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TITULO: CICLOOXIGENASA-1(COX-1)CANINA Y CICLOOXIGENASA-2(COX-2)

SOLICITANTE: G.D. SEARLE & CO.

INDICE DE DOCUMENTOS QUE SE ACOMPAÑAN

1 Copia certificada por la Oficina de Patentes de
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para la misma invención, de la cual REIVINDICAMOS
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This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

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[X] Additional inventors are being named on the 1 separately numbered sheets attached hereto

TITLE OF THE INVENTION (280 characters max)

Canine Cyclooxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2)

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ENCLOSED APPLICATION PARTS (check all that apply)

[X] Specification Number of Pages [ ] Small Entity Statement

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Respectfully submitted,

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CANINE CYCLOOXYGENASE -1 (COX-1) AND CYCLOOXYGENASE-2 (COX-2)

Field of the Invention

The present invention relates to genes that encode proteins of the canine cyclooxygenase-1 (COX-1) and the cyclooxygenase-2 (COX-2) enzymes, the canine COX-1 and canine COX-2 proteins, and assays for the evaluation of canine COX-1 and COX-2 activities. The invention also relates to nucleic acid molecules associated with these genes including their complements, homologues and fragments, and to methods of using these nucleic acid molecules, the enzymes and fragments of either.

Background

Millions of dogs are kept either as pets or for utilitarian uses (e.g., seeing-eye dogs or guard dogs) (Olson and Moulton, *J Reprod Fertil Suppl* 47:433-8 (1993)). It is well known that chronic inflammatory processes such as degenerative joint disease are common in canine species, ie. dogs, especially older dogs. There are, however, few drugs that can be successfully used in veterinary medicine for the treatment of inflammation (*Compendium of Veterinary Products*, Bennett 2d ed., 1993). Therefore, there is a large ongoing effort to find pharmaceutical agents which will impede or altogether stop the progress of such inflammatory disease processes in dogs, or at least ameliorate the symptoms of the inflammation such as pain and edema.

The non-steroidal anti-inflammatory drugs, or NSAIDs, have been investigated extensively for anti-inflammatory activity in humans and in a more limited manner, in dogs. Only two NSAIDs have been approved for use in dogs by the Food and Drug Administration, Committee on Veterinary Medicine (FDA/CVM), which has resulted in substantial off-label use in dogs of NSAIDs approved for humans. NSAID use in dogs has led to severe injury and even death due to gastrointestinal hemorrhage,

128-176

perforation and peritonitis, and kidney and liver toxicity. Although NSAID toxicities are also seen in humans, they are more pronounced in dogs due to inappropriately high doses from a lack of dosing information, as well as the inherently high degree of canine susceptibility to such adverse reactions. An alternative to NSAIDS, corticosteroids, has even more drastic side effects, especially when long term therapy is involved (MacDonald and Langston, Use of Corticosteroids and Non-steroidal Anti-inflammatory Agents, in: *Textbook of Veterinary Internal Medicine*, 284, Ettinger and Feldman, ed. (1995)).

The toxicity of NSAIDs is a result of their lack of specificity in prostaglandin inhibition. Prostaglandins, a group of compounds derived from arachidonic acid by the action of cyclooxygenase, are known to be involved in the inflammatory process. Some of these prostaglandins mediate inflammation; however, some of these prostaglandins also have a gastrointestinal protective effect. Previous NSAIDs were found to prevent prostaglandin release by inhibiting the enzyme cyclooxygenase (COX) (Rubin and Papich, *Curr. Vet. Ther.* X:47-54 (1989)). The recent discovery of an inducible enzyme associated with the inflammation (named cyclooxygenase II (COX-2), provides a viable target of inhibition which is more effectively reduces inflammation and produces fewer and less drastic side effects. It has now been shown that the COX-2 isozyme is responsible for the production of prostaglandins exclusively or primarily at inflammatory sites. Therefore, a new class of drugs that selectively inhibit the COX-2 isozyme, but not COX-1, can reduce inflammation without the side effects of gastrointestinal toxicity.

For many years, the only known cyclooxygenase was the COX-1 isoform. Since its discovery, the inducible form of cyclooxygenase-2 has been cloned, sequenced, and characterized from sheep, murine, and human sources. These purified enzymes have provided a means for assays and materials to identify and evaluate human pharmaceutical agents that selectively inhibit COX-2 or COX-2 activity over COX-1 or COX-1 activity. A substantially-purified canine form of the COX-1 and COX-2 enzymes would therefore provide a means to carry out assays and materials

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valuable in identifying drugs for use in dogs that can reduce inflammation without the toxic effects of current canine therapies. Thus, there is a substantial need in the art for the cloned, sequenced and characterized canine COX-1 and COX-2, and assays to screen and evaluate selective inhibitors of COX-2.

Summary of the Invention

The present invention provides a substantially-purified nucleic acid molecule which specifically hybridizes to SEQ ID: X or complement thereof, wherein the substantially-purified nucleic acid molecule encodes for a canine COX-2 protein or fragment thereof or complement of either. The present invention also provides a substantially-purified nucleic acid molecule which specifically hybridizes to SEQ ID: X or complement thereof, wherein the substantially-purified nucleic acid molecule encodes for a canine COX-1 protein or fragment thereof or complement of either. Substantially-purified protein sequences of canine COX-1 and COX-2 are also disclosed. This invention also provides assays and materials to identify and evaluate veterinary agents that are potent inhibitors of canine COX-2.

Brief Description of the Drawings

Fig. 1 diagramatically sets forth a comparison between the amino acid sequence which codes for canine COX-1 and the amino acid sequence which codes for human COX-1.

Fig. 2 diagramatically sets forth a comparison between the amino acid sequences of canine COX-2 and human COX-2.

Fig. 3 diagramatically sets forth a comparison between the nucleic acid sequences which code for human and canine COX-2, and the consensus sequence of both.

Fig. 4 diagramatically sets forth a comparison between the amino acid sequences of murine, rat, guineapig, rabbit, horse, canine, sheep, human and chicken COX-2.

180 178

SEQ NO: 1 – canine (*Canis familiaris*) COX-1 nucleic acid sequence

SEQ NO: 2 – canine (*Canis familiaris*) COX-1 amino acid sequence

SEQ NO: 3 – canine (*Canis familiaris*) COX-2 nucleic acid sequence

SEQ NO: 4 – canine (*Canis familiaris*) COX-2 amino acid sequence

SEQ NO: 5 – human (*Homo sapiens*) COX-1 nucleic acid sequence

SEQ NO: 6 – human (*Homo sapiens*) COX-1 amino acid sequence

SEQ NO: 7 – human (*Homo sapiens*) COX-2 nucleic acid sequence

SEQ NO: 8 - human (*Homo sapiens*) COX-2 amino acid sequence

SEQ NO: 9 - mouse (*Mus muscus*) COX-2 amino acid sequence

SEQ NO: 10 - rat (*Rattus norvegicus*) COX-2 amino acid sequence

SEQ NO: 11 - guinea pig (*Cavia porcellus*) COX-2 amino acid sequence

SEQ NO: 12 - rabbit (*Oryctolagus cuniculus*) COX-2 amino acid sequence

SEQ NO: 13 - horse (*Equus caballus*) COX-2 amino acid sequence

SEQ NO: 14 - sheep (*Ovis aries*) COX-2 amino acid sequence

SEQ NO: 15 - chicken (*Gallus gallus*) COX-2 amino acid sequence

These detailed descriptions are presented for illustrative purposes only and are not intended as a restriction on the scope of the invention. Rather, they are merely some of the embodiments that one skilled in the art would understand from the entire contents of this disclosure. All parts are by weight and temperatures are in Degrees centigrade unless otherwise indicated.

The following is a list of abbreviations and the corresponding meanings as used interchangeably herein:

IMDM = Iscove's modified Dulbecco's media

mg = milligram

ml = milliliter

mL = milliliter

181 . 179

μg = microgram

μl = microliter

ODNs= oligonucleotides

PCR= polymerase chain reaction

RP-HPLC = reverse phase high performance liquid chromatography

ug = microgram

ul = microliter

The following is a list definitions of various terms used herein:

The term "altered" means that expression differs from the expression response of cells or tissues not exhibiting the phenotype.

The term "amino acid(s)" means all naturally occurring L-amino acids.

The term "chromosome walking" means a process of extending a genetic map by successive hybridization steps.

The term "complete complementarity" means that every nucleotide of one molecule is complementary to a nucleotide of another molecule.

The term "degenerate" means that two nucleic acid molecules encode for the same amino acid sequences but comprise different nucleotide sequences.

The term "exogenous genetic material" means any genetic material, whether naturally occurring or otherwise, from any source that is capable of being inserted into any organism..

The term "expression response" means the mutation affecting the level or pattern of the expression encoded in part or whole by one or more nucleic acid molecules.

The term "fragment" means a nucleic acid molecule whose sequence is shorter than the target or identified nucleic acid molecule and having the identical, the substantial complement, or the substantial homologue of at least 10 contiguous nucleotides of the target or identified nucleic acid molecule.

The term "fusion molecule" means a protein-encoding molecule or fragment that upon expression, produces a fusion protein.

172 180

The term "fusion protein" means a protein or fragment thereof that comprises one or more additional peptide regions not derived from that protein.

The term "marker nucleic acid" means a nucleic acid molecule that is utilized to determine an attribute or feature (*e.g.*, presence or absence, location, correlation, etc.) of a molecule, cell, or tissue.

The term "mimetic" refers to a compound having similar functional and/or structural properties to another known compound or a particular fragment of that known compound.

The term "phenotype" means any of one or more characteristics of an organism, tissue, or cell.

The term "probe" means an agent that is utilized to determine an attribute or feature (e.g. presence or absence, location, correlation, etc.) of a molecule, cell, tissue, or organism.

The term "promoter" is used in an expansive sense to refer to the regulatory sequence(s) that control mRNA production.

The term "protein fragment" means a peptide or polypeptide molecule whose amino acid sequence comprises a subset of the amino acid sequence of that protein.

The term "protein molecule/peptide molecule" means any molecule that comprises five or more amino acids.

The term "recombinant" means any agent (e.g., DNA, peptide, etc.), that is, or results from, however indirectly, human manipulation of a nucleic acid molecule.

The term "selectable or screenable marker genes" means genes who's expression can be detected by a probe as a means of identifying or selecting for transformed cells.

The term "specifically bind" means that the binding of an antibody or peptide is not competitively inhibited by the presence of non-related molecules.

The term "specifically hybridizing" means that two nucleic acid molecules are capable of forming an anti-parallel, double-stranded nucleic acid structure.

The term "substantial complement" means that a nucleic acid sequence shares at least 80% sequence identity with the complement.

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The term "substantial fragment" means a fragment which comprises at least 100 nucleotides.

The term "substantial homologue" means that a nucleic acid molecule shares at least 80% sequence identity with another.

The term "substantially hybridizing" means that two nucleic acid molecules can form an anti-parallel, double-stranded nucleic acid structure under conditions (e.g. salt and temperature) that permit hybridization of sequences that exhibit 90% sequence identity or greater with each other and exhibit this identity for at least a contiguous 50 nucleotides of the nucleic acid molecules.

The term "substantially purified" means that one or more molecules that are or may be present in a naturally occurring preparation containing the target molecule will have been removed or reduced in concentration.

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Detailed Description of the Invention

I. Overview

The present invention stems in part from the isolation of cDNA molecules that encode canine COX-1 and canine COX-2. SEQ ID: 1 sets forth the nucleic acid sequence of canine COX-1 and SEQ ID: 2 sets forth the amino acid sequence of canine COX-1. SEQ ID: 3 sets forth the nucleic acid sequence of canine COX-2 and SEQ ID: 4 sets forth the amino acid sequence of canine COX-2. Figure 1 diagramatically sets forth a comparison between the amino acid sequences of human COX-1 and canine COX-1. Figure 2 diagramatically sets forth a comparison between the amino acid sequence which codes for human COX-2 and the amino acid sequence which codes for canine COX-2. Figure 3 diagramatically sets forth a comparison between the nucleic acid sequences of human and canine COX-2, and their consensus sequence. Figure 4 sets forth a multiple sequence alignment of the cyclooxygenase-2 isozymes from mouse, rat, guinea pit, rabbit, horse, canine, sheep, human, and chicken.

II. Molecules of the Present Invention

The methods of the present invention are particularly relevant in the monitoring of the expression of canine COX-2. The methods of the present invention are further relevant in screening compounds or compositions that selectively inhibit canine COX-2. The methods of the present invention are also relevant in the monitoring of the expression of canine COX-2 in canines with COX-2 related conditions, especially inflammatory joint disease and cancer. Molecules of the present invention are capable of being used to diagnose abnormal canine COX-2 expression. Molecules of the present invention can also be used as therapeutic agents.

The molecules of the present invention may be either naturally occurring or non-naturally occurring. As used herein, a naturally occurring molecule may be "substantially-purified," if desired, such that one or more molecules that is or may be present in a naturally-occurring preparation containing that molecule will have been removed or will be present at a lower concentration than that at which it would normally be found.

The molecules of the present invention will preferably be "biologically active" with respect to either a structural attribute, such as the capacity of a nucleic acid to hybridize to another nucleic acid molecule, or the ability of a protein to be bound by antibody (or to compete with another molecule for such binding) Alternatively, such an attribute may be catalytic, and thus involve the capacity of the agent to mediate a chemical reaction or response.

The molecules of the present invention comprise nucleic acid molecules, proteins, and organic molecules.

A. Nucleic Acid Molecules

A preferred class of agents of the present invention comprise canine COX-2 nucleic acid molecules. Such molecules may be either DNA or RNA.

In one embodiment, such nucleic acid molecules will encode all or a fragment of canine COX-2 protein, its "promoter" or flanking gene sequences. As used herein, the term "promoter" is used in an expansive sense to refer to the regulatory

sequence(s) that control mRNA production. Such sequences include RNA polymerase binding sites, enhancers, etc. All such canine COX-2 molecules may be used in a diagnostic or therapeutic context.

Fragment canine COX-2 nucleic acid molecules may encode significant portion(s) of, or indeed most of, the canine COX-2 protein. Preferably, a fragment canine COX-2 nucleic acid molecule is identical or complementary to at least 20 contiguous nucleotides in SEQ ID: 3. More preferably it comprises at least 25 nucleotides in SEQ ID: 3. Even more preferably it comprises at least 50 nucleotides in SEQ ID: 3. Most preferably, it comprises at least 100 nucleotides in SEQ ID: 3. In a preferred embodiment, a fragment canine COX-2 nucleic acid molecule comprises at least one nucleotide that is not found in a corresponding position in canine COX-2.

Canine COX-2 nucleic acid molecules and fragment canine COX-2 nucleic acid molecules can specifically hybridize with other nucleic acid molecules. As used herein, two nucleic acid molecules are said to be capable of specifically hybridizing to one another if the two molecules are capable of forming an anti-parallel, double-stranded nucleic acid structure. A nucleic acid molecule is said to be the "complement" of another nucleic acid molecule if they exhibit complete complementarity. As used herein, molecules are said to exhibit "complete complementarity" when every nucleotide of one of the molecules is complementary to a nucleotide of the other. Two molecules are said to be "minimally complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under at least conventional "low-stringency" conditions. Similarly, the molecules are said to be "complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under conventional "high-stringency" conditions. Conventional stringency conditions are described by Sambrook, *et al.*, *Molecular Cloning*, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Press, Cold Spring Harbor, New York (1989), and by Haymes, *et al. Nucleic Acid Hybridization, A Practical Approach*, IRL Press, Washington, DC (1985). Departures from complete complementarity are therefore permissible, as long

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as such departures do not completely preclude the capacity of the molecules to form a double-stranded structure.

In another preferred embodiment, the fragment canine COX-2 molecule exhibits canine COX-2 enzymatic activity.

Appropriate stringency conditions which promote DNA hybridization are, for example, 6.0 x sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0 x SSC at 50°C, are known to those skilled in the art or can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. For example, the salt concentration in the wash step can be selected from a low stringency of about 2.0 x SSC at 50°C to a high stringency of about 0.2 x SSC at 50°C. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22°C, to high stringency conditions at about 65°C. Both temperature and salt may be varied, or either the temperature or the salt concentration may be held constant while the other variable is changed.

In a preferred embodiment, a nucleic acid of the present invention will specifically hybridize to a nucleic acid molecule having SEQ ID: 3 or its complement under moderately stringent conditions, for example at about 2.0 x SSC and about 40°C.

In a particularly preferred embodiment, a nucleic acid of the present invention will specifically hybridize to SEQ ID: 3 or its complement under high stringency conditions. In one aspect of the present invention, a nucleic acid molecule of the present invention will comprise SEQ ID: 3 or its complement.

Fragment nucleic acid molecules can be determined and selected for such that under specified conditions, such as high stringency, they can be used to specifically hybridize to canine COX-2 and not, for example, to hybridize to canine COX-1.

In another aspect of the present invention, a nucleic acid molecule of the present invention shares between 100% and 90% sequence identity with the nucleic

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187 185

acid sequence set forth in SEQ ID: 3 or its complement. In a further aspect of the present invention, a nucleic acid molecule of the present invention shares between 100% and 95% sequence identity with the nucleic acid sequence set forth in SEQ ID: 3 or its complement. In a more preferred aspect of the present invention, a nucleic acid molecule of the present invention shares between 100% and 98% sequence identity with SEQ ID: 3 or its complement. In the most preferred aspect of the present invention, a nucleic acid molecule of the present invention shares between 100% and 99% sequence identity with SEQ ID: 3 or its complement.

The degeneracy of the genetic code, which allows different nucleic acid sequences to code for the same protein or peptide, is known in the literature. (U.S. Patent No. 4,757,006). As used herein, a nucleic acid molecule is degenerate of another nucleic acid molecule when the nucleic acid molecules encode for the same amino acid sequences, but comprise different nucleotide sequences. An aspect of the present invention is that the nucleic acid molecules of the present invention include nucleic acid molecules that are degenerate of SEQ ID: 3 and its complement.

Apart from their other uses such as those described below, the nucleic acid molecules of the present invention can be employed to obtain other canine COX-2 molecules. Nucleic acid molecules and fragments thereof of the present invention may be employed to obtain other nucleic acid molecules from dogs. Such nucleic acid molecules include the nucleic acid molecules that encode, the complete coding sequence of a protein and promoters and flanking sequences of such molecules. In addition, such nucleic acid molecules include nucleic acid molecules that encode for other isozymes or gene family members. Such molecules can be readily obtained by using the above-described nucleic acid molecules, or fragments thereof, to screen cDNA or genomic libraries obtained from species. Methods for forming such libraries are well known in the art.

Nucleic acid molecules and fragments thereof of the present invention may also be employed to obtain nucleic acid homologues. Such homologues include the

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183-186

nucleic acid molecule of other organisms (*e.g.*, monkey, cow, cat) including the nucleic acid molecules that encode, in whole or in part, protein homologues of plant species or other organisms, sequences of genetic elements such as promoters and transcriptional regulatory elements. Methods for forming such libraries are well known in the art. Such homologue molecules may differ in their nucleotide sequences from those found SEQ ID: 3 or complement thereof. The nucleic acid molecules of the present invention therefore also include molecules that, although capable of specifically hybridizing with the nucleic acid molecules may lack "complete complementarity."

In one embodiment, the nucleic acid molecules of the present invention include molecules that encode the canine COX-1 protein, its promoter, or flanking gene sequences. In a preferred embodiment, a canine nucleic acid of the present invention will specifically hybridize to a nucleic acid molecule having SEQ ID: 1 or its compliments. In one aspect of the present invention, a canine nucleic acid molecule of the present invention will comprise SEQ ID: 1 or its compliment.

Fragment canine COX-1 nucleic acid molecules may encode significant portion(s) of, or indeed most of, the canine COX-1 protein. Preferably, a fragment canine COX-1 nucleic acid molecule is identical or complementary to at least 20 contiguous nucleotides in SEQ ID: 1. More preferably it comprises at least 25 nucleotides in SEQ ID: 1. Even more preferably it comprises at least 50 nucleotides in SEQ ID: 1. Most preferably, it comprises at least 100 nucleotides in SEQ ID: 1. In a preferred embodiment, a fragment canine COX-1 nucleic acid molecule comprises at least one nucleotide that is not found in a corresponding position in canine COX-1. In another preferred embodiment, the fragment canine COX-1 molecule exhibits canine COX-1 enzymatic activity.

In another aspect of the present invention, a nucleic acid molecule of the present invention shares between 100% and 90% sequence identity with the canine COX-1 nucleic acid sequence set forth in SEQ ID: 1 or its complement. In a further

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184-187

aspect of the present invention, a nucleic acid molecule of the present invention shares between 100% and 95% sequence identity with the canine nucleic acid sequence set forth in SEQ ID: 1 or its complement. In a more preferred aspect of the present invention, a nucleic acid molecule of the present invention shares between 100% and 98% sequence identity with SEQ ID: 1 or its complement. In the most preferred aspect of the present invention, a nucleic acid molecule of the present invention shares between 100% and 99% sequence identity with SEQ ID: 1 or its complement.

The present invention also provides a substantially-purified canine COX-2 protein or fragment thereof encoded by a nucleic acid sequence which specifically hybridizes to SEQ ID: 4 or its complement.

The present invention also provides a substantially-purified canine COX-1 protein having SEQ ID: 2.

The present invention also provides a substantially-purified canine COX-2 protein or fragment thereof comprising at least 10 consecutive amino acids of SEQ ID: 4.

The present invention also provides an antibody capable of specifically binding to the substantially-purified canine COX-2 protein.

The present invention also provides a substantially-purified canine COX-2 protein or fragment thereof comprising at least 10 consecutive amino acids of SEQ ID: 4.

Nucleic acid molecules of the present invention may be used to find any canine COX-2 nucleic acid homologue. Nucleic acid molecules of the present invention may also be used to obtain any mammalian COX-2 homologue.

Any of a variety of methods may be used to obtain one or more of the above-described nucleic acid molecules (Zamechik *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* *83*:4143-4146 (1986); Goodchild *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* *85*:5507-5511 (1988); Wickstrom *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* *85*:1028-1032 (1988); Holt *et al.*, *Molec. Cell. Biol.* *8*:963-973 (1988); Gerwirtz *et al.*, *Science* 242:1303-1306

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190 188

(1988); Anfossi *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 86*:3379-3383 (1989); Becker *et al.*, *EMBO J. 8*:3685-3691 (1989)). Automated nucleic acid synthesizers may be employed for this purpose. In lieu of such synthesis, the disclosed nucleic acid molecules may be used to define a pair of primers that can be used with the polymerase chain reaction (Mullis *et al.*, *Cold Spring Harbor Symp. Quant. Biol. 51*:263-273 (1986); Erlich *et al.*, European Patent 50,424; European Patent 84,796, European Patent 258,017, European Patent 237,362; Mullis, European Patent 201,184; Mullis *et al.*, U.S. Patent 4,683,202; Erlich, U.S. Patent 4,582,788; and Saiki, R. *et al.*, U.S. Patent 4,683,194) to amplify and obtain any desired nucleic acid molecule or fragment.

The canine COX-2 promoter sequence(s) and canine COX-2 flanking sequences can also be obtained using the SEQ ID: 3 sequence provided herein. In one embodiment, such sequences are obtained by incubating oligonucleotide probes of canine COX-2 oligonucleotides with members of genomic canine libraries and recovering clones that hybridize to the probes. In a second embodiment, methods of "chromosome walking," or 3' or 5' RACE may be used (Frohman, M.A. *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 85*:8998-9002 (1988); Ohara, O. *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 86*:5673-5677 (1989)) to obtain such sequences.

B. Proteins and Peptides

A second class of preferred agents comprises the canine COX-1 and COX-2 proteins, and their peptide fragments, fusion proteins, and analogs. Canine COX-1 and COX-2 proteins may be produced via chemical synthesis, or more preferably, by expressing canine COX-1- or COX-2-encoding cDNA in a suitable bacterial or eukaryotic host. Most preferably, the subsequence of such cDNA that encodes canine COX-1 or COX-2 may be used for this purpose (SEQ ID: 1 or 3). Suitable methods for expression are described by Sambrook, J., *et al.*, (In: *Molecular Cloning, a Laboratory Manual, 2nd Edition, Cold Spring Harbor Press*, Cold Spring Harbor, New York (1989)), or similar texts.

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A "canine COX-2 fragment" is a peptide or polypeptide whose amino acid sequence comprises a subset of the amino acid sequence of canine COX-2 protein. Preferably a fragment canine COX-2 fragment molecule is identical or complementary to at least one region which corresponds to a contiguous 10 amino acids of SEQ ID: 4. More preferably, thus at least one region which corresponds to a contiguous 50 amino acids of SEQ ID: 4. Even more preferably, thus at least one region which responds to a contiguous 100 amino acids of SEQ ID: 4. A canine COX-2 protein or fragment thereof that comprises one or more additional non-canine COX-2 peptide regions is a "canine COX-2 fusion" protein. Such molecules may be derivatized to contain carbohydrate or other moieties (such as keyhole limpet hemocyanin, etc.). As in the case of canine COX-2 protein, the fragments and fusions of the present invention are preferably produced via recombinant means.

The analogs of the canine COX-2 molecules comprise canine COX-2 proteins, fragments or fusions in which non-essential, nor relevant, amino acid residues have been added, replaced, or deleted. An example of such an analog is the canine COX-2 protein of non-human species, such as primates, cows, cats, etc. Such analogs can readily be obtained by any of a variety of methods. Most preferably, as indicated above, the disclosed SEQ ID: 3 will be used to define a pair of primers that may be used to isolate the canine COX-2-encoding nucleic acid molecules from any desired species. Such molecules can be expressed to yield canine COX-2 analogs by recombinant means.

C. Antibodies Reactive Against canine COX-1 or canine COX-2

One aspect of the present invention concerns antibodies, single-chain antigen binding molecules, or other proteins that specifically bind to canine COX-2 protein and its analogs, fusions or fragments. Such antibodies are "anti-canine COX-2 antibodies," and may be used, for example to measure canine COX-1 or canine COX-2 protein. As used herein, an antibody or peptide is said to "specifically bind" to

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canine COX-1 or canine COX-2 if such binding is not competitively inhibited by the presence of non-canine COX molecules.

Nucleic acid molecules that encode all or part of the canine COX-2 protein can be expressed, via recombinant means, to yield canine COX-2 protein or peptides that can in turn be used to elicit antibodies that are capable of binding canine COX-2. Such antibodies may be used in immunodiagnostic assays. Such canine COX-2-encoding molecules, or their fragments may be a "fusion" molecule (i.e. a part of a larger nucleic acid molecule) such that, upon expression, a fusion protein is produced.

The antibodies that specifically bind canine COX-2 proteins and protein fragments may be polyclonal or monoclonal, and may comprise intact immunoglobulins, of antigen binding portions of immunoglobulins (such as (F(ab'), $F(ab')_2$) fragments, or single-chain immunoglobulins producable, for example, via recombinant means. It is understood that practitioners are familiar with the standard resource materials which describe specific conditions and procedures for the construction, manipulation and isolation of antibodies (*see*, for example, Harlow and Lane, *In Antibodies: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1988)).

Murine monoclonal antibodies are particularly preferred. BALB/c mice are preferred for this purpose, however, equivalent strains may also be used. The animals are preferably immunized with approximately 25 μg of purified canine COX-2 protein (or fragment thereof) that has been emulsified with a suitable adjuvant (such as TiterMax adjuvant (Vaxcel, Norcross, GA)). Immunization is preferably conducted at two intramuscular sites, one intraperitoneal site, and one subcutaneous site at the base of the tail. An additional i.v. injection of approximately 25 μg of antigen is preferably given in normal saline three weeks later. After approximately 11 days following the second injection, the mice may be bled and the blood screened for the presence of anti-canine COX-2 antibodies. Preferably, a direct binding ELISA is employed for this purpose.

Most preferably, the mouse having the highest antibody titer is given a third i.v. injection of approximately 25 μg of canine COX-2 protein, fragment, or fusion. The splenic leukocytes from this animal may be recovered 3 days later, and are then permitted to fuse, most preferably, using polyethylene glycol, with cells of a suitable myeloma cell line (such as, for example, the P3X63Ag8.653 myeloma cell line). Hybridoma cells are selected by culturing the cells under "HAT" (hypoxanthine-aminopterin-thymine) selection for about one week. The resulting clones may then be screened for their capacity to produce monoclonal antibodies ("mAbs) to canine COX-2 protein, preferably by direct ELISA.

In one embodiment, anti-canine COX-2 monoclonal antibodies are isolated using canine COX-2 fusions, or conjugates, as immunogens. Thus, for example, a group of mice can be immunized using a canine COX-2 fusion protein emulsified in Freund's complete adjuvant (approximately 50 μg of antigen per immunization). At three week intervals, an identical amount of antigen is emulsified in Freund's incomplete adjuvant and used to immunize the animals. Ten days following the third immunization, serum samples are taken and evaluated for the presence of antibody. If antibody titers are too low, a fourth booster can be employed. Polysera capable of binding canine COX-2 at 1:5,000 dilution can also be obtained using this method.

In a preferred procedure for obtaining monoclonal antibodies, the spleens of the above-described immunized mice are removed, disrupted, and immune splenocytes are isolated over a ficoll gradient. The isolated splenocytes are fused, using polyethylene glycol with BALB/c-derived HGPRT (hypoxanthine guanine phosphoribosyl transferase) deficient P3x63xAg8.653 plasmacytoma cells. The fused cells are plated into 96 well microtiter plates and screened for hybridoma fusion cells by their capacity to grow in culture medium supplemented with hypothanthine, aminopterin and thymidine for approximately 2-3 weeks. On average, out of every 10^6 spleen cells subjected to fusion yields a viable hybridoma. A typical spleen yields 5-10 x 10^7 spleen cells.

Hybridoma cells that arise from such incubation are preferably screened for their capacity to produce an immunoglobulin that binds to canine COX-2 protein. An indirect ELISA may be used for this purpose. In brief, the supernatants of hybridomas are incubated in microtiter wells that contain immobilized canine COX-2 protein. After washing, the titer of bound immunoglobulin can be determined using, for example, a goat anti-mouse antibody conjugated to horseradish peroxidase. After additional washing, the amount of immobilized enzyme is determined (for example, through the use of a chromogenic substrate). Such screening is performed as quickly as possible after the identification of the hybridoma in order to ensure that a desired clone is not overgrown by non-secreting neighbors. Preferably, the fusion plates are screened several times since the rates of hybridoma growth vary. In a preferred embodiment, a different antigenic form of canine COX-2 may be used to screen the hybridoma. Thus, for example, the splenocytes may be immunized with one canine COX-2 immunogen, but the resulting hybridomas can be screened using a different canine COX-2 immunogen.

As discussed below, such antibody molecules or their fragments may be used for diagnostic purposes. Where the antibodies are intended for diagnostic purposes, it may be desirable to derivatize them, for example with a ligand group (such as biotin) or a detectable marker group (such as fluorescent group, a radioisotope or an enzyme).

The ability to produce antibodies that bind canine COX-2 molecules permits the identification of mimetic compounds of canine COX-2. A "mimetic compound" of canine COX-2 is a compound that is not canine COX-2, or a fragment of canine COX-2, but which nonetheless exhibits an ability to specifically bind to anti-canine COX-2 antibodies. Such molecules can be used to elicit anti-canine COX-2 antibodies, and thus, may be used to assist diagnosis of canine COX-2 related disorders.

145/193

III. Uses of the Molecules of the Invention

Another aspect of the present invention provides plasmid DNA vectors for use in the expression of the canine COX-1 or COX-2 proteins. These vectors contain the DNA sequences described above which code for the polypeptides of the invention. Appropriate vectors which can transform eukaroytic cells, including mammalian cells and microorganisms capable of expressing the canine COX-1 or COX-2 proteins include expression vectors comprising nucleotide sequences coding for the canine COX-1 or COX-2 proteins joined to transcriptional and translational regulatory sequences which are selected according to the host cells used.

Vectors incorporating modified sequences as described above are included in the present invention and are useful in the production of the canine COX-1 or COX-2 polypeptides. The vector employed in the method also contains selected regulatory sequences in operative association with the DNA coding sequences of the invention which are capable of directing the replication and expression thereof in selected host cells.

Transfer of a nucleic acid that encodes for a protein can result in overexpression of that protein in a transformed cell. One or more of the proteins or fragments thereof encoded by nucleic acid molecules of the present invention may be overexpressed in a transformed cell. Particularly, any of the canine COX-1 or COX-2 proteins or fragments thereof may be overexpressed in a transformed cell. Such overexpression may be the result of transient or stable transfer of the exogenous genetic material. "Exogenous genetic material" is any genetic material, whether naturally occurring or otherwise, from any source that is capable of being inserted into any organism.

A construct or vector may include a promoter to express the protein or protein fragment of choice. Preferably, the promoter of the present invention is a hematopoietic stem cell-specific promoter. Promoters that can be used in the present invention include the glucose-6-phosphotase promoter (Yoshiuchi *et al.*, *J. Clin. Endocrin. Metab.* *83*:1016-1019 (1998)), interleukin-1 alpha promoter (Mori and

Prager, *Leuk. Lymphoma 26*:421-433 (1997)), CMV promoter (Tong *et al.*, *Anticancer Res. 18*:719-725 (1998); Norman *et al.*, *Vaccine 15*:801-803 (1997)); RSV promoter (Elshami *et al.*, *Cancer Gene Ther. 4*:213-221 (1997); Baldwin *et al.*, *Gene Ther. 4*:1142-1149 (1997)); SV40 promoter (Harms and Splitter, *Hum. Gene Ther. 6*:1291-1297 (1995)), CD11c integrin gene promoter (Corbi and Lopez-Rodriguez, *Leuk. Lymphoma 25*:415-425 (1997)), GM-CSF promoter (Shannon *et al.*, *Crit. Rev. Immunol. 17*:301-323 (1997)), interleukin-5R alpha promoter (Sun *et al.*, *Curr. Top. Microbiol. Immunol 211*:173-187 (1996)), interleukin-2 promoter (Serfing *et al.*, *Biochim. Biophys. Acta 1263*:181-200 (1995); O'Neill *et al.*, *Transplant Proc. 23*:2862-2866 (1991)), c-fos promoter (Janknecht, *Immunobiology 193*:137-142 (1995); Janknecht *et al.*, *Carcinogenesis 16*:443-450 (1995); Takai *et al.*, *Princess Takamatsu Symp. 22*:197-204 (1991)), h-ras promoter (Rachal *et al.*, *EXS 64*:330-342 (1993)), and DMD gene promoter (Ray *et al.*, *Adv. Exp. Med. Biol. 280*:107-111 (1990)).

Promoters suitable for expression of the canine COX-1 or COX-2 proteins or fragments of either of the present invention in bacteria have been described by Hawley and McClure, *Nucleic Acids Res. 11*:2237-2255 (1983), and Harley and Reynolds, *Nucleic Acids Res. 15*:2343-2361 (1987). Such promoters include, for example, the *rec*A promoter (Fernandez de Henestrosa *et al.*, *FEMS Microbiol. Lett. 147*:209-213 (1997); Nussbaumer *et al.*, *FEMS Microbiol. Lett. 118*:57-63 (1994); Weisemann *et al.*, *Biochimie 73*: 457-470 (1991)), the Ptac promoter (Hasan *et al.*, *Gene 56*:141-151 (1987); Marsh, *Nucleic Acids Res. 14*:3603 (1986)); and a Ptac-*rec*A hybrid promoter.

It is preferred that the particular promoter selected is capable of causing sufficient expression to result in the production of an effective amount of the canine COX-1 or COX-2 proteins or fragments of either to cause the desired phenotype.

Constructs or vectors may also include with the coding region of interest a nucleic acid sequence that acts, in whole or in part, to terminate transcription of that region.

197 195

Translational enhancers may also be incorporated as part of the vector DNA. DNA constructs could contain one or more 5' non-translated leader sequences that may serve to enhance expression of the gene products from the resulting mRNA transcripts. Such sequences may be derived from the promoter selected to express the gene or can be specifically modified to increase translation of the mRNA. Such regions may also be obtained from viral RNAs, from suitable eukaryotic genes, or from a synthetic gene sequence.

A vector or construct may also include a screenable marker. Screenable markers may be used to monitor expression. Exemplary screenable markers include β-glucuronidase encoded by the *uid*A gene (GUS) (Jefferson, *Plant Mol. Biol. Rep. 5:* 387-405 (1987); Jefferson *et al., EMBO J. 6:* 3901-3907 (1987)); β-lactamase (Sutcliffe *et al., Proc. Natl. Acad. Sci. (U.S.A.) 75*: 3737-3741 (1978)), luciferase (Clontech, Palo Alto, CA, USA) (Ow *et al., Science 234*: 856-859 (1986)) β-galactosidase (Clontech, Palo Alto, CA, USA); GST (Stratagene); Protein A (Calbiochem); blue fluorescent protein (Clontech, Palo Alto, CA, USA); and fluorescent green protein (Clontech, Palo Alto, CA, USA).

Included within the terms "selectable or screenable marker genes" are also genes which encode a secretable marker whose secretion can be detected as a means of identifying or selecting for transformed cells. Examples include markers which encode a secretable antigen that can be identified by antibody interaction, or even secretable enzymes which can be detected by catalytic reactions. Secretable proteins fall into a number of classes, including small, diffusible proteins which are detectable, (*e.g.*, by ELISA), small active enzymes which are detectable in extracellular solution (*e.g.*, α-amylase, β-lactamase, phosphinothricin transferase), or proteins which are inserted or trapped in the cell membrane (such as proteins which include a leader sequence). Other possible selectable and/or screenable marker genes are apparent to those of skill in the art.

As another aspect of the present invention, there is provided a method for producing the canine COX-1 or COX-2 proteins. Suitable cells or cell lines may be

198 196

mammalian, insect or yeast cells. For example, the canine COX-1 or COX-2 proteins or fragments of either of the present invention is expressed in a yeast cell, preferably *Saccharomyces cerevisiae*. The canine COX-1 or COX-2 proteins or fragments of either of the present invention can be expressed in *S. cerevisiae* by fusing it to the N-terminus of the URA3, CYC1 or ARG3 genes (Guarente and Ptashne, *Proc. Natl. Acad. Sci. (U.S.A.) 78*:2199-2203 (1981); Rose *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 78*:2460-2464 (1981); and Crabeel *et al.*, *EMBO J. 2*:205-212 (1983)). Alternatively, the canine COX-1 or COX-2 proteins or fragments of either of the present invention can be fused to either the PGK or TRP1 genes (Tuite *et al.*, *EMBO J. 1*:603-608 (1982); and Dobson *et al.*, *Nucleic Acids. Res. 11*:2287-2302 (1983)). More preferably, the canine COX-1 or COX-2 proteins or fragments of either of the present invention are expressed as a mature protein (Hitzeman *et al.*, *Nature 293*:717-722 (1981); Valenzuela *et al.*, *Nature 298*:347-350 (1982); and Derynck *et al.*, *Nucleic Acids Res. 11*:1819-1837 (1983)).

Native and engineered yeast promoters suitable for use in the present invention have been reviewed by Romanos *et al.*, *Yeast 8*:423-488 (1992) Most preferably, the canine COX-1 or COX-2 proteins or fragments of either of the present invention is secreted by the yeast cell (Blobel and Dobberstein, *J. Cell Biol. 67*:835-851 (1975); Kurjan and Herskowitz, *Cell 30*:933-943 (1982); Bostian *et al.*, *Cell 36*:741-751 (1984); Rothman and Orci, *Nature 355*:409-415 (1992); Julius *et al.*, *Cell 32*:839-852 (1983); and Julius *et al.*, *Cell 36*:309-318 (1984)).

Where desired, insect cells may be utilized as host cells in the method of the present invention. See, e.g., V. A. Luckow, *Protein Eng.* J. L. Cleland., Wiley-Liss, New York, NY: 183-2180 (1996) and references cited therein. In addition, general methods for expression of foreign genes in insect cells using baculovirus vectors are described in: O'Reilly *et al.*, *Baculovirus Expression Vectors: A Laboratory Manual*. New York, W.H. Freeman and Company (1992), and King and Possee, *The Baculovirus Expression System: A Laboratory Guide*, London, Chapman & Hall. An expression vector is constructed comprising a baculovirus transfer vector, in which a

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strong baculovirus promoter (such as the polyhedrin promoter) drives transcription of a eukaryotic secretion signal peptide coding region, which is translationally joined to the coding region for the multi-functional protein. For example, the plasmid pVL1393 (Invitrogen Corp., San Diego, California, U.S.A.) can be used. After construction of the vector carrying the gene encoding the multi-functional protein, two micrograms of this DNA is co-transfected with one microgram of baculovirus DNA into *Spodoptera frugiperda* insect cells, strain Sf9. Alternatively, recombinant baculoviruses can be created using a baculovirus shuttle vector system (Luckow *et al.*, *J. Virol. 67*: 4566-4579 (1993)), now marketed as the Bac-To-Bac™ Expression System (Life Technologies, Inc. Rockville, MD). Pure recombinant baculovirus carrying the multi-functional protein is used to infect cells cultured, for example, in Excell 401 serum-free medium (JRH Biosciences, Lenexa, Kansas) or Sf900-II (Life Technologies, Inc.). The multi-functional protein secreted into the medium can be recovered by standard biochemical approaches. Supernatants from mammalian or insect cells expressing the multi-functional proteins can be first concentrated using any of a number of commercial concentration units.

Most preferably, mammalian cells can be used to express the nucleic acid molecules of the present invention. Preferably, the nucleic acid molecules of the present invention are cloned into a suitable retroviral vector (*see, e.g.*, Dunbar *et al.*, *Blood 85*:3048-3057 (1995); Baum *et al.*, *J. Hematother. 5*: 323-329 (1996); Bregni *et al.*, *Blood 80*:1418-1422 (1992); Boris-Lawrie and Temin, *Curr. Opin. Genet. Dev. 3*:102-109 (1993); Boris-Lawrie and Temin, *Annal. New York Acad. Sci. 716*:59-71 (1994); Miller, *Current Top. Microbiol. Immunol. 158*:1-24 (1992)), adenovirus vector (Berkner, *BioTechniques 6*:616-629 (1988); Berkner, *Current Top. Microbiol. Immunol. 158*:39-66 (1992); Brody and Crystal, *Annal. New York Acad. Sci. 716*:90-103 (1994); Baldwin *et al.*, *Gene Ther. 4*:1142-1149 (1997)), RSV, MuSV, SSV, MuLV (Baum *et al.*, *J. Hematother. 5*: 323-329 (1996)), AAV (Chen *et al.*, *Gene Ther. 5*:50-58 (1998); Hallek *et al.*, *Cytokines Mol. Ther. 2*: 69-79 (1996)), AEV, AMV, or CMV (Griffiths *et al.*, *Biochem. J. 241*: 313-324 (1987)).

Methods and compositions for transforming a eukaryotic cell, bacteria and other microorganisms are known in the art (*see*, for example, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1989)).

Technology for introduction of DNA into cells is well known to those of skill in the art. Four general methods for delivering a gene into cells have been described: (1) chemical methods (Graham and van der Eb, *Virology 54*:536-539 (1973)); (2) physical methods such as microinjection (Capecchi, *Cell 22*:479-488 (1980)), electroporation (Wong and Neumann, *Biochem. Biophys. Res. Commun. 107*:584-587 (1982); Fromm *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 82*:5824-5828 (1985); U.S. Patent No. 5,384,253); and the gene gun (Johnston and Tang, *Methods Cell Biol. 43*:353-365 (1994)); (3) viral vectors (Clapp, *Clin. Perinatol. 20*:155-168 (1993); Lu *et al.*, *J. Exp. Med. 178*:2089-2096 (1993); Eglitis and Anderson, *Biotechniques, 6*:608-614 (1988)); and (4) receptor-mediated mechanisms (Curiel *et al.*, *Hum. Gen. Ther. 3*:147-154 (1992), Wagner *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 89*:6099-6103 (1992)).

Transformation can be achieved using methods based on calcium phosphate precipitation, polyethylene glycol treatment, electroporation, and combinations of these treatments (see for example Potrykus *et al.*, *Mol. Gen. Genet. 205*:193-200 (1986); Lorz *et al.*, *Mol. Gen. Genet. 199*:178 (1985); Fromm *et al.*, *Nature 319*:791 (1986); Uchimiya *et al.*, *Mol. Gen. Genet. 204*:204 (1986); Marcotte *et al.*, *Nature 335*:454-457 (1988)).

Assays for gene expression based on the transient expression of cloned nucleic acid constructs have been developed by introducing the nucleic acid molecules into cells by polyethylene glycol treatment, electroporation, or particle bombardment (Marcotte *et al.*, *Nature 335*: 454-457 (1988); McCarty *et al.*, *Cell 66:* 895-905 (1991); Hattori *et al.*, *Genes Dev. 6:* 609-618 (1992); Goff *et al.*, *EMBO J. 9:* 2517-2522 (1990)). Transient expression systems may be used to functionally dissect gene constructs.

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201 199

In one embodiment, the canine COX-2 molecules of the present invention are used to determine whether an individual has a mutation affecting the level (i.e., the concentration of canine COX-2 mRNA or protein in a sample, etc.) or pattern (i.e., the kinetics of expression, rate of decomposition, stability profile, etc.) of the canine COX-2 expression (collectively, the "canine COX-2 Response" of a cell or bodily fluid) (for example, a mutation in the canine COX-2 gene, or in a regulatory region(s) or other gene(s) that control or affect the expression of canine COX-2), and being predictive of dogs who would be predisposed to, for example inflammatory joint disease, and other disorders. Such COX-2 related disorders include, but are not limited to, arthritis, rheumatoid arthritis, spondyloarthropathies, gouty arthritis, osteoarthritis, systemic lupus erythematosus and juvenile arthritis, auto-immune disease, allograft rejection, asthma, bronchitis, tendinitis, bursitis, and skin-related conditions such as psoriasis, eczema, burns, and dermatitis, post-operative inflammation including from ophthalmic surgery such as cataract surgery and refractive surgery, gastrointestinal conditions such as inflammatory bowel syndrome, and ulcerative colitis, neoplasia, such as colorectal cancer, and cancer of the breast, lung, prostate, bladder, cervix, and skin, vascular diseases, migraine headaches, periateritis nodosa, thyroiditis, aplastic anemia, Hodgkin's disease, sclerodoma, rheumatic fever, type I diabetes, neuromuscular junction disease including multiple sclerosis, sarcoidosis, nephrotic syndrome, Behcet's syndrome, polymyositis, gingivitis, nephritis, hypersensitivity, conjunctivitis, swelling occuring after injury, myocardial ischemia, myochardial infarction, ophthalmic diseases, such as retinitis, retinopathies, uveitis, ocular photophobia, and of acute injury to the eye tissue, allergic rhinitis, respiratory distress syndrome, endotoxin shock syndrome, atherosclerosis, pulmonary inflammation such as from viral and bacterial infections and from cystic fibrosis, central nervous system disorders, such as cortical dementias including Alzheimer's disease, and central nervous system damage resulting from stroke, ischemia, and trauma.

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As used herein, the canine COX-2 Response manifested by a cell or bodily fluid is said to be "altered" if it differs from the canine COX-2 Response of cells or of bodily fluids of normal individuals. Such alteration may be manifested by either abnormally increased or abnormally diminished canine COX-2 Response. To determine whether a canine COX-2 Response is altered, the canine COX-2 Response manifested by the cell or bodily fluid of the canine is compared with that of a similar cell sample (or bodily fluid sample) of normal individuals. As will be appreciated, it is not necessary to re-determine the canine COX-2 Response of the cell sample (or bodily fluid sample) of normal dogs each time such a comparison is made; rather, the canine COX-2 Response of a particular canines may be compared with previously obtained values of normal dogs.

In one sub-embodiment, such an analysis is conducted by determining the presence and/or identity of polymorphism(s) in the canine COX-2 gene or its flanking regions which are associated with a disorder.

Any of a variety of molecules can be used to identify such polymorphism(s). In one embodiment, the canine COX-2 cDNA sequence (or a sub-sequence thereof) may be employed as a marker nucleic acid molecule to identify such polymorphism(s). Alternatively, such polymorphisms can be detected through the use of a marker nucleic acid molecule or a marker protein that is genetically linked to (i.e., a polynucleotide that co-segregates with) such polymorphism(s). In a preferred aspect of this embodiment, such marker nucleic acid molecules will have the nucleotide sequence of a polynucleotide that is closely genetically linked to such polymorphism(s). Polynucleotide markers that map to such locations are well known and can be employed to identify such polymorphism(s).

In an alternative embodiment, such polymorphisms can be detected through the use of a marker nucleic acid molecule that is physically linked to such polymorphism(s). For this purpose, marker nucleic acid molecules comprising a nucleotide sequence of a polynucleotide located within 1 mb of the polymorphism(s),

and more preferably within 100 kb of the polymorphism(s), and most preferably within 10 kb of the polymorphism(s) can be employed.

The genomes of animals and plants naturally undergo spontaneous mutation in the course of their continuing evolution (Gusella, *Ann. Rev. Biochem.* 55:831-854 (1986)).

A "polymorphism" in the canine COX-2 gene or its flanking regions is a variation or difference in the sequence of the canine COX-2 gene or its flanking regions that arises in some of the members of a species. The variant sequence and the "original" sequence co-exist in the species' population. In some instances, such co-existence is in stable or quasi-stable equilibrium.

A polymorphism is thus said to be "allelic," in that, due to the existence of the polymorphism, some members of a species may have the original sequence (i.e. the original "allele") whereas other members may have the variant sequence (i.e. the variant "allele"). In the simplest case, only one variant sequence may exist, and the polymorphism is thus said to be di-allelic. In other cases, the species' population may contain multiple alleles, and the polymorphism is termed tri-allelic, etc. A single gene may have multiple different unrelated polymorphisms. For example, it may have a di-allelic polymorphism at one site, and a multi-allelic polymorphism at another site.

The variation that defines the polymorphism may range from a single nucleotide variation to the insertion or deletion of extended regions within a gene. In some cases, the DNA sequence variations are in regions of the genome that are characterized by short tandem repeats (STRs) that include tandem di- or tri-nucleotide repeated motifs of nucleotides. Polymorphisms characterized by such tandem repeats are referred to as "variable number tandem repeat" ("VNTR") polymorphisms. VNTRs have been used in identity and paternity analysis (Weber, U.S. Patent 5,075,217; Armour, *et al.*, *FEBS Lett.* 307:113-115 (1992); Jones, *et al.*, *Eur. J. Haematol.* 39:144-147 (1987); Horn, *et al.*, PCT Application WO91/14003; Jeffreys, European Patent Application 370,719; Jeffreys, U.S. Patent 5,175,082); Jeffreys. *et al.*, *Amer. J. Hum. Genet.* 39:11-24 (1986); Jeffreys. *et al.*, *Nature* 316:76-79 (1985);

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Gray, *et al.*, *Proc. R. Acad. Soc. Lond. 243*:241-253 (1991); Moore, *et al.*, *Genomics 10*:654-660 (1991); Jeffreys, *et al.*, *Anim. Genet. 18*:1-15 (1987); Hillel, *et al.*, *Anim. Genet. 20*:145-155 (1989); Hillel, *et al.*, *Genet. 124*:783-789 (1990)).

The detection of polymorphic sites in a sample of DNA may be facilitated through the use of nucleic acid amplification methods. Such methods specifically increase the concentration of polynucleotides that span the polymorphic site, or include that site and sequences located either distally or proximally to it. Such amplified molecules can be readily detected by gel electrophoresis or other means.

The most preferred method of achieving such amplification employs the polymerase chain reaction ("PCR") (Mullis, *et al.*, *Cold Spring Harbor Symp. Quant. Biol. 51*:263-273 (1986); Erlich *et al.*, European Patent Appln. 50,424; European Patent Appln. 84,796, European Patent Application 258,017, European Patent Appln. 237,362; Mullis, European Patent Appln. 201,184; Mullis *et al.*, U.S. Patent No. 4,683,202; Erlich, U.S. Patent No. 4,582,788; and Saiki, *et al.*, U.S. Patent No. 4,683,194), using primer pairs that are capable of hybridizing to the proximal sequences that define a polymorphism in its double-stranded form.

In lieu of PCR, alternative methods, such as the "Ligase Chain Reaction" ("LCR") may be used (Barany, *Proc. Natl. Acad. Sci. (U.S.A.) 88*:189-193 (1991). LCR uses two pairs of oligonucleotide probes to exponentially amplify a specific target. The sequences of each pair of oligonucleotides is selected to permit the pair to hybridize to abutting sequences of the same strand of the target. Such hybridization forms a substrate for a template-dependent ligase. As with PCR, the resulting products thus serve as a template in subsequent cycles and an exponential amplification of the desired sequence is obtained.

LCR can be performed with oligonucleotides having the proximal and distal sequences of the same strand of a polymorphic site. In one embodiment, either oligonucleotide will be designed to include the actual polymorphic site of the polymorphism. In such an embodiment, the reaction conditions are selected such that the oligonucleotides can be ligated together only if the target molecule either contains

or lacks the specific nucleotide that is complementary to the polymorphic site present on the oligonucleotide. Alternatively, the oligonucleotides may be selected such that they do not include the polymorphic site (see, Segev, PCT Application WO 90/01069).

The "Oligonucleotide Ligation Assay" ("OLA") may alternatively be employed (Landegren, *et al., Science 241:*1077-1080 (1988)). The OLA protocol uses two oligonucleotides which are designed to be capable of hybridizing to abutting sequences of a single strand of a target. OLA, like LCR, is particularly suited for the detection of point mutations. Unlike LCR, however, OLA results in "linear" rather than exponential amplification of the target sequence.

Nickerson, *et al.* have described a nucleic acid detection assay that combines attributes of PCR and OLA (Nickerson, *et al., Proc. Natl. Acad. Sci. (U.S.A.) 87:*8923-8927 (1990)). In this method, PCR is used to achieve the exponential amplification of target DNA, which is then detected using OLA. In addition to requiring multiple, and separate, processing steps, one problem associated with such combinations is that they inherit all of the problems associated with PCR and OLA.

Schemes based on ligation of two (or more) oligonucleotides in the presence of nucleic acid having the sequence of the resulting "di-oligonucleotide", thereby amplifying the di-oligonucleotide, are also known (Wu, *et al., Genomics 4:*560 (1989)), and may be readily adapted to the purposes of the present invention.

Other known nucleic acid amplification procedures, such as allele-specific oligomers, branched DNA technology, transcription-based amplification systems, or isothermal amplification methods may also be used to amplify and analyze such polymorphisms (Malek, *et al.*, U.S. Patent 5,130,238; Davey, *et al.*, European Patent Application 329,822; Schuster *et al.*, U.S. Patent 5,169,766; Miller, *et al.*, PCT appln. WO 89/06700; Kwoh, *et al., Proc. Natl. Acad. Sci. (U.S.A.) 86:*1173 (1989); Gingeras, *et al.*, PCT application WO 88/10315; Walker, *et al., Proc. Natl. Acad. Sci. (U.S.A.) 89:*392-396 (1992)). All the foregoing nucleic acid amplification methods could be used.

The identification of a polymorphism in the canine COX-2 gene can be determined in a variety of ways. By correlating the presence or absence of inflammatory joint disease in an dog with the presence or absence of a polymorphism in the canine COX-2 gene or its flanking regions, it is possible to diagnose the predisposition of an asymptomatic dog to inflammatory joint disease or other diseases. If a polymorphism creates or destroys a restriction endonuclease cleavage site, or if it results in the loss or insertion of DNA (e.g., a VNTR polymorphism), it will alter the size or profile of the DNA fragments that are generated by digestion with that restriction endonuclease. As such, dogs that possess a variant sequence can be distinguished from those having the original sequence by restriction fragment analysis. Polymorphisms that can be identified in this manner are termed "restriction fragment length polymorphisms" ("RFLPs"). RFLPs have been widely used in human and animal genetic analyses (Glassberg, UK Patent Application 2135774; Skolnick, M.H. *et al.*, *Cytogen. Cell Genet.* 32:58-67 (1982); Botstein, *et al.*, *Ann. J. Hum. Genet.* 32:314-331 (1980); Fischer, *et al.* (PCT Application WO90/13668); Uhlen, PCT Application WO90/11369).

In accordance with this embodiment of the invention, a sample DNA is obtained from a dog's cells. In a preferred embodiment, the DNA sample is obtained from the dog's blood. However, any source of DNA may be used. The DNA is subjected to restriction endonuclease digestion. Canine COX-2 is used as a probe in accordance with the above-described RFLP methods.

The polymorphism obtained in this approach can then be cloned to identify the mutation at the coding region which alters the protein's structure, or at the regulatory region of the gene which affects its expression level. Changes involving promoter interactions with other regulatory proteins can be identified by, for example, gel shift.

Several different classes of polymorphisms may be identified through such methods. Examples of such classes include: (1) polymorphisms present in the canine COX-2 cDNA of different dogs; (2) polymorphisms in non-translated canine COX-2 gene sequences, including the promoter or other regulatory regions of the canine

COX-2 gene; (3) polymorphisms in genes whose products interact with canine COX-2 regulatory sequences; (4) polymorphisms in gene sequences whose products interact with the canine COX-2 protein, or to which the canine COX-2 protein binds.

In an alternate sub-embodiment, the evaluation is conducted using oligonucleotide "probes" whose sequence is complementary to that of a portion of canine COX-2 mRNA. Such molecules are then incubated with cell extracts of a patient under conditions sufficient to permit nucleic acid hybridization. For this sub-embodiment, cells of the trabecular meshworks are preferred. The detection of double-stranded probe-mRNA hybrid molecules is indicative of the presence of canine COX-2 mRNA; the amount of such hybrid formed is proportional to the amount of canine COX-2 mRNA. Thus, such probes may be used to ascertain the level and extent of canine COX-2 mRNA production in a patient's cells. Such nucleic acid hybridization may be conducted under quantitative conditions (thereby providing a numerical value of the amount of canine COX-2 mRNA present). Alternatively, the assay may be conducted as a qualitative assay that indicates either that canine COX-2 mRNA is present, or that its level exceeds a user set, predefined value.

In a second embodiment, the previously described "anti-canine COX-2 antibodies" are employed in an immunodiagnostic assay.

In one sub-embodiment of this aspect of the present invention, one can ascertain the canine COX-2 Response in a biopsy (or a macrophage or other blood cell sample), or other cell sample, or more preferably, in a sample of bodily fluid (especially, blood, serum, plasma, tears, etc.).

The anti-canine COX-2 antibodies of the present invention may thus be used in an immunoassay to assess the presence of canine COX-2. Any of a wide array of immunoassays formats may be used for this purpose (Fackrell, *Clin. Immunoassay* 8:213-219 (1985)), Yolken, *Rev. Infect. Dis.* 4:35 (1982); Collins, In: *Alternative Immunoassays*, John Wiley & Sons, NY (1985); Ngo, *et al.*, In: *Enzyme Mediated Immunoassay*, Plenum Press, NY (1985)).

The simplest immunoassay involves merely incubating an antibody that is capable of binding to a predetermined target molecule with a sample suspected to contain the target molecule. The presence of the target molecule is determined by the presence, and proportional to the concentration, of any antibody bound to the target molecule. In order to facilitate the separation of target-bound antibody from the unbound antibody initially present, a solid phase is typically employed. Thus, for example the sample can be passively bound to a solid support, and, after incubation with the antibody, the support can be washed to remove any unbound antibody.

In more sophisticated immunoassays, the concentration of the target molecule is determined by binding the antibody to a support, and then permitting the support to be in contact with a sample suspected of containing the target molecule. Target molecules that have become bound to the immobilized antibody can be detected in any of a variety of ways. For example, the support can be incubated in the presence of a labeled, second antibody that is capable of binding to a second epitope of the target molecule. Immobilization of the labeled antibody on the support thus requires the presence of the target, and is proportional to the concentration of the target in the sample. In an alternative assay, the target is incubated with the sample and with a known amount of labeled target. The presence of target molecule in the sample competes with the labeled target molecules for antibody binding sites. Thus, the amount of labeled target molecules that are able to bind the antibody is inversely proportional to the concentration of target molecule in the sample.

In general, immunoassay formats employ either radioactive labels ("RIAs") or enzyme labels ("ELISAs"). RIAs have the advantages of simplicity, sensitivity, and ease of use. Radioactive labels are of relatively small atomic dimension, and do not normally affect reaction kinetics. Such assays suffer, however, from the disadvantages that, due to radioisotopic decay, the reagents have a short shelf-life, require special handling and disposal, and entail the use of complex and expensive analytical equipment. RIAs are described in *Laboratory Techniques and Biochemistry in Molecular Biology*, by Work, *et al.*, North Holland Publishing Company, NY

(1978), with particular reference to the chapter entitled "An Introduction to Radioimmune Assay and Related Techniques" by Chard. ELISAs have the advantage that they can be conducted using inexpensive equipment, and with a myriad of different enzymes, such that a large number of detection strategies --colorimetric, pH, gas evolution, etc. -- can be used to quantitate the assay. In addition, the enzyme reagents have relatively long shelf-lives, and lack the risk of radiation contamination that attends to RIA use. ELISAs are described in *ELISA and Other Solid Phase Immunoassays* (Kemeny, *et al.*, Eds.), John Wiley & Sons, NY (1988).

Anti-canine COX-2 antibodies or canine COX-2 binding molecules may be administered to a dog, and their capacity to bind to canine COX-2 *in vivo* may be determined by ocular examination. Significantly, since such a diagnostic test is relatively rapid, immune responses that require significant time, such as the potential eliciting of anti-[anti-canine COX-2] antibodies, or the complexing of such antibodies with anti-canine COX-2 antibodies, is not important. In a preferred embodiment, the antibody will be fluorescently labeled, and will be provided to a dog by injection into the dog's circulatory system.

In another aspect of the present invention, a canine COX-2 protein or fragment thereof can be used in assays for screening test substances for the ability to modulate or maintain canine COX-2 activity. In a sub-embodiment, the test substance is an agonist, antagonist, or small molecule inhibitor of the canine COX-2 protein. In another sub-embodiment, the test substance may bind to canine COX-2 substrate. The test substance may also be an agonist, antagonist, or small molecule inhibitor of COX-1.

Assays for screening canine COX-2 activity include, for example, a colorimetric assay (Kaufman et al., *Enzyme* 12:537 (1971), Richards and Greengard, *Biochimica et Biophysica Acta* 304:842-850 (1973), McKnight *et al.*, *J. Biochem.* 267:25208-25212 (1992)) and a HPLC-based separation technique (Buse *et al. Am J. Physiol.* 272: E1080-1088 (1997), Nelson *et al.*, *Am. J. Physiol.* 272: E848-855 (1997)).

210 208

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

Example 1

cDNA Isolation

For construction of cDNA libraries for cloning canine COX-1 and COX-2, mRNA was prepared from canine kidney papillae and lipopolysaccharide (LPS)-stimulated canine mononuclear cells.

For the kidney cDNA library construction, total RNA was isolated from 200 micrograms (ug) of kidney tissue using 12 ml of TRIzol reagent (Life Technologies). RNA was prepared using the standard protocol provided by the manufacturer (Life Technologies). The protocol yielded about 200 ug of total RNA. The mRNA was then isolated using a Mini Oligo(dT) Cellulose Spin Column Kit (5 prime-3 prime). The yield was about 7 ug after a single oligo(dT) column purification.

For construction of a mononuclear cell cDNA library, mRNA was isolated directly from about 10^7 cells using the Poly(A) Pure mRNA Purification Kit (Ambion). After a single oligo(dT) purification step, 2 ug of mRNA were isolated.

Example 2

Gene Expression

Canine COX-1 was expressed using a full-length COX-1 cDNA containing the entire coding region, which was digested with SalI and NotI to generate ca.2600 base pair fragment for ligation into an insect cell expression vector, pFastBac 1 (Invitrogen), which had been digested with SalI and NotI. The resulting plasmid, pMON37775, was sequenced to confirm cloning.

An insect cell expression plasmid for canine COX-2 was created by ligating a COX-2 fragment generated by PCR fragment to a partial COX-2 cDNA. The 5' end of COX-2 was cloned from kidney double-stranded cDNA using PCR.. Both

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fragments were ligated into pFastBack 1, which had been digested with EcoRI and NotI. The resulting plasmid, pMON37776, was confirmed by sequencing.

Example 3

Selective COX-2 Inhibitor Screening Assay

The standard screening assay for inhibitor potency (IC50) was an ELISA method for detection of PGE2. IC50 values were determined by pre-incubation of the inhibitors with enzyme for 10 min. at 25 C in a buffer containing 50 mM potassium phosphate, pH 7.5, 2 mM phenol, 1 mM heme, and 300 mM epinephrine. Inhibitors were first made as a 10 mM stock in DMSO and further diluted into 1:9 (DMSO:ethanol). The reaction was allowed to proceed for 10 min. For time dependent inhibition, the initial rate of the reaction was measured by stopping the reaction at 15 sec. with the addition of 1/10 volume N HCl; the amount of prostaglandin formed was measured by ELISA (Cayman Chemical Co., Ann Arbor, MI). IC50s were determined from a 4 parameter log fit of the data using Grafit.

All references, patents or applications cited herein are incorporated by reference in their entirety as if written herein.

We claim:

1. A substantially-purified nucleic acid molecule selected from the groups of "DNAs" consisting of nucleic acid molecules comprising SEQ ID: 1 and its complement and internal fragments thereof wherein said SEQ ID: 1 encodes a canine COX-1 protein or fragment thereof.

2. The substantially-purified nucleic acid molecule according to claim 1, wherein said substantially-purified nucleic acid molecule encodes said canine COX-1 protein.

3. The substantially-purified nucleic acid molecule according to claim 1, wherein said nucleic acid is SEQ ID: 1 or complement thereof.

4. The substantially-purified nucleic acid molecule according to claim 1, wherein said nucleic acid molecule specifically hybridizes to a nucleic acid molecule that encodes canine COX-1 or complement thereof and fails to specifically hybridize to a nucleic acid molecule that encodes non-canine COX-1 or complement thereof.

5. The substantially-purified nucleic acid molecule according to claim 4, wherein said nucleic acid molecule specifically hybridizes to a nucleic acid molecule that encodes canine COX-1 or complement thereof under high stringency conditions and fails to specifically hybridize to a nucleic acid molecule that encodes non-canine COX-1 or complement thereof under high stringency conditions.

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6. A substantially-purified canine COX-1 nucleic acid molecule which comprises an nucleic acid sequence that is identical to at least 20 contiguous nucleotides of SEQ ID: 1 or its complement.

7. A substantially-purified canine COX-1 nucleic acid molecule according to claim 6, wherein said nucleic acid molecule comprises an nucleic acid sequence that is identical to at least 50 contiguous nucleotides of SEQ ID: 1 or its complement.

8. A substantially-purified canine COX-1 nucleic acid molecule according to claim 7, wherein said nucleic acid molecule comprises an nucleic acid sequence that is identical to at least 100 contiguous nucleotides of SEQ ID: 1 or its complement.

9. A substantially-purified canine COX-1 protein or fragment thereof encoded by a nucleic acid sequence which specifically hybridizes to SEQ ID: 1 or its complement.

10. A substantially-purified canine COX-1 protein or fragment thereof according to claim 9, where said nucleic acid sequence is selected from SEQ ID: 1 or its complement.

11. A substantially-purified canine COX-1 protein having SEQ ID: 2.

12. A substantially-purified canine COX-1 protein or fragment thereof comprising at least 10 consecutive amino acids of SEQ ID: 2.

13 A substantially-purified canine COX-1 protein or fragment thereof according to claim 12, wherein said canine COX-1 protein or fragment thereof is a fusion protein.

14. An antibody capable of specifically binding to the substantially-purified canine COX-1 protein of claim 11.

15. The antibody of claim 14, wherein said antibody is detectably labeled.

16. A substantially-purified nucleic acid molecule selected from the groups of "DNAs" consisting of nucleic acid molecules comprising SEQ ID: 3 and its complement and internal fragments thereof wherein said SEQ ID: 3 encodes a canine COX-2 protein or fragment thereof.

17. The substantially-purified nucleic acid molecule according to claim 16, wherein said substantially-purified nucleic acid molecule encodes said canine COX-2 protein.

18. The substantially-purified nucleic acid molecule according to claim 16, wherein said nucleic acid is SEQ ID: 3 or complement thereof.

19. The substantially-purified nucleic acid molecule according to claim 16, wherein said nucleic acid molecule specifically hybridizes to a nucleic acid molecule that encodes canine COX-2 or complement thereof and fails to specifically hybridize to a nucleic acid molecule that encodes non-canine COX-2 or complement thereof.

20. The substantially-purified nucleic acid molecule according to claim 19, wherein said nucleic acid molecule specifically hybridizes to a nucleic acid molecule that encodes canine COX-2 or complement thereof under high stringency conditions and fails to specifically hybridize to a nucleic acid molecule that encodes non-canine COX-2 or complement thereof under high stringency conditions.

21. A substantially-purified canine COX-2 nucleic acid molecule which comprises an nucleic acid sequence that is identical to at least 20 contiguous nucleotides of SEQ ID: 3 or its complement.

22. A substantially-purified canine COX-2 nucleic acid molecule according to claim 21, wherein said nucleic acid molecule comprises an nucleic acid sequence that is identical to at least 50 contiguous nucleotides of SEQ ID: 3 or its complement.

23. A substantially-purified canine COX-2 nucleic acid molecule according to claim 22, wherein said nucleic acid molecule comprises an nucleic acid sequence that is identical to at least 100 contiguous nucleotides of SEQ ID: 3 or its complement.

24. A substantially-purified canine COX-2 protein or fragment thereof encoded by a nucleic acid sequence which specifically hybridizes to SEQ ID: 4 or its complement.

25. A substantially-purified canine COX-2 protein or fragment thereof according to claim 24, where said nucleic acid sequence is selected from SEQ ID: 4 or its complement.

26. A substantially-purified canine COX-2 protein having SEQ ID: 4.

27. A substantially-purified canine COX-2 protein or fragment thereof comprising at least 10 consecutive amino acids of SEQ ID: 4.

28 A substantially-purified canine COX-2 protein or fragment thereof according to claim 27, wherein said canine COX-2 protein or fragment thereof is a fusion protein.

29. An antibody capable of specifically binding to the substantially-purified canine COX-2 protein of claim 26.

30. The antibody of claim 29, wherein said antibody is detectably labeled.

31. A transformed cell having a nucleic acid molecule which comprises:

(A) an exogenous promoter region which functions in a cell to cause the production of a mRNA molecule; which is linked to

(B) a structural nucleic acid molecule, wherein said structural nucleic acid molecule encodes a canine COX-1 gene or fragment thereof, which is linked to

(C) a 3' non-translated sequence that functions in a cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of said mRNA molecule.

32. The transformed cell according to claim 31, wherein said structural gene is in the antisense orientation.

33. A transformed cell having a nucleic acid molecule which comprises:

(A) an exogenous promoter region which functions in a cell to cause the production of a mRNA molecule; which is linked to

(B) a structural nucleic acid molecule, wherein said structural nucleic acid molecule encodes a canine COX-2 gene or fragment thereof, which is linked to

(C) a 3' non-translated sequence that functions in a cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of said mRNA molecule.

34. The transformed cell according to claim 33, wherein said structural gene is in the antisense orientation.

35. A method for determining a level or pattern of a canine COX-2 protein in a cell comprising:

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(A) incubating, under conditions permitting nucleic acid hybridization, a marker nucleic acid molecule, said marker nucleic molecule capable of specifically hybridizing to a nucleic acid molecule that encodes canine COX-2 or complement thereof under high stringency conditions and said marker nucleic acid molecule incapable of specifically hybridizing to a nucleic acid molecule that encodes canine COX-1 or complement thereof under high stringency conditions, with a complementary nucleic acid molecule obtained from said cell, wherein nucleic acid hybridization between said marker nucleic acid molecule, and said complementary nucleic acid molecule obtained from said cell permits the detection of canine COX-2;

(B) permitting hybridization between said marker nucleic acid molecule and said complementary nucleic acid molecule obtained from said cell; and

(C) detecting the level or pattern of said complementary nucleic acid, wherein the detection of said complementary nucleic acid is predictive of the level or pattern of said canine COX-2 protein.

36. The method of claim 35, wherein said level is predictive of said canine COX-2 protein.

37. The method of claim 35, wherein said pattern is predictive of said canine COX-2 protein.

38. The method of claim 35, wherein said level or pattern is detected by *in situ* hybridization.

39. A process for diagnosis or prognosis of COX-2 related conditions in a dog which comprises assaying the concentration of a molecule, whose concentration is dependent upon the expression of a canine COX-2 gene, said molecule being present in a sample of cells or bodily fluid of said dog, in comparison to the concentration of that molecule present in a sample of cells or bodily fluid of a dog that does not have an inflammatory condition and is

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not predisposed to developing inflammatory conditions, wherein a concentration of said molecule differs from that found in said dog who does not have inflammation and is not predisposed to having inflammation is diagnostic or prognostic of an inflammatory condition in said dog.

40. The method of claim 39, wherein said bodily fluid is selected from the group consisting of skeletal tissue, blood, lymph and serum.

41. The method of claim 39, wherein said molecule is a protein molecule expressed by said canine COX-2 gene.

42. The method of claim 39, wherein said molecule is an mRNA molecule encoded by said canine COX-2 gene, or a cDNA molecule encoded by said canine COX-2 gene.

43. The method of claim 42, wherein said concentration of said mRNA molecule is assayed by incubating a sample of said bodily fluid in the presence of a nucleic acid molecule that hybridizes to said mRNA molecule.

44. A method for diagnosing a COX-2 related condition in a dog which comprises the steps:

(A) incubating under conditions permitting nucleic acid hybridization: a marker nucleic acid molecule, said marker nucleic acid molecule comprising a nucleotide sequence of a polynucleotide that specifically hybridizes to a polynucleotide that is linked to a canine COX-2 gene, and a complementary nucleic acid molecule obtained from a cell or a bodily fluid of said dog, wherein nucleic acid hybridization between said marker nucleic acid molecule, and said complementary nucleic acid molecule obtained from said dog permits the detection of a polymorphism whose presence is predictive of a mutation affecting canine COX-2 response in said dog;

(B) permitting hybridization between said marker nucleic acid molecule and said complementary nucleic acid molecule obtained from said patient; and

(C) detecting the presence of said polymorphism, wherein the detection of said polymorphism is diagnostic of a COX-2 related condition.

45. The method of claim 44, wherein said canine COX-2 response affected by said mutation is canine COX-2 level.

46. The method of claim 44, wherein said canine COX-2 response affected by said mutation is canine COX-2 pattern.

47. The method of claim 44, wherein said molecule is an mRNA molecule encoded by said gene, or a cDNA molecule encoded by said gene.

48. The method of claim 44, wherein said disease state is inflammation

49. The method of claim 44, wherein said disease state is arthritis

50. The method of claim 44, wherein said disease state is cancer

51. The method of claim 44, wherein said disease state is neoplasia

52. The method of claim 44, wherein said disease state is a CNS disorder

53. A method of determining an association between a polymorphism and a trait comprising:

(A) hybridizing a nucleic acid molecule specific for the polymorphism to genetic material of a cell, wherein said nucleic acid molecule has a nucleic acid sequence of SEQ ID: 3 or complements thereof; and

(B) calculating the degree of association between the polymorphism and the trait.

54. The method of claim 53, wherein said marker nucleic acid molecule comprises a nucleotide sequence of a polynucleotide that is physically linked to within 1 mb of a sequence that specifically hybridizes to said gene.

55. The method of claim 53, wherein said marker nucleic acid molecule comprises a nucleotide sequence of a polynucleotide that is physically linked to within 100 kb to a sequence that specifically hybridizes to said gene.

56. The method of claim 53, wherein said marker nucleic acid molecule comprises a nucleotide sequence of a polynucleotide that is physically linked to within 10 kb to a sequence that specifically hybridizes to said gene.

57. A method of producing a cell containing an overexpressed canine COX-2 protein comprising:

(A) transforming said cell with a functional nucleic acid molecule, wherein said functional nucleic acid molecule comprises a promoter region, wherein said promoter region is linked to a structural region, wherein said structural region comprises a nucleic acid molecule having a nucleic acid sequence of SEQ ID: 3, wherein said structural region is linked to a 3' non-translated sequence that functions in said cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and wherein said functional nucleic acid molecule results in overexpression of said canine COX-2 protein; and

(B) culturing said transformed cell.

58. A method of producing a cell containing reduced levels of a canine COX-2 protein comprising:

(A) transforming said cell with a functional nucleic acid molecule, wherein said functional nucleic acid molecule comprises a promoter region, wherein said promoter region is linked to a structural region, wherein said structural region

comprises a nucleic acid molecule having a nucleic acid sequence of SEQ ID: 3, wherein said structural region is linked to a 3' non-translated sequence that functions in said cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and wherein said functional nucleic acid molecule results in co-suppression of the canine COX-2 protein; and

(B) culturing said transformed cell.

59. A method for reducing expression of a canine COX-2 protein in a cell comprising:

(A) transforming said cell with a nucleic acid molecule, said nucleic acid molecule having an exogenous promoter region which functions in a cell to cause the production of a mRNA molecule, wherein said exogenous promoter region is linked to a transcribed nucleic acid molecule having a transcribed strand and a non-transcribed strand, wherein the transcribed strand is complementary to a nucleic acid molecule having a nucleic acid sequence of SEQ ID: 3 or its complement and said transcribed strand is complementary to an endogenous mRNA molecule; and wherein the transcribed nucleic acid molecule is linked to a 3' non-translated sequence that functions in said cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and

(B) culturing said transformed cell.

60. A method of isolating a nucleic acid that encodes a canine COX-2 protein or fragment thereof comprising:

(A) incubating under conditions permitting nucleic acid hybridization, a first nucleic acid molecule comprising SEQ ID: 3 or the complement thereof with a complementary second nucleic acid molecule obtained from a cell;

(B) permitting hybridization between said first nucleic acid molecule and said second nucleic acid molecule obtained from said cell; and

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(C) isolating said second nucleic acid molecule.

61. A method of producing a cell containing an overexpressed canine COX-2 gene comprising:

(A) transforming said cell with a functional nucleic acid molecule, wherein said functional nucleic acid molecule comprises a promoter region, wherein said promoter region is linked to a structural region, wherein said structural region comprises a nucleic acid molecule having a nucleic acid sequence consisting of SEQ ID:3 or complements thereof; wherein said structural region is linked to a 3' non-translated sequence that functions in said cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and wherein said functional nucleic acid molecule results in overexpression of said canine COX-2 gene protein; and

(B) culturing said transformed cell.

62. A method of producing a cell containing reduced levels of a canine COX-2 gene comprising:

(A) transforming said cell with a functional nucleic acid molecule, wherein said functional nucleic acid molecule comprises a promoter region, wherein said promoter region is linked to a structural region, wherein said structural region comprises a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID: 3 or complements thereof; wherein said structural region is linked to a 3' non-translated sequence that functions in said cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and wherein said functional nucleic acid molecule results in co-suppression of the canine COX-2 gene; and

(B) culturing said transformed cell.

63. A method for reducing expression of a canine COX-2 gene in a cell comprising:

(A) transforming said cell with a nucleic acid molecule, said nucleic acid molecule having an exogenous promoter region which functions in a cell to cause the production of a mRNA molecule, wherein said exogenous promoter region is linked to a transcribed nucleic acid molecule having a transcribed strand and a non-transcribed strand, wherein the transcribed strand is complementary to a nucleic acid molecule having a nucleic acid selected from the group consisting of SEQ ID: 3 or complements thereof and said transcribed strand is complementary to an endogenous mRNA molecule; and wherein the transcribed nucleic acid molecule is linked to a 3' non-translated sequence that functions in said cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of a mRNA molecule; and

(B) culturing said transformed cell.

64. A substantially-purified nucleic acid molecule comprising a sequence which encodes a mammalian canine COX-2 protein.

65. A substantially-purified nucleic acid of claim 64 wherein said nucleic acid encodes a sequence selected from the group consisting of human, rat, or mouse canine COX-2.

66. A substantially-purified nucleic acid molecule of claim 64 wherein said nucleic acid sequence encodes human canine COX-2.

67. A substantially-purified nucleic acid molecule of claim 64 wherein said nucleic acid sequence encodes mouse canine COX-2.

68. A method of using a canine COX-2 protein or fragment thereof in an assay for screening test substances for the ability to modulate or maintain canine COX-2

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activity. comprising contacting a canine COX-2 protein or fragment with a test substance, and determining the presence or level of canine COX-2 activity compared to a control.

69. A method of identifying a compound or composition that preferentially or specifically effects canine COX-2 as compared to effecting canine COX-1 comprising performing the method of claim 50, and further comprising determining the presence or level of canine COX-1 activity.

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Abstract

The present invention relates to genes that encode proteins of the canine cyclooxygenase-1 (COX-1) and the cyclooxygenase-2 (COX-2) enzymes, the canine COX-1 and canine COX-2 proteins, and assays for the evaluation of canine COX-1 and COX-2 activities. The invention also relates to nucleic acid molecules associated with these genes including their complements, homologues and fragments, and to methods of using these nucleic acid molecules, the enzymes and fragments of either.

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Fig.1 Sequence alignment of human and canine COX-1

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Canine  MSRGSRLHRW PLLL--LL-L LLLPPPPVLP AE--ARTPAP VNPCCYYPCQ
        ::: :       :::  :: : ::::: :::  :.  : :  : ::::::::::
Human   MSR-S----- -LLLRFLLFL LLLPPLPVLL ADPGAPT--P VNPCCYYPCQ 41

        HQGICVRFGL DRYQCDCTRT GYSGPNCTIP ELWTWLRNSL RPSPSFLHFL
        :::::::::: :::::::::: :::::::::: ::::::::: :::::: :::
        HQGICVRFGL DRYQCDCTRT GYSGPNCTIP GLWTWLRNSL RPSPSFTHFL 91

        LTHGRWFWEF INATFIRDML MRLVLTARSN LIPSPPTYNI AHDYISWESF
        :::::::::: .::::::.:  :::::: ::: :::::::::  ::::::::::
        LTHGRWFWEF VNATFIREMR MRLVLTVRSN LIPSPPTYNS AHDYISWESF 141

        SNVSYYTRVL PSVPQDCPTP MGTKGKKQLP DAQLLGRRFL LRRKFIPDPQ
        ::::::::.: :::: ::::: :::::::::: ::::: :::: ::::::::::
        SNVSYYTRIL PSVPKDCPTP MGTKGKKQLP DAQLLARRFL LRRKFIPDPQ 191

        GTNLMFAFFA QHFTHQFFKT SGKMGPGFTK ALGHGVDLGH IYGDNLDRQY
        :::::::::: :::::::::: :::::::::: :::::::::: ::::::.:::
        GTNLMFAFFA QHFTHQFFKT SGKMGPGFTK ALGHGVDLGH IYGDNLERQY 241

        QLRLFKDGKL KYQVLDGEMY PPSVEEAPVL MHYPRGILPQ SQMAVGQEVF
        :::::::::: :::::::::: :::::::::: ::::::: :: ::::::::::
        QLRLFKDGKL KYQVLDGEMY PPSVEEAPVL MHYPRGIPPQ SQMAVGQEVF 250

        GLLPGLMLYA TLWLREHNRV CDLLKAEHPT WGDEQLFQTA RLILIGETIK
        :::::::::: :::::::::: :::::::::: :::::::::. ::::::::::
        GLLPGLMLYA TLWLREHNRV CDLLKAEHPT WGDEQLFQTT RLILIGETIK 300

        IVIEEYVQQL SGYFLQLKFD PELLFSAQFQ YRNRIAMEFN QLYHWHPLMP
        :::::::::: :::::::::: :::::  ::: ::::::::::  :::::::::
        IVIEEYVQQL SGYFLQLKFD PELLFGVQFQ YRNRIAMEFN HLYHWHPLMP 350

        DSFWVGSQEY SYEQFLFNTS MLTHYGIEAL VDAFSRQSAG RIGGGRNIDH
        ::: :::::: :::::::::: ::  ::.::: ::::::: :: :::::::.::
        DSFKVGSQEY SYEQFLFNTS MLVDYGVEAL VDAFSRQIAG RIGGGRNMDH 400

        HVLHVAVETI KESRELRLQP FNEYRKRFGM RPYMSFQELT GEKEMAAELE
        :.::::::. : .::::.:::: :::::::::: .:: :::::  ::::::::::
        HILHVAVDVI RESREMRLQP FNEYRKRFGM KPYTSFQELV GEKEMAAELE 450

        ELYGDIDALE FYPGLLLEKC HPNSIFGESM IEIGAPFSLK GLLGNPICSP
        :::::::::: :::::::::: :::::::::: :::::::::: ::::::::::
        ELYGDIDALE FYPGLLLEKC HPNSIFGESM IEIGAPFSLK GLLGNPICSP 500

        EYWKPSTFGG EMGFNMVKTA TLKKLVCLNT KTCPYVSFRV PDPHQDGGPG
        :::::::::: :.:::.:::: :::::::::: :::::::::: ::  :: ::
        EYWKPSTFGG EVGFNIVKTA TLKKLVCLNT KTCPYVSFRV PDASQDDGPA 550

        VERPSTEL
        ::::::::
        VERPSTEL
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Fig.2 Sequence alignment of human and canine COX-2

```
Canine  MLFRAVLLCA ALAVVRAANP CCSHPCQNQG ICMSTGFDQY KCDCTRTGFY
        :: ::.:::: ::.  .::: :::::::: : .::: ::::: ::::::::::
Human   MLARALLLCA VLALSHTANP CCSHPCQNRG VCMSVGFDQY KCDCTRTGFY 50

        GENCSTPEFL TRIKLYLKPT PNTVHYILTH FKGVWNIVNN IPFLRNTIMK
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        VKGKRQLPDS NEIVGKLLLR RKFIPDPQGS NMMFAFFAQH FTHQFFKTDH 200

        KRGPAFTNGL GHGVDLNHVY GETLDRQHKL RLFKDGKMKY QVIDGEVYPP
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        KRGPAFTNGL GHGVDLNHIY GETLARQRKL RLFKDGKMKY QIIDGEMYPP 250

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        :::::: ::: ::: ::::: :::::::::: :::::::::: ::::::::::
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        :::::::::  :: ::::::: :::::::::: :::::::::: ::::::::::
        VLKQENPEWG DEQLFQTSRL ILIGETIKIV IEDYVQHLSG YHFKLKFDPE 350

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        :::: ::::: ::.:::::::::: :::::::::: :: :: :::. ::::::::::
        LLFNKQFQYQ NRIAAEFNTL YHWHPLLPDT FQIHDQKYNY QQFIYNNSIL 400

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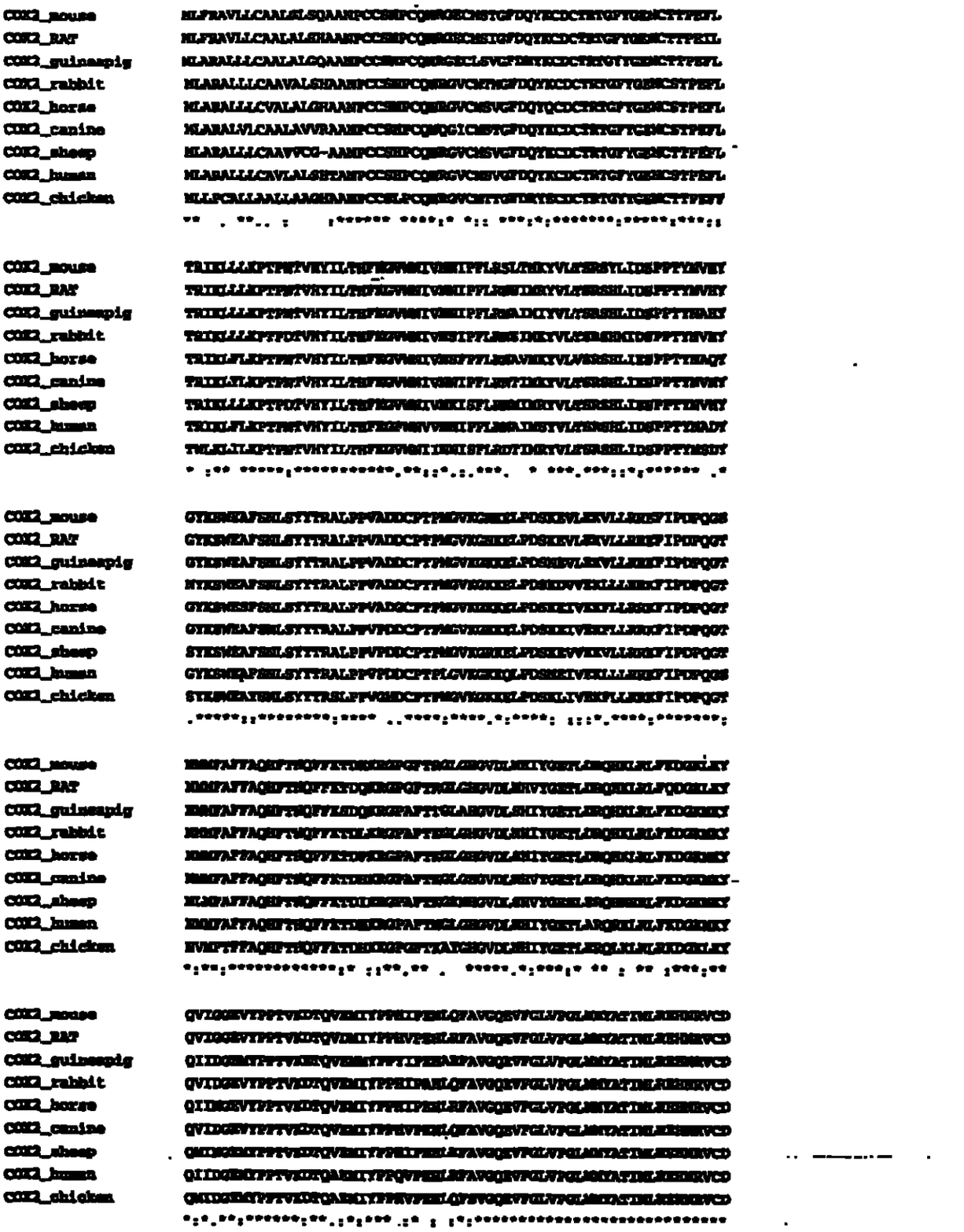
Fig.3 Multiple species sequence alignment of COX-2



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COX2_horse      MLARALLLCVALALGHAAMPCCSMPCQNRGVCMSVGFDQYQCDCTRTGFYGENCSTPEFL
COX2_canine     MLARALVLCAALAVVRAAMPCCSMPCQMQGICMSTGFDQYKCDCTRTGFYGENCSTPEFL
COX2_sheep      MLARALLLCAAVVCG-AAMPCCSMPCQNRGVCMSVGFDQYKCDCTRTGFYGENCTTPEFL
COX2_human      MLARALLLCAVLALSHTAMPCCSMPCQNRGVCMSVGFDQYKCDCTRTGFYGENCSTPEFL
COX2_chicken    MLLPCALLAALLAAGHAAMPCCSLPCQNRGVCMTTGFDRYECDCTRTGYYGENCTTPEFF
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COX2_mouse      TRIKLLLKPTPNTVHYILTHFKGVWNIVNNIPFLRSLTMKYVLTSRSYLIDSPPTYNVHY
COX2_RAT        TRIKLLLKPTPNTVHYILTHFKGVWNIVNNIPFLRNMIMRYVLTSRSHLIDSPPTYNVHY
COX2_guineapig  TRIKLLLKPTPNTVHYILTHFKGVWNIVNNIPFLRNAIMYVLTSRSHLIDSPPTYNAHY
COX2_rabbit     TRIKLLLKPTPDTVHYILTHFKGVWNIVNSIPFLRNSIMKYVLTSRSHMIDSPPTYNVHY
COX2_horse      TRIKLFLKPTPNTVHYILTHFKGVWNIVNSIPFLRNAVMKYVLVSRSHLIESPPTYNAQY
COX2_canine     TRIKLFLKPTPNTVHYILTHFKGVWNIVNNIPFLRNTIMKYVLTSRSHLIESPPTYNVHY
COX2_sheep      TRIKLLLKPTPDTVHYILTHFKGVWNIVNKISFLRNMIMRYVLTSRSHLIESPPTYNVHY
COX2_human      TRIKLFLKPTPNTVHYILTHFKGVWNVVNNIPFLRNAIMSYVLTSRSHLIDSPPTYNADY
COX2_chicken    TWLKLILKPTPNTVHYILTHFKGVWNIINNISFLRDTIMRYVLTSRSHLIDSPPTYNSDY
                * :** *****;***********.**;:*.:.***.  * ***.***;:*;****** .*

COX2_mouse      GYKSWEAFSNLSYYTRALPPVADDCPTPMGVKGKKELPDSKEVLEKVLLRREFIPDPQGS
COX2_RAT        GYKSWEAFSNLSYYTRALPPVADDCPTPMGVKGKKELPDSKEVLEKVLLRREFIPDPQGT
COX2_guineapig  GYKSWEAFSNLSYYTRALPPVADDCPTPMGVKGKKELPDSNEVLEKVLLRREFIPDPQGT
COX2_rabbit     NYKSWEAFSNLSYYTRALPPVADDCPTPMGVKGKKELPDSKDVVEKLLLRRKFIPDPQGT
COX2_horse      GYKSWESFSNLSYYTRALPPVADGCPTPMGVKGKKELPDSKEIVEKFLLRRKFIPDPQGT
COX2_canine     GYKSWEAFSNLSYYTRALPPVPDDCPTPMGVKGKKELPDSKEIVEKFLLRRKFIPDPQGT
COX2_sheep      SYKSWEAFSNLSYYTRALPPVPDDCPTPMGVKGKKELPDSKEVVEKVLLRRKFIPDPQGT
COX2_human      GYKSWEAFSNLSYYTRALPPVPDDCPTPLGVKGKKQLPDSNEIVEKLLLRRKFIPDPQGS
COX2_chicken    SYKSWEAYSNLSYYTRSLPPVGHDCPTPMGVKGKKELPDSKLIVEKFLLRRKFIPDPQGT
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COX2_mouse      NMMFAFFAQHFTHQFFKTDHKRGPGFTRGLGHGVDLNHIYGETLDRQHKLRLFKDGKLKY
COX2_RAT        NMMFAFFAQHFTHQFFKTDQKRGPGFTRGLGHGVDLNHVYGETLDRQHKLRLFQDGKLKY
COX2_guineapig  NMMFAFFAQHFTHQFFKSDQKRGPAFTTGLAHGVDLSHIYGETLDRQHKLRLFKDGKMKY
COX2_rabbit     NMMFAFFAQHFTHQFFKTDLKRGPAFTKGLGHGVDLNHIYGETLDRQHKLRLFKDGKMKY
COX2_horse      NMMFAFFAQHFTHQFFKTDPKRGPAFTKGLGHGVDLSHIYGETLDRQHKLRLFKDGKMKY
COX2_canine     NMMFAFFAQHFTHQFFKTDHKRGPAFTKGLGHGVDLNHVYGETLDRQHKLRLFKDGKMKY
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COX2_human      NMMFAFFAQHFTHQFFKTDHKRGPAFTNGLGHGVDLNHIYGETLARQHKLRLFKDGKMKY
COX2_chicken    NVMFTFFAQHFTHQFFKTDHKRGPGFTKAYGHGVDLNHIYGETLERQLKLRLRKDGKLKY
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COX2_RAT        QVIGGEVYPPTVKDTQVDMIYPPHVPENLRFAVGQEVFGLVPGLMMYATIWLREHNRVCD
COX2_guineapig  QIIDGEMYPPTVKETQVEMMYPPYIPEHARFAVGQEVFGLVPGLMMYATIWLREHNRVCD
COX2_rabbit     QVIDGEVYPPTVKDTQVEMIYPPHIPAHLQFAVGQEVFGLVPGLMMYATIWLREHNRVCD
COX2_horse      QIIDGEVYPPTVKDTQVEMIYPPHIPEHLRFAVGQEVFGLVPGLMMYATIWLREHNRVCD
COX2_canine     QVIDGEVYPPTVKDTQVEMIYPPHVPEHLQFAVGQEVFGLVPGLMMYATIWLREHNRVCD
COX2_sheep      QMIDGEMYPPTVKDTQVEMIYPPHIPEHLRFAVGQEVFGLVPGLMMYATIWLREHNRVCD
COX2_human      QIIDGEMYPPTVKDTQAEMIYPPQVPEHLRFAVGQEVFGLVPGLMMYATIWLREHNRVCD
COX2_chicken    QMIDGEMYPPTVKDTQAEMIYPPHVPEHLQFSVGQEVFGLVPGLMMYATIWLREHNRVCD
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COX2_mouse          ILKQENPEMGDEQLFQTSRLILIGETIKIVIEDYVQHLSGYHFKLKFDPELLFNQQFQYQ
COX2_RAT            ILKQENPEMDDEQLFQTSRLILIGETIKIVIEDYVQHLSGYHFKLKFDPELLFNQQFQYQ
COX2_guineapig      VLEQENPEMDDEQLFQTSRLILIGETIKIVIEDYVQHLSGYHFKLKFDPELLFNQQFQYQ
COX2_rabbit         VLKQENPEMDDEQLFQTSRLILIGETIKIVIEDYVQHLSGYHFKLKFDPELLFNQQFQYQ
COX2_horse          VLKQENPEMDDEQLFQTSRLILIGETIKIVIEDYVQHLSGYHFKLKFDPELLFNQQFQYQ
COX2_canine         VLKQENPEMDDEQLFQTSRLILIGETIKIVIEDYVQHLSGYHFKLKFDPELLFNQQFQYQ
COX2_sheep          VLKQENPEMGDEQLFQTSRLILIGETIKIVIEDYVQHLSGYHFKLKFDPELLFNQQFQYQ
COX2_human          VLKQENPEMGDEQLFQTSRLILIGETIKIVIEDYVQHLSGYHFKLKFDPELLFNEQFQYQ
COX2_chicken        VLKQENPEMDDEQLFQTTRLILIGETIKIVIEDYVQHLSGYHFKLKFDPELLFNQQFQYQ
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COX2_mouse          NRIASEFNTLYHWHPLLPDTFNIEDQEYSFKQFLYNNSILLEHGLTQFVESFTRQIAGRV
COX2_RAT            NRIASEFNTLYHWHPLLPDTFNIEDQEYTFKQFLYNNSILLEHGLAHFVESFTRQIAGRV
COX2_guineapig      NRIASEFNTLYHWHPLLPDTFQIDDQVYNFQQFLYNGSILVEHGLTQFVESFTRQIAGRV
COX2_rabbit         NRIAAEFNTLYHWHPLLPDTFQIDDQQYNFQQFLYNNSILLEHGLTQFVESFTRQIAGRV
COX2_horse          NRIAAEFNTLYHWHPLLPDTFQIDDQEYNFQQFLYNNSILLEHGLTQFVESFSRQIAGRV
COX2_canine         NRIAAEFNTLYHWHPLLPDTLQIDDQEYNFQQFIYNNSILLEHGLTQFVESFSRQIAGRV
COX2_sheep          NRIAAEFNTLYHWHPLLPDVFQIDGQEYNFQQFIYNNSVLLEHGVTQFVESFTRQIAGRV
COX2_human          NRIAAEFNTLYHWHPLLPDTFQIDDQKYNFQQFIYNNSILLEHGITQFVESFTRQIAGRV
COX2_chicken        NRIAAEFNTLYHWHPLLPDTFQIEDQETTFQQFLYNNSIMLEHGLSNFVESFSRQSAGRV
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COX2_mouse          AGGSNVPIAVQAVAKASIDQSREMKYQSLNEYRKRFSLKPYTSFEELTGEKEMAAELEAL
COX2_RAT            AGGSNVPIAVQAVAKASIDQSREMKYQSLNEYRKRFSLKPYTSFEELTGEKEMAAELEAL
COX2_guineapig      AGGSNVPLAVQKVAKASIDRSREMKYQSLNEYRKRFSLKPYTSFEELTGEKEMAAGLEAL
COX2_rabbit         AGGSNVPPAVQEVAKASIDQSREMKYQSLNEYRKRFLLKPYESFEELTGEKEMAAELEAL
COX2_horse          AGGSNVPAAAQKIAKASIDQSREMKYQSLNEYRKRFRLKPYESFEELTGEKEMAAELEAL
COX2_canine         AGGSNVPAAVQQVAKASIDQSREMKYQSLNEYRKRFRLKPYTSFEELTGEKEMAAGLEAL
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COX2_human          AGGSNVPPAVQKVEQASIDQSREMKYQSFNEYRKRFMLKPYESFEELTGEKEMAAELEAL
COX2_chicken        AGGSNVPAAVQKVAKASIDQSREMKYQSLNEYRKRFMLKPYESFEELTGEKEMAAELEEL
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COX2_human          YGDIDAVELYPALLVEKPKPDAIFGETMVEMGAPFSLKGLMGNVICSPAYWKPSTFGGEV
COX2_chicken        YGDIDAMELYPGLLVEKPKPGAIFGETMVELGAPFSLKGLMGNPICSPEYWKPSTFGGEV
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COX2_guineapig      GFQIVNTASIQSLICSNVEGCPVTAFNLPDPGLAKTVTIDDGSDSGLEDLGPTVLLKGR
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COX2_human          GFQLINTASIQSLICSNVEGCPFTSFSVPDPGLIKTVTIDDGSDSGLDDNPTVLLKER
COX2_chicken        GFKIINTASLQSLICSNVEGCPFTAFEVLEPEPT-EATIEVETSEETAMEDDNPTLLKEQ
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SO147601.080599

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Ala Glu Leu Glu Glu Leu Tyr Gly Asp Ile Asp Ala Leu Glu Phe Tyr
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Ser Met Ile Glu Ile Gly Ala Pro Phe Ser Leu Lys Gly Leu Leu Gly
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Asn Pro Ile Cys Ser Pro Glu Tyr Trp Lys Pro Ser Thr Phe Gly Gly
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Glu Met Gly Phe Asn Met Val Lys Thr Ala Thr Leu Lys Lys Leu Val
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Pro Pro Thr Tyr Asn Val Asn Tyr Gly Tyr Lys Ser Trp Glu Ala Phe
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Cys Pro Thr Pro Met Gly Val Lys Gly Lys Lys Glu Leu Pro Asp Ser
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Lys Glu Ile Val Glu Lys Phe Leu Leu Arg Arg Lys Phe Ile Pro Asp
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His Gln Phe Phe Lys Thr Asp His Lys Arg Gly Pro Ala Phe Thr Lys
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Cys Pro Phe Thr Ala Phe Ser Val Gln Asp Gly Gln Leu Thr Lys Thr
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Thr His Phe Leu Leu Thr His Gly Arg Trp Phe Trp Glu Phe Val Asn
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Ala Thr Phe Ile Arg Glu Met Arg Met Arg Leu Val Leu Thr Val Arg
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Ile Ser Trp Glu Ser Phe Ser Asn Val Ser Tyr Tyr Thr Arg Ile Leu
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Pro Ser Val Pro Lys Asp Cys Pro Thr Pro Met Gly Thr Lys Gly Lys
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Lys Gln Leu Pro Asp Ala Gln Leu Leu Ala Arg Arg Phe Leu Leu Arg
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Arg Lys Phe Ile Pro Asp Pro Gln Gly Thr Asn Leu Met Phe Ala Phe
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Phe Ala Gln His Phe Thr His Gln Phe Phe Lys Thr Ser Gly Lys Met
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Asp Gly Lys Leu Lys Tyr Gln Val Leu Asp Gly Glu Met Tyr Pro Pro
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Ser Val Glu Glu Ala Pro Val Leu Met His Tyr Pro Arg Gly Ile Pro
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2760

ctgtttatat ggctggtaac atgtaaaaac cccataaccc cgccaaaagg ggtcctaccc
2820

ttgaacataa agcaataacc aaaggagaaa agcccaaatt attggttcca aatttagggt
2880

ttaaactttt tgaagcaaac ttttttttag ccttgtgcac tgcagacctg gtactcagat
2940

tttgctatga ggttaatgaa gtaccaagct gtgcttgaat aacgatatgt tttctcagat
3000

tttctgttgt acagtttaat ttagcagtcc atatcacatt gcaaaagtag caatgacctc
3060

ataaaatacc tcttcaaaat gcttaaattc atttcacaca ttaattttat ctcagtcttg
3120

aagccaattc agtaggtgca ttggaatcaa gcctggctac ctgcatgctg ttcctttttct
3180

tttcttcttt tagccatttt gctaagagac acagtcttct caaacacttc gtttctccta
3240

ttttgtttta ctagttttaa gatcagagtt cactttcttt ggactctgcc tatatttttct
3300

tacctgaact tttgcaagtt ttcaggtaaa cctcagctca ggactgctat ttagctcctc
3360

ttaagaagat taaaaaaaaa aaaaaag
3387

<210> 8
<211> 604
<212> PRT
<213> Homo sapiens

<400> 8

Met Leu Ala Arg Ala Leu Leu Leu Cys Ala Val Leu Ala Leu Ser His
1 5 10 15

Thr Ala Asn Pro Cys Cys Ser His Pro Cys Gln Asn Arg Gly Val Cys
20 25 30

Met Ser Val Gly Phe Asp Gln Tyr Lys Cys Asp Cys Thr Arg Thr Gly
35 40 45

Phe Tyr Gly Glu Asn Cys Ser Thr Pro Glu Phe Leu Thr Arg Ile Lys
50 55 60

Leu Phe Leu Lys Pro Thr Pro Asn Thr Val His Tyr Ile Leu Thr His
65 70 75 80

Phe Lys Gly Phe Trp Asn Val Val Asn Asn Ile Pro Phe Leu Arg Asn
85 90 95

Ala Ile Met Ser Tyr Val Leu Thr Ser Arg Ser His Leu Ile Asp Ser
100 105 110

Pro Pro Thr Tyr Asn Ala Asp Tyr Gly Tyr Lys Ser Trp Glu Ala Phe
115 120 125

Ser Asn Leu Ser Tyr Tyr Thr Arg Ala Leu Pro Pro Val Pro Asp Asp

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    130                 135                 140

Cys Pro Thr Pro Leu Gly Val Lys Gly Lys Lys Gln Leu Pro Asp Ser
145                 150                 155                 160

Asn Glu Ile Val Glu Lys Leu Leu Leu Arg Arg Lys Phe Ile Pro Asp
                165                 170                 175

Pro Gln Gly Ser Asn Met Met Phe Ala Phe Phe Ala Gln His Phe Thr
            180                 185                 190

His Gln Phe Phe Lys Thr Asp His Lys Arg Gly Pro Ala Phe Thr Asn
        195                 200                 205

Gly Leu Gly His Gly Val Asp Leu Asn His Ile Tyr Gly Glu Thr Leu
    210                 215                 220

Ala Arg Gln Arg Lys Leu Arg Leu Phe Lys Asp Gly Lys Met Lys Tyr
225                 230                 235                 240

Gln Ile Ile Asp Gly Glu Met Tyr Pro Pro Thr Val Lys Asp Thr Gln
                245                 250                 255

Ala Glu Met Ile Tyr Pro Pro Gln Val Pro Glu His Leu Arg Phe Ala
            260                 265                 270

Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala
        275                 280                 285

Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Val Leu Lys Gln
    290                 295                 300

Glu His Pro Glu Trp Gly Asp Glu Gln Leu Phe Gln Thr Ser Arg Leu
305                 310                 315                 320

Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln
                325                 330                 335

His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu
            340                 345                 350

Phe Asn Lys Gln Phe Gln Tyr Gln Asn Arg Ile Ala Ala Glu Phe Asn
        355                 360                 365

Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Thr Phe Gln Ile His
    370                 375                 380

Asp Gln Lys Tyr Asn Tyr Gln Gln Phe Ile Tyr Asn Asn Ser Ile Leu
385                 390                 395                 400

Leu Glu His Gly Ile Thr Gln Phe Val Glu Ser Phe Thr Arg Gln Ile
                405                 410                 415

Ala Gly Arg Val Ala Gly Gly Arg Asn Val Pro Pro Ala Val Gln Lys
            420                 425                 430

Val Ser Gln Ala Ser Ile Asp Gln Ser Arg Gln Met Lys Tyr Gln Ser
        435                 440                 445

Phe Asn Glu Tyr Arg Lys Arg Phe Met Leu Lys Pro Tyr Glu Ser Phe
    450                 455                 460

Glu Glu Leu Thr Gly Glu Lys Glu Met Ser Ala Glu Leu Glu Ala Leu
465                 470                 475                 480

Tyr Gly Asp Ile Asp Ala Val Glu Leu Tyr Pro Ala Leu Leu Val Glu
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                485                 490                 495

Lys Pro Arg Pro Asp Ala Ile Phe Gly Glu Thr Met Val Glu Val Gly
            500                 505                 510

Ala Pro Phe Ser Leu Lys Gly Leu Met Gly Asn Val Ile Cys Ser Pro
        515                 520                 525

Ala Tyr Trp Lys Pro Ser Thr Phe Gly Gly Glu Val Gly Phe Gln Ile
    530                 535                 540

Ile Asn Thr Ala Ser Ile Gln Ser Leu Ile Cys Asn Asn Val Lys Gly
545                 550                 555                 560

Cys Pro Phe Thr Ser Phe Ser Val Pro Asp Pro Glu Leu Ile Lys Thr
                565                 570                 575

Val Thr Ile Asn Ala Ser Ser Ser Arg Ser Gly Leu Asp Asp Ile Asn
            580                 585                 590

Pro Thr Val Leu Leu Lys Glu Arg Ser Thr Glu Leu
        595                 600
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SUBSTITUTE SHEET (RULE 26)

<210> 9
<211> 604
<212> PRT
<213> Mus musculus

<400> 9

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Met Leu Phe Arg Ala Val Leu Leu Cys Ala Ala Leu Gly Leu Ser Gln
  1               5                  10                  15

Ala Ala Asn Pro Cys Cys Ser Asn Pro Cys Gln Asn Arg Gly Glu Cys
            20                  25                  30

Met Ser Thr Gly Phe Asp Gln Tyr Lys Cys Asp Cys Thr Arg Thr Gly
        35                  40                  45

Phe Tyr Gly Glu Asn Cys Thr Thr Pro Glu Phe Leu Thr Arg Ile Lys
    50                  55                  60

Leu Leu Leu Lys Pro Thr Pro Asn Thr Val His Tyr Ile Leu Thr His
65                  70                  75                  80

Phe Lys Gly Val Trp Asn Ile Val Asn Asn Ile Pro Phe Leu Arg Ser
                85                  90                  95

Leu Thr Met Lys Tyr Val Leu Thr Ser Arg Ser Tyr Leu Ile Asp Ser
            100                 105                 110

Pro Pro Thr Tyr Asn Val His Tyr Gly Tyr Lys Ser Trp Glu Ala Phe
        115                 120                 125

Ser Asn Leu Ser Tyr Tyr Thr Arg Ala Leu Pro Pro Val Ala Asp Asp
    130                 135                 140

Cys Pro Thr Pro Met Gly Val Lys Gly Asn Lys Glu Leu Pro Asp Ser
145                 150                 155                 160

Lys Glu Val Leu Glu Lys Val Leu Leu Arg Arg Glu Phe Ile Pro Asp
                165                 170                 175

Pro Gln Gly Ser Asn Met Met Phe Ala Phe Phe Ala Gln His Phe Thr
            180                 185                 190
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EP 9247

His Gln Phe Phe Lys Thr Asp His Lys Arg Gly Pro Gly Phe Thr Arg
195 200 205

Gly Leu Gly His Gly Val Asp Leu Asn His Ile Tyr Gly Glu Thr Leu
210 215 220

Asp Arg Gln His Lys Leu Arg Leu Phe Lys Asp Gly Lys Leu Lys Tyr
225 230 235 240

Gln Val Ile Gly Gly Glu Val Tyr Pro Pro Thr Val Lys Asp Thr Gln
245 250 255

Val Glu Met Ile Tyr Pro Pro His Ile Pro Glu Asn Leu Gln Phe Ala
260 265 270

Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala
275 280 285

Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Ile Leu Lys Gln
290 295 300

Glu His Pro Glu Trp Gly Asp Glu Gln Leu Phe Gln Thr Ser Arg Leu
305 310 315 320

Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln
325 330 335

His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu
340 345 350

Phe Asn Gln Gln Phe Gln Tyr Gln Asn Arg Ile Ala Ser Glu Phe Asn
355 360 365

Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Thr Phe Asn Ile Glu
370 375 380

Asp Gln Glu Tyr Ser Phe Lys Gln Phe Leu Tyr Asn Asn Ser Ile Leu
385 390 395 400

Leu Glu His Gly Leu Thr Gln Phe Val Glu Ser Phe Thr Arg Gln Ile
405 410 415

Ala Gly Arg Val Ala Gly Gly Arg Asn Val Pro Ile Ala Val Gln Ala
420 425 430

Val Ala Lys Ala Ser Ile Asp Gln Ser Arg Glu Met Lys Tyr Gln Ser
435 440 445

Leu Asn Glu Tyr Arg Lys Arg Phe Ser Leu Lys Pro Tyr Thr Ser Phe
450 455 460

Glu Glu Leu Thr Gly Glu Lys Glu Met Ala Ala Glu Leu Lys Ala Leu
465 470 475 480

Tyr Ser Asp Ile Asp Val Met Glu Leu Tyr Pro Ala Leu Leu Val Glu
485 490 495

Lys Pro Arg Pro Asp Ala Ile Phe Gly Glu Thr Met Val Glu Leu Gly
500 505 510

Ala Pro Phe Ser Leu Lys Gly Leu Met Gly Asn Pro Ile Cys Ser Pro
515 520 525

Gln Tyr Trp Lys Pro Ser Thr Phe Gly Gly Glu Val Gly Phe Lys Ile
530 535 540

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Ile Asn Thr Ala Ser Ile Gln Ser Leu Ile Cys Asn Asn Val Lys Gly
545 550 555 560

Cys Pro Phe Thr Ser Phe Asn Val Gln Asp Pro Gln Pro Thr Lys Thr
565 570 575

Ala Thr Ile Asn Ala Ser Ala Ser His Ser Arg Leu Asp Asp Ile Asn
580 585 590

Pro Thr Val Leu Ile Lys Arg Arg Ser Thr Glu Leu
595 600

<210> 10
<211> 604
<212> PRT
<213> Rattus norvegicus

<400> 10

Met Leu Phe Arg Ala Val Leu Leu Cys Ala Ala Leu Ala Leu Ser His
1 5 10 15

Ala Ala Asn Pro Cys Cys Ser Asn Pro Cys Gln Asn Arg Gly Glu Cys
20 25 30

Met Ser Ile Gly Phe Asp Gln Tyr Lys Cys Asp Cys Thr Arg Thr Gly
35 40 45

Phe Tyr Gly Glu Asn Cys Thr Thr Pro Glu Ile Leu Thr Arg Ile Lys
50 55 60

Leu Leu Leu Lys Pro Thr Pro Asn Thr Val His Tyr Ile Leu Thr His
65 70 75 80

Phe Lys Gly Val Trp Asn Ile Val Asn Asn Ile Pro Phe Leu Arg Asn
85 90 95

Ser Ile Met Arg Tyr Val Leu Thr Ser Arg Ser His Leu Ile Asp Ser
100 105 110

Pro Pro Thr Tyr Asn Val His Tyr Gly Tyr Lys Ser Trp Glu Ala Phe
115 120 125

Ser Asn Leu Ser Tyr Tyr Thr Arg Ala Leu Pro Pro Val Ala Asp Asp
130 135 140

Cys Pro Thr Pro Met Gly Val Lys Gly Asn Lys Glu Leu Pro Asp Ser
145 150 155 160

Lys Glu Val Leu Glu Lys Val Leu Leu Arg Arg Glu Phe Ile Pro Asp
165 170 175

Pro Gln Gly Thr Asn Met Met Phe Ala Phe Phe Ala Gln His Phe Thr
180 185 190

His Gln Phe Phe Lys Thr Asp Gln Lys Arg Gly Pro Gly Phe Thr Arg
195 200 205

Gly Leu Gly His Gly Val Asp Leu Asn His Val Tyr Gly Glu Thr Leu
210 215 220

Asp Arg Gln His Lys Leu Arg Leu Phe Gln Asp Gly Lys Leu Lys Tyr
225 230 235 240

249

Gln Val Ile Gly Gly Glu Val Tyr Pro Pro Thr Val Lys Asp Thr Gln
245 250 255

Val Asp Met Ile Tyr Pro Pro His Val Pro Glu His Leu Arg Phe Ala
260 265 270

Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala
275 280 285

Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Ile Leu Lys Gln
290 295 300

Glu His Pro Glu Trp Asp Asp Glu Arg Leu Phe Gln Thr Ser Arg Leu
305 310 315 320

Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln
325 330 335

His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu
340 345 350

Phe Asn Gln Gln Phe Gln Tyr Gln Asn Arg Ile Ala Ser Glu Phe Asn
355 360 365

Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Thr Phe Asn Ile Glu
370 375 380

Asp Gln Glu Tyr Thr Phe Lys Gln Phe Leu Tyr Asn Asn Ser Ile Leu
385 390 395 400

Leu Glu His Gly Leu Ala His Phe Val Glu Ser Phe Thr Arg Gln Ile
405 410 415

Ala Gly Arg Val Ala Gly Gly Arg Asn Val Pro Ile Ala Val Gln Ala
420 425 430

Val Ala Lys Ala Ser Ile Asp Gln Ser Arg Glu Met Lys Tyr Gln Ser
435 440 445

Leu Asn Glu Tyr Arg Lys Arg Phe Ser Leu Lys Pro Tyr Thr Ser Phe
450 455 460

Glu Glu Leu Thr Gly Glu Lys Glu Met Ala Ala Glu Leu Lys Ala Leu
465 470 475 480

Tyr His Asp Ile Asp Ala Met Glu Leu Tyr Pro Ala Leu Leu Val Glu
485 490 495

Lys Pro Arg Pro Asp Ala Ile Phe Gly Glu Thr Met Val Glu Leu Gly
500 505 510

Ala Pro Phe Ser Leu Lys Gly Leu Met Gly Asn Pro Ile Cys Ser Pro
515 520 525

Gln Tyr Trp Lys Pro Ser Thr Phe Gly Gly Glu Val Gly Phe Arg Ile
530 535 540

Ile Asn Thr Ala Ser Ile Gln Ser Leu Ile Cys Asn Asn Val Lys Gly
545 550 555 560

Cys Pro Phe Ala Ser Phe Asn Val Gln Asp Pro Gln Pro Thr Lys Thr
565 570 575

Ala Thr Ile Asn Ala Ser Ala Ser His Ser Arg Leu Asp Asp Ile Asn
580 585 590

250

Pro Thr Val Leu Ile Lys Arg Arg Ser Thr Glu Leu
595 600

<210> 11
<211> 604
<212> PRT
<213> Cavia porcellus

<400> 11

Met Leu Ala Arg Ala Leu Leu Leu Cys Ala Ala Leu Ala Leu Gly Gln
1 5 10 15

Ala Ala Asn Pro Cys Cys Ser Asn Pro Cys Gln Asn Arg Gly Glu Cys
20 25 30

Leu Ser Val Gly Phe Asp Arg Tyr Lys Cys Asp Cys Thr Arg Thr Gly
35 40 45

Tyr Tyr Gly Glu Asn Cys Thr Thr Pro Glu Phe Leu Thr Arg Ile Lys
50 55 60

Leu Leu Leu Lys Pro Thr Pro Asn Thr Val His Tyr Ile Leu Thr His
65 70 75 80

Phe Lys Gly Val Trp Asn Ile Val Asn Asn Ile Pro Phe Leu Arg Asn
85 90 95

Ala Ile Met Ile Tyr Val Leu Thr Ser Arg Ser His Leu Ile Asp Ser
100 105 110

Pro Pro Thr Tyr Asn Ala His Tyr Gly Tyr Lys Ser Trp Glu Ala Phe
115 120 125

Ser Asn Leu Ser Tyr Tyr Thr Arg Ala Leu Pro Pro Val Ala Asp Asp
130 135 140

Cys Pro Thr Pro Met Gly Val Lys Gly Lys Lys Glu Leu Pro Asp Ser
145 150 155 160

Asn Glu Val Leu Glu Lys Val Leu Leu Arg Arg Lys Phe Ile Pro Asp
165 170 175

Pro Gln Gly Thr Asn Met Met Phe Ala Phe Phe Ala Gln His Phe Thr
180 185 190

His Gln Phe Phe Lys Ser Asp Gln Lys Arg Gly Pro Ala Phe Thr Thr
195 200 205

Gly Leu Ala His Gly Val Asp Leu Ser His Ile Tyr Gly Glu Thr Leu
210 215 220

Asp Arg Gln His Lys Leu Arg Leu Phe Lys Asp Gly Lys Met Lys Tyr
225 230 235 240

Gln Ile Ile Asp Gly Glu Met Tyr Pro Pro Thr Val Lys Glu Thr Gln
245 250 255

Val Glu Met Met Tyr Pro Pro Tyr Ile Pro Glu His Ala Arg Phe Ala
260 265 270

Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala
275 280 285

Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Val Leu Lys Gln

253 01

290 295 300

Glu His Pro Glu Trp Asp Asp Glu Arg Leu Phe Gln Thr Ser Arg Leu
305 310 315 320

Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln
325 330 335

His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu
340 345 350

Phe Asn Gln Gln Phe Gln Tyr Gln Asn Arg Ile Ala Ser Glu Phe Asn
355 360 365

Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Thr Phe Gln Ile Asp
370 375 380

Asp Gln Val Tyr Asn Phe Gln Gln Phe Leu Tyr Asn Asn Ser Ile Leu
385 390 395 400

Val Glu His Gly Leu Thr Gln Phe Val Glu Ser Phe Thr Lys Gln Ile
405 410 415

Ala Gly Arg Val Ala Gly Gly Arg Asn Val Pro Leu Ala Val Gln Arg
420 425 430

Val Ala Lys Ala Ser Ile Glu His Ser Arg Lys Met Lys Tyr Gln Ser
435 440 445

Leu Asn Glu Tyr Arg Lys Arg Phe Leu Met Lys Pro Tyr Thr Ser Phe
450 455 460

Glu Glu Leu Thr Gly Glu Lys Glu Met Ala Ala Gly Leu Glu Ala Leu
465 470 475 480

Tyr Gly Asp Ile Asp Ala Met Glu Leu Tyr Pro Ala Leu Leu Val Glu
485 490 495

Lys Pro Arg Pro Asp Ala Ile Phe Gly Glu Thr Met Val Glu Met Gly
500 505 510

Ala Pro Phe Ser Leu Lys Gly Leu Met Gly Asn Pro Ile Cys Ser Pro
515 520 525

His Tyr Trp Lys Pro Ser Thr Phe Gly Gly Glu Val Gly Phe Gln Ile
530 535 540

Val Asn Thr Ala Ser Ile Gln Ser Leu Ile Cys Asn Asn Val Lys Gly
545 550 555 560

Cys Pro Val Thr Ala Phe Asn Leu Pro Asp Pro Gln Leu Ala Lys Thr
565 570 575

Val Thr Ile Asn Ala Ser Ala Ser His Ser Arg Leu Glu Asp Leu Ser
580 585 590

Pro Thr Val Leu Leu Lys Gly Arg Ser Thr Glu Leu
595 600

<210> 12
<211> 604
<212> PRT
<213> Oryctolagus cuniculus

<400> 12

254252

Met Leu Ala Arg Ala Leu Leu Leu Cys Ala Ala Val Ala Leu Ser His
1 5 10 15

Ala Ala Asn Pro Cys Cys Ser Asn Pro Cys Gln Asn Arg Gly Val Cys
20 25 30

Met Thr Met Gly Phe Asp Gln Tyr Lys Cys Asp Cys Thr Arg Thr Gly
35 40 45

Phe Tyr Gly Glu Asn Cys Ser Thr Pro Glu Phe Leu Thr Arg Ile Lys
50 55 60

Leu Leu Leu Lys Pro Thr Pro Asp Thr Val His Tyr Ile Leu Thr His
65 70 75 80

Phe Lys Gly Val Trp Asn Ile Val Asn Ser Ile Pro Phe Leu Arg Asn
85 90 95

Ser Ile Met Lys Tyr Val Leu Thr Ser Arg Ser His Met Ile Asp Ser
100 105 110

Pro Pro Thr Tyr Asn Val His Tyr Asn Tyr Lys Ser Trp Glu Ala Phe
115 120 125

Ser Asn Leu Ser Tyr Tyr Thr Arg Ala Leu Pro Pro Val Ala Asp Asp
130 135 140

Cys Pro Thr Pro Met Gly Val Lys Gly Lys Lys Glu Leu Pro Asp Ser
145 150 155 160

Lys Asp Val Val Glu Lys Leu Leu Leu Arg Arg Lys Phe Ile Pro Asp
165 170 175

Pro Gln Gly Thr Asn Met Met Phe Ala Phe Phe Ala Gln His Phe Thr
180 185 190

His Gln Phe Phe Lys Thr Asp Leu Lys Arg Gly Pro Ala Phe Thr Lys
195 200 205

Gly Leu Gly His Gly Val Asp Leu Asn His Ile Tyr Gly Glu Thr Leu
210 215 220

Asp Arg Gln His Lys Leu Arg Leu Phe Lys Asp Gly Lys Met Lys Tyr
225 230 235 240

Gln Val Ile Asp Gly Glu Val Tyr Pro Pro Thr Val Lys Asp Thr Gln
245 250 255

Val Glu Met Ile Tyr Pro Pro His Ile Pro Ala His Leu Gln Phe Ala
260 265 270

Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala
275 280 285

Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Val Leu Lys Gln
290 295 300

Glu His Pro Glu Trp Asp Asp Glu Gln Leu Phe Gln Thr Ser Arg Leu
305 310 315 320

Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln
325 330 335

His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu
340 345 350

Phe Asn Gln Gln Phe Gln Tyr Gln Asn Arg Ile Ala Ala Glu Phe Asn
355 360 365

Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Thr Phe Gln Ile Asp
370 375 380

Asp Gln Gln Tyr Asn Tyr Gln Gln Phe Leu Tyr Asn Asn Ser Ile Leu
385 390 395 400

Leu Glu His Gly Leu Thr Gln Phe Val Glu Ser Phe Thr Arg Gln Ile
405 410 415

Ala Gly Arg Val Ala Gly Gly Arg Asn Val Pro Pro Ala Val Gln Lys
420 425 430

Val Ala Lys Ala Ser Ile Asp Gln Ser Arg Gln Met Lys Tyr Gln Ser
435 440 445

Leu Asn Glu Tyr Arg Lys Arg Phe Leu Leu Lys Pro Tyr Glu Ser Phe
450 455 460

Glu Glu Leu Thr Gly Glu Lys Glu Met Ala Ala Glu Leu Glu Ala Leu
465 470 475 480

Tyr Gly Asp Ile Asp Ala Val Glu Leu Tyr Pro Ala Leu Leu Val Glu
485 490 495

Arg Pro Arg Pro Asp Ala Ile Phe Gly Glu Ser Met Val Glu Met Gly
500 505 510

Ala Pro Phe Ser Leu Lys Gly Leu Met Gly Asn Pro Ile Cys Ser Pro
515 520 525

Asn Tyr Trp Lys Pro Ser Thr Phe Gly Gly Glu Val Gly Phe Lys Ile
530 535 540

Val Asn Thr Ala Ser Ile Gln Ser Leu Ile Cys Asn Asn Val Lys Gly
545 550 555 560

Cys Pro Phe Thr Ser Phe Asn Val Pro Asp Pro Gln Leu Thr Lys Thr
565 570 575

Val Thr Ile Asn Ala Ser Ala Ser His Ser Arg Leu Glu Asp Ile Asn
580 585 590

Pro Thr Val Leu Leu Lys Gly Arg Ser Thr Glu Leu
595 600

<210> 13
<211> 604
<212> PRT
<213> Equus caballus

<400> 13

Met Leu Ala Arg Ala Leu Leu Leu Cys Val Ala Leu Ala Leu Gly His
1 5 10 15

Ala Ala Asn Pro Cys Cys Ser Asn Pro Cys Gln Asn Arg Gly Val Cys
20 25 30

Met Ser Val Gly Phe Asp Gln Tyr Gln Cys Asp Cys Thr Arg Thr Gly
35 40 45

Phe Tyr Gly Glu Asn Cys Ser Thr Pro Glu Phe Leu Thr Arg Ile Lys
50 55 60

Leu Phe Leu Lys Pro Thr Pro Asn Thr Val His Tyr Ile Leu Thr His
65 70 75 80

Phe Lys Gly Val Trp Asn Ile Val Asn Ser Phe Pro Phe Leu Arg Asn
85 90 95

Ala Val Met Lys Tyr Val Leu Val Ser Arg Ser His Leu Ile Glu Ser
100 105 110

Pro Pro Thr Tyr Asn Ala Gln Tyr Gly Tyr Lys Ser Trp Glu Ser Phe
115 120 125

Ser Asn Leu Ser Tyr Tyr Thr Arg Ala Leu Pro Pro Val Ala Asp Gly
130 135 140

Cys Pro Thr Pro Met Gly Val Lys Gly Lys Lys Glu Leu Pro Asp Ser
145 150 155 160

Lys Glu Ile Val Glu Lys Phe Leu Leu Arg Arg Lys Phe Ile Pro Asp
165 170 175

Pro Gln Gly Thr Asn Met Met Phe Ala Phe Phe Ala Gln His Phe Thr
180 185 190

His Gln Phe Phe Lys Thr Asp Pro Lys Arg Gly Pro Ala Phe Thr Lys
195 200 205

Gly Leu Gly His Gly Val Asp Leu Ser His Ile Tyr Gly Glu Thr Leu
210 215 220

Asp Arg Gln His Lys Leu Arg Leu Phe Lys Asp Gly Lys Met Lys Tyr
225 230 235 240

Gln Ile Ile Asn Gly Glu Val Tyr Pro Pro Thr Val Lys Asp Thr Gln
245 250 255

Val Glu Met Ile Tyr Pro Pro His Ile Pro Glu His Leu Arg Phe Ala
260 265 270

Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala
275 280 285

Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Val Leu Lys Gln
290 295 300

Glu His Pro Glu Trp Asp Asp Glu Arg Leu Phe Gln Thr Ser Arg Leu
305 310 315 320

Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln
325 330 335

His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu
340 345 350

Phe Asn Gln Gln Phe Gln Tyr Gln Asn Arg Ile Ala Ala Glu Phe Asn
355 360 365

Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Thr Phe Gln Ile Asp
370 375 380

Asp Gln Glu Tyr Asn Phe Gln Gln Phe Leu Tyr Asn Asn Ser Ile Leu
385 390 395 400

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Leu Glu His Gly Leu Thr Gln Phe Val Glu Ser Phe Ser Arg Gln Ile
405 410 415

Ala Gly Arg Val Ala Gly Gly Arg Asn Val Pro Ala Ala Ala Gln Lys
420 425 430

Ile Ala Lys Ala Ser Ile Asp Gln Ser Arg Glu Met Lys Tyr Gln Ser
435 440 445

Leu Asn Glu Tyr Arg Lys Arg Phe Arg Leu Thr Pro Tyr Lys Ser Phe
450 455 460

Glu Glu Leu Thr Gly Glu Lys Glu Met Ala Ala Glu Leu Glu Ala Leu
465 470 475 480

Tyr Gly Asp Ile Asp Ala Met Glu Leu Tyr Pro Ala Leu Leu Val Glu
485 490 495

Lys Pro Arg Pro Asp Ala Ile Phe Gly Glu Thr Met Val Glu Leu Gly
500 505 510

Ala Pro Phe Ser Leu Lys Gly Leu Leu Gly Asn Pro Ile Cys Ser Pro
515 520 525

Asp Tyr Trp Lys Pro Ser Thr Phe Gly Gly Glu Val Gly Phe Lys Ile
530 535 540

Ile Asn Thr Ala Ser Ile Gln Ser Leu Ile Cys Asn Asn Val Lys Gly
545 550 555 560

Cys Pro Phe Thr Ala Phe Ser Val Gln Asp Pro Gln Leu Ser Lys Ala
565 570 575

Val Thr Ile Asn Ala Ser Ala Ser His Ser Gly Leu Asp Asp Val Asn
580 585 590

Pro Thr Val Leu Leu Lys Glu Arg Ser Thr Glu Leu
595 600

<210> 14
<211> 604
<212> PRT
<213> Ovis aries

<223> unsure at all Xaa locations
<400> 14

Met Leu Ala Arg Ala Leu Leu Leu Cys Ala Ala Val Val Cys Gly Xaa
1 5 10 15

Ala Ala Asn Pro Cys Cys Ser His Pro Cys Gln Asn Arg Gly Val Cys
20 25 30

Met Ser Val Gly Phe Asp Gln Tyr Lys Cys Asp Cys Thr Arg Thr Gly
35 40 45

Phe Tyr Gly Glu Asn Cys Thr Thr Pro Glu Phe Leu Thr Arg Ile Lys
50 55 60

Leu Leu Leu Lys Pro Thr Pro Asp Thr Val His Tyr Ile Leu Thr His
65 70 75 80

Phe Lys Gly Val Trp Asn Ile Val Asn Lys Ile Ser Phe Leu Arg Asn
85 90 95

5 304 750 A1 '080 5

Met Ile Met Arg Tyr Val Leu Thr Ser Arg Ser His Leu Ile Glu Ser
100 105 110

Pro Pro Thr Tyr Asn Val His Tyr Ser Tyr Lys Ser Trp Glu Ala Phe
115 120 125

Ser Asn Leu Ser Tyr Tyr Thr Arg Ala Leu Pro Pro Val Pro Asp Asp
130 135 140

Cys Pro Thr Pro Met Gly Val Lys Gly Arg Lys Glu Leu Pro Asp Ser-
145 150 155 160

Lys Glu Val Val Lys Lys Val Leu Leu Arg Arg Lys Phe Ile Pro Asp
165 170 175

Pro Gln Gly Thr Asn Leu Met Phe Ala Phe Phe Ala Gln His Phe Thr
180 185 190

His Gln Phe Phe Lys Thr Asp Ile Glu Arg Gly Pro Ala Phe Thr Lys
195 200 205

Gly Lys Asn His Gly Val Asp Leu Ser His Val Tyr Gly Glu Ser Leu
210 215 220

Glu Arg Gln His Asn Arg Arg Leu Phe Lys Asp Gly Lys Met Lys Tyr
225 230 235 240

Gln Met Ile Asn Gly Glu Met Tyr Pro Pro Thr Val Lys Asp Thr Gln
245 250 255

Val Glu Met Ile Tyr Pro Pro His Ile Pro Glu His Leu Lys Phe Ala
260 265 270

Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala
275 280 285

Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Val Leu Lys Gln
290 295 300

Glu His Pro Glu Trp Gly Asp Glu Gln Leu Phe Gln Thr Ser Arg Leu
305 310 315 320

Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln
325 330 335

His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu
340 345 350

Phe Asn Gln Gln Phe Gln Tyr Gln Asn Arg Ile Ala Ala Glu Phe Asn
355 360 365

Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Val Phe Gln Ile Asp
370 375 380

Gly Gln Glu Tyr Asn Tyr Gln Gln Phe Ile Tyr Asn Asn Ser Val Leu
385 390 395 400

Leu Glu His Gly Val Thr Gln Phe Val Glu Ser Phe Thr Arg Gln Ile
405 410 415

Ala Gly Arg Val Ala Gly Arg Arg Asn Leu Pro Ala Ala Val Glu Lys
420 425 430

Val Ser Lys Ala Ser Leu Asp Gln Ser Arg Glu Met Lys Tyr Gln Ser
435 440 445

Phe Asn Glu Tyr Arg Lys Arg Phe Leu Leu Lys Pro Tyr Glu Ser Phe
450 455 460

Glu Glu Leu Thr Gly Glu Lys Glu Met Ala Ala Glu Leu Glu Ala Leu
465 470 475 480

Tyr Gly Asp Ile Asp Ala Met Glu Leu Tyr Pro Ala Leu Leu Val Glu
485 490 495

Lys Pro Ala Pro Asp Ala Ile Phe Gly Glu Thr Met Val Glu Ala Gly
500 505 510

Ala Pro Phe Ser Leu Lys Gly Leu Met Gly Asn Pro Ile Cys Ser Pro
515 520 525

Glu Tyr Trp Lys Pro Ser Thr Phe Gly Gly Glu Val Gly Phe Lys Ile
530 535 540

Ile Asn Thr Ala Ser Ile Gln Ser Leu Ile Cys Ser Asn Val Lys Gly
545 550 555 560

Cys Pro Phe Thr Ser Phe Ser Val Gln Asp Ala His Leu Thr Lys Thr
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Val Thr Ile Asn Ala Ser Ser Ser His Ser Gly Leu Asp Asp Ile Asn
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Pro Thr Val Leu Leu Lys Glu Arg Ser Thr Glu Leu
595 600

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<211> 604
<212> PRT
<213> Gallus gallus

<223> unsure at all Xaa locations
<400> 15

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Ala Ala Asn Pro Cys Cys Ser Leu Pro Cys Gln Asn Arg Gly Val Cys
20 25 30

Met Thr Thr Gly Phe Asp Arg Tyr Glu Cys Asp Cys Thr Arg Thr Gly
35 40 45

Tyr Tyr Gly Glu Asn Cys Thr Thr Pro Glu Phe Phe Thr Trp Leu Lys
50 55 60

Leu Ile Leu Lys Pro Thr Pro Asn Thr Val His Tyr Ile Leu Thr His
65 70 75 80

Phe Lys Gly Val Trp Asn Ile Ile Asn Asn Ile Ser Phe Leu Arg Asp
85 90 95

Thr Ile Met Arg Tyr Val Leu Thr Ser Arg Ser His Leu Ile Asp Ser
100 105 110

Pro Pro Thr Tyr Asn Ser Asp Tyr Ser Tyr Lys Ser Trp Glu Ala Tyr
115 120 125

Ser Asn Leu Ser Tyr Tyr Thr Arg Ser Leu Pro Pro Val Gly His Asp
130 135 140

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Cys Pro Thr Pro Met Gly Val Lys Gly Lys Lys Glu Leu Pro Asp Ser
145 150 155 160

Lys Leu Ile Val Glu Lys Phe Leu Leu Arg Arg Lys Phe Ile Pro Asp
165 170 175

Pro Gln Gly Thr Asn Val Met Phe Thr Phe Phe Ala Gln His Phe Thr
180 185 190

His Gln Phe Phe Lys Thr Asp His Lys Lys Gly Pro Gly Phe Thr Lys
195 200 205

Ala Tyr Gly His Gly Val Asp Leu Asn His Ile Tyr Gly Glu Thr Leu
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Glu Arg Gln Leu Lys Leu Arg Leu Arg Lys Asp Gly Lys Leu Lys Tyr
225 230 235 240

Gln Met Ile Asp Gly Glu Met Tyr Pro Pro Thr Val Lys Asp Thr Gln
245 250 255

Ala Glu Met Ile Tyr Pro Pro His Val Pro Glu His Leu Gln Phe Ser
260 265 270

Val Gly Gln Glu Val Phe Gly Leu Val Pro Gly Leu Met Met Tyr Ala
275 280 285

Thr Ile Trp Leu Arg Glu His Asn Arg Val Cys Asp Val Leu Lys Gln
290 295 300

Glu His Pro Glu Trp Asp Asp Glu Gln Leu Phe Gln Thr Thr Arg Leu
305 310 315 320

Ile Leu Ile Gly Glu Thr Ile Lys Ile Val Ile Glu Asp Tyr Val Gln
325 330 335

His Leu Ser Gly Tyr His Phe Lys Leu Lys Phe Asp Pro Glu Leu Leu
340 345 350

Phe Asn Gln Arg Phe Gln Tyr Gln Asn Arg Ile Ala Ala Glu Phe Asn
355 360 365

Thr Leu Tyr His Trp His Pro Leu Leu Pro Asp Thr Phe Gln Ile His
370 375 380

Asn Gln Glu Tyr Thr Phe Gln Gln Phe Leu Tyr Asn Asn Ser Ile Met
385 390 395 400

Leu Glu His Gly Leu Ser His Met Val Lys Ser Phe Ser Lys Gln Ser
405 410 415

Ala Gly Arg Val Ala Gly Gly Lys Asn Val Pro Ala Ala Val Gln Lys
420 425 430

Val Ala Lys Ala Ser Ile Asp Gln Ser Arg Gln Met Arg Tyr Gln Ser
435 440 445

Leu Asn Glu Tyr Arg Lys Arg Phe Met Leu Lys Pro Phe Lys Ser Phe
450 455 460

Glu Glu Leu Thr Gly Glu Lys Glu Met Ala Ala Glu Leu Glu Glu Leu
465 470 475 480

Tyr Gly Asp Ile Asp Ala Met Glu Leu Tyr Pro Gly Leu Leu Val Glu
485 490 495

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Lys Pro Arg Pro Gly Ala Ile Phe Gly Glu Thr Met Val Glu Ile Gly
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Ala Pro Phe Ser Leu Lys Gly Leu Met Gly Asn Thr Ile Cys Ser Pro
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Glu Tyr Trp Lys Pro Ser Thr Phe Gly Gly Lys Val Gly Phe Glu Ile
    530                 535                 540

Ile Asn Thr Ala Ser Leu Gln Lys Leu Ile Cys Asn Asn Val Lys Gly
545                 550                 555                 560

Cys Pro Phe Thr Ala Phe His Val Leu Asn Pro Glu Pro Thr Xaa Glu
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Ala Thr Ile Asn Val Ser Thr Ser Asn Thr Ala Met Glu Asp Ile Asn
            580                 585                 590

Pro Thr Leu Leu Leu Lys Glu Gln Ser Ala Glu Leu
        595                 600
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(TRADUCCION DEL INGLES AL ESPAÑOL)

LOS ESTADOS UNIDOS DE AMERICA

A TODOS A QUIENES ESTAS PRESENTES LLEGAREN:

DEPARTAMENTO DE COMERCIO DE LOS ESTADOS UNIDOS

Oficina de Patentes y Marcas Comerciales de los Estados Unidos

Agosto 16 del año 2000

ESTA ES PARA CERTIFICAR QUE ANEXA A LA PRESENTE ESTA UNA FIEL COPIA DE LOS REGISTROS DE LA OFICINA DE PATENTES Y MARCAS COMERCIALES DE LOS ESTADOS UNIDOS DE AQUELOS PAPELES DE LA SOLICITUD DE PATENTE ABAJO IDENTIFICADA QUE LOGRA LOS REQUERIMIENTOS PARA QUE LE SEA OTORGADA UNA FECHA DE PRESENTACIÓN BAJO 35 USC 111.

SOLICITUD NUMERO: 60/147,601

FECHA DE PRESENTACION: Agosto 06 del año 2000

Por Autorización del

COMISIONADO DE PATENTES Y MARCAS COMERCIALES

Firma Ilegible (Firmado)

H. PHILLIPS

Funcionario de Certificaciones

SELLO EN RELIEVE.

++

REIVINDICACIONES

Reivindicamos:

1 Una molécula de asiduo nucleico sustancialmente purificada seleccionada de los grupo de "DNAs" consistente de moléculas de ácido nucleico que comprenden SEQ ID:1 y su

complemento y fragmentos internos de éstas en las cuales dichas SEQ ID:1 codifican una proteína canina COX-1 o fragmento de ésta.

2. La molécula de ácido nucleico sustancialmente purificada de acuerdo con la reivindicación 1, en la cual dicha molécula de ácido nucleico sustancialmente purificada codifica dicha proteína canina COX-1.

3. La molécula de ácido nucleico sustancialmente purificada de acuerdo con la reivindicación 1, en la cual dicho ácido nucleico es SEQ ID:1 o complemento de éste.

4. La molécula de ácido nucleico sustancialmente purificada de acuerdo con la reivindicación 1, en la cual dicha molécula de ácido nucleico específicamente hibridiza a una molécula de ácido nucleico que codifica COX-1 canina o complemento de éste y cae para específicamente hibridizar a una molécula de ácido nucleico que no codifica COX-1 canina o complemento de ésta.

5. La molécula de ácido nucleico sustancialmente purificada de acuerdo con la reivindicación 4, en la cual dicha molécula de ácido nucleico específicamente hibridiza a una molécula de ácido nucleico que codifica COX-1 canina o complemento de éste bajo altas condiciones severas y cae para específicamente hibridizar a una molécula de ácido nucleico que no codifica COX-1 canina o complemento de ésta bajo altas condiciones severas.

6. Una molécula de ácido nucleico COX-1 canina sustancialmente purificada la cual comprende una secuencia

de ácido nucleico que es idéntica a por lo menos 20 nucleotides contiguos de SEQ ID:1 o su complemento.

7. Una molécula de ácido nucleico COX-1 canina sustancialmente purificada de acuerdo con la reivindicación 6, en la cual dicha molécula de ácido nucleico comprende una secuencia de ácido nucleico que es idéntica a por lo menos 50 nucleotides contiguos de SEQ ID:1 o su complemento.

8. Una molécula de ácido nucleico COX-1 canina sustancialmente purificada de acuerdo con la reivindicación 7, en la cual dicha molécula de ácido nucleico comprende una secuencia de ácido nucleico que es idéntica a por lo menos 100 nucleotides contiguos de SEQ ID:1 o su complemento.

9. Una proteína COX-1 canina sustancialmente purificada o fragmento de ésta codificada por una secuencia de ácido nucleico la cual específicamente hibridiza a SEQ ID:1 o su complemento.

10. Una proteína COX-1 canina sustancialmente purificada o fragmento de ésta de acuerdo con la reivindicación 9, en la cual dicha secuencia de ácido nucleico es seleccionada de SEQ ID:1 o su complemento.

11. Una proteína COX-1 canina sustancialmente purificada que tiene SEQ ID:2.

12. Una proteína COX-1 canina sustancialmente purificada o fragmento de ésta que comprende por lo menos 10 amino ácidos consecutivos de SEQ ID:2.

263

13. Una proteína COX-1 canina sustancialmente purificada o fragmento de ésta de acuerdo con la reivindicación 12, en la cual dicha secuencia proteína COX-1 canina o fragmento de ésta es una proteína de fusión.

14. Un anticuerpo capaz de específicamente unir a la sustancialmente purificada proteína COX-1 canina de la reivindicación 11.

15. El anticuerpo de la reivindicación 14, en el cual dicho anticuerpo es detectablemente rotulado.

16. Una molécula de ácido nucleico sustancialmente purificada de los grupos de "DNAs" consistente de moléculas de ácido nucleico comprende SEQ ID:3 y su complemento y fragmentos internos de ésta en la cual dicha SEQ ID:1 codifica una proteína COX-2 canina o fragmento de ésta.

17. La molécula de ácido nucleico sustancialmente purificada de acuerdo con la reivindicación 16, en la cual dicho ácido nucleico sustancialmente purificado codifica dicha proteína COX-2 canina.

18. La molécula de ácido nucleico sustancialmente purificada de acuerdo con la reivindicación 16, en la cual dicho ácido nucleico es SEQ ID:3 o complemento de ésta.

19. La molécula de ácido nucleico sustancialmente purificada de acuerdo con la reivindicación 16, en la cual dicha molécula de ácido nucleico específicamente hibridiza a una molécula de ácido nucleico que codifica COX-2 canina o complemento de ésta y cae para específicamente hibridizar a una molécula de

264

ácido nucleico que no codifica COX-2 canina o complemento de ésta.

20. La molécula de ácido nucleico sustancialmente purificada de acuerdo con la reivindicación 19, en la cual dicha molécula de ácido nucleico es pecificamente hibridiza a una molécula de ácido nucleico que codifica COX-2 canina o complemento de ésta bajo altas condiciones severas y cae para especificamente hibridizar a una molécula de ácido nucleico que no codifica COX-2 canina o complemento de ésta bajo altaas condiciones severas.

21. Una molécula de ácido nucleico COX-2 canina sustancialmente purificada la cual comprende una secuencia de ácido nucleico que es idéntica a por lo menos 20 nucleotides contiguos de SEQ ID:3 o su complemento.

22. Una molécula de ácido nucleico COX-2 canina sustancialmente purificada de acuerdo con la reivindicación 21, en la cual dicha molécula de ácido nucleico comprende una secuencia de ácido nucleico que es idéntica a por lo menos 50 nucleotides contiguos de SEQ ID:3 o su complemento.

23. Una molécula de ácido nucleico COX-2 canina sustancialmente purificada de acuerdo con la reivindicación 22, en la cual dicha molécula de ácido nucleico comprende una secuencia de ácido nucleico que es idéntica a por lo menos 100 nucleotides contiguos de SEQ ID:3 o su complemento.

24. Una proteina COX-2 canina sustancialmente purificada o fragmento de ésta codificada por una secuencia de ácido

nucleico la cual especificamente hibridiza a SEQ ID:4 o su complemento.

25. Una proteína COX-2 canina sustancialmente purificada o fragmento de ésta de acuerdo con la reivindicación 24, donde dicha secuencia de ácido nucleico es seleccionada de SEQ ID:4 o su complemento.

26. Una proteína COX-2 canina sustancialmente purificada que tiene SEQ ID:4.

27. Una proteína COX-2 canina sustancialmente purificada o fragmento de ésta que comprende por lo menos 10 amino ácidos consecutivos de SEQ ID:4.

28. Una proteína COX-2 canina sustancialmente purificada o fragmento de ésta de acuerdo con la reivindicación 27, en la cual dicha proteína COX-2 canina o fragmento de ésta es una proteína de fusión.

29. Un anticuerpo capaz de especificamente unir a la proteína COX-2 canina sustancialmente purificada de la reivindicación 26.

30. El anticuerpo de la reivindicación 29, en el cual dicho anticuerpo es detectablemente rotulado.

31. Una célula transformada que tiene una molécula de ácido nucleico la cual comprende: 31

266

35. Un método para determinar un nivel o patrón de proteína COX-2 canina en una célula comprende:

(A) incubar, bajo condiciones permitidas hirbidización de ácido nucleico, un marcador de ácido nucleico, dicho marcador de molécula nucleica capaz de especificamente hibridizar a una molécula de ácido nucleico que codifica COX-2 canina o complemento de ésta bajo altas condiciones de severidad y dicha molécula de marcador de ácido nucleico incapaz de especificamente hibridizar a una molécula de ácido nucleico que codifica COX-1 canina o complemento de ésta bajo altas condiciones de severidad, con una molécula de ácido nucleico complementaria obtenida de dicha célula, en la cual la hibridización de ácido nucleico entre dicho marcador de molécula de ácido nucleico y dicha molécula de ácido nucleico complementaria obtenida de dicha célula permite la detección de COX-2 canina;

(B) permitir la hibridización entre dicho marcador de molécula de ácido nucleico y dicha molécula de ácido nucleico complementaria obtenida de dicha célula; y

(C) detectar el nivel o patrón de dicho ácido nucleico complementario en el cual la detección de dicho ácido nucleico complementario es predictivo del nivel o patrón de dicha proteína COX-2 canina.

36. El método de la reivindicación 35, en el cual dicho nivel es predictivo de dicha proteína COX-2 canina.

37. El método de la reivindicación 35, en el cual dicho patrón es predictivo de dicha proteína COX-2 canina.

38. El método de la reivindicación 35, en el cual dicho nivel o patrón es detectado por hibridación in situ.

267

39. Un proceso para la diagnosis o prognosis de condiciones relacionadas de COX-2 en un perro el cual comprende ensayar la concentración de una molécula, cuya concentración es dependiente sobre la expresión de un gen COX-2 canino, dicha molécula estando presente en una muestra de células o fluido corporal de dicho perro, en comparación con la concentración de la molécula presente en una muestra de células o fluido del cuerpo de un perro que no tiene una condición inflamatoria y no está predispuesto a desarrollar condiciones inflamatorias, en el cual una concentración de dicha molécula difiere de aquella encontrada en dicho perro que no tiene inflamación y no está predispuesto a tener inflamación es diagnóstico o prognóstico de una condición inflamatoria en dicho perro.

40. El método de la reivindicación 39, en el cual dicho fluido corporal es seleccionado del grupo consistente de tejido esquelético, sangre, linfa y suero.

41. El método de la reivindicación 39, en el cual dicha molécula es una molécula de proteína expresada por dicho gen COX-2 canino.

es una molécula de proteína expresada por dicho gen COX-2

42. El método de la reivindicación 39, en el cual dicha molécula es una molécula mRNA codificada por dicho gen COX-2 canino, o una molécula cDNA codificada por dicho gen COX-2 canino.

43. El método de la reivindicación 39, en el cual dicha concentración de dicha molécula mRNA es ensayada por incubación de una muestra de dicho fluido corporal en la presencia de una molécula de ácido nucleico que hibridiza a dicha molécula mRNA.

44. Un método para diagnosticar una condición relacionada COX-2 en un perro el cual comprende las etapas:

(A) incubar bajo condiciones permitiendo al ácido nucleico hibridización: un marcador de molécula de ácido nucleico, dicho marcador de molécula de ácido nucleico comprende una secuencia nucleotida de un polinucleotida que específicamente hibridiza a un polinucleotida que está elaborado a un gen COX-2 canino, y una molécula de ácido nucleico complementaria obtenida de una célula o un fluido corporal de dicho perro, en la cual la hirbidización de ácido nucleico entre dicho marcador de molécula de ácido nucleico, y dicha molécula de ácido nucleico complementario obtenida de dicho perro permite la detección de un polimorfismo cuya presencia es predictiva de una mutación que afecta la respuesta de COX-2 canina en dicho perro.

(B) permitir la hirbidización entre dicho marcador de molécula de ácido nucleico y dicha molécula de ácido nucleico complementaria obtenida de dicho paciente; y

(C) detectar la presencia de dicho polimorfismo, en el cual la detección de dicho polimorfismo es diagnóstico de una condición relacionada de COX-2.

45. El método de la reivindicación 44, en el cual dicha respuesta de COX-2 canina afectada por dicha mutación es nivel canino COX-2.

46. El método de la reivindicación 44, en el cual dicha respuesta COX-2 canina afectada por dicha mutación es patrón COX-2 canino.

47. El método de la reivindicación 44, en el cual dicha molécula es una molécula mRNA codificada por dicho gen, o una molécula cDNA codificada por dicho gen.

48. El método de la reivindicación 44, en el cual dicho estado de enfermedad es inflamación.

49. El método de la reivindicación 44, en el cual dicho estado de enfermedad es artritis.

50. El método de la reivindicación 44, en el cual dicho estado de enfermedad es cancer.

51. El método de la reivindicación 44, en el cual dicho estado de enfermedad es neopiasia.

52. El método de la reivindicación 44, en el cual dicho estado de enfermedad es un desorden CNS.

53. Un método de determinar una asociación entre un polifórmismo y un rasgo comprende:

(A) hibridizar una molécula de ácido nucleico especifica para el polifórmismo para material genético de una célula, en el cual dicha molécula de ácido nucleico tiene una secuencia de SEQ ID: 3 o complemento de ésta; y

(B) calcular el grado de asociación entre e polifórmismo y el rasgo.

54. El método de la reivindicación 53, en el cual dicho marcador de molécula de ácido nucleico comprende una secuencia nucleotida de 1 polinucleotido que está físicamente eslabonado a dentro de 1 mb de una secuencia que específicamente hibridiza a dicho gen.

55. El método de la reivindicación 53, en el cual dicho marcador de molécula de ácido nucleico comprende una secuencia nucleotida de 1 polinucleotido que está físicamente eslabonado a dentro de 100 kb a una secuencia que específicamente hibridiza a dicho gen.

56. El método de la reivindicación 53, en el cual dicho marcador de molécula de ácido nucleico comprende una secuencia nucleotida de 1 polinucleotido que está físicamente eslabonado a dentro de 10 kb a una secuencia que específicamente hibridiza a dicho gen.

57. Un método de producir una célula que contiene una proteína COX-2 canina sobre expresada comprende:

(A) transformar dicha célula con una molécula de ácido nucleico funcional, en la cual dicha molécula de ácido nucleico funcional comprende una región promotora, en la cual dicha región promotora está eslabonada a una región estructural, en la cual dicha región estructural comprende una molécula de ácido nucleico que tiene una secuencia de ácido nucleico de SEQ ID:3, en la cual dicha región estructural está eslabonada a una secuencia no traducida 3' que funciona en dicha célula para causar la terminación de transcripción y adición de ribonucleotides poliadenilatados a un extremo 3' de molécula mRNA; y en la cual dicha molécula de ácido nucleico funcional resulta en sobre expresión de dicha proteína COX-2 canina; y

(B) cultivar dicha célula transformada.

58. Un método de producir una célula que contiene niveles reducidos de una proteína COX-2 canina comprende:

(A) transformar dicha célula con una molécula de ácido nucleico funcional, en la cual dicha molécula de ácido nucleico funcional comprende una región promotora, en la cual dicha

271

región promotora está eslabonada a una región estructural, en la cual dicha región estructural comprende una molécula de ácido nucleico que tiene una secuencia de ácido nucleico de SEQ ID:3, en la cual dicha región estructural está eslabonada a una secuencia no traducida 3' que funciona en dicha célula para causar la terminación de transcripción y adición de ribonucleotides poliadenilatados a un extremo 3' de molécula mRNA; y en la cual dicha molécula de ácido nucleico funcional resulta en una co-supresión de la proteína COX-2 canina; y

(B) cultivar dicha célula transformada.

59. Un método para reducir la expresión de una proteína COX-2 canina en una célula comprende:

(A) transformar dicha célula con una molécula de ácido nucleico, en la cual dicha molécula de ácido nucleico tiene una región promotora exogenosa la cual funciona en una célula para causar la producción de una molécula mRNA, en la cual región promotora exogenosa está eslabonada a una molécula de ácido nucleico transcrita que tiene hebra transcrita y una hebra no transcrita, en la cual la hebra transcrita es complementaria a una molécula de ácido nucleico que tiene una secuencia de ácido nucleico de SEQ ID:3 o su complemento y dicha hebra transcrita es complementaria a una molécula mRNA endogenosa; y en la cual la molécula de ácido nucleico transcrita está eslabonada a una secuencia no traducida 3' que funciona en dicha célula para causar la terminación de transcripción y adición de ribonucleotides poliadenilatados a un extremo 3' de molécula mRNA; y

(B) cultivar dicha célula transformada.

60. Un método de aislar un ácido nucléico que codifica una proteína COX-2 canina o fragmento de ésta comprende:

(A) incubar bajo condiciones permitidas hibridación de ácido nucleico, una primera molécula de ácido nucleico comprende SEQ ID:3 o el complemento de ésta con una segunda molécula de ácido nucleico complementaria obtenida de una célula;

(B) permitir la hibridación entre dicha primera molécula de ácido nucleico y dicha segunda molécula de ácido nucleico obtenida de dicha célula; y

(C) aislar dicha segunda molécula de ácido nucleico.

61. Un método de producir una célula contiene un gen COX-2 canino sobre expresado que comprende:

(A) transformar dicha célula con una molécula de ácido nucleico funcional, en la cual dicha molécula de ácido nucleico funcional comprende una región promotora, en la cual dicha región promotora está eslabonada a una región estructural, en la cual dicha región estructural comprende una molécula de ácido nucleico que tiene una secuencia de ácido nucleico consistente de SEQ ID:3 o complementos de ésta; en la cual dicha región estructural está eslabonada a una secuencia no traducida 3' que funciona en dicha célula para causar la terminación de transcripción y adición de ribonucleotides poliadenilatados a un extremo 3' de molécula mRNA; y en la cual dicha molécula de ácido nucleico funcional resulta en sobre expresión de dicha gen de proteína COX-2 canina; y

(B) cultivar dicha célula transformada.

62. Un método de producir una célula contiene niveles reducidos de gen COX-2 canino comprende:

(A) transformar dicha célula con una molécula de ácido nucleico funcional, en la cual dicha molécula de ácido nucleico funcional comprende una región promotora, en la cual dicha región promotora está eslabonada a una región estructural, en la cual dicha región estructural comprende una molécula de

ácido nucleico que tiene una secuencia de ácido nucleico de seleccionada del grupo consistente de SEQ ID:3 o complementos de ésta; en la cual dicha región estructural está eslabonada a una secuencia no traducida 3' que funciona en dicha célula para causar la terminación de transcripción y adición de ribonucleotides poliadenilatados a un extremo 3' de molécula mRNA; y en la cual dicha molécula de ácido nucleico funcional resulta en co-supresión del gen COX-2 canino; y

(B) cultivar dicha célula transformada.

63. Un método para reducir la expresión de un gen COX-2 canino en una célula comprende:

(A) transformar dicha célula con una molécula de ácido nucleico, en la cual dicha molécula de ácido nucleico tiene una región promotora exogenosa la cual funciona en una célula para causar la transformación de una molécula mRNA, en la cual dicha región promotora exogenosa está eslabonada a una molécula de ácido nucleico transcrita que tiene una hebra transcrita y una hebra no transcrita, en la cual la hebra transcrita es complementaria a una molécula de ácido nucleico que tiene un ácido nucleico seleccionado del grupo consistente de SEQ ID:3 o complementos de éste y dicha hebra transcrita es complementaria a una molécula mRNA endogenosa; y en la cual la molécula de ácido nucleico transcrita está eslabonada a una secuencia no traducida 3' que funciona en dicha célula para causar la terminación de transcripción y adición de ribonucleotidas poliadenilatados a un extremo 3' de molécula mRNA; y

(B) cultivar dicha célula transformada.

64. Una molécula de ácido nucleico sustancialmente purificada comprende un secuencia la cual codifica una proteína COX-2 canina de mamífero.

65. El ácido nucleico sustancialmente purificado de la reivindicación 64 en el cual dicho ácido nucleico codifica una secuencia seleccionada del grupo consistente de COX-2 canino, humano, de rata, o ratón.

66. Una molécula de ácido nucleico sustancialmente purificada de la reivindicación 64 en la cual dicha secuencia de ácido nucleico codifica COX-2 canino humano.

67. Una molécula de ácido nucleico sustancialmente purificada de la reivindicación 64 en la cual dicha secuencia de ácido nucleico codifica COX-2 canino de ratón.

68. Un método de usar una proteína COX-2 canina o fragmento de ésta en un ensayo para tamizar sustancias de prueba para la habilidad de modular o mantener actividad COX-2 canina, comprende la contracción de una proteína COX-2 canina o fragmento con una sustancia de prueba, y determinar la presencia o nivel de actividad COX-2 canina comparada con un control.

69. Un método de identificar un compuesto o composición que preferencial o específicamente efectua COX-2 canina tal como se comparó para efectuar COX-1 canina comprende llevar a cabo el método de la reivindicación 50, y además comprende determinar la presencia o nivel de actividad COX-1 canina.

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Resumen

La presente invención se refiere a genes que codifican proteínas de enzimas de la ciclooxigenasa-1 (COX-1) y la ciclooxigenasa-2 (COX-2), las proteínas COX-1 canina y COX-2 canina, y ensayos para la evaluación de actividades COX-1 canina y COX-2. La invención también se refiere a molécula de ácido nucleico asociadas con esos genes que incluyen sus complementos, homólogos y fragmentos, y para métodos de usar esas moléculas de ácido nucleico, las enzimas y fragmentos de esos.

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ES TRADUCCIÓN FIEL Y COMPLETA DE LOS DOCUMENT S ADJUNTOS.

276

Industria y Comercio
SUPERINTENDENCIA

2do. EXAMEN DE FORMA PATENTE DE INVENCION

Folio 279

Radicación no 00 - 59230

Concepto Técnico no 1863 Clasificación Internacional de Patentes C12N 9/00

Practicado el examen de acuerdo a lo establecido en el Art. 38 de la Decisión 486 se determinó que esta solicitud de Patente de Invención:

SI [✔]	NO [ ]	Contiene un resumen de la invención (Art.26-e).
SI [✔]	NO [ ]	Contiene el título o nombre de la invención (Art.27-d).
SI [✔]	NO [ ]	Contiene la descripción de la invención (Art.26-b).
SI [ ]	NO [ ]	NO ES APLICABLE [✔] Contiene los dibujos necesarios para comprender la invención (Art.26-d)
SI [✔]	NO [ ]	Contiene una o más reivindicaciones. (Art.26-c).
SI [ ]	NO [ ]	NO ES APLICABLE [✔] Han sido desarrollados los productos y/o procedimientos a partir de recursos genéticos o de sus productos derivados de los que cualquiera de los Países Miembros es país de origen. (Art. 26- h).
SI [ ]	NO [ ]	NO ES APLICABLE [✔]Los productos y/o procedimientos han sido obtenidos o desarrollados a partir de los conocimientos tradicionales de las comunidades indígenas, afroamericanas, o locales de los países miembros, de acuerdo a lo establecido en la Decisión 391 y sus modificaciones y reglamentaciones vigentes.(Art.26-I).
SI [✔]	NO [ ]	La invención se refiere a un producto relativo a un material biológico. (Art.26-J).
SI [✔]	NO [ ]	La prioridad coincide con la materia reivindicada.
SI [✔]	**NO [ ]**	**CUMPLE LOS REQUISITOS TÉCNICOS**

OBSERVACIONES Y OTROS: SI [✔] NO [ ] ver hoja(s) anexa(s)

- Anexa copia certificada de la solicitud de la patente Británica del 6 de agosto del 1999, folios del 175 al 262, con su respectiva traducción. Extemporanea.
- Anexa un nuevo capitulo reivindicatorio correspondiente a los folios del 155 al 172

EXAMINADOR TÉCNICO
202048

Bogotá D. C., 23 de enro del 2003

Industria y Comercio
SUPERINTENDENCIA

277

Industria y Comercio
SUPERINTENDENCIA

Folio 280

2020
Bogotá DC

Doctor
RAMIRO CASTRO DUQUE
Apoderado

REFERENCIA:

Radicación No.	00-59230
Trámite	002
Evento	001
Actuación	416
Oficio No.	2356
Folios	001

Teniendo en cuenta que la solicitud de privilegio de patente que se tramita bajo el expediente indicado en la referencia, reúne los requisitos de forma establecidos en los artículos 26 y 27 de la Decisión 486 de la Comisión de la Comunidad Andina, y en las demás disposiciones vigentes sobre la materia, se envía el extracto de la solicitud a la Oficina de Comunicaciones para efecto de su publicación en la Gaceta de Propiedad Industrial, conforme lo establece el artículo 40 de la misma Decisión.

No obstante lo anterior, no se tendrá en cuenta la prioridad invocada ya que no la presentó dentro del plazo de tres (3) meses, contados a partir de la fecha de depósito de la solicitud.

Cúmplase
Dado en Bogotá DC, a los 29 ENE. 2003

ALIX CARMENZA CÉSPEDES DE VERGEL
Jefe de la División de Nuevas Creaciones

528

202048

278

Industria y Comercio
SUPERINTENDENCIA

DIVISION DE NUEVAS CREACIONES

RADICACIÓN EXPEDIENTE No. **00-059230**

EL EXTRACTO DE LA PRESENTE SOLICITUD DE **PATENTE DE INVENCION**

FUE PUBLICADO EN LA GACETA DE PROPIEDAD INDUSTRIAL No **528**, DE

FECHA 30 DE **MAYO** DE **2003**, EL TERMINO HABIL SEÑALADO POR LA LEY

EXPIRA EL **29** DE **AGOSTO** DE **2003**.

NOTA: Dentro del plazo de los seis (6) meses siguientes a partir de la fecha de publicación en la gaceta de Propiedad Industrial, deberá solicitar y cancelar el examen de patentabilidad so pena de que la solicitud caiga en abandono, de acuerdo con lo establecido en el Artículo 44 de la Decisión 486/2000.

SE PRESENTARON OPOSICIONES POR PARTE DE TERCEROS.

SI _____ NO_____**X**_____

Bogotá D.C., Diciembre de 2003

279

SUPERINTENDENCIA DE INDUSTRIA Y COMERCI

RADICACIÓN: 0-59230 EDICTO No. 816

LA SECRETARÍA GENERAL AD-HOC
HACE SABER:

Que esta Superintendencia profirió Resolución No. 35904 de 22-DIC-2003. Que el contenido de la parte resolutiva es como sigue: El Superintendente de Industria y Comercio.

RESUELVE

ARTICULO PRIMERO: Declarar abandonada la solicitud de privilegio de patente de invención contenida en el expediente de la referencia.

ARTICULO SEGUNDO: Notificar personalmente el contenido de la presente resolución al doctor Ramiro Castro Duque, o a quien haga sus veces, quien actúa como apoderado de G.D. Searle & Co., entregándole copia de la misma, advirtiéndole que contra ella procede el recurso de reposición interpuesto ante el Superintendente de Industria y Comercio, al momento de la notificación o dentro de los 5 días hábiles siguientes a ella.

NOTIFÍQUESE Y CUMPLASE
Dada en Bogotá, D.C., a los 22 DIC. 2003

EL SUPERINTENDENTE DE INDUSTRIA Y COMERCIO,

JAIME RUBIO ESCOBAR

Para notificar a los interesados, se fija el presente EDICTO en lugar visible al público en la SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO, por el término de (10) diez días hábiles contados a partir de las 8:30 a.m. de hoy.

Secretaría General Ad-Hoc 15-ENE-2004 Este edicto se desfija hoy 28-ENE-2004 a las cinco (5:00 p.m.)
Secretaría General Ad-Hoc
EDICTO No. 816