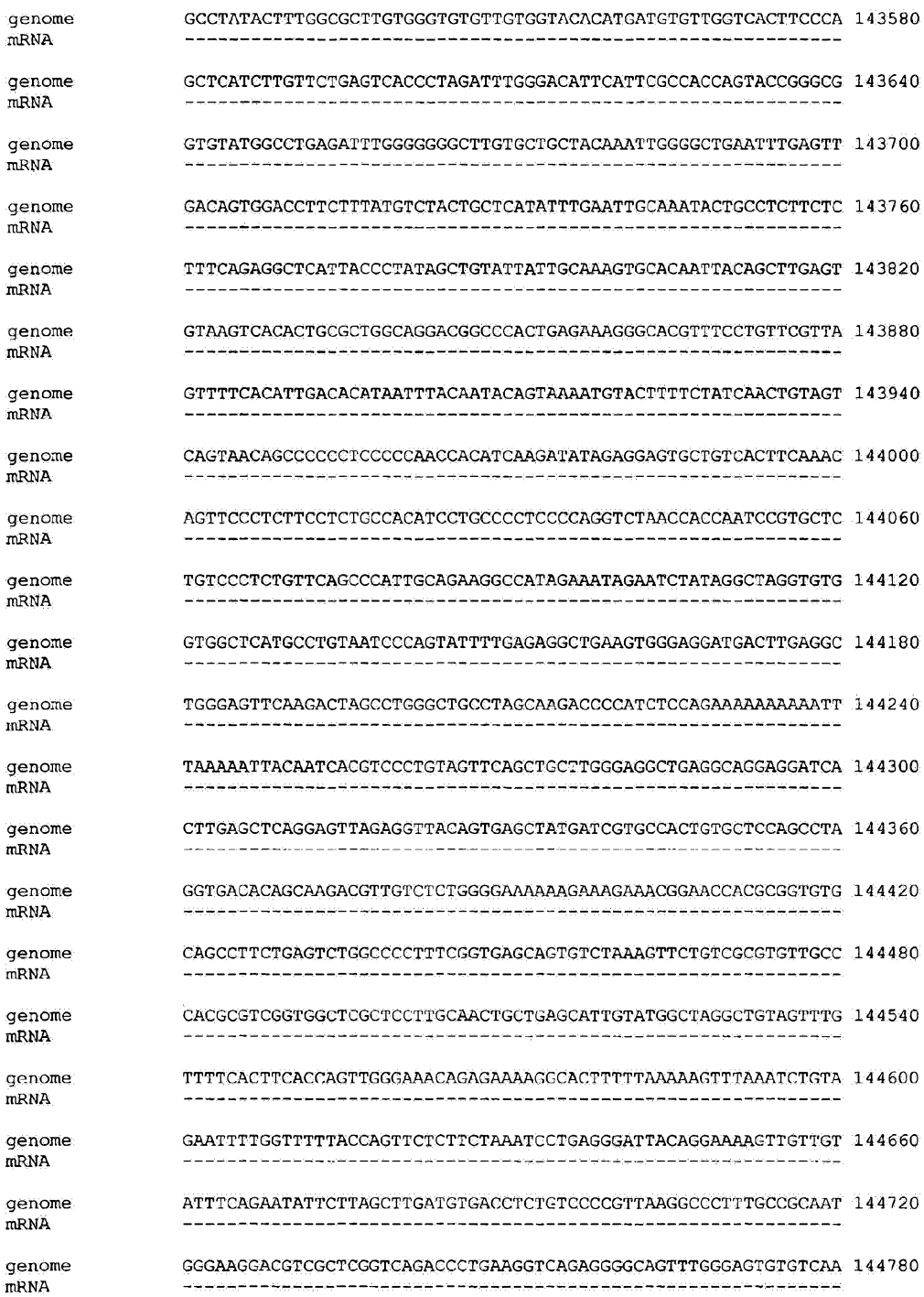
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Figure 1

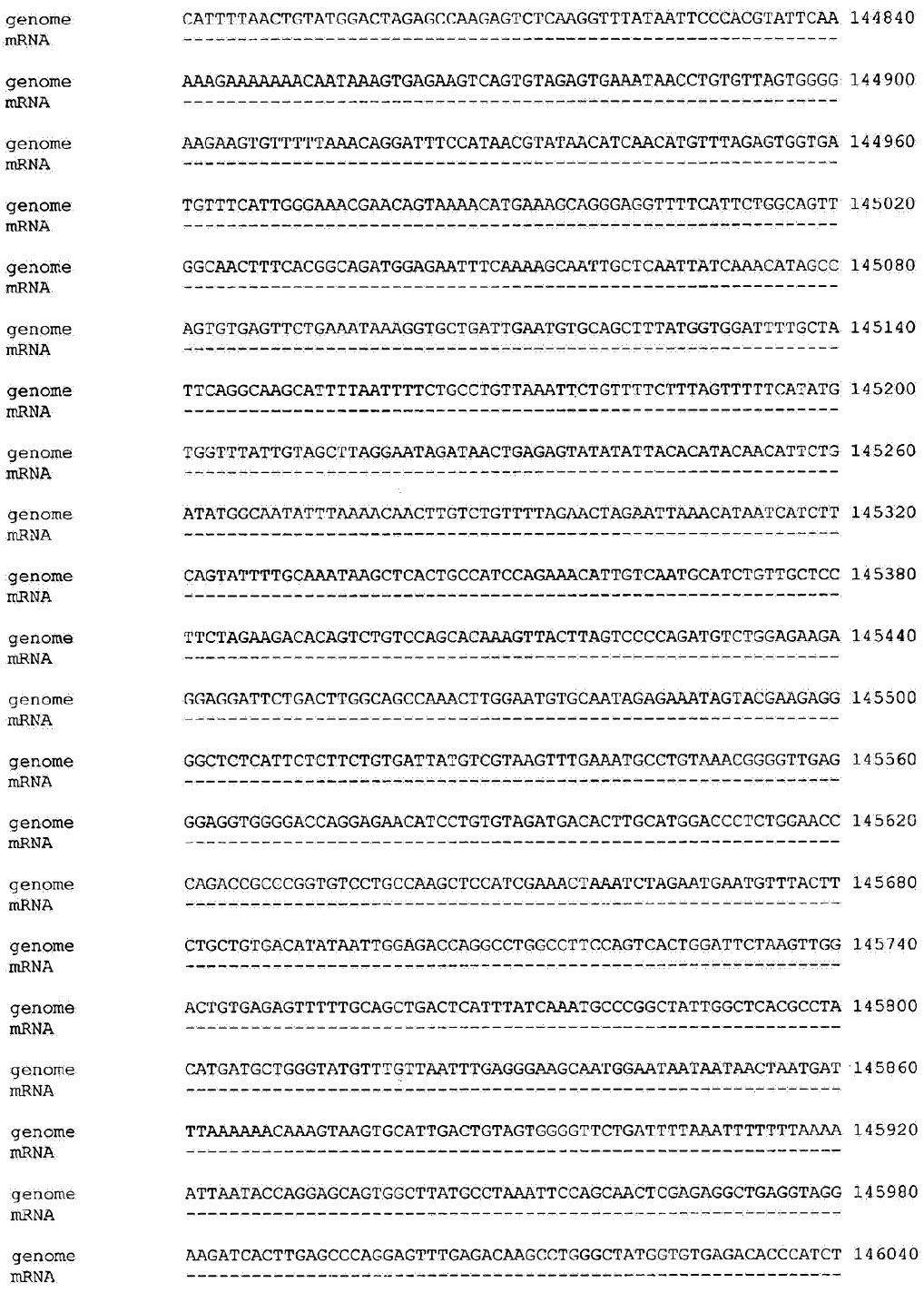
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Figure 1

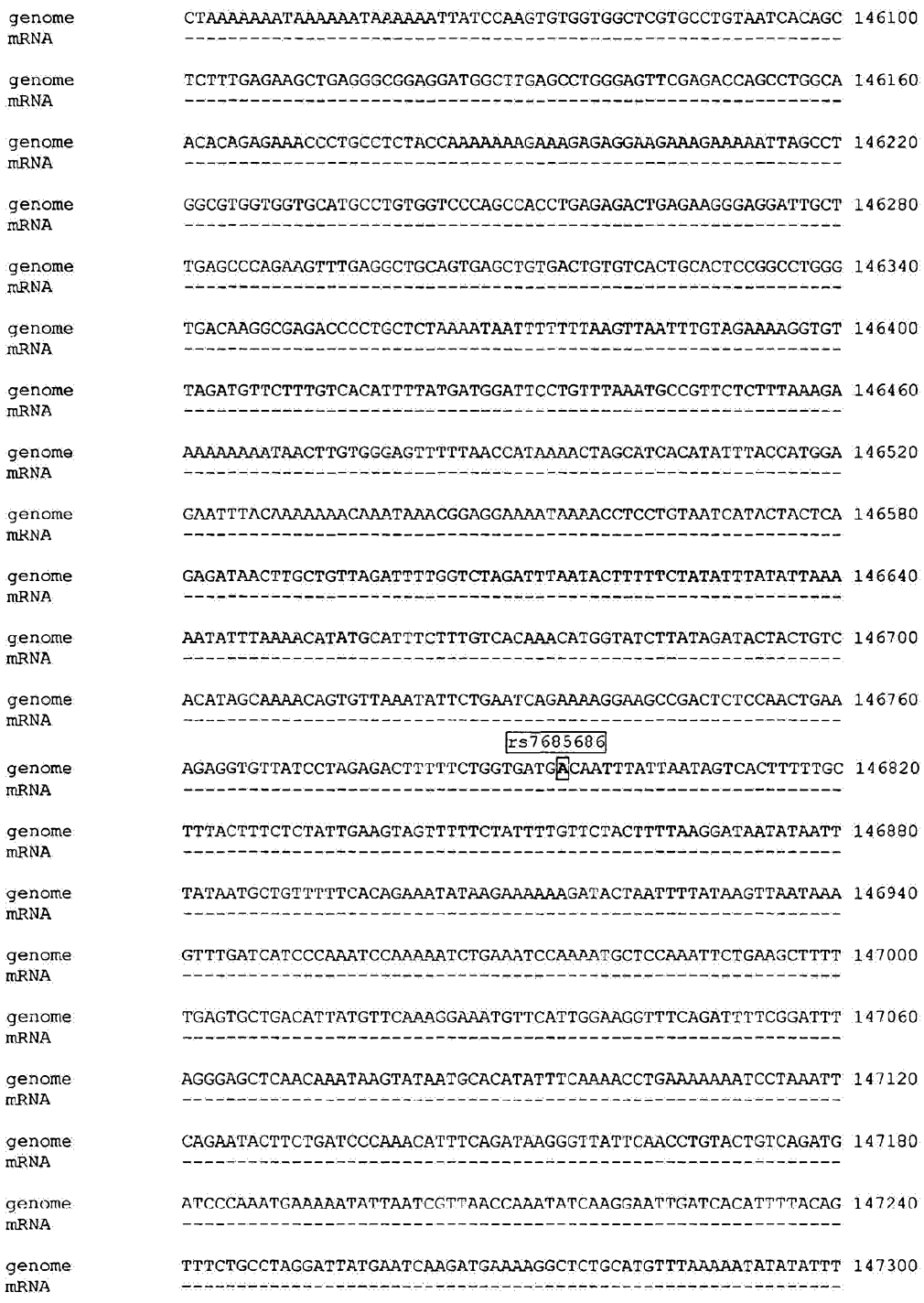
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Figure 1

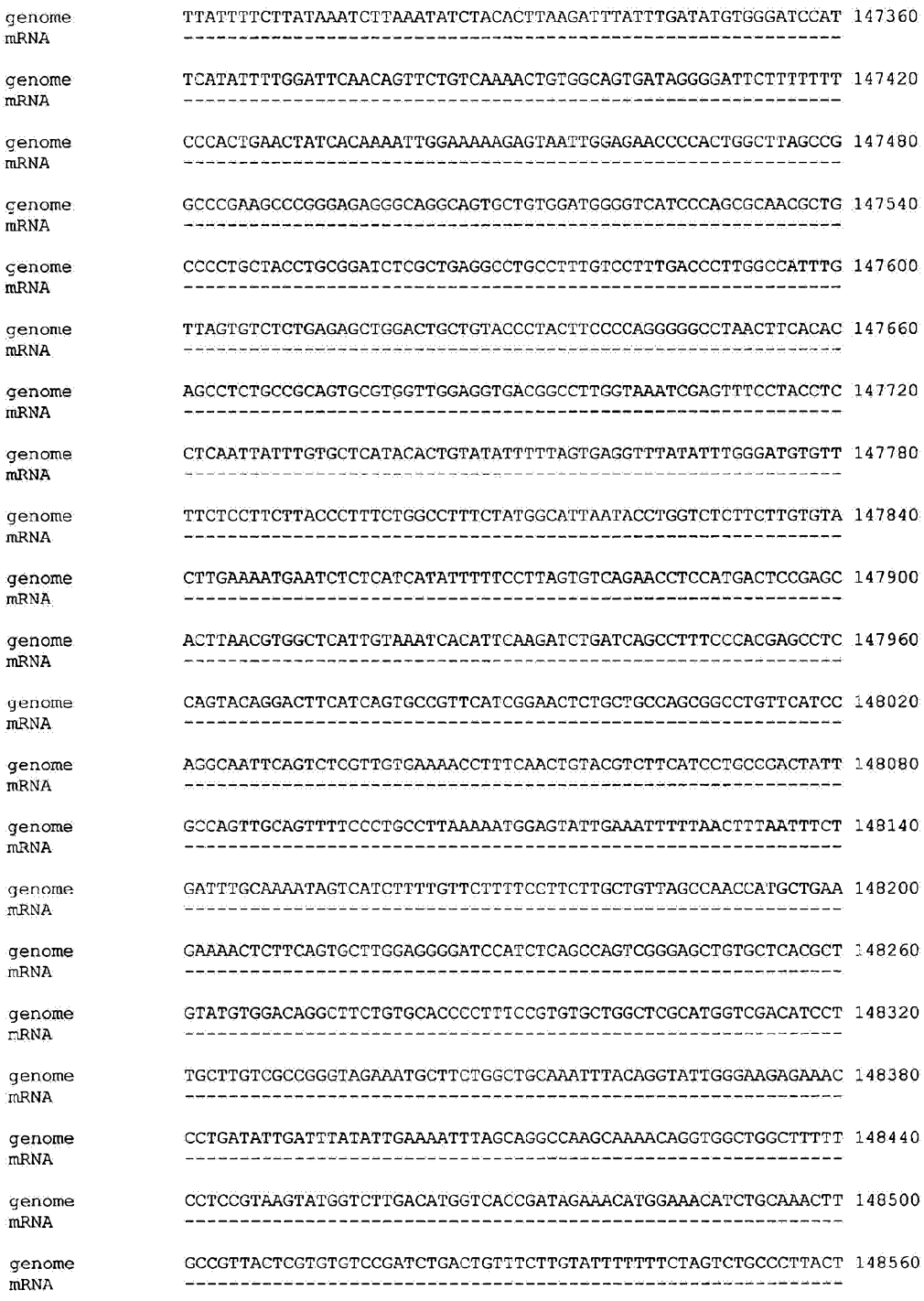
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Figure 1

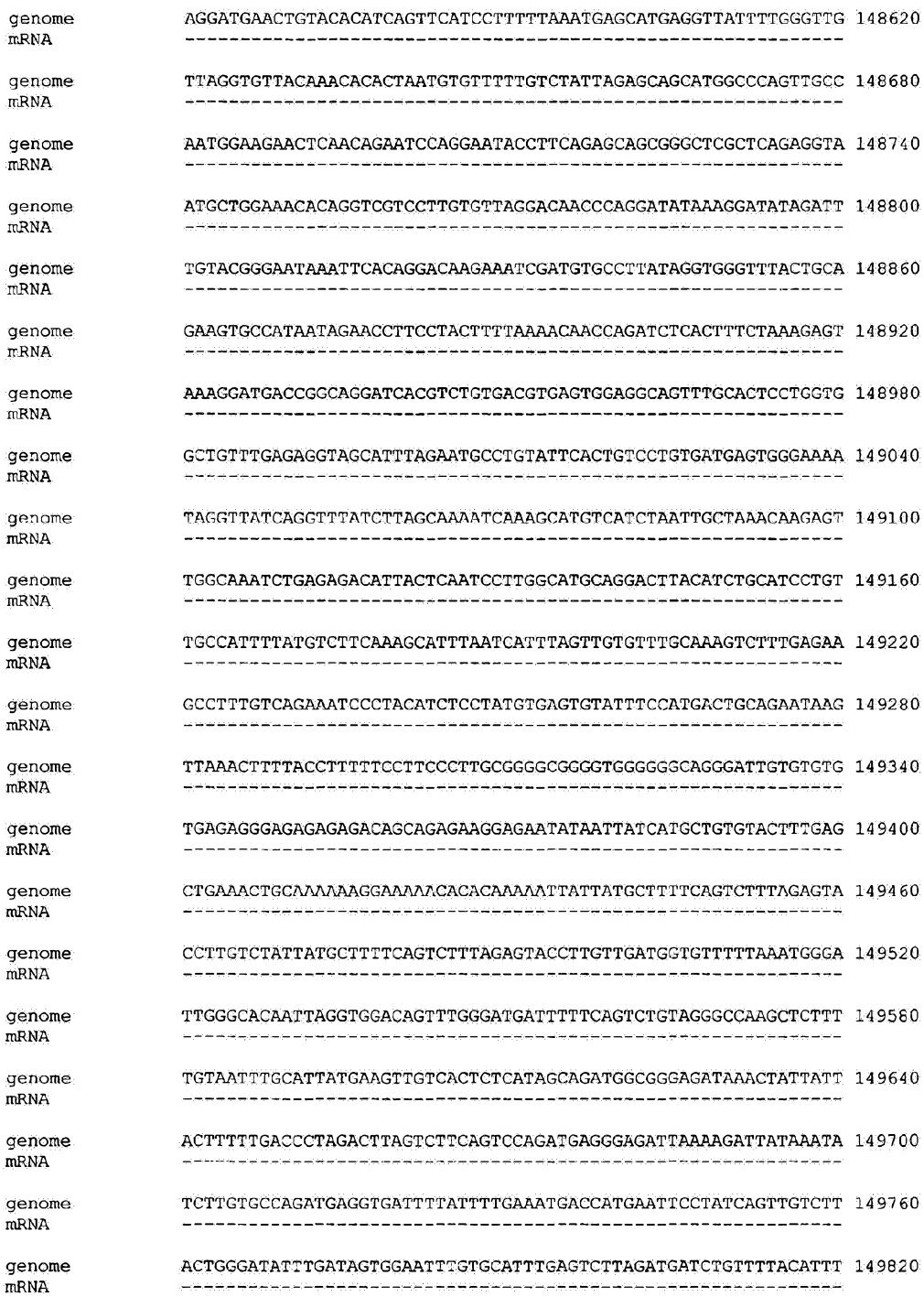
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Figure 1

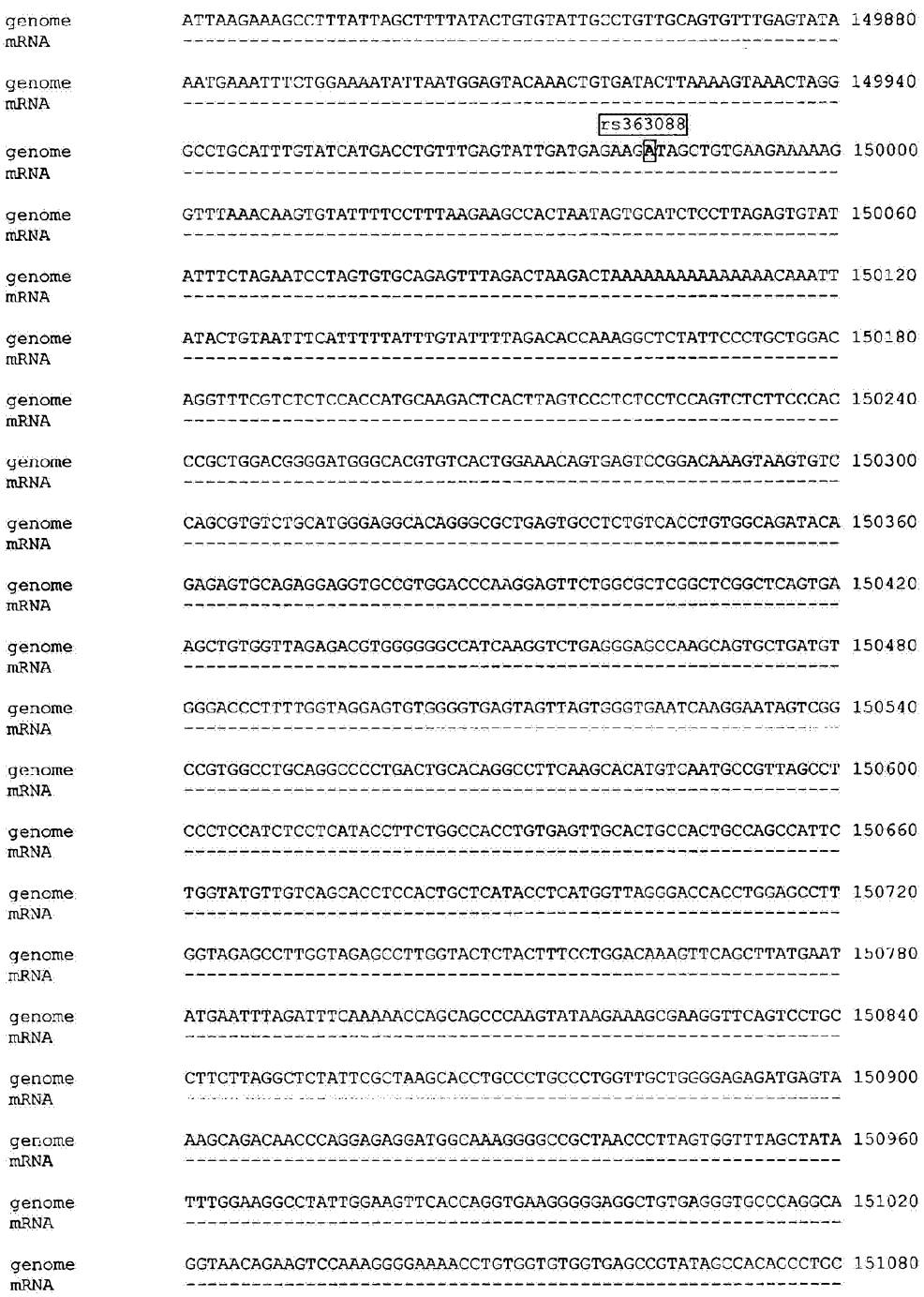
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Figure 1

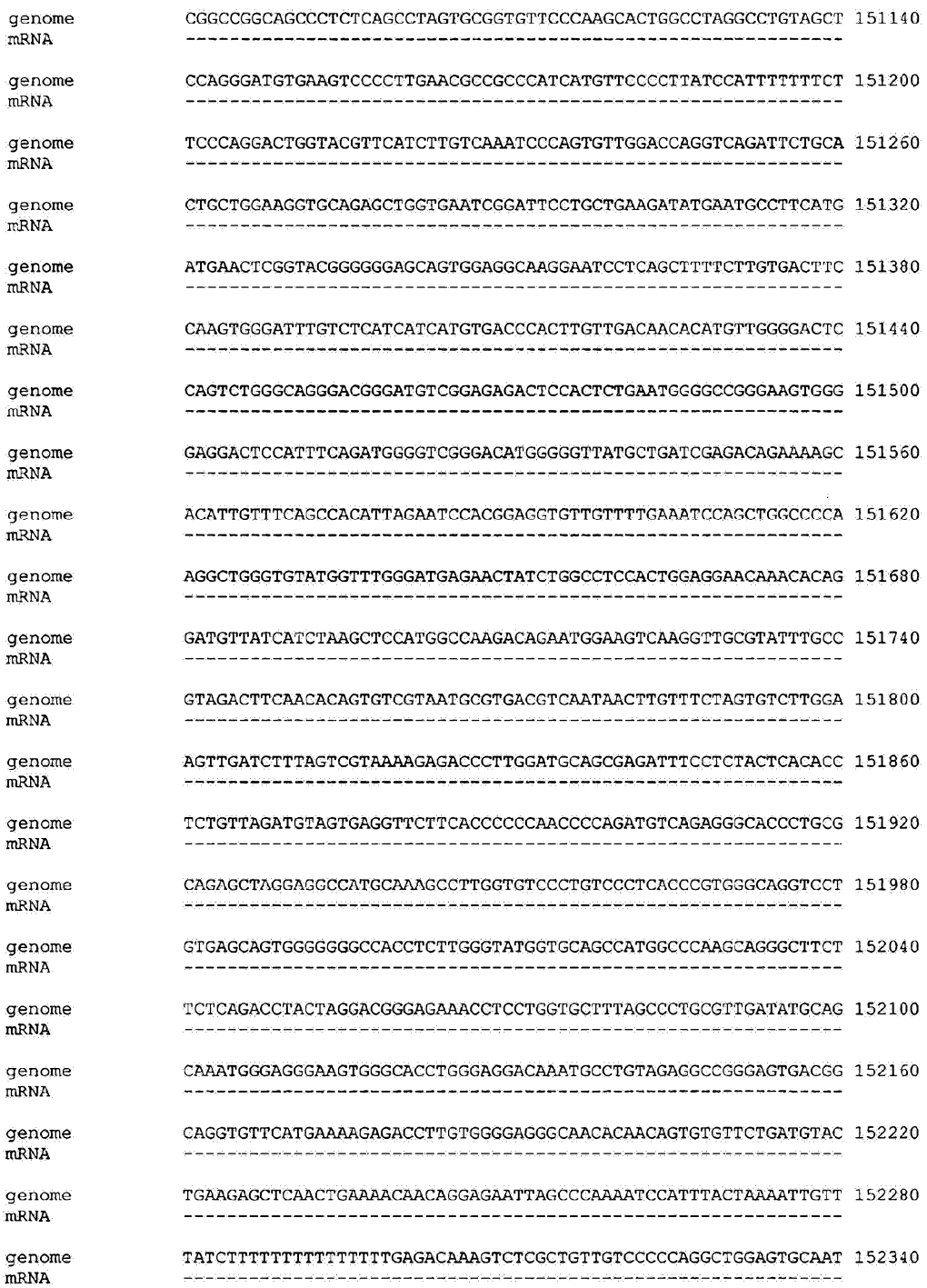
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Figure 1

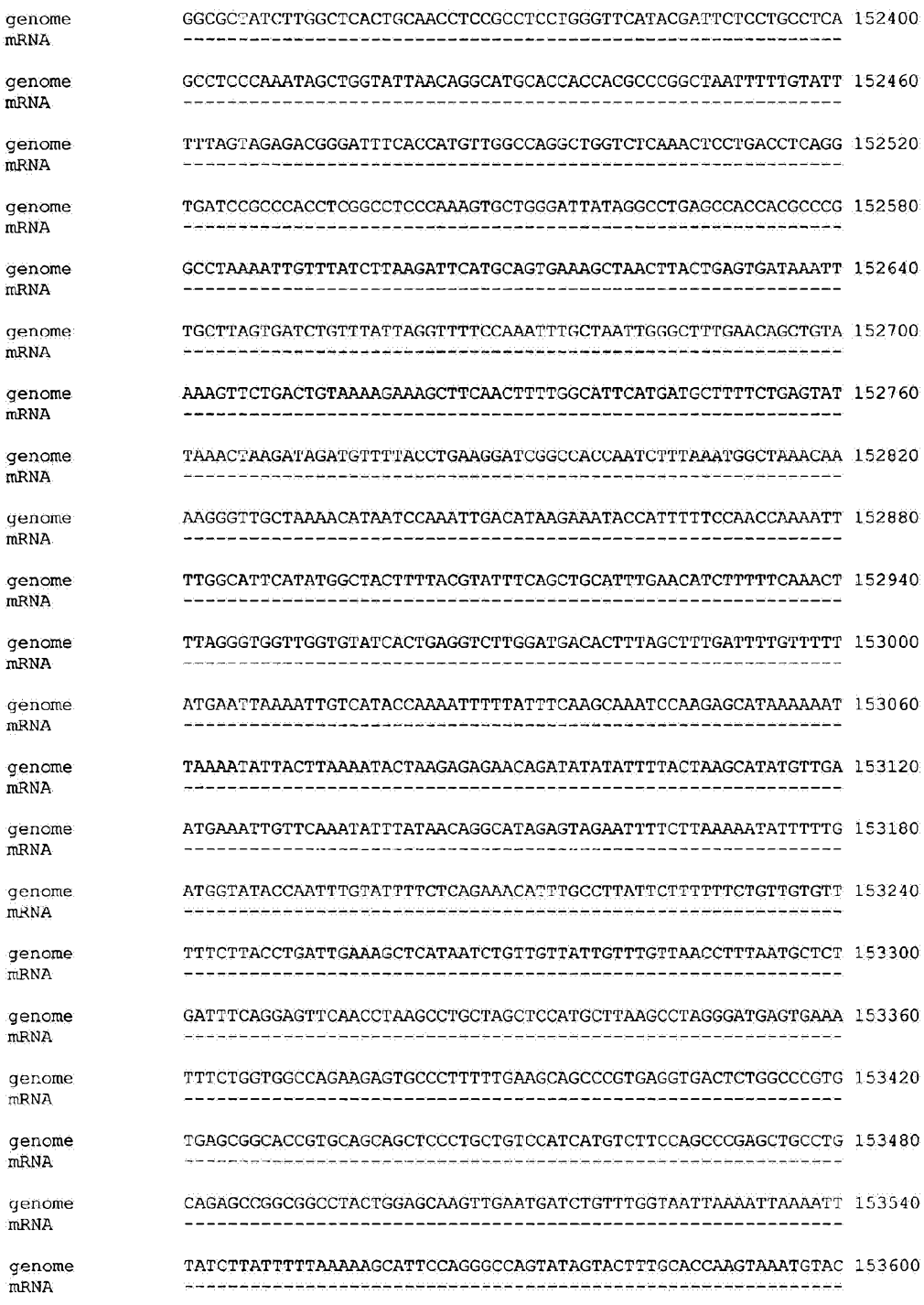
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Figure 1

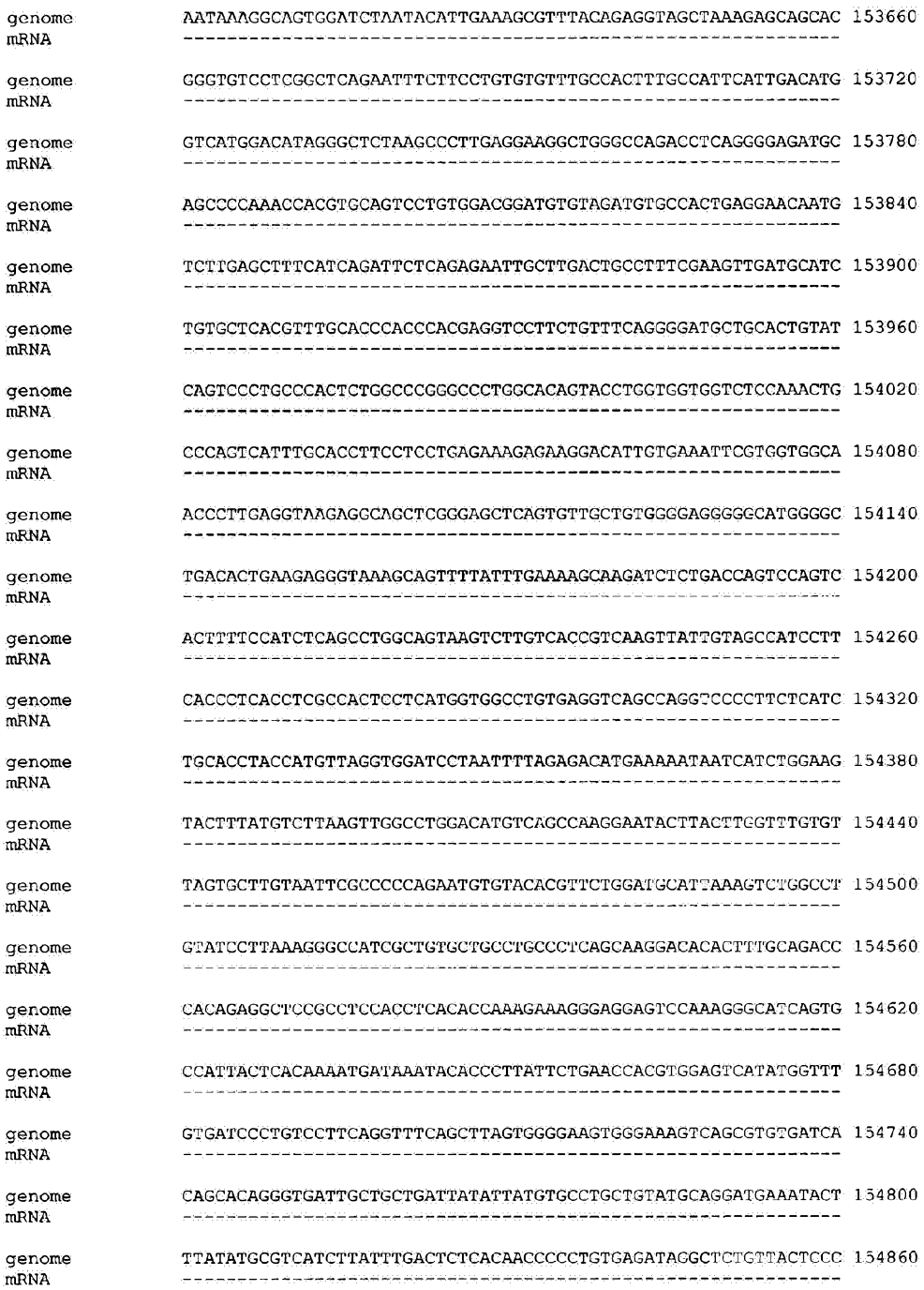
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Figure 1

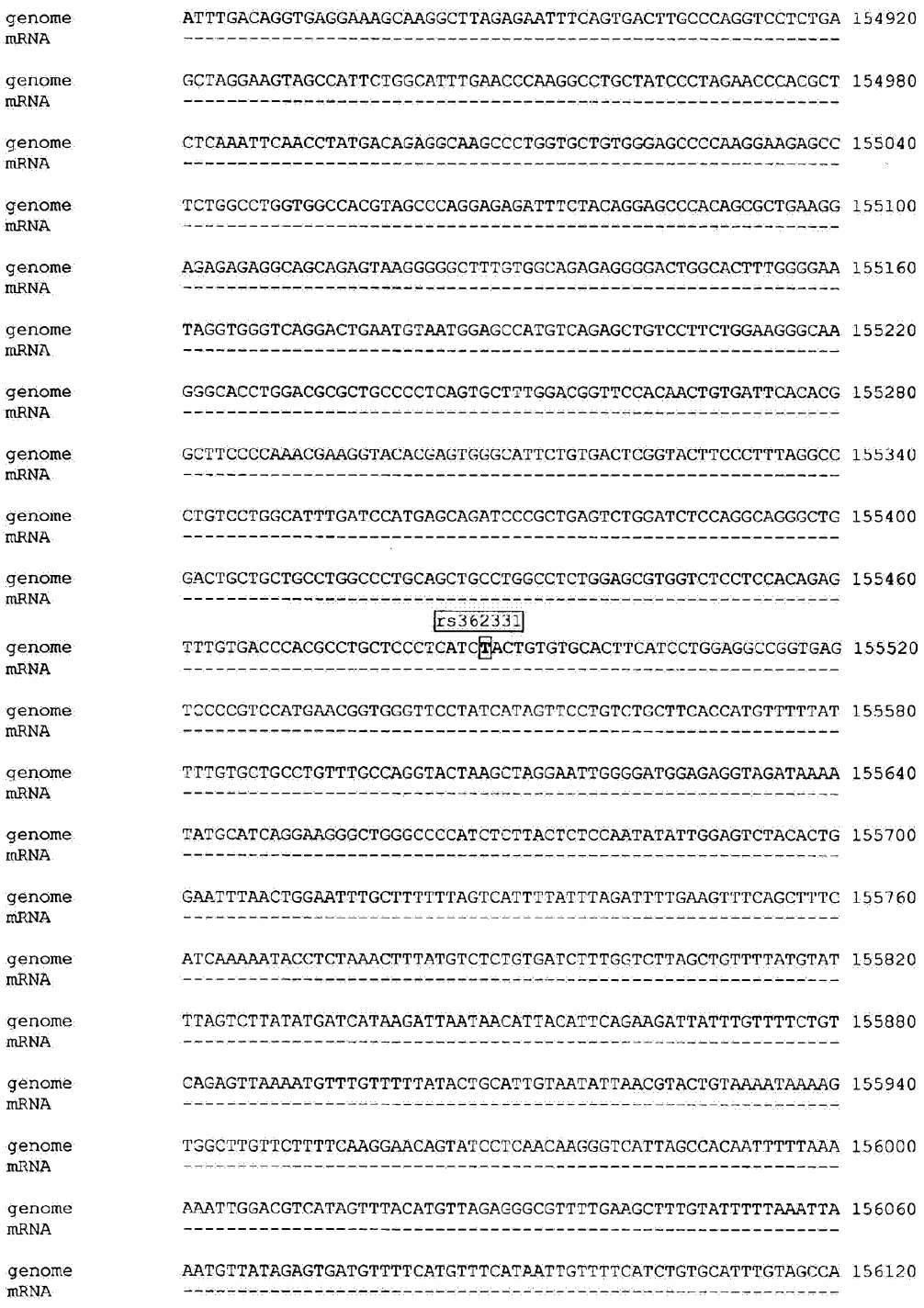
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Figure 1

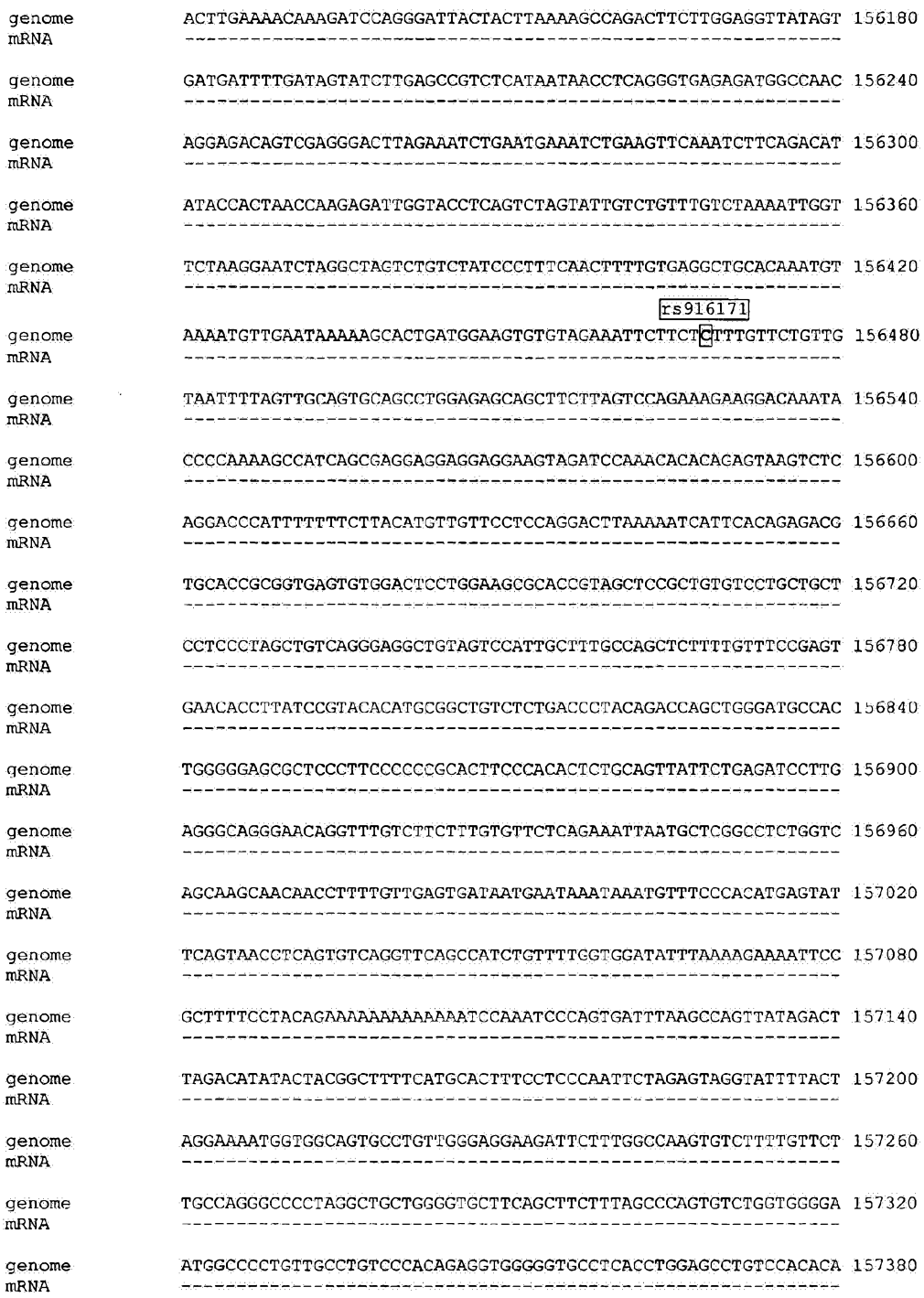
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Figure 1

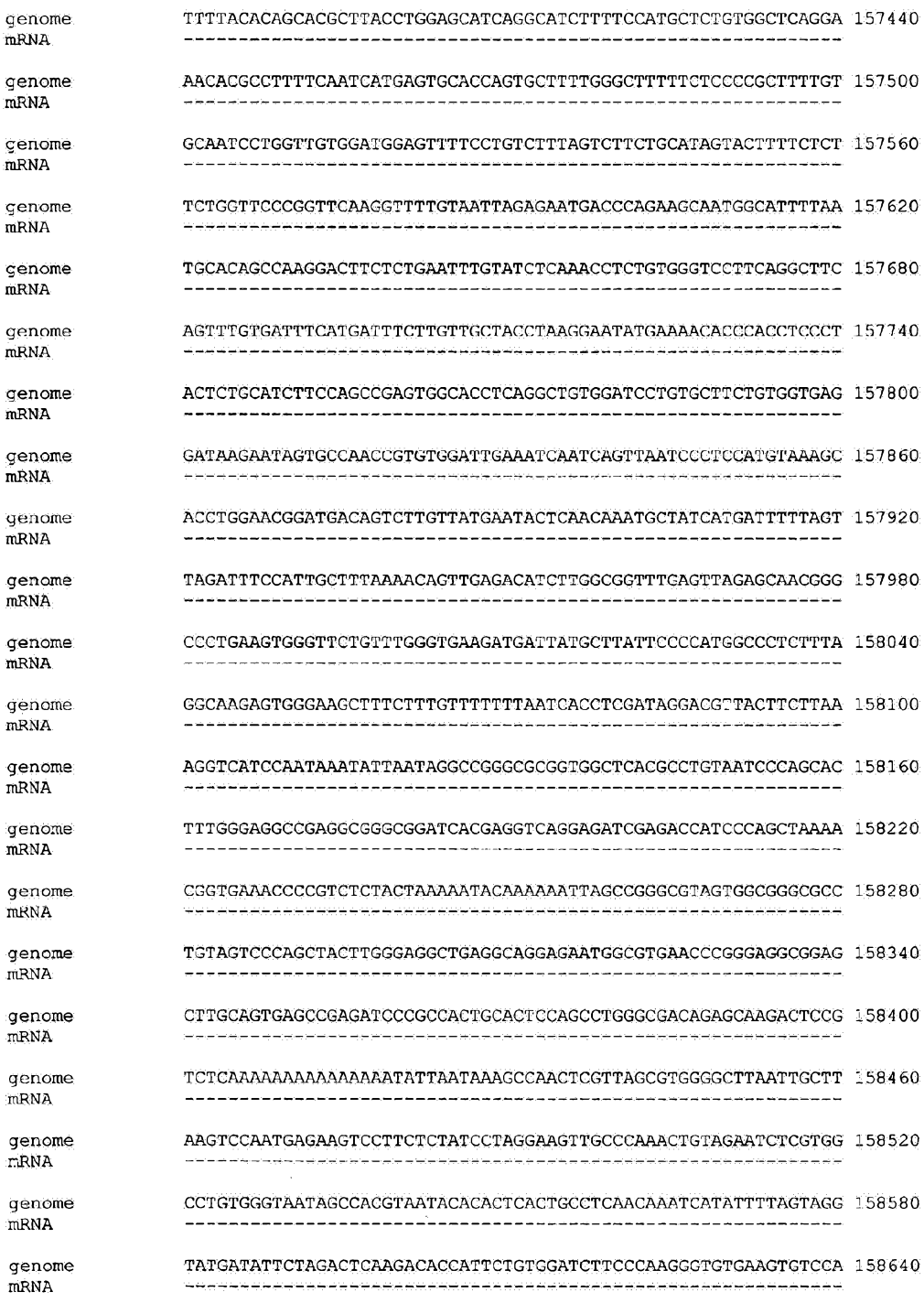
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Figure 1

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Figure 1

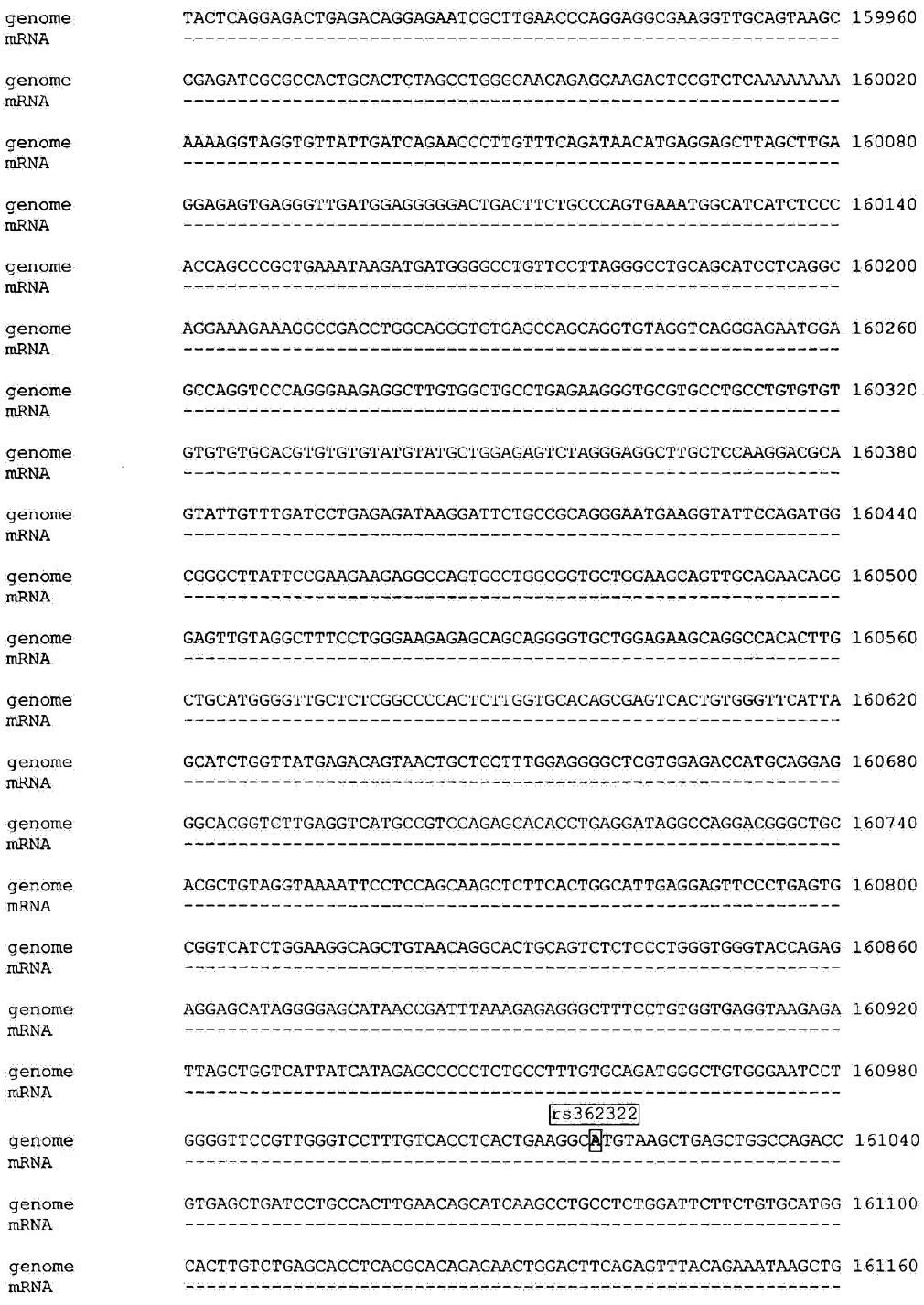
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Figure 1

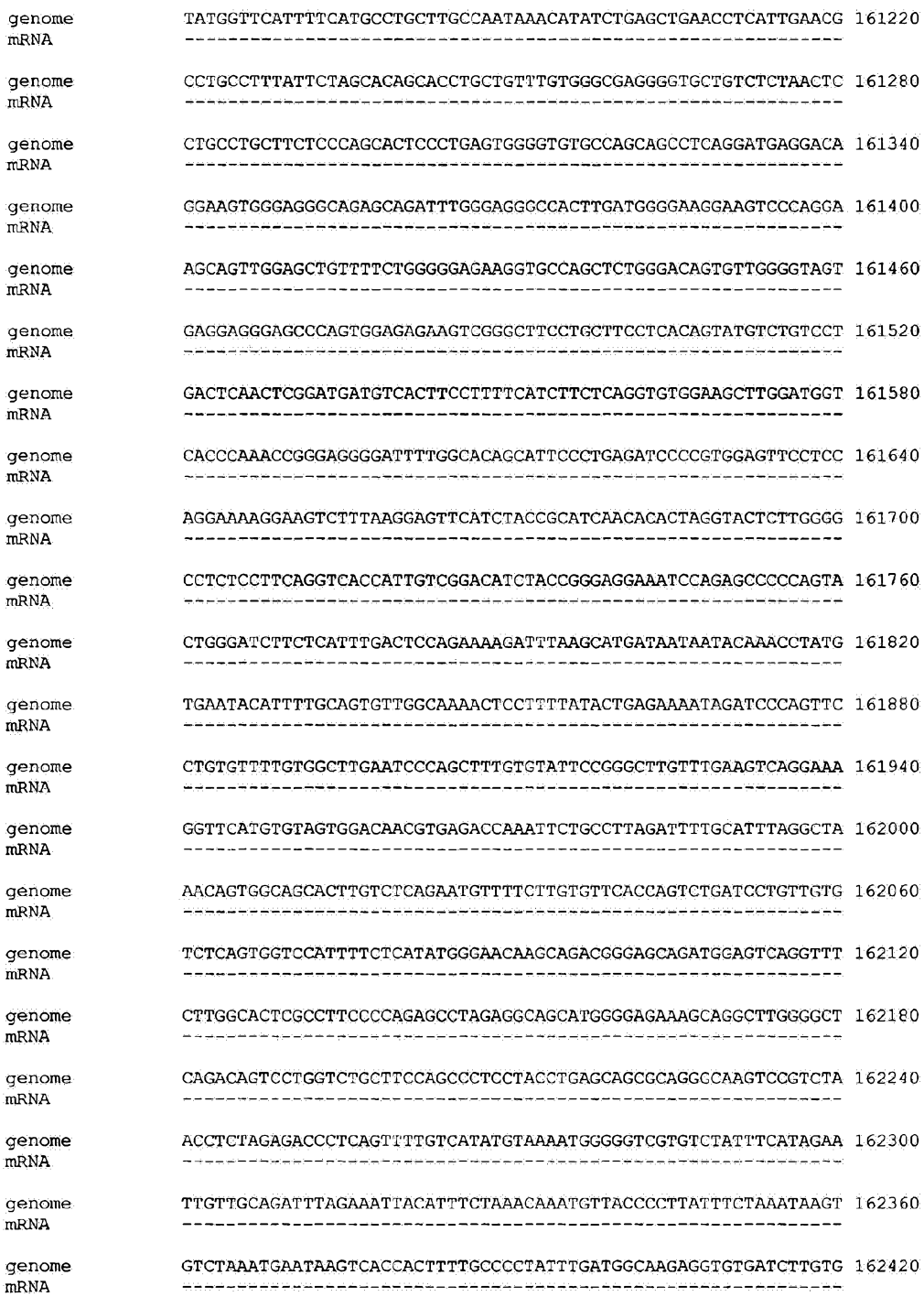
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Figure 1

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Figure 1

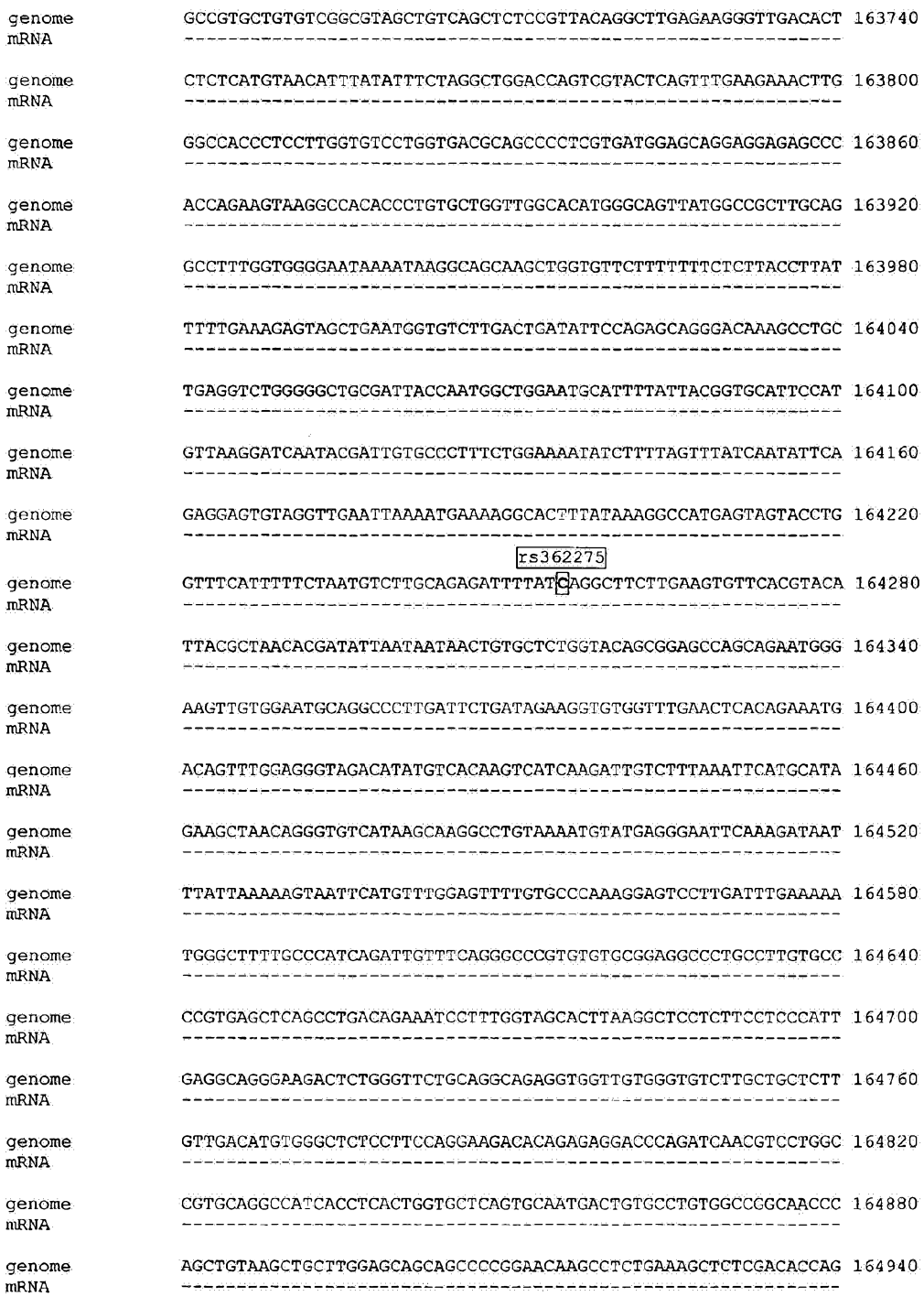
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Figure 1

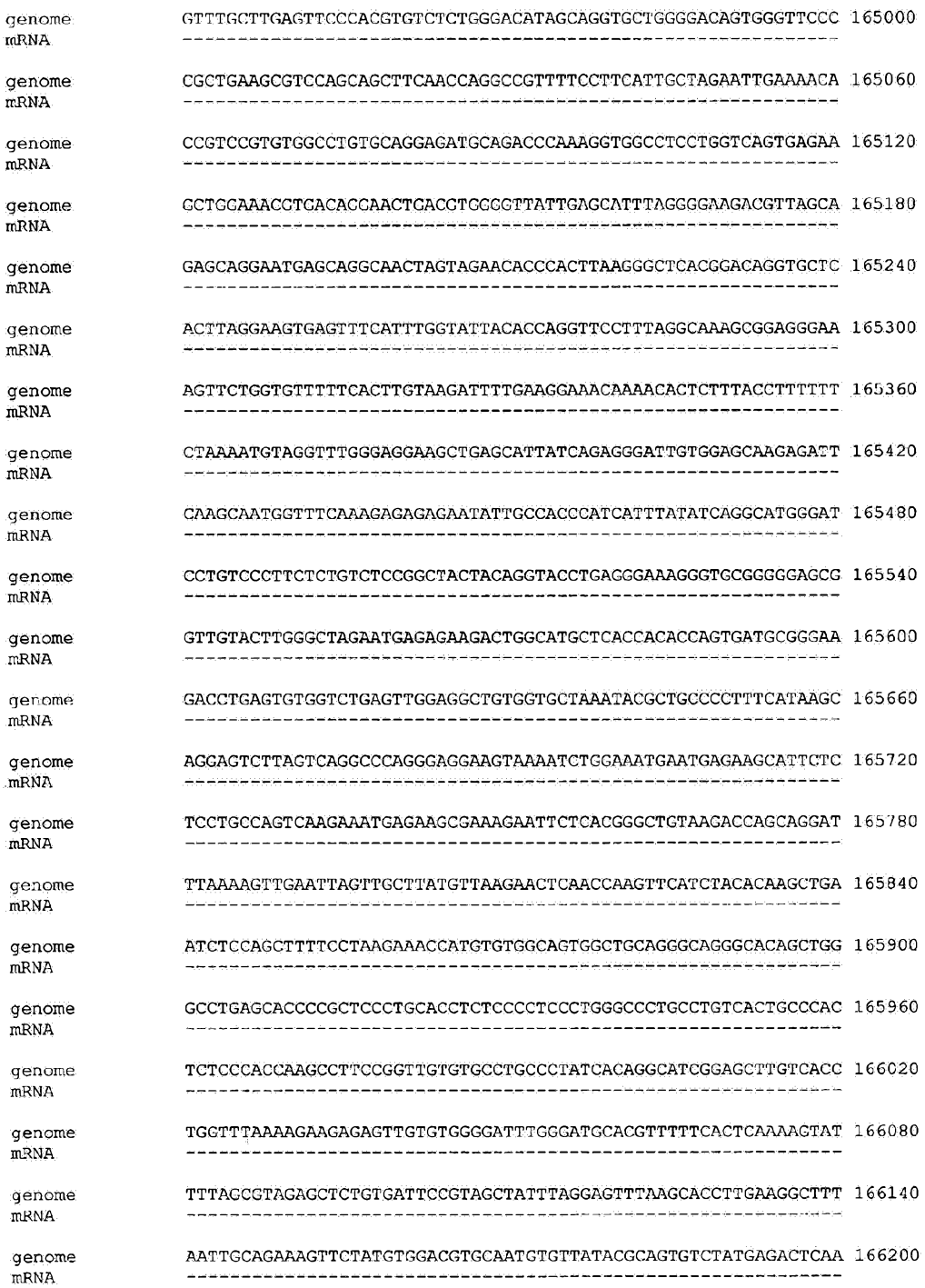
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Figure 1

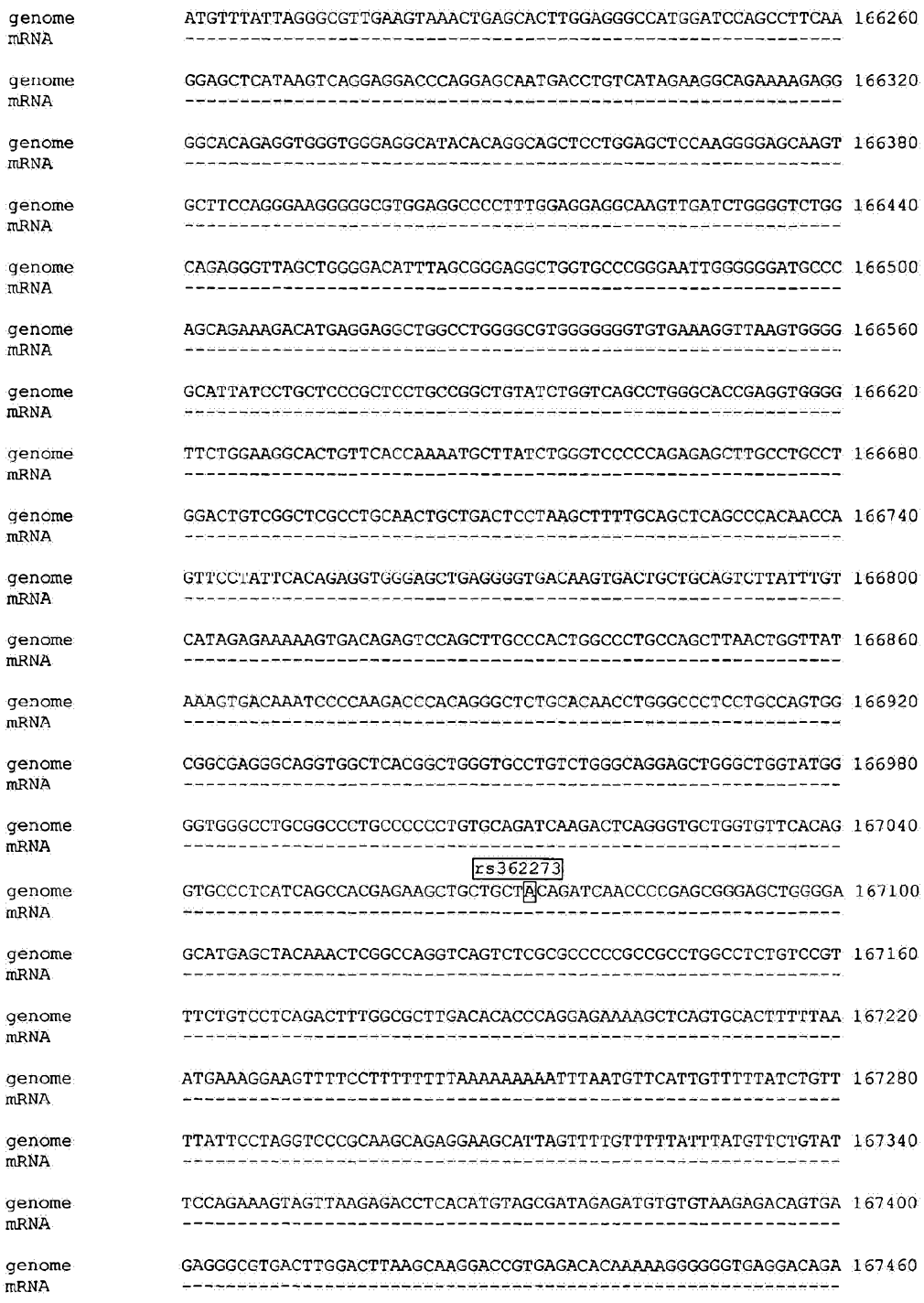
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Figure 1

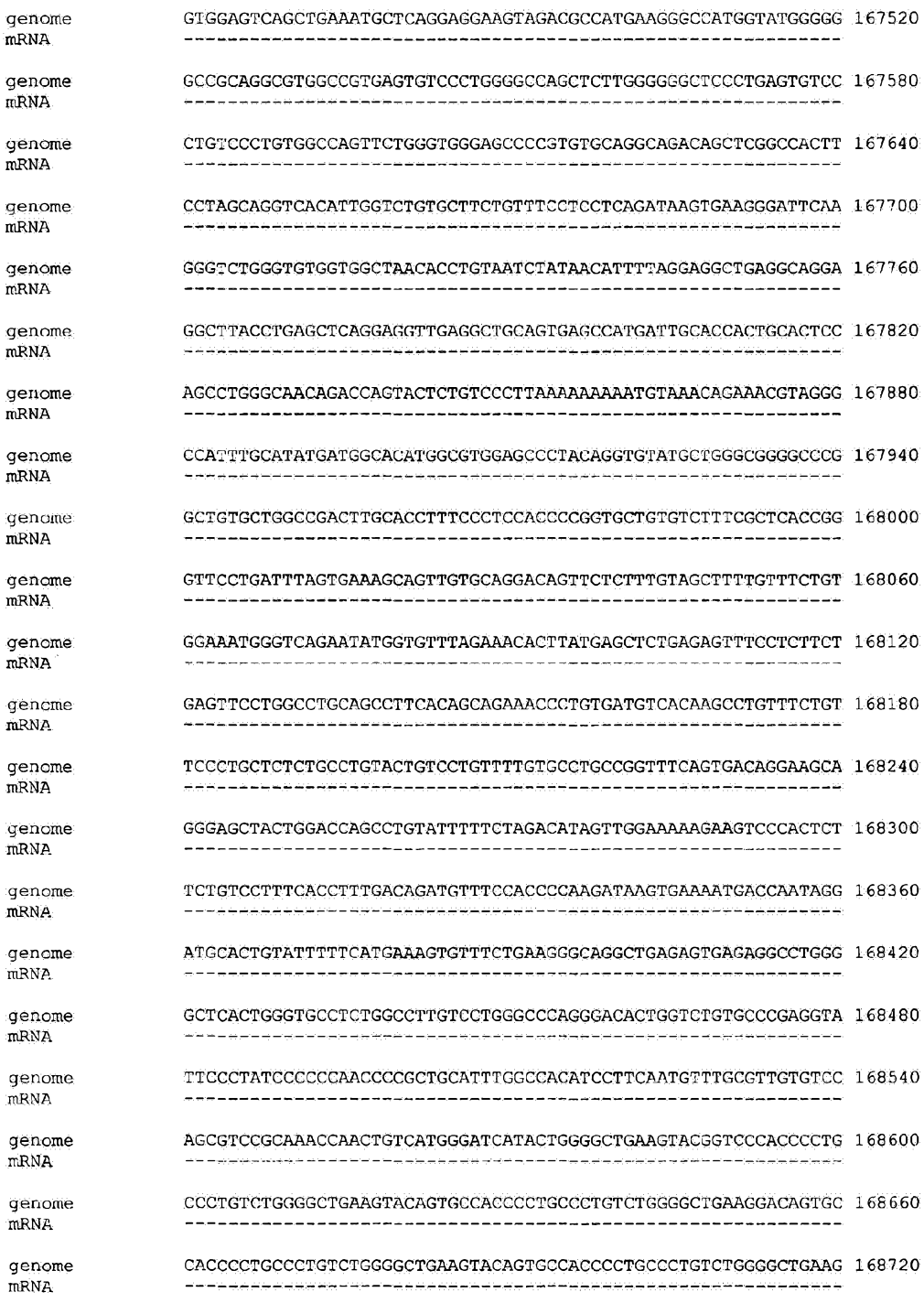
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Figure 1

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Figure 1

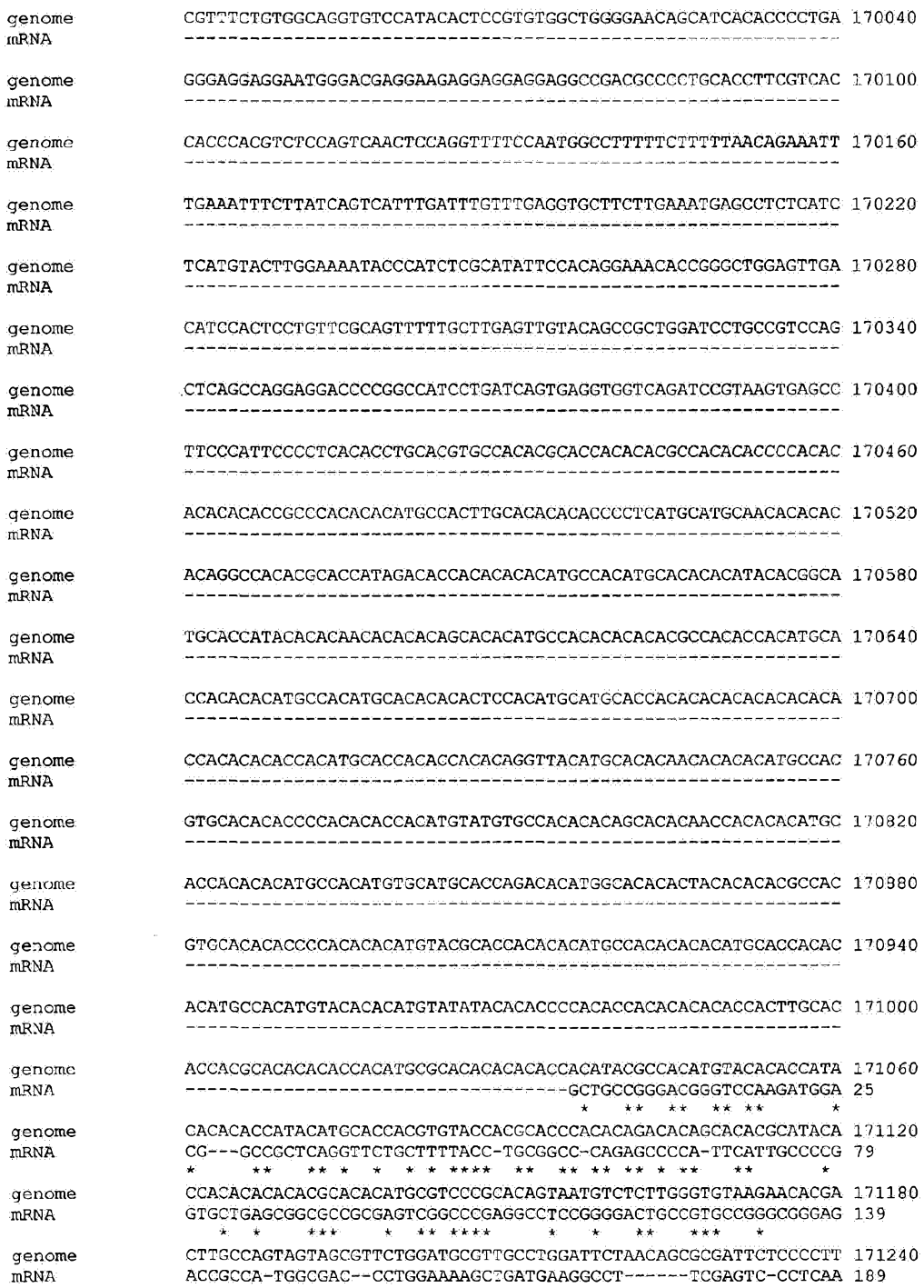
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Figure 1

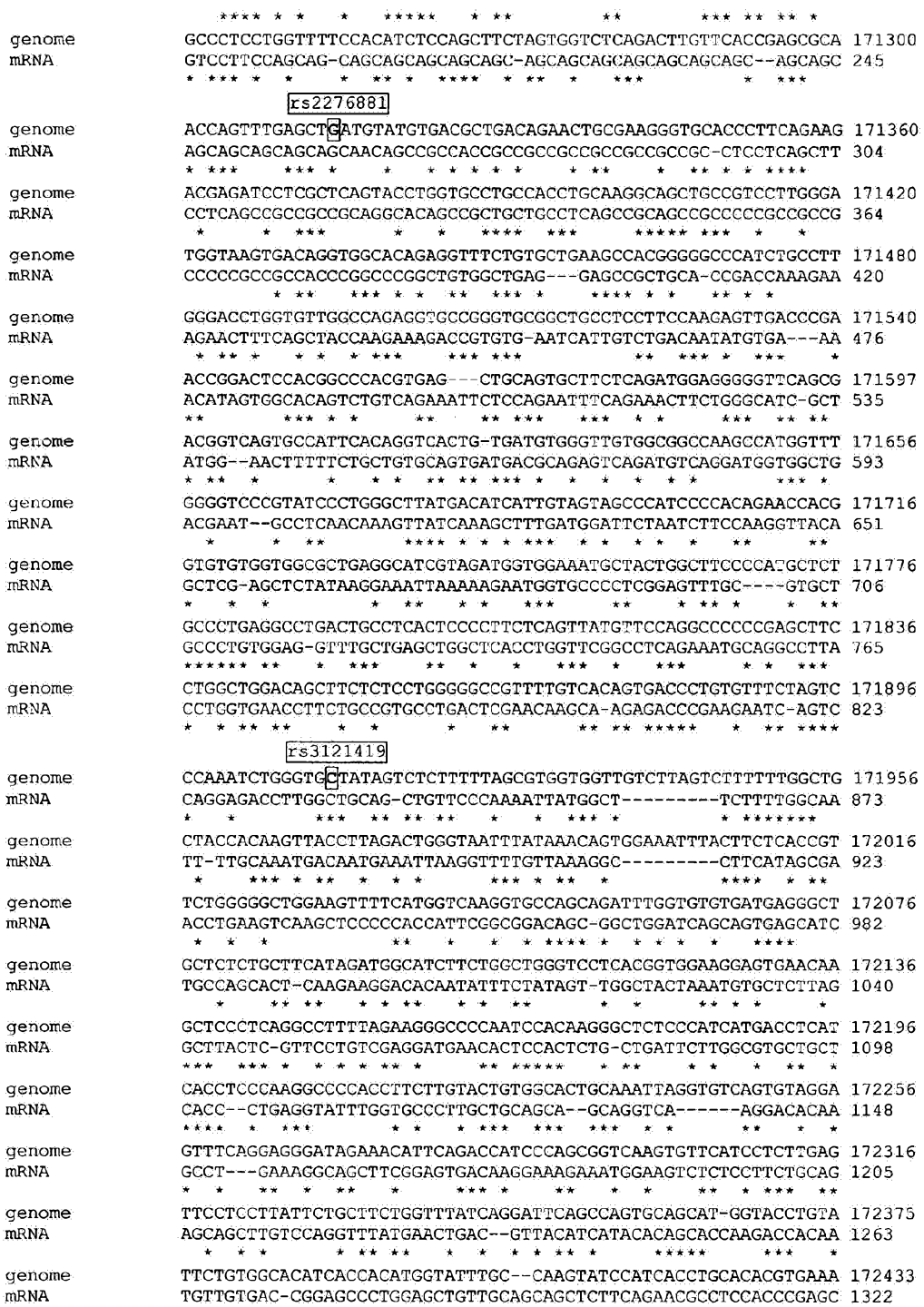
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Figure 1

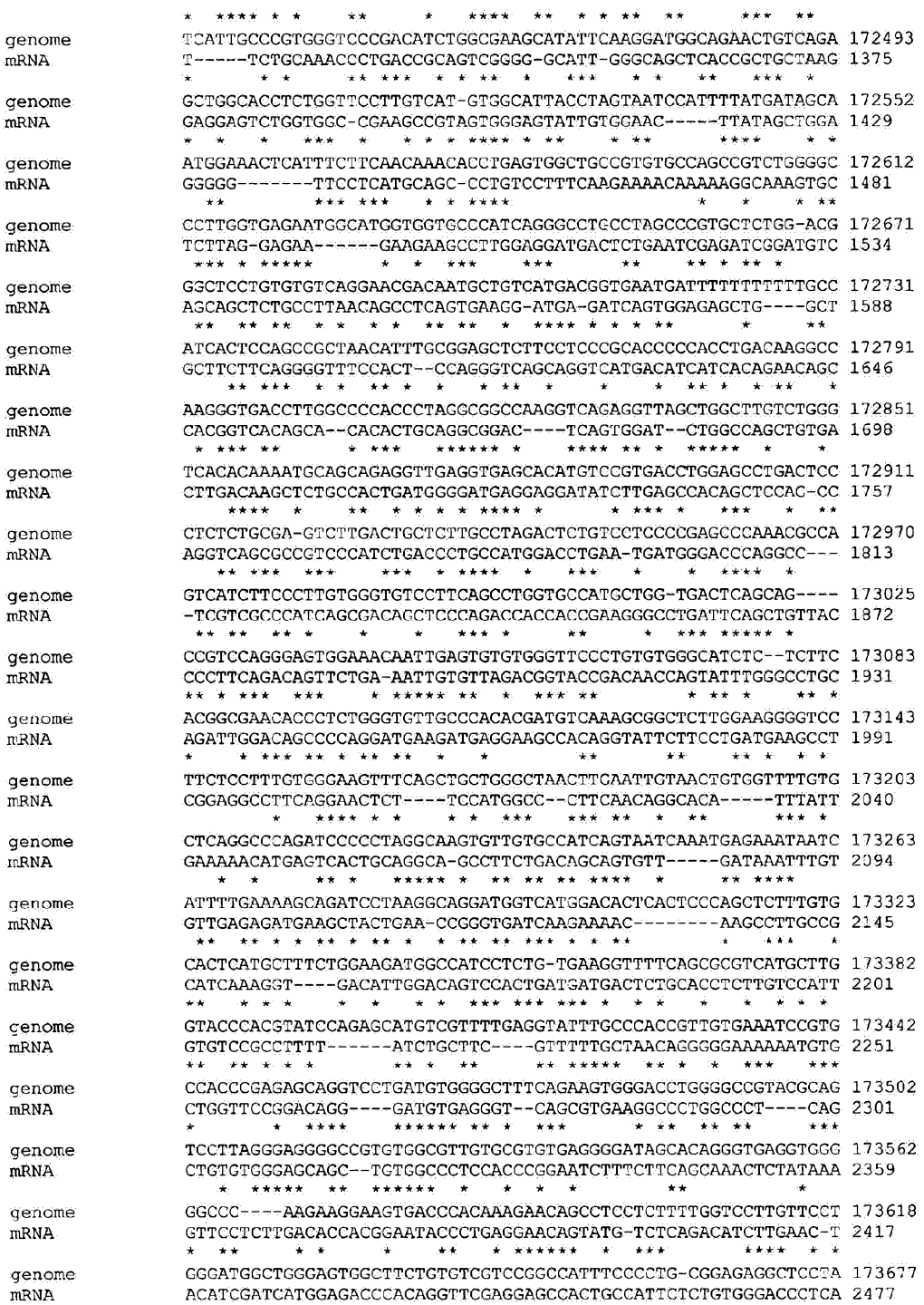
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Figure 1

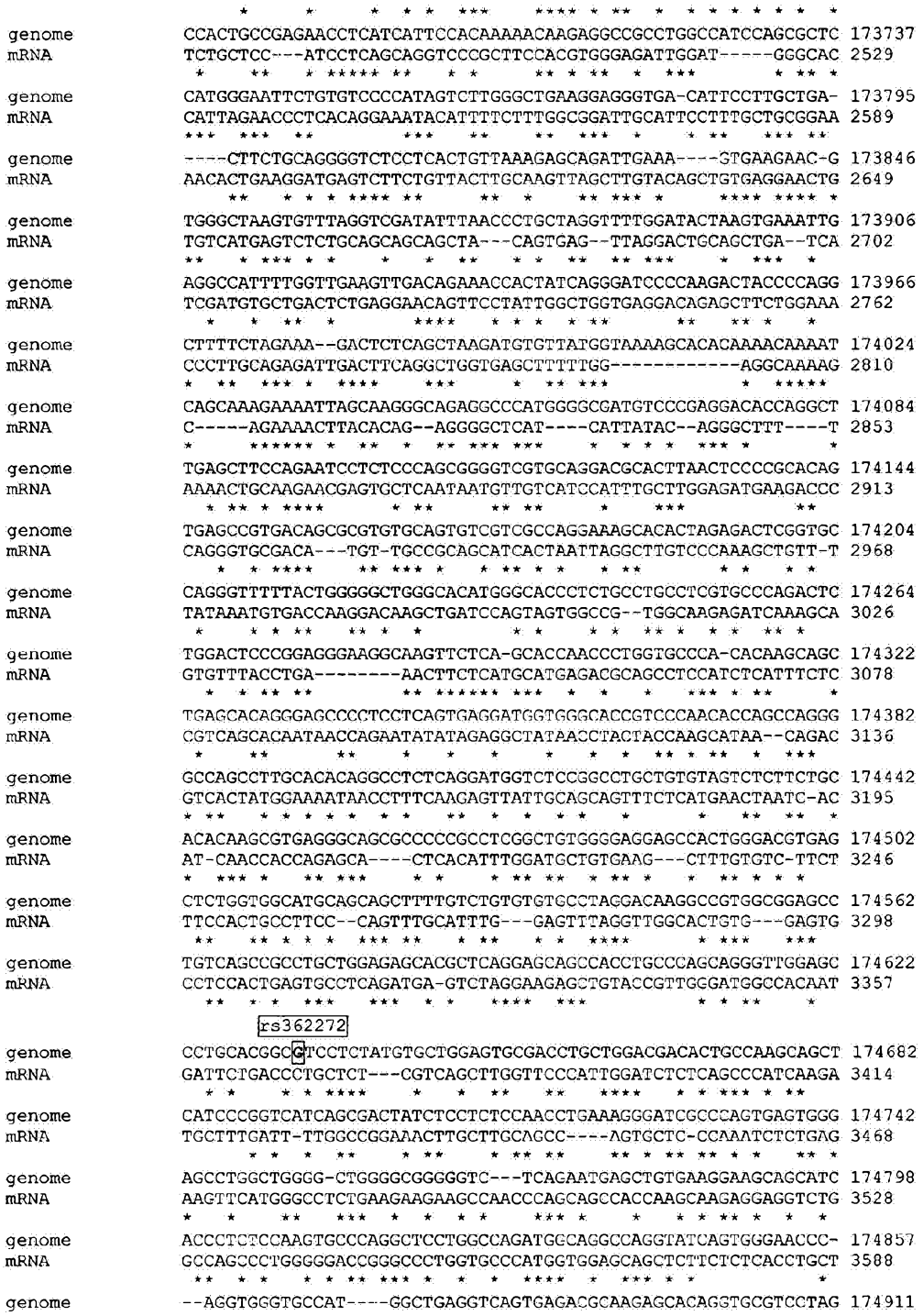
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Figure 1

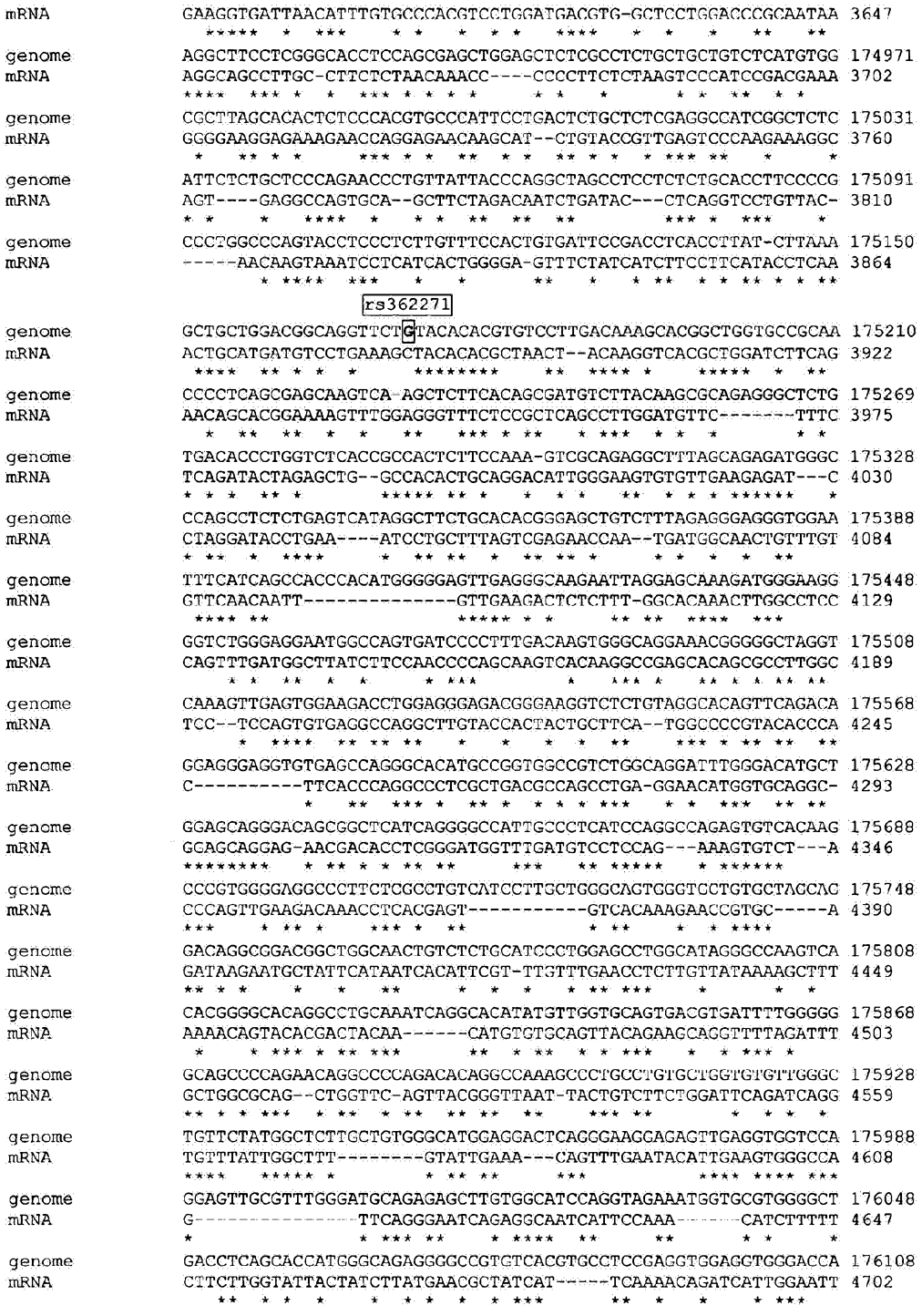
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Figure 1

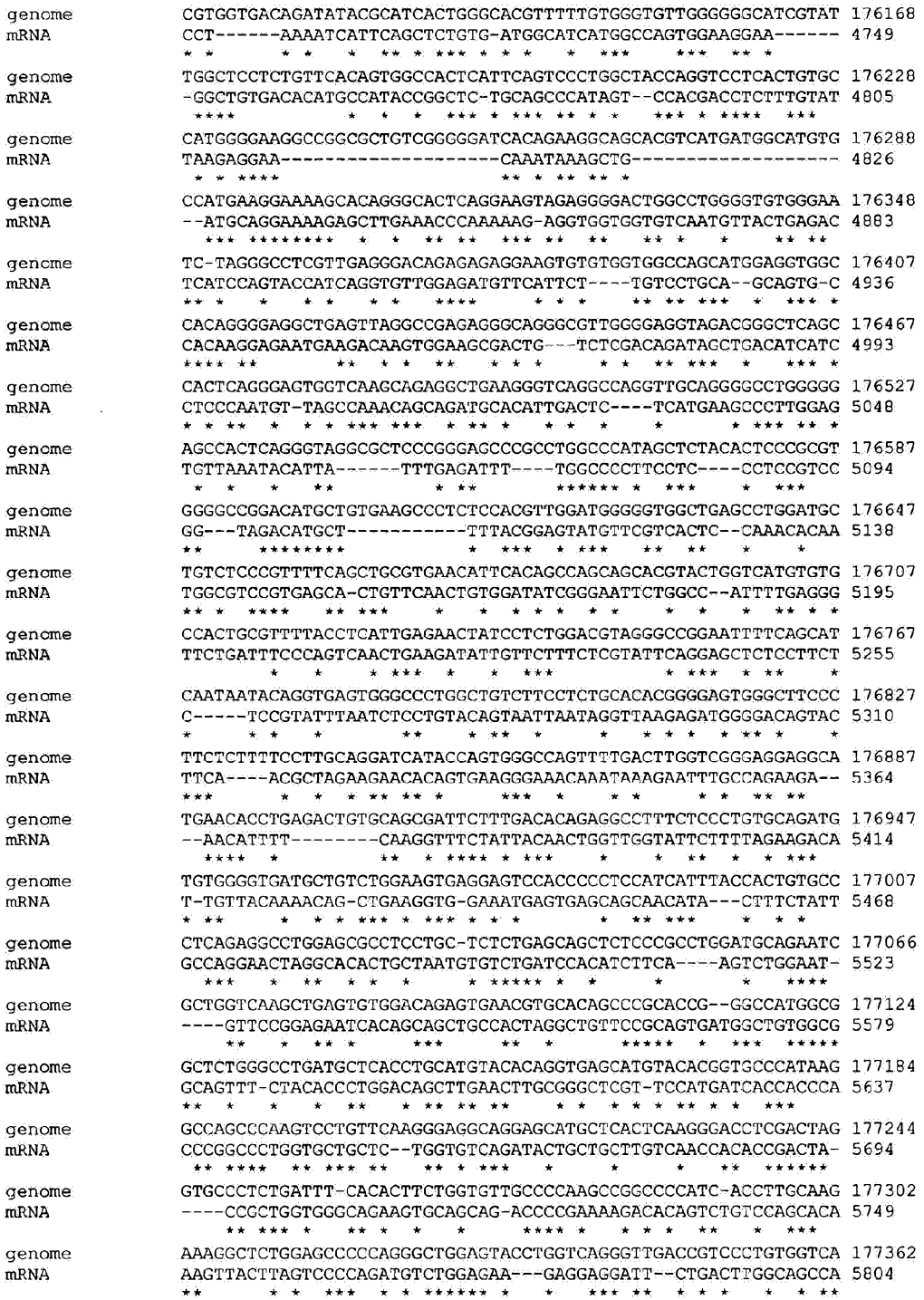
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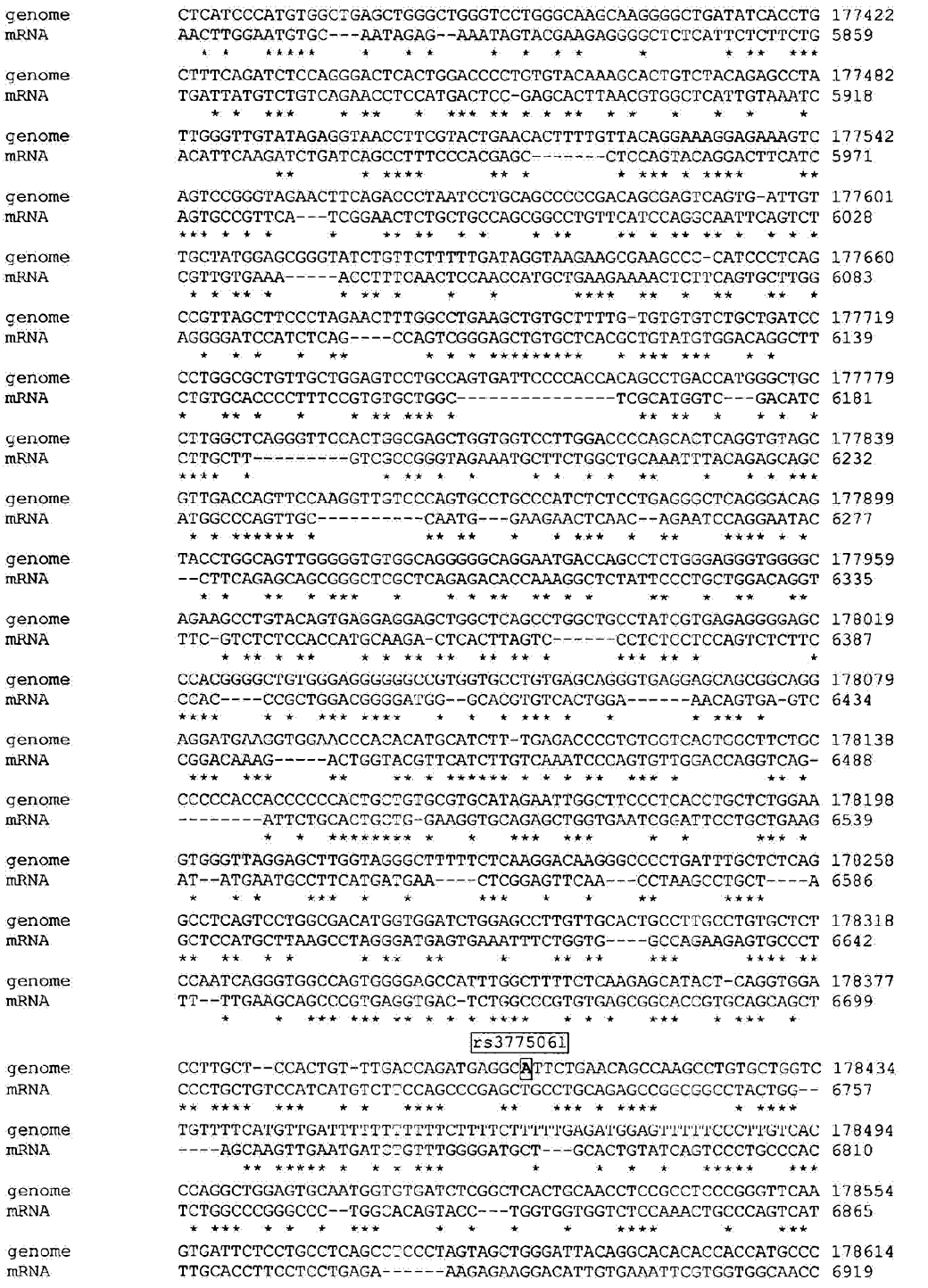
Figure 1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genome GTGATTCTCCTGCCTCAGCCTCCCTAGTAGCTGGGATTACAGGCACACACCACCATGCCC 178614
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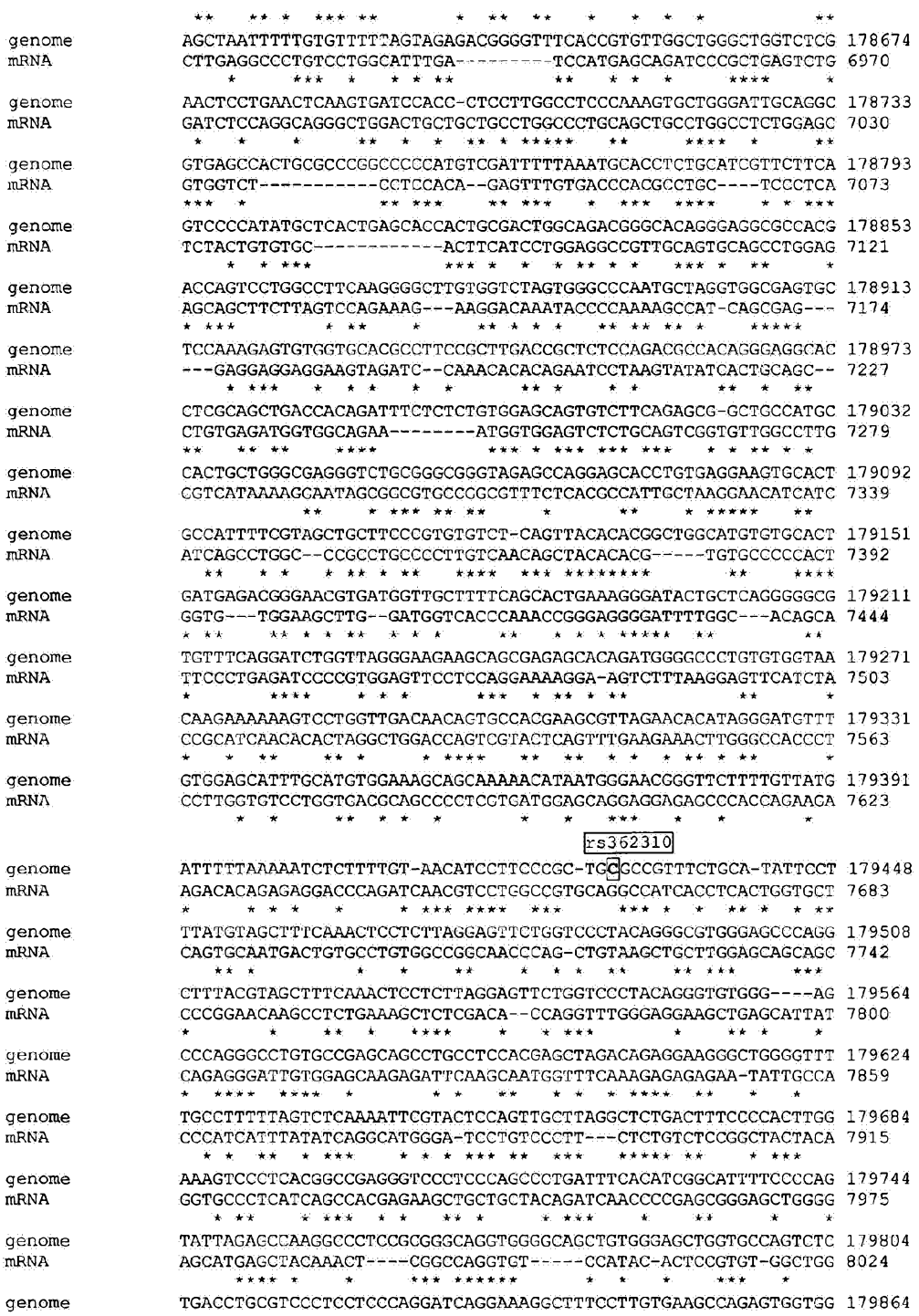
Figure 1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genome TCCAAAGAGTGTGGTGCACGCCTTCCGCTTGACCGCTCTCCAGACGCCACAGGGAGGCAC 178973
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Figure 1

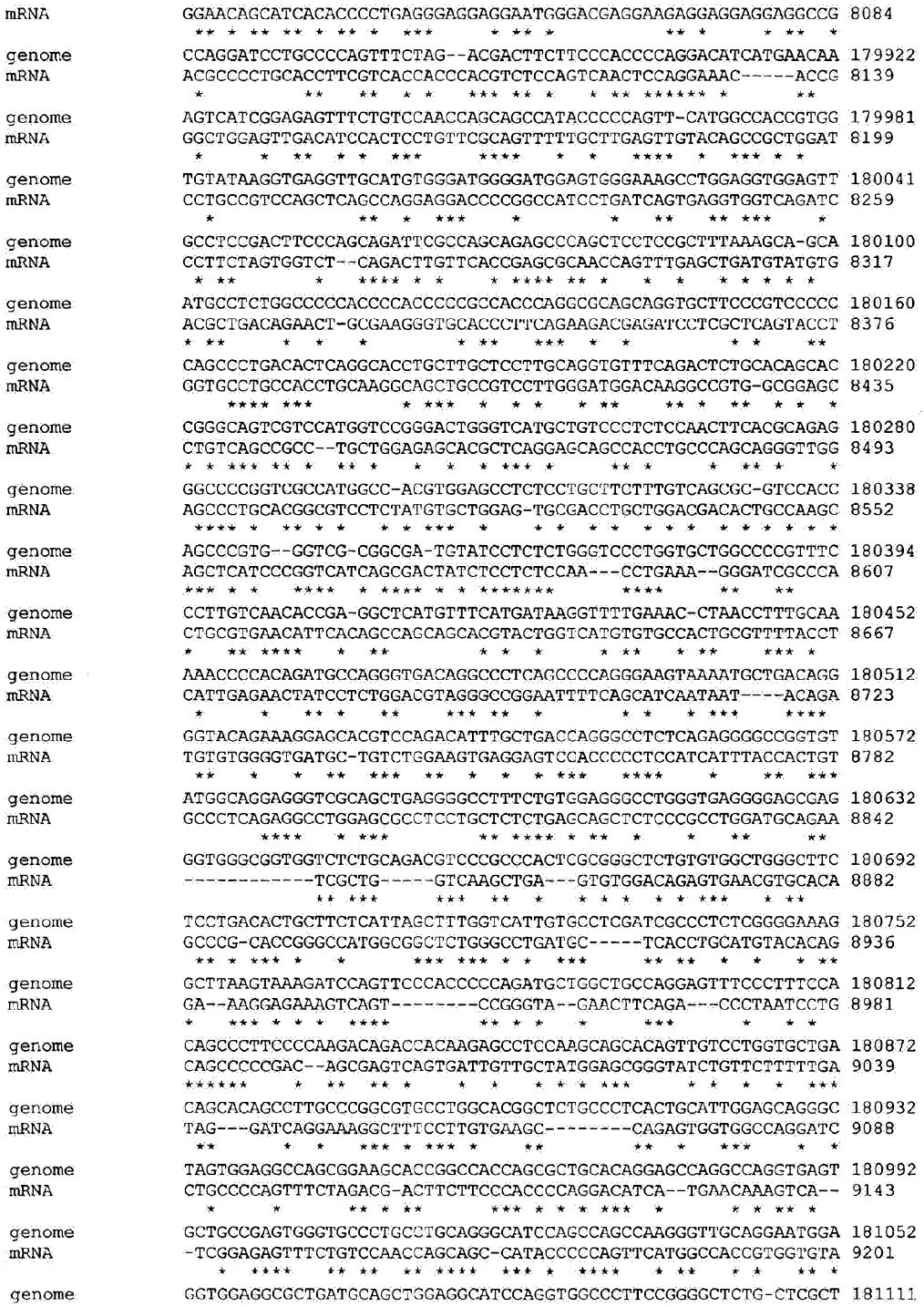
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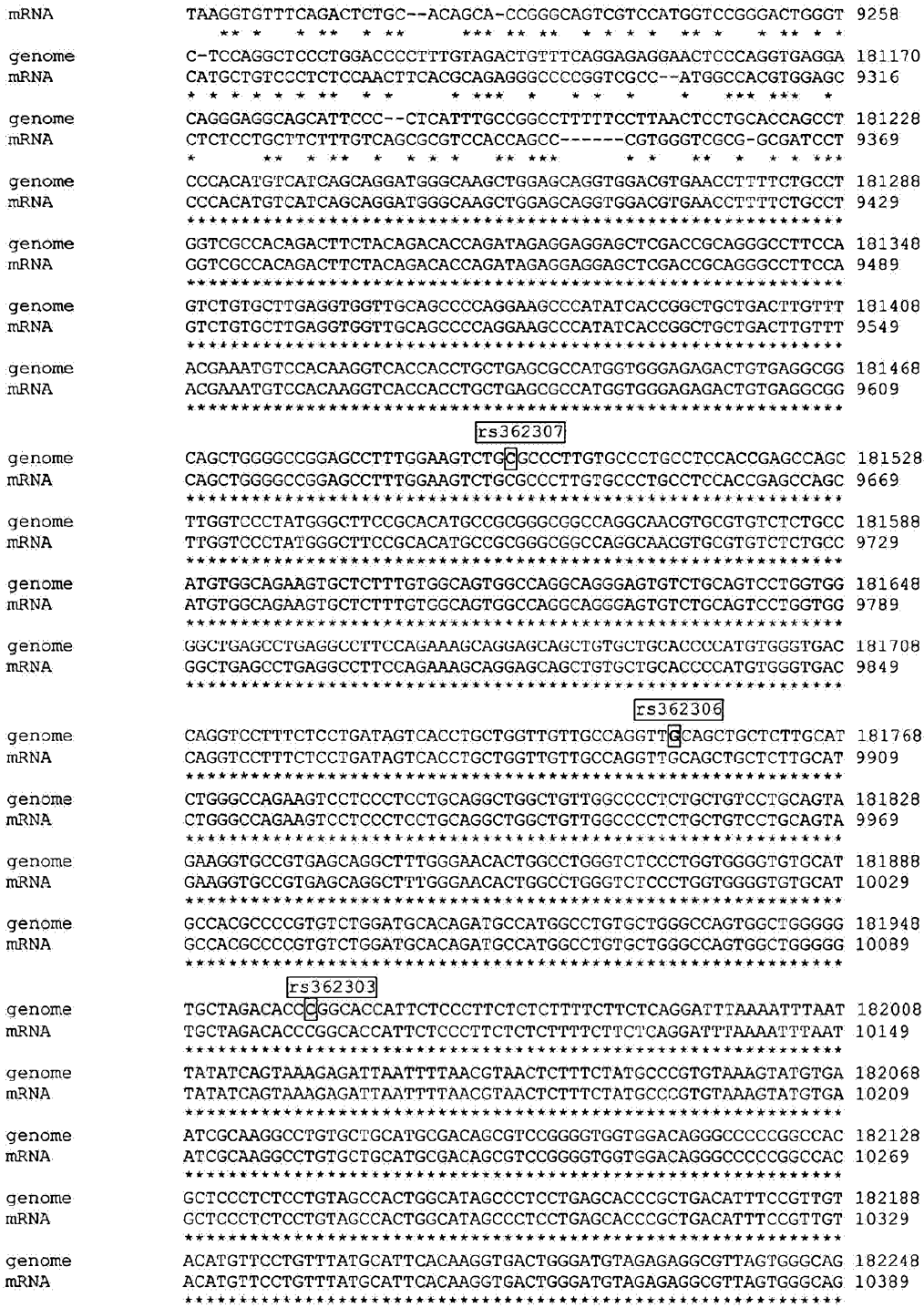
Figure 1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genome ACATGTTCCTGTTTATGCATTCACAAGGTGACTGGGATGTAGAGAGGCGTTAGTGGGCAG 182248
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Figure 1

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mRNA    GTGGCCACAGCAGGACTGAGGACAGGCCCCCCATTATCCTAGGGGTGCGCTCACCTGCAGC 10449
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genome  CCTCCCCCGCAGGTTATGTCAGCAGCTCTGAGACAGCAGTATCACAGGCCAGATGTTGTT 183508
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Figure 1

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Figure 1

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Figure 1

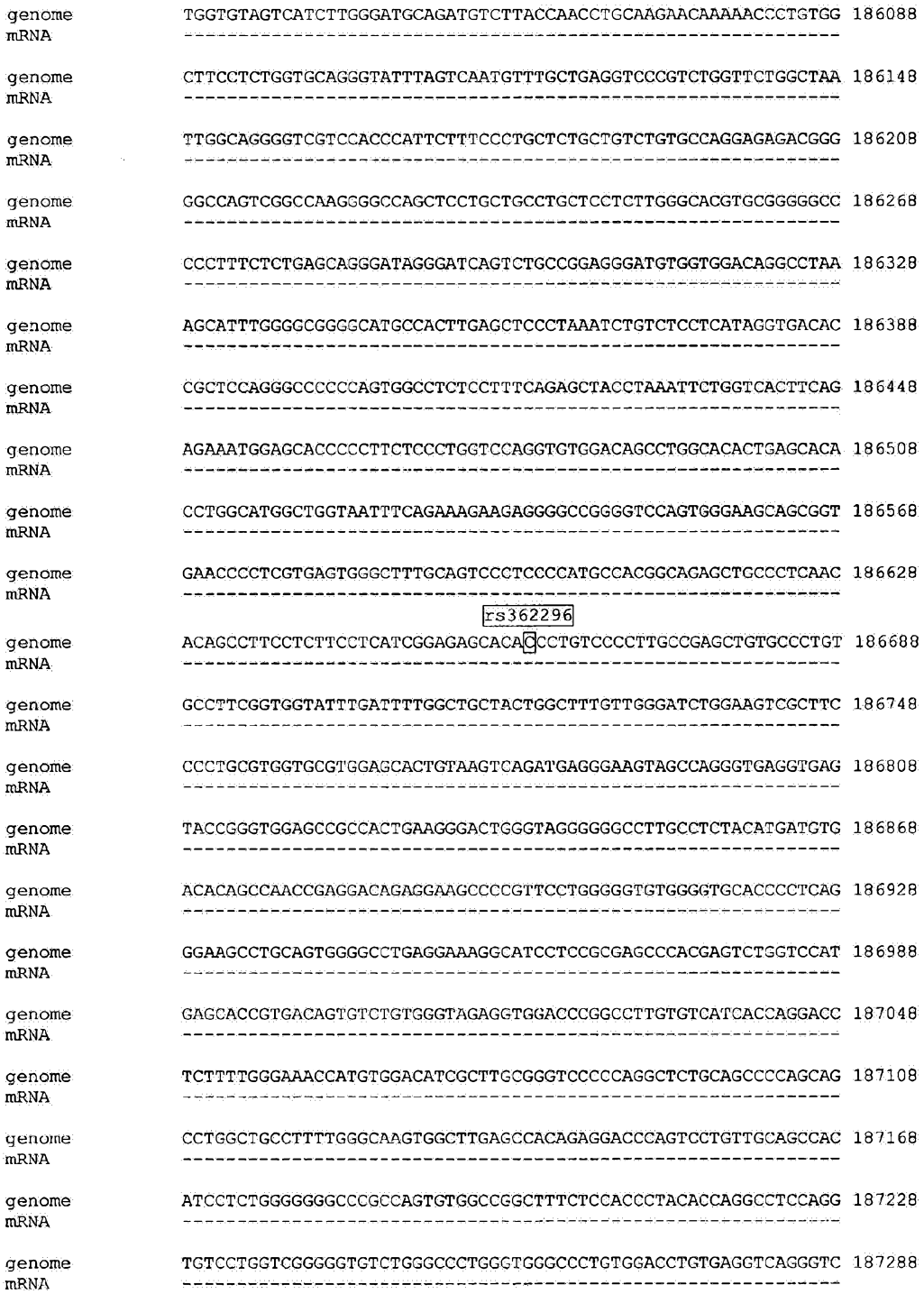
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Figure 1

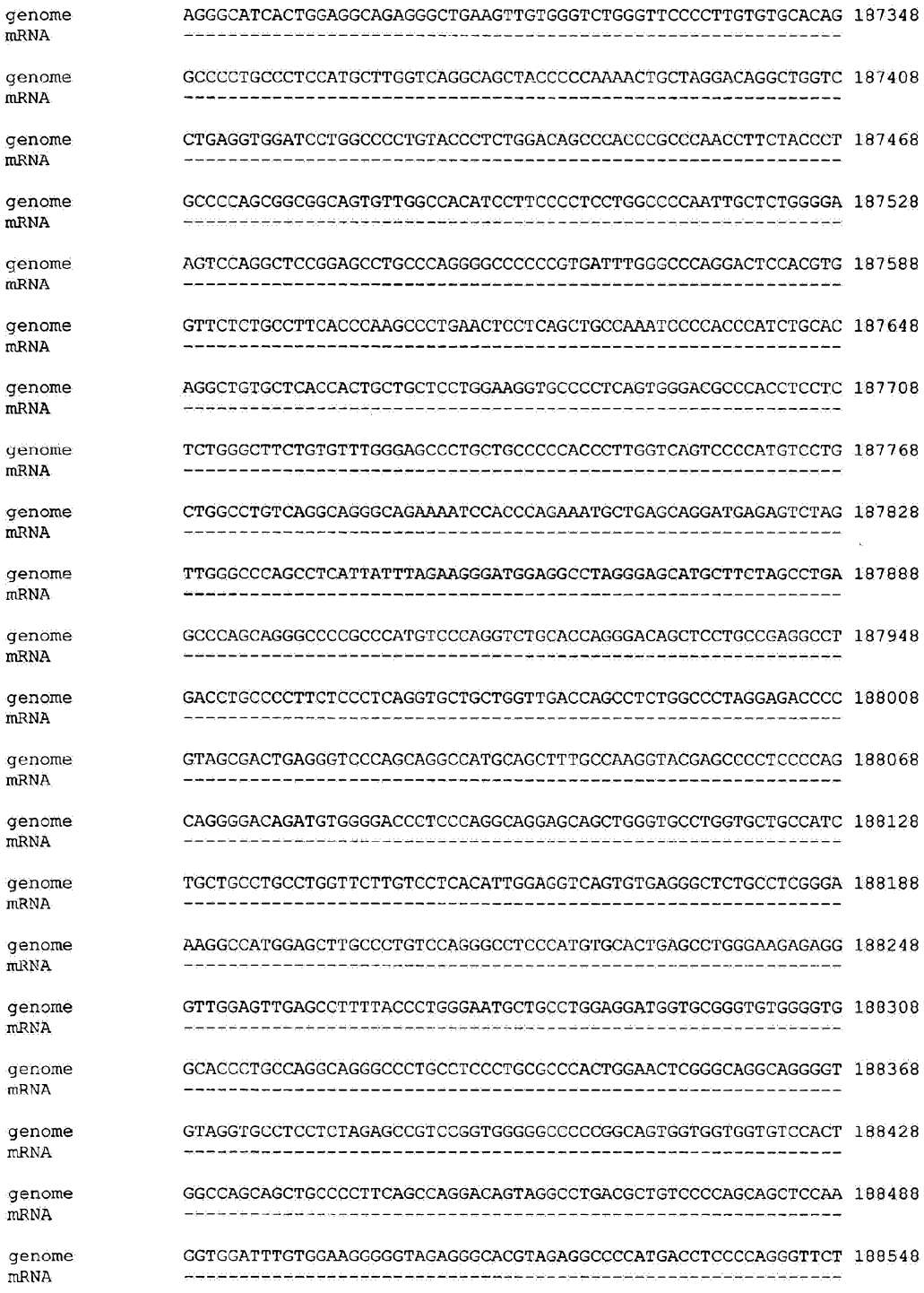
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Figure 1

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Figure 1

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Figure 1

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Figure 1

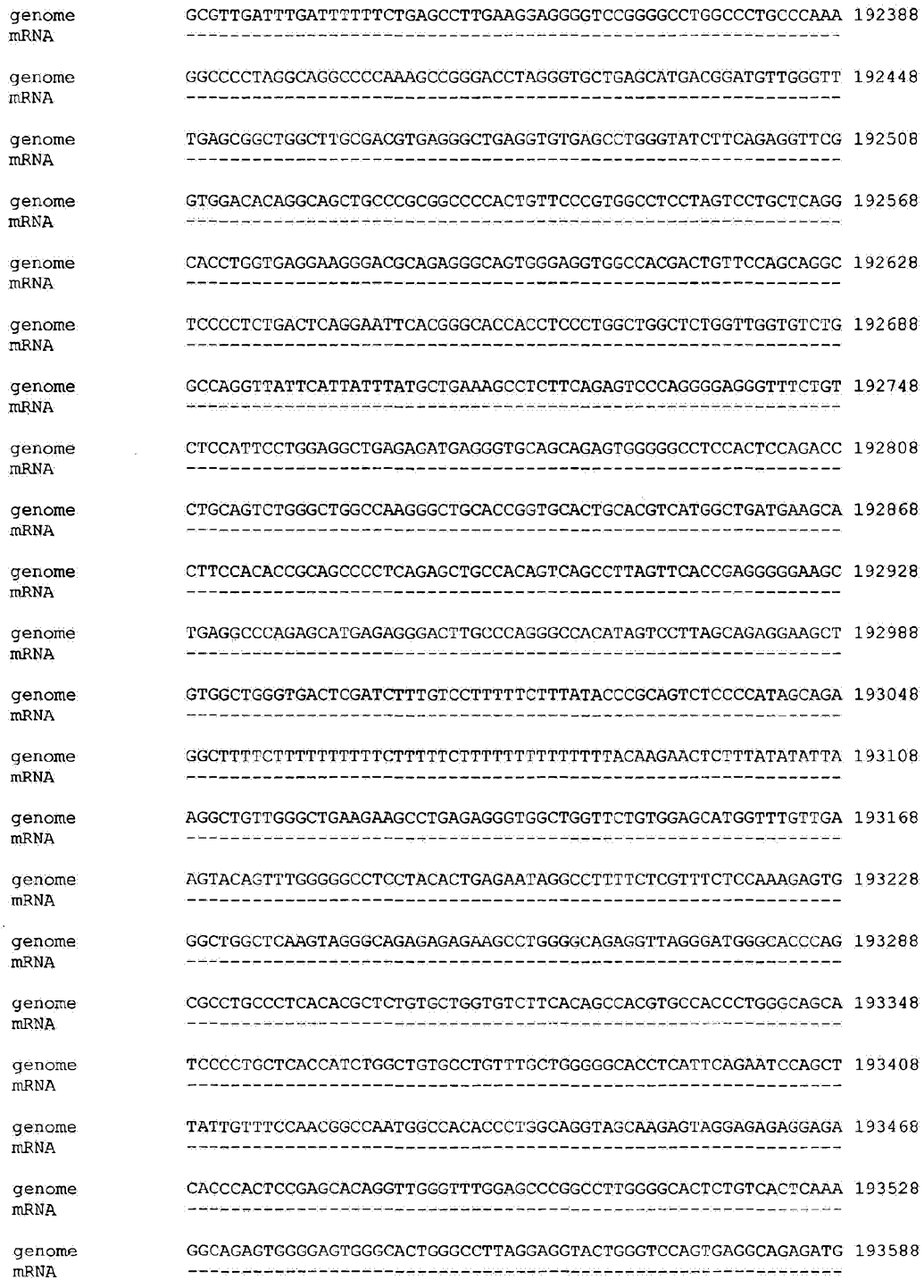
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Figure 1

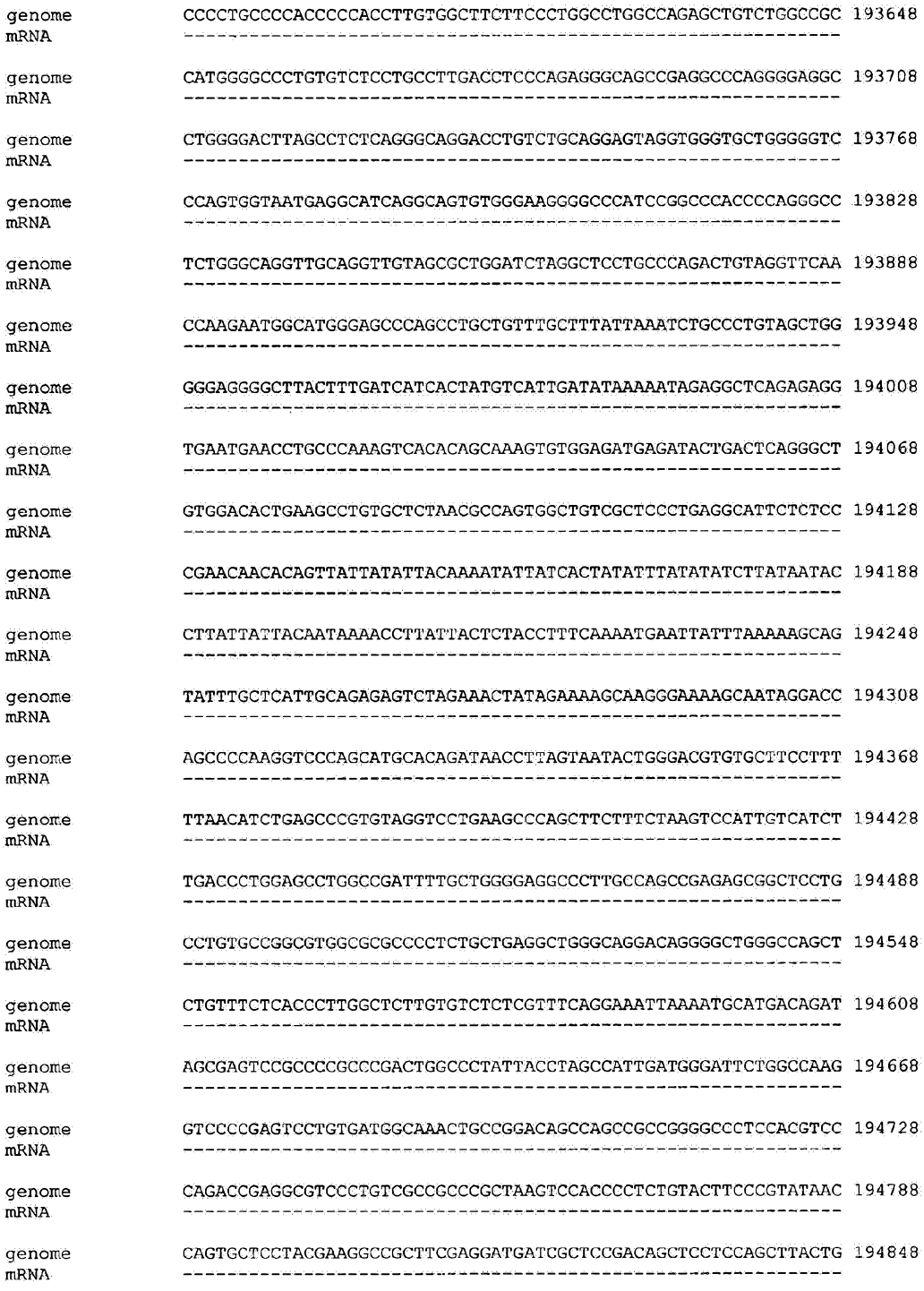
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Figure 1

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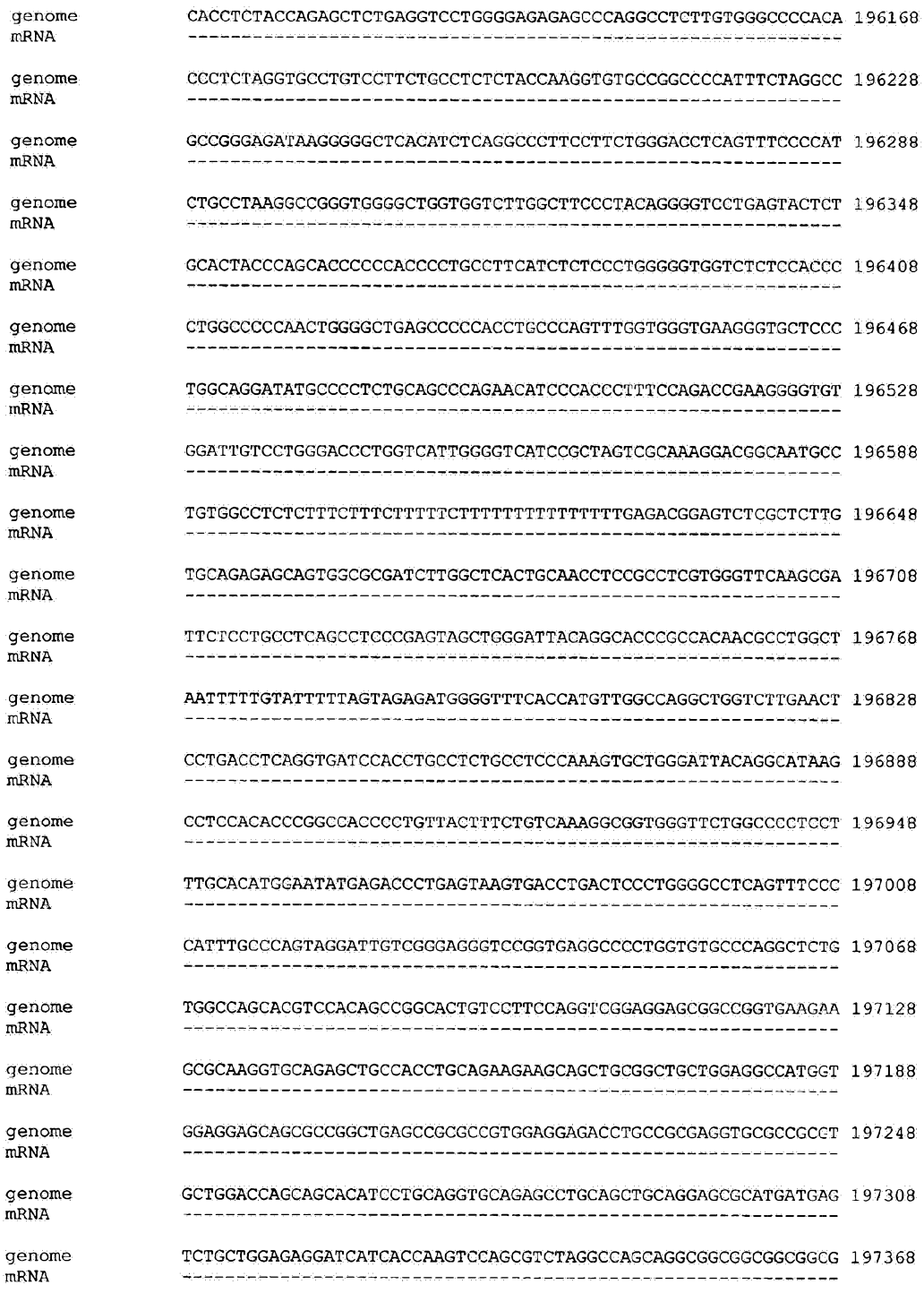
Figure 1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genome GGAGGAGCAGCGCCGGCTGAGCCGCGCCGTGGAGGAGACCTGCCGCGAGGTGCGCCGCCT 197248
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genome GCTGGACCAGCAGCACATCCTGCAGGTGCAGAGCCTGCAGCTGCAGGAGCGCATGATGAG 197308
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Figure 1

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mRNA
genome CATGAATGCCCTTCCTCGGACACAGGCCAGGGCCTCTGGGGTTCACTCCGAGTAAGAACG 197608
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mRNA
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mRNA
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mRNA
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mRNA

Figure 1

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mRNA
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Figure 1

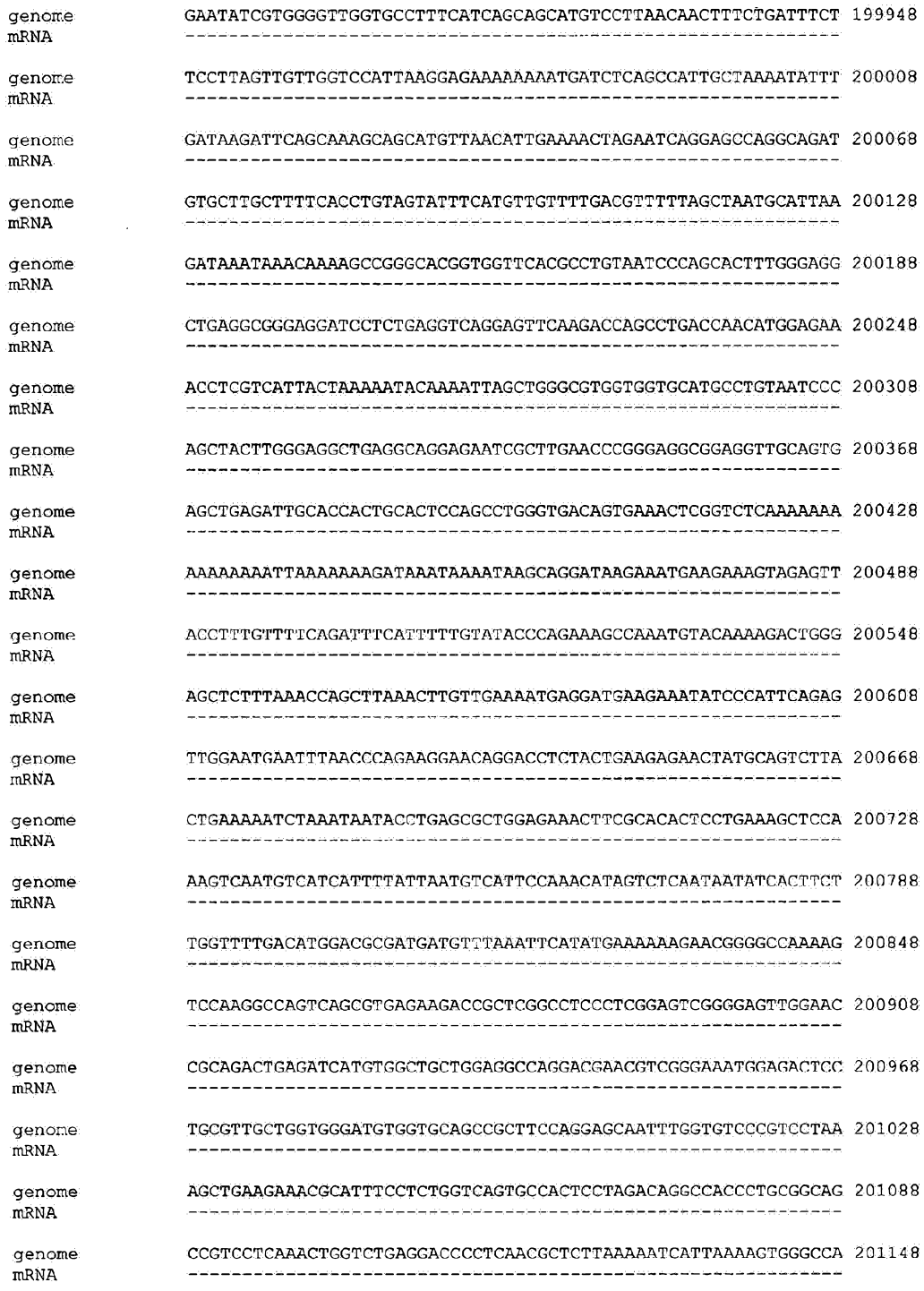
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Figure 1

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