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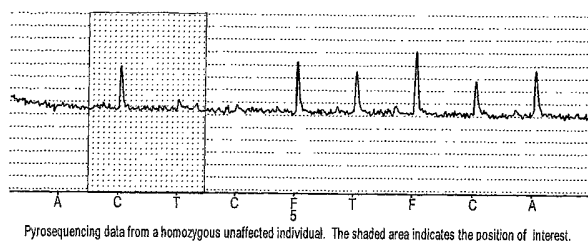
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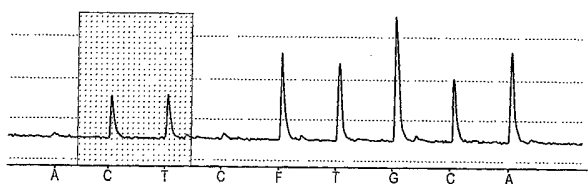
(54) Title: A MUTANT MET AND USES THEREFOR

(57) Abstract: The present invention provides compositions and methods for detecting mutations in the proto-oncogene Met.



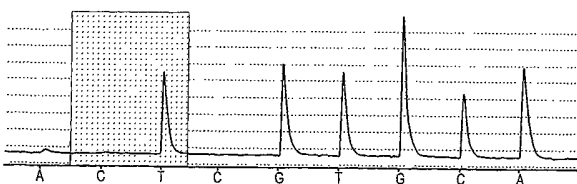
Pyrosequencing data from a homozygous unaffected individual. The shaded area indicates the position of interest.

3A



Pyrosequencing data from a heterozygous individual. The shaded area indicates the position of interest.

3B



Pyrosequencing data from a homozygous affected individual. The shaded area indicates the position of interest.

3C

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A MUTANT MET AND USES THEREFOR

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 60/602,778, filed August 18, 2004, the disclosure of which is hereby incorporated by
5 reference in its entirety for all purposes.

BACKGROUND OF THE INVENTION

[0002] Cancer is increasingly common in canines. Canine cancers include solid tumors and lymphomas such as, *e.g.*, cancers of the mammary glands, prostate, oropharynx, skin, gastrointestinal tract, lungs and bone. A number of canine cancers are highly aggressive.

10 [0003] Recently, studies have been directed toward elucidating the biological mechanism underlying canine cancers. Dysregulation of growth factor receptors (also known as receptor tyrosine kinases, RTKs) is a common mechanism by which normal cells can undergo malignant transformation. One particular RTK termed Met is over-expressed in 60-100% of human OSA, and is known to be aberrantly expressed in canine OSA (*see, e.g.*, Ferracini *et al.*, *J Orthop Res* 18, 253-6 (2000); Ferracini *et al.*, *Oncogene* 10, 739-49 (1995); Ferracini *et al.*, *J Cell Physiol* 184, 191-6 (2000); and Scotlandi *et al.*, *Am J Pathol* 149, 1209-19 (1996)).

[0004] The proto-oncogene Met was originally identified as the transforming gene of a human OSA cell line (MNNG-HOS) (Cooper *et al.* *Nature* 311, 29-33 (1984)). The ligand for Met is hepatocyte growth factor (HGF, also known as scatter factor) (Nakamura, *Prog Growth Factor Res* 3, 67-85 (1991); Nakamura *et al.*, *Nature* 342, 440-3 (1989); Shimomura *et al.*, *J Biol Chem* 268, 22927-32 (1993)). Met-HGF interactions promote an array of cellular responses such as proliferation, scattering (motility), and branching morphogenesis (Kan *et al.*, *Biochem Biophys Res Commun* 174, 331-7 (1991); Montesano *et al.*, *Cell* 67, 901-8 (1991); Weidner *et al.*, *J Cell Biol* 111, 2097-108 (1990); Grant *et al.*, *PNAS USA* 90, 1937-41 (1993); Birchmeier and Gherardi, *Trends Cell Biol* 8, 404-10 (1998)). Binding of
25 HGF to Met initiates receptor dimerization and autophosphorylation at multiple tyrosine residues, resulting in a cascade of downstream signaling events including phosphorylation of adaptor proteins (Gab-1, Grb2, Shc, c-Cbl) and activation of PI3K, ERK1/2, FAK and PLC γ (Jiang *et al.*, *Crit Rev Oncol Hematol* 29, 209-48 (1999); Maulik *et al.*, *Cytokine Growth Factor Rev* 13, 41-59 (2002)). These signals are important in mediating a wide range of
30 biological activities, including embryological development, wound healing, tissue

regeneration, angiogenesis, invasion, and morphogenic differentiation (Jiang *et al.*, *Crit Rev Oncol Hematol* 29, 209-48 (1999); Maulik *et al.*, *Cytokine Growth Factor Rev* 13, 41-59 (2002); Birchmeier and Gherardi, *Trends Cell Biol* 8, 404-10 (1998)).

[0005] Increasing evidence suggests that both Met and HGF are dysregulated in a variety of canine, murine, and human cancers through mutation, overexpression, or co-expression of Met and HGF (Birchmeier *et al.*, *Nat Rev Mol Cell Biol* 4, 915-25 (2003); Jeffers *et al.*, *Proc Natl Acad Sci U S A* 94, 11445-50 (1997); Jeffers *et al.*, *Mol Cell Biol* 16, 1115-25 (1996); Jeffers *et al.*, *Oncogene* 13, 853-6 (1996); Jeffers *et al.*, *PNAS USA* 95, 14417-22 (1998); Pennacchietti *et al.*, *Cancer Cell* 3, 347-61 (2003); Scarpino *et al.*, *J Pathol* 202, 352-8 (2004); Schmidt *et al.*, *Cancer Res* 58, 1719-22 (1998); Schmidt *et al.*, *Nat Genet* 16, 68-73 (1997); Ma *et al.*, *Cancer Res* 63, 6272-81 (2003); Lindor *et al.*, *Genet Test* 5, 101-6 (2001); Lee *et al.*, *Oncogene* 19, 4947-53 (2000); Di Renzo *et al.*, *Oncogene* 19, 1547-55 (2000); Park *et al.*, *Cancer Res* 59, 307-10 (1999); Ferracini *et al.*, *Oncogene* 10, 739-49 (1995); Ferracini *et al.*, *J Cell Physiol* 184, 191-6 (2000); Scotlandi *et al.*, *Am J Pathol* 149, 1209-19 (1996); Tsao *et al.*, *Lung Cancer* 20, 1-16 (1998); Ruco *et al.*, *J Pathol* 180, 266-70 (1996); Ruco *et al.*, *J Pathol* 194, 4-8 (2001)). It is also important to note that aberrant Met expression and/or function through mutation is associated with high tumor grade and a poor prognosis in a variety of human cancers (Di Renzo *et al.*, *Clin Cancer Res* 1, 147-54 (1995); Baykal *et al.*, *Gynecol Oncol* 88, 123-9 (2003); Carneiro, F. and Sobrinho-Simoes, M., *Cancer* 88, 238-40 (2000); Nakajima *et al.*, *Cancer* 85, 1894-902 (1999); Takeuchi *et al.*, *Clin Cancer Res* 9, 1480-8 (2003); Di Renzo *et al.*, *Oncogene* 7, 2549-53 (1992)). Together, this data suggests that inappropriate expression or function of Met contributes to both tumor development and tumor progression.

[0006] Met dysregulation has also been implicated in canine cancer, specifically osteosarcoma (OSA). OSA is the most common bone tumor in dogs, representing approximately 85% of all bone tumors (Withrow *et al.*, *Clin Orthop*, 159-68 (1991); Withrow and MacEwen, *Small animal clinical oncology*, xvii, 736 (W.B. Saunders, Philadelphia, 2001)). Both large and giant breed dogs are at a higher risk for the development of OSA, with certain breeds such as Rotweillers and Irish Wolfhounds over-represented (Withrow *et al.*, *Clin Orthop*, 159-68 (1991); Withrow and MacEwen, 2001, *supra*).

[0007] OSA is a very aggressive tumor, causing lysis of the affected bone leading to a progressive lameness. While less than 10% of dogs have radiographically detectable pulmonary metastases at the time of presentation, over 90% have microscopic metastatic disease. Treatment involves removal of the primary tumor through either limb amputation or

limb spare surgery. Amputation alone leads to a median survival time of 3-4 months, with nearly all dogs succumbing to metastasis within 1 year (Withrow *et al.*, *Clin Orthop*, 159-68 (1991); Withrow. and MacEwen, 2001, *supra*). Survival times are extended to 8-12 months if adjuvant chemotherapy with cisplatin, adriamycin, or carboplatin is used (reviewed in (Withrow and MacEwen, 2001, *supra*); Chun and de Lorimier, *Vet Clin North Am Small Anim Pract* 33, 491-516, vi (2003))). However, less than 20% of patients will survive longer than 2 years. Recent efforts at co-administering adriamycin and a platinum compound have not improved survival times (Chun and de Lorimier, 2003, *supra*). In one report, 5 of 7 canine osteosarcoma (OSA) tumor samples exhibited high levels of Met expression as assessed by Northern analysis (Ferracini *et al.*, *J Orthop Res* 18, 253-6 (2000)). In addition, a lung metastasis from one dog expressed Met at a higher level than the primary tumor. These results are similar to findings in human OSA in which 60%-95% of primary tumors and 80-100% of recurrences (local and distant) exhibit excessive Met expression (Ferracini *et al.*, *Oncogene* 10, 739-49 (1995); Ferracini *et al.*, *J Cell Physiol* 184, 191-6 (2000); Scotlandi *et al.*, *Am J Pathol* 149, 1209-19 (1996)). Furthermore, Met was found to be overexpressed in multiple human OSA cell lines, and HGF stimulation induced phosphorylation, scattering and proliferation of these cells, although this effect varied in degree among the cell lines tested (Coltella *et al.*, *Faseb J* 17, 1162-4 (2003)). Lastly, three canine OSA cell lines were recently demonstrated to express Met and to respond to HGF stimulation leading to Met phosphorylation (MacEwen *et al.*, *Clin Exp Metastasis* 20, 421-30 (2003)).

[0008] Clearly, novel therapeutic approaches for the treatment of canine cancer are needed if significant improvements in clinical outcome are to occur.

[0009] It is therefore clear that there is a need in the art for more effective therapeutic and diagnostic approaches for treating and preventing canine cancers. The present invention addresses these and other needs.

BRIEF SUMMARY OF THE INVENTION

[0010] The present invention provides compositions and methods for detecting mutant Met sequences associated with cancer in canines.

[0011] One embodiment of the invention provides isolated polynucleotides comprising the sequence provided in SEQ ID NO:1, 2, or a complement thereof, expression vectors comprising a polynucleotide of claim 1, operably linked to an expression control sequence, and host cells (*i.e.* mammalian cells) comprising the expression vectors. The invention also

provides isolated polypeptides comprising an amino acid sequence encoded by a SEQ ID NOS: 1 and 2. The invention also provides isolated nucleic acids comprising the sequence set forth in SEQ ID NOS: 3-6. The invention further provides isolated polypeptides comprising the amino acid sequence set forth in SEQ ID NO:7.

- 5 **[0012]** Another embodiment of the invention provides methods for detecting mutations associated with neoplasia in a canine by detecting a sequence comprising a G to A substitution at position 2896 of a gene encoding the Met receptor tyrosine kinase or a G to A substitution at position 1630 in a biological sample from the canine (*e.g.*, a wild canine such as a wolf, a fox, or a coyote or a domesticated canine such as a Rottweiler, a Golden
- 10 Retriever, a Boxer, a Sharpei, a Burmese Mountain Dog, a Flat-coated Retriever, a Labrador Retriever, an Irish Wolfhound, or an American Pit Bull Terrier). The mutant Met sequence may comprise SEQ ID NO:1, 2, or a complement thereof. In some embodiments, the mutant Met sequence is detected by specifically amplifying a nucleic acid comprising the sequence from a biological sample from the canine and detecting the amplified nucleic acids, thereby
- 15 detecting the mutation. The Met sequence may be specifically amplified using primers comprising the sequences set forth in SEQ ID NOS: 3 and 4 or 5 and 6. The mutation may be detected by contacting the amplified nucleic acids with a restriction enzyme (*e.g.*, Dde I) or by sequencing the amplified product.

- [0013]** A further embodiment of the invention provides isolated polynucleotides capable of
- 20 distinguishing between the sequence provided in SEQ ID NO:1 or a complement thereof and a nucleic acid encoding a wild type Met receptor tyrosine kinase protein and isolated polynucleotides capable of distinguishing between the sequence provided in SEQ ID NO:2 or a complement thereof and a nucleic acid encoding a wild type Met receptor tyrosine kinase protein.

- 25 **[0014]** These and other embodiments of the invention are further illustrated by the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

- [0015]** Figure 1 depicts an alignment of canine Met wild type and mutant G1630A nucleotide sequences.
- 30 **[0016]** Figure 2 depicts an alignment of canine Met wild type and mutant D544N amino acid sequences.
- [0017]** Figure 3 illustrates data from pyrosequencing to detect the D544N mutation.

DETAILED DESCRIPTION OF THE INVENTION

I. Introduction

[0018] The present invention is based on the identification of mutations in the canine Met gene that are associated with neoplasia in canines. More particularly, the invention is based on the identification of two different mutations in the canine Met proto-oncogene, one of which is present in 60-80% of Rottweillers, a breed predisposed to OSA. Without being bound by theory, we hypothesize that these mutations in Met result in a lower threshold for activation of Met, resulting in a predisposition to malignant transformation. We further hypothesize that both mutations will result in abnormally prolonged Met signal transduction in response to HGF stimulation, thereby contributing to malignant transformation.

[0019] Thus, the invention provides compositions comprising isolated polynucleotides encoding mutant Met sequences and polypeptides encoded by such polynucleotides. The invention further provides methods, and kits for identifying mutant Met sequences and canines that are at risk for cancer (*i.e.*, carriers of the mutant Met). Mutant Met sequences as diagnostic and prognostic markers for neoplastic disease (*e.g.*, to predict how aggressively metastatic a tumor may be). Cells transfected with the mutant Met sequences can be used in development of therapy for cancer, *e.g.* to screen for antineoplastic agents. Canines identified as carriers of the mutant Met can be used as models for study of neoplastic disease and development of therapy for such diseases. Canines identified as carriers of the mutant Met can also be removed from breeding populations to enhance the overall health of dog breeds, both domesticated and wild.

II. Definitions

[0020] Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Generally, the nomenclature used herein and the laboratory procedures in cell culture, molecular genetics, organic chemistry and nucleic acid chemistry and hybridization described below are those well known and commonly employed in the art. Standard techniques are used for nucleic acid and peptide synthesis. Generally, enzymatic reactions and purification steps are performed according to the manufacturer's specifications. The techniques and procedures are generally performed according to conventional methods in the art and various general references (*see generally*, Sambrook *et al.* MOLECULAR CLONING:

A LABORATORY MANUAL, 2d ed. (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.), which are provided throughout this document. The nomenclature used herein and the laboratory procedures in analytical chemistry, and organic synthetic described below are those well known and commonly employed in the art. Standard techniques, or

5 modifications thereof, are used for chemical syntheses and chemical analyses.

[0021] The terms "c-Met," and "Met," refer to both mutant and wild type receptor tyrosine kinase found in, *inter alia*, canines, mice, and humans. The mouse and human Met genes encode a 190 Kd receptor composed of two disulfide-linked chains, an extracellular 50kd α -chain, and a transmembrane 145kd β -chain which shows tyrosine kinase activity (Giordano *et al.*, *Nature* 339, 155-6 (1989); Gonzatti-Haces *et al.*, *PNAS USA* 85, 21-5 (1988)). Both

10 chains are derived from a 170kd precursor that is glycosylated and cleaved to give the mature heterodimer (Giordano *et al.*, *Nature* 339, 155-6 (1989)). Canine Met is 1382 amino acids in length and exhibits 88% homology with the human sequence and 87% homology with the murine sequence.

15 [0022] The terms "c-Met," "mutant c-Met," "Met," and "mutant Met" also refer to nucleic acids comprising a sequence set forth in Genbank Accession No. AY543631 or SEQ ID NOS:1 or 2 or complements thereof and to polypeptides encoded by such sequences (*i.e.*, a protein encoded by SEQ ID NO:1 or 2 or a polypeptide comprising the sequence set forth in SEQ ID NO:7). "c-Met," "mutant c-Met," "Met," and "mutant Met" also refer to nucleic

20 acids comprising a sequence that specifically hybridize under stringent hybridization conditions to a sequence set forth in Genbank Accession No. AY543631 or SEQ ID NOS:1 or 2 or complements thereof as well as nucleic acids having a sequence that has greater than about 95%, preferably greater than about 96%, 97%, 98%, 99%, or higher nucleotide sequence identity, preferably over a region of at least about 25, 50, 100, 200, 500, 1000, or

25 more nucleotides, to a sequence set forth in Genbank Accession No. AY543631 or SEQ ID NOS:1 or 2 or a complement thereof. A Met polynucleotide or polypeptide sequence is typically from a mammal including, but not limited to, domestic dogs and wild dogs (*e.g.*, any member of the family Canidae). Typically, the mammals are of the genus *Canis* such as, for example, *C. lupus*, *C. lupus familiaris*, *C. lupus dingo*, *C. lupus mogollonensis*, *C. lupus baileyi* *C. rufus*, *C. simensis*). The Met nucleic acids and Met proteins of the invention

30 include both naturally occurring or recombinant molecules. Mutant Met sequences include, *e.g.*, sequences that comprise a mutation associated with neoplasia including, *e.g.*, nucleotide sequences comprising SEQ ID NOS: 1, 2 or complements thereof. Mutant Met sequences

also include, *e.g.*, amino acid sequences encoded by the sequence comprising SEQ ID NOS: 1, 2 or complements thereof.

[0023] The terms “isolated,” “purified,” or “biologically pure” refer to material that is substantially or essentially free from components that normally accompany it as found in its native state. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein that is the predominant species present in a preparation is substantially purified. In particular, an isolated mutant Met nucleic acid is separated from open reading frames that flank the mutant Met gene and encode proteins other than mutant Met. The term “purified” denotes that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel. Particularly, it means that the nucleic acid or protein is at least 85% pure, more preferably at least 95% pure, and most preferably at least 99% pure.

[0024] The terms “nucleic acid” and “polynucleotide” are used interchangeably herein to refer to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs).

[0025] Unless otherwise indicated, a particular nucleic acid sequence also encompasses conservatively modified variants thereof (*e.g.*, degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al.*, *Nuc. Acid Res.* 19:5081 (1991); Ohtsuka *et al.*, *J. Biol. Chem.* 260:2605-2608 (1985); Rossolini *et al.*, *Mol. Cell. Probes* 8:91-98 (1994)). The term nucleic acid is used interchangeably with gene, cDNA, mRNA, oligonucleotide, and polynucleotide.

[0026] The terms “polypeptide,” “peptide” and “protein” are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally

occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer.

[0027] The term “amino acid” refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, *e.g.*, hydroxyproline, γ -carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, *i.e.*, an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, *e.g.*, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (*e.g.*, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

[0028] “Conservatively modified variants” applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are “silent variations,” which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence.

[0029] As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a “conservatively modified variant” where the alteration results in the substitution

of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologues, and alleles of the invention.

5 [0030] The following eight groups each contain amino acids that are conservative substitutions for one another:

- 1) Alanine (A), Glycine (G);
- 2) Aspartic acid (D), Glutamic acid (E);
- 3) Asparagine (N), Glutamine (Q);
- 10 4) Arginine (R), Lysine (K);
- 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);
- 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W);
- 7) Serine (S), Threonine (T); and
- 8) Cysteine (C), Methionine (M)

15 (see, *e.g.*, Creighton, Proteins (1984)).

[0031] “Amplifying” refers to submitting a solution to conditions sufficient to allow for amplification of a target polynucleotide *i.e.*, a mutant Met sequence) if all of the components of the reaction are intact. Components of an amplification reaction include, *e.g.*, primers, a polynucleotide template, polymerase, nucleotides, and the like.

20 [0032] The term “subsequence” refers to a sequence of nucleotides that are contiguous within a second sequence but does not include all of the nucleotides of the second sequence.

[0033] A “target” or “target sequence” refers to a single or double stranded polynucleotide sequence sought to be amplified in an amplification reaction. Two target sequences are different if they comprise non-identical polynucleotide sequences.

25 [0034] The term “recombinant” when used with reference, *e.g.*, to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within the native (non-recombinant) form
30 of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all.

[0035] The term “heterologous” when used with reference to portions of a nucleic acid indicates that the nucleic acid comprises two or more subsequences that are not found in the same relationship to each other in nature. For instance, the nucleic acid is typically

recombinantly produced, having two or more sequences from unrelated genes arranged to make a new functional nucleic acid, *e.g.*, a promoter from one source and a coding region from another source. Similarly, a heterologous protein indicates that the protein comprises two or more subsequences that are not found in the same relationship to each other in nature (e.g., a fusion protein).

[0036] The terms “identical” or percent “identity,” in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (*i.e.*, 60% identity, preferably 65%, 70%, 75%, 80%, 85%, 90%, or 95% identity over a specified region such as the intracellular juxtamembrane domain of the Met gene or another region of SEQ ID NOS:1 or 2), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Such sequences are then said to be “substantially identical.” This definition also refers to the complement of a test sequence. Preferably, the identity exists over a region that is at least about 25 amino acids or nucleotides in length, or more preferably over a region that is 50-100 amino acids or nucleotides in length.

[0037] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters. For sequence comparison of nucleic acids and proteins to mutant Met nucleic acids and proteins, the BLAST and BLAST 2.0 algorithms and the default parameters discussed below are used.

[0038] A “comparison window”, as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970),

by the search for similarity method of Pearson & Lipman, *PNAS. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (see, *e.g.*, Current Protocols in Molecular Biology (Ausubel *et al.*, eds. 1995 supplement)).

[0039] A preferred example of algorithm that is suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.*, *Nuc. Acids Res.* 25:3389-3402 (1977) and Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990), respectively. BLAST and BLAST 2.0 are used, with the parameters described herein, to determine percent sequence identity for the nucleic acids and proteins of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al.*, *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a word length (W) of 11, an expectation (E) of 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a word length of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, *PNAS USA* 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

[0040] The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, *e.g.*, Karlin & Altschul, *PNAS USA* 90:5873-5787 (1993)). One

measure of similarity provided by the BLAST algorithm is the smallest sum probability ($P(N)$), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

[0041] An indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the antibodies raised against the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules or their complements hybridize to each other under stringent conditions, as described below. Yet another indication that two nucleic acid sequences are substantially identical is that the same primers can be used to amplify the sequence.

[0042] The phrase “selectively (or specifically) hybridizes to” refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent hybridization conditions when that sequence is present in a complex mixture (*e.g.*, total cellular or library DNA or RNA).

[0043] The phrase “stringent hybridization conditions” refers to conditions under which a probe will hybridize to its target subsequence, typically in a complex mixture of nucleic acid, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Probes, “Overview of principles of hybridization and the strategy of nucleic acid assays” (1993). Generally, stringent conditions are selected to be about 5-10 °C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. The T_m is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m , 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30 °C for short probes (*e.g.*, 10 to 50 nucleotides).

and at least about 60 °C for long probes (*e.g.*, greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For high stringency hybridization, a positive signal is at least two times background, preferably 10 times background hybridization. Exemplary high stringency or stringent

5 hybridization conditions include: 50% formamide, 5x SSC and 1% SDS incubated at 42° C or 5x SSC and 1% SDS incubated at 65° C, with a wash in 0.2x SSC and 0.1% SDS at 65° C.

[0044] Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides that they encode are substantially identical. This occurs, for example, when a copy of a nucleic acid is created using the maximum codon

10 degeneracy permitted by the genetic code. In such cases, the nucleic acids typically hybridize under moderately stringent hybridization conditions. Exemplary “moderately stringent hybridization conditions” include a hybridization in a buffer of 40% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 1X SSC at 45°C. A positive hybridization is at least twice background. Those of ordinary skill will readily recognize that alternative hybridization and

15 wash conditions can be utilized to provide conditions of similar stringency. For PCR, a temperature of about 36 °C is typical for low stringency amplification, although annealing temperatures may vary between about 32 °C and 48 °C depending on primer length. For high stringency PCR amplification, a temperature of about 62 °C is typical, although high stringency annealing temperatures can range from about 50 °C to about 65 °C, depending on

20 the primer length and specificity. Typical cycle conditions for both high and low stringency amplifications include a denaturation phase of 90 °C - 95 °C for 30 sec - 2 min., an annealing phase lasting 30 sec. - 2 min., and an extension phase of about 72 °C for 1 - 2 min.

[0045] The phrase “selectively associates with” refers to the ability of a nucleic acid to “selectively hybridize” with another as defined above, or the ability of an antibody to

25 “selectively (or specifically) bind to a protein, as defined above.

[0046] “Biological sample” as used herein is a sample of biological tissue or fluid that is suspected of containing a nucleic acid encoding a mutant Met polypeptide. These samples can be tested by the methods described herein and include body fluids such as whole blood, serum, plasma, lymph fluids, and various external secretions of the respiratory, intestinal and

30 genitourinary tracts, tears, saliva, milk, white blood cells, and the like; and tissue samples including tumor samples and bone samples. The samples may be fresh, frozen, ore preserved in a fixative such as paraffin. A biological sample is obtained from any mammal including, *e.g.*, any species of the family *Canis*. Suitable *Canis* species include, *e.g.*, wild canines such as, *e.g.*, wolves, foxes, and coyotes, and domesticated canines such as, *e.g.*, Rottweilers,

Golden Retrievers, Boxers, Sharpeis, Burmese Mountain Dogs, Flat-coated Retrievers, Laborador Retrievers, Irish Wolfhounds, or American Pit Bull Terriers) . A biological sample may be suspended or dissolved in liquid materials such as buffers, extractants, solvents and the like.

5 **III. Nucleic Acids Encoding Mutant Met**

A. General Recombinant DNA Methods

[0047] This invention relies on routine techniques in the field of recombinant genetics. Generally, the nomenclature and the laboratory procedures in recombinant DNA technology described below are those well known and commonly employed in the art. Standard
10 techniques are used for cloning, DNA and RNA isolation, amplification and purification. Generally enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like are performed according to the manufacturer's specifications. Basic texts disclosing the general methods of use in this invention include Sambrook *et al.*,
Molecular Cloning, A Laboratory Manual (2nd ed. 1989); Kriegler, Gene Transfer and
15 Expression: A Laboratory Manual (1990); and Current Protocols in Molecular Biology (Ausubel *et al.*, eds., 1994)).

[0048] Oligonucleotides that are not commercially available can be chemically synthesized according to the solid phase phosphoramidite triester method first described by Beaucage & Caruthers, Tetrahedron Letts. 22:1859-1862 (1981), using an automated synthesizer, as
20 described in Van Devanter *et. al.*, Nucleic Acids Res. 12:6159-6168 (1984). Purification of oligonucleotides is by either native acrylamide gel electrophoresis or by anion-exchange HPLC as described in Pearson & Reanier, J. Chrom. 255:137-149 (1983).

[0049] The sequence of the cloned genes and synthetic oligonucleotides can be verified after cloning using, *e.g.*, the chain termination method for sequencing double-stranded
25 templates of Wallace *et al.*, Gene 16:21-26 (1981).

B. Cloning methods for the isolation of nucleotide sequences encoding mutant Met

[0050] In general, the nucleic acid sequences encoding mutant Met and related nucleic acid sequence homologues are cloned from cDNA and genomic DNA libraries or isolated using
30 amplification techniques with oligonucleotide primers. For example, mutant Met sequences are typically isolated from nucleic acid (genomic or cDNA) libraries by hybridizing with a

nucleic acid probe, the sequence of which can be derived from SEQ ID NO:1, or a subsequence thereof. Mutant Met RNA and genomic DNA can be isolated from any canine including a dog such as, *e.g.*, a Rottweiler or a Golden Retriever. Methods for making and screening cDNA libraries and genomic DNA libraries are well known (see, *e.g.*, Gubler & Hoffman, *Gene* 25:263-269 (1983); Sambrook *et al.*, *supra*; Ausubel *et al.*, *supra*; Benton & Davis, *Science* 196:180-182 (1977); and Grunstein *et al.*, *PNAS USA*, 72:3961-3965 (1975)).

[0051] Nucleic acids encoding mutant Met can also be isolated from expression libraries using antibodies as probes. Such polyclonal or monoclonal antibodies can be raised using, for example, the polypeptides encoded by the sequence of SEQ ID NOS:1 or 2.

[0052] Mutant Met polymorphic variants, alleles, and interspecies homologues that are substantially identical to mutant Met can be isolated using mutant Met nucleic acid probes and oligonucleotides under stringent hybridization conditions, by screening libraries. Alternatively, expression libraries can be used to clone mutant Met polymorphic variants, alleles, and interspecies homologues, by detecting expressed homologues immunologically with antisera or purified antibodies made against the core domain of mutant Met which also recognize and selectively bind to the mutant Met homologue.

[0053] An alternative method of isolating mutant Met nucleic acids and their homologues combines the use of synthetic oligonucleotide primers and amplification of an RNA or DNA template (see U.S. Patents 4,683,195 and 4,683,202; PCR Protocols: A Guide to Methods and Applications (Innis *et al.*, eds, 1990)). The primers can be used, *e.g.*, to amplify either the full length sequence or a probe of one to several hundred nucleotides, which is then used to screen a cDNA library for full-length mutant Met. For example, nucleic acids encoding mutant Met or fragments thereof may be obtained by amplification of a dog cDNA library or reverse transcribed from dog RNA using isolated nucleic acid primer pairs having the following sequences: 5' primer: 5' gcaatccacgagcccatgag 3' and 3' primer: 5' gtagtgggcttacacttcggg 3'; or 5' primer: 5' gtcctgcagtcagtgctctccg 3' and 3' primer: 5' ggtgcagctctcattccaagg 3'. Methods such as polymerase chain reaction (PCR) and ligase chain reaction (LCR) can be used to amplify nucleic acid sequences of mutant Met directly from mRNA, from cDNA, from genomic libraries or cDNA libraries. Degenerate oligonucleotides can be designed to amplify mutant Met homologues using the sequences provided herein. Restriction endonuclease sites can be incorporated into the primers. Polymerase chain reaction or other in vitro amplification methods may also be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of mutant Met encoding mRNA in

physiological samples, for nucleic acid sequencing, or for other purposes. Genes amplified by the PCR reaction can be purified from agarose gels and cloned into an appropriate vector.

[0054] Gene expression of mutant Met can also be analyzed by techniques known in the art, *e.g.*, reverse transcription and amplification of mRNA, isolation of total RNA or poly A⁺ RNA, northern blotting, dot blotting, in situ hybridization, RNase protection, probing DNA microchip arrays, and the like.

[0055] Synthetic oligonucleotides can be used to construct recombinant mutant Met genes for use as probes or for expression of protein. This method is performed using a series of overlapping oligonucleotides usually 40-120 bp in length, representing both the sense and non-sense strands of the gene. These DNA fragments are then annealed, ligated and cloned. Alternatively, amplification techniques can be used with precise primers to amplify a specific subsequence of the mutant Met gene. The specific subsequence is then ligated into an expression vector. mutant Met chimeras can be made, which combine, *e.g.*, a portion of mutant Met with a portion of a heterologous mutant Met to create a chimeric, functional mutant Met.

C. Expression of mutant Met in Recombinant Cells

[0056] To obtain high level expression of a cloned gene, such as those cDNAs encoding mutant Met, one typically subclones a mutant Met sequence (*i.e.*, SEQ ID NOS:1 or 2 or a subsequence thereof) into an expression vector that is subsequently transfected into a mammalian cells. The expression vector typically contains a strong promoter or a promoter/enhancer to direct transcription, a transcription/translation terminator, and for a nucleic acid encoding a protein, a ribosome binding site for translational initiation. The promoter is perably linked to the nucleic acid sequence encoding mutant Met (*e.g.*, SEQ ID NOS:1 or 2 or a subsequence thereof). Eukaryotic expression systems for mammalian cells are well known in the art and described, *e.g.*, in Sambrook *et al.* and Ausubel *et al.* and are also commercially available. Preferably the mammalian cells are cells that do not endogenously express Met, HGF, or combinations thereof. Particularly preferred cells include, for example, MBCK cells, NIH 3T3 cells, or Jurkat cells.

[0057] The particular expression vector used to transport the genetic information into the cell is not particularly critical. Any of the conventional vectors used for expression in eukaryotic cells may be used. Preferred vectors include vectors that comprise a strong promoter/enhancer region such as, *e.g.*, pCI-neo, or vectors that comprise multiple cloning

sites followed by an internal ribosome entry site (IRES) and/or a sequence encoding a marker protein such as, *e.g.*, pIRES2-EGFP. The vectors also typically comprise a gene encoding antibiotic resistance to permit selection of cells have been transformed with the vector. The particular antibiotic resistance gene chosen is not critical, any of the many resistance genes known in the art are suitable. Preferably the antibiotic resistance gene confers resistance to neomycin.

[0058] Standard transfection methods are used to produce cell lines that express large quantities of mutant Met protein, which are then purified using standard techniques (see, *e.g.*, Colley *et al.*, J. Biol. Chem. 264:17619-17622 (1989); Guide to Protein Purification, in Methods in Enzymology, vol. 182 (Deutscher, ed., 1990)). Transformation of cells is performed according to standard techniques (see, *e.g.*, Morrison, J. Bact. 132:349-351 (1977); Clark-Curtiss & Curtiss, Methods in Enzymology 101:347-362 (Wu *et al.*, eds, 1983). For example, any of the well known procedures for introducing foreign nucleotide sequences into host cells may be used. These include the use of calcium phosphate transfection, polybrene, protoplast fusion, electroporation, liposomes, microinjection, plasma vectors, viral vectors and any of the other well known methods for introducing cloned genomic DNA, cDNA, synthetic DNA or other foreign genetic material into a host cell (see, *e.g.*, Sambrook *et al.*, *supra*). It is only necessary that the particular genetic engineering procedure used be capable of successfully introducing at least one gene into the host cell capable of expressing mutant Met.

[0059] After the expression vector is introduced into the cells, the transfected cells are cultured under conditions favoring expression of mutant Met. The transfected cells are then used in functional assays (*i.e.*, signal transduction assays, cell scattering and migration assays, and focus formation assays) to analyze the functional biological consequences of the particular mutant Met. Mutant Met can also be recovered from the culture using standard techniques identified below.

D. Purification of Mutant Met Protein

[0060] Either naturally occurring or recombinant mutant Met (*i.e.*, Met encoded by the sequence set forth in SEQ ID NOS:1 OR 2) can be purified for use in functional assay.

Naturally occurring mutant Met is purified, *e.g.*, from a biological sample from a canine or any other source of a mutant Met homologue. Recombinant mutant Met can also be purified from any suitable expression system as described herein.

[0061] Once expressed, mutant Met may be purified to substantial purity by standard techniques known in the art, including, for example, size differential filtration, solubility fractionation, selective precipitation with such substances as ammonium sulfate; column chromatography, immunopurification methods, and others (see, *e.g.*, Scopes, Protein Purification: Principles and Practice (1982); U.S. Patent No. 4,673,641; Ausubel *et al.*, *supra*; and Sambrook *et al.*, *supra*).

[0062] A number of procedures can be employed when recombinant mutant Met is being purified. For example, proteins having established molecular adhesion properties can be reversibly fused to mutant Met. With the appropriate ligand, mutant Met can be selectively adsorbed to a purification column and then freed from the column in a relatively pure form. The fused protein is then removed by enzymatic activity. Finally mutant Met could be purified using immunoaffinity columns.

IV. Detection of Mutant Met Nucleic Acid Sequences

[0063] Canine can be tested to determine whether they are carriers of mutant Met, *i.e.*, whether they are homozygous or heterozygous for mutant Met. Determination of the presence or absence of a particular mutant Met gene in the canine is generally performed by analyzing a nucleic acid sample that is obtained from a canine (*e.g.*, a mammal of the genus *canis*) to be analyzed. Often, the nucleic acid sample comprises genomic DNA. It is also possible to analyze RNA samples for the presence of the Met mutations described herein.

[0064] Detection techniques for evaluating nucleic acids for the presence of a single base change involve procedures well known in the field of molecular genetics. Further, many of the methods involve amplification of nucleic acids. Ample guidance for performing the methods is provided in the art. Exemplary references include manuals such as PCR Technology: PRINCIPLES AND APPLICATIONS FOR DNA AMPLIFICATION (ed. H. A. Erlich, Freeman Press, NY, N.Y., 1992); PCR PROTOCOLS: A GUIDE TO METHODS AND APPLICATIONS (eds. Innis, *et al.*, Academic Press, San Diego, Calif., 1990); CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Ausubel, 1994-1999, including supplemental updates through April 2004; Sambrook & Russell, *Molecular Cloning, A Laboratory Manual* (3rd Ed, 2001).

[0065] Although the methods typically employ PCR steps, other amplification protocols may also be used. Suitable amplification methods include ligase chain reaction (*see, e.g.*, Wu & Wallace, *Genomics* 4:560-569, 1988); strand displacement assay (*see, e.g.*, Walker *et al.*,

PNAS USA 89:392-396, 1992; U.S. Pat. No. 5,455,166); and several transcription-based amplification systems, including the methods described in U.S. Pat. Nos. 5,437,990; 5,409,818; and 5,399,491; the transcription amplification system (TAS) (Kwoh *et al.*, *PNAS USA* 86:1173-1177, 1989); and self-sustained sequence replication (3SR) (Guatelli *et al.*, *PNAS USA* 87:1874-1878, 1990; WO 92/08800). Alternatively, methods that amplify the probe to detectable levels can be used, such as Q β -replicase amplification (Kramer & Lizardi, *Nature* 339:401-402, 1989; Lomeli *et al.*, *Clin. Chem.* 35:1826-1831, 1989). A review of known amplification methods is provided, for example, by Abramson and Myers in *Curr. Op. Biotech.* 4:41-47, 1993.

10 A. PCR

[0066] PCR can be used to detect carriers of mutant Met by amplification of nucleic acids encoding mutant Met. A general overview of the applicable technology can be found in PCR Protocols: A Guide to Methods and Applications (Innis *et al.* eds. (1990)) and PCR

Technology: Principles and Applications for DNA Amplification (Erlich, *ed.* (1992)). In addition, amplification technology is described in U.S. Patent Nos. 4,683,195 and 4,683,202.

[0067] PCR permits the copying, and resultant amplification of a target nucleic acid, *e.g.*, a nucleic acid encoding mutant Met. In general, PCR and other methods of amplification use primers which anneal to either end of the DNA of interest. For example, nucleic acids encoding mutant Met or fragments thereof may be amplified using an isolated nucleic acid primer pair having the following sequences: 5' primer: 5' gcaatccacgagcccatgag 3' and 3' primer: 5' gtagtgggcttacactcggg 3'; or 5' primer: 5' gtcctgcagtcagtgctctccg 3' and 3' primer: 5' ggtgcagctctcattccaagg 3'.. Amplification of DNA encoding mutant Met from a biological sample from a subject (*i.e.*, a canine) suspected of being a mutant Met carrier indicates that the subject is a carrier for mutant Met.

[0068] Target nucleic acid sequences may be double or single-stranded DNA or RNA from any biological sample from a canine suspected of being a carrier of mutant Met. Preferably, the target template is an isolated DNA sequence. Target DNA sequences may be isolated using a variety of techniques. For example, methods are known for lysing organisms and preparing extracts or purifying DNA. See, Current Protocols in Molecular Biology Volumes 1-3, John Wiley & Sons, Inc. (Ausubel *et al.*, eds., 1994-1998) (hereinafter "Ausubel *et al.*"). Also, total RNA or polyA⁺ RNA can be reverse transcribed to produce cDNA that can serve as the target DNA.

[0069] Typical PCR reaction components include, *e.g.*, a target sequence, oligonucleotide primers, oligonucleotide probes, buffers (*e.g.*, borate, phosphate, carbonate, barbital, Tris, etc. based buffers), salts (*e.g.*, NaCl or KCl), a source of magnesium ions, dNTP's, and a nucleic acid polymerase (*e.g.*, Taq DNA polymerase). PCR reactions can also include additional agents such as DMSO and stabilizing agents (*e.g.*, gelatin, bovine serum albumin, and non-ionic detergents (*e.g.* Tween-20)).

[0070] The oligonucleotides (*i.e.*, primers and probes) can be prepared by any suitable method, including chemical synthesis. Alternatively, they can be purchased through commercial sources. Methods of synthesizing oligonucleotides are well known in the art (*see, e.g.* Narang *et al.*, *Meth. Enzymol.* 68:90-99, 1979; Brown *et al.*, *Meth. Enzymol.* 68:109-151, 1979; Beaucage *et al.*, *Tetrahedron Lett.* 22:1859-1862, 1981; and the solid support method of U.S. Pat. No. 4,458,066). These oligonucleotides can be labeled with radioisotopes, chemiluminescent moieties, or fluorescent moieties. Such labels are useful for the characterization and detection of amplification products using the methods and compositions of the present invention.

[0071] The primer are typically about 15 to about 60 nucleotides in length and are typically present in the PCR reaction mixture at a concentration of between about 0.1 and about 1.0 μM or about about 0.1 to about 0.75 μM . Typically the magnesium ion is present at about a 0.5 to 2.5 mM excess over the concentration of deoxynucleotide triphosphates (dNTPs). dNTPs typically are added to the reaction to a final concentration of about 20 μM to about 300 μM . Typically, each of the four dNTPs (G, A, C, T) are present at equivalent concentrations. (*See, Innis et al.*).

[0072] A variety of DNA dependent polymerases are commercially available that will function using the methods and compositions of the present invention. For example, Taq DNA Polymerase may be used to amplify the mutant Met sequences. Taq DNA polymerase which may be the native enzyme purified from *Thermus aquaticus* and/or a genetically engineered form of the enzyme. Other commercially available polymerase enzymes include, *e.g.*, Taq polymerases marketed by Promega or Pharmacia. Other examples of thermostable DNA polymerases that could be used in the invention include DNA polymerases obtained from, *e.g.*, *Thermus* and *Pyrococcus* species. Concentration ranges of the polymerase may range from 1-5 units per reaction mixture. The reaction mixture is typically between 20 and 100 μl .

[0073] One of skill in the art will recognize that buffer conditions, salt concentrations, magnesium ion concentrations, and dNTP concentrations can be designed to allow for the

function of all reactions of interest, *i.e.*, to support the amplification reaction as well as any subsequent restriction enzyme reactions. A particular set of reaction components can be tested for its ability to support various reactions by testing the components both individually and in combination. The optimal reaction conditions can vary depending on the nature of the target nucleic acid(s) and the primers being used, among other parameters.

B. Detection of amplified products

[0074] Amplified products can be detected using any means known in the art, including, *e.g.*, restriction fragment length polymorphism (RFLP) analysis; denaturing gel electrophoresis, direct sequencing, and HPLC-based analysis, hybridization using sequence-specific oligonucleotides, primer extension, sequence-specific ligation, sequencing, or electrophoretic separation techniques, *e.g.*, singled-stranded conformational polymorphism (SSCP) and heteroduplex analysis. Exemplary assays include 5' nuclease assays, template-directed dye-terminator incorporation, molecular beacon allele-specific oligonucleotide assays, single-base extension assays, and SNP scoring by real-time pyrophosphate sequences. Analysis of amplified sequences can be performed using various technologies such as microchips, fluorescence polarization assays, and matrix-assisted laser desorption ionization (MALDI) mass spectrometry. In addition to these frequently used methodologies for analysis of nucleic acid samples to detect single base changes, any method known in the art can be used to detect the presence of the mutant Met mutations described herein.

1. RFLP Analysis

[0075] In some embodiments, a mutant Met gene is detected using restriction fragment length polymorphism (RFLP) analysis. For example, Met is amplified from a biological sample from a canine suspected of having the Met mutation described herein (*i.e.*, using primers comprising the sequence set forth in SEQ ID NO: 3 and 4). The amplification products of Met are digested with a restriction enzyme (*e.g.*, *Dde* I) that digests the mutant Met, but not the wild type Met. The restriction fragments are then analyzed using gel electrophoresis. For example, dogs heterozygous for a mutant Met mutation associated with neoplasia have a 192 bp undigested wild type fragment and two digested fragments of 104 bp and 88 bp.

2. DNA Sequencing and single base extensions

[0076] The mutant Met genes can also be detected by direct sequencing, *e.g.*, to detect the G to A substitution at position 2896 of Met (*i.e.*, the G966S mutation set forth in SEQ ID NO:1) and the G to A substitution at position 1630 (*i.e.*, the D544N mutation set forth in SEQ ID NO:2) of Met. For example, Met is amplified from a biological sample from a canine suspected of having the Met mutation described herein (*i.e.*, using primers comprising the sequence set forth in SEQ ID NO: 5 and 6) and the amplification product is sequenced. Suitable sequencing methods include *e.g.*, dideoxy sequencing-based methods and Maxam and Gilbert sequence (*see, e.g.*, Sambrook and Russell, *supra*).

[0077] Other detection methods include pyrosequencing of oligonucleotide-length products (*see, e.g.*, Langae *et al.*, *Mutat Res.* 573(1-2):96-102 (2005); Diggle and Clarke, *Mol Biotechnol.* 28(2):129-37 (2004); Shi, *Am J Pharmacogenomics.* 2(3):197-205 (2002); Drmanac *et al.*, *Eng Biotechnol.* 77:75-101 (2002); Franca *et al.*, *Q Rev Biophys.* 35(2):169-200 (2002); Fakhrai-Rad *et al.*, *Hum Mutat.* 19(5):479-85 (2002); and Ronaghi *et al.*, *Genome Res.* 11(1):3-11 (2001). Primers are typically used in conjunction with a labeled M13 (*e.g.*, biotin-labeled) primer to generate a pyrosequencing product for each sample under evaluation. A sequencing primer is hybridized to a single stranded, PCR amplified, DNA template (*e.g.*, SEQ ID NO: 1 or 2), and incubated with the enzymes, DNA polymerase, ATP sulfurylase, luciferase and apyrase, and the substrates, adenosine 5' phosphosulfate (APS) and luciferin. The first of four deoxynucleotide triphosphates (dNTP) is added to the reaction. DNA polymerase catalyzes the incorporation of the deoxynucleotide triphosphate into the DNA strand, if it is complementary to the base in the template strand. Each incorporation event is accompanied by release of pyrophosphate (PPi) in a quantity equimolar to the amount of incorporated nucleotide. ATP sulfurylase quantitatively converts PPi to ATP in the presence of adenosine 5' phosphosulfate. This ATP drives the luciferase-mediated conversion of luciferin to oxyluciferin that generates visible light in amounts that are proportional to the amount of ATP. The light produced in the luciferase-catalyzed reaction is detected by a charge coupled device (CCD) camera and seen as a peak in a pyrogram™. Each light signal is proportional to the number of nucleotides incorporated. Apyrase, a nucleotide degrading enzyme, continuously degrades unincorporated dNTPs and excess ATP. When degradation is complete, another dNTP is added. Addition of dNTPs is performed one at a time. Deoxyadenosine alpha-thio triphosphate (dATPαS) is typically used as a substitute for the natural deoxyadenosine triphosphate (dATP) since it is efficiently used

by the DNA polymerase, but not recognized by the luciferase. As the process continues, the complementary DNA strand is built up and the nucleotide sequence is determined from the signal peak in the pyrogram.

[0078] The following primers and probe can be used to assess samples for the D544N

5 mutation in cMET exon 4: forward (CACCAGGATCCCCTGAA); reverse (TTGCAGGCAGACAGACCT); and probe (CCAGGTGCACACATCTAT). An M13 specific leader sequence (AGCGCATAACAATTTTCACACAGG) can be added to the 5' end of the forward primer.

10 [0079] Another similar method for characterizing single base changes does not require use of a complete PCR, but typically uses only the extension of a primer by a single, fluorescence-labeled dideoxynucleic acid molecule (ddNTP) that is complementary to the nucleotide to be investigated. The nucleotide at the site of the mutant Met mutation can be identified via detection of a primer that has been extended by one base and is fluorescently labeled (*e.g.*, Kobayashi *et al.*, *Mol. Cell. Probes*, 9:175-182, 1995).

15 3. HPLC

[0080] Target mutant Met sequences can be differentiated using high performance liquid chromatography (HPLC) based methods including denaturing HPLC (dHPLC) as described in *e.g.*, Premstaller and Oefner, *LC-GC Europe* 1-9 (July 2002); Bennet *et al.*, *BMC Genetics* 2:17 (2001); Schrimi *et al.*, *Biotechniques* 28(4):740 (2000); and Nairz *et al.*, *PNAS USA* 20 99(16):10575-10580 (2002); and ion-pair reversed phase HPLC-electrospray ionization mass spectrometry (ICEMS) as described in *e.g.*, Oberacher *et al.*, *Hum. Mutat.* 21(1):86 (2003).

[0081] Partially denaturing HPLC analysis compares two or more sets of amplified products (*e.g.*, a wild-type Met amplicon and a mutant Met amplicon). The amplified products are denatured (*e.g.*, at about 95°C) and allowed to reanneal by gradually lowering 25 the temperature from about 95°C to about 30°C. In the presence of a Met mutation the original homoduplex products are reformed along with heteroduplex products comprising the sense and anti-sense strands of either homoduplex. The homoduplexes and heteroduplexes are loaded onto an HPLC apparatus at a partially denaturing temperature of about 50°C to about 70°C and can be distinguished based on their elution profile. Completely denaturing 30 HPLC analysis compares two or more sets of amplicons (*e.g.*, primer extension products). The amplified products are loaded onto an HPLC apparatus at a completely denaturing temperature of about 70°C to about 80°C. Specific sequence variants are eluted from the

column by varying the temperature of the column and sequence variants are distinguished based on their order of elution from the column.

[0082] Ion-pair reversed phase HPLC-electrospray ionization mass spectrometry (ICEMS) uses a combination of HPLC under completely denaturing conditions and ICEMS to resolve differences between nucleic acid sequences.

4. Denaturing Gradient Gel Electrophoresis

[0083] Amplification products generated using the polymerase chain reaction can be analyzed by the use of denaturing gradient gel electrophoresis. Different Met mutations can be identified based on the different sequence-dependent melting properties and electrophoretic migration of DNA in solution (*see, e.g.,* Erlich, ed., PCR TECHNOLOGY, PRINCIPLES AND APPLICATIONS FOR DNA AMPLIFICATION, W. H. Freeman and Co, New York, 1992, Chapter 7).

5. Single-Strand Conformation Polymorphism Analysis

[0084] Target mutant Met sequences can also be differentiated using single-strand conformation polymorphism analysis, which identifies base differences by alteration in electrophoretic migration of single stranded PCR products, as described, *e.g.,* in Orita *et al.*, PNAS USA. 86, 2766-2770 (1989). Amplified PCR products can be generated using methods known in the art, and heated or otherwise denatured, to form single stranded amplification products. Single-stranded nucleic acids may refold or form secondary structures which are partially dependent on the base sequence. The different electrophoretic mobilities of single-stranded amplification products can be related to base-sequence difference between wild type and mutant Met sequences.

[0085] Methods for detecting single base changes often employ labeled oligonucleotides. Oligonucleotides can be labeled by incorporating a label detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. Useful labels include fluorescent dyes, radioactive labels, *e.g.,* ^{32}P , electron-dense reagents, enzyme, such as peroxidase or alkaline phosphatase, biotin, or haptens and proteins for which antisera or monoclonal antibodies are available. Labeling techniques are well known in the art (*see, e.g.,* Current Protocols in Molecular Biology, *supra*; Sambrook & Russell, *supra*).

6. Sequence Specific Hybridization

[0086] A technique commonly referred to as allele specific oligonucleotide hybridization (ASO) (*e.g.*, Stoneking *et al.*, *Am. J. Hum. Genet.* 48:70-382, 1991; Saiki *et al.*, *Nature* 324, 163-166, 1986; EP 235,726; and WO 89/11548) can be used to detect mutant Met genes. Two DNA molecules differing by one base (*e.g.*, wild type or mutant Met) are distinguished by hybridizing an oligonucleotide probe that is specific for one of the variants (*e.g.*, wild type) to an amplified product obtained from amplifying the nucleic acid sample. The probes are designed to differentially hybridize to one variant versus another. The presence of a mutant Met mutation is determined by measuring the amount of sequence-specific oligonucleotide probe that is hybridized to the sample. Principles and guidance for designing such probes is available in the art (*see, e.g.*, Jeffrys and Mays, *Genome Res.* 13(1): 2316-2324 (2003) and Howell *et al.*, *Nature Biotech* 17(1): 87-88 (1999)). Hybridization conditions should be sufficiently stringent that there is a significant difference in hybridization intensity between alleles, and preferably an essentially binary response, whereby a probe hybridizes to only one of the sequences. Typically, the oligonucleotide probe is labeled with a label such as a fluorescent label. For example, a mutant Met-specific oligonucleotide is applied to immobilized oligonucleotides representing mutant Met sequences. After stringent hybridization and washing conditions, fluorescence intensity is measured for each mutant Met oligonucleotide.

[0087] Suitable assay formats for detecting hybrids formed between probes and target nucleic acid sequences in a sample are known in the art and include the immobilized target (dot-blot) format and immobilized probe (reverse dot-blot or line-blot) assay formats. Dot blot and reverse dot blot assay formats are described in U.S. Pat. Nos. 5,310,893; 5,451,512; 5,468,613; and 5,604,099; WO 95/11995 and WO 95/11995.

7. Sequence-Specific Amplification

[0088] Mutations are also commonly detected using sequence-specific amplification or primer extension methods. These reactions typically involve use of primers that are designed to specifically target a polymorphism via a mismatch at the 3' end of a primer. The presence of a mismatch effects the ability of a polymerase to extend a primer when the polymerase lacks error-correcting activity. For example, to detect a mutant Met sequence using a mutant Met-specific amplification- or extension-based method, a primer complementary to the wild type or mutant Met gene is designed such that the 3' terminal nucleotide hybridizes at the

mutation site. The presence of the mutant Met mutation can be determined by the ability of the primer to initiate extension. If the 3' terminus is mismatched, the extension is impeded. Thus, for example, if a primer matches the mutant Met mutation at the 3' end, the primer matches and will be efficiently extended. Sequence -specific amplification- or extension-
5 based methods are described in, for example, WO 93/22456; U.S. Pat. Nos. 5,137,806; 5,595,890; 5,639,611; and 4,851,331.

[0089] In an alternative probe-less method, the amplified nucleic acid is detected by monitoring the increase in the total amount of double-stranded DNA in the reaction mixture, is described, *e.g.*, in U.S. Pat. No. 5,994,056; and European Patent Publication Nos. 487,218
10 and 512,334. The detection of double-stranded target DNA relies on the increased fluorescence various DNA-binding dyes, *e.g.*, SYBR Green, exhibit when bound to double-stranded DNA.

[0090] As appreciated by one in the art, sequence-specific amplification methods, can be performed in reaction that employ multiple sequence -specific primers to target particular
15 mutations. Primers for such multiplex applications are generally labeled with distinguishable labels or are selected such that the amplification products produced from the target sequences are distinguishable by size. Thus, for example, the presence of both a wild type and mutant Met gene in a single sample can be identified using a single amplification by gel analysis of the amplification product.

[0091] As in the case of sequence-specific probes, a sequence -specific oligonucleotide primer may be exactly complementary to one of the Met mutants in the hybridizing region or may have some mismatches at positions other than the 3' terminus of the oligonucleotide, which mismatches occur away from the site of the Met mutation.

5'-nuclease assay

[0092] Genotyping can also be performed using a "TaqMan®" or "5'-nuclease assay", as described in U.S. Pat. Nos. 5,210,015; 5,487,972; and 5,804,375; and Holland *et al.*, *PNAS USA* 88:7276-7280 (1988) or using a combined high-temperature reverse-transcription-polymerase chain reaction (RT-PCR), as described in U.S. Pat. Nos. 5,310,652; 5,322,770; 5,561,058; 5,641,864; and 5,693,517.

30 V. Immunological Detection of Mutant Met Polypeptides

[0093] In addition to the identification of carriers of mutant Met by detection of mutant Met nucleotides, Met polypeptides encoded by the sequence set forth in SEQ ID NOS:1 or 2

can be detected using immunoassays known in the art. For example, polyclonal or monoclonal antibodies that specifically bind to mutant Met, but not to wild-type Met can be used to detect mutant Met in a biological sample from a canine suspected of being a carrier of mutant Met. A general overview of the applicable technology can be found in Harlow &

5 Lane, *Antibodies: A Laboratory Manual* (1988).

[0094] Methods of producing polyclonal and monoclonal antibodies that specifically bind mutant Met, or immunogenic fragments of mutant Met, are known to those of skill in the art (see, *e.g.*, Coligan, *Current Protocols in Immunology* (1991); Harlow & Lane, *supra*; Goding, *Monoclonal Antibodies: Principles and Practice* (2d ed. 1986); Kohler & Milstein, *Nature* 10 256:495-497 (1975); Kozbor *et al.*, *Immunology Today* 4: 72 (1983); Cole *et al.*, pp. 77-96 in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc. (1985)). Such techniques include antibody preparation by selection of antibodies from libraries of recombinant antibodies in phage or similar vectors, as well as preparation of polyclonal and monoclonal antibodies by immunizing rabbits or mice (see, *e.g.*, Huse *et al.*, *Science* 246:1275-1281 15 (1989); Ward *et al.*, *Nature* 341:544-546 (1989)).

[0095] A number of immunogens comprising portions of mutant Met may be used to produce antibodies specifically reactive with mutant Met or homologues thereof. For example, recombinant mutant Met polypeptide (encoded by a sequence comprising SEQ ID NOS:1 or 2) or antigenic fragment thereof, can be isolated as described herein. Recombinant 20 protein can be expressed in eukaryotic or prokaryotic cells as described above, and purified as generally described above for use as an immunogen. Alternatively, a synthetic peptide (*e.g.*, PYPSLLSSQDNIDGEGDT) derived from the sequences disclosed herein and conjugated to a carrier protein can be used as an immunogen. Naturally occurring Met protein may also be used either in pure or impure form.

25 [0096] The phrase “specifically (or selectively) binds” to an antibody or “specifically (or selectively) immunoreactive with,” when referring to a protein or peptide, refers to a binding reaction that is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least two times the background and do not 30 substantially bind in a significant amount to other proteins present in the sample. Specific binding to an antibody under such conditions may require an antibody that is selected for its specificity for a particular protein. For example, polyclonal and monoclonal antibodies and raised to mutant Met can be selected to obtain only those polyclonal and monoclonal antibodies that are specifically immunoreactive with mutant Met (*e.g.*, a mutant Met encoded

by the sequence set forth in SEQ ID NOS:1 or 2) and not with other proteins (*e.g.*, wild type Met). This selection may be achieved by subtracting out antibodies that cross-react with molecules such as mutant Met from other species. In addition, polyclonal and monoclonal antibodies raised to mutant Met polymorphic variants, alleles, orthologs, and conservatively modified variants can be selected to obtain only those antibodies that recognize specific fragments of mutant Met. For example polyclonal antibodies raised to can be selected to obtain only those antibodies that recognize polypeptides encoded by a nucleic acid amplified by primers comprising the sequences set forth in SEQ ID NOS:3 and 4 or 5 and 6, but not other mutant Met fragments. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular mutant Met. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (see, *e.g.*, Harlow & Lane, *Antibodies, A Laboratory Manual* (1988) for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity). Typically a specific or selective reaction will be at least twice background signal or noise and more typically more than 10 to 100 times background.

[0097] Immunoassays known in the art can be used to assess the binding specificity and binding affinity of antibodies that specifically bind to mutant Met. Typically, polyclonal antisera with a titer of 10^4 or greater are selected and tested for their cross reactivity against non-mutant Met proteins, using a competitive binding immunoassay. Specific polyclonal antisera and monoclonal antibodies will usually bind with a K_d of at least about 0.1 mM, more usually at least about 1 μ M, preferably at least about 0.1 μ M or better, and most preferably, 0.01 μ M or better. Antibodies specific only for a particular mutant Met homologue, such as the canine mutant Met encoded by a sequence comprising SEQ ID NOS:1 OR 2, can also be made, by subtracting out other cross-reacting homologues from a species such as a non-human mammal. For a review of immunological and immunoassay procedures, see *Basic and Clinical Immunology* (Stites & Terr eds., 7th ed. 1991). Moreover, the immunoassays of the present invention can be performed in any of several configurations, which are reviewed extensively in *Enzyme Immunoassay* (Maggio, ed., 1980); and Harlow & Lane, *supra*. For a review of the general immunoassays, see also *Methods in Cell Biology: Antibodies in Cell Biology*, volume 37 (Asai, ed. 1993); *Basic and Clinical Immunology* (Stites & Terr, eds., 7th ed. 1991). Immunoassays for detecting mutant Met or immunogenic fragments thereof in samples may be either competitive or noncompetitive. Noncompetitive immunoassays are assays in which the amount of antigen is directly measured. In

competitive assays, the amount of the mutant Met present in the sample is measured indirectly by measuring the amount of known, added (exogenous) mutant Met displaced (competed away) from an anti-mutant Met antibody by the unknown mutant Met present in a sample. Western blot (immunoblot) analysis can also be used to detect and quantify the presence of the mutant Met polypeptides in the sample.

VI. Identification of Inhibitors of Met Signal Transduction

[0098] Inhibitors of Met signal transduction can be identified as potential therapeutic or diagnostic agents (*e.g.*, as anti-neoplastic agents). Assays to measure Met signal transduction are well known in the art (*see, e.g.*, Hov *et al.*, *Clin Cancer Res.* 10(19):6686-94 (2004)). For example, cells transfected with wild-type or mutant canine Met can be used to identify compounds that inhibit Met signal transduction.

[0099] In certain embodiments, inhibitors of Met signal transduction can be identified using high throughput screening (HTS) methods. For example, combinatorial libraries of compounds can be screened for an ability to inhibit Met signal transduction. For example, peptides that antagonize binding of HGF to Met (*see, e.g.*, WO 04/078778); trastuzumab, imatinib, bevacizumab, and gefitinib inhibitors (*see, e.g.*, Christensen *et al.*, *Cancer Lett.* 225(1):1-26 (2005)); or indolinones or their derivative (*see, e.g.*, U.S. Patent Nos. 6,855,730; 6,797,725; 6,777,417; 6,762,180; 6,716,870; 6,710,067; 6,706,709; 6,696,448; 6,689,806; 6,683,082; 6,653,308; 6,642,232; 6,638,965; 6,599,902; 6,579,897; 6,573,293; 6,545,035; 6,531,502; 6,486,185; 6,465,507; 6,451,838; 6,395,734; 6,350,754; 6,319,918; 6,316,429; 6,130,238; 6,114,371; 6,051,593; 4,882,329; 4,137,331; 4,053,613; 4,053,483; 4,020,179) may be screened for their ability to inhibit Met signal transduction. Conventionally, new chemical entities with useful properties are generated by identifying a chemical compound (called a "lead compound") with some desirable property or activity, *e.g.*, inhibiting activity, creating variants of the lead compound, and evaluating the property and activity of those variant compounds. Often, high throughput screening (HTS) methods are employed for such an analysis.

[0046] In one preferred embodiment, high throughput screening methods involve providing a library containing a large number of potential therapeutic compounds (candidate compounds). Such "combinatorial chemical libraries" are then screened in one or more assays to identify those library members (particular chemical species or subclasses) that

display a desired characteristic activity. The compounds thus identified can serve as conventional "lead compounds" or can themselves be used as potential or actual therapeutics.

[0047] A combinatorial chemical library is a collection of diverse chemical compounds generated by either chemical synthesis or biological synthesis by combining a number of chemical "building blocks" such as reagents. For example, a linear combinatorial chemical library, such as a polypeptide (*e.g.*, mutein) library, is formed by combining a set of chemical building blocks called amino acids in every possible way for a given compound length (*i.e.*, the number of amino acids in a polypeptide compound). Millions of chemical compounds can be synthesized through such combinatorial mixing of chemical building blocks (Gallop *et al.*, *J. Med. Chem.* 37(9):1233-1251 (1994)).

[0048] Preparation and screening of combinatorial chemical libraries is well known to those of skill in the art. Such combinatorial chemical libraries include, but are not limited to, peptide libraries (see, *e.g.*, U.S. Patent No. 5,010,175, Furka, *Pept. Prot. Res.* 37:487-493 (1991), Houghton *et al.*, *Nature*, 354:84-88 (1991)), peptoids (PCT Publication No WO 91/19735), encoded peptides (PCT Publication WO 93/20242), random bio-oligomers (PCT Publication WO 92/00091), benzodiazepines (U.S. Pat. No. 5,288,514), diversomers such as hydantoins, benzodiazepines and dipeptides (Hobbs *et al.*, *PNAS USA* 90:6909-6913 (1993)), vinylogous polypeptides (Hagihara *et al.*, *J. Amer. Chem. Soc.* 114:6568 (1992)), nonpeptidal peptidomimetics with a Beta-D-Glucose scaffolding (Hirschmann *et al.*, *J. Amer. Chem. Soc.* 114:9217-9218 (1992)), analogous organic syntheses of small compound libraries (Chen *et al.*, *J. Amer. Chem. Soc.* 116:2661 (1994)), oligocarbamates (Cho, *et al.*, *Science* 261:1303 (1993)), and/or peptidyl phosphonates (Campbell *et al.*, *J. Org. Chem.* 59:658 (1994)). See, generally, Gordon *et al.*, *J. Med. Chem.* 37:1385 (1994), carbohydrate libraries (see, *e.g.*, Liang *et al.*, *Science* 274:1520-1522 (1996), and U.S. Patent No. 5,593,853), and small organic molecule libraries including, for example libraries of indolinones (U.S. Patent No. 6,147,106), benzodiazepines, (U.S. Patent No. 5,288,514); isoprenoids (U.S. Patent No. 5,569,588); thiazolidinones and metathiazanones (U.S. Patent No. 5,549,974); pyrrolidines (U.S. Patent Nos. 5,525,735 and 5,519,134); and morpholino compounds (U.S. Patent No. 5,506,337).

[0049] Devices for the preparation of combinatorial libraries are commercially available (see, *e.g.*, 357 MPS, 390 MPS, Advanced Chem Tech, Louisville KY, Symphony, Rainin, Woburn, MA, 433A Applied Biosystems, Foster City, CA, 9050 Plus, Millipore, Bedford, MA).

[0050] A number of well known robotic systems have also been developed for solution phase chemistries. These systems include automated workstations like the automated synthesis apparatus developed by Takeda Chemical Industries, LTD. (Osaka, Japan) and many robotic systems utilizing robotic arms (Zymate II, Zymark Corporation, Hopkinton, Mass.; Orca, Hewlett-Packard, Palo Alto, Calif.), which mimic the manual synthetic operations performed by a chemist. The above devices, with appropriate modification, are suitable for use with the present invention. In addition, numerous combinatorial libraries are themselves commercially available (see, *e.g.*, ComGenex, Princeton, N.J., Asinex, Moscow, Ru, Tripos, Inc., St. Louis, MO, ChemStar, Ltd, Moscow, RU, 3D Pharmaceuticals, Exton, PA, Martek Biosciences, Columbia, MD, etc.).

[0051] The assays to identify compounds that inhibit Met signal transduction are amenable to high throughput screening. High throughput assays for evaluating the presence, absence, quantification, or other properties of particular nucleic acids or protein products are well known to those of skill in the art. Similarly, binding assays and reporter gene assays are similarly well known. Thus, *e.g.*, U.S. Patent No. 5,559,410 discloses high throughput screening methods for proteins, U.S. Patent No. 5,585,639 discloses high throughput screening methods for nucleic acid binding (*i.e.*, in arrays), while U.S. Patent Nos. 5,576,220 and 5,541,061 disclose high throughput methods of screening for ligand/antibody binding.

[0052] In addition, high throughput screening systems are commercially available (see, *e.g.*, Zymark Corp., Hopkinton, MA; Air Technical Industries, Mentor, OH; Beckman Instruments, Inc. Fullerton, CA; Precision Systems, Inc., Natick, MA, etc.). These systems typically automate procedures, including sample and reagent pipeting, liquid dispensing, timed incubations, and final readings of the microplate in detector(s) appropriate for the assay. These configurable systems provide high throughput and rapid start up as well as a high degree of flexibility and customization. The manufacturers of such systems provide detailed protocols for various high throughput systems. Thus, *e.g.*, Zymark Corp. provides technical bulletins describing screening systems for detecting the modulation of gene transcription, ligand binding, and the like.

VII. Kits

[0100] The present invention also provides kits for detecting mutant Met nucleotides or peptides. Such kits typically comprise two or more components necessary for amplifying mutant Met nucleotides or for detecting mutant Met polypeptides. Components may be

compounds, reagents, containers and/or equipment. In some embodiments, one container within a kit may contain a control Met sequence (*e.g.*, a wild type Met sequence or SEQ ID NO: 1 or 2) and another container within a kit may contain a set of primers, *e.g.*, SEQ ID NOS: 3 and 4 and/or SEQ ID NOS: 5 and 6. Some kits may further comprise a restriction enzyme (*e.g.*, *Dde* I) that digests a Met sequence amplified by the primers. In some embodiments, one container within a kit may contain a control Met polypeptide and another container within a kit may contain a polyclonal or monoclonal antibody that specifically binds to mutant Met. In addition, the kits comprise instructions for use, *i.e.*, instructions for using the primers in amplification and/or detection reactions as described herein or instructions for using the antibodies in immunoassays as described herein.

[0101] The kits may further comprise any of the extraction, amplification, detection reaction components or buffers described herein.

EXAMPLES

The following examples are provided to illustrate, but not to limit the claimed invention.

15 Example 1: Cloning of Met, HGF, and HGFA

[0102] We cloned canine Met, HGF and HGF activator (Genbank Accession Nos. AY543631, AY543632, AY532633, respectively), to develop reagents appropriate for RT-PCR, immunoprecipitation and Western blotting. Canine Met is 1382 amino acids in length and exhibits 88% homology with the human sequence and 87% homology with the murine sequence; HGF is 731 amino acids in length and exhibits 90% homology with the human sequence and 91% homology with the murine sequence; HGF activator is 655 amino acids in length and exhibits 81% homology with the human sequence and 74% homology with the murine sequence. We then evaluated the expression of Met and HGF mRNA in 4 canine OSA cell lines (OSCA2, OSCA8, OSCA11M and D17). All were found to express Met and HGF mRNA and all expressed HGFA mRNA with the exception of the D17 cell line.

Example 2: Identification of the canine Met D544N mutation

[0103] We next evaluated the phosphorylation status of Met in the OSA lines and determined if recombinant human HGF (rhHGF) was capable of eliciting an appropriate response. rhHGF induced phosphorylation of canine Met in all cell lines tested in a dose dependent fashion, with maximal stimulation occurring at 50 ng/ml. Interestingly, the D17 line exhibited a low level of basal phosphorylation in the absence of HGF stimulation.

Subsequent evaluation of Met in this line demonstrated the presence of a point mutation in the extracellular domain leading to an amino acid change consisting of Asp544Asn (D544N). It is possible that this mutation results in the basal phosphorylation of Met in this cell line.

[0104] We next screened a number of additional canine tumor cell lines for the presence of

5 the D544N mutation. The human osteosarcoma cell line, U2OS, was purchased from American Type Culture Collection (Manassas, VA) and maintained in McCoy's 5 medium supplied with 10% fetal bovine serum, penicillin, streptomycin, and L-glutamine. Four canine osteosarcoma (OSA) cell lines, OSA2, OSA8, OSA11M and D17 and one hemangiosarcoma line, SB-HSA-2, were provided by Dr. Jaime Modiano (AMC Cancer
10 Research Institute, Denver, CO). Three histiocytic sarcoma cell lines, Nike 1.1, 030210.4 and DH82, and the myeloid leukemia line, ML3, were provided by Dr. Peter Moore (School of Veterinary Medicine, UC Davis). Two mast cell tumor cell lines, C2 and BR, were provided by Dr. Warren Gold (Cardiovascular Research Institute, UCSF). The additional canine OSA cell lines 348617, 344706, 293069, 344886, 346092, 346381, 348529, and
15 344692 were provided by Dr. Bernard Seguin (School of Veterinary Medicine, UC Davis). The canine melanoma cell lines 321251, 322354, 323610, 325086, 326960, 326937, 344512, and 338301, were provided by Dr. Michael Kent (School of Veterinary Medicine, UC Davis). All canine cell lines were maintained in RPMI 1640 supplemented with 10% fetal bovine serum, non-essential amino acids, sodium pyruvate, HEPES, penicillin, streptomycin and L-
20 glutamine. All cell lines tested expressed message for canine Met, including osteosarcomas, mast cell tumors, hemangiosarcoma, melanomas and histiocytic sarcomas. Additionally, 19 of the 25 lines tested expressed message for both HGF and HGFA indicating a potential role for autocrine Met stimulation in these cells.

[0105] To assess samples for the D544N mutation in cMET exon 4, a pyrosequencing
25 assay was developed. Forward (CACCAGGATCCCACTGAA) and reverse (TTGCAGGCAGACAGACCT) primers were designed flanking the mutation of interest. An M13 specific leader sequence (AGCGCATAACAATTTACACAGG) was added to the 5' end of the forward primer. These primers were used in conjunction with a biotin labeled M13 primer to generate pyrosequencing product for each sample under evaluation. Using the
30 pyrosequencing probe (CCAGGTGCACACATCTAT), sample sequence was determined at the nucleotide position of interest. Homozygous wild type pyrosequencing data is shown in Figure 3A. The heterozygous state is indicated in Figure 3B while the homozygous affected state is depicted in Figure 3C.

[0106] We also validated the ability of several phospho-Met specific antibodies to specifically bind canine Met. Specifically, we tested the anti-pY¹⁰⁰³, anti-pY¹³⁴⁹, anti-pY^{1230/34/35}, and anti-pY¹³⁶⁵, all from Biosource and found that each of these antibodies was capable of specifically binding (*i.e.*, recognizing the specific phospho-motifs) to canine Met after rhHGF stimulation.

Example 3: Identification of the Canine Met G966S Mutation and Analysis of Mutation Incidence and Expression Patterns of Met in Canine OSA Samples

[0107] We identified a mutation in the intracellular juxtamembrane domain in one of the OSA cell lines two canine OSA cell lines OSA11M and OSA2. This mutation is present in leads nucleotide substitution at position 2896 (G2896A), resulting in an amino acid change of glutamine to serine at position 966 (G966S) located in the juxtamembrane domain of Met, encoded by exon 14. The presence of the nucleotide change introduced a restriction cutting site for DdeI permitting rapid identification of the mutation through a simple PCR-restriction digest assay. Canine Met was used as a template to design a forward primer (gcaatccacgagcccatgag) for use on genomic DNA, with the reverse primer (gtagtgggcttacacttcggg) placed in the 3' end of exon 14. Following PCR, DdeI digest is performed and the products are then resolved on a 3.5% agarose gel by electrophoresis. Wild-type samples remain uncut, heterozygous samples demonstrate 3 distinct bands (192 bp uncut, 104 bp and 88 bp cut fragments), while homozygous samples demonstrate only the cut fragments.

[0108] We found that approximately 80% of Rottweillers carry this mutation, compared to less than 5% of all other breeds evaluated. These data suggest that dysfunction of Met may play a role in the predisposition of some dog breeds to the development of neoplasia.

To confirm these results, we screened all of our available canine OSA cell lines for the G966S mutation. 7 of the 23 lines (30%) were found to be either heterozygous (n=6) or homozygous (n=1) for the G966S mutation. To confirm these results, of the PCR products were directly sequenced and the presence of the mutation was confirmed in all 7 samples. 6/7 cell lines were derived from Rottweillers, while the remaining line was derived from a mixed breed dog.

[0109] We next screened random genomic DNA from random dog blood samples obtained from the Veterinary Medical Teaching Hospital at UC Davis, as we reasoned that such samples would provide a reasonable pool of a variety of dog breeds. Of the 433 samples screened, 4.8% (21/433) were found to carry the G966S mutation. The positive samples were

all confirmed by direct sequencing to be either heterozygous (n=15) or homozygous (n=6) for the mutation, thus confirming that the G966S change is indeed germline in nature, and not the consequence of a somatic change associated with tumorigenesis. 11/21 positive dogs were Rottweillers, and of the 10 that were not, 4 were Rottweiler mixes.

5 [0110] Based on the VMTH blood sample screening and the screening of OSA cell lines in which 22/28 samples possessing the mutation were derived from Rottweillers or Rottweiler mixes, we suspected that a high percentage of Rottweillers may be carrying the G966S change in their germline. To explore this possible connection, blood samples and/or genomic DNA were solicited from Rottweiler breeders as well as from Dr. Jaime Modiano (AMC
10 Cancer Research Center) who had collected samples for a separate project. Rottweillers were screened for the G966S mutation as follows. Briefly, peripheral blood mononuclear cells were collected and the resultant genomic DNA was extracted using the DNeasy kit (Qiagen, Valencia, CA). PCR was performed using the primer pair sense 5'gcaatccacgagcccatgag3' and antisense 5'gtagtgggcttacacttcggg3' for 40 cycles (94°C 30 sec, 60°C 30 sec, 72°C 90
15 sec). The intact PCR product was 192 bp in length; this was digested with DdeI and the digest products will be evaluated by agarose gel electrophoresis. Digestion of the mutant allele yields two products (104 bp and 88 bp) while the wild-type allele remains intact. 78% (68/87) of Rottweillers were found to carry the mutation. Within this population, 42 were heterozygous while 26 were homozygous for the mutation. Based on these results,
20 Rottweillers appear to carry a germline mutation in Met and it is possible that this mutation may play a role in their high rates of cancer.

[0111] To directly evaluate tumor specimens for the presence of the G966S mutation, archived paraffin embedded OSA tumor specimens will be obtained. A survey of the VMTH pathology data base has revealed at least 100 available OSA specimens. Briefly, 25 µ
25 sections of tumor will be collected into microcentrifuge tubes, the paraffin will be removed using xylene, and genomic DNA will be obtained according to a previously established protocol (Downing *et al.*, *Am J Vet Res* 63, 1718-1723 (2002)). PCR and restriction digestion analysis will be performed as described above. These experiments will determine whether dogs that do not carry the germ line G966S mutation develop this mutation during
30 tumorigenesis.

Example 4: Response of OSA cells to stress and hypoxia

[0112] As it recently has been demonstrated that stress and/or hypoxia can lead to upregulation of Met expression, we sought to evaluate the effects of such conditions on

canine OSA cells in culture. To evaluate the ability of OSA cells to scatter and migrate, we cultured cells in medium containing fetal bovine serum or no fetal bovine serum (stressful condition), then stimulated with HGF. The OSA cells began to show evidence of morphology change (scattering) upon growth factor deprivation. This was further enhanced when HGF was added to the tissue culture medium, demonstrating that similar to human OSA cells, canine OSA cells respond to cell stress by scattering and migrating in the presence of HGF.

[0113] To evaluate the effects of hypoxia on Met gene expression, we cultured two different canine OSA cell lines in the presence of cobalt chloride, an agent that mimics hypoxia. Using semi-quantitative RT-PCR, it can be seen that the addition of cobalt chloride to the tissue culture medium leads to upregulation of Met gene expression. Together, these results demonstrate that the biological response of Met to stress and hypoxia in canine tumor cells is similar to that found in human cancer cells. Given the substantial data indicating a role of Met in a variety of human cancers, it is likely that dysregulation of Met will play a similar role in canine cancers.

Example 5: D544N Mutation Incidence and Expression patterns of Met in Canine OSA Samples.

[0114] To screen for the D544N mutation, we will use the genomic DNA isolated from the peripheral blood and tumor specimens detailed above. We have developed a pyrosequencing assay (see Figure 1) that is capable of detecting the point mutation. We will use this assay to first evaluate the tumor specimens to determine whether any evidence of the D544N change exists. Should no tumors be found to possess this mutation, then only a limited number of peripheral blood samples will be analyzed for evidence of a germ line mutation.

[0115] To determine the relationship of Met expression in primary OSA tumor samples to tumor behavior, we have generated an anti-canine Met antibody, *i.e.*, a rabbit polyclonal anti-Met antibody directed against the carboxy terminus of canine Met (peptide sequence PYPSSLSSQDNIDGEGDT). Once the antibody has been verified to work appropriately in formalin fixed pellets made from the D17 canine OSA line, we will analyze the expression of Met on canine OSA tumor samples. Briefly, paraffin sections will be boiled at 95°C for 5 minutes in the Dako Antigen Retrieval Solution (DakoCytomation, Carpinteria, CA), to unmask antigen and routine immunohistochemical (IHC) analysis will be performed for Met using the Biosource antibody and the Vectastain ABC Kit (Vector Labs, Burlingame, CA). The D17 cell pellet will be used as the positive control, and pooled normal rabbit IgG will be

used for the negative control. Additionally, we will use the Met peptide (provided by Biosource) to block staining to demonstrate specificity of the reaction. To determine the relationship between Met expression and overall survival, the OSA samples will be scored for positive staining on a scale of 0-5 (0=no positive cells) and a breakpoint between high and low Met staining will be identified. The survival distribution will be plotted using the method of Kaplan and Meier and the log-rank test will be used to determine if there is a significant difference. Data analyses will be performed using commercial statistical software (StatView, SAS Institute, Inc). A p-value <0.05 will be used to indicate statistical significance.

10 Example 6: Effect of Met mutations on Met signal transduction.

6A. Studies using Canine Cell Lines

[0116] To determine whether HGF stimulation of canine Met induces appropriate Met autophosphorylation and downstream signal transduction, Met expressing tumor cell lines were treated with recombinant human HGF and the phosphorylation of Met on several specific tyrosines as well as phosphorylation of downstream signaling components were evaluated. Figure 1a demonstrates Met phosphorylation in canine OSA and melanoma cell lines after rhHGF stimulation. It was then necessary to determine whether all critical tyrosine residues were phosphorylated after rhHGF exposure, as it was possible that the recombinant human protein was only inducing partial or incomplete signal transduction. Using the OSA8 canine OSA cell line, it was determined that tyrosines 1003, 1230/34/35, 1349, and 1365 were all phosphorylated appropriately. The D17 canine OSA cell line showed basal levels of Met phosphorylation even after 24 hours of serum starvation. Although this could be attributed to autocrine HGF and HGFA production, the D17 cell line also has the D544N mutation.

25 [0117] The activation of downstream signal transduction components Gab-1, Erk1/2, and Akt were then examined. In each of the canine cell lines tested, Gab-1 was phosphorylated after stimulation of cells with rhHGF. The D17 cell line showed basal phosphorylation of Gab-1 in the absence of HGF stimulation. Not all cell lines evaluated demonstrated an apparent correlation of rhHGF stimulation with phosphorylation of Erk1/2; some of these showed high basal phosphorylation even in the face of 12 hours of serum starvation. Levels of Akt phosphorylation also did not appear to change substantially after rhHGF stimulation in some of the cell lines. It is possible that in these cases, other growth factor pathways contribute to this high level of basal phosphorylation. However, some of the other cell lines

evaluated (Mel 3 and Mel 8) demonstrated inducible Erk and Akt phosphorylation upon rhHGF stimulation.

6B. Studies Using Cell Lines Stably Transfected with canine Met constructs

[0118] The following studies will analyze Met signal transduction clarify the biochemical

5 consequences of each mutation and provide a framework for potential therapeutic intervention. As previously discussed, recent evidence suggests that mutations in Met do not lead to constitutive activation of the receptor but instead result in a lower threshold for activation and prolonged signal transduction by preventing c-cbl induced Met ubiquitination and subsequent degradation (Lee *et al.*, *Oncogene* 19, 4947-53 (2000); Chiara *et al.*, *J Biol*
10 *Chem* 278, 29352-8 (2003)). To investigate the functional consequences of mutant canine Met, we stably transfected the full length cDNAs containing these mutations into suitable cell lines that do not express HGF or Met. The full length products will be generated from the following tissues: wild-type canine Met from normal canine liver, D544N mutant from the D17 canine OSA cells (ATCC), and G966S mutant from the OSCA 8 canine OSA cell line
15 (provided by Dr. Jaime Modiano). To generate the known human R9886, P1009S, and M1251T Met mutants on the canine Met background, we employed site-directed mutagenesis of wild-type canine Met using the QuikChange XL mutagenesis kit (Stratagene, La Jolla, CA). The human Met mutants serve as a reference for changes in both Met signal transduction and Met biological responses as all of these mutations are known to alter Met
20 signal transduction and Met induced migration/scattering. The following primers were used to generate wild-type canine Met from normal canine liver:

ATGAAGGCTCCTGCTGTGCTTGACCTGGCATCCTTG (forward) and gggaccaa
TCATGTGTCCCCCTCGCCATCAATGTTATCTTGTG (reverse).

[0119] Once the constructs were generated by RT-PCR, they were subcloned into the

25 pIRES2-EGFP vector (Clontech) that possesses a neomycin resistance gene for selection of transfectants and a strong CMV enhancer/promoter region.

[0120] The stably transfected cells will be used in studies to assess the effect of the mutations on Met signal transduction including downstream signaling molecules: Gab-1, Erk1/2, and Akt. Furthermore, these stable transfectants will be used to study the biological
30 effects of the mutations on cell migration and scattering.

[0121] We have screened a number of cell lines for Met and HGF expression and have found that Jurkat cells do not express Met or HGF, while NIH3T3 and MDCK (canine kidney) cells both express Met but not HGF. To prevent confounding signal transduction results due to the expression of wild-type Met, the constructs generated in the section above

will be stably transfected into Jurkat cells. Approximately 10×10^6 cells will be serum starved overnight then either left untreated or exposed to 5 ng/ml (sub-optimal) or 50 ng/ml (optimal) rhHGF for 15 minutes. Cells will then be collected, lysed in protein lysis buffer containing both protease and phosphatase inhibitors, and Met will be immunoprecipitated using the anti-mouse Met polyclonal rabbit antibody (Upstate) or the anti-canine Met polyclonal rabbit antibody (made by Biosource). After SDS polyacrylamide gel electrophoresis (PAGE) of the immunoprecipitates, Western blotting will be performed using the previously validated phospho-specific Met antibodies (anti-pY¹⁰⁰³, anti-pY¹³⁴⁹, anti-pY^{1230/34/35}, and anti-pY¹³⁶⁵) to directly assess the phosphorylation state of the Met mutants in the presence or absence of HGF. The blots will then be stripped and re-probed for total Met. These experiments will provide information regarding whether either of the canine Met mutants possess baseline autophosphorylation in the absence of HGF stimulation and whether the threshold for phosphorylation of Met has been lowered by the presence of either mutation. To determine if either of the canine Met mutations results in extended Met phosphorylation upon stimulation, we will perform the same experiment as detailed above but instead of collecting the cells 15 minutes after stimulation, cells will be washed out of HGF, re-plated in medium lacking FBS, and collected at 6, 12, and 24 hours.

[0122] In addition to the studies described above, we will also evaluate the effect of Met mutations on three specific downstream signaling molecules: Gab-1, a major adaptor molecule for Met that is phosphorylated after HGF stimulation; Erk1/2, members of the MAPK signaling pathway that are important mediators of cell growth and survival for growth factor receptors; and Akt, a downstream target of PI3-kinase that is phosphorylated upon growth factor binding. These pathways all play a role in HGF mediated Met signaling and it is possible that the canine Met mutations alter their function (Birchmeier *et al.*, *Nat Rev Mol Cell Biol* 4, 915-25 (2003)). Approximately 10×10^6 stably transfected Jurkat cells will be serum starved overnight then either left untreated or exposed to 5 ng/ml (sub-optimal) or 50 ng/ml (optimal) rhHGF for 15 minutes and collected immediately or at 2, 12, and 24 hours. After lysis, protein will be quantitated, SDS-PAGE will be performed, and Western blotting for phospho-Gab-1, Erk1/2, and Akt will be performed. The blots will be stripped and re-probed for total Gab-1, Erk1/2, and Akt (all antibodies from Cell Signaling Technology) to control for protein loading.

Example 7: Determine the effects of Met mutations on cell biology

[0123] To further characterize the identified Met mutations, scattering and migration assays and focus formations assays can be performed.

7A. Effect of Met mutations on scattering and migration

5 [0124] NIH-3T3 cells were stably transfected with constructs for wild-type canine Met, R988C canine Met, M1251T canine Met (both mimic human Met mutants that exhibits transforming activity and is found in human cancers) and the canine D544N and G966S Met mutants. Cells were allowed to grow to confluence in tissue culture, then defects were created manually to create a "gap" in the cells. The cells were then left in medium alone or
10 treated with recombinant human HGF (rhHGF) at 50 ng/ml. Cells were also treated with a Met inhibitor in the absence or presence of rhHGF. Cultures were evaluated 24 hours later by staining the cells with crystal violet and digital imaging. The NIH-3T3 lines stably transfected with R988C, M1251T, D544N, and G966S Met all exhibited higher baseline levels of migration in the absence of rhHGF which was markedly increased in the presence of
15 rhHGF when compared to NIH-3T3 expressing wild-type Met. Therefore, the canine D544N and G966S Met mutants exhibit greater migratory capacity than its wild type counterpart.

7B. Assess tumorigenic potential of Met mutations

[0125] To determine whether the mutant forms of Met are likely to be tumorigenic, the stably transfected NIH3T3 cells will be cultured at low FBS concentrations in soft agar as
20 previously described (Jeffers *et al.*, *PNAS USA* 94, 11445-50 (1997); Lee *et al.*, *Oncogene* 19, 4947-53 (2000)). Foci of transformed cells will be counted at 2, 4, and 6 weeks after staining with Giemsa dye. The P1009S and M1251T mutants are known to induce focus formation of NIH3T3 cells in the soft agar assay and will serve as a positive control (Jeffers *et al.*, 1997, *supra*; Lee *et al.*, 2000, *supra*).

25 [0126] It is to be understood that the above description is intended to be illustrative and not restrictive. Many embodiments will be apparent to those of skill in the art upon reading the above description. The scope of the invention should, therefore, be determined not with reference to the above description, but should instead be determined with reference to the appended claims, along with the full scope of equivalents to which such claims are entitled.
30 The disclosures of all Accession Nos., articles and references, including patent applications, patents and PCT publications, are incorporated herein by reference for all purposes.

WHAT IS CLAIMED IS:

- 1 1. A method for detecting a mutation associated with neoplasia in a
2 canine, said method comprising detecting a sequence comprising a G to A substitution at
3 position 2896 of a gene encoding the Met receptor tyrosine kinase in a biological sample
4 from the canine.
- 1 2. The method of claim 1, wherein said canine is domesticated canine.
- 1 3. The method of claim 2, wherein said domesticated canine is a
2 Rottweiler.
- 1 4. The method of claim , wherein the sequence comprises the sequence
2 set forth in SEQ ID NO:1 or a complement thereof.
- 1 5. The method of claim 1, wherein the sequence is detected by
2 (a) specifically amplifying a nucleic acid comprising the sequence in the
3 biological sample, thereby amplifying nucleic acids comprising the mutation; and
4 (b) detecting the amplified nucleic acids, thereby detecting the mutation.
- 1 6. The method of claim 5, wherein the nucleic acids are specifically
2 amplified using primers comprising the sequences set forth in SEQ ID NOS: 3 and 4.
- 1 7. The method of claim 5, wherein the mutation is detected by contacting
2 the amplified nucleic acids with a restriction enzyme.
- 1 8. The method of claim 7, wherein the restriction enzyme is *Dde* I.
- 1 9. The method of claim 5, wherein the amplified nucleic acids are
2 detected by sequencing.
- 1 10. A kit for detecting a mutation associated with neoplasia, said kit
2 comprising:
3 (a) an isolated polynucleotide comprising the sequence provided in SEQ ID
4 NO:1 or a complement thereof; and
5 (b) primers that specifically amplify the sequence provided in SEQ ID NO: 1
6 or a complement thereof.

- 1 11. The kit of claim 10, wherein the primers comprise the sequences set
2 forth in SEQ ID NOS: 3 and 4.
- 1 12. The kit of claim 11, further comprising a restriction enzyme.
- 1 13. The kit of claim 12, wherein the restriction enzyme is *Dde* I.
- 1 14. A method for detecting a mutation associated with neoplasia in a
2 canine, said method comprising detecting a sequence comprising a G to A substitution at
3 position 1630 of a gene encoding the Met receptor tyrosine kinase in a biological sample
4 from the canine.
- 1 15. The method of claim 14, wherein said canine is domesticated canine.
- 1 16. The method of claim 15, wherein said domesticated canine is a
2 Rottweiler.
- 1 17. The method of claim 14, wherein the sequence comprises the sequence
2 set forth in SEQ ID NO:2 or a complement
- 1 18. The method of claim 14, wherein the sequence is detected by
2 (a) specifically amplifying a nucleic acid comprising the sequence in the
3 biological sample, thereby amplifying nucleic acids comprising the mutation; and
4 (b) detecting the amplified nucleic acids, thereby detecting the mutation.
- 1 19. The method of claim 18, wherein the nucleic acids are specifically
2 amplified using primers comprising the sequences set forth in SEQ ID NOS: 5 and 6.
- 1 20. The method of claim 18, wherein the amplified nucleic acids are
2 detected by sequencing.
- 1 21. A kit for detecting a mutation associated with neoplasia, said kit
2 comprising:
3 (a) an isolated polynucleotide comprising the sequence provided in SEQ ID
4 NO:2 or a complement thereof; and
5 (b) primers that specifically amplify the sequence provided in SEQ ID NO: 2
6 or a complement thereof.

- 1 22. The kit of claim 21, wherein the primers comprise the sequences set
2 forth in SEQ ID NOS: 5 and 6.
- 1 23. An isolated polynucleotide comprising the sequence provided in SEQ
2 ID NOS:1, 2, , or a complement thereof.
- 1 24. An isolated polynucleotide comprising the sequence provided in SEQ
2 ID NOS: 3, 4, 5, or 6.
- 1 25. An expression vector comprising a polynucleotide of claim 23,
2 operably linked to an expression control sequence.
- 1 26. A host cell comprising an expression vector according to claim 25.
- 1 27. The host cell of claim 26, wherein the cell is a mammalian cell.
- 1 28. An isolated polypeptide comprising an amino acid sequence encoded
2 by a polynucleotide of claim 23.
- 1 29. An isolated polypeptide comprising an amino acid sequence set forth
2 in SEQ ID NO: 7.
- 1 30. An isolated polynucleotide capable of distinguishing between the
2 sequence provided in SEQ ID NO:1 or a complement thereof and a nucleic acid encoding a
3 wild type Met receptor tyrosine kinase protein.
- 1 31. An isolated polynucleotide capable of distinguishing between the
2 sequence provided in SEQ ID NO:2 or a complement thereof and a nucleic acid encoding a
3 wild type Met receptor tyrosine kinase protein.

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Alignment of Canine MET wild type and mutant (G1630A) nucleotide sequences

WT Met	1	ATGAAGGCTC	CTGCTGTGCT	TGCACCTGGC	ATCCTTGTGC	TTCTGTTTAC
MUTANT MET	1	ATGAAGGCTC	CTGCTGTGCT	TGCACCTGGC	ATCCTTGTGC	TTCTGTTTAC
WT Met	51	CTTGGTGCAG	AAGAGCTATG	GGGAGTGCAA	AGAGGCACTA	GTAAAGTCTG
MUTANT MET	51	CTTGGTGCAG	AAGAGCTATG	GGGAGTGCAA	AGAGGCACTA	GTAAAGTCTG
WT Met	101	AGATGAATGT	GAACATGAAG	TATCAGCTTC	CCAAC TTCAC	TGCCGAAACA
MUTANT MET	101	AGATGAATGT	GAACATGAAG	TATCAGCTTC	CCAAC TTCAC	TGCCGAAACA
WT Met	151	CCCATCCAGA	ATGTTGTTTT	ACACAAGCAT	CATATTTACC	TTGGTGCAGT
MUTANT MET	151	CCCATCCAGA	ATGTTGTTTT	ACACAAGCAT	CATATTTACC	TTGGTGCAGT
WT Met	201	TAAC TATATT	TACGTTTTAA	ATGACAAAGA	CCTTCAGAAG	GTTGCTGAGT
MUTANT MET	201	TAAC TATATT	TACGTTTTAA	ATGACAAAGA	CCTTCAGAAG	GTTGCTGAGT
WT Met	251	ACAAGACTGG	GCCCGTGCTG	GAACACCCAG	ATTGTTCCCC	ATGTCAGGAC
MUTANT MET	251	ACAAGACTGG	GCCCGTGCTG	GAACACCCAG	ATTGTTCCCC	ATGTCAGGAC
WT Met	301	TGCAGCCACA	AAGCCAATTT	ATCAGGTGGT	GTTTGGGAAG	ATAACATCAA
MUTANT MET	301	TGCAGCCACA	AAGCCAATTT	ATCAGGTGGT	GTTTGGGAAG	ATAACATCAA
WT Met	351	CATGGCTCTG	CTTGTTGACA	CATACTACGA	TGACCAACTC	ATTAGCTGTG
MUTANT MET	351	CATGGCTCTG	CTTGTTGACA	CATACTACGA	TGACCAACTC	ATTAGCTGTG
WT Met	401	GCAGTGTCCA	CAGAGGGACC	TGCCAGCGAC	ATATCCTTCC	ACCCAGCAAT
MUTANT MET	401	GCAGTGTCCA	CAGAGGGACC	TGCCAGCGAC	ATATCCTTCC	ACCCAGCAAT
WT Met	451	ATTGCTGACA	TACAGTCGGA	AGTGCATTGC	ATGTACTCCT	CACAGGCAGA
MUTANT MET	451	ATTGCTGACA	TACAGTCGGA	AGTGCATTGC	ATGTACTCCT	CACAGGCAGA
WT Met	501	CGAAGAGCCC	AGCCAGTGCC	CTGACTGTGT	GGTGAGTGCT	CTAGGAACCA
MUTANT MET	501	CGAAGAGCCC	AGCCAGTGCC	CTGACTGTGT	GGTGAGTGCT	CTAGGAACCA
WT Met	551	AAGTCCTGAT	ATCAGAAAAG	GACCGGTTCA	TCAACTTCTT	CGTAGGCAAT
MUTANT MET	551	AAGTCCTGAT	ATCAGAAAAG	GACCGGTTCA	TCAACTTCTT	CGTAGGCAAT
WT Met	601	ACCATAAATT	CCTCGGACCA	TCCAGATCAT	TCATTGCATT	CGATATCGGT
MUTANT MET	601	ACCATAAATT	CCTCGGACCA	TCCAGATCAT	TCATTGCATT	CGATATCGGT
WT Met	651	GAGAAGGCTA	AAGGAAACGC	AAGATGGGTT	CAAGTTTTTG	ACAGACCAGT
MUTANT MET	651	GAGAAGGCTA	AAGGAAACGC	AAGATGGGTT	CAAGTTTTTG	ACAGACCAGT
WT Met	701	CTTACATTGA	TGTTCTACCG	GAGTTCAGAG	ACTCCTACCC	CATTAAATAT
MUTANT MET	701	CTTACATTGA	TGTTCTACCG	GAGTTCAGAG	ACTCCTACCC	CATTAAATAT
WT Met	751	GTCCACGCCT	TTGAAAGCAA	CCACTTATT	TACTTTTTGA	CAGTCCAGCG
MUTANT MET	751	GTCCACGCCT	TTGAAAGCAA	CCACTTATT	TACTTTTTGA	CAGTCCAGCG
WT Met	801	AGAAACTCTA	GATGCTCAGA	CTTTTCACAC	GAGAATAATC	AGGTTCTGTT
MUTANT MET	801	AGAAACTCTA	GATGCTCAGA	CTTTTCACAC	GAGAATAATC	AGGTTCTGTT
WT Met	851	CTGTAGACTC	TGGATTGCAT	TCCTACATGG	AAATGCCTCT	GGAGTGTATT
MUTANT MET	851	CTGTAGACTC	TGGATTGCAT	TCCTACATGG	AAATGCCTCT	GGAGTGTATT
WT Met	901	CTCACGGAAA	AGAGAAGAAA	GAGATCCACA	AGGGAGGAAG	TGTTTAATAT
MUTANT MET	901	CTCACGGAAA	AGAGAAGAAA	GAGATCCACA	AGGGAGGAAG	TGTTTAATAT
	951	TCTCCAAGCT	GCATATGTCA	GTAAGCCTGG	GGCCCATCTC	GCTAAACAAA

FIG. 1

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WT Met							
MUTANT MET	951	TCTCCAAGCT	GCATATGTCA	GTAAGCCTGG	GGCCCATCTC	GCTAAACAAA	
WT Met	1001	TAGGTGCCAA	CCTGAATGAT	GACATTCTCT	ATGGAGTGTT	CGCACAAAGC	
MUTANT MET	1001	TAGGTGCCAA	CCTGAATGAT	GACATTCTCT	ATGGAGTGTT	CGCACAAAGC	
WT Met	1051	AAGCCAGATT	CTGCTGAACC	AATGAATCGC	TCTGCCGTCT	GTGCGTTCCC	
MUTANT MET	1051	AAGCCAGATT	CTGCTGAACC	AATGAATCGC	TCTGCCGTCT	GTGCGTTCCC	
WT Met	1101	TATCAAATAT	GTCATGAAT	TCTTCAACAA	GATCGTCAAC	AAAAACAATG	
MUTANT MET	1101	TATCAAATAT	GTCATGAAT	TCTTCAACAA	GATCGTCAAC	AAAAACAATG	
WT Met	1151	TGAGATGTCT	TCAGCACTTT	TATGGACCCA	ACCACGAACA	CTGCTTTAAT	
MUTANT MET	1151	TGAGATGTCT	TCAGCACTTT	TATGGACCCA	ACCACGAACA	CTGCTTTAAT	
WT Met	1201	AGGACACTTT	TGAGAAATTC	ATCGGGCTGT	GAAGCGCGCA	ATGATGAATA	
MUTANT MET	1201	AGGACACTTT	TGAGAAATTC	ATCGGGCTGT	GAAGCGCGCA	ATGATGAATA	
WT Met	1251	TCGAACGGAG	TTCACCTACAG	CTTTGCAGCG	CGTTGACTTA	TTCATGGGCC	
MUTANT MET	1251	TCGAACGGAG	TTCACCTACAG	CTTTGCAGCG	CGTTGACTTA	TTCATGGGCC	
WT Met	1301	AGTTCAACCA	AGTCCTCTTA	ACGTCTATAT	CCACCTTCAT	CAAAGGAGAC	
MUTANT MET	1301	AGTTCAACCA	AGTCCTCTTA	ACGTCTATAT	CCACCTTCAT	CAAAGGAGAC	
WT Met	1351	CTCACCATTG	CTAATCTTGG	GACGTCCGAG	GGTCGCTTCA	TGCAGGTCGT	
MUTANT MET	1351	CTCACCATTG	CTAATCTTGG	GACGTCCGAG	GGTCGCTTCA	TGCAGGTCGT	
WT Met	1401	GGTTTCTCGA	TCAGGATTGT	CGACCCCTCA	CGTGAACCTC	CGCCTGGACT	
MUTANT MET	1401	GGTTTCTCGA	TCAGGATTGT	CGACCCCTCA	CGTGAACCTC	CGCCTGGACT	
WT Met	1451	CCCACCCCGT	GTCTCCAGAA	GCAATTGTGG	AGCACCCACT	AAACCAAAAC	
MUTANT MET	1451	CCCACCCCGT	GTCTCCAGAA	GCAATTGTGG	AGCACCCACT	AAACCAAAAC	
WT Met	1501	GGCTACACAC	TCGTTGTCAC	TGGGAAGAAG	ATCACCAGGA	TCCCACTGAA	
MUTANT MET	1501	GGCTACACAC	TCGTTGTCAC	TGGGAAGAAG	ATCACCAGGA	TCCCACTGAA	
WT Met	1551	TGGCTTAGGC	TGTGAGCATT	TTCAGTCCTG	CAGTCAGTGT	CTCTCCGCCC	
MUTANT MET	1551	TGGCTTAGGC	TGTGAGCATT	TTCAGTCCTG	CAGTCAGTGT	CTCTCCGCCC	
WT Met	1601	CTCCCTTTGT	GCAGTGTGGC	TGGTGCCACG	ATAGATGTGT	GCACCTGGAG	
MUTANT MET	1601	CTCCCTTTGT	GCAGTGTGGC	TGGTGCCACA	ATAGATGTGT	GCACCTGGAG	
WT Met	1651	GAATGTCCCA	CTGGAGCGTG	GACTCAGGAG	GTCTGTCTGC	CTGCAATCTA	
MUTANT MET	1651	GAATGTCCCA	CTGGAGCGTG	GACTCAGGAG	GTCTGTCTGC	CTGCAATCTA	
WT Met	1701	TGAGGTTTTT	CCAAC TAGTG	CACCCCTGGA	AGGAGGGACA	GTGCTGACTG	
MUTANT MET	1701	TGAGGTTTTT	CCAAC TAGTG	CACCCCTGGA	AGGAGGGACA	GTGCTGACTG	
WT Met	1751	TATGTGGCTG	GGACTTCGGA	TTCAGGAGGA	ATAATAAATT	TGATTTAAAG	
MUTANT MET	1751	TATGTGGCTG	GGACTTCGGA	TTCAGGAGGA	ATAATAAATT	TGATTTAAAG	
WT Met	1801	AAAACCAAAG	TTTTCTTTGG	AAATGAGAGC	TGCACCTTGA	CCTTAAGTGA	
MUTANT MET	1801	AAAACCAAAG	TTTTCTTTGG	AAATGAGAGC	TGCACCTTGA	CCTTAAGTGA	
WT Met	1851	GAGCACAACA	AATATGCTGA	AATGCACAGT	TGGCCCTGCA	GTGAACGAGC	
MUTANT MET	1851	GAGCACAACA	AATATGCTGA	AATGCACAGT	TGGCCCTGCA	GTGAACGAGC	
WT Met	1901	ATTTCAATAT	ATCCATAATT	ATTTCAAATG	GTCGAGGGAC	AGCACAATAT	
MUTANT MET	1901	ATTTCAATAT	ATCCATAATT	ATTTCAAATG	GTCGAGGGAC	AGCACAATAT	
WT Met	1951	AGTACATTTT	CGTATGTGGA	TCCTATTATA	ACAAGTATTT	CTCCAAGTTA	

FIG. 1 (cont.)**SUBSTITUTE SHEET (RULE 26)**

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MUTANT	MET	1951	AGTACATTTT	CGTATGTGGA	TCCTATTATA	ACAAGTATTT	CTCCAAGTTA
WT Met		2001	TGGTCCCAAG	AATGGTGGCA	CCTTGCTCAC	TTAACTGGA	AAATACCTCA
MUTANT	MET	2001	TGGTCCCAAG	AATGGTGGCA	CCTTGCTCAC	TTAACTGGA	AAATACCTCA
WT Met		2051	ACAGTGGGAA	TTCTAGACAC	ATTTCAATGG	GTGGAAAAAC	ATGTACTTTA
MUTANT	MET	2051	ACAGTGGGAA	TTCTAGACAC	ATTTCAATGG	GTGGAAAAAC	ATGTACTTTA
WT Met		2101	AAAAGTGTGT	CAGATAGTAT	TCTCGAATGT	TATACCCAG	CTCAAGCCAC
MUTANT	MET	2101	AAAAGTGTGT	CAGATAGTAT	TCTCGAATGT	TATACCCAG	CTCAAGCCAC
WT Met		2151	TGCAACTGAG	TTTCTATTA	AATTGAAAAT	TGACCTAGCC	AACCGAGAGA
MUTANT	MET	2151	TGCAACTGAG	TTTCTATTA	AATTGAAAAT	TGACCTAGCC	AACCGAGAGA
WT Met		2201	TGAACAGCTT	CAGTTACCAG	GAAGACCCCA	TTGTCTATGC	AATTCATCCA
MUTANT	MET	2201	TGAACAGCTT	CAGTTACCAG	GAAGACCCCA	TTGTCTATGC	AATTCATCCA
WT Met		2251	ACGAAATCTT	TTATTAGTGG	TGGGAGCACA	ATAACAGCTG	TTGGAAAAAA
MUTANT	MET	2251	ACGAAATCTT	TTATTAGTGG	TGGGAGCACA	ATAACAGCTG	TTGGAAAAAA
WT Met		2301	CCTGAATTCA	GTGAGTGTCC	TGAGGATGGT	AATAGATGTC	CATGAAACAA
MUTANT	MET	2301	CCTGAATTCA	GTGAGTGTCC	TGAGGATGGT	AATAGATGTC	CATGAAACAA
WT Met		2351	GAAGGAACTT	TACAGTGGCA	TGTCAACATC	GCTCTAATTC	AGAGATAATC
MUTANT	MET	2351	GAAGGAACTT	TACAGTGGCA	TGTCAACATC	GCTCTAATTC	AGAGATAATC
WT Met		2401	TGTTGTACGA	CTCCTTCACT	GCAACAGCTG	AATCTGCAAC	TCCCTCTGAA
MUTANT	MET	2401	TGTTGTACGA	CTCCTTCACT	GCAACAGCTG	AATCTGCAAC	TCCCTCTGAA
WT Met		2451	AACCAAAGCC	TTTTTCATGT	TAGATGGGAT	CCATTCCAAA	TACTTTGATC
MUTANT	MET	2451	AACCAAAGCC	TTTTTCATGT	TAGATGGGAT	CCATTCCAAA	TACTTTGATC
WT Met		2501	TCATTTATGT	ACATAATCCT	GTGTTTAAGC	CTTTTGAAAA	GCCAGTGATG
MUTANT	MET	2501	TCATTTATGT	ACATAATCCT	GTGTTTAAGC	CTTTTGAAAA	GCCAGTGATG
WT Met		2551	ATCTCAATAG	GCAATGAAAA	TGTACTGGAA	ATTAAGGGAA	ATGATATTGA
MUTANT	MET	2551	ATCTCAATAG	GCAATGAAAA	TGTACTGGAA	ATTAAGGGAA	ATGATATTGA
WT Met		2601	CCCTGAAGCA	GTTAAAGGCG	AAGTGTTAAA	AGTTGGAAAT	AAGAGCTGTG
MUTANT	MET	2601	CCCTGAAGCA	GTTAAAGGCG	AAGTGTTAAA	AGTTGGAAAT	AAGAGCTGTG
WT Met		2651	AGACTATCTA	CTCAGATTCT	AAAGCCGTTT	TATGCAAGGT	CCCCAATGAC
MUTANT	MET	2651	AGACTATCTA	CTCAGATTCT	AAAGCCGTTT	TATGCAAGGT	CCCCAATGAC
WT Met		2701	CTGCTGAAAT	TGAACAACGA	GCTAAATATA	GACTGGAAGC	AAGCAGTTTC
MUTANT	MET	2701	CTGCTGAAAT	TGAACAACGA	GCTAAATATA	GACTGGAAGC	AAGCAGTTTC
WT Met		2751	TTCAACCGTC	CTTGGAAGG	TAATAGTTCA	ACCAGATCAG	AATTTACAG
MUTANT	MET	2751	TTCAACCGTC	CTTGGAAGG	TAATAGTTCA	ACCAGATCAG	AATTTACAG
WT Met		2801	GATTGATTGC	TGGTGTATC	TCAATATCAA	CAATAGTCTT	ATTATTACTC
MUTANT	MET	2801	GATTGATTGC	TGGTGTATC	TCAATATCAA	CAATAGTCTT	ATTATTACTC
WT Met		2851	GGACTTTTCC	TGTGGCTGAA	AAGGAAAAAG	CAAATTAAAG	ATCTGGGCAG
MUTANT	MET	2851	GGACTTTTCC	TGTGGCTGAA	AAGGAAAAAG	CAAATTAAAG	ATCTGGGCAG
WT Met		2901	TGAATTAGTT	CGCTATGATG	CAAGAGTACA	CACTCCTCAT	TTGGATAGGC
MUTANT	MET	2901	TGAATTAGTT	CGCTATGATG	CAAGAGTACA	CACTCCTCAT	TTGGATAGGC
WT Met		2951	TTGTAAGTGC	CCGAAGTGTA	AGCCCAACTA	CAGAAATGGT	TTCAAATGAA
MUTANT	MET	2951	TTGTAAGTGC	CCGAAGTGTA	AGCCCAACTA	CAGAAATGGT	TTCAAATGAA

FIG. 1 (cont.)

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WT Met	3001	TCTGTAGACT	ACCGAGCTAC	TTTCCAGAA	GACCAGTTTC	CTAATTCATC
MUTANT MET	3001	TCTGTAGACT	ACCGAGCTAC	TTTCCAGAA	GACCAGTTTC	CTAATTCATC
WT Met	3051	TCAGAAATGGA	TCATGCAGAC	AAGTACAATA	TCCTCTGACG	GACCTGTCCC
MUTANT MET	3051	TCAGAAATGGA	TCATGCAGAC	AAGTACAATA	TCCTCTGACG	GACCTGTCCC
WT Met	3101	CCATGCTTAC	TAGTGGGGAC	TCTGATATAT	CCAGTCCATT	ATTGCAAAAT
MUTANT MET	3101	CCATGCTTAC	TAGTGGGGAC	TCTGATATAT	CCAGTCCATT	ATTGCAAAAT
WT Met	3151	ACTGTCCACA	TTGACCTCAG	TGCTCTAAAT	CCAGAGCTGG	TGCAGGCAGT
MUTANT MET	3151	ACTGTCCACA	TTGACCTCAG	TGCTCTAAAT	CCAGAGCTGG	TGCAGGCAGT
WT Met	3201	CCAGCATGTA	GTGATTGGGC	CCAGTAGCCT	GATTGTGCAT	TTCAATGAAG
MUTANT MET	3201	CCAGCATGTA	GTGATTGGGC	CCAGTAGCCT	GATTGTGCAT	TTCAATGAAG
WT Met	3251	TCATAGGAAG	AGGACATTTT	GGTGTGTAT	ACCATGGGAC	TTTGTGGAC
MUTANT MET	3251	TCATAGGAAG	AGGACATTTT	GGTGTGTAT	ACCATGGGAC	TTTGTGGAC
WT Met	3301	AATGACGACA	AAAAAATTCA	CTGTGCTGTG	AAATCCCTGA	ATAGAATCAC
MUTANT MET	3301	AATGACGACA	AAAAAATTCA	CTGTGCTGTG	AAATCCCTGA	ATAGAATCAC
WT Met	3351	TGACATAGGA	GAAGTTTCCC	AGTTTCTGAC	CGAGGGAATC	ATCATGAAAG
MUTANT MET	3351	TGACATAGGA	GAAGTTTCCC	AGTTTCTGAC	CGAGGGAATC	ATCATGAAAG
WT Met	3401	ATTTTAGTCA	TCCAAACGTA	CTCTCACTCT	TGGGAATCTG	CCTTCGAAGT
MUTANT MET	3401	ATTTTAGTCA	TCCAAACGTA	CTCTCACTCT	TGGGAATCTG	CCTTCGAAGT
WT Met	3451	GAGGGGTCTC	CACTGGTGGT	CCTACCATAC	ATGAAACATG	GAGATCTTCG
MUTANT MET	3451	GAGGGGTCTC	CACTGGTGGT	CCTACCATAC	ATGAAACATG	GAGATCTTCG
WT Met	3501	AAATTTTCATT	AGAAATGAGA	CTCATAACCC	AACGTAAAA	GATCTTATTG
MUTANT MET	3501	AAATTTTCATT	AGAAATGAGA	CTCATAACCC	AACGTAAAA	GATCTTATTG
WT Met	3551	GCTTTGGTCT	TCAAGTAGCC	AAAGGCATGA	AATATCTTGC	AAGCAAAAAG
MUTANT MET	3551	GCTTTGGTCT	TCAAGTAGCC	AAAGGCATGA	AATATCTTGC	AAGCAAAAAG
WT Met	3601	TTTGTCCACA	GAGACTTGGC	TGCAAGAAAC	TGTATGCTGG	ATGAAAAATT
MUTANT MET	3601	TTTGTCCACA	GAGACTTGGC	TGCAAGAAAC	TGTATGCTGG	ATGAAAAATT
WT Met	3651	CACTGTCAAG	GTTGCTGATT	TTGGTCTTGC	CAGAGACATG	TATGATAAAG
MUTANT MET	3651	CACTGTCAAG	GTTGCTGATT	TTGGTCTTGC	CAGAGACATG	TATGATAAAG
WT Met	3701	AATACTACAG	CGTACACAAC	AAAACAGGCG	CCAAACTACC	AGTGAAGTGG
MUTANT MET	3701	AATACTACAG	CGTACACAAC	AAAACAGGCG	CCAAACTACC	AGTGAAGTGG
WT Met	3751	ATGGCTTTAG	AAAGTCTGCA	AACTCAGAAG	TTTACCACCA	AGTCAGATGT
MUTANT MET	3751	ATGGCTTTAG	AAAGTCTGCA	AACTCAGAAG	TTTACCACCA	AGTCAGATGT
WT Met	3801	GTGGTCCTTT	GGCGTGCTCC	TCTGGGAACT	GATGACAAGA	GGAGCACCAC
MUTANT MET	3801	GTGGTCCTTT	GGCGTGCTCC	TCTGGGAACT	GATGACAAGA	GGAGCACCAC
WT Met	3851	CTTATCCTGA	CGTCAACACC	TTTGATATAA	CAGTTTACTT	GTTGCAAGGC
MUTANT MET	3851	CTTATCCTGA	CGTCAACACC	TTTGATATAA	CAGTTTACTT	GTTGCAAGGC
WT Met	3901	AGAAGGCTCC	TGCAACCCGA	ATACTGCCCA	GATCCCTTAT	ACGAAGTGAT
MUTANT MET	3901	AGAAGGCTCC	TGCAACCCGA	ATACTGCCCA	GATCCCTTAT	ACGAAGTGAT
WT Met	3951	GCTAAAATGC	TGGCACCCTA	GAGCTGAACT	GCGCCCATCT	TTTTCTGAAC
MUTANT MET	3951	GCTAAAATGC	TGGCACCCTA	GAGCTGAACT	GCGCCCATCT	TTTTCTGAAC

FIG. 1 (cont.)

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WT Met	4001	TGGTCTCCAG	GATATCAGCA	ATATTCTCTA	CTTTCATTGG	GGAGCACTAT
MUTANT MET	4001	TGGTCTCCAG	GATATCAGCA	ATATTCTCTA	CTTTCATTGG	GGAGCACTAT
WT Met	4051	GTCCATGTGA	ACGCCACTTA	TGTGAATGTC	AAATGTGTTG	CTCCATACCC
MUTANT MET	4051	GTCCATGTGA	ACGCCACTTA	TGTGAATGTC	AAATGTGTTG	CTCCATACCC
WT Met	4101	TTCTCTCTTG	TCATCACAAG	ATAACATTGA	TGGCGAGGGG	GACACATGA
MUTANT MET	4101	TTCTCTCTTG	TCATCACAAG	ATAACATTGA	TGGCGAGGGG	GACACATGA

FIG. 1 (cont.)

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Alignment of Canine c-MET wild-type and mutant (D544N) amino acid sequences

WT Met	1	MKAPAVLAPG	ILVLLFTLVQ	KSYGECKEAL	VKSEMNVNMK	YQLPNFTAET
MUTANT MET	1	MKAPAVLAPG	ILVLLFTLVQ	KSYGECKEAL	VKSEMNVNMK	YQLPNFTAET
WT Met	51	PIQNVVLHKK	HIYLGAVNYI	YVLNDKDLQK	VAEYKTGPVL	EHPDCSPCQD
MUTANT MET	51	PIQNVVLHKK	HIYLGAVNYI	YVLNDKDLQK	VAEYKTGPVL	EHPDCSPCQD
WT Met	101	CSHKANLSSG	VWEDNINMAL	LVDTYYDDQL	ISCGSVHRGT	CQRHILPPSN
MUTANT MET	101	CSHKANLSSG	VWEDNINMAL	LVDTYYDDQL	ISCGSVHRGT	CQRHILPPSN
WT Met	151	IADIQSEVHC	MYSSQADEEP	SQCPDCVSA	LGTKVLISEK	DRFINFFVGN
MUTANT MET	151	IADIQSEVHC	MYSSQADEEP	SQCPDCVSA	LGTKVLISEK	DRFINFFVGN
WT Met	201	TINSSDHPDH	SLHSISVRR	KETQDGFKFL	TDQSYIDVLP	EFRDSYPIKY
MUTANT MET	201	TINSSDHPDH	SLHSISVRR	KETQDGFKFL	TDQSYIDVLP	EFRDSYPIKY
WT Met	251	VHAFESNHFI	YFLTVQRETL	DAQTFHTRII	RFCSDVSLGH	SYMEMPLCCI
MUTANT MET	251	VHAFESNHFI	YFLTVQRETL	DAQTFHTRII	RFCSDVSLGH	SYMEMPLCCI
WT Met	301	LTEKRRKRST	REEVFNILOA	AYVSKPGAHL	AKQIGANLND	DILYGVFAQS
MUTANT MET	301	LTEKRRKRST	REEVFNILOA	AYVSKPGAHL	AKQIGANLND	DILYGVFAQS
WT Met	351	KPDSAEPMNR	SAVCAFPIKY	VNEFFNKIVN	KNNVRCLQHF	YGNPNEHCFN
MUTANT MET	351	KPDSAEPMNR	SAVCAFPIKY	VNEFFNKIVN	KNNVRCLQHF	YGNPNEHCFN
WT Met	401	RTLLRNSSGC	EARNDEYRTE	FTTALQRVDL	FMGQFNQVLL	TSISTFIKGD
MUTANT MET	401	RTLLRNSSGC	EARNDEYRTE	FTTALQRVDL	FMGQFNQVLL	TSISTFIKGD
WT Met	451	LTIANLGTSE	GRFMQVVSR	SGLSTPHVNF	RLDSHPVSPE	AIVEHPLNQ
MUTANT MET	451	LTIANLGTSE	GRFMQVVSR	SGLSTPHVNF	RLDSHPVSPE	AIVEHPLNQ
WT Met	501	GYTLVVTGKK	ITRIPLNGLG	CEHFQSCSQ	LSAPPFVQCG	WCHDRCVHLE
MUTANT MET	501	GYTLVVTGKK	ITRIPLNGLG	CEHFQSCSQ	LSAPPFVQCG	WCHNRCVHLE
WT Met	551	ECPTGAWTQE	VCLPAIYEVF	PTSAPLEGGT	VLTVCGWDFG	FRNKNKFDLK
MUTANT MET	551	ECPTGAWTQE	VCLPAIYEVF	PTSAPLEGGT	VLTVCGWDFG	FRNKNKFDLK
WT Met	601	KTKVFLGNES	CTLTLSESTT	NMLKCTVGPA	VNEHFNISII	ISNGRGTAQY
MUTANT MET	601	KTKVFLGNES	CTLTLSESTT	NMLKCTVGPA	VNEHFNISII	ISNGRGTAQY
WT Met	651	STFSYVDPII	TSISPSYGPK	NGGTLTLTG	KYLNSGNSRH	ISMGGKTCTL
MUTANT MET	651	STFSYVDPII	TSISPSYGPK	NGGTLTLTG	KYLNSGNSRH	ISMGGKTCTL
WT Met	701	KSVSDSILEC	YTPAQATATE	FPIKLKIDLA	NREMNSFSYQ	EDPIVYAIHP
MUTANT MET	701	KSVSDSILEC	YTPAQATATE	FPIKLKIDLA	NREMNSFSYQ	EDPIVYAIHP
WT Met	751	TKSFISGGST	ITAVGKNLNS	VSVLRMVIDV	HETRRNFTVA	CQHRNSSEII
MUTANT MET	751	TKSFISGGST	ITAVGKNLNS	VSVLRMVIDV	HETRRNFTVA	CQHRNSSEII
WT Met	801	CCTTPSLQQL	NLQLPLKTKA	FFMLDGIHSK	YFDLIYVHNP	VFKPFEKPVM
MUTANT MET	801	CCTTPSLQQL	NLQLPLKTKA	FFMLDGIHSK	YFDLIYVHNP	VFKPFEKPVM
WT Met	851	ISIGNENVLE	IKGNDIDPEA	VKGEVLKVG	KSCETIYSDS	KAVLCKVPND
MUTANT MET	851	ISIGNENVLE	IKGNDIDPEA	VKGEVLKVG	KSCETIYSDS	KAVLCKVPND
WT Met	901	LLKLNNELNI	EWKQAVSSTV	LGKVIVQPDQ	NFTGLIAGVI	SISTIVLLLL
MUTANT MET	901	LLKLNNELNI	EWKQAVSSTV	LGKVIVQPDQ	NFTGLIAGVI	SISTIVLLLL
WT Met	951	GLFLWLKRKK	QIKDLGSELV	RYDARVHTPH	LDRLVSARSV	SPTTEMVSNE
MUTANT MET	951	GLFLWLKRKK	QIKDLGSELV	RYDARVHTPH	LDRLVSARSV	SPTTEMVSNE

FIG. 2

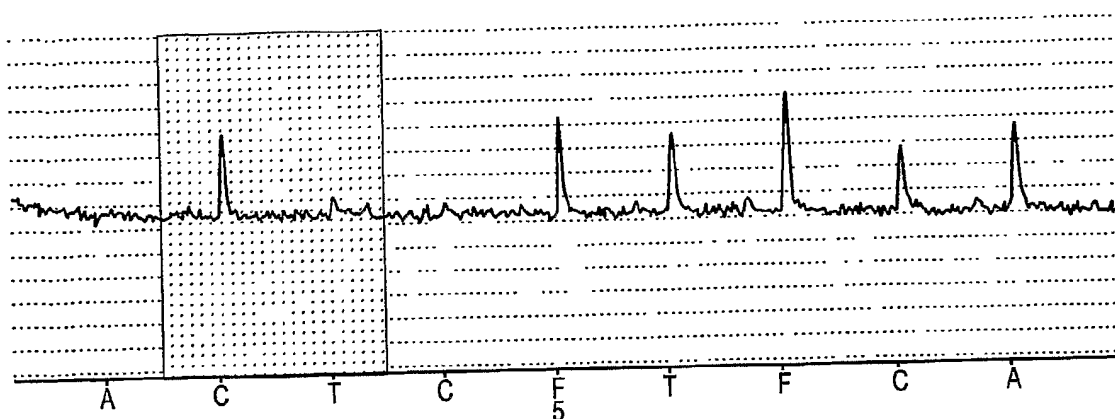
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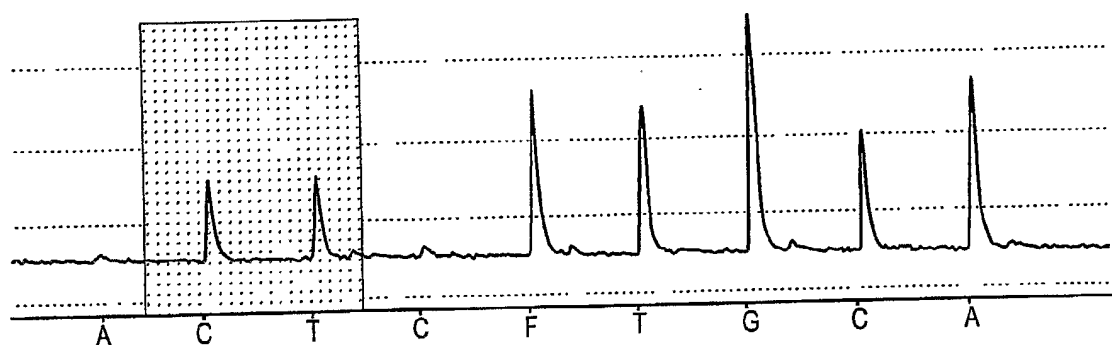
WT Met	1001	SVDYRATFPE	DQFPNSSQNG	SCRQVQYPLT	DLSPMLTSGD	SDISSPLLQN
MUTANT MET	1001	SVDYRATFPE	DQFPNSSQNG	SCRQVQYPLT	DLSPMLTSGD	SDISSPLLQN
WT Met	1051	TVHIDLSALN	PELVQAVQHV	VIGPSSLIVH	FNEVIGRGHF	GCVYHGTLLD
MUTANT MET	1051	TVHIDLSALN	PELVQAVQHV	VIGPSSLIVH	FNEVIGRGHF	GCVYHGTLLD
WT Met	1101	NDDKKIHCAV	KSLNRITDIG	EVSQFLTEGI	IMKDFSHPNV	LSLLGICLRS
MUTANT MET	1101	NDDKKIHCAV	KSLNRITDIG	EVSQFLTEGI	IMKDFSHPNV	LSLLGICLRS
WT Met	1151	EGSPLVVL PY	MKHGDLRNFI	RNETHNPTVK	DLIGFGLQVA	KGMKYLASKK
MUTANT MET	1151	EGSPLVVL PY	MKHGDLRNFI	RNETHNPTVK	DLIGFGLQVA	KGMKYLASKK
WT Met	1201	FVHRDLAARN	CMLDEKFTVK	VADFGLARDM	YDKEYYSVHN	KTGAKLPVKW
MUTANT MET	1201	FVHRDLAARN	CMLDEKFTVK	VADFGLARDM	YDKEYYSVHN	KTGAKLPVKW
WT Met	1251	MAESLQTQK	FTTKSDVWSF	GVLLWELMTR	GAPPYPDVNT	FDITVYLLQG
MUTANT MET	1251	MAESLQTQK	FTTKSDVWSF	GVLLWELMTR	GAPPYPDVNT	FDITVYLLQG
WT Met	1301	RRLQPEYCP	DPLYEVMLKC	WHPRAELRPS	FSELVSRISA	IFSTFIGEHY
MUTANT MET	1301	RRLQPEYCP	DPLYEVMLKC	WHPRAELRPS	FSELVSRISA	IFSTFIGEHY
WT Met	1351	VHVNATYVNV	KCVAPYPSLL	SSQDNIDGEG	DT*	
MUTANT MET	1351	VHVNATYVNV	KCVAPYPSLL	SSQDNIDGEG	DT*	

FIG. 2 (cont.)

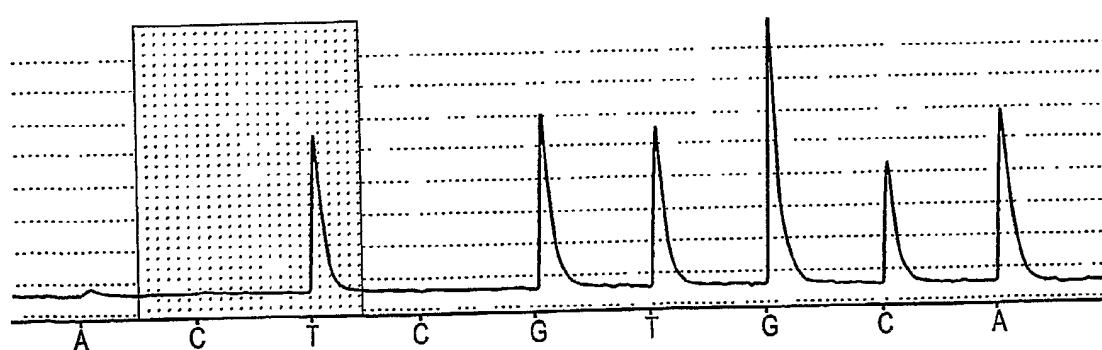
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Pyrosequencing data from a homozygous unaffected individual. The shaded area indicates the position of interest.

FIG. 3A

Pyrosequencing data from a heterozygous individual. The shaded area indicates the position of interest.

FIG. 3B

Pyrosequencing data from a homozygous affected individual. The shaded area indicates the position of interest.

FIG. 3C

INFORMAL SEQUENCE LISTING

SEQ ID NO:1

Canine Met G966S (G2896A) mutant sequence

ATGAAGGCTC CTGCTGTGCT TGCACCTGGC ATCCTTGTGC TTCTGTTTAC CTTGGTGCAG AAGAGCTATG GGGAGTGCAA AGAGGCACTA
 GTAAAGTCTG AGATGAATGT GAACATGAAG TATCAGCTTC CCAACTTCAC TGCCGAAACA CCCATCCAGA ATGTTGTTTT ACACAAGCAT
 CATATTTACC TTGGTGCACT TAACATATATT TACGTTTTAA ATGACAAAGA CCTTCAGAAG GTTGCTGAGT ACAAGACTGG GCGCGTGCTG
 GAACACCCAG ATTGTTCCCC ATGTCAGGAC TGCAGCCACA AAGCCAATTT ATCAGGTGGT GTTTGGGAAG ATAACATCAA CATGGCTCTG
 CTTGTTGACA CATACTACGA TGACCAACTC ATTAGCTGTG GCAGTGTCCA CAGAGGGACC TGCCAGCGAC ATATCCTTCC ACCCAGCAAT
 ATTGCTGACA TACAGTCGGA AGTGCATTGC ATGTACTCCT CACAGGCAGA CGAAGAGCCC AGCCAGTGCC CTGACTGTGT GGTGAGTGCT
 CTAGGAACCA AAGTCTGAT ATCAGAAAAG GACCGGTTCA TCAACTTCTT CGTAGGCAAT ACCATAAATT CCTCGGACCA TCCAGATCAT
 TCATTGCATT CGATATCGGT GAGAAGGCTA AAGGAAACGC AAGATGGGTT CAAGTTTTTG ACAGACCACT CTTACATTGA TGTCTACCG
 GAGTTCAGAG ACTCCTACCC CATTAAATAT GTCCACGCCT TTGAAAGCAA CCACTTTATT TACTTTTTGA CAGTCCAGCG AGAAACTCTA
 GATGCTCAGA CTTTTCACAC GAGAATAATC AGGTTCGTGT CTGTAGACTC TGGATTGCAT TCCTACATGG AAATGCCTCT GGAGTGATT
 CTCACGAAA AGAGAAGAAA GAGATCCACA AGGGAGGAAG TGTTAATAT TCTCCAAGCT GCATATGTCA GTAAGCCTGG GCGCCATCTC
 GCTAAACAAA TAGGTGCCAA CCTGAATGAT GACATTCTCT ATGGAGTGT CGCACAAAGC AAGCCAGATT CTGCTGAACC AATGAATCGC
 TCTGCCGTCT GTGCGTTCCC TATCAATAT GTCAATGAAT TCTTCAACAA GATCGTCAAC AAAACAATG TGAGATGTCT TCAGCACTTT
 TATGGACCCA ACCACGAACA CTGCTTTAAT AGGACACTTT TGAGAAATTC ATCGGGCTGT GAAGCGCGCA ATGATGAATA TCGAACGGAG
 TTCCTACAG CTTTGACGCG CGTTGACTTA TTCATGGGCC AGTTCAACCA AGTCTCTTA ACGTCTATAT CCACCTTCAT CAAAGGAGAC
 CTCACCATTT CTAATCTTGG GACGTCGAG GGTGCTTCA TGCAGGTGCT GGTTCCTCGA TCAGGATTGT CGACCCCTCA CGTGAATTC
 CGCTGGACT CCCACCCCGT GTCTCCAGAA GCAATTGTGG AGCACCCACT AAACCAAAAC GGCTACACAC TCGTTGTAC TGGGAAGAAG
 ATCACCAGGA TCCCACTGAA TGGCTTAGGC TGTGAGCATT TTCAGTCTG CAGTCAGTGT CTCTCCGCC CTCCCTTTGT GCAGTGTGGC
 TGGTGCCACG ATAGATGTGT GCACCTGGAG GAATGTCCCA CTGGAGCGTG GACTCAGGAG GTCTGTCTGC CTGCAATCTA TGAGGTTTTT
 CCAACTAGTG CACCCCTGGA AGGAGGGACA GTGCTGACTG TATGTGGCTG GGACTTCGGA TTCAGGAGGA ATAATAAATT TGATTTAAAG
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 GTGAACGAGC ATTTCAATAT ATCCATAATT ATTTCAAATG GTCGAGGAC AGCACAATAT AGTACATTTT CGTATGTGGA TCCTATTATA
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 ATTTCAATGG GTGAAAAAAC ATGTACTTTA AAAAGTGTGT CAGATAGTAT TCTCGAATGT TATACCCAG CTCAAGCCAC TGCAACTGAG
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 CTGCTGAAAT TGAACAACGA GCTAAATATA GAGTGAAGC AAGCAGTTTC TTCAACCGTC CTGGAAAAAG TAATAGTTCA ACCAGATCAG
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 ACTGTCCACA TTGACCTCAG TGCTCTAAAT CCAGAGCTGG TGCAGGCAGT CCAGCATGTA GTGATTGGGC CCAGTAGCCT GATTGTGCAT
 TTCAATGAAG TCATAGGAAG AGGACATTTT GGGTGTGTAT ACCATGGGAC TTTGTGGAC AATGACGACA AAAAAATTC CTGTGCTGTG
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 CTCTCACTCT TGGGAATCTG CCTTCGAAGT GAGGGGTCTC CACTGGTGGT CCTACCATAC ATGAAACATG GAGATCTTCG AAATTTCTATT
 AGAAATGAGA CTCATAACCC AACTGTAAAA GATCTTATTG GCTTTGGTCT TCAAGTAGCC AAAGGCATGA AATATCTTGC AAGCAAAAAAG
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 TATGATAAAG AATACTACAG CGTACACAAC AAAACAGGCG CCAAACTACC AGTGAAGTGG ATGGCTTTAG AAAGTCTGCA AACTCAGAAG
 TTTACCACCA AGTCAGATGT GTGGTCTTT GCGTGCTCC TCTGGAACT GATGACAAGA GGAGCACCAC CTTATCCTGA CGTCAACACC

TTTGATATAA CAGTTTACTT GTTGAAGGC AGAAGGCTCC TGCAACCCGA ATACTGCCCA GATCCCTTAT ACGAAGTGAT GCTAAAATGC
 TGGCACCCCTA GAGCTGAAC TGGCCCATCT TTTTCTGAAC TGGTCTCCAG GATATCAGCA ATATTCTCTA CTTTCATTGG GGAGCACTAT
 GTCCATGTGA ACGCCACTTA TGTGAATGTC AAATGTGTTG CTCATACCC TTCTCTCTTG TCATCACAAG ATAACATTGA TGGCGAGGGG
 GACACATGA

SEQ ID NO:2

Canine Met Gl630A mutant sequence

1 ATGAAGGCTC CTGCTGTGCT TGCACCTGGC ATCCTTGTGC TTCTGTTTAC
 51 CTTGGTGCAG AAGAGCTATG GGGAGTGCAA AGAGGCACTA GTAAAGTCTG
 101 AGATGAATGT GAACATGAAG TATCAGCTTC CCAACTTCAC TGCCGAAACA
 151 CCCATCCAGA ATGTTGTTTT ACACAAGCAT CATATTTACC TTGGTGCAGT
 201 TAACTATATT TACGTTTTAA ATGACAAAGA CCTTCAGAAG GTTGCTGAGT
 251 ACAAGACTGG GCCCGTGCTG GAACACCCAG ATTGTTCCCC ATGTCAGGAC
 301 TGCAGCCACA AAGCCAATTT ATCAGGTGGT GTTTGGGAAG ATAACATCAA
 351 CATGGCTCTG CTTGTTGACA CATACTACGA TGACCAACTC ATTAGCTGTG
 401 GCAGTGTCCA CAGAGGGACC TGCCAGCGAC ATATCCTTCC ACCCAGCAAT
 451 ATTGCTGACA TACAGTCGGA AGTGCATTGC ATGTACTCCT CACAGGCAGA
 501 CGAAGAGCCC AGCCAGTGCC CTGACTGTGT GGTGAGTGCT CTAGGAACCA
 551 AAGTCCTGAT ATCAGAAAAG GACCGGTTCA TCAACTTCTT CGTAGGCAAT
 601 ACCATAAATT CCTCGGACCA TCCAGATCAT TCATTGCATT CGATATCGGT
 651 GAGAAGGCTA AAGGAAACGC AAGATGGGT CAAGTTTTTG ACAGACCAGT
 701 CTTACATTGA TGTTCTACCG GAGTTCAGAG ACTCCTACCC CATTAATAT
 751 GTCCACGCCT TTGAAAGCAA CCACTTTATT TACTTTTTGA CAGTCCAGCG
 801 AGAACTCTA GATGCTCAGA CTTTTCACAC GAGAATAATC AGGTTCTGTT
 851 CTGTAGACTC TGGATTGCAT TCCTACATGG AAATGCCTCT GGAGTGTATT
 901 CTCACGGAAA AGAGAAGAAA GAGATCCACA AGGGAGGAAG TGTTTAATAT
 951 TCTCCAAGTT GCATATGTCA GTAAGCCTGG GGCCCATCTC GCTAAACAAA
 1001 TAGGTGCCAA CCTGAATGAT GACATTCTCT ATGGAGTGTT CGCACAAAGC
 1051 AAGCCAGATT CTGCTGAACC AATGAATCGC TCTGCCGTCT GTGCGTTCCC
 1101 TATCAAATAT GTCAATGAAT TCTTCAACAA GATCGTCAAC AAAACAATG
 1151 TGAGATGTCT TCAGCACTTT TATGGACCCA ACCACGAACA CTGCTTTAAT
 1201 AGGACACTTT TGAGAAATTC ATCGGGCTGT GAAGCGCGCA ATGATGAATA
 1251 TCGAACGGAG TTTACTACAG CTTTGCAGCG CGTTGACTTA TTCATGGGCC
 1301 AGTTCAACCA AGTCCTCTTA ACGTCTATAT CCACCTTCAT CAAAGGAGAC
 1351 CTCACCATTG CTAATCTTGG GACGTCCGAG GGTCGCTTCA TGCAGGTCGT
 1401 GGTTCCTCGA TCAGGATTGT CGACCCCTCA CGTGAACCTC CGCCTGGACT
 1451 CCCACCCCGT GTCTCCAGAA GCAATTGTGG AGCACCCTC AAACCAAAAC
 1501 GGCTACACAC TCGTTGTCTC TGGGAAGAAG ATCACCAGGA TCCCATTGAA
 1551 TGGCTTAGGC TGTGAGCATT TTCAGTCCTG CAGTCAGTGT CTCTCCGCC
 1601 CTCCCTTTGT GCAGTGTGGC TGGTGCCACA ATAGATGTGT GCACCTGGAG
 1651 GAATGTCCCA CTGGAGCGTG GACTCAGGAG GTCTGTCTGC CTGCAATCTA
 1701 TGAGGTTTTT CCAACTAGTG CACCCCTGGA AGGAGGGACA GTGCTGACTG
 1751 TATGTGGCTG GGACTTCGGA TTCAGGAGGA ATAATAAATT TGATTTAAAG
 1801 AAAACCAAAG TTTTCCTTGG AAATGAGAGC TGCACCTTGA CCTTAAGTGA
 1851 GAGCACAACA AATATGCTGA AATGCACAGT TGGCCCTGCA GTGAACGAGC
 1901 ATTTCAATAT ATCCATAATT ATTTCAAATG GTCGAGGGAC AGCACAATAT
 1951 AGTACATTTT CGTATGTGGA TCCTATTATA ACAAGTATTT CTCCAAGTTA
 2001 TGGTCCCAAG AATGGTGGCA CCTTGCTCAC TTTAACTGGA AAATACCTCA
 2051 ACAGTGGGAA TTCTAGACAC ATTTCAATGG GTGGAAAAAC ATGTACTTTA
 2101 AAAAGTGTGT CAGATAGTAT TCTCGAATGT TATACCCAG CTCAAGCCAC
 2151 TGCAACTGAG TTTCTATTAT AATTGAAAAT TGACCTAGCC AACCAGAGAG
 2201 TGAACAGCTT CAGTTACCAG GAAGACCCCA TTGTCTATGC AATTCATCCA
 2251 ACGAAATCTT TTATTAGTGG TGGGAGCACA ATAACAGCTG TTGGAAAAAA
 2301 CCTGAATTCA GTGAGTGTCC TGAGGATGGT AATAGATGTC CATGAAACAA
 2351 GAAGGAACCT TACAGTGGCA TGTCACATC GCTCTAATTC AGAGATAATC
 2401 TGTGTACGA CTCCTTCACT GCAACAGCTG AATCTGCAAC TCCCTCTGAA
 2451 AACCAAAGCC TTTTTCATGT TAGATGGGAT CCATTCCAAA TACTTTGATC
 2501 TCATTTATGT ACATAATCCT GTGTTTAAAG CTTTGTAAAA GCCAGTGATG
 2551 ATCTCAATAG GCAATGAAAA TGTACTGGAA ATTAAGGGAA ATGATATTGA

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2601 CCCTGAAGCA GTTAAAGGCG AAGTGTTAAA AGTTGGAAAT AAGAGCTGTG
2651 AGACTATCTA CTCAGATTCT AAAGCCGTTT TATGCAAGGT CCCCAGTAC
2701 CTGCTGAAAT TGAACAACGA GCTAAATATA GAGTGGAAAG AAGCAGTTTC
2751 TTCAACCGTC CTTGGAAAAAG TAATAGTTCA ACCAGATCAG AATTTACACAG
2801 GATTGATTGC TGGTGTATC TCAATATCAA CAATAGTCTT ATTATTACTC
2851 GGAATTTTCC TGTGGCTGAA AAGGAAAAAG CAAATTAAAG ATCTGGGCAG
2901 TGAATTAGTT CGCTATGATG CAAGAGTACA CACTCCTCAT TTGGATAGGC
2951 TTGTAAGTGC CCGAAGTGTA AGCCCAACTA CAGAAATGGT TTCAAATGAA
3001 TCTGTAGACT ACCGAGCTAC TTTTCCAGAA GACCAGTTTC CTAATTCATC
3051 TCAGAATGGA TCATGCAGAC AAGTACAATA TCCTCTGACG GACCTGTCCC
3101 CCATGCTTAC TAGTGGGGAC TCTGATATAT CCAGTCCATT ATTGCAAAAT
3151 ACTGTCCACA TTGACCTCAG TGCTCTAAAT CCAGAGCTGG TGCAGGCAGT
3201 CCAGCATGTA GTGATTGGGC CCAGTAGCCT GATTGTGCAT TTCAATGAAG
3251 TCATAGGAAG AGGACATTTT GGGTGTGTAT ACCATGGGAC TTTGTTGGAC
3301 AATGACGACA AAAAAATTCA CTGTGCTGTG AAATCCCTGA ATAGAATCAC
3351 TGACATAGGA GAAGTTTCCC AGTTTCTGAC CGAGGGAATC ATCATGAAAG
3401 ATTTTAGTCA TCCAAACGTA CTCTCACTCT TGGGAATCTG CCTTCGAAGT
3451 GAGGGGTCTC CACTGGTGGT CCTACCATAC ATGAAACATG GAGATCTTCG
3501 AAATTTTCAAT AGAAATGAGA CTCATAACCC AACTGTAAAA GATCTTATTG
3551 GCTTTGGTCT TCAAGTAGCC AAAGGCATGA AATATCTTGC AAGCAAAAAG
3601 TTTGTCCACA GAGACTTGGC TGCAAGAAAC TGTATGCTGG ATGAAAAATT
3651 CACTGTCAAG GTTGCTGATT TTGGTCTTGC CAGAGACATG TATGATAAAG
3701 AATACTACAG CGTACACAAC AAAACAGGCG CCAACTACC AGTGAAGTGG
3751 ATGGCTTTAG AAAGTCTGCA AACTCAGAAG TTTACCACCA AGTCAGATGT
3801 GTGGTCCTTT GGCGTGCTCC TCTGGGAACT GATGACAAGA GGAGCACCAC
3851 CTTATCCTGA CGTCAACACC TTTGATATAA CAGTTTACTT GTTGCAAGGC
3901 AGAAGGCTCC TGCAACCCGA ATACTGCCCA GATCCCTTAT ACGAAGTGAT
3951 GCTAAAATGC TGGCACCTTA GAGCTGAACT GCGCCCATCT TTTTCTGAAC
4001 TGGTCTCCAG GATATCAGCA ATATTCTCTA CTTTCATTGG GGAGCACTAT
4051 GTCCATGTGA ACGCCACTTA TGTGAATGTC AAATGTGTTG CTCCATACCC
4101 TTCTCTCTTG TCATCACAAG ATAACATTGA TGGCGAGGGG GACACATGA

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SEQ ID NO:3

forward primer for amplification of Canine Met G966S (G2896A) mutant sequence

gcaatccacgagcccatgag

SEQ ID NO:4

reverse primer for amplification of Canine Met G966S (G2896A) mutant sequence

gtagttgggcttacacttcggg

SEQ ID NO:5

forward primer for amplification of Canine Met D544N mutant sequence

gtcctgcagtcagtgctctctccg

SEQ ID NO:6

reverse primer for amplification of Canine Met D544N mutant sequence

ggtgcagctctcatttccaagg

SEQ ID NO:7

Canine Met D544N mutant sequence

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1  MKAPAVLAPG ILVLLFTLVQ KSYGECKEAL VKSEMNVNMK YQLPNFTAET
51  PIQNVVLHKH HIYLGAVNYI YVLNDKDLQK VAEYKTGPVL EHPDCSPCQD
101 CSHKANLSGG VWEDNINMAL LVDITYDDQL ISCGSVHRGT CQRHILPPSN
151 IADIQSEVHC MYSSQADEEP SQCPDCVVS LGTKVLISEK DRFINFFVGN
201 TINSSDHPDH SLHSISVRR LKETQDGFKFL TDQSYIDVLP EFRDSYPIKY
251 VHAFESNHFI YFLTVQRETL DAQTFHTRII RFCSVDSGLH SYMEMPLECI
301 LTEKRRKRST REEVFNILQA AYSKPGAHL AKQIGANLND DILYGVFAQS
351 KPDSAEPMNR SAVCAFPYKY VNEFFNKIVN KNNVRCLQHF YGPNHEHCEN

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401  RTLLRNSSGC  EARNDEYRTE  FTTALQRVDL  FMGQFNQVLL  TSISTFIKGD
451  LTIANLGTSE  GRFMQVVVSR  SGLSTPHVNF  RLDSPVVSPE  AIVEHPLNQN
501  GYTLVVTGKK  ITRIPLNGLG  CEHFQSCSQC  LSAPPFVQCG  WCHNRCVHLE
551  ECPTGAWTQE  VCLPAIYEVF  PTSAPLEGGT  VLTVCGWDFG  FRRNNKFDLK
601  KTKVFLGNES  CCTLSESTT  NMLKCTVGPA  VNEHFNISII  ISNGRGTAQY
651  STFSYVDPII  TSISPSYGPK  NGGTLTTLTG  KYLNSGNSRH  ISMGGKTCTL
701  KSVSDSILEC  YTPAQATATE  FPIKLKIDLA  NREMNSFSYQ  EDPIVYAIHP
751  TKSFISGGST  ITAVGKNLNS  VSVLRMVIDV  HETRRNFTVA  CQHRNSSEII
801  CCTTPSLQQL  NLQLPLKTKA  FFMLDGIHSK  YFDLIYVHNP  VFKPFKEKPV
851  ISIGNENVLE  IKGNDIDPEA  VKGEVLKVG  KSCETIYSDS  KAVLCKVPND
901  LLKLNNELNI  EWKQAVSSTV  LGKVIVQPDQ  NFTGLIAGVI  SISTIVLLLL
951  LLKLWLRKK  QIKDLGSELV  RYDARVHTPH  LDRLVSARSV  SPTTEMVSNE
1001 SVDYRATFPE  DQFPNSSQNG  SCRQVQYPLT  DLSPMLTSGD  SDISSPLLQN
1051 TVHIDLALN  PELVQAVQHV  VIGPSSLIVH  FNEVIGRGHF  GCVYHGTLDD
1101 NDDKKIHCAV  KSLNRITDIG  EVSQFLTEGI  IMKDFSHPNV  LSLLGICLRS
1151 EGSPLVLPY  MKHGDLRNFI  RNETHNPTVK  DLIGFGLQVA  KGMKYLASKK
1201 FVHRDLAARN  CMLDEKFTVK  VADFGGLARM  YDKEYYSVHN  KTGAKLPVKW
1251 MALESLQTQK  FTTKSDVWSF  GVLLWELMTR  GAPYPDVNT  FDITVYLLQG
1301 RRLQLQPEYCP  DPLYEVMLKC  WHPRAELRPS  FSELVSRISA  IFSTFIGEHY
1351 VHVNATYVNV  KCVAPYPSLL  SSQDNIDGEG  DT*

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SEQ ID NO:8

wild-type Met nucleotide sequence

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1   ATGAAGGCTC  CTGCTGTGCT  TGCACCTGGC  ATCCTTGTC  TTCTGTTTAC
51  CTTGGTGCAG  AAGAGCTATG  GGGAGTGCAA  AGAGGCACTA  GTAAAGTCTG
101 AGATGAATGT  GAACATGAAG  TATCAGCTTC  CCAACTTCAC  TGCCGAAACA
151 CCCATCCAGA  ATGTTGTTTT  ACACAAGCAT  CATATTTACC  TTGGTGCAGT
201 TAACTATATT  TACGTTTTAA  ATGACAAAGA  CCTTCAGAAG  GTTGCTGAGT
251 ACAAGACTGG  GCCCGTGCTG  GAACACCCAG  ATTGTTCCCC  ATGTCAGGAC
301 TGCAGCCACA  AAGCCAATTT  ATCAGGTGGT  GTTTGGGAAG  ATAACATCAA
351 CATGGCTCTG  CTTGTTGACA  CATACTACGA  TGACCAACTC  ATTAGCTGTG
401 GCAGTGCCA  CAGAGGGACC  TGCCAGCGAC  ATATCCTTCC  ACCCAGCAAT
451 ATTGCTGACA  TACAGTCGGA  AGTGCATTGC  ATGTACTCCT  CACAGGCAGA
501 CGAAGAGCCC  AGCCAGTGCC  CTGACTGTGT  GGTGAGTGCT  CTAGGAACCA
551 AAGTCCTGAT  ATCAGAAAAG  GACCGGTTCA  TCAACTTCTT  CGTAGGCAAT
601 ACCATAAATT  CCTCGGACCA  TCCAGATCAT  TCATTGCATT  CGATATCGGT
651 GAGAAGGCTA  AAGGAAACGC  AAGATGGGTT  CAAGTTTTTG  ACAGACCAGT
701 CTTACATTGA  TGTTCTACCG  GAGTTCAGAG  ACTCCTACCC  CATTAAATAT
751 GTCCACGCCT  TTGAAAGCAA  CCACTTTATT  TACTTTTTGA  CAGTCCAGCG
801 AGAAACTCTA  GATGCTCAGA  CTTTTCACAC  GAGAATAATC  AGGTTCTGTT
851 CTGTAGACTC  TGGATTGCAT  TCCTACATGG  AAATGCCTCT  GGAGTGTATT
901 CTCACGAAA  AGAGAAGAAA  GAGATCCACA  AGGGAGGAAG  TGTTTAATAT
951 TCTCCAAGCT  GCATATGTCA  GTAAGCCTGG  GGCCCATCTC  GCTAAACAAA
1001 TAGGTGCCAA  CCTGAATGAT  GACATTCTCT  ATGGAGTGTT  CGCACAAAGC
1051 AAGCCAGATT  CTGCTGAACC  AATGAATCGC  TCTGCCGTCT  GTGCGTTCCC
1101 TATCAAATAT  GTCAATGAAT  TCTTCAACAA  GATCGTCAAC  AAAACAATG
1151 TGAGATGTCT  TCAGCACTTT  TATGGACCCA  ACCACGAACA  CTGCTTTAAT
1201 AGGACACTTT  TGAGAAATTC  ATCGGGCTGT  GAAGCGCGCA  ATGATGAATA
1251 TCGAACGGAG  TTTACTACAG  CTTTGCAGCG  CGTTGACTTA  TTCATGGGCC
1301 AGTTCAACCA  AGTCCTCTTA  ACGTCTATAT  CCACCTTCAT  CAAAGGAGAC
1351 CTCACCATTG  CTAATCTTGG  GACGTCCGAG  GGTGCTTCA  TGCAGTGGT
1401 GGTTCCTCGA  TCAGGATTGT  CGACCCCTCA  CGTGAACCTC  CGCAGTGGACT
1451 CCCACCCCGT  GTCTCCAGAA  CGAATTGTGG  AGCACCCACT  AAACCAAAAC
1501 GGCTACACAC  TCGTTGTCAC  TGGGAAGAAG  ATCACCAGGA  TCCCACTGAA
1551 TGGCTTAGGC  TGTGAGCATT  TTCAGTCCTG  CAGTCAGTGT  CTCTCCGCCC
1601 CTCCCTTTGT  GCAGTGTGGC  TGGTGCCACG  ATAGATGTGT  GCACCTGGAG
1651 GAATGTCCCA  CTGGAGCGTG  GACTCAGGAG  GTCTGTCTGC  CTGCAATCTA
1701 TGAGGTTTTT  CCAACTAGTG  CACCCCTGGA  AGGAGGGACA  GTGCTGACTG
1751 TATGTGGCTG  GGACTTCGGA  TTCAGGAGGA  ATAATAAATT  TGATTTAAAG
1801 AAAACCAAAG  TTTTCCTTGG  AAATGAGAGC  TGCACCTTGA  CCTTAAGTGA

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1851 GAGCACAACA AATATGCTGA AATGCACAGT TGGCCCTGCA GTGAACGAGC
1901 ATTTCAATAT ATCCATAATT ATTTCAAATG GTCGAGGGAC AGCACAATAT
1951 AGTACATTTT CGTATGTGGA TCCTATTATA ACAAGTATTT CTCCAAGTTA
2001 TGGTCCCAAG AATGGTGGCA CCTTGCTCAC TTTAACTGGA AAATACCTCA
2051 ACAGTGGGAA TTCTAGACAC ATTTCAATGG GTGGAAAAAC ATGTACTTTA
2101 AAAAGTGTGT CAGATAGTAT TCTCGAATGT TATACCCAG CTCAAGCCAC
2151 TGCAACTGAG TTTCTTATTA AATTGAAAAA TGACCTAGCC AACCAGAGAGA
2201 TGAACAGCTT CAGTTACCAG GAAGACCCCA TTGTCTATGC AATTCATCCA
2251 ACGAAATCTT TTATTAGTGG TGGGAGCACA ATAACAGCTG TTGGAAAAAA
2301 CCTGAATTCA GTGAGTGTCC TGAGGATGGT AATAGATGTC CATGAAACAA
2351 GAAGGAACTT TACAGTGGCA TGTCAACATC GCTCTAATTC AGAGATAATC
2401 TGTTGTACGA CTCCTTCACT GCAACAGCTG AATCTGCAAC TCCCTCTGAA
2451 AACCAAAGCC TTTTTCATGT TAGATGGGAT CCATTCCAAA TACTTTGATC
2501 TCATTTATGT ACATAATCCT GTGTTTAAAG CTTTGTAAAA GCCAGTGATG
2551 ATCTCAATAG GCAATGAAAA TGTACTGGAA ATTAAGGGAA ATGATATTGA
2601 CCCTGAAGCA GTTAAAGGCG AAGTGTTAAA AGTTGGAAAT AAGAGCTGTG
2651 AGACTATCTA CTCAGATTCT AAAGCCGTTT TATGCAAGGT CCCCATGAC
2701 CTGCTGAAAT TGAACAACGA GCTAAATATA GAGTGAAGC AAGCAGTTTC
2751 TTCAACCGTC CTTGGAAAAG TAATAGTTCA ACCAGATCAG AATTTACAG
2801 GATTGATTGC TGGTGTATC TCAATATCAA CAATAGTCTT ATTATTACTC
2851 GGACTTTTCC TGTGGCTGAA AAGGAAAAAG CAAATTAAG ATCTGGGCAG
2901 TGAATTAGTT CGCTATGATG CAAGAGTACA CACTCCTCAT TTGGATAGGC
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3001 TCTGTAGACT ACCGAGCTAC TTTTCCAGAA GACCAGTTTC CTAATTCATC
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3151 ACTGTCCACA TTGACCTCAG TGCTCTAAAT CCAGAGCTGG TGCAGGCAGT
3201 CCAGCATGTA GTGATTGGGC CCAGTAGCCT GATTGTGCAT TTCAATGAAG
3251 TCATAGGAAG AGGACATTTT GGGTGTGTAT ACCATGGGAC TTTGTTGGAC
3301 AATGACGACA AAAAAATTCA CTGTGCTGTG AAATCCCTGA ATAGAATCAC
3351 TGACATAGGA GAAGTTTCCC AGTTTCTGAC CGAGGGAATC ATCATGAAAG
3401 ATTTTAGTCA TCCAAACGTA CTCTCACTCT TGGGAATCTG CCTTCGAAGT
3451 GAGGGGTCTC CACTGGTGGT CCTACCATAC ATGAAACATG GAGATCTTCG
3501 AAATTTTATT AGAAATGAGA CTCATAACCC AACTGTAAAA GATCTTATTG
3551 GCTTTGGTCT TCAAGTAGCC AAAGGCATGA AATATCTTGC AAGCAAAAAG
3601 TTTGTCCACA GAGACTTGGC TGCAAGAAAC TGTATGCTGG ATGAAAAAAT
3651 CACTGTCAAG GTTGCTGATT TTGGTCTTGC CAGAGACATG TATGATAAAG
3701 AATACTACAG CGTACACAAC AAAACAGGCG CCAAACTACC AGTGAAGTGG
3751 ATGGCTTTAG AAAGTCTGCA AACTCAGAAG TTTACCACCA AGTCAGATGT
3801 GTGGTCCTTT GCGTGCTCC TCTGGGAAC TATGACAAGA GGAGCACCAC
3851 CTTATCCTGA CGTCAACACC TTTGATATAA CAGTTTACTT GTTGCAAGGC
3901 AGAAGGCTCC TGCAACCCGA ATACTGCCCA GATCCCTTAT ACGAAGTGAT
3951 GCTAAAATGC TGGCACCCCTA GAGCTGAACT GCGCCCATCT TTTTCTGAAC
4001 TGGTCTCCAG GATATCAGCA ATATTCTCTA CTTTCATTGG GGAGCACTAT
4051 GTCCATGTGA ACGCCACTTA TGTGAATGTC AAATGTGTTG CTCCATACCC
4101 TTCTCTCTTG TCATCACAAG ATAACATTGA TGGCGAGGGG GACACATGA

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SEQ ID NO:9

wild-type Met amino acid sequence

Dog Met	1	MKAPAVLAPG	ILVLLFTLVQ	KSYGECKEAL	VKSEMNVNMK	YQLPNFTAET
Dog Met	51	PIQNVVLHKH	HIYLGAVNYI	YVLNDKDLQK	VAEYKTGPVL	EHPDCSPCQD
Dog Met	101	CSHKANLSSG	VWEDNINMAL	LVDYTYDDQL	ISCGSVHRGT	CQRHILPPSN
Dog Met	151	IADIQSEVHC	MYSSQADEEP	SQCPDCVSA	LGTKVLISEK	DRFINFFVGN
Dog Met	201	TINSSDHPDH	SLHSISVRL	KETQDGFKFL	TDQSYIDVLP	EFRDSYPIKY
Dog Met	251	VHAFESNHFI	YFLTQVRETL	DAQTFHTRII	RFCSVDSGLH	SYMEMPLECTI
Dog Met	301	LTEKRRKRST	REEVFNILQA	AYVSKPGAHL	AKQIGANLND	DILYGVFAQS
Dog Met	351	KPDSAEPNMR	SAVCAFPKIY	VNEFFNKIVN	KNNVRCLQHF	YGNPNEHCEN
Dog Met	401	RTLLRNSSGC	EARNDEYRTE	FTTALQVRDL	FMGQFNQVLL	TSISTFIKGD
Dog Met	451	LTIANLGTSE	GRFMQVVSR	SGLSTPHVNF	RLDSHPVSPE	AIVEHPLNQN
Dog Met	501	GYTLVVTGKK	ITRIPLNGLG	CEHFQSCSQC	LSAPPFVQCG	WCHDRCVHLE
Dog Met	551	ECPTGAWTQE	VCLPAIYEVF	PTSAPLEGGT	VLTVCGWDFG	FRRNNKFDLK

Dog Met	601	KTKVFLGNES	CTLTLSESTT	NMLKCTVGPA	VNEHFNISII	ISNGRGTAQY
Dog Met	651	STFSYVDPII	TSISPSYGPK	NGGTLLTLTG	KYLNSGNSRH	ISMGGKTCTL
Dog Met	701	KSVSDSILEC	YTPAQATATE	FPIKLIKIDLA	NREMNSFSYQ	EDPIVYAIHP
Dog Met	751	TKSFISGGST	ITAVGKNLNS	VSVLRMVIDV	HETRRNFTVA	CQHRSNSEII
Dog Met	801	CCTTPSLQQL	NLQLPLKTKA	FFMLDGIHSK	YFDLIYVHNP	VFKPFEKPVM
Dog Met	851	ISIGNENVLE	IKGNDIDPEA	VKGEVLKVG	KSCETIYSDS	KAVLCKVPND
Dog Met	901	LLKLNNELNI	EWKQAVSSTV	LGKVIVQPDQ	NFTGLIAGVI	SISTIVLLLL
Dog Met	951	GLFLWLKRKK	QIKDLGSELV	RYDARVHTPH	LDRLVSARSV	SPTTEMVSNE
Dog Met	1001	SVDYRATFPE	DQFPNSSQNG	SCRQVQYPLT	DLSPMLTSGD	SDISSPLLQN
Dog Met	1051	TVHIDLSALN	PELVQAVQHV	VIGPSSLIVH	FNEVIGRGHF	GCVYHGTLDD
Dog Met	1101	NDDKKIHCAV	KSLNRITDIG	EVSQFLTEGI	IMKDFSHPNV	LSLLGICLRS
Dog Met	1151	EGSPLVVLPI	MKHGDLRNFI	RNETHNPTVK	DLIGFGLQVA	KGMKYLASKK
Dog Met	1201	FVHRDLAARN	CMLDEKFTVK	VADFGLARDM	YDKEYYSVHN	KTGAKLPVKW
Dog Met	1251	MALESLQTQK	FTTKSDVWSF	GVLLWELMTR	GAPPYPDVNT	FDITVYLLQG
Dog Met	1301	RRLQLQPEYCP	DPLYEVMKLC	WHPRAELRPS	FSELVSRISA	IFSTFIGEHY
Dog Met	1351	VHVNATYVNV	KCVAPYPSLL	SSQDNIDGEG	DT*	