



(86) Date de dépôt PCT/PCT Filing Date: 2000/09/28
(87) Date publication PCT/PCT Publication Date: 2001/04/05
(85) Entrée phase nationale/National Entry: 2002/03/25
(86) N° demande PCT/PCT Application No.: US 2000/026592
(87) N° publication PCT/PCT Publication No.: 2001/023565
(30) Priorités/Priorities: 1999/09/28 (60/156,921) US;
1999/10/12 (60/158,818) US

(51) Cl.Int.⁷/Int.Cl.⁷ C12N 15/12, C07K 14/705, C12Q 1/68,
C12N 15/11, C07K 16/28, G01N 33/50

(71) Demandeur/Applicant:
GENTEST CORPORATION, US

(72) Inventeurs/Inventors:
STOCKER, PENNY J., US;
STEIMEL-CRESPI, DOROTHY T., US;
CRESPI, CHARLES L., US

(74) Agent: SMART & BIGGAR

(54) Titre : P-GLYCOPROTEINES PROVENANT DE MACACA FASCICULARIS ET UTILISATIONS
CORRESPONDANTES

(54) Title: P-GLYCOPROTEINS FROM MACACA FASCICULARIS AND USES THEREOF

(57) **Abrégé/Abstract:**

The invention pertains to cynomologous monkey P-glycoproteins and related P-glycoproteins which include cynomologous-specific amino acids, as well as nucleic acids which encode those polypeptides. The present invention also includes fragments and biologically functional variants of the cynomologous monkey P-glycoprotein. The invention further relates to methods of using such cynomologous monkey P-glycoprotein nucleic acids and polypeptides, especially in methods for determining bioavailability of drugs and for screening for inhibitors of cynomologous PGP. Also included are cynomologous PGP inhibitors which inhibit cynomologous PGP activity by inhibiting the expression or function of cynomologous PGP.



(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
5 April 2001 (05.04.2001)

PCT

(10) International Publication Number
WO 01/23565 A1

(51) International Patent Classification⁷: **C12N 15/12**,
C07K 14/705, 16/28, C12N 15/11, G01N 33/50, C12Q
1/68

STEIMEL-CRESPI, Dorothy, T.; 4 Evans Road, Mar-
blehead, MA 01945 (US). CRESPI, Charles, L.; 4 Evans
Road, Marblehead, MA 01945 (US).

(21) International Application Number: PCT/US00/26592

(74) Agent: VAN AMSTERDAM, John, R.; Wolf, Greenfield
& Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210
(US).

(22) International Filing Date:
28 September 2000 (28.09.2000)

(81) Designated States (*national*): AU, CA, JP.

(25) Filing Language: English

(84) Designated States (*regional*): European patent (AT, BE,
CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC,
NL, PT, SE).

(26) Publication Language: English

(30) Priority Data:
60/156,921 28 September 1999 (28.09.1999) US
60/158,818 12 October 1999 (12.10.1999) US

Published:

- With international search report.
- Before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments.

(71) Applicant: GENTEST CORPORATION [US/US]; 6
Henshaw Street, Woburn, MA 01801 (US).

(72) Inventors: STOCKER, Penny, J.; Apartment 2, 119
Forest Hills Street, Jamaica Plain, MA 02130 (US).

*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*

(54) Title: P-GLYCOPROTEINS FROM MACACA FASCICULARIS AND USES THEREOF

(57) Abstract: The invention pertains to cynomologous monkey P-glycoproteins and related P-glycoproteins which include cynomologous-specific amino acids, as well as nucleic acids which encode those polypeptides. The present invention also includes fragments and biologically functional variants of the cynomologous monkey P-glycoprotein. The invention further relates to methods of using such cynomologous monkey P-glycoprotein nucleic acids and polypeptides, especially in methods for determining bioavailability of drugs and for screening for inhibitors of cynomologous PGP. Also included are cynomologous PGP inhibitors which inhibit cynomologous PGP activity by inhibiting the expression or function of cynomologous PGP.

WO 01/23565 A1

- 1 -

P-GLYCOPROTEINS FROM MACACA FASCICULARIS AND USES THEREOF

Field of the Invention

The invention pertains to P-glycoproteins of cynomologous monkey (*Macaca*
5 *fascicularis*).

Background of the Invention

P-glycoprotein (PGP; also known as multidrug transporter, MDR1) is a member of the ABC transporter superfamily and is expressed in the human intestine, liver and other tissues.
10 This enzyme serves as an efflux pump exporting small molecules across the cell membrane. It has been known for several years that high level expression of PGP is a mechanism for tumor resistance to cancer chemotherapy. Intestinal expression of PGP may affect the oral bioavailability of drug molecules that are substrates for this transporter. PGP can efficiently efflux drugs back into the intestinal lumen and thus reduce the amount of drug that enters into
15 circulation.

The measurement of interaction with PGP can provide a better understanding of the reasons why particular drugs demonstrate low or high bioavailability. Interaction with PGP can be studied using either direct assays of drug transport in polarized cell systems or with indirect assays such as drug-stimulated ATPase activity and inhibition of the transport of
20 fluorescent substrates.

Therefore there is a need for additional PGP polypeptides, preferably which are closely related to the human PGP, for use in the foregoing drug assays.

Summary of the Invention

25 Nucleic acids encoding the P-glycoprotein of cynomologous monkey (*Macaca fascicularis*) have now been identified, isolated, cloned and sequenced. This PGP is closely related (has a high degree of identity) to the human PGP. The invention provides isolated nucleic acid molecules, unique fragments of those molecules, expression vectors containing the foregoing, and host cells transfected with those molecules. The invention also provides
30 isolated polypeptides and inhibitors of the foregoing nucleic acids and polypeptides which reduce drug transport. The PGP nucleic acids and polypeptides are useful in assays for evaluating bioavailability of drugs, as well as for the optimization or discovery of drugs. In

- 2 -

addition, the foregoing can be used in the diagnosis or treatment of conditions characterized by PGP activity and can be used in methods in which it is therapeutically useful to increase or decrease PGP activity.

According to one aspect of the invention, isolated nucleic acid molecules are provided
5 selected from the group consisting of (a) nucleic acid molecules that code for the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, (b) allelic variants of (a), and (c) complements of (a) or (b). In certain embodiments, the isolated nucleic acid molecule codes for SEQ ID NO:2 or SEQ ID NO:4. In other embodiments, the isolated nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO:2 or SEQ ID NO:4.

10 According to another aspect of the invention, isolated P-glycoprotein polypeptides or fragments thereof are provided which include at least one amino acid of a cynomologous P-glycoprotein selected from the group consisting of amino acids 12, 24, 30, 74, 78, 86, 89, 90, 91, 92, 95, 97, 99, 102, 103, 104, 185, 324, 363, 518, 635, 650, 656, 659, 677, 730, 738, 742, 745, 761, 765, 835, 851, 921, 967, 1003, 1027, 1038, 1048, 1103, 1128, 1168 and 1277 of
15 SEQ ID NO:2 and amino acids 93, 94 and 95 of SEQ ID NO:4, wherein the P-glycoprotein is identical to a human P-glycoprotein except for the at least one amino acid of a cynomologous P-glycoprotein. In certain embodiments, the human P-glycoprotein is selected from the group of SEQ ID NO:5 and SEQ ID NO:6.

According to yet another aspect of the invention, isolated P-glycoprotein polypeptides
20 or fragments thereof which include at least one amino acid of a cynomologous P-glycoprotein selected from the group consisting of amino acids 3, 6, 8, 10, 13, 17, 19, 20, 21, 26, 30, 36, 38, 48, 52, 56, 64, 74, 78, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 98, 100, 101, 102, 103, 104, 105, 106, 110, 113, 145, 190, 197, 210, 231, 319, 324, 327, 345, 363, 395, 451, 455, 456, 468, 473, 494, 518, 530, 631, 641, 642, 648, 650, 655, 656, 664, 665, 672, 673, 674, 675,
25 683, 687, 689, 691, 692, 694, 701, 705, 715, 729, 730, 734, 742, 743, 745, 754, 757, 765, 835, 912, 918, 921, 940, 941, 944, 966, 967, 968, 970, 972, 981, 1008, 1015, 1023, 1024, 1048, 1093, 1096, 1103, 1128, 1142, 1146, 1147, 1156, 1160, 1163, 1166, 1250 and 1271 of SEQ ID NO:2 and amino acids 93 and 94 of SEQ ID NO:4, wherein the P-glycoprotein is identical to a dog P-glycoprotein except for the at least one amino acid of a cynomologous
30 P-glycoprotein. In some embodiments, the dog P-glycoprotein is selected from the group of SEQ ID NO:7 and SEQ ID NO:8.

In preferred embodiments, the isolated P-glycoprotein polypeptides or fragments

- 3 -

thereof include an amino acid sequence selected from the group consisting of SEQ ID NO:2, fragments of SEQ ID NO:2, SEQ ID NO:4 and fragments of SEQ ID NO:4.. Yet other polypeptides include combinations of the foregoing dog, human and cynomologous PGP polypeptides.

5 According to still other embodiments of the invention, isolated nucleic acid molecules are provide which encode the foregoing isolated P-glycoprotein polypeptides or fragments thereof. Also included expression vectors comprising the foregoing isolated nucleic acid molecules operably linked to a promoter, as well as host cells transformed or transfected with the expression vectors.

10 In another aspect of the invention, agents which selectively binds the isolated PGP polypeptides are provided. Preferably the agent does not bind a human or dog P-glycoprotein, except those provided herein. In certain embodiments, the agent is a polypeptide preferably one selected from the group consisting of monoclonal antibodies, polyclonal antibodies, Fab antibody fragments, F(ab)₂ antibody fragments and antibody fragments including a CDR3
15 region. Also provided are agents which selectively binds the foregoing isolated nucleic acid molecules, preferably antisense nucleic acid molecules which selectively binds to the isolated nucleic acid molecule.

 According to another aspect of the invention, methods for predicting the bioavailability of a compound are provided. The methods include measuring the
20 transmembrane transport of a test compound by a first P-glycoprotein, comparing the transmembrane transport of the test compound by the first P-glycoprotein and a second P-glycoprotein to predict the bioavailability of the test compound, wherein the relative amount or rate of transport by the first P-glycoprotein and the second P-glycoprotein is predictive of bioavailability of the test compound. In certain embodiments the first P-
25 glycoprotein is selected from the group consisting of dog P-glycoproteins and primate P-glycoproteins, preferably one of the foregoing polypeptides. In other embodiments the second P-glycoprotein is a human P-glycoprotein.

 In still other aspects of the invention, methods for inhibiting P-glycoprotein transporter activity in a mammalian cell are provided. The methods include contacting the
30 mammalian cell with an amount of one of the foregoing agents effective to inhibit P-glycoprotein transporter activity in the mammalian cell.

 Also included in the invention are methods for increasing bioavailability of a drug in a

- 4 -

subject. The methods include administering to a subject in need of such treatment one of the foregoing agents in an amount effective to increasing bioavailability of a drug. The inhibitor can be administered prior to administering the drug, or concurrently with the drug.

Also provided are methods for increasing P-glycoprotein transporter activity in a cell.

5 These methods include contacting the cell with a molecule selected from the group consisting of the foregoing nucleic acid molecules, in an amount effective to increase P-glycoprotein transporter activity in the cell. The cell can be contacted under conditions whereby the P-glycoprotein is expressed.

According to yet another aspect of the invention, methods for identifying lead
10 compounds for a pharmacological agent useful in the treatment of disease associated with P-glycoprotein transporter activity are provided. The methods include providing a cell or other membrane-encapsulated space comprising a P-glycoprotein as provided herein; contacting the cell or other membrane-encapsulated space with a candidate pharmacological agent under conditions which, in the absence of the candidate pharmacological agent, cause a
15 first amount of P-glycoprotein transporter activity; and determining a second amount of P-glycoprotein transporter activity as a measure of the effect of the pharmacological agent on the P-glycoprotein transporter activity, wherein a second amount of P-glycoprotein transporter activity which is less than the first amount indicates that the candidate pharmacological agent is a lead compound for a pharmacological agent which reduces P-glycoprotein transporter
20 activity and wherein a second amount of P-glycoprotein transporter activity which is greater than the first amount indicates that the candidate pharmacological agent is a lead compound for a pharmacological agent which increases P-glycoprotein transporter activity. The methods can further include a step of loading the cell or other membrane-encapsulated space with a detectable compound, wherein the compound is detected as a measure of the P-glycoprotein
25 transporter activity.

Also included are methods for identifying compounds which selectively bind a P-glycoprotein. The methods include contacting a P-glycoprotein provided herein with a compound, and determining the binding of the compound to the P-glycoprotein. The methods can further include determining the effect of the compound on the P-glycoprotein transporter
30 activity of the P-glycoprotein or determining the effect of the compound on the ATPase activity of the P-glycoprotein.

Additional methods provided according to the invention include methods for

- 5 -

determining ATPase activity of a P-glycoprotein. The methods include contacting a host cell as provided above, or a membrane fraction thereof, with a test drug, and measuring ATPase activity of the P-glycoprotein. In certain embodiments, the step of measuring ATPase activity is performed at least twice at different times. Also provided methods for determining

5 transmembrane transport of a compound by a P-glycoprotein. The methods include contacting a host cell provided above, or a membrane fraction thereof, with a test drug, and measuring transport of the test drug under sink conditions in at least one direction of transport selected from the group consisting of the apical to basolateral direction and the basolateral to apical direction. In certain embodiments the step of measuring transport of the test drug is

10 performed at least twice at different times.

These and other aspects of the invention are described in greater detail below.

Brief Description of the Sequences

SEQ ID NO:1 is the nucleotide sequence encoding cynomologous monkey

15 P-glycoprotein.

SEQ ID NO:2 is the amino acid sequence of a cynomologous monkey P-glycoprotein encoded by SEQ ID NO:1.

SEQ ID NO:3 is the nucleotide sequence of a cynomologous monkey P-glycoprotein allele having a 9 nucleotide insert relative to SEQ ID NO:1.

20 SEQ ID NO:4 is the amino acid sequence of a cynomologous monkey P-glycoprotein allelic variant encoded by SEQ ID NO:3, having a 3 amino acid insert.

SEQ ID NO:5 is the amino acid sequence of a human P-glycoprotein having Genbank accession number M14758.

25 SEQ ID NO:6 is the amino acid sequence of a human P-glycoprotein having Genbank accession numbers AF016535 or NM_000927.

SEQ ID NO:7 is the amino acid sequence of a dog P-glycoprotein having Genbank accession number AF045016.

SEQ ID NO:8 is the amino acid sequence of a dog P-glycoprotein having Genbank accession numbers AF092810.

30 SEQ ID NO:9 is the nucleotide sequence of a primer based on the human PGP nucleotide sequence.

SEQ ID NO:10 is the nucleotide sequence of a primer based on the human PGP

- 6 -

nucleotide sequence.

SEQ ID NO:11 is the nucleotide sequence of a primer based on the human PGP nucleotide sequence.

5 SEQ ID NO:12 is the nucleotide sequence of a primer based on the cynomologous PGP nucleotide sequence.

SEQ ID NO:13 is the nucleotide sequence of a primer based on the cynomologous PGP nucleotide sequence.

SEQ ID NO:14 is the nucleotide sequence of a primer based on the T7 promoter nucleotide sequence.

10 SEQ ID NO:15 is the nucleotide sequence of a primer based on the cynomologous and human PGP nucleotide sequences.

SEQ ID NO:16 is the nucleotide sequence of a primer based on the cynomologous and human PGP nucleotide sequences.

15 SEQ ID NO:17 is the nucleotide sequence of a primer based on the cynomologous PGP nucleotide sequence.

SEQ ID NO:18 is the nucleotide sequence of a primer based on the cynomologous PGP nucleotide sequence.

Detailed Description of the Invention

20 The present invention in one aspect involves the identification of cDNAs encoding cynomologous monkey P-glycoproteins, referred to herein as cynomologous PGP. The nucleotide sequences of the cynomologous PGP are presented as SEQ ID Nos:1 and 3, and the amino acid sequences of the cynomologous PGP are presented as SEQ ID Nos:2 and 4. The nucleotide and amino acid sequences of a cynomologous PGP allelic variant (SEQ ID NOS:3 and 4) have inserts of 9 nucleotides and 3 amino acids, respectively, but are otherwise identical to SEQ ID NOS:1 and 2. The closely related human PGP was deposited in GenBank under accession number M14758. Whereas much of the polypeptides presented herein is identical to human PGP, cynomologous PGP has several single amino acid differences and a N-terminal domain of about 19-34 amino acids that is about 36-58% identical to human PGP.

30 The insert present in the allelic variant referred to above (SEQ ID NOS:3 and 4) is located near the end of this cynomologous-specific domain. Surprisingly, the N-terminal domain of the cynomologous PGP that differs from the human amino acid sequence is located in a

- 7 -

portion of the molecule in which the cynomologous and human amino acid sequences are otherwise 100% identical. This species difference in the very highly conserved protein domains of the P-glycoprotein is entirely unexpected.

The invention involves in one aspect cynomologous PGP nucleic acids and
5 polypeptides, as well as therapeutics relating thereto. The invention also embraces isolated functionally equivalent variants, useful analogs and fragments of the foregoing nucleic acids and polypeptides; complements of the foregoing nucleic acids; and molecules which selectively bind the foregoing nucleic acids and polypeptides.

The cynomologous PGP nucleic acids and polypeptides of the invention are isolated.
10 As used herein with respect to nucleic acids, the term "isolated" means: (i) amplified *in vitro* by, for example, polymerase chain reaction (PCR); (ii) recombinantly produced by cloning; (iii) purified, as by cleavage and gel separation; or (iv) synthesized by, for example, chemical synthesis. An isolated nucleic acid is one which is readily manipulable by recombinant DNA techniques well known in the art. Thus, a nucleotide sequence contained in a vector in which
15 5' and 3' restriction sites are known or for which polymerase chain reaction (PCR) primer sequences have been disclosed is considered isolated but a nucleic acid sequence existing in its native state in its natural host is not. An isolated nucleic acid may be substantially purified, but need not be. For example, a nucleic acid that is isolated within a cloning or expression vector is not pure in that it may comprise only a tiny percentage of the material in
20 the cell in which it resides. Such a nucleic acid is isolated, however, as the term is used herein because it is readily manipulable by standard techniques known to those of ordinary skill in the art. An isolated nucleic acid as used herein is not a naturally occurring chromosome.

As used herein with respect to polypeptides, "isolated" means separated from its native environment and present in sufficient quantity to permit its identification or use. Isolated,
25 when referring to a protein or polypeptide, means, for example: (i) selectively produced by expression cloning or (ii) purified as by chromatography or electrophoresis. Isolated proteins or polypeptides may be, but need not be, substantially pure. The term "substantially pure" means that the proteins or polypeptides are essentially free of other substances with which they may be found in nature or *in vivo* systems to an extent practical and appropriate for their
30 intended use. Substantially pure polypeptides may be produced by techniques well known in the art. Because an isolated protein may be admixed with a pharmaceutically acceptable carrier in a pharmaceutical preparation, the protein may comprise only a small percentage by

- 8 -

weight of the preparation. The protein is nonetheless isolated in that it has been separated from the substances with which it may be associated in living systems, i.e. isolated from other proteins.

As used herein a cynomologous PGP nucleic acid refers to an isolated nucleic acid molecule which codes for a cynomologous PGP polypeptide. Such nucleic acid molecules code for cynomologous PGP polypeptides which include the sequence of SEQ ID NOs:2 and 4, and fragments thereof. The nucleic acid molecules include the nucleotide sequences of SEQ ID Nos:1 and 3, and nucleotide sequences which differ from the sequences of SEQ ID NOs:1 and 3 in codon sequence due to the degeneracy of the genetic code. The cynomologous PGP nucleic acids of the invention also include alleles of the foregoing nucleic acids, as well as fragments of the foregoing nucleic acids. Such fragments can be used, for example, as probes in hybridization assays and as primers in a polymerase chain reaction (PCR). Preferred cynomologous PGP nucleic acids include the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:3. Complements of the foregoing nucleic acids also are embraced by the invention.

As used herein "cynomologous PGP activity" refers to an ability of a PGP polypeptide to export small molecules across the cell membrane. A molecule which inhibits cynomologous PGP activity (an antagonist) is one which inhibits export of small molecules via PGP and a molecule which increases cynomologous PGP activity (an agonist) is one which increases export of small molecules via PGP. Changes in cynomologous PGP activity can be measured by assays such as those disclosed herein, including efflux of fluorescent compounds from cells.

Alleles of the cynomologous PGP nucleic acids of the invention can be identified by conventional techniques. For example, alleles of cynomologous PGP can be isolated by hybridizing a probe which includes at least a fragment of SEQ ID NO:1 or SEQ ID NO:3 under stringent conditions with a cDNA library and selecting positive clones. Thus, an aspect of the invention is those nucleic acid sequences which code for cynomologous PGP polypeptides and which hybridize to a nucleic acid molecule consisting of SEQ ID NO:1 or SEQ ID NO:3 under stringent conditions. The term "stringent conditions" as used herein refers to parameters with which the art is familiar. Nucleic acid hybridization parameters may be found in references which compile such methods, e.g. *Molecular Cloning: A Laboratory Manual*, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold

- 9 -

Spring Harbor, New York, 1989, or *Current Protocols in Molecular Biology*, F.M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. More specifically, stringent conditions, as used herein, refers, for example, to hybridization at 65°C in hybridization buffer (3.5 x SSC, 0.02% Ficoll, 0.02% polyvinyl pyrrolidone, 0.02% Bovine Serum Albumin, 2.5mM NaH₂PO₄(pH7), 0.5% SDS, 2mM EDTA). SSC is 0.15M sodium chloride/0.15M sodium citrate, pH7; SDS is sodium dodecyl sulphate; and EDTA is ethylenediaminetetracetic acid. After hybridization, the membrane upon which the DNA is transferred is washed at 2 x SSC at room temperature and then at 0.1 - 0.5 x SSC/0.1 x SDS at temperatures up to 68°C.

There are other conditions, reagents, and so forth which can be used, which result in a similar degree of stringency. The skilled artisan will be familiar with such conditions, and thus they are not given here. It will be understood, however, that the skilled artisan will be able to manipulate the conditions in a manner to permit the clear identification of alleles of cynomologous PGP nucleic acids of the invention. The skilled artisan also is familiar with the methodology for screening cells and libraries for expression of such molecules which then are routinely isolated, followed by isolation of the pertinent nucleic acid molecule and sequencing.

In screening for cynomologous PGP nucleic acids, a Southern blot may be performed using the foregoing stringent conditions, together with a radioactive probe. After washing the membrane to which the DNA is finally transferred, the membrane can be placed against X-ray film to detect the radioactive signal.

The cynomologous PGP nucleic acids of the invention also include degenerate nucleic acids which include alternative codons to those present in the native materials. For example, serine residues are encoded by the codons TCA, AGT, TCC, TCG, TCT and AGC. Each of the six codons is equivalent for the purposes of encoding a serine residue. Thus, it will be apparent to one of ordinary skill in the art that any of the serine-encoding nucleotide triplets may be employed to direct the protein synthesis apparatus, *in vitro* or *in vivo*, to incorporate a serine residue into an elongating cynomologous PGP polypeptide. Similarly, nucleotide sequence triplets which encode other amino acid residues include, but are not limited to: CCA, CCC, CCG and CCT (proline codons); CGA, CGC, CGG, CGT, AGA and AGG (arginine codons); ACA, ACC, ACG and ACT (threonine codons); AAC and AAT (asparagine codons); and ATA, ATC and ATT (isoleucine codons). Other amino acid residues may be encoded similarly by multiple nucleotide sequences. Thus, the invention

- 10 -

embraces degenerate nucleic acids that differ from the biologically isolated nucleic acids in codon sequence due to the degeneracy of the genetic code.

The invention also provides modified nucleic acid molecules which include additions, substitutions and deletions of one or more nucleotides. In preferred embodiments, these
5 modified nucleic acid molecules and/or the polypeptides they encode retain at least one activity or function of the unmodified nucleic acid molecule and/or the polypeptides, such as transporter activity, etc. In certain embodiments, the modified nucleic acid molecules encode modified polypeptides, preferably polypeptides having conservative amino acid substitutions as are described elsewhere herein. The modified nucleic acid molecules are structurally
10 related to the unmodified nucleic acid molecules and in preferred embodiments are sufficiently structurally related to the unmodified nucleic acid molecules so that the modified and unmodified nucleic acid molecules hybridize under stringent conditions known to one of skill in the art.

For example, modified nucleic acid molecules which encode polypeptides having
15 single amino acid changes can be prepared. Each of these nucleic acid molecules can have one, two or three nucleotide substitutions exclusive of nucleotide changes corresponding to the degeneracy of the genetic code as described herein. Likewise, modified nucleic acid molecules which encode polypeptides having two amino acid changes can be prepared which have, e.g., 2-6 nucleotide changes. Numerous modified nucleic acid molecules like these will
20 be readily envisioned by one of skill in the art, including for example, substitutions of nucleotides in codons encoding amino acids 2 and 3, 2 and 4, 2 and 5, 2 and 6, and so on. In the foregoing example, each combination of two amino acids is included in the set of modified nucleic acid molecules, as well as all nucleotide substitutions which code for the amino acid substitutions. Additional nucleic acid molecules that encode polypeptides having
25 additional substitutions (i.e., 3 or more), additions or deletions (e.g., by introduction of a stop codon or a splice site(s)) also can be prepared and are embraced by the invention as readily envisioned by one of ordinary skill in the art. Any of the foregoing nucleic acids or polypeptides can be tested by routine experimentation for retention of structural relation or activity to the nucleic acids and/or polypeptides disclosed herein.

30 The invention also provides isolated fragments of SEQ ID NO:1 and SEQ ID NO:3. The fragments can be used as probes in Southern blot assays to identify such nucleic acids, or can be used in amplification assays such as those employing PCR. Smaller fragments are

- 11 -

those comprising 12, 13, 14, 15, 16, 17, 18, 20, 22, 25, 30, 40, 50, or 75 nucleotides, and every integer therebetween, and are useful e.g. as primers for nucleic acid amplification procedures. As known to those skilled in the art, larger probes such as 200, 250, 300, 400 or more nucleotides are preferred for certain uses such as Southern blots, while smaller
5 fragments will be preferred for uses such as PCR. Fragments also can be used to produce fusion proteins for generating antibodies or determining binding of the polypeptide fragments. Likewise, fragments can be employed to produce non-fused fragments of the cynomologous PGP polypeptides, useful, for example, in the preparation of antibodies, in immunoassays, and the like. The foregoing nucleic acid fragments further can be used as antisense molecules to
10 inhibit the expression of cynomologous PGP nucleic acids and polypeptides, particularly for therapeutic purposes as described in greater detail below.

The invention also includes functionally equivalent variants of the cynomologous PGP, which include variant nucleic acids and polypeptides which retain one or more of the functional properties of the cynomologous PGP. Preferably such variants include the
15 cynomologous-specific N-terminal domain (e.g., amino acids 86 - 104 of SEQ ID NO:2 or amino acids 86 - 101 of SEQ ID NO:4). For example, variants include a fusion protein which includes the extracellular and transmembrane domains of the cynomologous PGP which retains the ability to transport molecules. Still other functionally equivalent variants include truncations, deletions, point mutations, or additions of amino acids to the sequence of SEQ ID
20 NOs:2 or 4 which retain functions of SEQ ID NOs:2 or 4. Functionally equivalent variants also include a cynomologous PGP which has had a portion of the N-terminus removed or replaced by a similar domain from another P-glycoprotein (e.g. a "domain-swapping" variant). Other functionally equivalent variants will be known to one of ordinary skill in the art, as will methods for preparing such variants. The activity of a functionally equivalent
25 variant can be determined using the methods provided herein, and in references that have described assays using P-glycoproteins of other species. Such variants are useful, *inter alia*, for evaluating bioavailability of drugs, in assays for identification of compounds which bind and/or regulate the transporter function of the cynomologous PGP, and for determining the portions of the cynomologous PGP which are required for transporter activity.

30 Variants which are non-functional also can be prepared as described above. Such variants are useful, for example, as negative controls in experiments testing transporter activity.

- 12 -

A cynomologous PGP nucleic acid, in one embodiment, is operably linked to a gene expression sequence which directs the expression of the cynomologous PGP nucleic acid within a eukaryotic or prokaryotic cell. The "gene expression sequence" is any regulatory nucleotide sequence, such as a promoter sequence or promoter-enhancer combination, which facilitates the efficient transcription and translation of the cynomologous PGP nucleic acid to which it is operably linked. The gene expression sequence may, for example, be a mammalian or viral promoter, such as a constitutive or inducible promoter. Constitutive mammalian promoters include, but are not limited to, the promoters for the following genes: hypoxanthine phosphoribosyl transferase (HPTR), adenosine deaminase, pyruvate kinase, β -actin promoter and other constitutive promoters. Exemplary viral promoters which function constitutively in eukaryotic cells include, for example, promoters from the simian virus, papilloma virus, adenovirus, human immunodeficiency virus (HIV), Rous sarcoma virus, cytomegalovirus, the long terminal repeats (LTR) of Moloney murine leukemia virus and other retroviruses, and the thymidine kinase promoter of herpes simplex virus. Other constitutive promoters are known to those of ordinary skill in the art. The promoters useful as gene expression sequences of the invention also include inducible promoters. Inducible promoters are expressed in the presence of an inducing agent. For example, the metallothionein promoter is induced to promote transcription and translation in the presence of certain metal ions. Other inducible promoters are known to those of ordinary skill in the art.

In general, the gene expression sequence shall include, as necessary, 5' non-transcribing and 5' non-translating sequences involved with the initiation of transcription and translation, respectively, such as a TATA box, capping sequence, CAAT sequence, and the like. Especially, such 5' non-transcribing sequences will include a promoter region which includes a promoter sequence for transcriptional control of the operably joined cynomologous PGP nucleic acid. The gene expression sequences optionally includes enhancer sequences or upstream activator sequences as desired.

The cynomologous PGP nucleic acid sequence and the gene expression sequence are said to be "operably linked" when they are covalently linked in such a way as to place the transcription and/or translation of the cynomologous PGP coding sequence under the influence or control of the gene expression sequence. If it is desired that the cynomologous PGP sequence be translated into a functional protein, two DNA sequences are said to be

- 13 -

operably linked if induction of a promoter in the 5' gene expression sequence results in the transcription of the cynomologous PGP sequence and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region to direct the transcription of the
5 cynomologous PGP sequence, or (3) interfere with the ability of the corresponding RNA transcript to be translated into a protein. Thus, a gene expression sequence would be operably linked to a cynomologous PGP nucleic acid sequence if the gene expression sequence were capable of effecting transcription of that cynomologous PGP nucleic acid sequence such that the resulting transcript might be translated into the desired protein or polypeptide.

10 The cynomologous PGP nucleic acid molecules and the cynomologous PGP polypeptides (including the cynomologous PGP inhibitors described below) of the invention can be delivered to the eukaryotic or prokaryotic cell alone or in association with a vector. In its broadest sense, a "vector" is any vehicle capable of facilitating: (1) delivery of a cynomologous PGP nucleic acid or polypeptide to a target cell, (2) uptake of a cynomologous
15 PGP nucleic acid or polypeptide by a target cell, or (3) expression of a cynomologous PGP nucleic acid molecule or polypeptide in a target cell. Preferably, the vectors transport the cynomologous PGP nucleic acid or polypeptide into the target cell with reduced degradation relative to the extent of degradation that would result in the absence of the vector. Optionally, a "targeting ligand" can be attached to the vector to selectively deliver the vector to a cell
20 which expresses on its surface the cognate receptor (e.g. a receptor, an antigen recognized by an antibody) for the targeting ligand. In this manner, the vector (containing a cynomologous PGP nucleic acid or a cynomologous PGP polypeptide) can be selectively delivered to a specific cell. In general, the vectors useful in the invention are divided into two classes: biological vectors and chemical/physical vectors. Biological vectors are more useful for
25 delivery/uptake of cynomologous PGP nucleic acids to/by a target cell. Chemical/physical vectors are more useful for delivery/uptake of cynomologous PGP nucleic acids or cynomologous PGP proteins to/by a target cell.

Biological vectors include, but are not limited to, plasmids, phagemids, viruses, other vehicles derived from viral or bacterial sources that have been manipulated by the insertion or
30 incorporation of the nucleic acid sequences of the invention, and free nucleic acid fragments which can be linked to the nucleic acid sequences of the invention. Viral vectors are a preferred type of biological vector and include, but are not limited to, nucleic acid sequences

- 14 -

from the following viruses: retroviruses, such as Moloney murine leukemia virus; Harvey murine sarcoma virus; murine mammary tumor virus; Rous sarcoma virus; adenovirus; adeno-associated virus; SV40-type viruses; polyoma viruses; poxviruses; retroviruses; Epstein-Barr viruses; papilloma viruses; herpes virus; vaccinia virus; and polio virus. One can readily
5 employ other vectors not named but known in the art.

Preferred viral vectors are based on non-cytopathic eukaryotic viruses in which non-essential genes have been replaced with the gene of interest. Non-cytopathic viruses include retroviruses, the life cycle of which involves reverse transcription of genomic viral RNA into DNA with subsequent proviral integration into host cellular DNA. In general, the retroviruses
10 are replication-deficient (i.e., capable of directing synthesis of the desired proteins, but incapable of manufacturing an infectious particle). Such genetically altered retroviral expression vectors have general utility for the high-efficiency transduction of genes *in vivo*. Standard protocols for producing replication-deficient retroviruses (including the steps of incorporation of exogenous genetic material into a plasmid, transfection of a packaging cell
15 line with plasmid, production of recombinant retroviruses by the packaging cell line, collection of viral particles from tissue culture media, and infection of the target cells with viral particles) are provided in Kriegler, M., "*Gene Transfer and Expression, A Laboratory Manual*," W.H. Freeman C.O., New York (1990) and Murry, E.J. Ed. "*Methods in Molecular Biology*," vol. 7, Humana Press, Inc., Clifton, New Jersey (1991).

20 Another preferred virus for certain applications is the adeno-associated virus, a double-stranded DNA virus. The adeno-associated virus can be engineered to be replication-deficient and is capable of infecting a wide range of cell types and species. It further has advantages, such as heat and lipid solvent stability; high transduction frequencies in cells of diverse lineages; and lack of superinfection inhibition thus allowing multiple series of transductions.
25 Reportedly, the adeno-associated virus can integrate into human cellular DNA in a site-specific manner, thereby minimizing the possibility of insertional mutagenesis and variability of inserted gene expression. In addition, wild-type adeno-associated virus infections have been followed in tissue culture for greater than 100 passages in the absence of selective pressure, implying that the adeno-associated virus genomic integration is a relatively stable
30 event. The adeno-associated virus can also function in an extrachromosomal fashion.

Expression vectors containing all the necessary elements for expression are commercially available and known to those skilled in the art. See, e.g., Sambrook et al.,

- 15 -

Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, 1989. Cells are genetically engineered by the introduction into the cells of heterologous DNA (RNA) encoding a cynomologous PGP polypeptide or fragment or variant thereof. That heterologous DNA (RNA) is placed under operable control of transcriptional elements to permit the expression of the heterologous DNA in the host cell.

Preferred systems for mRNA expression in mammalian cells are those such as pRc/CMV (available from Invitrogen, Carlsbad, CA) that contain a selectable marker such as a gene that confers G418 resistance (which facilitates the selection of stably transfected cell lines) and the human cytomegalovirus (CMV) enhancer-promoter sequences. Additionally, suitable for expression in primate or canine cell lines is the pCEP4 vector (Invitrogen), which contains an Epstein Barr virus (EBV) origin of replication, facilitating the maintenance of plasmid as a multicopy extrachromosomal element. Another expression vector is the pEF-BOS plasmid containing the promoter of polypeptide Elongation Factor 1 α , which stimulates efficiently transcription *in vitro*. The plasmid is described by Mishizuma and Nagata (*Nuc. Acids Res.* 18:5322, 1990), and its use in transfection experiments is disclosed by, for example, Demoulin (*Mol. Cell. Biol.* 16:4710-4716, 1996). Still another preferred expression vector is an adenovirus, described by Stratford-Perricaudet, which is defective for E1 and E3 proteins (*J. Clin. Invest.* 90:626-630, 1992).

In addition to the biological vectors, chemical/physical vectors may be used to deliver a cynomologous PGP nucleic acid or polypeptide to a target cell and facilitate uptake thereby. As used herein, a "chemical/physical vector" refers to a natural or synthetic molecule, other than those derived from bacteriological or viral sources, capable of delivering the isolated cynomologous PGP nucleic acid or polypeptide to a cell.

A preferred chemical/physical vector of the invention is a colloidal dispersion system. Colloidal dispersion systems include lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system of the invention is a liposome. Liposomes are artificial membrane vesicles which are useful as a delivery vector *in vivo* or *in vitro*. It has been shown that large unilamellar vesicles (LUV), which range in size from 0.2 - 4.0 μ can encapsulate large macromolecules. RNA, DNA, and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, et al., *Trends Biochem. Sci.*, v. 6, p. 77 (1981)). In order for a liposome to be an efficient nucleic acid transfer vector, one or more of the following characteristics should be

- 16 -

present: (1) encapsulation of the nucleic acid of interest at high efficiency with retention of biological activity; (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous contents of the vesicle to the target cell cytoplasm at high efficiency; and (4) accurate and effective expression of genetic information.

5 Liposomes may be targeted to a particular tissue by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein. Ligands which may be useful for targeting a liposome to a particular cell will depend on the particular cell or tissue type. Additionally when the vector encapsulates a nucleic acid, the vector may be coupled to a nuclear targeting peptide, which will direct the cynomologous PGP nucleic acid to the
10 nucleus of the host cell.

 Liposomes are commercially available from Gibco BRL, for example, as LIPOFECTIN™ and LIPOFECTACE™, which are formed of cationic lipids such as N-[1-(2, 3 dioleyloxy)-propyl]-N, N, N-trimethylammonium chloride (DOTMA) and dimethyl
15 dioctadecylammonium bromide (DDAB). Methods for making liposomes are well known in the art and have been described in many publications.

 Other exemplary compositions that can be used to facilitate uptake by a target cell of the cynomologous PGP nucleic acids include calcium phosphate and other chemical
mediators of intracellular transport, microinjection compositions, electroporation and
homologous recombination compositions (e.g., for integrating a cynomologous PGP nucleic
20 acid into a preselected location within a target cell chromosome).

 The invention also embraces so-called expression kits, which allow the artisan to prepare a desired expression vector or vectors. Such expression kits include at least separate
portions of the previously discussed coding sequences. Other components may be added, as
desired, as long as the previously mentioned sequences, which are required, are included.

25 It will also be recognized that the invention embraces the use of the cynomologous PGP cDNA sequences in expression vectors, as well as to transfect host cells and cell lines, be these prokaryotic (e.g., *E. coli*), or eukaryotic (e.g., COS cells, yeast expression systems and recombinant baculovirus expression in insect cells). Especially useful are mammalian cells
such as human, pig, goat, primate, etc. They may be of a wide variety of tissue types, and
30 include primary cells and cell lines. Specific examples include intestinal cells and liver cells. The expression vectors require that the pertinent sequence, i.e., those nucleic acids described
supra, be operably linked to a promoter.

- 17 -

The invention also provides isolated cynomologous PGP polypeptides which include the amino acid sequences of SEQ ID NO:2 and SEQ ID NO:4, and fragments thereof, encoded by the cynomologous PGP nucleic acids described above. Cynomologous PGP polypeptides also embrace alleles, functionally equivalent variants and analogs (those non-allelic polypeptides which vary in amino acid sequence from the disclosed cynomologous PGP polypeptides by 1, 2, 3, 4, 5, or more amino acids) provided that such polypeptides retain cynomologous PGP activity. Non-functional variants also are embraced by the invention; these are useful as antagonists of transporter function, as negative controls in assays, and the like. Such alleles, variants, analogs and fragments are useful, for example, alone or as fusion proteins for a variety of purposes including as a component of assays.

Fragments of a polypeptide preferably are those fragments which retain a distinct functional capability of the cynomologous PGP polypeptide, in particular as a transporter of various molecules. Other functional capabilities which can be retained in a fragment of a cynomologous PGP polypeptide include interaction with antibodies and interaction with other polypeptides (such as would be found in a protein complex). Those skilled in the art are well versed in methods for selecting fragments which retain a functional capability of the cynomologous PGP. Confirmation of the functional capability of the fragment can be carried out by synthesis of the fragment and testing of the capability according to standard methods. For example, to test the transporter activity of a cynomologous PGP fragment, one inserts or expresses the fragment in a cell in which molecular transport can be measured. Such methods, which are standard in the art, are described further herein.

The invention embraces variants of the cynomologous PGP polypeptides described above. As used herein, a "variant" of a cynomologous PGP polypeptide is a polypeptide which contains one or more modifications to the primary amino acid sequence of a cynomologous PGP polypeptide. Modifications which create a cynomologous PGP variant can be made to a cynomologous PGP polypeptide for a variety of reasons, including 1) to reduce or eliminate an activity of a cynomologous PGP polypeptide, such as transport; 2) to enhance a property of a cynomologous PGP polypeptide, such as protein stability in an expression system or the stability of protein-protein binding; 3) to provide a novel activity or property to a cynomologous PGP polypeptide, such as addition of an antigenic epitope or addition of a detectable moiety; or 4) to establish that an amino acid substitution does or does not affect molecular transport activity. Modifications to a cynomologous PGP polypeptide

- 18 -

are typically made to the nucleic acid which encodes the cynomologous PGP polypeptide, and can include deletions, point mutations, truncations, amino acid substitutions and additions of amino acids or non-amino acid moieties. Alternatively, modifications can be made directly to the polypeptide, such as by cleavage, addition of a linker molecule, addition of a detectable moiety, such as biotin, addition of a fatty acid, and the like. Modifications also embrace fusion proteins comprising all or part of the cynomologous PGP amino acid sequence. One of skill in the art will be familiar with methods for predicting the effect on protein conformation of a change in protein sequence, and can thus “design” a variant cynomologous PGP according to known methods. One example of such a method is described by Dahiyat and Mayo in *Science* 278:82-87, 1997, whereby proteins can be designed *de novo*. The method can be applied to a known protein to vary a only a portion of the polypeptide sequence. By applying the computational methods of Dahiyat and Mayo, specific variants of a cynomologous PGP polypeptide can be proposed and tested to determine whether the variant retains a desired conformation.

Variants include cynomologous PGP polypeptides which are modified specifically to alter a feature of the polypeptide unrelated to its physiological activity. For example, cysteine residues can be substituted or deleted to prevent unwanted disulfide linkages. Similarly, certain amino acids can be changed to enhance expression of a cynomologous PGP polypeptide by eliminating proteolysis by proteases in an expression system (e.g., dibasic amino acid residues in yeast expression systems in which KEX2 protease activity is present).

Mutations of a nucleic acid which encode a cynomologous PGP polypeptide preferably preserve the amino acid reading frame of the coding sequence, and preferably do not create regions in the nucleic acid which are likely to hybridize to form secondary structures, such as hairpins or loops, which can be deleterious to expression of the variant polypeptide.

Mutations can be made by selecting an amino acid substitution, or by random mutagenesis of a selected site in a nucleic acid which encodes the polypeptide. Variant polypeptides are then expressed and tested for one or more activities to determine which mutation provides a variant polypeptide with a desired property. Further mutations can be made to variants (or to non-variant cynomologous PGP polypeptides) which are silent as to the amino acid sequence of the polypeptide, but which provide preferred codons for translation in a particular host. The preferred codons for translation of a nucleic acid in, e.g.,

- 19 -

E. coli, are well known to those of ordinary skill in the art. Still other mutations can be made to the noncoding sequences of a cynomologous PGP gene or cDNA clone to enhance expression of the polypeptide.

The activity of variants of cynomologous PGP polypeptides can be tested by cloning
5 the gene encoding the variant cynomologous PGP polypeptide into a bacterial or mammalian expression vector, introducing the vector into an appropriate host cell, expressing the variant cynomologous PGP polypeptide, and testing for a functional capability of the cynomologous PGP polypeptides as disclosed herein. For example, the variant cynomologous PGP polypeptide can be tested for ability to provide molecular transport (e.g., efflux), as set forth
10 below in the examples. Preparation of other variant polypeptides may favor testing of other activities, as will be known to one of ordinary skill in the art.

The skilled artisan will also realize that conservative amino acid substitutions may be made in cynomologous PGP polypeptides to provide functionally equivalent variants of the foregoing polypeptides, i.e, variants which retain the functional capabilities of the
15 cynomologous PGP polypeptides. As used herein, a “conservative amino acid substitution” refers to an amino acid substitution which does not alter the relative charge or size characteristics of the polypeptide in which the amino acid substitution is made. Variants can be prepared according to methods for altering polypeptide sequence known to one of ordinary skill in the art such as are found in references which compile such methods, e.g. *Molecular*
20 *Cloning: A Laboratory Manual*, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or *Current Protocols in Molecular Biology*, F.M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. Exemplary functionally equivalent variants of the cynomologous PGP polypeptides include conservative amino acid substitutions of SEQ ID NO:2 or SEQ ID NO:4. Conservative substitutions of
25 amino acids include substitutions made amongst amino acids within the following groups: (a) M, I, L, V; (b) F, Y, W; (c) K, R, H; (d) A, G; (e) S, T; (f) Q, N; and (g) E, D.

Conservative amino-acid substitutions in the amino acid sequence of cynomologous PGP polypeptide to produce functionally equivalent variants of cynomologous PGP typically are made by alteration of the nucleic acid sequence encoding cynomologous PGP
30 polypeptides (e.g., SEQ ID NOs:1 or 3). Such substitutions can be made by a variety of methods known to one of ordinary skill in the art. For example, amino acid substitutions may be made by PCR-directed mutation, site-directed mutagenesis according to the method of

- 20 -

Kunkel (Kunkel, *Proc. Nat. Acad. Sci. U.S.A.* 82: 488-492, 1985), or by chemical synthesis of a gene encoding a cynomologous PGP polypeptide. The activity of functionally equivalent fragments of cynomologous PGP polypeptides can be tested by cloning the gene encoding the altered cynomologous PGP polypeptide into a bacterial or mammalian expression vector, introducing the vector into an appropriate host cell, expressing the altered cynomologous PGP polypeptide, and testing for the ability of the cynomologous PGP polypeptide to mediate transmembrane transport of compounds. Peptides which are chemically synthesized can be tested directly for function.

A variety of methodologies well-known to the skilled practitioner can be utilized to obtain isolated cynomologous PGP molecules. The polypeptide may be purified from cells which naturally produce the polypeptide by chromatographic means or immunological recognition. Alternatively, an expression vector may be introduced into cells to cause production of the polypeptide. In another method, mRNA transcripts may be microinjected or otherwise introduced into cells to cause production of the encoded polypeptide. Translation of mRNA in cell-free extracts such as the reticulocyte lysate system also may be used to produce polypeptide. Those skilled in the art also can readily follow known methods for isolating cynomologous PGP polypeptides. These include, but are not limited to, immunochromatography, HPLC, size-exclusion chromatography, ion-exchange chromatography and immune-affinity chromatography.

The invention as described herein has a number of uses, some of which are described elsewhere herein. For example, the invention permits isolation of the cynomologous PGP polypeptide molecules by e.g., expression of a recombinant nucleic acid to produce large quantities of polypeptide which may be isolated using standard protocols. As another example, the isolation of the cynomologous PGP gene makes it possible for cynomologous PGP to be used in methods for assaying of molecular transport, such as drug bioavailability studies. These methods involve determining transport of a drug by a first species' PGP (e.g., cynomologous, dog) in comparison to transport of the drug by other species' PGP (e.g., human) as a method for determining or predicting the bioavailability of the drug. Thus the results of whole animal studies on the metabolism of a drug can be evaluated in view of the relative rates or amounts of P-glycoprotein transport of the drug. For example, if a drug administered to a dog has good oral bioavailability and low transport by dog PGP, one can predict that the oral bioavailability of the drug in humans will be good if the transport by

- 21 -

human PGP is also low. Conversely, if the transport of the drug by human PGP is high, then the bioavailability of the drug would be predicted to be low.

The invention also embraces agents which bind selectively to the cynomologous PGP nucleic acid molecules or polypeptides as well as agents which bind to variants and fragments
5 of the polypeptides and nucleic acids as described herein. The agents include polypeptides which bind to cynomologous PGP, and antisense nucleic acids, both of which are described in greater detail below. The agents can inhibit or increase cynomologous PGP activity (antagonists and agonists, respectively).

Some of the agents are inhibitors. A cynomologous PGP inhibitor is an agent that
10 inhibits cynomologous PGP mediated transport of molecules across a cell membrane. Efflux assays can be performed to screen and/or determine whether a cynomologous PGP inhibitor has the ability to inhibit cynomologous PGP activity, and whether the inhibition is selective. An exemplary assay of efflux is described below in the Examples.

In one embodiment the cynomologous PGP inhibitor is an antisense oligonucleotide
15 that selectively binds to a cynomologous PGP nucleic acid molecule, to reduce the expression of cynomologous PGP (or other species' PGPs) in a cell. This is desirable in virtually any medical condition wherein a reduction of PGP transporter activity is desirable, e.g., to increase retention of cytotoxic agents in a cell.

As used herein, the term "antisense oligonucleotide" or "antisense" describes an
20 oligonucleotide that is an oligoribonucleotide, oligodeoxyribonucleotide, modified oligoribonucleotide, or modified oligodeoxyribonucleotide which hybridizes under physiological conditions to DNA comprising a particular gene or to an mRNA transcript of that gene and, thereby, inhibits the transcription of that gene and/or the translation of that mRNA. The antisense molecules are designed so as to interfere with transcription or
25 translation of a target gene upon hybridization with the target gene or transcript. Those skilled in the art will recognize that the exact length of the antisense oligonucleotide and its degree of complementarity with its target will depend upon the specific target selected, including the sequence of the target and the particular bases which comprise that sequence. It is preferred that the antisense oligonucleotide be constructed and arranged so as to bind
30 selectively with the target under physiological conditions, i.e., to hybridize substantially more to the target sequence than to any other sequence in the target cell under physiological conditions. Based upon SEQ ID NOs:1 or 3, or upon allelic or homologous genomic and/or

- 22 -

cDNA sequences, one of skill in the art can easily choose and synthesize any of a number of appropriate antisense molecules for use in accordance with the present invention. In order to be sufficiently selective and potent for inhibition, such antisense oligonucleotides should comprise at least 10 and, more preferably, at least 15 consecutive bases which are

5 complementary to the target, although in certain cases modified oligonucleotides as short as 7 bases in length have been used successfully as antisense oligonucleotides (Wagner et al., *Nature Biotechnol.* 14:840-844, 1996). Most preferably, the antisense oligonucleotides comprise a complementary sequence of 20-30 bases. Although oligonucleotides may be chosen which are antisense to any region of the gene or mRNA transcripts, in preferred

10 embodiments the antisense oligonucleotides correspond to N-terminal or 5' upstream sites such as translation initiation, transcription initiation or promoter sites. In addition, 3'-untranslated regions may be targeted. Targeting to mRNA splicing sites has also been used in the art but may be less preferred if alternative mRNA splicing occurs. In addition, the antisense is targeted, preferably, to sites in which mRNA secondary structure is not expected

15 (see, e.g., Sainio et al., *Cell Mol. Neurobiol.* 14(5):439-457, 1994) and at which polypeptides are not expected to bind. Thus, the present invention also provides for antisense oligonucleotides which are complementary to allelic or homologous cDNAs and genomic DNAs corresponding to cynomolgous PGP nucleic acid containing SEQ ID NOs:1 or 3.

In one set of embodiments, the antisense oligonucleotides of the invention may be

20 composed of "natural" deoxyribonucleotides, ribonucleotides, or any combination thereof. That is, the 5' end of one native nucleotide and the 3' end of another native nucleotide may be covalently linked, as in natural systems, via a phosphodiester internucleoside linkage. These oligonucleotides may be prepared by art recognized methods which may be carried out manually or by an automated synthesizer. They also may be produced recombinantly by

25 vectors.

In preferred embodiments, however, the antisense oligonucleotides of the invention also may include "modified" oligonucleotides. That is, the oligonucleotides may be modified in a number of ways which do not prevent them from hybridizing to their target but which enhance their stability or targeting or which otherwise enhance their therapeutic effectiveness.

30 The term "modified oligonucleotide" as used herein describes an oligonucleotide in which (1) at least two of its nucleotides are covalently linked via a synthetic internucleoside

- 23 -

linkage (i.e., a linkage other than a phosphodiester linkage between the 5' end of one nucleotide and the 3' end of another nucleotide) and/or (2) a chemical group not normally associated with nucleic acids has been covalently attached to the oligonucleotide. Preferred synthetic internucleoside linkages are phosphorothioates, alkylphosphonates, 5 phosphorodithioates, phosphate esters, alkylphosphonothioates, phosphoramidates, carbamates, carbonates, phosphate triesters, acetamides, carboxymethyl esters and peptides.

The term "modified oligonucleotide" also encompasses oligonucleotides with a covalently modified base and/or sugar. For example, modified oligonucleotides include oligonucleotides having backbone sugars which are covalently attached to low molecular 10 weight organic groups other than a hydroxyl group at the 3' position and other than a phosphate group at the 5' position. Thus modified oligonucleotides may include a 2'-O-alkylated ribose group. In addition, modified oligonucleotides may include sugars such as arabinose instead of ribose. The present invention, thus, contemplates pharmaceutical preparations containing modified antisense molecules that are complementary to and 15 hybridizable with, under physiological conditions, nucleic acids encoding cynomolgous PGP polypeptides, together with pharmaceutically acceptable carriers.

Agents which bind cynomolgous PGP also include binding peptides and other molecules which bind to the cynomolgous PGP polypeptide and complexes containing the cynomolgous PGP polypeptide. When the binding molecules are inhibitors, the molecules 20 bind to and inhibit the activity of cynomolgous PGP. To determine whether a cynomolgous PGP binding agent binds to cynomolgous PGP any known binding assay may be employed. For example, the binding agent may be immobilized on a surface and then contacted with a labeled cynomolgous PGP polypeptide. The amount of cynomolgous PGP which interacts with the cynomolgous PGP binding agent or the amount which does not bind to the 25 cynomolgous PGP binding agent may then be quantitated to determine whether the cynomolgous PGP binding agent binds to cynomolgous PGP.

The cynomolgous PGP binding agents include molecules of numerous size and type that bind selectively or preferentially to cynomolgous PGP polypeptides, and complexes of both cynomolgous PGP polypeptides and their binding partners. These molecules may be 30 derived from a variety of sources. For example, cynomolgous PGP binding agents can be provided by screening degenerate peptide libraries which can be readily prepared in solution, in immobilized form or as phage display libraries. Combinatorial libraries also can be

- 24 -

synthesized of peptides containing one or more amino acids. Libraries further can be synthesized of peptoids and non-peptide synthetic moieties.

Phage display can be particularly effective in identifying binding peptides useful according to the invention. Briefly, one prepares a phage library (using e.g. m13, fd, or
5 lambda phage), displaying inserts from 4 to about 80 amino acid residues using conventional procedures. The inserts may represent, for example, a completely degenerate or biased array. One then can select phage-bearing inserts which bind to the cynomologous PGP polypeptide. This process can be repeated through several cycles of reselection of phage that bind to the cynomologous PGP polypeptide. Repeated rounds lead to enrichment of phage bearing
10 particular sequences. DNA sequence analysis can be conducted to identify the sequences of the expressed polypeptides. The minimal linear portion of the sequence that binds to the cynomologous PGP polypeptide can be determined. One can repeat the procedure using a biased library containing inserts containing part or all of the minimal linear portion plus one or more additional degenerate residues upstream or downstream thereof. Yeast two-hybrid
15 screening methods also may be used to identify polypeptides that bind to the cynomologous PGP polypeptides. Thus, the cynomologous PGP polypeptides of the invention, or a fragment thereof, can be used to screen peptide libraries, including phage display libraries, to identify and select peptide binding partners of the cynomologous PGP polypeptides of the invention. Such molecules can be used, as described, for screening assays, for purification protocols, for
20 interfering directly with the functioning of cynomologous PGP and for other purposes that will be apparent to those of ordinary skill in the art.

Therefore the invention generally provides efficient methods of identifying pharmacological agents or lead compounds for agents useful in the treatment of conditions associated with aberrant PGP activity and the compounds and agents so identified. Generally,
25 the screening methods involve assaying for compounds which inhibit or enhance transport of molecules through cynomologous PGP. Such methods are adaptable to automated, high throughput screening of compounds. Examples of such methods are described in US patent 5,429,921.

A variety of assays for pharmacological agents are provided, including, labeled *in*
30 *vitro* protein binding assays, efflux assays using detectable molecules, etc. For example, protein binding screens are used to rapidly examine the binding of candidate pharmacological agents to a cynomologous PGP. The candidate pharmacological agents can be derived from,

- 25 -

for example, combinatorial peptide libraries. Convenient reagents for such assays are known in the art. An exemplary cell-based assay of efflux involves contacting a cell having a cynomologous PGP with a candidate pharmacological agent under conditions whereby the efflux of a detectably labeled molecule can occur. Specific conditions are well known in the art and are described, for example, in Sharom et al., *Biochem. Pharmacol.* 58:571-586, 1999, and references cited therein. A reduction in the efflux in the presence of the candidate pharmacological agent indicates that the candidate pharmacological agent reduces the efflux activity of cynomologous PGP. An increase in the efflux in the presence of the candidate pharmacological agent indicates that the candidate pharmacological agent increases the efflux activity of cynomologous PGP.

Cynomologous PGP used in the methods of the invention can be added to an assay mixture as an isolated polypeptide (where binding of a candidate pharmaceutical agent is to be measured) or as a cell or other membrane-encapsulated space which includes a cynomologous PGP polypeptide. In the latter assay configuration, the cell or other membrane-encapsulated space can contain the cynomologous PGP as a preloaded polypeptide or as a nucleic acid (e.g. a cell transfected with an expression vector containing a cynomologous PGP). In the assays described herein, the cynomologous PGP polypeptide can be produced recombinantly, or isolated from biological extracts, but preferably is synthesized *in vitro*. Cynomologous PGP polypeptides encompass chimeric proteins comprising a fusion of a cynomologous PGP polypeptide with another polypeptide, e.g., a polypeptide capable of providing or enhancing protein-protein binding, or enhancing stability of the cynomologous PGP polypeptide under assay conditions. A polypeptide fused to a cynomologous PGP polypeptide or fragment thereof may also provide means of readily detecting the fusion protein, e.g., by immunological recognition or by fluorescent labeling.

The assay mixture also comprises a candidate pharmacological agent. Typically, a plurality of assay mixtures are run in parallel with different agent concentrations to obtain a different response to the various concentrations. Typically, one of these concentrations serves as a negative control, i.e., at zero concentration of agent or at a concentration of agent below the limits of assay detection. Candidate agents encompass numerous chemical classes, although typically they are organic compounds. Preferably, the candidate pharmacological agents are small organic compounds, i.e., those having a molecular weight of more than 50 yet less than about 2500. Candidate agents comprise functional chemical groups necessary for

- 26 -

structural interactions with polypeptides, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups and more preferably at least three of the functional chemical groups. The candidate agents can comprise cyclic carbon or heterocyclic structure and/or aromatic or polyaromatic structures substituted with one or more of the above-identified functional groups. Candidate agents also can be biomolecules such as peptides, saccharides, fatty acids, sterols, isoprenoids, purines, pyrimidines, derivatives or structural analogs of the above, or combinations thereof and the like. Where the agent is a nucleic acid, the agent typically is a DNA or RNA molecule, although modified nucleic acids having non-natural bonds or subunits are also contemplated.

Candidate agents are obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides, synthetic organic combinatorial libraries, phage display libraries of random peptides, and the like. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural and synthetically produced libraries and compounds can be readily modified through conventional chemical, physical, and biochemical means. Further, known pharmacological agents may be subjected to directed or random chemical modifications such as acylation, alkylation, esterification, amidification, etc. to produce structural analogs of the agents.

Therefore, a source of candidate agents are libraries of molecules based on known P-glycoprotein inhibitors, in which the structure of the inhibitor is changed at one or more positions of the molecule to contain more or fewer chemical moieties or different chemical moieties. The structural changes made to the molecules in creating the libraries of analog inhibitors can be directed, random, or a combination of both directed and random substitutions and/or additions. One of ordinary skill in the art in the preparation of combinatorial libraries can readily prepare such libraries based on existing P-glycoprotein inhibitors.

A variety of other reagents also can be included in the mixture. These include reagents such as salts, buffers, neutral proteins (e.g., albumin), detergents, etc. which may be used to facilitate optimal protein-protein and/or protein-nucleic acid binding. Such a reagent may also reduce non-specific or background interactions of the reaction components. Other

- 27 -

reagents that improve the efficiency of the assay such as protease inhibitors, nuclease inhibitors, antimicrobial agents, and the like may also be used.

The mixture of the foregoing assay materials is incubated under conditions whereby, but for the presence of the candidate pharmacological agent, the cynomologous PGP mediates the efflux of a control amount of a compound such as a drug. For determining the binding of a candidate pharmaceutical agent to a cynomologous PGP, the mixture is incubated under conditions which permit binding. The order of addition of components, incubation temperature, time of incubation, and other parameters of the assay may be readily determined.

Such experimentation merely involves optimization of the assay parameters, not the fundamental composition of the assay. Incubation temperatures typically are between 4°C and 40°C. Incubation times preferably are minimized to facilitate rapid, high throughput screening, and typically are between 1 minute and 10 hours.

After incubation, the level of efflux or the level of specific binding between the cynomologous PGP polypeptide and the candidate pharmaceutical agent is detected by any convenient method available to the user. For cell free binding type assays, a separation step is often used to separate bound from unbound components. The separation step may be accomplished in a variety of ways. Conveniently, at least one of the components is immobilized on a solid substrate, from which the unbound components may be easily separated. The solid substrate can be made of a wide variety of materials and in a wide variety of shapes, e.g., microtiter plate, microbead, dipstick, resin particle, etc. The substrate preferably is chosen to maximize signal to noise ratios, primarily to minimize background binding, as well as for ease of separation and cost.

Separation may be effected for example, by removing a bead or dipstick from a reservoir, emptying or diluting a reservoir such as a microtiter plate well, rinsing a bead, particle, chromatographic column or filter with a wash solution or solvent. The separation step preferably includes multiple rinses or washes. For example, when the solid substrate is a microtiter plate, the wells may be washed several times with a washing solution, which typically includes those components of the incubation mixture that do not participate in specific bindings such as salts, buffer, detergent, non-specific protein, etc. Where the solid substrate is a magnetic bead, the beads may be washed one or more times with a washing solution and isolated using a magnet.

Detection may be effected in any convenient way for cell-based assays such as a

- 28 -

transmembrane transport assay. The transport of a directly or indirectly detectable product, e.g., a fluorescent molecule such as calcein AM or rhodamine 123, is preferred. For cell free binding assays, one of the components usually comprises, or is coupled to, a detectable label. A wide variety of labels can be used, such as those that provide direct detection (e.g.,
5 radioactivity, luminescence, optical or electron density, etc). or indirect detection (e.g., epitope tag such as the FLAG epitope, enzyme tag such as horseradish peroxidase, etc.). The label may be bound to a cynomologous PGP polypeptide or the candidate pharmacological agent.

A variety of methods may be used to detect the label, depending on the nature of the
10 label and other assay components. For example, the label may be detected while bound to the solid substrate or subsequent to separation from the solid substrate. Labels may be directly detected through optical or electron density, radioactive emissions, nonradiative energy transfers, etc. or indirectly detected with antibody conjugates, streptavidin-biotin conjugates, etc. Methods for detecting the labels are well known in the art.

The cynomologous PGP binding agent may also be an antibody or a functionally
15 active antibody fragment. Antibodies are well known to those of ordinary skill in the science of immunology. As used herein, the term "antibody" means not only intact antibody molecules but also fragments of antibody molecules retaining cynomologous PGP binding ability. Such fragments are also well known in the art and are regularly employed both *in*
20 *vitro* and *in vivo*. In particular, as used herein, the term "antibody" means not only intact immunoglobulin molecules but also the well-known active fragments $F(ab')_2$, and Fab. $F(ab')_2$, and Fab fragments which lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl et al., *J. Nucl. Med.* 24:316-325 (1983)).

Monoclonal antibodies may be made by any of the methods known in the art utilizing
25 cynomologous PGP, or a fragment thereof, as an immunogen. Alternatively the antibody may be a polyclonal antibody specific for cynomologous PGP which inhibits cynomologous PGP activity. The preparation and use of polyclonal antibodies is also known to one of ordinary skill in the art.

Significantly, as is well known in the art, only a small portion of an antibody
30 molecule; the paratope, is involved in the binding of the antibody to its epitope (see, in general, Clark, W.R. (1986) *The Experimental Foundations of Modern Immunology* Wiley &

- 29 -

Sons, Inc., New York; Roitt, I. (1991) *Essential Immunology*, 7th Ed., Blackwell Scientific Publications, Oxford). The pFc' and Fc regions, for example, are effectors of the complement cascade but are not involved in antigen binding. An antibody from which the pFc' region has been enzymatically cleaved, or which has been produced without the pFc' region, designated an F(ab')₂ fragment, retains both of the antigen binding sites of an intact antibody. Similarly, an antibody from which the Fc region has been enzymatically cleaved, or which has been produced without the Fc region, designated an Fab fragment, retains one of the antigen binding sites of an intact antibody molecule. Proceeding further, Fab fragments consist of a covalently bound antibody light chain and a portion of the antibody heavy chain denoted Fd. The Fd fragments are the major determinant of antibody specificity (a single Fd fragment may be associated with up to ten different light chains without altering antibody specificity) and Fd fragments retain epitope-binding ability in isolation.

Within the antigen-binding portion of an antibody, as is well-known in the art, there are complementarity determining regions (CDRs), which directly interact with the epitope of the antigen, and framework regions (FRs), which maintain the tertiary structure of the paratope (see, in general, Clark, 1986; Roitt, 1991). In both the heavy chain Fd fragment and the light chain of IgG immunoglobulins, there are four framework regions (FR1 through FR4) separated respectively by three complementarity determining regions (CDR1 through CDR3). The CDRs, and in particular the CDR3 regions, and more particularly the heavy chain CDR3, are largely responsible for antibody specificity.

In general, intact antibodies are said to contain "Fc" and "Fab" regions. The Fc regions are involved in complement activation and are not involved in antigen binding. An antibody from which the Fc' region has been enzymatically cleaved, or which has been produced without the Fc' region, designated an "F(ab')₂" fragment, retains both of the antigen binding sites of the intact antibody. Similarly, an antibody from which the Fc region has been enzymatically cleaved, or which has been produced without the Fc region, designated an "Fab" fragment, retains one of the antigen binding sites of the intact antibody. Fab' fragments consist of a covalently bound antibody light chain and a portion of the antibody heavy chain, denoted "Fd." The Fd fragments are the major determinants of antibody specificity (a single Fd fragment may be associated with up to ten different light chains without altering antibody specificity). Isolated Fd fragments retain the ability to specifically bind to antigen epitopes.

- 30 -

The sequences of the antigen-binding Fab' portion of the anti-cynomologous PGP monoclonal antibodies identified as being useful according to the invention in the assays provided above, as well as the relevant FR and CDR regions, can be determined using amino acid sequencing methods that are routine in the art. It is well established that non-CDR regions of a mammalian antibody may be replaced with corresponding regions of non-specific or hetero-specific antibodies while retaining the epitope specificity of the original antibody. This technique is useful for the development and use of "humanized" antibodies in which non-human CDRs are covalently joined to human FR and/or Fc/pFc' regions to produce a functional antibody. Techniques to humanize antibodies are particularly useful when non-human animal (e.g., murine) antibodies which inhibit cynomologous PGP activity are identified. These non-human animal antibodies can be humanized for use in the treatment of a human subject in the methods according to the invention. Examples of methods for humanizing a murine antibody are provided in U.S. patents 4,816,567, 5,225,539, 5,585,089, 5,693,762 and 5,859,205. Other antibodies, including fragments of intact antibodies with antigen-binding ability, are often referred to as "chimeric" antibodies.

Thus, as will be apparent to one of ordinary skill in the art, the present invention also provides for F(ab')₂, and Fab fragments of an anti-cynomologous PGP monoclonal antibody; chimeric antibodies in which the Fc and/or FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions of an anti-cynomologous PGP antibody have been replaced by homologous human or non-human sequences; chimeric F(ab')₂ fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions of an anti-cynomologous PGP antibody have been replaced by homologous human or non-human sequences; and chimeric Fab fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences.

According to the invention cynomologous PGP inhibitors also include "dominant negative" polypeptides derived from SEQ ID NOs:2 or 4. A dominant negative polypeptide is an inactive variant of a polypeptide, which, by interacting with the cellular machinery, displaces an active polypeptide from its interaction with the cellular machinery or competes with the active polypeptide, thereby reducing the effect of the active polypeptide. For example, a dominant negative receptor which binds a ligand but does not transmit a signal in response to binding of the ligand can reduce the biological effect of expression of the ligand.

The end result of the expression of a dominant negative cynomologous PGP

- 31 -

polypeptide of the invention in a cell is a reduction in PGP activity such as molecular transport. One of ordinary skill in the art can assess the potential for a dominant negative variant of a cynomologous PGP polypeptide, and using standard mutagenesis techniques to create one or more dominant negative variant polypeptides. For example, given the teachings
5 contained herein of a cynomologous PGP polypeptide, one of ordinary skill in the art can modify the sequence of the cynomologous PGP polypeptide by site-specific mutagenesis, scanning mutagenesis, partial gene deletion or truncation, and the like. See, e.g., U.S. Patent No. 5,580,723 and Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, 1989. The skilled artisan then can test the
10 population of mutagenized polypeptides for diminution in cynomologous PGP activity and/or for retention of such an activity. Other similar methods for creating and testing dominant negative variants of a cynomologous PGP polypeptide will be apparent to one of ordinary skill in the art.

Each of the compositions of the invention is useful for a variety of therapeutic and
15 non-therapeutic purposes. For example, the cynomologous PGP nucleic acids of the invention are useful as oligonucleotide probes. Such oligonucleotide probes can be used herein to identify genomic or cDNA library clones possessing an identical or substantially similar nucleic acid sequence. A suitable oligonucleotide or set of oligonucleotides, which is capable of hybridizing under stringent hybridization conditions to the desired sequence, a variant or
20 fragment thereof, or an anti-sense complement of such an oligonucleotide or set of oligonucleotides, can be synthesized by means well known in the art (see, for example, *Synthesis and Application of DNA and RNA*, S.A. Narang, ed., 1987, Academic Press, San Diego, CA) and employed as a probe to identify and isolate the desired sequence, variant or fragment thereof by techniques known in the art. Techniques of nucleic acid hybridization
25 and clone identification are disclosed by Sambrook, et al., *Molecular Cloning, A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory Press, Plainview, NY (1989). To facilitate the detection of a desired nucleic acid sequence, or variant or fragment thereof, whether for cloning purposes or for the mere detection of the presence of the sequence, the above-described probes may be labeled with a detectable group. Such a detectable group may
30 be any material having a detectable physical or chemical property. Such materials have been well-developed in the field of nucleic acid hybridization and, in general, many labels useful in such methods can be applied to the present invention. Particularly useful are radioactive

- 32 -

labels. Any radioactive label may be employed which provides for an adequate signal and has a sufficient half-life. If single stranded, the oligonucleotide may be radioactively labeled using kinase reactions. Alternatively, oligonucleotides are also useful as nucleic acid hybridization probes when labeled with a non-radioactive marker such as biotin, an enzyme or a fluorescent group. See, for example, Leary, J.J., et al., *Proc. Natl. Acad. Sci. (USA)* 80:4045 (1983); Renz, M. et al., *Nucl. Acids Res.* 12:3435 (1984); and Renz, M., *EMBO J.* 6:817 (1983).

Additionally, complements of the cynomologous PGP nucleic acids can be useful as antisense oligonucleotides, e.g., by delivering the antisense oligonucleotide to an animal to induce a cynomologous PGP “knockout” phenotype. The administration of antisense RNA probes to block gene expression is discussed in Lichtenstein, C., *Nature* 333:801-802 (1988).

Alternatively, the cynomologous PGP nucleic acid of the invention can be used to prepare a non-human transgenic animal. A “transgenic animal” is an animal having cells that contain DNA which has been artificially inserted into a cell, which DNA becomes part of the genome of the animal which develops from that cell. Preferred transgenic animals are primates, mice, rats, cows, pigs, horses, goats, sheep, dogs and cats. Animals suitable for transgenic experiments can be obtained from standard commercial sources such as Charles River (Wilmington, MA), Taconic (Germantown, NY), Harlan Sprague Dawley (Indianapolis, IN), etc. Transgenic animals having a particular property associated with a particular disease can be used to study the affects of a variety of drugs and treatment methods on the disease, and thus serve as genetic models for the study of a number of human diseases. The invention, therefore, contemplates the use of cynomologous PGP knockout and transgenic animals as models for the study of disorders involving transport of molecules across cell membranes. A variety of methods known to one of ordinary skill in the art are available for the production of transgenic animals associated with this invention.

Inactivation or replacement of the endogenous PGP/MDR1 gene can be achieved by a homologous recombination system using embryonic stem cells. The resultant transgenic non-human mammals having a PGP^{-/-} knockout phenotype may be made transgenic for the cynomologous PGP and used as a model for screening compounds as modulators (agonists or antagonists/inhibitors) of the cynomologous PGP. In this manner, such therapeutic drugs can be identified.

Additionally, a normal or mutant version of cynomologous PGP can be inserted into

- 33 -

the germ line to produce transgenic animals which constitutively or inducibly express the normal or mutant form of cynomologous PGP. These animals are useful in studies to define the role and function of cynomologous PGP in cells.

The compositions of the invention are also useful for therapeutic purposes.

5 Accordingly the invention encompasses a method for inhibiting cynomologous PGP activity in a mammalian cell. The invention further provides methods for reducing or increasing cynomologous PGP activity in a cell. In one embodiment, the method involves contacting the mammalian cell with an amount of a cynomologous PGP nucleic acid or polypeptide effective to inhibit molecular transport out of the mammalian cell. Such methods are useful *in vitro* for
10 the purpose of, for example, elucidating the mechanisms involved in drug resistance and reduced drug bioavailability.

The invention also encompasses a method for increasing PGP expression in a cell or subject. The amount of cynomologous PGP can be increased in such cell or subject by contacting the cell with, or administering to the subject, a PGP nucleic acid or a PGP
15 polypeptide of the invention to the subject in an amount effective to increase transmembrane transport in the cell or the subject. An increase in PGP activity can be measured by the assays described herein, e.g., assays of transmembrane transport.

The preparations of the invention are administered in effective amounts. An effective amount is that amount of a pharmaceutical preparation that alone, or together with further
20 doses, produces the desired response. Such amounts will depend, of course, on the particular condition being treated, the severity of the condition, the individual patient parameters including age, physical condition, size and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. It is preferred generally that a maximum
25 dose be used, that is, the highest safe dose according to sound medical judgment. It will be understood by those of ordinary skill in the art, however, that a patient may insist upon a lower dose or tolerable dose for medical reasons, psychological reasons or for virtually any other reasons.

Generally, doses of active compounds would be from about 0.01 mg/kg per day to
30 1000 mg/kg per day. It is expected that doses ranging from 50-500 mg/kg will be suitable and in one or several administrations per day. Lower doses will result from other forms of administration, such as intravenous administration. In the event that a response in a subject is

- 34 -

insufficient at the initial doses applied, higher doses (or effectively higher doses by a different, more localized delivery route) may be employed to the extent that patient tolerance permits. Multiple doses per day are contemplated to achieve appropriate systemic levels of compound, although fewer doses typically will be given when compounds are prepared as
5 slow release or sustained release medications.

When administered, the pharmaceutical preparations of the invention are applied in pharmaceutically-acceptable amounts and in pharmaceutically-acceptably compositions. Such preparations may routinely contain salts, buffering agents, preservatives, compatible carriers, and optionally other therapeutic agents. When used in medicine, the salts should be
10 pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically-acceptable salts thereof and are not excluded from the scope of the invention. Such pharmacologically and pharmaceutically-acceptable salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulfuric, nitric, phosphoric, maleic, acetic, salicylic, citric, formic, malonic, succinic, and the
15 like. Also, pharmaceutically-acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts.

The cynomologous PGP inhibitors or cynomologous PGP nucleic acids and polypeptides useful according to the invention may be combined, optionally, with a pharmaceutically-acceptable carrier. The term "pharmaceutically-acceptable carrier" as used
20 herein means one or more compatible solid or liquid fillers, diluents or encapsulating substances which are suitable for administration into a human. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being co-mingled with the molecules of the present invention, and with
25 each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficacy.

The pharmaceutical compositions may contain suitable buffering agents, including: acetic acid in a salt; citric acid in a salt; and phosphoric acid in a salt.

The pharmaceutical compositions also may contain, optionally, suitable preservatives,
30 such as: benzalkonium chloride; chlorobutanol; parabens and thimerosal.

A variety of administration routes are available. The particular mode selected will depend, of course, upon the particular compound selected, the severity of the condition being

- 35 -

treated and the dosage required for therapeutic efficacy. The methods of the invention, generally speaking, may be practiced using any mode of administration that is medically acceptable, meaning any mode that produces effective levels of the active compounds without causing clinically unacceptable adverse effects. Such modes of administration include oral, 5 rectal, topical, nasal, interdermal, or parenteral routes. The term "parenteral" includes subcutaneous, intravenous, intrathecal, intramuscular, or infusion. Intravenous or intramuscular routes are not particularly suitable for long-term therapy and prophylaxis.

The pharmaceutical compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well-known in the art of pharmacy. All methods 10 include the step of bringing the active agent into association with a carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing the active compound into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product.

Compositions suitable for oral administration may be presented as discrete units, such 15 as capsules, tablets, lozenges, each containing a predetermined amount of the active compound. Other compositions include suspensions in aqueous liquids or non-aqueous liquids such as a syrup, elixir or an emulsion.

Compositions suitable for parenteral administration conveniently comprise a sterile aqueous preparation of the cynomologous PGP inhibitor or cynomologous PGP nucleic acids 20 and polypeptides, which is preferably isotonic with the blood of the recipient. This aqueous preparation may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation also may be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that 25 may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono-or di-glycerides. In addition, fatty acids such as oleic acid may be used in the preparation of injectables. Carrier formulation suitable for oral, subcutaneous, intravenous, intrathecal, 30 intramuscular, etc. administrations can be found in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA.

Other delivery systems can include time-release, delayed release or sustained release

- 36 -

delivery systems such as the biological/chemical vectors is discussed above. Such systems can avoid repeated administrations of the active compound, increasing convenience to the subject and the physician. Many types of release delivery systems are available and known to those of ordinary skill in the art. Use of a long-term sustained release implant may be desirable. Long-term release, as used herein, means that the implant is constructed and arranged to deliver therapeutic levels of the active ingredient for at least 30 days, and preferably 60 days. Long-term sustained release implants are well-known to those of ordinary skill in the art and include some of the release systems described above.

The invention will be more fully understood by reference to the following examples.

These examples, however, are merely intended to illustrate the embodiments of the invention and are not to be construed to limit the scope of the invention.

Examples

Example 1: Isolation of cynomolgous monkey P-glycoprotein

cDNA libraries were prepared using cynomolgous monkey (*Macaca fascicularis*) mRNA according to standard procedures. The libraries were screened for P-glycoprotein clones using a human P-glycoprotein DNA probe. Clones were isolated, purified and sequenced in accordance with standard procedures.

Preparation of Library

A custom Lambda ZAP II cDNA library from Cynomolgous monkey liver was prepared by Stratagene. This template was used to obtain clones 72/73 and 79/77 1500.

Anticipating that the monkey would show substantial homology to the human PGP, initial primers were designed from the human PGP (Genbank Accession Number M14758).

Primers were also made based on the Lambda ZAP II vector sequences. Later primers were designed based on the monkey sequence or a combination of the monkey/human sequences. All primers used are listed below.

Primer	Sequence	Source	SEQ NO	nucleotides
PS070	ctg gac ttc ctc tca tga tgc tgg tgt	Human PGP	9	612-638 F
PS072	gac agc tat tcg aag agt ggg cac aaa c	"	10	1550-1577 F
PS073	ggc cat ggc acc aaa gac aac agc	"	11	3362-3385 R

- 37 -

PS074	ttg gac aca gaa agt gaa gca gt	Cyno Monkey	12	2105-2127 F
		PGP		(human seq)
PS075	ctg agc atg gat cgg aaa ac	“	13	2798-2817 R
				(human seq)
PS077	ttg taa tac gac tca cta tag ggc gaa t	T7 primer	14	“Based on
				Stratagene’s
				seq.
PS078	ctt ttc gag atg ggt aac tga agt gaa c	Cyno/human	15	1613-1641 R
		PGP		
PS079	aga agg tgc tgg gaa gat cgc tac tga a	“	16	3094-3121 F
PS080	gcc taa agc cga aca cat	Cyno Monkey	17	3498-3515 F
		PGP		
PS081	cta tta agt ctg cat tct gga	“	18	4134-4154 R

All PCR reactions were done using a Perkin Elmer 9700 Thermocycler. PCR products were analyzed on an agarose gel, and promising bands were purified by the use of Qiaquick Gel Extraction Kit (Qiagen) according to the manufacturer’s instructions. These bands were

5 ligated into pCR 2.1 and transformed into INVaF using Invitrogen’s TA Cloning protocol according to the manufacturer’s instructions. White colonies were picked and analyzed by restriction digest. DNA was prepared from promising clones using the Promega Wizard Plus Miniprep DNA Purification System according to the manufacturer's instructions and sequenced with an ABI 377 sequencer.

10

Clone 72/73

Using primers ps072 and ps073, a ~1.8 kb fragment was obtained following 38 cycles of PCR (94°C for 5 m; followed by 38 cycles of 94° for 30 s, 63°C for 45 s, 72°C for 60 s; ending with 72°C for 7 m) using Klentaq. This was sequenced using m13F and m13R

15 primers. Further sequencing primers, ps074 and ps075 were designed based on the sequence obtained. This resulted in a total of ~1.85 kb sequenced corresponding to human PGP 1553-3361.

Clone 79/77 C1500

- 38 -

Using primers ps079 and ps077, a ~1.5 kb fragment was obtained following 38 cycles of PCR (94°C for 5 m; followed by 38 cycles of 94°C for 30 s, 63°C for 45 s, 72°C for 60 s; ending with 72°C for 7 m) using Klentaq. This was sequenced using m13F and m13R as primers. Further sequencing primers, ps080 and ps081 were designed based on the sequence obtained. Sequence corresponding to the human pgp 3102 – 4525 was obtained, which included the stop codon for the cynomologous monkey PGP cDNA.

Clone 70/78C

Nucleobond RNA Maxi kit from Clontech was used according to the manufacturers to prepare total RNA from Cynomologous monkey liver. Single stranded cDNA was prepared from this RNA using the Superscript Kit from GIBCO BRL Life Technologies according to the manufacturer's instructions. This was used as a template for clones 70/78C. Using primers ps070 and ps078, a ~1.0 kb fragment was obtained from the liver cDNA following 38 cycles of PCR (94°C for 5 m; followed by 38 cycles of 94°C for 30 s, 65°C for 45 s, 72°C for 60 s; ending with 72°C for 7 m) using Klentaq. This was sequenced using m13F and m13R as primers. Sequence corresponding to the human pgp was obtained, which corresponded to human pgp 670 – 1638.

Clone 88/U 750

Nucleobond RNA Maxi kit from Clontech was used according to the manufacturer's instructions to prepare total RNA from Cynomologous monkey liver. Using the SMART Race cDNA Amplification Kit from Clontech, first strand cDNA was prepared from this RNA. The Universal Primer provided with this kit and the gene specific primer, ps088 were used with this template for 40 cycles of Touchdown PCR (94°C for 5 m; followed by 5 cycles of 94°C for 30 s, 72°C for 120 s, 94°C for 30 s; 5 cycles of 94°C for 30 s, 70°C for 45s, 72°C for 120 s; 30 cycles of 94°C for 30 s, 68°C for 45 s, 72°C for 120 s; ending with 72°C for 7 m) using Advantage 2 Taq. A 750 nucleotide fragment was obtained and sequenced with m13F and m13R primers. This represented the 5' end of the cDNA including the start codon.

It showed good homology to the human sequence from 275 – 775 (the start codon for the human sequence being at 433).

Assembly of complete cDNA

- 39 -

Clone AB

Clone 88/U 750 was digested with NaeI / SacI. A 339 nucleotide fragment was isolated on a gel, and purified using the Qiaquick Gel Extraction Kit (Qiagen) according to the manufacturer's instructions. Clone 70/78C was digested with SacI / EcoRI, a 916 nucleotide
5 fragment was isolated on a gel, and purified using the Qiaquick Gel Extraction Kit (Qiagen) according to the manufacturer's instructions. These were ligated together into pUC 19 SmaI / EcoRI / SAP.

Clone CD

10 Clone 72/73 was digested with EcoRI / KpnI. A 1.6 kb fragment was isolated on a gel, and purified using the Qiaquick Gel Extraction Kit (Qiagen) according to the manufacturer's instructions. Clone 79/77 C1500 was digested with KpnI / DraI. A 1.1 kb fragment was isolated on a gel, and purified using the Qiaquick Gel Extraction Kit (Qiagen) according to the manufacturer's instructions. These were ligated into pUC 19 EcoRI / SmaI /
15 SAP.

Complete cDNA

Clone AB was digested with Hinc II / EcoRI. A 1.3 kb fragment was isolated on a gel, and purified using the Qiaquick Gel Extraction Kit (Qiagen) according to the manufacturer's
20 instructions.

Clone CD was digested with EcoRI / EheI. The 5.2 kb fragment was isolated on a gel, and purified using the Qiaquick Gel Extraction Kit (Qiagen) according to the manufacturer's instructions.

These were ligated together, transformed into SCS-1 Competent Cells (Stratagene)
25 according to the manufacturer's instructions. Promising clones were identified by restriction digest. The identity of the final clone was confirmed by sequencing.

The nucleotide sequence of the cynomologous P-glycoprotein is presented as SEQ ID NO:1. The coding sequence consists of nucleotides 100-3940, producing a polypeptide of
30 1280 amino acids (SEQ ID NO:2).

Sequencing of additional clones from libraries of individual cynomologous monkeys indicated the presence of a polymorphism comprising an unexpected 9 base pair insert in the

- 40 -

cDNA that is also present in the genomic sequence from the same individual monkey. The polymorphism resulted in an insertion of three amino acids after amino acid 92. The nucleotide sequence of this allelic variant is presented as SEQ ID NO:3. The coding sequence consists of nucleotides 100-3949, producing a polypeptide of 1283 amino acids (SEQ ID NO:4). The cynomologous PGP cDNA (SEQ ID NO:3) cloned in plasmid pUC19 was deposited with ATCC (deposit number PTA-809).

Example 2: Activity of cynomologous P-glycoprotein

Materials and Methods

Cynomologous monkey PGP cDNA (SEQ ID NO:3) is introduced into a clonal population of LLC-PK1 cells in a vector that confers resistance to hygromycin B. LLC-PK1 cells are obtained from the American Type Culture Collections and are propagated in Medium 199 supplemented to 7% with fetal bovine serum. LLC-PK1 cells are recloned prior to transfection in order to assure homogeneity of the cell population. Briefly, cynomologous monkey PGP cDNA is incorporated into the p222CMV vector. This vector is derived from the p220.2 episomal vector system based on the OriP sequences for Epstein Barr virus and the EBNA-1 gene product (Sugden et al., *Mol. Cell Biol.* 5:410-413, 1985; Yates et al., *Nature (Lond.)* 313: 812-815, 1985). The PGP cDNA is under the control of the cytomegalovirus (CMV) immediate early promoter. The vector confers resistance to hygromycin B. Cells (in 0.4 mL) and DNA (10 to 20 µg) were transfected by electroporation using a BTX Electro cell manipulator model 600 using a 2 mm gap cell, 100V, 2500 µF capacitance and 72 ohm resistance. After electroporation, the cells are plated in multiwell plates (48 well, Corning Costar) at 10% of confluence. One to two days after transfection hygromycin B is introduced at a final concentration of 400 to 600 µg/ml. Cells are refed every 2 to 4 days and are propagated in 400 to 600 µg/ml hygromycin B for 6 to 8 days at which point the bulk of the wild type cells are detached. The hygromycin B is reduced to 100 µg/ml and maintained in this concentration of hygromycin B. After 14 to 18 days the wells are inspected and wells containing single colonies are trypsinized and scaled up to bulk cultures. Expression of PGP is measured by the polarization of vinblastine (0.1 uM) transport in Transwells™.

LLC-PK1 cell based transport studies are conducted in 24 well Transwells™ (Corning Costar, Catalog number 3415). Transwells™ are prepared by the addition of 0.6 mL media to the basolateral space and 0.1 mL media to the apical space. Cells are seeded at 4×10^4 cells

- 41 -

per insert (typically in 0.05 mL to 0.15 mL), refed with fresh media every 2 to 4 days and used for transport studies 4 to 8 days post seeding. Transport assays are conducted in Hank's balanced saline (HBSS) buffered with 10 mM HEPES (pH 7 to 7.2). Cell monolayers are rinsed with HBSS prior to use in transport assays. Transport is measured under sink
5 conditions in both the apical to basolateral (A to B) and basolateral to apical (B to A) directions. At least duplicate monolayers are used per determination. At the desired time points, samples are withdrawn from the receiver chamber (apical or basolateral chambers). Quantitation of the amount of compound transported is by liquid scintillation counting (vinblastine) or HPLC with UV or mass spectrometric detection.

10 Cynomologous PGP cDNA is expressed in insect cells using a baculovirus vector. Membranes are prepared according to the method of (Sarkadi et al., *J. Biol. Chem.* 267: 4854-4858, 1992) and stored at -80°C until use. ATPase assays are conducted in 96 well microtiter plates. The assays are conducted using a modification of the methods of (Sarkadi et al., 1992 and Druekes et al., *Anal. Biochem.* 230: 173-177, 1995).

15 A detailed method for each well of a 96 well plate is contained below:
A 0.06 ml reaction mixture containing 40 µg membranes, 20 µM Verapamil (positive control) or test drug, and 3-5 mM MgATP, in buffer containing 50 mM Tris-MES, 2 mM EGTA, 50 mM KCl, 2 mM dithiothreitol, and 5 mM sodium azide, is incubated at 37°C for 20 min. An identical reaction mixture containing 100 µM sodium orthovanadate is assayed in parallel.
20 Orthovanadate inhibits PGP by trapping MgADP in the nucleotide binding site. Thus, ATPase activity measured in the presence of orthovanadate represents non-PGP ATPase activity and can be subtracted from the activity generated without orthovanadate to yield vanadate-sensitive ATPase activity. The reaction is stopped by the addition of 30 µl of 10 % SDS + Antifoam A. Two additional reaction mixtures (+ and - orthovanadate) but without
25 MgATP, are also prepared and incubated with the others, and then supplemented with SDS and MgATP, to represent time = 0 min of reaction. The incubations are followed with addition of 200 µl of 35 mM ammonium molybdate in 15 mM zinc acetate:10 % ascorbic acid (1:4) and incubated for an additional 20 min at 37°C. The liberation of inorganic phosphate is detected by its absorbance at 800 nm and quantitated by comparing the absorbance to a
30 phosphate standard curve.

Ligand binding assays and assays for measuring inhibition of fluorescent dye uptake are preformed as described by Sharom et al. (*Biochem. Pharmacol.* 58:571-586, 1999).

- 42 -

I. Stable PGP Expression in LLC-PK1 Cells.

Functional expression of cynomologous monkey PGP is measured by the polarization of transport of vinblastine. Control cells typically demonstrate a B to A / A to B ratio of
5 between 1 and 3. PGP transfected cells demonstrate a much higher ratio. The expression of cDNA-derived cynomologous monkey is stable.

II. Activation of ATPase Activity in PGP Membranes.

The stimulation of ATPase assay provides a rapid measure of the concentration
10 dependence of any interaction of a drug with PGP. The liberated inorganic phosphate is measured by a simple spectrophotometric assay performed in a microtiter plate format. The testing of multiple drug concentrations allows estimation of the affinity of the drug for PGP and whether saturation of the response was observed.

III. Drug Transport Across Cell Monolayers.

The ATPase assay does not directly measure drug transport. In order to examine the concordance between activation of ATPase and actual transport, the rates of transport of the drugs are measured in control LLC-PK1 and cynomologous monkey PGP cell monolayers.
For each drug concentration, four measurements are made:

A:	A to B	Control cells
B:	B to A	Control cells
C:	A to B	PGP cells
D:	B to A	PGP cells

The polarization of transport is calculated in control cells (B/A) and PGP cells (D/C).
25 The intrinsic activity (IA) of PGP is calculated as the sum of the amount PGP facilitated B to A transport in PGP cells relative to control cells (D minus B) and the amount that PGP impeded A to B transport in PGP cells relative to control cells (A minus C). The intrinsic clearance of PGP is calculated from a plot of the concentration dependence data by either calculating the slope of the line under non-saturating conditions or from the calculated
30 apparent Km and Vmax values when saturation is observed. Intrinsic clearance is expressed as mL/m²/min.

The ATPase data provides useful concentration response data. For example, the

- 43 -

apparent Km values for some compounds are in good agreement between the ATPase and transport systems. However, other drugs activate ATPase activity but transport by PGP is not detectable. At the least, ATPase assay can identify a concentration range below which the response to transport by PGP was linear with respect to drug concentration. This should allow simplification of the experimental design for measuring the intrinsic clearance of PGP, an important consideration if large numbers of compounds are to be tested.

IV. Bioavailability

Bioavailability studies are performed by performing one or more of the assays described above with two or more different PGP types. The different PGP types can be by different species (e.g., dog and human, cynomologous monkey and human, dog and cynomologous monkey, etc.) or can be different alleles of the same species. The results of these assays are compared to determine or estimate the bioavailability of a drug in individuals of the different species or in individuals that express different PGP alleles. The results of one determination also may be compared to a previously determined value of, e.g., ATPase or transport, as an historical control.

Each of the foregoing patents, patent applications and references is hereby incorporated by reference. While the invention has been described with respect to certain embodiments, it should be appreciated that many modifications and changes may be made by those of ordinary skill in the art without departing from the spirit of the invention. It is intended that such modification, changes and equivalents fall within the scope of the following claims.

What is claimed is:

SEQUENCE LISTING

5	<110> GENTEST CORPORATION	
	<120> P-GLYCOPROTEINS AND USES THEREOF	
10	<130> G0307/7014WO	
	<150> US 60/156,921	
	<151> 1999-09-28	
15	<150> US 60/158,818	
	<151> 1999-10-12	
	<160> 18	
20	<170> FastSEQ for Windows Version 3.0	
	<210> 1	
	<211> 4186	
25	<212> DNA	
	<213> Macaca fascicularis	
	<220>	
	<221> CDS	
30	<222> (100)...(3940)	
	<400> 1	
	ggccgctgtt cgtttccgct aggtctttcc actaaagtcg gagtatcttc ttccaaaatt	60
	tcacgacttg gtggccgttc caaggagcgc gaggtcggg atg gat ctt gaa ggg	114
35		Met Asp Leu Glu Gly
		1 5
	gac cgc aat gga gga gca gag aag aag aac ttt ttt aaa ctg aac aat	162
40	Asp Arg Asn Gly Gly Ala Glu Lys Lys Asn Phe Phe Lys Leu Asn Asn	
		10 15 20
	aaa agt aaa aaa gat aag aag gaa agg aaa cca act gtc agt gta ttt	210
	Lys Ser Lys Lys Asp Lys Lys Glu Arg Lys Pro Thr Val Ser Val Phe	
45		25 30 35
	tca atg ttt cgc tat tca aat tgg ctt gac aag ttg tat atg gtg gtg	258
	Ser Met Phe Arg Tyr Ser Asn Trp Leu Asp Lys Leu Tyr Met Val Val	
		40 45 50
50	gga act ttg gct gcc atc atc cat gga gct gga ctt cct ctc atg atg	306
	Gly Thr Leu Ala Ala Ile Ile His Gly Ala Gly Leu Pro Leu Met Met	
		55 60 65
	ctg gtg ttt gga gac atg acg gat acc ttt gca aat gca gga aat tta	354
55	Leu Val Phe Gly Asp Met Thr Asp Thr Phe Ala Asn Ala Gly Asn Leu	
		70 75 80 85
	gga gat tta gga gct ctg ttg act aat agc agt aat atc act gat aca	402
60	Gly Asp Leu Gly Ala Leu Leu Thr Asn Ser Ser Asn Ile Thr Asp Thr	
		90 95 100

5	gtg	ccc	gtc	atg	aat	ctg	gag	gaa	gat	atg	acc	agg	tat	gcc	tat	tat	450
	Val	Pro	Val	Met	Asn	Leu	Glu	Glu	Asp	Met	Thr	Arg	Tyr	Ala	Tyr	Tyr	
				105					110					115			
10	tac	agt	gga	att	ggg	gct	ggg	gtg	ctg	gtt	gct	gct	tac	att	cag	gtt	498
	Tyr	Ser	Gly	Ile	Gly	Ala	Gly	Val	Leu	Val	Ala	Ala	Tyr	Ile	Gln	Val	
			120					125					130				
15	tca	ttt	tgg	tgc	ctg	gca	gct	gga	aga	caa	ata	cac	aaa	att	aga	aaa	546
	Ser	Phe	Trp	Cys	Leu	Ala	Ala	Gly	Arg	Gln	Ile	His	Lys	Ile	Arg	Lys	
		135					140					145					
20	cag	ttt	ttt	cat	gct	ata	atg	cga	cag	gag	ata	ggc	tgg	ttt	gat	gtg	594
	Gln	Phe	Phe	His	Ala	Ile	Met	Arg	Gln	Glu	Ile	Gly	Trp	Phe	Asp	Val	
	150					155					160					165	
25	cac	gat	gtt	ggg	gag	ctt	aac	acc	cgg	ctt	aca	gat	gat	gtc	tcc	aag	642
	His	Asp	Val	Gly	Glu	Leu	Asn	Thr	Arg	Leu	Thr	Asp	Asp	Val	Ser	Lys	
					170					175					180		
30	att	aat	gaa	gga	att	ggg	gac	aaa	att	gga	atg	ttc	ttt	cag	tca	atg	690
	Ile	Asn	Glu	Gly	Ile	Gly	Asp	Lys	Ile	Gly	Met	Phe	Phe	Gln	Ser	Met	
			185					190						195			
35	gca	aca	ttt	ttc	act	ggg	ttt	ata	gta	gga	ttt	aca	cgt	ggg	tgg	aag	738
	Ala	Thr	Phe	Phe	Thr	Gly	Phe	Ile	Val	Gly	Phe	Thr	Arg	Gly	Trp	Lys	
			200					205					210				
40	cta	acc	ctt	gtg	att	ttg	gcc	atc	agt	cct	gtt	ctt	gga	ctg	tca	gct	786
	Leu	Thr	Leu	Val	Ile	Leu	Ala	Ile	Ser	Pro	Val	Leu	Gly	Leu	Ser	Ala	
		215					220					225					
45	gca	gtc	tgg	gca	aag	ata	ctg	tct	tca	ttt	act	gat	aaa	gaa	ctc	tta	834
	Ala	Val	Trp	Ala	Lys	Ile	Leu	Ser	Ser	Phe	Thr	Asp	Lys	Glu	Leu	Leu	
	230					235					240					245	
50	gct	tat	gca	aaa	gct	gga	gca	gta	gct	gaa	gag	gtc	ttg	gca	gca	att	882
	Ala	Tyr	Ala	Lys	Ala	Gly	Ala	Val	Ala	Glu	Glu	Val	Leu	Ala	Ala	Ile	
				250						255					260		
55	aga	act	gtg	att	gca	ttt	gga	gga	caa	aag	aaa	gaa	ctc	gaa	agg	tac	930
	Arg	Thr	Val	Ile	Ala	Phe	Gly	Gly	Gln	Lys	Lys	Glu	Leu	Glu	Arg	Tyr	
				265					270					275			
60	aac	aaa	aat	tta	gaa	gaa	gct	aaa	aga	att	ggg	ata	aag	aaa	gct	att	978
	Asn	Lys	Asn	Leu	Glu	Glu	Ala	Lys	Arg	Ile	Gly	Ile	Lys	Lys	Ala	Ile	
			280					285					290				
65	aca	gcc	aat	att	tct	ata	ggg	gct	gct	ttc	ctg	ctt	atc	tat	gca	tct	1026
	Thr	Ala	Asn	Ile	Ser	Ile	Gly	Ala	Ala	Phe	Leu	Leu	Ile	Tyr	Ala	Ser	
		295					300					305					
70	tat	gct	ctg	gcc	ttc	tgg	tat	ggg	acc	acc	ttg	gtc	ctc	tca	aag	gaa	1074
	Tyr	Ala	Leu	Ala	Phe	Trp	Tyr	Gly	Thr	Thr	Leu	Val	Leu	Ser	Lys	Glu	
	310					315					320					325	
75	tat	tct	att	gga	caa	gta	ctc	act	gta	ttc	ttt	tct	gta	tta	att	ggg	1122
	Tyr	Ser	Ile	Gly	Gln	Val	Leu	Thr	Val	Phe	Phe	Ser	Val	Leu	Ile	Gly	
					330					335						340	

		gct	ttt	agt	gtt	gga	cag	gca	tct	cca	agc	att	gaa	gca	ttt	gca	aat	1170
		Ala	Phe	Ser	Val	Gly	Gln	Ala	Ser	Pro	Ser	Ile	Glu	Ala	Phe	Ala	Asn	
					345					350						355		
5		gca	aga	gga	gca	gct	ttt	gaa	atc	ttc	aag	ata	att	gat	aat	aag	cca	1218
		Ala	Arg	Gly	Ala	Ala	Phe	Glu	Ile	Phe	Lys	Ile	Ile	Asp	Asn	Lys	Pro	
				360					365					370				
10		agt	att	gac	agc	tat	tcg	aag	agt	ggg	cac	aaa	cca	gat	aat	att	aag	1266
		Ser	Ile	Asp	Ser	Tyr	Ser	Lys	Ser	Gly	His	Lys	Pro	Asp	Asn	Ile	Lys	
			375					380					385					
15		gga	aat	ttg	gaa	ttc	aga	aat	gtt	cac	ttc	agt	tac	cca	tct	cga	aaa	1314
		Gly	Asn	Leu	Glu	Phe	Arg	Asn	Val	His	Phe	Ser	Tyr	Pro	Ser	Arg	Lys	
		390					395					400					405	
20		gaa	gtt	aag	atc	ttg	aag	ggc	ctg	aac	ctg	aag	gtg	cag	agt	ggg	cag	1362
		Glu	Val	Lys	Ile	Leu	Lys	Gly	Leu	Asn	Leu	Lys	Val	Gln	Ser	Gly	Gln	
						410					415					420		
25		acg	gtg	gcc	ctg	gtt	gga	aac	agc	ggc	tgt	ggg	aag	agc	aca	acg	gtc	1410
		Thr	Val	Ala	Leu	Val	Gly	Asn	Ser	Gly	Cys	Gly	Lys	Ser	Thr	Thr	Val	
					425					430					435			
30		cag	ctg	atg	cag	agg	ctt	tat	gac	ccc	aca	gag	ggc	atg	gtc	agt	gtt	1458
		Gln	Leu	Met	Gln	Arg	Leu	Tyr	Asp	Pro	Thr	Glu	Gly	Met	Val	Ser	Val	
				440					445					450				
35		gat	gga	cag	gat	att	agg	acc	ata	aac	gta	agg	ttt	cta	cgg	gaa	atc	1506
		Asp	Gly	Gln	Asp	Ile	Arg	Thr	Ile	Asn	Val	Arg	Phe	Leu	Arg	Glu	Ile	
			455					460					465					
40		atc	ggc	gtg	gtg	agt	cag	gaa	cct	gta	ttg	ttt	gcc	acc	acg	ata	gct	1554
		Ile	Gly	Val	Val	Ser	Gln	Glu	Pro	Val	Leu	Phe	Ala	Thr	Thr	Ile	Ala	
		470					475					480					485	
45		gaa	aac	att	cgc	tat	ggc	cgt	gaa	gat	gtc	acc	atg	gat	gag	att	gag	1602
		Glu	Asn	Ile	Arg	Tyr	Gly	Arg	Glu	Asp	Val	Thr	Met	Asp	Glu	Ile	Glu	
					490						495					500		
50		aaa	gct	gtc	aag	gaa	gcc	aat	gcc	tat	gac	ttt	atc	atg	aaa	ctg	cct	1650
		Lys	Ala	Val	Lys	Glu	Ala	Asn	Ala	Tyr	Asp	Phe	Ile	Met	Lys	Leu	Pro	
					505					510					515			
55		cag	aaa	ttt	gac	acc	ctg	gtt	gga	gag	aga	ggg	gcc	cag	ctg	agt	ggc	1698
		Gln	Lys	Phe	Asp	Thr	Leu	Val	Gly	Glu	Arg	Gly	Ala	Gln	Leu	Ser	Gly	
				520					525					530				
60		ggg	cag	aag	cag	agg	atc	gcc	att	gca	cgt	gcc	ctg	gtt	cgc	aac	ccc	1746
		Gly	Gln	Lys	Gln	Arg	Ile	Ala	Ile	Ala	Arg	Ala	Leu	Val	Arg	Asn	Pro	
			535					540					545					
65		aag	atc	ctc	ctg	ctg	gac	gag	gcc	acg	tca	gcc	ttg	gac	aca	gaa	agt	1794
		Lys	Ile	Leu	Leu	Leu	Asp	Glu	Ala	Thr	Ser	Ala	Leu	Asp	Thr	Glu	Ser	
		550					555					560					565	
70		gaa	gca	gtg	gtt	cag	gtg	gct	ctg	gat	aag	gcc	aga	aaa	ggc	cgg	acc	1842
		Glu	Ala	Val	Val	Gln	Val	Ala	Leu	Asp	Lys	Ala	Arg	Lys	Gly	Arg	Thr	
					570						575					580		

5	acc	att	gtg	ata	gct	cat	cgt	ttg	tct	acg	gtt	cgt	aat	gcc	gac	gtc	1890
	Thr	Ile	Val	Ile	Ala	His	Arg	Leu	Ser	Thr	Val	Arg	Asn	Ala	Asp	Val	
				585					590					595			
10	atc	gct	ggg	ttc	gat	gat	gga	gtc	att	gtg	gag	aaa	gga	aat	cat	gat	1938
	Ile	Ala	Gly	Phe	Asp	Asp	Gly	Val	Ile	Val	Glu	Lys	Gly	Asn	His	Asp	
			600					605					610				
15	gag	ctc	atg	aaa	gag	aaa	ggc	att	tac	ttc	aaa	ctt	gtc	aca	atg	cag	1986
	Glu	Leu	Met	Lys	Glu	Lys	Gly	Ile	Tyr	Phe	Lys	Leu	Val	Thr	Met	Gln	
			615				620					625					
20	aca	gca	gga	aat	gaa	att	gaa	tta	gaa	aat	gca	gct	gat	gaa	tcc	aaa	2034
	Thr	Ala	Gly	Asn	Glu	Ile	Glu	Leu	Glu	Asn	Ala	Ala	Asp	Glu	Ser	Lys	
	630					635					640					645	
25	agt	gaa	att	gat	acc	ttg	gaa	atg	tct	tca	cat	gat	tca	gga	tcc	agt	2082
	Ser	Glu	Ile	Asp	Thr	Leu	Glu	Met	Ser	Ser	His	Asp	Ser	Gly	Ser	Ser	
					650					655					660		
30	cta	ata	aga	aaa	aga	tcc	act	cgt	agg	agt	gtc	cgt	gga	tca	caa	ggc	2130
	Leu	Ile	Arg	Lys	Arg	Ser	Thr	Arg	Arg	Ser	Val	Arg	Gly	Ser	Gln	Gly	
				665					670					675			
35	caa	gac	aga	aag	ctt	agt	acc	aaa	gag	gct	ctg	gat	gaa	agt	ata	cct	2178
	Gln	Asp	Arg	Lys	Leu	Ser	Thr	Lys	Glu	Ala	Leu	Asp	Glu	Ser	Ile	Pro	
			680					685					690				
40	cca	gtt	tcc	ttt	tgg	agg	att	atg	aag	cta	aat	tta	act	gag	tgg	cct	2226
	Pro	Val	Ser	Phe	Trp	Arg	Ile	Met	Lys	Leu	Asn	Leu	Thr	Glu	Trp	Pro	
			695				700					705					
45	tat	ttt	gtt	gtt	ggg	gta	ttt	tgt	gcc	att	ata	aat	gga	ggg	ctg	caa	2274
	Tyr	Phe	Val	Val	Gly	Val	Phe	Cys	Ala	Ile	Ile	Asn	Gly	Gly	Leu	Gln	
	710					715					720					725	
50	cca	gca	ttt	gca	gta	ata	ttt	tca	aag	att	ata	ggg	att	ttt	aca	aga	2322
	Pro	Ala	Phe	Ala	Val	Ile	Phe	Ser	Lys	Ile	Ile	Gly	Ile	Phe	Thr	Arg	
					730						735				740		
55	aat	gat	gat	gcc	gaa	aca	aaa	cga	cag	aat	agt	aac	ttg	ttt	tca	cta	2370
	Asn	Asp	Asp	Ala	Glu	Thr	Lys	Arg	Gln	Asn	Ser	Asn	Leu	Phe	Ser	Leu	
				745					750					755			
60	ttg	ttt	cta	gtc	ctt	gga	att	gtt	tct	ttt	att	aca	ttt	ttc	ctt	cag	2418
	Leu	Phe	Leu	Val	Leu	Gly	Ile	Val	Ser	Phe	Ile	Thr	Phe	Phe	Leu	Gln	
			760					765					770				
65	ggc	ttc	aca	ttt	ggc	aaa	gct	gga	gag	atc	ctc	acc	aag	cgg	ctc	cga	2466
	Gly	Phe	Thr	Phe	Gly	Lys	Ala	Gly	Glu	Ile	Leu	Thr	Lys	Arg	Leu	Arg	
			775				780					785					
70	tac	atg	gtt	ttc	cga	tcc	atg	ctc	aga	cag	gat	gtg	agc	tgg	ttt	gat	2514
	Tyr	Met	Val	Phe	Arg	Ser	Met	Leu	Arg	Gln	Asp	Val	Ser	Trp	Phe	Asp	
	790					795					800					805	
75	gac	cct	aaa	aac	acc	act	gga	gca	ttg	act	acc	agg	ctc	gcc	aat	gat	2562
	Asp	Pro	Lys	Asn	Thr	Thr	Gly	Ala	Leu	Thr	Thr	Arg	Leu	Ala	Asn	Asp	
					810					815					820		

60

gct Ala	gct Ala	caa Gln	gtt Val 825	aaa Lys	ggg Gly	gct Ala	ata Ile	ggt Gly 830	tcc Ser	agg Arg	ctt Leu	gct Ala	ata Ile 835	att Ile	acc Thr	2610
cag Gln	aat Asn	ata Ile 840	gca Ala	aat Asn	ctt Leu	ggg Gly	aca Thr 845	gga Gly	ata Ile	att Ile	ata Ile	tcc Ser 850	tta Leu	atc Ile	tat Tyr	2658
ggt Gly	tgg Trp 855	caa Gln	ctg Leu	aca Thr	ctg Leu	tta Leu 860	ctc Leu	tta Leu	gca Ala	att Ile	gta Val 865	ccc Pro	atc Ile	att Ile	gca Ala	2706
ata Ile 870	gca Ala	gga Gly	gtt Val	gtt Val	gaa Glu 875	atg Met	aaa Lys	atg Met	ttg Leu	tct Ser 880	gga Gly	caa Gln	gca Ala	ctg Leu	aaa Lys 885	2754
gat Asp	aag Lys	aaa Lys	gaa Glu	cta Leu 890	gaa Glu	ggt Gly	gct Ala	ggg Gly	aag Lys 895	atc Ile	gct Ala	act Thr	gaa Glu	gca Ala 900	ata Ile	2802
gaa Glu	aac Asn	ttc Phe	cga Arg 905	act Thr	gtt Val	gtt Val	tct Ser	ttg Leu 910	act Thr	cag Gln	gag Glu	cag Gln	aag Lys 915	ttt Phe	gaa Glu	2850
cat His	atg Met	tat Tyr 920	gat Asp	cag Gln	agt Ser	ttg Leu	cag Gln 925	gta Val	cca Pro	tac Tyr	aga Arg	aac Asn 930	tct Ser	ttg Leu	agg Arg	2898
aaa Lys	gca Ala 935	cac His	atc Ile	ttt Phe	gga Gly	atc Ile 940	acg Thr	ttt Phe	tcc Ser	ttc Phe	acg Thr 945	cag Gln	gca Ala	atg Met	atg Met	2946
tat Tyr 950	ttt Phe	tcc Ser	tat Tyr	gct Ala	gga Gly 955	tgt Cys	ttc Phe	cgg Arg	ttt Phe	gga Gly 960	gcc Ala	tac Tyr	ttg Leu	gtg Val	gca Ala 965	2994
cat His	agt Ser	ctc Leu	atg Met	agc Ser 970	ttt Phe	gag Glu	gat Asp	gtt Val	ctg Leu 975	tta Leu	gta Val	ttt Phe	tca Ser	gct Ala 980	gtt Val	3042
gtc Val	ttt Phe	ggt Gly	gcc Ala 985	atg Met	gcc Ala	gtg Val	ggg Gly	caa Gln 990	gtc Val	agt Ser	tca Ser	ttt Phe	gct Ala 995	cct Pro	gac Asp	3090
tat Tyr	gcc Ala 1000	aaa Lys	gcc Ala	aaa Lys	gta Val	tca Ser	gca Ala 1005	gcc Ala	cac His	atc Ile	atc Ile	atg Met 1010	atc Ile	att Ile	gaa Glu	3138
aaa Lys 1015	acc Thr	cct Pro	ttg Leu	att Ile	gac Asp	agc Ser	tac Tyr	agc Ser	aca Thr	gaa Glu	ggc Gly	cta Leu	aag Lys	ccg Pro	aac Asn	3186
aca Thr 030	ttg Leu	gaa Glu	gga Gly	aat Asn	gtc Val 1035	aca Thr	ttt Phe	aat Asn	gaa Glu	gtt Val 1040	gta Val	ttc Phe	aac Asn	tat Tyr	ccc Pro 1045	3234
acc Thr	cga Arg	ctg Leu	gac Asp	atc Ile 1050	cca Pro	gtg Val	ctt Leu	cag Gln	ggg Gly	ctg Leu	agc Ser	ctg Leu	gaa Glu	gtg Val	aag Lys	3282

	aag ggc cag acg ctg gcc ctg gtg ggc agc agt ggc tgt ggg aag agc	3330
	Lys Gly Gln Thr Leu Ala Leu Val Gly Ser Ser Gly Cys Gly Lys Ser	
	1065 1070 1075	
5	acg gtg gtc cag ctc ctg gag cgg ttc tat gac ccc ttg gcg ggg aaa	3378
	Thr Val Val Gln Leu Leu Glu Arg Phe Tyr Asp Pro Leu Ala Gly Lys	
	1080 1085 1090	
10	gtg ctg ctt gac ggc aaa gaa ata aag caa ctg aat gtt cag tgg ctc	3426
	Val Leu Leu Asp Gly Lys Glu Ile Lys Gln Leu Asn Val Gln Trp Leu	
	1095 1100 1105	
15	cga gca cac ctg ggc atc gtg tcc cag gag ccc atc ctg ttt gac tgc	3474
	Arg Ala His Leu Gly Ile Val Ser Gln Glu Pro Ile Leu Phe Asp Cys	
	1110 1115 1120 1125	
20	agc att agt gag aac att gcc tat gga gac aac agc cgg gtg gtg tca	3522
	Ser Ile Ser Glu Asn Ile Ala Tyr Gly Asp Asn Ser Arg Val Val Ser	
	1130 1135 1140	
25	cag gaa gag atc gtg agg gca gcc aag gag gcc aat ata cac gcc ttc	3570
	Gln Glu Glu Ile Val Arg Ala Ala Lys Glu Ala Asn Ile His Ala Phe	
	1145 1150 1155	
30	atc gag tca ctg cct aat aaa tat agc acc aga gta gga gac aaa gga	3618
	Ile Glu Ser Leu Pro Asn Lys Tyr Ser Thr Arg Val Gly Asp Lys Gly	
	1160 1165 1170	
35	act cag ctc tct ggt ggc cag aaa caa cgc att gcc ata gct cgt gcc	3666
	Thr Gln Leu Ser Gly Gly Gln Lys Gln Arg Ile Ala Ile Ala Arg Ala	
	1175 1180 1185	
40	ctt gtt aga cag cct cat att ttg ctt ttg gat gaa gcc aca tca gct	3714
	Leu Val Arg Gln Pro His Ile Leu Leu Leu Asp Glu Ala Thr Ser Ala	
	1190 1195 1200 1205	
45	ctg gat aca gaa agt gaa aag gtt gtc caa gaa gcc ctg gac aaa gcc	3762
	Leu Asp Thr Glu Ser Glu Lys Val Val Gln Glu Ala Leu Asp Lys Ala	
	1210 1215 1220	
50	aga gaa ggc cgt acc tgc att gtg att gct cac cgc ctg tcc acc atc	3810
	Arg Glu Gly Arg Thr Cys Ile Val Ile Ala His Arg Leu Ser Thr Ile	
	1225 1230 1235	
55	cag aat gca gac tta ata gtg gtg ttt cag aat ggc aga gtc aag gag	3858
	Gln Asn Ala Asp Leu Ile Val Val Phe Gln Asn Gly Arg Val Lys Glu	
	1240 1245 1250	
60	cac ggc aca cat cag cag ctg ctg gca cag aaa ggc atc tat ttt tca	3906
	His Gly Thr His Gln Gln Leu Leu Ala Gln Lys Gly Ile Tyr Phe Ser	
	1255 1260 1265	
65	atg gtc agt gtc cag gct gga gca aag cgc cag t gaactgtgac	3950
	Met Val Ser Val Gln Ala Gly Ala Lys Arg Gln	
	270 1275 1280	
70	tgtatgagat gttaaataatt ttttaataatt tgtgttttaa tatggcattt attcaaagtt	4010
	aaaaagcaag tacttataga attatgaaga gttatctgtt taacatttcc tcaaccaagt	4070
	tcagagtctt cagacactcg taattaaagg aagagagcga gagacatcat caagtggaga	4130

gaaataatgg tttaaattgc attataaatt ttataacaga gttaaagtag attttt

<210> 2

<211> 1280

5

<212> PRT

<213> Macaca fascicularis

<400> 2

10	Met	Asp	Leu	Glu	Gly	Asp	Arg	Asn	Gly	Gly	Ala	Glu	Lys	Lys	Asn	Phe
	1				5				10						15	
	Phe	Lys	Leu	Asn	Asn	Lys	Ser	Lys	Lys	Asp	Lys	Lys	Glu	Arg	Lys	Pro
				20					25					30		
	Thr	Val	Ser	Val	Phe	Ser	Met	Phe	Arg	Tyr	Ser	Asn	Trp	Leu	Asp	Lys
			35					40					45			
15	Leu	Tyr	Met	Val	Val	Gly	Thr	Leu	Ala	Ala	Ile	Ile	His	Gly	Ala	Gly
		50					55					60				
	Leu	Pro	Leu	Met	Met	Leu	Val	Phe	Gly	Asp	Met	Thr	Asp	Thr	Phe	Ala
	65					70				75						80
	Asn	Ala	Gly	Asn	Leu	Gly	Asp	Leu	Gly	Ala	Leu	Leu	Thr	Asn	Ser	Ser
20					85					90					95	
	Asn	Ile	Thr	Asp	Thr	Val	Pro	Val	Met	Asn	Leu	Glu	Glu	Asp	Met	Thr
				100					105					110		
	Arg	Tyr	Ala	Tyr	Tyr	Tyr	Ser	Gly	Ile	Gly	Ala	Gly	Val	Leu	Val	Ala
			115					120					125			
25	Ala	Tyr	Ile	Gln	Val	Ser	Phe	Trp	Cys	Leu	Ala	Ala	Gly	Arg	Gln	Ile
		130					135					140				
	His	Lys	Ile	Arg	Lys	Gln	Phe	Phe	His	Ala	Ile	Met	Arg	Gln	Glu	Ile
	145					150					155					160
	Gly	Trp	Phe	Asp	Val	His	Asp	Val	Gly	Glu	Leu	Asn	Thr	Arg	Leu	Thr
30					165					170					175	
	Asp	Asp	Val	Ser	Lys	Ile	Asn	Glu	Gly	Ile	Gly	Asp	Lys	Ile	Gly	Met
				180					185					190		
	Phe	Phe	Gln	Ser	Met	Ala	Thr	Phe	Phe	Thr	Gly	Phe	Ile	Val	Gly	Phe
			195					200					205			
35	Thr	Arg	Gly	Trp	Lys	Leu	Thr	Leu	Val	Ile	Leu	Ala	Ile	Ser	Pro	Val
		210					215					220				
	Leu	Gly	Leu	Ser	Ala	Ala	Val	Trp	Ala	Lys	Ile	Leu	Ser	Ser	Phe	Thr
	225					230					235					240
	Asp	Lys	Glu	Leu	Leu	Ala	Tyr	Ala	Lys	Ala	Gly	Ala	Val	Ala	Glu	Glu
40					245					250					255	
	Val	Leu	Ala	Ala	Ile	Arg	Thr	Val	Ile	Ala	Phe	Gly	Gly	Gln	Lys	Lys
				260					265					270		
	Glu	Leu	Glu	Arg	Tyr	Asn	Lys	Asn	Leu	Glu	Glu	Ala	Lys	Arg	Ile	Gly
			275					280					285			
45	Ile	Lys	Lys	Ala	Ile	Thr	Ala	Asn	Ile	Ser	Ile	Gly	Ala	Ala	Phe	Leu
		290					295					300				
	Leu	Ile	Tyr	Ala	Ser	Tyr	Ala	Leu	Ala	Phe	Trp	Tyr	Gly	Thr	Thr	Leu
	305					310					315					320
	Val	Leu	Ser	Lys	Glu	Tyr	Ser	Ile	Gly	Gln	Val	Leu	Thr	Val	Phe	Phe
50					325					330					335	
	Ser	Val	Leu	Ile	Gly	Ala	Phe	Ser	Val	Gly	Gln	Ala	Ser	Pro	Ser	Ile
				340					345					350		
	Glu	Ala	Phe	Ala	Asn	Ala	Arg	Gly	Ala	Ala	Phe	Glu	Ile	Phe	Lys	Ile
			355					360					365			
55	Ile	Asp	Asn	Lys	Pro	Ser	Ile	Asp	Ser	Tyr	Ser	Lys	Ser	Gly	His	Lys
		370					375					380				
	Pro	Asp	Asn	Ile	Lys	Gly	Asn	Leu	Glu	Phe	Arg	Asn	Val	His	Phe	Ser
	385					390					395					400
	Tyr	Pro	Ser	Arg	Lys	Glu	Val	Lys	Ile	Leu	Lys	Gly	Leu	Asn	Leu	Lys
60					405					410					415	

8

	Val	Gln	Ser	Gly	Gln	Thr	Val	Ala	Leu	Val	Gly	Asn	Ser	Gly	Cys	Gly
				420					425					430		
	Lys	Ser	Thr	Thr	Val	Gln	Leu	Met	Gln	Arg	Leu	Tyr	Asp	Pro	Thr	Glu
			435					440					445			
5	Gly	Met	Val	Ser	Val	Asp	Gly	Gln	Asp	Ile	Arg	Thr	Ile	Asn	Val	Arg
		450					455					460				
	Phe	Leu	Arg	Glu	Ile	Ile	Gly	Val	Val	Ser	Gln	Glu	Pro	Val	Leu	Phe
	465					470					475					480
10	Ala	Thr	Thr	Ile	Ala	Glu	Asn	Ile	Arg	Tyr	Gly	Arg	Glu	Asp	Val	Thr
					485					490					495	
	Met	Asp	Glu	Ile	Glu	Lys	Ala	Val	Lys	Glu	Ala	Asn	Ala	Tyr	Asp	Phe
				500					505					510		
	Ile	Met	Lys	Leu	Pro	Gln	Lys	Phe	Asp	Thr	Leu	Val	Gly	Glu	Arg	Gly
			515					520					525			
15	Ala	Gln	Leu	Ser	Gly	Gly	Gln	Lys	Gln	Arg	Ile	Ala	Ile	Ala	Arg	Ala
		530					535					540				
	Leu	Val	Arg	Asn	Pro	Lys	Ile	Leu	Leu	Leu	Asp	Glu	Ala	Thr	Ser	Ala
	545					550					555					560
20	Leu	Asp	Thr	Glu	Ser	Glu	Ala	Val	Val	Gln	Val	Ala	Leu	Asp	Lys	Ala
					565					570					575	
	Arg	Lys	Gly	Arg	Thr	Thr	Ile	Val	Ile	Ala	His	Arg	Leu	Ser	Thr	Val
				580					585					590		
	Arg	Asn	Ala	Asp	Val	Ile	Ala	Gly	Phe	Asp	Asp	Gly	Val	Ile	Val	Glu
			595					600					605			
25	Lys	Gly	Asn	His	Asp	Glu	Leu	Met	Lys	Glu	Lys	Gly	Ile	Tyr	Phe	Lys
		610					615					620				
	Leu	Val	Thr	Met	Gln	Thr	Ala	Gly	Asn	Glu	Ile	Glu	Leu	Glu	Asn	Ala
	625					630					635					640
30	Ala	Asp	Glu	Ser	Lys	Ser	Glu	Ile	Asp	Thr	Leu	Glu	Met	Ser	Ser	His
					645					650					655	
	Asp	Ser	Gly	Ser	Ser	Leu	Ile	Arg	Lys	Arg	Ser	Thr	Arg	Arg	Ser	Val
				660					665					670		
	Arg	Gly	Ser	Gln	Gly	Gln	Asp	Arg	Lys	Leu	Ser	Thr	Lys	Glu	Ala	Leu
			675					680					685			
35	Asp	Glu	Ser	Ile	Pro	Pro	Val	Ser	Phe	Trp	Arg	Ile	Met	Lys	Leu	Asn
		690					695					700				
	Leu	Thr	Glu	Trp	Pro	Tyr	Phe	Val	Val	Gly	Val	Phe	Cys	Ala	Ile	Ile
	705					710					715					720
40	Asn	Gly	Gly	Leu	Gln	Pro	Ala	Phe	Ala	Val	Ile	Phe	Ser	Lys	Ile	Ile
					725					730					735	
	Gly	Ile	Phe	Thr	Arg	Asn	Asp	Asp	Ala	Glu	Thr	Lys	Arg	Gln	Asn	Ser
				740					745					750		
	Asn	Leu	Phe	Ser	Leu	Leu	Phe	Leu	Val	Leu	Gly	Ile	Val	Ser	Phe	Ile
			755					760					765			
45	Thr	Phe	Phe	Leu	Gln	Gly	Phe	Thr	Phe	Gly	Lys	Ala	Gly	Glu	Ile	Leu
		770					775					780				
	Thr	Lys	Arg	Leu	Arg	Tyr	Met	Val	Phe	Arg	Ser	Met	Leu	Arg	Gln	Asp
	785					790					795					800
50	Val	Ser	Trp	Phe	Asp	Asp	Pro	Lys	Asn	Thr	Thr	Gly	Ala	Leu	Thr	Thr
					805					810					815	
	Arg	Leu	Ala	Asn	Asp	Ala	Ala	Gln	Val	Lys	Gly	Ala	Ile	Gly	Ser	Arg
				820				825						830		
	Leu	Ala	Ile	Ile	Thr	Gln	Asn	Ile	Ala	Asn	Leu	Gly	Thr	Gly	Ile	Ile
			835					840					845			
55	Ile	Ser	Leu	Ile	Tyr	Gly	Trp	Gln	Leu	Thr	Leu	Leu	Leu	Leu	Ala	Ile
		850					855					860				
	Val	Pro	Ile	Ile	Ala	Ile	Ala	Gly	Val	Val	Glu	Met	Lys	Met	Leu	Ser
	865					870					875					880
60	Gly	Gln	Ala	Leu	Lys	Asp	Lys	Lys	Glu	Leu	Glu	Gly	Ala	Gly	Lys	Ile
					885					890					895	

9

Ala Thr Glu Ala Ile Glu Asn Phe Arg Thr Val Val Ser Leu Thr Gln
 900 905 910
 Glu Gln Lys Phe Glu His Met Tyr Asp Gln Ser Leu Gln Val Pro Tyr
 915 920 925
 5 Arg Asn Ser Leu Arg Lys Ala His Ile Phe Gly Ile Thr Phe Ser Phe
 930 935 940
 Thr Gln Ala Met Met Tyr Phe Ser Tyr Ala Gly Cys Phe Arg Phe Gly
 945 950 955 960
 10 Ala Tyr Leu Val Ala His Ser Leu Met Ser Phe Glu Asp Val Leu Leu
 965 970 975
 Val Phe Ser Ala Val Val Phe Gly Ala Met Ala Val Gly Gln Val Ser
 980 985 990
 Ser Phe Ala Pro Asp Tyr Ala Lys Ala Lys Val Ser Ala Ala His Ile
 995 1000 1005
 15 Ile Met Ile Ile Glu Lys Thr Pro Leu Ile Asp Ser Tyr Ser Thr Glu
 1010 1015 1020
 Gly Leu Lys Pro Asn Thr Leu Glu Gly Asn Val Thr Phe Asn Glu Val
 1025 1030 1035 1040
 20 Val Phe Asn Tyr Pro Thr Arg Leu Asp Ile Pro Val Leu Gln Gly Leu
 1045 1050 1055
 Ser Leu Glu Val Lys Lys Gly Gln Thr Leu Ala Leu Val Gly Ser Ser
 1060 1065 1070
 Gly Cys Gly Lys Ser Thr Val Val Gln Leu Leu Glu Arg Phe Tyr Asp
 1075 1080 1085
 25 Pro Leu Ala Gly Lys Val Leu Leu Asp Gly Lys Glu Ile Lys Gln Leu
 1090 1095 1100
 Asn Val Gln Trp Leu Arg Ala His Leu Gly Ile Val Ser Gln Glu Pro
 1105 1110 1115 1120
 30 Ile Leu Phe Asp Cys Ser Ile Ser Glu Asn Ile Ala Tyr Gly Asp Asn
 1125 1130 1135
 Ser Arg Val Val Ser Gln Glu Glu Ile Val Arg Ala Ala Lys Glu Ala
 1140 1145 1150
 Asn Ile His Ala Phe Ile Glu Ser Leu Pro Asn Lys Tyr Ser Thr Arg
 1155 1160 1165
 35 Val Gly Asp Lys Gly Thr Gln Leu Ser Gly Gly Gln Lys Gln Arg Ile
 1170 1175 1180
 Ala Ile Ala Arg Ala Leu Val Arg Gln Pro His Ile Leu Leu Leu Asp
 1185 1190 1195 1200
 40 Glu Ala Thr Ser Ala Leu Asp Thr Glu Ser Glu Lys Val Val Gln Glu
 1205 1210 1215
 Ala Leu Asp Lys Ala Arg Glu Gly Arg Thr Cys Ile Val Ile Ala His
 1220 1225 1230
 Arg Leu Ser Thr Ile Gln Asn Ala Asp Leu Ile Val Val Phe Gln Asn
 1235 1240 1245
 45 Gly Arg Val Lys Glu His Gly Thr His Gln Gln Leu Leu Ala Gln Lys
 1250 1255 1260
 Gly Ile Tyr Phe Ser Met Val Ser Val Gln Ala Gly Ala Lys Arg Gln
 1265 1270 1275 1280

50 <210> 3
 <211> 4195
 <212> DNA
 <213> Macaca fascicularis

55 <220>
 <221> CDS
 <222> (100)...(3949)

60 <400> 3
 ggccgctggtt cgtttccgct aggtctttcc actaaagtcg gagtatcttc ttccaaaatt

60

10

	tcacgacttg	gtggccgttc	caaggagcgc	gaggtcggg	atg	gat	ctt	gaa	ggg								114
					Met	Asp	Leu	Glu	Gly								
					1				5								
5	gac	cgc	aat	gga	gga	gca	gag	aag	aag	aac	ttt	ttt	aaa	ctg	aac	aat	162
	Asp	Arg	Asn	Gly	Gly	Ala	Glu	Lys	Lys	Asn	Phe	Phe	Lys	Leu	Asn	Asn	
					10					15						20	
10	aaa	agt	aaa	aaa	gat	aag	aag	gaa	agg	aaa	cca	act	gtc	agt	gta	ttt	210
	Lys	Ser	Lys	Lys	Asp	Lys	Lys	Glu	Arg	Lys	Pro	Thr	Val	Ser	Val	Phe	
					25				30							35	
15	tca	atg	ttt	cgc	tat	tca	aat	tgg	ctt	gac	aag	ttg	tat	atg	gtg	gtg	258
	Ser	Met	Phe	Arg	Tyr	Ser	Asn	Trp	Leu	Asp	Lys	Leu	Tyr	Met	Val	Val	
					40				45							50	
20	gga	act	ttg	gct	gcc	atc	atc	cat	gga	gct	gga	ctt	cct	ctc	atg	atg	306
	Gly	Thr	Leu	Ala	Ala	Ile	Ile	His	Gly	Ala	Gly	Leu	Pro	Leu	Met	Met	
					55				60							65	
25	ctg	gtg	ttt	gga	gac	atg	acg	gat	acc	ttt	gca	aat	gca	gga	aat	tta	354
	Leu	Val	Phe	Gly	Asp	Met	Thr	Asp	Thr	Phe	Ala	Asn	Ala	Gly	Asn	Leu	
											80					85	
30	gga	gat	tta	gga	gct	ctg	ttg	ttt	aac	aac	act	aat	agc	agt	aat	atc	402
	Gly	Asp	Leu	Gly	Ala	Leu	Leu	Phe	Asn	Asn	Thr	Asn	Ser	Ser	Asn	Ile	
					90					95						100	
35	act	gat	aca	gtg	ccc	gtc	atg	aat	ctg	gag	gaa	gat	atg	acc	agg	tat	450
	Thr	Asp	Thr	Val	Pro	Val	Met	Asn	Leu	Glu	Glu	Asp	Met	Thr	Arg	Tyr	
					105					110						115	
40	gcc	tat	tat	tac	agt	gga	att	ggt	gct	ggg	gtg	ctg	ggt	gct	gct	tac	498
	Ala	Tyr	Tyr	Tyr	Ser	Gly	Ile	Gly	Ala	Gly	Val	Leu	Val	Ala	Ala	Tyr	
					120				125							130	
45	att	cag	gtt	tca	ttt	tgg	tgc	ctg	gca	gct	gga	aga	caa	ata	cac	aaa	546
	Ile	Gln	Val	Ser	Phe	Trp	Cys	Leu	Ala	Ala	Gly	Arg	Gln	Ile	His	Lys	
					135				140							145	
50	att	aga	aaa	cag	ttt	ttt	cat	gct	ata	atg	cga	cag	gag	ata	ggc	tgg	594
	Ile	Arg	Lys	Gln	Phe	Phe	His	Ala	Ile	Met	Arg	Gln	Glu	Ile	Gly	Trp	
											160					165	
55	ttt	gat	gtg	cac	gat	gtt	ggg	gag	ctt	aac	acc	cgg	ctt	aca	gat	gat	642
	Phe	Asp	Val	His	Asp	Val	Gly	Glu	Leu	Asn	Thr	Arg	Leu	Thr	Asp	Asp	
					170					175						180	
60	gtc	tcc	aag	att	aat	gaa	gga	att	ggt	gac	aaa	att	gga	atg	ttc	ttt	690
	Val	Ser	Lys	Ile	Asn	Glu	Gly	Ile	Gly	Asp	Lys	Ile	Gly	Met	Phe	Phe	
					185					190						195	
55	cag	tca	atg	gca	aca	ttt	ttc	act	ggg	ttt	ata	gta	gga	ttt	aca	cgt	738
	Gln	Ser	Met	Ala	Thr	Phe	Phe	Thr	Gly	Phe	Ile	Val	Gly	Phe	Thr	Arg	
					200				205							210	
60	ggt	tgg	aag	cta	acc	ctt	gtg	att	ttg	gcc	atc	agt	cct	gtt	ctt	gga	786
	Gly	Trp	Lys	Leu	Thr	Leu	Val	Ile	Leu	Ala	Ile	Ser	Pro	Val	Leu	Gly	
					215					220						225	

11

	ctg	tca	gct	gca	gtc	tgg	gca	aag	ata	ctg	tct	tca	ttt	act	gat	aaa	834
	Leu	Ser	Ala	Ala	Val	Trp	Ala	Lys	Ile	Leu	Ser	Ser	Phe	Thr	Asp	Lys	
	230					235					240					245	
5	gaa	ctc	tta	gct	tat	gca	aaa	gct	gga	gca	gta	gct	gaa	gag	gtc	ttg	882
	Glu	Leu	Leu	Ala	Tyr	Ala	Lys	Ala	Gly	Ala	Val	Ala	Glu	Glu	Val	Leu	
					250					255					260		
10	gca	gca	att	aga	act	gtg	att	gca	ttt	gga	gga	caa	aag	aaa	gaa	ctc	930
	Ala	Ala	Ile	Arg	Thr	Val	Ile	Ala	Phe	Gly	Gly	Gln	Lys	Lys	Glu	Leu	
				265					270					275			
15	gaa	agg	tac	aac	aaa	aat	tta	gaa	gaa	gct	aaa	aga	att	ggg	ata	aag	978
	Glu	Arg	Tyr	Asn	Lys	Asn	Leu	Glu	Glu	Ala	Lys	Arg	Ile	Gly	Ile	Lys	
			280					285					290				
20	aaa	gct	att	aca	gcc	aat	att	tct	ata	ggt	gct	gct	ttc	ctg	ctt	atc	1026
	Lys	Ala	Ile	Thr	Ala	Asn	Ile	Ser	Ile	Gly	Ala	Ala	Phe	Leu	Leu	Ile	
		295					300					305					
	tat	gca	tct	tat	gct	ctg	gcc	ttc	tgg	tat	ggg	acc	acc	ttg	gtc	ctc	1074
	Tyr	Ala	Ser	Tyr	Ala	Leu	Ala	Phe	Trp	Tyr	Gly	Thr	Thr	Leu	Val	Leu	
	310					315					320					325	
25	tca	aag	gaa	tat	tct	att	gga	caa	gta	ctc	act	gta	ttc	ttt	tct	gta	1122
	Ser	Lys	Glu	Tyr	Ser	Ile	Gly	Gln	Val	Leu	Thr	Val	Phe	Phe	Ser	Val	
					330					335					340		
30	tta	att	ggg	gct	ttt	agt	gtt	gga	cag	gca	tct	cca	agc	att	gaa	gca	1170
	Leu	Ile	Gly	Ala	Phe	Ser	Val	Gly	Gln	Ala	Ser	Pro	Ser	Ile	Glu	Ala	
				345					350					355			
35	ttt	gca	aat	gca	aga	gga	gca	gct	ttt	gaa	atc	ttc	aag	ata	att	gat	1218
	Phe	Ala	Asn	Ala	Arg	Gly	Ala	Ala	Phe	Glu	Ile	Phe	Lys	Ile	Ile	Asp	
			360					365					370				
40	aat	aag	cca	agt	att	gac	agc	tat	tcg	aag	agt	ggg	cac	aaa	cca	gat	1266
	Asn	Lys	Pro	Ser	Ile	Asp	Ser	Tyr	Ser	Lys	Ser	Gly	His	Lys	Pro	Asp	
		375					380					385					
	aat	att	aag	gga	aat	ttg	gaa	ttc	aga	aat	gtt	cac	ttc	agt	tac	cca	1314
	Asn	Ile	Lys	Gly	Asn	Leu	Glu	Phe	Arg	Asn	Val	His	Phe	Ser	Tyr	Pro	
	390					395					400					405	
45	tct	cga	aaa	gaa	gtt	aag	atc	ttg	aag	ggc	ctg	aac	ctg	aag	gtg	cag	1362
	Ser	Arg	Lys	Glu	Val	Lys	Ile	Leu	Lys	Gly	Leu	Asn	Leu	Lys	Val	Gln	
					410					415					420		
50	agt	ggg	cag	acg	gtg	gcc	ctg	gtt	gga	aac	agc	ggc	tgt	ggg	aag	agc	1410
	Ser	Gly	Gln	Thr	Val	Ala	Leu	Val	Gly	Asn	Ser	Gly	Cys	Gly	Lys	Ser	
				425					430					435			
55	aca	acg	gtc	cag	ctg	atg	cag	agg	ctt	tat	gac	ccc	aca	gag	ggc	atg	1458
	Thr	Thr	Val	Gln	Leu	Met	Gln	Arg	Leu	Tyr	Asp	Pro	Thr	Glu	Gly	Met	
			440					445					450				
60	gtc	agt	gtt	gat	gga	cag	gat	att	agg	acc	ata	aac	gta	agg	ttt	cta	1506
	Val	Ser	Val	Asp	Gly	Gln	Asp	Ile	Arg	Thr	Ile	Asn	Val	Arg	Phe	Leu	
		455					460					465					

12

		cgg	gaa	atc	atc	ggt	gtg	gtg	agt	cag	gaa	cct	gta	ttg	ttt	gcc	acc	1554
		Arg	Glu	Ile	Ile	Gly	Val	Val	Ser	Gln	Glu	Pro	Val	Leu	Phe	Ala	Thr	
		470					475					480					485	
5		acg	ata	gct	gaa	aac	att	cgc	tat	ggt	cgt	gaa	gat	gtc	acc	atg	gat	1602
		Thr	Ile	Ala	Glu	Asn	Ile	Arg	Tyr	Gly	Arg	Glu	Asp	Val	Thr	Met	Asp	
						490					495					500		
10		gag	att	gag	aaa	gct	gtc	aag	gaa	gcc	aat	gcc	tat	gac	ttt	atc	atg	1650
		Glu	Ile	Glu	Lys	Ala	Val	Lys	Glu	Ala	Asn	Ala	Tyr	Asp	Phe	Ile	Met	
					505					510					515			
15		aaa	ctg	cct	cag	aaa	ttt	gac	acc	ctg	ggt	gga	gag	aga	ggg	gcc	cag	1698
		Lys	Leu	Pro	Gln	Lys	Phe	Asp	Thr	Leu	Val	Gly	Glu	Arg	Gly	Ala	Gln	
				520					525					530				
20		ctg	agt	ggt	ggg	cag	aag	cag	agg	atc	gcc	att	gca	cgt	gcc	ctg	ggt	1746
		Leu	Ser	Gly	Gly	Gln	Lys	Gln	Arg	Ile	Ala	Ile	Ala	Arg	Ala	Leu	Val	
			535					540					545					
25		cgc	aac	ccc	aag	atc	ctc	ctg	ctg	gac	gag	gcc	acg	tca	gcc	ttg	gac	1794
		Arg	Asn	Pro	Lys	Ile	Leu	Leu	Leu	Asp	Glu	Ala	Thr	Ser	Ala	Leu	Asp	
		550					555					560					565	
30		aca	gaa	agt	gaa	gca	gtg	ggt	cag	gtg	gct	ctg	gat	aag	gcc	aga	aaa	1842
		Thr	Glu	Ser	Glu	Ala	Val	Val	Gln	Val	Ala	Leu	Asp	Lys	Ala	Arg	Lys	
						570					575					580		
35		ggt	cgg	acc	acc	att	gtg	ata	gct	cat	cgt	ttg	tct	acg	gtt	cgt	aat	1890
		Gly	Arg	Thr	Thr	Ile	Val	Ile	Ala	His	Arg	Leu	Ser	Thr	Val	Arg	Asn	
					585					590					595			
40		gcc	gac	gtc	atc	gct	ggt	ttc	gat	gat	gga	gtc	att	gtg	gag	aaa	gga	1938
		Ala	Asp	Val	Ile	Ala	Gly	Phe	Asp	Asp	Gly	Val	Ile	Val	Glu	Lys	Gly	
				600					605					610				
45		aat	cat	gat	gag	ctc	atg	aaa	gag	aaa	ggc	att	tac	ttc	aaa	ctt	gtc	1986
		Asn	His	Asp	Glu	Leu	Met	Lys	Glu	Lys	Gly	Ile	Tyr	Phe	Lys	Leu	Val	
			615					620					625					
50		aca	atg	cag	aca	gca	gga	aat	gaa	att	gaa	tta	gaa	aat	gca	gct	gat	2034
		Thr	Met	Gln	Thr	Ala	Gly	Asn	Glu	Ile	Glu	Leu	Glu	Asn	Ala	Ala	Asp	
		630					635					640					645	
55		gaa	tcc	aaa	agt	gaa	att	gat	acc	ttg	gaa	atg	tct	tca	cat	gat	tca	2082
		Glu	Ser	Lys	Ser	Glu	Ile	Asp	Thr	Leu	Glu	Met	Ser	Ser	His	Asp	Ser	
						650					655					660		
60		gga	tcc	agt	cta	ata	aga	aaa	aga	tcc	act	cgt	agg	agt	gtc	cgt	gga	2130
		Gly	Ser	Ser	Leu	Ile	Arg	Lys	Arg	Ser	Thr	Arg	Arg	Ser	Val	Arg	Gly	
					665					670					675			
65		tca	caa	ggc	caa	gac	aga	aag	ctt	agt	acc	aaa	gag	gct	ctg	gat	gaa	2178
		Ser	Gln	Gly	Gln	Asp	Arg	Lys	Leu	Ser	Thr	Lys	Glu	Ala	Leu	Asp	Glu	
				680					685					690				
70		agt	ata	cct	cca	gtt	tcc	ttt	tgg	agg	att	atg	aag	cta	aat	tta	act	2226
		Ser	Ile	Pro	Pro	Val	Ser	Phe	Trp	Arg	Ile	Met	Lys	Leu	Asn	Leu	Thr	
			695					700					705					

13

		gag	tgg	cct	tat	ttt	gtt	gtt	ggt	gta	ttt	tgt	gcc	att	ata	aat	gga	2274
		Glu	Trp	Pro	Tyr	Phe	Val	Val	Gly	Val	Phe	Cys	Ala	Ile	Ile	Asn	Gly	
		710					715					720					725	
5		ggt	ctg	caa	cca	gca	ttt	gca	gta	ata	ttt	tca	aag	att	ata	ggg	att	2322
		Gly	Leu	Gln	Pro	Ala	Phe	Ala	Val	Ile	Phe	Ser	Lys	Ile	Ile	Gly	Ile	
						730					735					740		
10		ttt	aca	aga	aat	gat	gat	gcc	gaa	aca	aaa	cga	cag	aat	agt	aac	ttg	2370
		Phe	Thr	Arg	Asn	Asp	Asp	Ala	Glu	Thr	Lys	Arg	Gln	Asn	Ser	Asn	Leu	
					745					750					755			
15		ttt	tca	cta	ttg	ttt	cta	gtc	ctt	gga	att	gtt	tct	ttt	att	aca	ttt	2418
		Phe	Ser	Leu	Leu	Phe	Leu	Val	Leu	Gly	Ile	Val	Ser	Phe	Ile	Thr	Phe	
				760				765						770				
20		ttc	ctt	cag	ggc	ttc	aca	ttt	ggc	aaa	gct	gga	gag	atc	ctc	acc	aag	2466
		Phe	Leu	Gln	Gly	Phe	Thr	Phe	Gly	Lys	Ala	Gly	Glu	Ile	Leu	Thr	Lys	
			775					780					785					
25		cgg	ctc	cga	tac	atg	gtt	ttc	cga	tcc	atg	ctc	aga	cag	gat	gtg	agc	2514
		Arg	Leu	Arg	Tyr	Met	Val	Phe	Arg	Ser	Met	Leu	Arg	Gln	Asp	Val	Ser	
		790					795					800					805	
30		tgg	ttt	gat	gac	cct	aaa	aac	acc	act	gga	gca	ttg	act	acc	agg	ctc	2562
		Trp	Phe	Asp	Asp	Pro	Lys	Asn	Thr	Thr	Gly	Ala	Leu	Thr	Thr	Arg	Leu	
						810					815					820		
35		gcc	aat	gat	gct	gct	caa	gtt	aaa	ggg	gct	ata	ggt	tcc	agg	ctt	gct	2610
		Ala	Asn	Asp	Ala	Ala	Gln	Val	Lys	Gly	Ala	Ile	Gly	Ser	Arg	Leu	Ala	
					825					830					835			
40		ata	att	acc	cag	aat	ata	gca	aat	ctt	ggg	aca	gga	ata	att	ata	tcc	2658
		Ile	Ile	Thr	Gln	Asn	Ile	Ala	Asn	Leu	Gly	Thr	Gly	Ile	Ile	Ile	Ser	
				840					845					850				
45		tta	atc	tat	ggt	tgg	caa	ctg	aca	ctg	tta	ctc	tta	gca	att	gta	ccc	2706
		Leu	Ile	Tyr	Gly	Trp	Gln	Leu	Thr	Leu	Leu	Leu	Leu	Ala	Ile	Val	Pro	
			855					860					865					
50		atc	att	gca	ata	gca	gga	gtt	gtt	gaa	atg	aaa	atg	ttg	tct	gga	caa	2754
		Ile	Ile	Ala	Ile	Ala	Gly	Val	Val	Glu	Met	Lys	Met	Leu	Ser	Gly	Gln	
		870					875					880					885	
55		gca	ctg	aaa	gat	aag	aaa	gaa	cta	gaa	ggt	gct	ggg	aag	atc	gct	act	2802
		Ala	Leu	Lys	Asp	Lys	Lys	Glu	Leu	Glu	Gly	Ala	Gly	Lys	Ile	Ala	Thr	
					890						895					900		
60		gaa	gca	ata	gaa	aac	ttc	cga	act	gtt	gtt	tct	ttg	act	cag	gag	cag	2850
		Glu	Ala	Ile	Glu	Asn	Phe	Arg	Thr	Val	Val	Ser	Leu	Thr	Gln	Glu	Gln	
					905					910					915			
65		aag	ttt	gaa	cat	atg	tat	gat	cag	agt	ttg	cag	gta	cca	tac	aga	aac	2898
		Lys	Phe	Glu	His	Met	Tyr	Asp	Gln	Ser	Leu	Gln	Val	Pro	Tyr	Arg	Asn	
				920					925					930				
70		tct	ttg	agg	aaa	gca	cac	atc	ttt	gga	atc	acg	ttt	tcc	ttc	acg	cag	2946
		Ser	Leu	Arg	Lys	Ala	His	Ile	Phe	Gly	Ile	Thr	Phe	Ser	Phe	Thr	Gln	
			935					940					945					

14

	gca atg atg tat ttt tcc tat gct gga tgt ttc cgg ttt gga gcc tac	2994
	Ala Met Met Tyr Phe Ser Tyr Ala Gly Cys Phe Arg Phe Gly Ala Tyr	
	950 955 960 965	
5	ttg gtg gca cat agt ctc atg agc ttt gag gat gtt ctg tta gta ttt	3042
	Leu Val Ala His Ser Leu Met Ser Phe Glu Asp Val Leu Leu Val Phe	
	970 975 980	
10	tca gct gtt gtc ttt ggt gcc atg gcc gtg ggg caa gtc agt tca ttt	3090
	Ser Ala Val Val Phe Gly Ala Met Ala Val Gly Gln Val Ser Ser Phe	
	985 990 995	
15	gct cct gac tat gcc aaa gcc aaa gta tca gca gcc cac atc atc atg	3138
	Ala Pro Asp Tyr Ala Lys Ala Lys Val Ser Ala Ala His Ile Ile Met	
	1000 1005 1010	
20	atc att gaa aaa acc cct ttg att gac agc tac agc aca gaa ggc cta	3186
	Ile Ile Glu Lys Thr Pro Leu Ile Asp Ser Tyr Ser Thr Glu Gly Leu	
	1015 1020 1025	
	aag ccg aac aca ttg gaa gga aat gtc aca ttt aat gaa gtt gta ttc	3234
	Lys Pro Asn Thr Leu Glu Gly Asn Val Thr Phe Asn Glu Val Val Phe	
	030 1035 1040 1045	
25	aac tat ccc acc cga ctg gac atc cca gtg ctt cag ggg ctg agc ctg	3282
	Asn Tyr Pro Thr Arg Leu Asp Ile Pro Val Leu Gln Gly Leu Ser Leu	
	1050 1055 1060	
30	gaa gtg aag aag ggc cag acg ctg gcc ctg gtg ggc agc agt ggc tgt	3330
	Glu Val Lys Lys Gly Gln Thr Leu Ala Leu Val Gly Ser Ser Gly Cys	
	1065 1070 1075	
35	ggg aag agc acg gtg gtc cag ctc ctg gag cgg ttc tat gac ccc ttg	3378
	Gly Lys Ser Thr Val Val Gln Leu Leu Glu Arg Phe Tyr Asp Pro Leu	
	1080 1085 1090	
40	gcg ggg aaa gtg ctg ctt gac ggc aaa gaa ata aag caa ctg aat gtt	3426
	Ala Gly Lys Val Leu Leu Asp Gly Lys Glu Ile Lys Gln Leu Asn Val	
	1095 1100 1105	
	cag tgg ctc cga gca cac ctg ggc atc gtg tcc cag gag ccc atc ctg	3474
	Gln Trp Leu Arg Ala His Leu Gly Ile Val Ser Gln Glu Pro Ile Leu	
	110 1115 1120 1125	
45	ttt gac tgc agc att agt gag aac att gcc tat gga gac aac agc cgg	3522
	Phe Asp Cys Ser Ile Ser Glu Asn Ile Ala Tyr Gly Asp Asn Ser Arg	
	1130 1135 1140	
50	gtg gtg tca cag gaa gag atc gtg agg gca gcc aag gag gcc aat ata	3570
	Val Val Ser Gln Glu Glu Ile Val Arg Ala Ala Lys Glu Ala Asn Ile	
	1145 1150 1155	
55	cac gcc ttc atc gag tca ctg cct aat aaa tat agc acc aga gta gga	3618
	His Ala Phe Ile Glu Ser Leu Pro Asn Lys Tyr Ser Thr Arg Val Gly	
	1160 1165 1170	
60	gac aaa gga act cag ctc tct ggt ggc cag aaa caa cgc att gcc ata	3666
	Asp Lys Gly Thr Gln Leu Ser Gly Gly Gln Lys Gln Arg Ile Ala Ile	
	1175 1180 1185	

	gct	cgt	gcc	ctt	gtt	aga	cag	cct	cat	att	ttg	ctt	ttg	gat	gaa	gcc	3714
	Ala	Arg	Ala	Leu	Val	Arg	Gln	Pro	His	Ile	Leu	Leu	Leu	Asp	Glu	Ala	
	190					1195					1200				1205		
5	aca	tca	gct	ctg	gat	aca	gaa	agt	gaa	aag	gtt	gtc	caa	gaa	gcc	ctg	3762
	Thr	Ser	Ala	Leu	Asp	Thr	Glu	Ser	Glu	Lys	Val	Val	Gln	Glu	Ala	Leu	
				1210					1215					1220			
10	gac	aaa	gcc	aga	gaa	ggc	cgt	acc	tgc	att	gtg	att	gct	cac	cgc	ctg	3810
	Asp	Lys	Ala	Arg	Glu	Gly	Arg	Thr	Cys	Ile	Val	Ile	Ala	His	Arg	Leu	
			1225				1230						1235				
15	tcc	acc	atc	cag	aat	gca	gac	tta	ata	gtg	gtg	ttt	cag	aat	ggc	aga	3858
	Ser	Thr	Ile	Gln	Asn	Ala	Asp	Leu	Ile	Val	Val	Phe	Gln	Asn	Gly	Arg	
		1240				1245						1250					
20	gtc	aag	gag	cac	ggc	aca	cat	cag	cag	ctg	ctg	gca	cag	aaa	ggc	atc	3906
	Val	Lys	Glu	His	Gly	Thr	His	Gln	Gln	Leu	Leu	Ala	Gln	Lys	Gly	Ile	
	1255					1260						1265					
25	tat	ttt	tca	atg	gtc	agt	gtc	cag	gct	gga	gca	aag	cgc	cag	t		3949
	Tyr	Phe	Ser	Met	Val	Ser	Val	Gln	Ala	Gly	Ala	Lys	Arg	Gln			
	270				1275					1280							
30	gaactgtgac	tgtatgagat	gttaaataatt	ttttaataatt	tgtgttttaaa	tatggcattt											4009
	attcaaagtt	aaaaagcaag	tacttataga	attatgaaga	gttatctgtt	taacattttcc											4069
	tcaaccaagt	tcagagtctt	cagacactcg	taattaaagg	aagagagcga	gagacatcat											4129
	caagtggaga	gaaataatgg	tttaaattgc	attataaatt	ttataacaga	gttaaagtag											4189
	atTTTT																
35	<210>	4															
	<211>	1283															
	<212>	PRT															
	<213>	Macaca fascicularis															
40	<400>	4															
	Met	Asp	Leu	Glu	Gly	Asp	Arg	Asn	Gly	Gly	Ala	Glu	Lys	Lys	Asn	Phe	
	1				5					10					15		
	Phe	Lys	Leu	Asn	Asn	Lys	Ser	Lys	Lys	Asp	Lys	Lys	Glu	Arg	Lys	Pro	
				20					25					30			
	Thr	Val	Ser	Val	Phe	Ser	Met	Phe	Arg	Tyr	Ser	Asn	Trp	Leu	Asp	Lys	
			35				40						45				
	Leu	Tyr	Met	Val	Val	Gly	Thr	Leu	Ala	Ala	Ile	Ile	His	Gly	Ala	Gly	
	50						55					60					
45	Leu	Pro	Leu	Met	Met	Leu	Val	Phe	Gly	Asp	Met	Thr	Asp	Thr	Phe	Ala	
	65					70				75						80	
	Asn	Ala	Gly	Asn	Leu	Gly	Asp	Leu	Gly	Ala	Leu	Leu	Phe	Asn	Asn	Thr	
				85					90								

16

	Ile	Gly	Met	Phe	Phe	Gln	Ser	Met	Ala	Thr	Phe	Phe	Thr	Gly	Phe	Ile
			195					200					205			
	Val	Gly	Phe	Thr	Arg	Gly	Trp	Lys	Leu	Thr	Leu	Val	Ile	Leu	Ala	Ile
		210					215					220				
5	Ser	Pro	Val	Leu	Gly	Leu	Ser	Ala	Ala	Val	Trp	Ala	Lys	Ile	Leu	Ser
	225					230					235					240
	Ser	Phe	Thr	Asp	Lys	Glu	Leu	Leu	Ala	Tyr	Ala	Lys	Ala	Gly	Ala	Val
					245					250					255	
10	Ala	Glu	Glu	Val	Leu	Ala	Ala	Ile	Arg	Thr	Val	Ile	Ala	Phe	Gly	Gly
				260					265					270		
	Gln	Lys	Lys	Glu	Leu	Glu	Arg	Tyr	Asn	Lys	Asn	Leu	Glu	Glu	Ala	Lys
			275					280					285			
	Arg	Ile	Gly	Ile	Lys	Lys	Ala	Ile	Thr	Ala	Asn	Ile	Ser	Ile	Gly	Ala
		290					295					300				
15	Ala	Phe	Leu	Leu	Ile	Tyr	Ala	Ser	Tyr	Ala	Leu	Ala	Phe	Trp	Tyr	Gly
	305					310					315					320
	Thr	Thr	Leu	Val	Leu	Ser	Lys	Glu	Tyr	Ser	Ile	Gly	Gln	Val	Leu	Thr
					325					330					335	
20	Val	Phe	Phe	Ser	Val	Leu	Ile	Gly	Ala	Phe	Ser	Val	Gly	Gln	Ala	Ser
				340					345					350		
	Pro	Ser	Ile	Glu	Ala	Phe	Ala	Asn	Ala	Arg	Gly	Ala	Ala	Phe	Glu	Ile
			355					360					365			
	Phe	Lys	Ile	Ile	Asp	Asn	Lys	Pro	Ser	Ile	Asp	Ser	Tyr	Ser	Lys	Ser
		370					375					380				
25	Gly	His	Lys	Pro	Asp	Asn	Ile	Lys	Gly	Asn	Leu	Glu	Phe	Arg	Asn	Val
	385					390					395					400
	His	Phe	Ser	Tyr	Pro	Ser	Arg	Lys	Glu	Val	Lys	Ile	Leu	Lys	Gly	Leu
				405						410					415	
30	Asn	Leu	Lys	Val	Gln	Ser	Gly	Gln	Thr	Val	Ala	Leu	Val	Gly	Asn	Ser
				420					425					430		
	Gly	Cys	Gly	Lys	Ser	Thr	Thr	Val	Gln	Leu	Met	Gln	Arg	Leu	Tyr	Asp
			435					440					445			
	Pro	Thr	Glu	Gly	Met	Val	Ser	Val	Asp	Gly	Gln	Asp	Ile	Arg	Thr	Ile
		450					455					460				
35	Asn	Val	Arg	Phe	Leu	Arg	Glu	Ile	Ile	Gly	Val	Val	Ser	Gln	Glu	Pro
	465					470					475					480
	Val	Leu	Phe	Ala	Thr	Thr	Ile	Ala	Glu	Asn	Ile	Arg	Tyr	Gly	Arg	Glu
				485					490						495	
40	Asp	Val	Thr	Met	Asp	Glu	Ile	Glu	Lys	Ala	Val	Lys	Glu	Ala	Asn	Ala
				500					505					510		
	Tyr	Asp	Phe	Ile	Met	Lys	Leu	Pro	Gln	Lys	Phe	Asp	Thr	Leu	Val	Gly
			515					520					525			
	Glu	Arg	Gly	Ala	Gln	Leu	Ser	Gly	Gly	Gln	Lys	Gln	Arg	Ile	Ala	Ile
		530					535					540				
45	Ala	Arg	Ala	Leu	Val	Arg	Asn	Pro	Lys	Ile	Leu	Leu	Leu	Asp	Glu	Ala
	545					550					555					560
	Thr	Ser	Ala	Leu	Asp	Thr	Glu	Ser	Glu	Ala	Val	Val	Gln	Val	Ala	Leu
				565						570					575	
50	Asp	Lys	Ala	Arg	Lys	Gly	Arg	Thr	Thr	Ile	Val	Ile	Ala	His	Arg	Leu
				580					585					590		
	Ser	Thr	Val	Arg	Asn	Ala	Asp	Val	Ile	Ala	Gly	Phe	Asp	Asp	Gly	Val
			595				600						605			
	Ile	Val	Glu	Lys	Gly	Asn	His	Asp	Glu	Leu	Met	Lys	Glu	Lys	Gly	Ile
		610				615						620				
55	Tyr	Phe	Lys	Leu	Val	Thr	Met	Gln	Thr	Ala	Gly	Asn	Glu	Ile	Glu	Leu
	625					630					635					640
	Glu	Asn	Ala	Ala	Asp	Glu	Ser	Lys	Ser	Glu	Ile	Asp	Thr	Leu	Glu	Met
				645						650					655	
60	Ser	Ser	His	Asp	Ser	Gly	Ser	Ser	Leu	Ile	Arg	Lys	Arg	Ser	Thr	Arg
				660					665					670		

17

	Arg	Ser	Val	Arg	Gly	Ser	Gln	Gly	Gln	Asp	Arg	Lys	Leu	Ser	Thr	Lys
			675					680					685			
	Glu	Ala	Leu	Asp	Glu	Ser	Ile	Pro	Pro	Val	Ser	Phe	Trp	Arg	Ile	Met
		690					695					700				
5	Lys	Leu	Asn	Leu	Thr	Glu	Trp	Pro	Tyr	Phe	Val	Val	Gly	Val	Phe	Cys
	705					710					715					720
	Ala	Ile	Ile	Asn	Gly	Gly	Leu	Gln	Pro	Ala	Phe	Ala	Val	Ile	Phe	Ser
					725					730					735	
10	Lys	Ile	Ile	Gly	Ile	Phe	Thr	Arg	Asn	Asp	Asp	Ala	Glu	Thr	Lys	Arg
				740					745					750		
	Gln	Asn	Ser	Asn	Leu	Phe	Ser	Leu	Leu	Phe	Leu	Val	Leu	Gly	Ile	Val
			755					760					765			
	Ser	Phe	Ile	Thr	Phe	Phe	Leu	Gln	Gly	Phe	Thr	Phe	Gly	Lys	Ala	Gly
		770					775					780				
15	Glu	Ile	Leu	Thr	Lys	Arg	Leu	Arg	Tyr	Met	Val	Phe	Arg	Ser	Met	Leu
	785					790					795					800
	Arg	Gln	Asp	Val	Ser	Trp	Phe	Asp	Asp	Pro	Lys	Asn	Thr	Thr	Gly	Ala
					805					810					815	
20	Leu	Thr	Thr	Arg	Leu	Ala	Asn	Asp	Ala	Ala	Gln	Val	Lys	Gly	Ala	Ile
				820					825					830		
	Gly	Ser	Arg	Leu	Ala	Ile	Ile	Thr	Gln	Asn	Ile	Ala	Asn	Leu	Gly	Thr
			835					840					845			
	Gly	Ile	Ile	Ile	Ser	Leu	Ile	Tyr	Gly	Trp	Gln	Leu	Thr	Leu	Leu	Leu
		850					855				860					
25	Leu	Ala	Ile	Val	Pro	Ile	Ile	Ala	Ile	Ala	Gly	Val	Val	Glu	Met	Lys
	865					870					875					880
	Met	Leu	Ser	Gly	Gln	Ala	Leu	Lys	Asp	Lys	Lys	Glu	Leu	Glu	Gly	Ala
					885					890					895	
30	Gly	Lys	Ile	Ala	Thr	Glu	Ala	Ile	Glu	Asn	Phe	Arg	Thr	Val	Val	Ser
				900					905					910		
	Leu	Thr	Gln	Glu	Gln	Lys	Phe	Glu	His	Met	Tyr	Asp	Gln	Ser	Leu	Gln
			915					920					925			
	Val	Pro	Tyr	Arg	Asn	Ser	Leu	Arg	Lys	Ala	His	Ile	Phe	Gly	Ile	Thr
		930					935					940				
35	Phe	Ser	Phe	Thr	Gln	Ala	Met	Met	Tyr	Phe	Ser	Tyr	Ala	Gly	Cys	Phe
	945					950					955					960
	Arg	Phe	Gly	Ala	Tyr	Leu	Val	Ala	His	Ser	Leu	Met	Ser	Phe	Glu	Asp
					965					970					975	
40	Val	Leu	Leu	Val	Phe	Ser	Ala	Val	Val	Phe	Gly	Ala	Met	Ala	Val	Gly
				980					985					990		
	Gln	Val	Ser	Ser	Phe	Ala	Pro	Asp	Tyr	Ala	Lys	Ala	Lys	Val	Ser	Ala
			995					1000					1005			
	Ala	His	Ile	Ile	Met	Ile	Ile	Glu	Lys	Thr	Pro	Leu	Ile	Asp	Ser	Tyr
		1010						1015				1020				
45	Ser	Thr	Glu	Gly	Leu	Lys	Pro	Asn	Thr	Leu	Glu	Gly	Asn	Val	Thr	Phe
	1025					1030					1035					1040
	Asn	Glu	Val	Val	Phe	Asn	Tyr	Pro	Thr	Arg	Leu	Asp	Ile	Pro	Val	Leu
					1045					1050					1055	
50	Gln	Gly	Leu	Ser	Leu	Glu	Val	Lys	Lys	Gly	Gln	Thr	Leu	Ala	Leu	Val
			1060						1065					1070		
	Gly	Ser	Ser	Gly	Cys	Gly	Lys	Ser	Thr	Val	Val	Gln	Leu	Leu	Glu	Arg
			1075					1080					1085			
	Phe	Tyr	Asp	Pro	Leu	Ala	Gly	Lys	Val	Leu	Leu	Asp	Gly	Lys	Glu	Ile
		1090					1095					1100				
55	Lys	Gln	Leu	Asn	Val	Gln	Trp	Leu	Arg	Ala	His	Leu	Gly	Ile	Val	Ser
	1105					1110					1115					1120
	Gln	Glu	Pro	Ile	Leu	Phe	Asp	Cys	Ser	Ile	Ser	Glu	Asn	Ile	Ala	Tyr
					1125					1130					1135	
60	Gly	Asp	Asn	Ser	Arg	Val	Val	Ser	Gln	Glu	Glu	Ile	Val	Arg	Ala	Ala
				1140					1145					1150		

18

Lys Glu Ala Asn Ile His Ala Phe Ile Glu Ser Leu Pro Asn Lys Tyr
 1155 1160 1165
 Ser Thr Arg Val Gly Asp Lys Gly Thr Gln Leu Ser Gly Gly Gln Lys
 1170 1175 1180
 5 Gln Arg Ile Ala Ile Ala Arg Ala Leu Val Arg Gln Pro His Ile Leu
 1185 1190 1195 1200
 Leu Leu Asp Glu Ala Thr Ser Ala Leu Asp Thr Glu Ser Glu Lys Val
 1205 1210 1215
 10 Val Gln Glu Ala Leu Asp Lys Ala Arg Glu Gly Arg Thr Cys Ile Val
 1220 1225 1230
 Ile Ala His Arg Leu Ser Thr Ile Gln Asn Ala Asp Leu Ile Val Val
 1235 1240 1245
 Phe Gln Asn Gly Arg Val Lys Glu His Gly Thr His Gln Gln Leu Leu
 1250 1255 1260
 15 Ala Gln Lys Gly Ile Tyr Phe Ser Met Val Ser Val Gln Ala Gly Ala
 1265 1270 1275 1280
 Lys Arg Gln

 20 <210> 5
 <211> 1280
 <212> PRT
 <213> Homo sapiens

 25 <400> 5
 Met Asp Leu Glu Gly Asp Arg Asn Gly Gly Ala Lys Lys Lys Asn Phe
 1 5 10 15
 Phe Lys Leu Asn Asn Lys Ser Glu Lys Asp Lys Lys Glu Lys Lys Pro
 20 25 30
 30 Thr Val Ser Val Phe Ser Met Phe Arg Tyr Ser Asn Trp Leu Asp Lys
 35 40 45
 Leu Tyr Met Val Val Gly Thr Leu Ala Ala Ile Ile His Gly Ala Gly
 50 55 60
 Leu Pro Leu Met Met Leu Val Phe Gly Glu Met Thr Asp Ile Phe Ala
 65 70 75 80
 35 Asn Ala Gly Asn Leu Glu Asp Leu Met Ser Asn Ile Thr Asn Arg Ser
 85 90 95
 Asp Ile Asn Asp Thr Gly Phe Phe Met Asn Leu Glu Glu Asp Met Thr
 100 105 110
 40 Arg Tyr Ala Tyr Tyr Tyr Ser Gly Ile Gly Ala Gly Val Leu Val Ala
 115 120 125
 Ala Tyr Ile Gln Val Ser Phe Trp Cys Leu Ala Ala Gly Arg Gln Ile
 130 135 140
 His Lys Ile Arg Lys Gln Phe Phe His Ala Ile Met Arg Gln Glu Ile
 145 150 155 160
 45 Gly Trp Phe Asp Val His Asp Val Gly Glu Leu Asn Thr Arg Leu Thr
 165 170 175
 Asp Asp Val Ser Lys Ile Asn Glu Val Ile Gly Asp Lys Ile Gly Met
 180 185 190
 50 Phe Phe Gln Ser Met Ala Thr Phe Phe Thr Gly Phe Ile Val Gly Phe
 195 200 205
 Thr Arg Gly Trp Lys Leu Thr Leu Val Ile Leu Ala Ile Ser Pro Val
 210 215 220
 Leu Gly Leu Ser Ala Ala Val Trp Ala Lys Ile Leu Ser Ser Phe Thr
 225 230 235 240
 55 Asp Lys Glu Leu Leu Ala Tyr Ala Lys Ala Gly Ala Val Ala Glu Glu
 245 250 255
 Val Leu Ala Ala Ile Arg Thr Val Ile Ala Phe Gly Gly Gln Lys Lys
 260 265 270
 60 Glu Leu Glu Arg Tyr Asn Lys Asn Leu Glu Glu Ala Lys Arg Ile Gly

19

		275		280		285											
	Ile	Lys	Lys	Ala	Ile	Thr	Ala	Asn	Ile	Ser	Ile	Gly	Ala	Ala	Phe	Leu	
		290					295					300					
5	Leu	Ile	Tyr	Ala	Ser	Tyr	Ala	Leu	Ala	Phe	Trp	Tyr	Gly	Thr	Thr	Leu	
	305					310					315					320	
	Val	Leu	Ser	Gly	Glu	Tyr	Ser	Ile	Gly	Gln	Val	Leu	Thr	Val	Phe	Phe	
					325					330					335		
	Ser	Val	Leu	Ile	Gly	Ala	Phe	Ser	Val	Gly	Gln	Ala	Ser	Pro	Ser	Ile	
				340					345					350			
10	Glu	Ala	Phe	Ala	Asn	Ala	Arg	Gly	Ala	Ala	Tyr	Glu	Ile	Phe	Lys	Ile	
			355					360					365				
	Ile	Asp	Asn	Lys	Pro	Ser	Ile	Asp	Ser	Tyr	Ser	Lys	Ser	Gly	His	Lys	
	370						375					380					
15	Pro	Asp	Asn	Ile	Lys	Gly	Asn	Leu	Glu	Phe	Arg	Asn	Val	His	Phe	Ser	
	385					390					395					400	
	Tyr	Pro	Ser	Arg	Lys	Glu	Val	Lys	Ile	Leu	Lys	Gly	Leu	Asn	Leu	Lys	
				405						410					415		
	Val	Gln	Ser	Gly	Gln	Thr	Val	Ala	Leu	Val	Gly	Asn	Ser	Gly	Cys	Gly	
				420					425					430			
20	Lys	Ser	Thr	Thr	Val	Gln	Leu	Met	Gln	Arg	Leu	Tyr	Asp	Pro	Thr	Glu	
			435					440					445				
	Gly	Met	Val	Ser	Val	Asp	Gly	Gln	Asp	Ile	Arg	Thr	Ile	Asn	Val	Arg	
	450						455					460					
25	Phe	Leu	Arg	Glu	Ile	Ile	Gly	Val	Val	Ser	Gln	Glu	Pro	Val	Leu	Phe	
	465					470					475					480	
	Ala	Thr	Thr	Ile	Ala	Glu	Asn	Ile	Arg	Tyr	Gly	Arg	Glu	Asn	Val	Thr	
				485						490					495		
	Met	Asp	Glu	Ile	Glu	Lys	Ala	Val	Lys	Glu	Ala	Asn	Ala	Tyr	Asp	Phe	
				500					505					510			
30	Ile	Met	Lys	Leu	Pro	His	Lys	Phe	Asp	Thr	Leu	Val	Gly	Glu	Arg	Gly	
			515					520					525				
	Ala	Gln	Leu	Ser	Gly	Gly	Gln	Lys	Gln	Arg	Ile	Ala	Ile	Ala	Arg	Ala	
	530						535					540					
35	Leu	Val	Arg	Asn	Pro	Lys	Ile	Leu	Leu	Leu	Asp	Glu	Ala	Thr	Ser	Ala	
	545					550					555					560	
	Leu	Asp	Thr	Glu	Ser	Glu	Ala	Val	Val	Gln	Val	Ala	Leu	Asp	Lys	Ala	
				565						570					575		
	Arg	Lys	Gly	Arg	Thr	Thr	Ile	Val	Ile	Ala	His	Arg	Leu	Ser	Thr	Val	
				580					585					590			
40	Arg	Asn	Ala	Asp	Val	Ile	Ala	Gly	Phe	Asp	Asp	Gly	Val	Ile	Val	Glu	
			595					600					605				
	Lys	Gly	Asn	His	Asp	Glu	Leu	Met	Lys	Glu	Lys	Gly	Ile	Tyr	Phe	Lys	
	610						615					620					
45	Leu	Val	Thr	Met	Gln	Thr	Ala	Gly	Asn	Glu	Val	Glu	Leu	Glu	Asn	Ala	
	625					630					635					640	
	Ala	Asp	Glu	Ser	Lys	Ser	Glu	Ile	Asp	Ala	Leu	Glu	Met	Ser	Ser	Asn	
				645						650					655		
	Asp	Ser	Arg	Ser	Ser	Leu	Ile	Arg	Lys	Arg	Ser	Thr	Arg	Arg	Ser	Val	
				660					665					670			
50	Arg	Gly	Ser	Gln	Ala	Gln	Asp	Arg	Lys	Leu	Ser	Thr	Lys	Glu	Ala	Leu	
			675					680					685				
	Asp	Glu	Ser	Ile	Pro	Pro	Val	Ser	Phe	Trp	Arg	Ile	Met	Lys	Leu	Asn	
	690						695					700					
55	Leu	Thr	Glu	Trp	Pro	Tyr	Phe	Val	Val	Gly	Val	Phe	Cys	Ala	Ile	Ile	
	705					710					715					720	
	Asn	Gly	Gly	Leu	Gln	Pro	Ala	Phe	Ala	Ile	Ile	Phe	Ser	Lys	Ile	Ile	
				725						730					735		
	Gly	Val	Phe	Thr	Arg	Ile	Asp	Asp	Pro	Glu	Thr	Lys	Arg	Gln	Asn	Ser	
				740					745					750			
60	Asn	Leu	Phe	Ser	Leu	Leu	Phe	Leu	Ala	Leu	Gly	Ile	Ile	Ser	Phe	Ile	

20

		755				760				765						
	Thr	Phe	Phe	Leu	Gln	Gly	Phe	Thr	Phe	Gly	Lys	Ala	Gly	Glu	Ile	Leu
		770					775					780				
5	Thr	Lys	Arg	Leu	Arg	Tyr	Met	Val	Phe	Arg	Ser	Met	Leu	Arg	Gln	Asp
	785					790					795					800
	Val	Ser	Trp	Phe	Asp	Asp	Pro	Lys	Asn	Thr	Thr	Gly	Ala	Leu	Thr	Thr
					805					810					815	
	Arg	Leu	Ala	Asn	Asp	Ala	Ala	Gln	Val	Lys	Gly	Ala	Ile	Gly	Ser	Arg
				820					825					830		
10	Leu	Ala	Val	Ile	Thr	Gln	Asn	Ile	Ala	Asn	Leu	Gly	Thr	Gly	Ile	Ile
		835						840					845			
	Ile	Ser	Phe	Ile	Tyr	Gly	Trp	Gln	Leu	Thr	Leu	Leu	Leu	Leu	Ala	Ile
	850						855					860				
15	Val	Pro	Ile	Ile	Ala	Ile	Ala	Gly	Val	Val	Glu	Met	Lys	Met	Leu	Ser
	865					870					875					880
	Gly	Gln	Ala	Leu	Lys	Asp	Lys	Lys	Glu	Leu	Glu	Gly	Ala	Gly	Lys	Ile
					885					890					895	
	Ala	Thr	Glu	Ala	Ile	Glu	Asn	Phe	Arg	Thr	Val	Val	Ser	Leu	Thr	Gln
				900					905					910		
20	Glu	Gln	Lys	Phe	Glu	His	Met	Tyr	Ala	Gln	Ser	Leu	Gln	Val	Pro	Tyr
		915						920					925			
	Arg	Asn	Ser	Leu	Arg	Lys	Ala	His	Ile	Phe	Gly	Ile	Thr	Phe	Ser	Phe
	930						935					940				
25	Thr	Gln	Ala	Met	Met	Tyr	Phe	Ser	Tyr	Ala	Gly	Cys	Phe	Arg	Phe	Gly
	945					950					955					960
	Ala	Tyr	Leu	Val	Ala	His	Lys	Leu	Met	Ser	Phe	Glu	Asp	Val	Leu	Leu
				965						970					975	
	Val	Phe	Ser	Ala	Val	Val	Phe	Gly	Ala	Met	Ala	Val	Gly	Gln	Val	Ser
			980					985						990		
30	Ser	Phe	Ala	Pro	Asp	Tyr	Ala	Lys	Ala	Lys	Ile	Ser	Ala	Ala	His	Ile
		995						1000					1005			
	Ile	Met	Ile	Ile	Glu	Lys	Thr	Pro	Leu	Ile	Asp	Ser	Tyr	Ser	Thr	Glu
	1010						1015					1020				
35	Gly	Leu	Met	Pro	Asn	Thr	Leu	Glu	Gly	Asn	Val	Thr	Phe	Gly	Glu	Val
	1025					1030					1035					1040
	Val	Phe	Asn	Tyr	Pro	Thr	Arg	Pro	Asp	Ile	Pro	Val	Leu	Gln	Gly	Leu
				1045						1050					1055	
	Ser	Leu	Glu	Val	Lys	Lys	Gly	Gln	Thr	Leu	Ala	Leu	Val	Gly	Ser	Ser
			1060						1065					1070		
40	Gly	Cys	Gly	Lys	Ser	Thr	Val	Val	Gln	Leu	Leu	Glu	Arg	Phe	Tyr	Asp
		1075						1080					1085			
	Pro	Leu	Ala	Gly	Lys	Val	Leu	Leu	Asp	Gly	Lys	Glu	Ile	Lys	Arg	Leu
	1090						1095					1100				
45	Asn	Val	Gln	Trp	Leu	Arg	Ala	His	Leu	Gly	Ile	Val	Ser	Gln	Glu	Pro
	1105					1110					1115					1120
	Ile	Leu	Phe	Asp	Cys	Ser	Ile	Ala	Glu	Asn	Ile	Ala	Tyr	Gly	Asp	Asn
				1125						1130					1135	
	Ser	Arg	Val	Val	Ser	Gln	Glu	Glu	Ile	Val	Arg	Ala	Ala	Lys	Glu	Ala
			1140						1145					1150		
50	Asn	Ile	His	Ala	Phe	Ile	Glu	Ser	Leu	Pro	Asn	Lys	Tyr	Ser	Thr	Lys
		1155						1160					1165			
	Val	Gly	Asp	Lys	Gly	Thr	Gln	Leu	Ser	Gly	Gly	Gln	Lys	Gln	Arg	Ile
	1170						1175					1180				
55	Ala	Ile	Ala	Arg	Ala	Leu	Val	Arg	Gln	Pro	His	Ile	Leu	Leu	Leu	Asp
	1185					1190					1195					1200
	Glu	Ala	Thr	Ser	Ala	Leu	Asp	Thr	Glu	Ser	Glu	Lys	Val	Val	Gln	Glu
				1205						1210					1215	
	Ala	Leu	Asp	Lys	Ala	Arg	Glu	Gly	Arg	Thr	Cys	Ile	Val	Ile	Ala	His
			1220						1225					1230		
60	Arg	Leu	Ser	Thr	Ile	Gln	Asn	Ala	Asp	Leu	Ile	Val	Val	Phe	Gln	Asn

21

1235 1240 1245
 Gly Arg Val Lys Glu His Gly Thr His Gln Gln Leu Leu Ala Gln Lys
 1250 1255 1260
 Gly Ile Tyr Phe Ser Met Val Ser Val Gln Ala Gly Thr Lys Arg Gln
 5 1265 1270 1275 1280

 <210> 6
 <211> 1279
 <212> PRT
 10 <213> Homo sapiens

 <400> 6
 Met Asp Leu Glu Gly Asp Arg Asn Gly Gly Ala Lys Lys Lys Asn Phe
 1 5 10 15
 15 Phe Lys Leu Asn Asn Lys Ser Glu Lys Asp Lys Lys Glu Lys Lys Pro
 20 Thr Val Ser Val Phe Ser Met Phe Arg Tyr Ser Asn Trp Leu Asp Lys
 35 40 45
 20 Leu Tyr Met Val Val Gly Thr Leu Ala Ala Ile Ile His Gly Ala Gly
 50 55 60
 Leu Pro Leu Met Met Leu Val Phe Gly Glu Met Thr Asp Ile Phe Ala
 65 70 75 80
 Asn Ala Gly Asn Leu Glu Asp Leu Met Ser Asn Ile Thr Asn Arg Ser
 85 90 95
 25 Asp Ile Asn Asp Thr Gly Phe Phe Met Asn Leu Glu Glu Asp Met Thr
 100 105 110
 Arg Tyr Ala Tyr Tyr Tyr Ser Gly Ile Gly Ala Gly Val Leu Val Ala
 115 120 125
 30 Ala Tyr Ile Gln Val Ser Phe Trp Cys Leu Ala Ala Gly Arg Gln Ile
 130 135 140
 His Lys Ile Arg Lys Gln Phe Phe His Ala Ile Met Arg Gln Glu Ile
 145 150 155 160
 Gly Trp Phe Asp Val His Asp Val Gly Glu Leu Asn Thr Arg Leu Thr
 165 170 175
 35 Asp Asp Val Ser Lys Ile Asn Glu Gly Ile Gly Asp Lys Ile Gly Met
 180 185 190
 Phe Phe Gln Ser Met Ala Thr Phe Phe Thr Gly Phe Ile Val Gly Phe
 195 200 205
 40 Thr Arg Gly Trp Lys Leu Thr Leu Val Ile Leu Ala Ile Ser Pro Val
 210 215 220
 Leu Gly Leu Ser Ala Ala Val Trp Ala Lys Ile Leu Ser Ser Phe Thr
 225 230 235 240
 Asp Lys Glu Leu Leu Ala Tyr Ala Lys Ala Gly Ala Val Ala Glu Glu
 245 250 255
 45 Val Leu Ala Ala Ile Arg Thr Val Ile Ala Phe Gly Gly Gln Lys Lys
 260 265 270
 Glu Leu Glu Arg Tyr Asn Lys Asn Leu Glu Glu Ala Lys Arg Ile Gly
 275 280 285
 50 Ile Lys Lys Ala Ile Thr Ala Asn Ile Ser Ile Gly Ala Ala Phe Leu
 290 295 300
 Leu Ile Tyr Ala Ser Tyr Ala Leu Ala Phe Trp Tyr Gly Thr Thr Leu
 305 310 315 320
 Val Leu Ser Gly Glu Tyr Ser Ile Gly Gln Val Leu Thr Val Phe Ser
 325 330 335
 55 Val Leu Ile Gly Ala Phe Ser Val Gly Gln Ala Ser Pro Ser Ile Glu
 340 345 350
 Ala Phe Ala Asn Ala Arg Gly Ala Ala Tyr Glu Ile Phe Lys Ile Ile
 355 360 365
 60 Asp Asn Lys Pro Ser Ile Asp Ser Tyr Ser Lys Ser Gly His Lys Pro
 370 375 380

22

	Asp	Asn	Ile	Lys	Gly	Asn	Leu	Glu	Phe	Arg	Asn	Val	His	Phe	Ser	Tyr
	385					390					395					400
	Pro	Ser	Arg	Lys	Glu	Val	Lys	Ile	Leu	Lys	Gly	Leu	Asn	Leu	Lys	Val
					405					410					415	
5	Gln	Ser	Gly	Gln	Thr	Val	Ala	Leu	Val	Gly	Asn	Ser	Gly	Cys	Gly	Lys
				420					425					430		
	Ser	Thr	Thr	Val	Gln	Leu	Met	Gln	Arg	Leu	Tyr	Asp	Pro	Thr	Glu	Gly
			435					440					445			
10	Met	Val	Ser	Val	Asp	Gly	Gln	Asp	Ile	Arg	Thr	Ile	Asn	Val	Arg	Phe
	450						455					460				
	Leu	Arg	Glu	Ile	Ile	Gly	Val	Val	Ser	Gln	Glu	Pro	Val	Leu	Phe	Ala
	465					470					475					480
	Thr	Thr	Ile	Ala	Glu	Asn	Ile	Arg	Tyr	Gly	Arg	Glu	Asn	Val	Thr	Met
					485					490						495
15	Asp	Glu	Ile	Glu	Lys	Ala	Val	Lys	Glu	Ala	Asn	Ala	Tyr	Asp	Phe	Ile
				500					505					510		
	Met	Lys	Leu	Pro	His	Lys	Phe	Asp	Thr	Leu	Val	Gly	Glu	Arg	Gly	Ala
		515						520					525			
20	Gln	Leu	Ser	Gly	Gly	Gln	Lys	Gln	Arg	Ile	Ala	Ile	Ala	Arg	Ala	Leu
	530						535					540				
	Val	Arg	Asn	Pro	Lys	Ile	Leu	Leu	Leu	Asp	Glu	Ala	Thr	Ser	Ala	Leu
	545					550					555					560
	Asp	Thr	Glu	Ser	Glu	Ala	Val	Val	Gln	Val	Ala	Leu	Asp	Lys	Ala	Arg
					565					570						575
25	Lys	Gly	Arg	Thr	Thr	Ile	Val	Ile	Ala	His	Arg	Leu	Ser	Thr	Val	Arg
				580					585					590		
	Asn	Ala	Asp	Val	Ile	Ala	Gly	Phe	Asp	Asp	Gly	Val	Ile	Val	Glu	Lys
			595					600					605			
30	Gly	Asn	His	Asp	Glu	Leu	Met	Lys	Glu	Lys	Gly	Ile	Tyr	Phe	Lys	Leu
	610						615					620				
	Val	Thr	Met	Gln	Thr	Ala	Gly	Asn	Glu	Val	Glu	Leu	Glu	Asn	Ala	Ala
	625					630					635					640
	Asp	Glu	Ser	Lys	Ser	Glu	Ile	Asp	Ala	Leu	Glu	Met	Ser	Ser	Asn	Asp
					645					650					655	
35	Ser	Arg	Ser	Ser	Leu	Ile	Arg	Lys	Arg	Ser	Thr	Arg	Arg	Ser	Val	Arg
				660					665					670		
	Gly	Ser	Gln	Ala	Gln	Asp	Arg	Lys	Leu	Ser	Thr	Lys	Glu	Ala	Leu	Asp
			675					680					685			
40	Glu	Ser	Ile	Pro	Pro	Val	Ser	Phe	Trp	Arg	Ile	Met	Lys	Leu	Asn	Leu
	690						695					700				
	Thr	Glu	Trp	Pro	Tyr	Phe	Val	Val	Gly	Val	Phe	Cys	Ala	Ile	Ile	Asn
	705					710					715					720
	Gly	Gly	Leu	Gln	Pro	Ala	Phe	Ala	Ile	Ile	Phe	Ser	Lys	Ile	Ile	Gly
					725						730					735
45	Val	Phe	Thr	Arg	Ile	Asp	Asp	Pro	Glu	Thr	Lys	Arg	Gln	Asn	Ser	Asn
				740					745					750		
	Leu	Phe	Ser	Leu	Leu	Phe	Leu	Ala	Leu	Gly	Ile	Ile	Ser	Phe	Ile	Thr
			755					760					765			
50	Phe	Phe	Leu	Gln	Gly	Phe	Thr	Phe	Gly	Lys	Ala	Gly	Glu	Ile	Leu	Thr
	770						775					780				
	Lys	Arg	Leu	Arg	Tyr	Met	Val	Phe	Arg	Ser	Met	Leu	Arg	Gln	Asp	Val
	785					790					795					800
	Ser	Trp	Phe	Asp	Asp	Pro	Lys	Asn	Thr	Thr	Gly	Ala	Leu	Thr	Thr	Arg
					805					810					815	
55	Leu	Ala	Asn	Asp	Ala	Ala	Gln	Val	Lys	Gly	Ala	Ile	Gly	Ser	Arg	Leu
				820					825					830		
	Ala	Val	Ile	Thr	Gln	Asn	Ile	Ala	Asn	Leu	Gly	Thr	Gly	Ile	Ile	Ile
			835					840					845			
60	Ser	Phe	Ile	Tyr	Gly	Trp	Gln	Leu	Thr	Leu	Leu	Leu	Leu	Ala	Ile	Val
	850						855						860			

5	Pro 865	Ile	Ile	Ala	Ile	Ala	Gly	Val	Val	Glu	Met	Lys	Met	Leu	Ser	Gly
	Gln	Ala	Leu	Lys	Asp	Lys	Lys	Glu	Leu	Glu	Gly	Ala	Gly	Lys	Ile	Ala
					885					890					895	
	Thr	Glu	Ala	Ile	Glu	Asn	Phe	Arg	Thr	Val	Val	Ser	Leu	Thr	Gln	Glu
10				900					905					910		
	Gln	Lys	Phe	Glu	His	Met	Tyr	Ala	Gln	Ser	Leu	Gln	Val	Pro	Tyr	Arg
			915					920					925			
	Asn	Ser	Leu	Arg	Lys	Ala	His	Ile	Phe	Gly	Ile	Thr	Phe	Ser	Phe	Thr
15							935					940				
	Gln	Ala	Met	Met	Tyr	Phe	Ser	Tyr	Ala	Gly	Cys	Phe	Arg	Phe	Gly	Ala
						950					955					960
	Tyr	Leu	Val	Ala	His	Lys	Leu	Met	Ser	Phe	Glu	Asp	Val	Leu	Leu	Val
20					965					970					975	
	Phe	Ser	Ala	Val	Val	Phe	Gly	Ala	Met	Ala	Val	Gly	Gln	Val	Ser	Ser
				980					985					990		
	Phe	Ala	Pro	Asp	Tyr	Ala	Lys	Ala	Lys	Ile	Ser	Ala	Ala	His	Ile	Ile
25				995				1000					1005			
	Met	Ile	Ile	Glu	Lys	Thr	Pro	Leu	Ile	Asp	Ser	Tyr	Ser	Thr	Glu	Gly
		1010					1015					1020				
	Leu	Met	Pro	Asn	Thr	Leu	Glu	Gly	Asn	Val	Thr	Phe	Gly	Glu	Val	Val
30						1030					1035					1040
	Phe	Asn	Tyr	Pro	Thr	Arg	Pro	Asp	Ile	Pro	Val	Leu	Gln	Gly	Leu	Ser
					1045					1050					1055	
	Leu	Glu	Val	Lys	Lys	Gly	Gln	Thr	Leu	Ala	Leu	Val	Gly	Ser	Ser	Gly
35				1060					1065					1070		
	Cys	Gly	Lys	Ser	Thr	Val	Val	Gln	Leu	Leu	Glu	Arg	Phe	Tyr	Asp	Pro
			1075					1080					1085			
	Leu	Ala	Gly	Lys	Val	Leu	Leu	Asp	Gly	Lys	Glu	Ile	Lys	Arg	Leu	Asn
40				1090				1095					1100			
	Val	Gln	Trp	Leu	Arg	Ala	His	Leu	Gly	Ile	Val	Ser	Gln	Glu	Pro	Ile
						1110					1115					1120
	Leu	Phe	Asp	Cys	Ser	Ile	Ala	Glu	Asn	Ile	Ala	Tyr	Gly	Asp	Asn	Ser
45				1125						1130					1135	
	Arg	Val	Val	Ser	Gln	Glu	Glu	Ile	Val	Arg	Ala	Ala	Lys	Glu	Ala	Asn
				1140					1145					1150		
	Ile	His	Ala	Phe	Ile	Glu	Ser	Leu	Pro	Asn	Lys	Tyr	Ser	Thr	Lys	Val
50				1155				1160					1165			
	Gly	Asp	Lys	Gly	Thr	Gln	Leu	Ser	Gly	Gly	Gln	Lys	Gln	Arg	Ile	Ala
						1175					1180					
	Ile	Ala	Arg	Ala	Leu	Val	Arg	Gln	Pro	His	Ile	Leu	Leu	Leu	Asp	Glu
55					1190						1195					1200
	Ala	Thr	Ser	Ala	Leu	Asp	Thr	Glu	Ser	Glu	Lys	Val	Val	Gln	Glu	Ala
					1205					1210						

24

	1			5				10			15					
	Lys	Met	Gly	Lys	Lys	Ser	Lys	Lys	Glu	Lys	Lys	Glu	Lys	Lys	Pro	Thr
				20					25					30		
5	Val	Ser	Thr	Phe	Ala	Met	Phe	Arg	Tyr	Ser	Asn	Trp	Leu	Asp	Arg	Leu
			35					40					45			
	Tyr	Met	Leu	Val	Gly	Thr	Met	Ala	Ala	Ile	Ile	His	Gly	Ala	Ala	Leu
		50					55					60				
	Pro	Leu	Met	Met	Leu	Val	Phe	Gly	Asn	Met	Thr	Asp	Ser	Phe	Ala	Asn
	65					70					75					80
10	Ala	Gly	Ile	Ser	Arg	Asn	Lys	Thr	Phe	Pro	Val	Ile	Ile	Asn	Glu	Ser
				85					90						95	
	Ile	Thr	Asn	Asn	Thr	Gln	His	Phe	Ile	Asn	His	Leu	Glu	Glu	Glu	Met
				100					105					110		
	Thr	Thr	Tyr	Ala	Tyr	Tyr	Tyr	Ser	Gly	Ile	Gly	Ala	Gly	Val	Leu	Val
15			115					120					125			
	Ala	Ala	Tyr	Ile	Gln	Val	Ser	Phe	Trp	Cys	Leu	Ala	Ala	Gly	Arg	Gln
		130					135					140				
	Ile	Leu	Lys	Ile	Arg	Lys	Gln	Phe	Phe	His	Ala	Ile	Met	Arg	Gln	Glu
	145					150					155					160
20	Ile	Gly	Trp	Phe	Asp	Val	His	Asp	Val	Gly	Glu	Leu	Asn	Thr	Arg	Leu
				165						170					175	
	Thr	Asp	Asp	Val	Ser	Lys	Ile	Asn	Glu	Gly	Ile	Gly	Asp	Lys	Val	Gly
				180					185					190		
	Met	Phe	Phe	Gln	Ser	Ile	Ala	Thr	Phe	Phe	Thr	Gly	Phe	Ile	Val	Gly
25			195					200					205			
	Phe	Thr	Pro	Gly	Trp	Lys	Leu	Thr	Leu	Val	Ile	Leu	Ala	Ile	Ser	Pro
		210					215					220				
	Val	Leu	Gly	Leu	Ser	Ala	Ala	Ile	Trp	Ala	Lys	Ile	Leu	Ser	Ser	Phe
	225					230					235					240
30	Thr	Asp	Lys	Glu	Leu	Leu	Ala	Tyr	Ala	Lys	Ala	Gly	Ala	Val	Ala	Glu
				245						250					255	
	Glu	Val	Leu	Ala	Ala	Ile	Arg	Thr	Val	Ile	Ala	Phe	Gly	Gly	Gln	Lys
				260					265					270		
	Lys	Glu	Leu	Glu	Arg	Tyr	Asn	Lys	Asn	Leu	Glu	Glu	Ala	Lys	Arg	Ile
35			275					280					285			
	Gly	Ile	Lys	Lys	Ala	Ile	Thr	Ala	Asn	Ile	Ser	Ile	Gly	Ala	Ala	Phe
		290					295						300			
	Leu	Leu	Ile	Tyr	Ala	Ser	Tyr	Ala	Leu	Ala	Phe	Trp	Tyr	Gly	Thr	Ser
	305					310					315					320
40	Leu	Val	Leu	Ser	Ser	Glu	Tyr	Thr	Ile	Gly	Gln	Val	Leu	Thr	Val	Phe
				325						330					335	
	Phe	Ser	Val	Leu	Ile	Gly	Ala	Phe	Ser	Ile	Gly	Gln	Ala	Ser	Pro	Ser
				340					345					350		
	Ile	Glu	Ala	Phe	Ala	Asn	Ala	Arg	Gly	Ala	Ala	Tyr	Glu	Ile	Phe	Lys
45			355					360					365			
	Ile	Ile	Asp	Asn	Lys	Pro	Ser	Ile	Asp	Ser	Tyr	Ser	Lys	Ser	Gly	His
		370					375					380				
	Lys	Pro	Asp	Asn	Ile	Lys	Gly	Asn	Leu	Glu	Phe	Lys	Asn	Val	His	Phe
	385					390					395					400
50	Ser	Tyr	Pro	Ser	Arg	Lys	Glu	Val	Lys	Ile	Leu	Lys	Gly	Leu	Asn	Leu
				405						410					415	
	Lys	Val	Gln	Ser	Gly	Gln	Thr	Val	Ala	Leu	Val	Gly	Asn	Ser	Gly	Cys
				420					425					430		
	Gly	Lys	Ser	Thr	Thr	Val	Gln	Leu	Met	Gln	Arg	Leu	Tyr	Asp	Pro	Thr
55			435					440					445			
	Asp	Gly	Met	Val	Cys	Ile	Asp	Gly	Gln	Asp	Ile	Arg	Thr	Ile	Asn	Val
		450					455					460				
	Arg	His	Leu	Arg	Glu	Ile	Thr	Gly	Val	Val	Ser	Gln	Glu	Pro	Val	Leu
	465					470					475					480
60	Phe	Ala	Thr	Thr	Ile	Ala	Glu	Asn	Ile	Arg	Tyr	Gly	Arg	Glu	Asn	Val

25

				485				490					495			
	Thr	Met	Asp	Glu	Ile	Glu	Lys	Ala	Val	Lys	Glu	Ala	Asn	Ala	Tyr	Asp
				500						505				510		
5	Phe	Ile	Met	Lys	Leu	Pro	Asn	Lys	Phe	Asp	Thr	Leu	Val	Gly	Glu	Arg
			515					520					525			
	Gly	Ala	Arg	Leu	Ser	Gly	Gly	Gln	Lys	Gln	Arg	Ile	Ala	Ile	Ala	Arg
		530					535					540				
	Ala	Leu	Val	Arg	Asn	Pro	Lys	Ile	Leu	Leu	Leu	Asp	Glu	Ala	Thr	Ser
	545					550					555					560
10	Ala	Leu	Asp	Thr	Glu	Ser	Glu	Ala	Val	Val	Gln	Val	Ala	Leu	Asp	Lys
				565						570					575	
	Ala	Arg	Lys	Gly	Arg	Thr	Thr	Ile	Val	Ile	Ala	His	Arg	Leu	Ser	Thr
				580					585					590		
15	Val	Arg	Asn	Ala	Asp	Val	Ile	Ala	Gly	Phe	Asp	Asp	Gly	Val	Ile	Val
			595					600					605			
	Glu	Lys	Gly	Asn	His	Asp	Glu	Leu	Met	Lys	Glu	Lys	Gly	Ile	Tyr	Phe
		610					615					620				
	Lys	Leu	Val	Thr	Met	Gln	Thr	Arg	Gly	Asn	Glu	Ile	Glu	Leu	Glu	Asn
	625					630					635					640
20	Ala	Thr	Gly	Glu	Ser	Lys	Ser	Glu	Ser	Asp	Ala	Leu	Glu	Met	Ser	Pro
				645						650					655	
	Lys	Asp	Ser	Gly	Ser	Ser	Leu	Ile	Lys	Arg	Arg	Ser	Thr	Arg	Arg	Ser
				660					665					670		
25	Ile	His	Ala	Pro	Gln	Gly	Gln	Asp	Arg	Lys	Leu	Gly	Thr	Lys	Glu	Asp
			675					680					685			
	Leu	Asn	Glu	Asn	Val	Pro	Ser	Val	Ser	Phe	Trp	Arg	Ile	Leu	Lys	Leu
		690					695					700				
	Asn	Ser	Thr	Glu	Trp	Pro	Tyr	Phe	Val	Val	Gly	Ile	Phe	Cys	Ala	Ile
	705					710					715					720
30	Ile	Asn	Gly	Gly	Leu	Gln	Pro	Ala	Phe	Ser	Ile	Ile	Phe	Ser	Arg	Ile
				725						730					735	
	Ile	Gly	Ile	Phe	Thr	Arg	Asp	Glu	Asp	Pro	Glu	Thr	Lys	Arg	Gln	Asn
				740					745					750		
35	Ser	Asn	Met	Phe	Ser	Val	Leu	Phe	Leu	Val	Leu	Gly	Ile	Ile	Ser	Phe
			755					760					765			
	Ile	Thr	Phe	Phe	Leu	Gln	Gly	Phe	Thr	Phe	Gly	Lys	Ala	Gly	Glu	Ile
		770					775					780				
	Leu	Thr	Lys	Arg	Leu	Arg	Tyr	Met	Val	Phe	Arg	Ser	Met	Leu	Arg	Gln
						790					795					800
40	Asp	Val	Ser	Trp	Phe	Asp	Asp	Pro	Lys	Asn	Thr	Thr	Gly	Ala	Leu	Thr
				805						810					815	
	Thr	Arg	Leu	Ala	Asn	Asp	Ala	Ala	Gln	Val	Lys	Gly	Ala	Ile	Gly	Ser
				820					825					830		
45	Arg	Leu	Ala	Val	Ile	Thr	Gln	Asn	Ile	Ala	Asn	Leu	Gly	Thr	Gly	Ile
			835					840					845			
	Ile	Ile	Ser	Leu	Ile	Tyr	Gly	Trp	Gln	Leu	Thr	Leu	Leu	Leu	Leu	Ala
		850					855					860				
	Ile	Val	Pro	Ile	Ile	Ala	Ile	Ala	Gly	Val	Val	Glu	Met	Lys	Met	Leu
						870					875					880
50	Ser	Gly	Gln	Ala	Leu	Lys	Asp	Lys	Lys	Glu	Leu	Glu	Gly	Ala	Gly	Lys
				885						890					895	
	Ile	Ala	Thr	Glu	Ala	Ile	Glu	Asn	Phe	Arg	Thr	Val	Val	Ser	Leu	Thr
				900					905					910		
55	Arg	Glu	Gln	Lys	Phe	Glu	Tyr	Met	Tyr	Ala	Gln	Ser	Leu	Gln	Val	Pro
			915					920					925			
	Tyr	Arg	Asn	Ser	Leu	Arg	Lys	Ala	His	Ile	Phe	Gly	Val	Ser	Phe	Ser
		930					935					940				
	Ile	Thr	Gln	Ala	Met	Met	Tyr	Phe	Ser	Tyr	Ala	Gly	Cys	Phe	Arg	Phe
						950					955					960
60	Gly	Ala	Tyr	Leu	Val	Ala	Asn	Glu	Phe	Met	Asn	Phe	Gln	Asp	Val	Leu

26

				965					970					975		
	Leu	Val	Phe	Ser	Ala	Ile	Val	Phe	Gly	Ala	Met	Ala	Val	Gly	Gln	Val
				980					985					990		
5	Ser	Ser	Phe	Ala	Pro	Asp	Tyr	Ala	Lys	Ala	Lys	Val	Ser	Ala	Ala	His
			995					1000					1005			
	Val	Ile	Met	Ile	Ile	Glu	Lys	Ser	Pro	Leu	Ile	Asp	Ser	Tyr	Ser	Pro
		1010					1015					1020				
	His	Gly	Leu	Lys	Pro	Asn	Thr	Leu	Glu	Gly	Asn	Val	Thr	Phe	Asn	Glu
	1025					1030					1035					1040
10	Val	Val	Phe	Asn	Tyr	Pro	Thr	Arg	Pro	Asp	Ile	Pro	Val	Leu	Gln	Gly
				1045						1050					1055	
	Leu	Ser	Leu	Glu	Val	Lys	Lys	Gly	Gln	Thr	Leu	Ala	Leu	Val	Gly	Ser
				1060					1065					1070		
	Ser	Gly	Cys	Gly	Lys	Ser	Thr	Val	Val	Gln	Leu	Leu	Glu	Arg	Phe	Tyr
15			1075					1080					1085			
	Asp	Pro	Leu	Ala	Gly	Ser	Val	Leu	Ile	Asp	Gly	Lys	Glu	Ile	Lys	His
		1090					1095					1100				
	Leu	Asn	Val	Gln	Trp	Leu	Arg	Ala	His	Leu	Gly	Ile	Val	Ser	Gln	Glu
	1105					1110					1115					1120
20	Pro	Ile	Leu	Phe	Asp	Cys	Ser	Ile	Ala	Glu	Asn	Ile	Ala	Tyr	Gly	Asp
				1125						1130					1135	
	Asn	Ser	Arg	Val	Val	Ser	His	Glu	Glu	Ile	Met	Gln	Ala	Ala	Lys	Glu
				1140					1145					1150		
	Ala	Asn	Ile	His	His	Phe	Ile	Glu	Thr	Leu	Pro	Glu	Lys	Tyr	Asn	Thr
25			1155					1160					1165			
	Arg	Val	Gly	Asp	Lys	Gly	Thr	Gln	Leu	Ser	Gly	Gly	Gln	Lys	Gln	Arg
		1170					1175					1180				
	Ile	Ala	Ile	Ala	Arg	Ala	Leu	Val	Arg	Gln	Pro	His	Ile	Leu	Leu	Leu
	1185					1190					1195					1200
30	Asp	Glu	Ala	Thr	Ser	Ala	Leu	Asp	Thr	Glu	Ser	Glu	Lys	Val	Val	Gln
				1205						1210					1215	
	Glu	Ala	Leu	Asp	Lys	Ala	Arg	Glu	Gly	Arg	Thr	Cys	Ile	Val	Ile	Ala
				1220					1225					1230		
	His	Arg	Leu	Ser	Thr	Ile	Gln	Asn	Ala	Asp	Leu	Ile	Val	Val	Phe	Gln
35			1235					1240					1245			
	Asn	Gly	Lys	Val	Lys	Glu	His	Gly	Thr	His	Gln	Gln	Leu	Leu	Ala	Gln
		1250					1255					1260				
	Lys	Gly	Ile	Tyr	Phe	Ser	Met	Ile	Ser	Val	Gln	Ala	Gly	Ala	Lys	Arg
40						1270					1275					1280
			<210>	8												
			<211>	368												
			<212>	PRT												

27

	Arg	Ser	Ile	His	Ala	Pro	Gln	Gly	Gln	Asp	Arg	Lys	Leu	Gly	Thr	Lys
			115					120					125			
	Glu	Asp	Leu	Asn	Glu	Asn	Val	Pro	Pro	Val	Ser	Phe	Trp	Arg	Ile	Leu
		130					135					140				
5	Lys	Leu	Asn	Ser	Thr	Glu	Trp	Pro	Tyr	Phe	Val	Val	Gly	Ile	Phe	Cys
	145					150				155						160
	Ala	Ile	Ile	Asn	Gly	Gly	Leu	Gln	Pro	Ala	Phe	Ser	Ile	Ile	Phe	Ser
				165						170					175	
10	Arg	Ile	Ile	Gly	Ile	Phe	Thr	Arg	Asp	Glu	Asp	Pro	Glu	Thr	Lys	Arg
				180					185				190			
	Gln	Ile	Ser	Asn	Met	Phe	Ser	Val	Leu	Phe	Leu	Val	Leu	Gly	Ile	Ile
			195					200					205			
	Ser	Phe	Ile	Thr	Phe	Phe	Leu	Gln	Gly	Phe	Thr	Phe	Gly	Lys	Ala	Gly
		210					215					220				
15	Glu	Ile	Leu	Thr	Lys	Arg	Leu	Arg	Tyr	Met	Val	Phe	Arg	Ser	Met	Leu
	225					230					235					240
	Arg	Gln	Asp	Val	Ser	Trp	Phe	Asp	Asp	Leu	Lys	Asn	Thr	Thr	Gly	Ala
				245						250					255	
20	Leu	Thr	Thr	Arg	Leu	Ala	Asn	Asp	Ala	Ala	Gln	Val	Lys	Gly	Ala	Ile
				260					265					270		
	Gly	Ser	Arg	Leu	Ala	Val	Ile	Thr	Gln	Asn	Ile	Ala	Asn	Leu	Gly	Thr
			275					280					285			
	Gly	Ile	Ile	Ile	Ser	Leu	Ile	Tyr	Gly	Trp	Gln	Leu	Thr	Leu	Leu	Leu
		290					295					300				
25	Leu	Ala	Ile	Val	Pro	Ile	Ile	Ala	Ile	Ala	Gly	Val	Val	Glu	Met	Lys
	305					310					315					320
	Met	Leu	Ser	Gly	Gln	Ala	Leu	Lys	Asp	Lys	Lys	Glu	Leu	Glu	Gly	Ala
				325					330						335	
30	Gly	Lys	Ile	Ala	Thr	Glu	Ala	Ile	Glu	Asn	Phe	Arg	Thr	Val	Val	Ser
				340				345					350			
	Leu	Thr	Gln	Glu	Gln	Lys	Phe	Glu	His	Met	Tyr	Ala	Gln	Ser	Leu	Gln
			355					360					365			

35

<210> 9
 <211> 27
 <212> DNA
 <213> Homo sapiens

40

<400> 9
 ctggacttcc tctcatgatg ctggtgt

27

45

<210> 10
 <211> 28
 <212> DNA
 <213> Homo sapiens

50

<400> 10
 gacagctatt cgaagagtgg gcacaaac

28

55

<210> 11
 <211> 24
 <212> DNA
 <213> Homo sapiens

60

<400> 11
 ggccatggca ccaaagacaa cagc

24

60

<210> 12
 <211> 23
 <212> DNA

	<213> Macaca fascicularis	
	<400> 12	
5	ttggacacag aaagtgaagc agt	23
	<210> 13	
	<211> 20	
	<212> DNA	
10	<213> Macaca fascicularis	
	<400> 13	
	ctgagcatgg atcggaaaac	20
	<210> 14	
15	<211> 28	
	<212> DNA	
	<213> Artificial Sequence	
	<220>	
20	<223> Synthetic oligonucleotide	
	<400> 14	
	ttgtaatacg actcactata gggcgaat	28
	<210> 15	
25	<211> 28	
	<212> DNA	
	<213> Artificial Sequence	
	<220>	
30	<223> Synthetic oligonucleotide based on Macaca fascicularis and Homo sapiens	
	<400> 15	
35	cttttcgaga tgggtaactg aagtgaac	28
	<210> 16	
	<211> 28	
	<212> DNA	
40	<213> Artificial Sequence	
	<220>	
	<223> Synthetic oligonucleotide based on Macaca fascicularis and Homo sapiens	
45	<400> 16	
	agaaggtgct gggaagatcg ctactgaa	28
	<210> 17	
50	<211> 18	
	<212> DNA	
	<213> Macaca fascicularis	
	<400> 17	
55	gcctaaagcc gaacacat	18
	<210> 18	
	<211> 21	
	<212> DNA	
60	<213> Macaca fascicularis	

<400> 18
ctattaagtc tgcattctgg a

- 44 -

CLAIMS

1. An isolated nucleic acid molecule selected from the group consisting of
 - (a) nucleic acid molecules that code for the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4,
 - (b) allelic variants of (a), and
 - (c) complements of (a) or (b).
2. The isolated nucleic acid molecule of claim 1, wherein the isolated nucleic acid molecule codes for SEQ ID NO:2.
3. The isolated nucleic acid molecule of claim 1, wherein the isolated nucleic acid molecule codes for SEQ ID NO:4.
4. The isolated nucleic acid molecule of claim 1, wherein the isolated nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 3.
5. An isolated P-glycoprotein polypeptide or fragment thereof which comprises at least one amino acid of a cynomologous P-glycoprotein selected from the group consisting of amino acids 12, 24, 30, 74, 78, 86, 89, 90, 91, 92, 95, 97, 99, 102, 103, 104, 185, 324, 363, 518, 635, 650, 656, 659, 677, 730, 738, 742, 745, 761, 765, 835, 851, 921, 967, 1003, 1027, 1038, 1048, 1103, 1128, 1168 and 1277 of SEQ ID NO: 2 and amino acids 93, 94 and 95 of SEQ ID NO: 4, wherein the P-glycoprotein is identical to a human P-glycoprotein except for the at least one amino acid of a cynomologous P-glycoprotein and wherein the P-glycoprotein polypeptide or fragment thereof is at least 10 amino acids in length.
6. The isolated P-glycoprotein polypeptide or fragment thereof of claim 5, wherein the human P-glycoprotein is selected from the group of SEQ ID NO:5 and SEQ ID NO:6.
7. An isolated P-glycoprotein polypeptide or fragment thereof which comprises at least one amino acid of a cynomologous P-glycoprotein selected from the group consisting of amino acids 3, 6, 8, 10, 13, 17, 19, 20, 21, 26, 30, 36, 38, 48, 52, 56, 64, 74, 78, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 98, 100, 101, 102, 103, 104, 105, 106, 110, 113, 145, 190, 197, 210, 231, 319, 324, 327, 345, 363, 395, 451, 455, 456, 468, 473, 494, 518, 530, 631, 641, 642, 648, 650, 655, 656, 664, 665, 672, 673, 674, 675, 683, 687, 689, 691, 692, 694, 701, 705, 715, 729, 730, 734,

AMENDED SHEET

Empfangszeit 11. Okt. 22:57

- 45 -

742, 743, 745, 754, 757, 765, 835, 912, 918, 921, 940, 941, 944, 966, 967, 968, 970, 972, 981, 1008, 1015, 1023, 1024, 1048, 1093, 1096, 1103, 1128, 1142, 1146, 1147, 1156, 1160, 1163, 1166, 1250 and 1271 of SEQ ID NO: 2 and amino acids 93 and 94 of SEQ ID NO: 4, wherein the P-glycoprotein is identical to a dog P-glycoprotein except for the at least one amino acid of a cynomologous P-glycoprotein and wherein the P-glycoprotein polypeptide or fragment thereof is at least 10 amino acids in length.

8. The isolated P-glycoprotein polypeptide or fragment thereof of claim 7, wherein the dog P-glycoprotein is selected from the group of SEQ ID NO:7 and SEQ ID NO:8.

9. The isolated P-glycoprotein polypeptide or fragment thereof of claim 5 or 7, wherein the amino acid sequence of the polypeptide or fragment thereof is an amino acid sequence selected from the group consisting of SEQ ID NO:2, fragments of SEQ ID NO:2, SEQ ID NO:4 and fragments of SEQ ID NO:4.

10. An isolated nucleic acid molecule which encodes the isolated P-glycoprotein polypeptide or fragment thereof of any of claims 5-9.

11. An expression vector comprising the isolated nucleic acid molecule of claim 1 operably linked to a promoter.

12. An expression vector comprising the isolated nucleic acid molecule of claim 10 operably linked to a promoter.

13. A host cell transformed or transfected with the expression vector of claim 11.

14. A host cell transformed or transfected with the expression vector of claim 12.

WOLF GREENFIELD

Fax:6177202441

Oct 17 2001 16:49 P.10

- 45/1 -

15. An agent which selectively binds the isolated polypeptide of claim 5, wherein the agent does not bind human P-glycoprotein.

16. (cancelled)

Empf. AMENDED SHEET

- 46 -

17. The agent of claim 15, wherein the agent is a polypeptide.
18. The agent of claim 17, wherein the polypeptide is selected from the group consisting of monoclonal antibodies, polyclonal antibodies, Fab antibody fragments, F(ab)₂ antibody fragments and antibody fragments including a CDR3 region.
19. An agent which selectively binds the isolated nucleic acid molecule of claim 1 or claim 10, wherein the agent does not bind a nucleic acid molecule consisting of nucleotides 88-111 or nucleotides 100-117 of SEQ ID NO: 1.
20. (cancelled)
21. A method for predicting the bioavailability of a compound, comprising measuring the transmembrane transport of a test compound by a first P-glycoprotein, comparing the transmembrane transport of the test compound by the first P-glycoprotein and a second P-glycoprotein to predict the bioavailability of the test compound, wherein the relative amount or rate of transport by the first P-glycoprotein and the second P-glycoprotein is predictive of bioavailability of the test compound.
22. The method of claim 21, wherein the first P-glycoprotein is selected from the group consisting of dog P-glycoproteins and primate P-glycoproteins.
23. The method of claim 21, wherein the first P-glycoprotein is the polypeptide of claims 5 or 7.
24. The method of claim 21, wherein the second P-glycoprotein is a human P-glycoprotein.
25. A method for inhibiting P-glycoprotein transporter activity in a mammalian cell comprising

- 47 -

contacting the mammalian cell with an amount of the agent of claim 19 effective to inhibit P-glycoprotein transporter activity in the mammalian cell.

26. A method for increasing bioavailability of a drug in a subject comprising
5 administering to a subject in need of such treatment the agent of claim 19 in an amount effective to increasing bioavailability of a drug.

27. The method of claim 26, wherein the inhibitor is administered prior to administering the drug.

10

28. The method of claim 26, wherein the inhibitor is administered concurrently with the drug.

29. A method for increasing P-glycoprotein transporter activity in a cell comprising
15 contacting the cell with a molecule selected from the group consisting of the nucleic acid molecule of claim 1 and the nucleic acid molecule of claim 10, in an amount effective to increase P-glycoprotein transporter activity in the cell.

30. A method for identifying lead compounds for a pharmacological agent useful in the
20 treatment of disease associated with P-glycoprotein transporter activity comprising providing a cell or other membrane-encapsulated space comprising a P-glycoprotein as claimed in claim 5 or claim 7;

contacting the cell or other membrane-encapsulated space with a candidate pharmacological agent under conditions which, in the absence of the candidate
25 pharmacological agent, cause a first amount of P-glycoprotein transporter activity; determining a second amount of P-glycoprotein transporter activity as a measure of the effect of the pharmacological agent on the P-glycoprotein transporter activity, wherein a second amount of P-glycoprotein transporter activity which is less than the first amount indicates that the candidate pharmacological agent is a lead compound for a pharmacological
30 agent which reduces P-glycoprotein transporter activity and wherein a second amount of P-glycoprotein transporter activity which is greater than the first amount indicates that the candidate pharmacological agent is a lead compound for a pharmacological agent which

- 48 -

increases P-glycoprotein transporter activity.

31. The method of claim 30, further comprising the step of loading the cell or other membrane-encapsulated space with a detectable compound, wherein the compound is detected
5 as a measure of the P-glycoprotein transporter activity.

32. A method for identifying compounds which selectively bind a P-glycoprotein comprising,

contacting the P-glycoprotein claimed in claim 5 or claim 7 with a compound,

10 determining the binding of the compound to the P-glycoprotein.

33. The method of claim 32 further comprising determining the effect of the compound on the P-glycoprotein transporter activity of the P-glycoprotein.

15 34. The method of claim 32 further comprising determining the effect of the compound on the ATPase activity of the P-glycoprotein.

35. A method for determining ATPase activity of a P-glycoprotein comprising

contacting the host cell of claim 12 or 14, or a membrane fraction thereof, with

20 a test drug, and

measuring ATPase activity of the P-glycoprotein.

36. The method of claim 35, wherein the step of measuring ATPase activity is performed at least twice at different times.

25 37. A method for determining transmembrane transport of a compound by a P-glycoprotein, comprising

contacting the host cell of claim 12 or 14, or a membrane fraction thereof, with

a test drug, and

30 measuring transport of the test drug under sink conditions in at least one direction of transport selected from the group consisting of the apical to basolateral direction and the basolateral to apical direction.

- 49 -

38. The method of claim 37, wherein the step of measuring transport of the test drug is performed at least twice at different times.