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Ser/thr Kinase 14790 Expression on HBV+ Liver

(57) Abstract: The invention provides an isolated nucleic acid molecule, designated as a 14790 kinase nucleic acid molecule, which encodes a novel protein kinase. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing kinase nucleic acid molecules, host cells into which the expression vectors have been introduced, and nonhuman transgenic animals in which a kinase gene has been introduced or disrupted. The invention still further provides isolated 14790 proteins, fusion proteins, antigenic peptides and anti-kinase antibodies. Diagnostic, screening and therapeutic methods utilizing compositions of the invention are also provided.
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.
14790, A NOVEL PROTEIN KINASE MOLECULE AND USES THEREFORE

Background of the Invention

Phosphate tightly associated with protein has been known since the late nineteenth century. Since then, a variety of covalent linkages of phosphate to proteins have been found. The most common involve esterification of phosphate to serine, threonine, and tyrosine with smaller amounts being linked to lysine, arginine, histidine, aspartic acid, glutamic acid, and cysteine. The occurrence of phosphorylated proteins indicates the existence of one or more protein kinases capable of phosphorylating amino acid residues on proteins, and also of protein phosphatases capable of hydrolyzing phosphorylated amino acid residues on proteins.

Kinases play a critical role in the mechanism of intracellular signal transduction. They act on the hydroxyamino acids of target proteins to catalyze the transfer of a high energy phosphate group from adenosine triphosphate (ATP). This process is known as protein phosphorylation. Along with phosphatases, which remove phosphates from phosphorylated proteins, kinases participate in reversible protein phosphorylation. Reversible phosphorylation acts as the main strategy for regulating protein activity in eukaryotic cells.

Kinases vary widely in their selectivity and specificity of target proteins. They still may, however, comprise the largest known enzyme superfamily. Protein kinases can be divided into two main groups based on either amino acid sequence similarity or specificity for either serine/threonine or tyrosine residues. Serine/threonine specific kinases are often referred to as STKs while tyrosine specific kinases are referred to as PTKs. A small number of dual-specificity kinases are structurally like the serine/threonine-specific group. Within the broad classification, kinases can be further sub-divided into families whose members share a higher degree of catalytic domain amino acid sequence identity and also have similar biochemical properties. Most protein kinase family members also share structural features outside the kinase domain that reflect their particular cellular roles. These include regulatory domains that control kinase activity or interaction with other proteins (Hanks, S.K. et al. (1988) Science 241: 42-52).

Almost all kinases contain a catalytic domain composed of 250-300 conserved amino acids. This catalytic domain may be viewed as composed of 11 subdomains. Some of these subdomains apparently contain distinct amino acid motifs which confer specificity as a STK or PTK or both. Kinases may also contain additional amino acid sequences, usually between 5 and 100 residues, flanking or occurring within the catalytic domain. These residues apparently act to regulate kinase activity and to determine substrate specificity. (Reviewed in Hardie, G. and Hanks, S. (1995) The Protein Kinase Facts Book, Vol I:7-20 Academic Press, San Diego, Calif.).

Approximately one third of the known oncogenes encode PTKs. PTKs may occur as either transmembrane or soluble proteins. Transmembrane PTKs act as receptors for many growth factors. Interaction of a growth factor to its cognate receptor initiates the phosphorylation of specific tyrosine residues in the receptor itself as well as in certain second messenger proteins. Growth factors found to associate with such PTK receptors include epidermal growth factor, platelet-derived growth factor, fibroblast growth factor, hepatocyte growth factor, insulin and insulin-like growth factors, nerve growth factor, vascular endothelial growth factor, and macrophage colony stimulating factor.

Soluble PTKs often interact with the cytosolic domains of plasma membrane receptors. Receptors that signal through such PTKs include cytokine, hormone, and
antigen-specific lymphocytic receptors. Many PTKs were identified as oncogene products by the observation that PTK activation was no longer subject to normal cellular controls. Also, increased tyrosine phosphorylation activity is often observed in cellular transformation, or oncogenesis, (Carbonneau, H. and Tonks, N. K. (1992) Annu. Rev. Cell Biol. 8:463-93.) PTK regulation may therefore be an important strategy in controlling some types of cancer.

One example of regulation of a cellular function by reversible protein phosphorylation is in the case of eukaryotic initiation factor-2 (eIF-2). When phosphorylated by an eIF-2 kinase at the alpha subunit, eIF-2 is inhibited from continuing to participate in the initiation of protein translation, which leads to termination of protein synthesis. Thus inhibitors of eIF-2 phosphorylation are expected to be anti-proliferative in nature. This regulation of eIF-2 apparently plays a role in eukaryotic cells under viral infection, nutritional deprivation, and heat shock conditions. Phosphorylation of eIF-2 apparently also plays a role in programmed cell death.

Phosphorylation of eIF-2 may be controlled by regulating the eIF-2 kinase, which is activated by double-stranded RNA (dsRNA). Double-stranded RNA is induced by interferon and represents an interferon mediated response to viral infection. Thus inhibitors of eIF-2 phosphorylation are expected to also be anti-viral in nature.

**Summary of the Invention**

The present invention is based, at least in part, on the discovery of novel kinases referred to herein as 14790 proteins with similarities to known eIF-2 kinases, as well as nucleic acid molecules encoding the kinase. The kinase nucleic acid and protein molecules of the present invention are useful as modulating agents in regulating a variety of cellular processes, e.g., regulation of cell cycle, including cell proliferation, differentiation, growth and division. In particular, the kinase and its related nucleic acids will be advantageous in the regulation of any cellular function uncontrolled proliferation and differentiation, such as in cases of cancer or liver fibrosis. Other situations where the kinases of the invention are of particular advantage are in cases of autoimmune disorders or undesired inflammation.
Additionally, the kinases of the present invention, similar to known eIF-2 kinases, may play a role in cellular metabolism in response to conditions such as viral infection, nutritional deprivation, and heat shock conditions. As such, inhibitory modulators of the kinases are expected to be of benefit as anti-viral agents. A kinase of the invention has been observed to be up-regulated in HBV infected liver cells. Thus modulators of the kinases of the invention may be used in cases of HBV infection.

The kinases of the invention are also expected to play a role in cardiac cellular processes, either independent of, or in connection with, programmed cell death (apoptosis).

Thus, in one aspect, this invention provides isolated nucleic acid molecules encoding 14790 proteins or biologically active portions thereof, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection of kinase-encoding nucleic acids.

In one embodiment, a kinase encoding a nucleic acid molecule of the invention is at least 60%, 65% 70%, 75%, 77%, 80%, 82%, 85%, 87%, 90%, 92%, 95%, 97%, 98%, 99% or greater homology to a nucleotide sequence (e.g., to the entire length of the nucleotide sequence) including SEQ ID NO:1 or a complement thereof.

In a preferred embodiment, the isolated nucleic acid molecule includes the nucleotide sequence shown SEQ ID NO:1 or a coding region of SEQ ID NO:1 (i.e. SEQ ID NO:3 without the terminal codon), or a complement thereof. In another embodiment, the nucleic acid molecule includes the 5' UTR and the coding region of SEQ ID NO:1. In yet another embodiment, the nucleic acid molecule includes the coding region of SEQ ID NO:3 and the 3' UTR of SEQ ID NO:1. In another preferred embodiment, the nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:1 or the coding region of SEQ ID NO:1. In another preferred embodiment, the nucleic acid molecule comprises a fragment of at least 30 nucleotides of the nucleotide sequence of SEQ ID NO:1 or the coding region of SEQ ID NO:1, or a complement thereof.

In another embodiment, a kinase nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:2. In a preferred embodiment, a kinase nucleic acid
molecule includes a nucleotide sequence encoding a protein having an amino acid sequence at least 50%, 54%, 55%, 60%, 62%, 65%, 70%, 75%, 78%, 80%, 85%, 86%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homologous to an amino acid sequence including SEQ ID NO:2 (e.g., the entire amino acid sequence of SEQ ID NO:2).

In another preferred embodiment, an isolated nucleic acid molecule encodes the amino acid sequence of a human kinase, preferably with similarities to known eIF-2 kinases. In yet another preferred embodiment, the nucleic acid molecule includes a nucleotide sequence encoding a protein which includes the amino acid sequence of SEQ ID NO: 2. In yet another preferred embodiment, the nucleic acid molecule includes a nucleotide sequence encoding a protein having the amino acid sequence of SEQ ID NO: 2.

Another embodiment of the invention features nucleic acid molecules, preferably kinase nucleic acid molecules, which specifically detect kinase nucleic acid molecules relative to other nucleic acid molecules. In one embodiment, the nucleic acid molecules of the invention may be used to detect and/or differentiate between different eIF-2 kinase encoding nucleic acids. For example, in one embodiment, such a nucleic acid molecule is at least 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, or 5500 nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:1 or a complement thereof.

In other preferred embodiments, the nucleic acid molecule encodes a naturally occurring allelic variant of a polypeptide which includes the amino acid sequence of SEQ ID NO:2, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule which includes SEQ ID NO:1 or the coding region of SEQ ID NO:1 under stringent conditions.

Another embodiment of the invention provides an isolated nucleic acid molecule which is antisense to the nucleic acid molecule of the invention, e.g., the coding strand of a nucleic acid molecule of the invention.

Another aspect of the invention provides a vector comprising the nucleic acid molecule. In certain embodiments, the vector is a recombinant expression vector. In another embodiment, the invention provides a host cell containing a vector of the invention.
The invention also provides a method for producing a kinase, preferably a protein, by culturing in a suitable medium, a host cell, e.g., a mammalian host cell such as a non-human mammalian cell, of the invention containing a recombinant expression vector, such that the protein is produced.

Another aspect of this invention features isolated or recombinant proteins and polypeptides. In one preferred embodiment, the isolated protein, preferably a 14790 protein includes at least one N-glycosylation site; at least one cGMP-dependent protein kinase phosphorylation site; at least one protein kinase C phosphorylation site; at least one casein kinase II phosphorylation site; at least one tyrosine kinase phosphorylation site; at least one N-myristoylation site; at least one amidation site; at least one protein kinase ATP-binding region signature; and at least one Ser/Thr protein kinase active-site signature; and at least one DNA polymerase family B signature.

In another embodiment, the isolated protein, preferably a 14790 protein, includes an amino acid sequence which is of at least 51%, 55%, 60%, 65%, 70%, 75%, 80%, 81%, 85%, 90%, 95%, 99% or greater homology to an amino acid sequence including SEQ ID NO:2. Preferably, the proteins are kinases.

In yet another embodiment, the isolated protein, preferably a 14790 protein, is expressed and/or functions in cells of the hepatic system. Preferably, such proteins act as kinases.

In an even further embodiment, the isolated protein, preferably a 14790 protein, plays a role in signalling pathways associated with cellular growth, e.g., signalling pathways associated with cell cycle regulation and act as kinases.

In another embodiment, the isolated protein, preferably a 14790 protein, includes at least one N-glycosylation site; at least one cGMP-dependent protein kinase phosphorylation site; at least one protein kinase C phosphorylation site; at least one casein kinase II phosphorylation site; at least one tyrosine kinase phosphorylation site; at least one N-myristoylation site; at least one amidation site; at least one protein kinase ATP-binding region signature; at least one Ser/Thr protein kinase active-site signature; and at least one
DNA polymerase family B signature and is able to phosphorylate the core protein of Hepatitis B Virus (HBV).

In another embodiment, the isolated protein, preferably a 14790 protein, is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1. Preferably, the proteins are kinases.

In another embodiment, the isolated protein, preferably a 14790 protein, has an amino acid sequence homologous to the amino acid sequence of SEQ ID NO:2. In a preferred embodiment, the protein, preferably a 14790 protein, has an amino acid sequence at least about 50%, 55%, 59%, 60%, 65%, 70%, 75%, 80%, 81%, 85%, 90%, 95%, 98% or greater homology to an amino acid sequence including SEQ ID NO:2 (e.g., the entire amino acid sequence of SEQ ID NO:2). In another embodiment, the invention features fragments of the proteins having the amino acid sequence of SEQ ID NO:2, wherein the fragment comprises at least 15, 17, 19 or 21 amino acids (e.g., contiguous amino acids) of the amino acid sequence of SEQ ID NO:2. In another embodiment, the protein, preferably a 14790 protein, has the amino acid sequence of SEQ ID NO:2.

Another embodiment of the invention features an isolated protein, preferably a 14790 protein, which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 50%, 54%, 55%, 60%, 62%, 65%, 70%, 75%, 78%, 80%, 85%, 86%, 90%, 95%, 97%, 98% or greater homology to a nucleotide sequence (e.g., to the entire length of the nucleotide sequence) including SEQ ID NO:1 or the coding region of SEQ ID NO:1 a complement thereof. This invention further features an isolated protein, preferably a 14790 protein, which is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or a complement thereof.

The proteins of the present invention or biologically active portions thereof, can be operatively linked to a non-14790 polypeptide (e.g., heterologous amino acid sequences) to form fusion proteins. The invention further features antibodies, such as monoclonal or polyclonal antibodies, that specifically bind proteins of the invention, preferably 14790 proteins. In addition, the 14790 proteins or biologically active portions thereof can be
incorporated into pharmaceutical compositions, which optionally include pharmaceutically acceptable carriers.

In another aspect, the present invention provides a method for detecting the presence of a kinase nucleic acid molecule, protein or polypeptide in a biological sample by contacting the biological sample with one or more agent(s) capable of detecting a kinase nucleic acid molecule, protein or polypeptide such that the presence of a kinase nucleic acid molecule, protein or polypeptide is detected in the biological sample. Examples of agents for the detection of kinases and nucleic acids that encode them are well known in the art.

In another aspect, the present invention provides a method for detecting the presence of kinase activity in a sample by contacting the biological sample with one or more agent(s) capable of detecting 14790 activity. Such agents are known in the art. Examples of agents useful for the detection of eIF-2 kinase activity or the like include peptides or proteins containing eIF-2 phosphorylation target sequences.

In another aspect, the invention provides a method for modulating 14790 activity in a cell by contacting a cell capable of expressing kinase with an agent that modulates kinase activity. In one embodiment, the modulating agent inhibits kinase activity. In another embodiment, the modulating agent stimulates or increases kinase activity. In one embodiment of an inhibitory modulator, the agent is an antibody that specifically binds to a 14790 protein. In another embodiment, the modulating agent regulates expression of kinase by modulating transcription of a kinase gene or translation of a kinase mRNA. In yet another embodiment, the agent is a nucleic acid molecule having a nucleotide sequence that is antisense to the coding strand of a kinase mRNA or a kinase gene.

In one embodiment, the methods of the present invention are used to treat a subject having a disorder characterized by aberrant 14790 protein activity or nucleic acid expression by administering an agent which is a kinase modulator to the subject. In one embodiment, the kinase modulator is a protein. In another embodiment the kinase related modulator is a kinase nucleic acid molecule. In yet another embodiment, the kinase modulator is a peptide, peptidomimetic, or other small molecule. In a preferred embodiment, the disorder characterized by aberrant 14790 protein or nucleic acid
expression is a disorder characterized by enhanced viral replication, e.g. during infection with HBV.

The present invention also provides a diagnostic assay for identifying the presence or absence of a genetic alteration characterized by at least one of (i) aberrant modification or mutation of a gene encoding a 14790 protein; (ii) mis-regulation of the gene; and (iii) aberrant post-translational modification of a 14790 protein, wherein a wild-type form of the gene encodes a protein with a kinase activity.

In another aspect the invention provides a method for identifying a compound that binds to or modulates the activity of a 14790 protein, by providing an indicator composition comprising a 14790 protein having kinase activity, contacting the indicator composition with a test compound, and determining the effect of the test compound on kinase activity in the indicator composition to identify a compound that modulates the activity of a 14790 protein.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

**Brief Description of the Drawings**

Figures 1a-e depict the cDNA sequence (SEQ ID NO:1) and predicted amino acid sequence (SEQ ID NO:2) of human 14790 kinase. The nucleotide sequence corresponds to the 5525 nucleic acids of SEQ ID NO:1 which include nucleic acids 1-4947 of the coding region (SEQ ID NO:3), the 5' UTR of 62 nucleic acids, and the 3' UTR of 513 nucleic acids. The amino acid sequence corresponds to amino acids 1 to 1649 of SEQ ID NO:2.

Figures 2 a-c shows a multiple sequence alignment of the amino acid sequence of SEQ ID NO:2 in comparison with a known mouse and human kinase.

Figure 3 is a graph of the relative gene expression of 14790 RNA relative to a no template control in a panel of HBV positive liver using liver PIT as a reference sample using real-time quantitative RT-PCR Taq Man analysis.
Figure 4 is a graph of the relative gene expression of 14790 RNA relative to a no template control in a panel of HepG2.2.15 (HBV-expressing line) using liver PIT as a reference sample using real-time quantitative RT-PCR Taq Man analysis.

Figure 5 is a bar graph of data illustrating the relative gene expression in normal human tissue of 14790 RNA relative to a no template control in a panel of human tissues or cells, detected using real-time quantitative RT-PCR Taq Man analysis using thyroid as a reference. The graph indicates significant expression in prostate, colon, trachea, fetal heart and lymph nodes.

Figure 6 depicts the structural components of the protein of the amino acid sequence of SEQ ID NO:2 as generated from Protean software.

Figures 7 a-n illustrate data generated using the 14790 protein. A hydropathy plot of human 14790 shows relative hydrophobic residues above the dashed horizontal line, and relative hydrophilic residues below the dashed horizontal line. The location of the transmembrane domains, and the extracellular and intracellular loops is also indicated. The cysteine residues (cys) and N-glycosylation (Ngly) sites are indicated by short vertical lines just below the hydropathy trace. The numbers corresponding to the amino acid sequence of human 14790 are indicated. The signal peptide predictions and the transmembrane regions as predicted by MEMSTAT are also shown. Results from the Prosite database of protein families and domains identify biologically significant sites. PFAM search results are shown. PFAM search results depict an alignment of protein kinase domain of human 14790 with a consensus amino acid sequence derived from a hidden Markov model. The upper sequence is the consensus amino acid sequence, while the lower amino acid sequence corresponds to a portion of SEQ ID NO:2. Finally, results from the ProDom protein domain database identify homologous domains. The lower sequence is the consensus amino acid sequence, while the upper amino acid sequence corresponds to a portion of SEQ ID NO:2.

**Detailed Description of the Invention**

The present invention is based, at least in part, on the discovery of a novel 14790 molecule, referred to herein as a "kinase" or "14790 kinase" or “14790” nucleic acid and a
polypeptide molecule, which play a role in or function in signalling pathways associated with cellular growth. In one embodiment, the molecule modulates the activity of one or more proteins involved in cellular growth or differentiation, e.g., hepatic cell growth or differentiation. In another embodiment, the molecule of the present invention has its mRNA induced in liver cells infected with HBV. In another embodiment, the molecule of the present invention encodes a polypeptide that is able to phosphorylate the core protein of HBV.

In a preferred embodiment, the molecules are protein kinases which are expressed and/or function in cells of the hepatic system, e.g., cells of the liver and the associated blood vessels of the liver.

As used herein, the term "protein kinase" includes a protein or polypeptide which is capable of modulating its own phosphorylation state or the phosphorylation state of another protein or polypeptide. Protein kinases can have a specificity for (i.e., a specificity to phosphorylate) serine/threonine residues, tyrosine residues, or both serine/threonine and tyrosine residues, e.g., the dual specificity kinases. As referred to herein, protein kinases may include a catalytic domain of about 150-400 amino acid residues in length, preferably about 170-300 amino acid residues in length, or more preferably about 190-300 amino acid residues in length, which includes preferably 5-20, more preferably 5-15, or preferably 11 highly conserved motifs or subdomains separated by sequences of amino acids with reduced or minimal conservation. Specificity of a protein kinase for phosphorylation of either tyrosine or serine/threonine can be predicted by the sequence of two of the subdomains (VIb and VIII) in which different residues are conserved in each class (as described in, for example, Hanks et al. (1988) Science 241:42-52) the contents of which are incorporated herein by reference). These subdomains are also described in further detail herein.

Protein kinases play a role in signalling pathways associated with cellular growth. For example, protein kinases are involved in the regulation of signal transmission from cellular receptors, e.g., growth-factor receptors; entry of cells into mitosis; and the regulation of cytoskeleton function, e.g., actin bundling. Thus, the molecules of the present invention may be involved in: 1) the regulation of transmission of signals from cellular
receptors, e.g., cardiac cell growth factor receptors; 2) the modulation of the entry of cells, e.g., cardiac precursor cells, into mitosis; 3) the modulation of cellular differentiation; 4) the modulation of cell death; and 5) the regulation of cytoskeleton function, e.g., actin bundling.

Inhibition or over stimulation of the activity of protein kinases involved in signaling pathways associated with cellular growth can lead to perturbed cellular growth, which can in turn lead to cellular growth related disorders. As used herein, a "cellular growth related disorder" includes a disorder, disease, or condition characterized by a deregulation, e.g., an upregulation or a downregulation, of cellular growth. Cellular growth deregulation may be due to a deregulation of cellular proliferation, cell cycle progression, cellular differentiation and/or cellular hypertrophy. Examples of cellular growth related disorders include cardiovascular disorders such as heart failure, hypertension, atrial fibrillation, dilated cardiomyopathy, idiopathic cardiomyopathy, or angina; proliferative disorders or differentiative disorders such as cancer, e.g., melanoma, prostate cancer, cervical cancer, breast cancer, colon cancer, or sarcoma or fibrotic lesions seen in liver fibrosis.

The present invention is based, at least in part, on the discovery of novel molecules, referred to herein as protein and nucleic acid molecules, which comprise a family of molecules having certain conserved structural and functional features. The term "family" when referring to the protein and nucleic acid molecules of the invention is intended to mean two or more proteins or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide sequence homology as defined herein. Such family members can be naturally or non-naturally occurring and can be from either the same or different species. For example, a family can contain a first protein of human origin, as well as other, distinct proteins of human origin or alternatively, can contain homologues of non-human origin. Members of a family may also have common functional characteristics.

One embodiment of the invention features a nucleic acid molecule, preferably a human 14790 molecule, which was identified from a cDNA library. The nucleic acid and protein molecule of the invention is described in further detail in the following subsections.
A. The 14790 Kinase Nucleic Acid and Protein Molecules

In one embodiment, the isolated proteins of the present invention, preferably 14790 proteins, are identified based on the presence of at least one "Ser/Thr kinase site" and at least one "ATP-binding region." As used herein, the term "Ser/Thr kinase site" includes an amino acid sequence of about 200-400 amino acid residues in length, preferably 200-300 amino acid residues in length, and more preferably 250-300 amino acid residues in length, which is conserved in kinases which phosphorylate serine and threonine residues and found in the catalytic domain of Ser/Thr kinases. Preferably, the Ser/Thr kinase site includes the following amino acid consensus sequence X₉-g-X-G-X₄-V-X₁₂-K-X-(10-19)-E-X₆₆-h-X₈-h-r-D-X-K-X₂-N-X₁₇-K-X₂-D-f-g-X₂₁-p-X₁₃-w-X₃-g-X₅₅-R-X₁₄-h-X₃ (where invariant residues are indicated by upper case letters and nearly invariant residues are indicated by lower case letters). The nearly invariant residues are usually found in most Ser/Thr kinase sites, but can be replaced by other amino acids which, preferably, have similar characteristics. For example, a nearly invariant hydrophobic amino acid in the above amino acid consensus sequence would most likely be replaced by another hydrophobic amino acid. Ser/Thr kinase domains are described in, for example, Levin D.E. et al. (1990) Proc. Natl. Acad. Sci. USA 87:8272-76, the contents of which are incorporated herein by reference.

As used herein, the term "ATP-binding region" includes an amino acid sequence of about 5-40, preferably 5-25, and more preferably 5-15 amino acid residues in length, present in enzymes which activate their substrates by phosphorylation, and involved in binding adenosine triphosphate (ATP). ATP-binding regions preferably include the following amino acid consensus sequence: G-X-G-X-G-X(15-23)-K. ATP-binding regions are described in, for example, Samuel K.P. et al. (1987) FEBS Let. 218(1): 81-86, the contents of which are incorporated herein by reference. Amino acid residues 596-604 of the 14790 kinase comprise an ATP-binding region.

Isolated proteins of the present invention, preferably 14790 proteins, have an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:2 or are encoded by a nucleotide sequence sufficiently homologous to SEQ ID NO:1. As used herein, the term "sufficiently homologous" refers to a first amino acid or nucleotide
sequence which contains a sufficient or minimum number of identical or equivalent (e.g., an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural domains or motifs and/or a common functional activity. For example, amino acid or nucleotide sequences which share common structural domains have at least 30%, 40%, or 50% homology, preferably 60% homology, more preferably 70%-80%, and even more preferably 90-95% homology across the amino acid sequences of the domains and contain at least one and preferably two structural domains or motifs, are defined herein as sufficiently homologous. Furthermore, amino acid or nucleotide sequences which share at least 30%, 40%, or 50%, preferably 60%, more preferably 70-80%, or 90-95% homology and share a common functional activity are defined herein as sufficiently homologous.

In one embodiment, a 14790 protein includes at least one transmembrane domain. As used herein, the term "transmembrane domain" includes an amino acid sequence of about 15 amino acid residues in length that spans a phospholipid membrane. More preferably, a transmembrane domain includes about at least 18, 20, 22, 24, 25, 30, 35 or 40 amino acid residues and spans a phospholipid membrane. Transmembrane domains are rich in hydrophobic residues, and typically have an α-helical structure. In a preferred embodiment, at least 50%, 60%, 70%, 80%, 90%, 95% or more of the amino acids of a transmembrane domain are hydrophobic, e.g., leucines, isoleucines, tyrosines, or tryptophans. Transmembrane domains are described in, for example, http://pfam.wustl.edu/cgi-bin/getdesc?name=7tm-1, and Zagotta W.N. et al., (1996) Annual Rev. Neurosci. 19: 235-63, the contents of which are incorporated herein by reference.

In a preferred embodiment, a 14790 polypeptide or protein has at least one transmembrane domain or a region which includes at least 18, 20, 22, 24, 25, 30, 35 or 40 amino acid residues and has at least about 60%, 70% 80% 90% 95%, 99%, or 100% homology with a "transmembrane domain," e.g., at least one transmembrane domain of human 14790 (e.g., amino acid residues 1522-1538 of SEQ ID NO:2).

In another embodiment, a 14790 protein includes at least one “non-transmembrane domain.” As used herein, “non-transmembrane domains” are domains that reside outside
of the membrane. When referring to plasma membranes, non-transmembrane domains include extracellular domains (i.e., outside of the cell) and intracellular domains (i.e., within the cell). When referring to membrane-bound proteins found in intracellular organelles (e.g., mitochondria, endoplasmic reticulum, peroxisomes and microsomes), non-transmembrane domains include those domains of the protein that reside in the cytosol (i.e., the cytoplasm), the lumen of the organelle, or the matrix or the intermembrane space (the latter two relate specifically to mitochondria organelles). The C-terminal amino acid residue of a non-transmembrane domain is adjacent to an N-terminal amino acid residue of a transmembrane domain in a naturally-occurring 14790, or 14790-like protein.

In a preferred embodiment, a 14790 polypeptide or protein has a "non-transmembrane domain" or a region which includes at least about 100-1550, preferably about 100-120 or 1550-1560, more preferably about 105-115 or 1550-1555 amino acid residues, and has at least about 60%, 70% 80% 90% 95%, 99% or 100% homology with a "non-transmembrane domain", e.g., a non-transmembrane domain of human 14790 (e.g., residues 1-1521 and 1539-1649 of SEQ ID NO:2). Preferably, a non-transmembrane domain is capable of catalytic activity (e.g., catalyzing a kinase reaction).

A non-transmembrane domain located at the N-terminus of a 14790 protein or polypeptide is referred to herein as a "N-terminal non-transmembrane domain." As used herein, an "N-terminal non-transmembrane domain" includes an amino acid sequence having about 1400-1600, preferably about 1500-1550, more preferably about 1510-1530, and even more preferably about 1520-1525 amino acid residues in length and is located outside the boundaries of a membrane. For example, an N-terminal non-transmembrane domain is located at about amino acid residues 1-1521 of SEQ ID NO:2.

Similarly, a non-transmembrane domain located at the C-terminus of a 14790 protein or polypeptide is referred to herein as a "C-terminal non-transmembrane domain." As used herein, an "C-terminal non-transmembrane domain" includes an amino acid sequence having about 50-150, preferably about 75-125, more preferably about 90-120, and even more preferably about 105-115 amino acid residues in length and is located outside the boundaries of a membrane. For example, a C-terminal non-transmembrane domain is located at about amino acid residues 1539-1649 of SEQ ID NO:2.
As used herein, the term “eukaryotic protein kinase domain” with regard to 14790 includes at least one kinase domain having an amino acid sequence of about 30-700 amino acid residues and having a bit score for the alignment of the sequence to the kinase domain (HMM) of at least about 20. Preferably, a kinase domain includes at least about 35, 70, 110, or 200 amino acid residues, and may have a bit score for the alignment of the sequence to the kinase domain (HMM) of at least about 20, 50, 70, 130 or may have a combined score of 280 or greater. The kinase domain (HMM) as shown in Figure 7 has been assigned the PFAM Accession PF00069 (http://genome.wustl.edu/Pfam/).

As used interchangeably herein a “kinase activity”, “biological activity of kinase” or "functional activity of kinase", refers to an activity exerted by a 14790 protein, polypeptide or nucleic acid molecule on a kinase responsive cell or a 14790 protein substrate, as determined in vivo, or in vitro, according to standard techniques. The biological activity of kinase is described herein.

Accordingly, another embodiment of the invention features isolated 14790 proteins and polypeptides having a kinase activity. Preferred proteins are 14790 proteins having at least one N-glycosylation site; at least one cGMP-dependent protein kinase phosphorylation site; at least one protein kinase C phosphorylation site; at least one casein kinase II phosphorylation site; at least one tyrosine kinase phosphorylation site; at least one N-myristoylation site; at least one amidation site; at least one protein kinase ATP-binding region signature; and at least one Ser/Thr protein kinase active-site signature; and at least one DNA polymerase family B signature and, preferably, a kinase activity. Additional preferred proteins have at least one N-glycosylation site; at least one cGMP-dependent protein kinase phosphorylation site; at least one protein kinase C phosphorylation site; at least one casein kinase II phosphorylation site; at least one tyrosine kinase phosphorylation site; at least one N-myristoylation site; at least one amidation site; at least one protein kinase ATP-binding region signature; and at least one Ser/Thr protein kinase active-site signature; and at least one DNA polymerase family B signature and are, preferably, encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under
stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or the coding region thereof.

The nucleotide sequence of the isolated human kinase cDNA and the predicted amino acid sequence of the human 14790 polypeptide are shown in Figure 1 and in SEQ ID NOs:1 and 2, respectively. A plasmid containing the nucleotide sequence encoding human kinase was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on _____ and assigned Accession Number ______. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

The kinase gene, which is approximately 5525 nucleotides in length, encodes a protein having a molecular weight of approximately 181.5 kD and which is approximately 1649 amino acid residues in length. The kinase gene is expressed predominantly in skeletal muscle, brain and liver.

Various aspects of the invention are described in further detail in the following subsections:

I. Isolated 14790 Nucleic Acid Molecules

One aspect of the invention pertains to isolated nucleic acid molecules that encode 14790 proteins or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify kinase-encoding nucleic acids (e.g., kinase mRNA) and fragments for use as PCR primers for the amplification or mutation of kinase nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (e.g., cDNA or genomic DNA) and RNA molecules (e.g., mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

An "isolated" nucleic acid molecule is one which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. For example,
with regards to genomic DNA, the term "isolated" includes nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated kinase nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, e.g., a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1 or coding region thereof, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. For example, using all or portion of the nucleic acid sequence of SEQ ID NO:1, or the coding region thereof, as a hybridization probe, kinase nucleic acid molecules can be isolated using standard hybridization and cloning techniques (e.g., as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. Molecular Cloning: A Laboratory Manual. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID NO:1, or the coding region thereof, can be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the sequence of SEQ ID NO:1, or the coding region thereof.

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis.
Furthermore, oligonucleotides corresponding to nucleotide sequences can be prepared by standard synthetic techniques, e.g., using an automated DNA synthesizer.

In a preferred embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:1. The sequence of SEQ ID NO:1 corresponds to the partial human kinase cDNA. This cDNA comprises sequences encoding the human 14790 protein (i.e., "the coding region", from nucleotides 1-4947), as well as 5' untranslated sequences (62 nucleotides) and 3' untranslated sequences (513 nucleotides). Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:1 (e.g., nucleotides 1-4947).

In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1, or the coding region thereof, or a portion of any of these nucleotide sequences. A nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID NO:1, or the coding region thereof, is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1, or the coding region thereof, such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1, or the coding region thereof, thereby forming a stable duplex.

In still another preferred embodiment, an isolated nucleic acid molecule of the present invention comprises a nucleotide sequence which is at least about 50%, 54%, 55%, 60%, 62%, 65%, 70%, 75%, 78%, 80%, 85%, 86%, 90%, 95%, 97%, 98% or more homologous to the nucleotide sequence (e.g., to the entire length of the nucleotide sequence) shown in SEQ ID NO:1, or the coding region thereof, or a portion of any of these nucleotide sequences.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO:1, or the coding region thereof, for example a fragment which can be used as a probe or primer or a fragment encoding a biologically active portion of a 14790 protein. The nucleotide sequence determined from the cloning of the kinase gene allows for the generation of probes and primers designed for use in identifying and/or cloning other kinase family members, as well as kinase homologues from other species. The probe/primer typically comprises substantially purified
oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence
that hybridizes under stringent conditions to at least about 12 or 15, preferably about 20 or
25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 consecutive nucleotides of a
sense sequence of SEQ ID NO:1, of an anti-sense sequence of SEQ ID NO:1, or the coding
region thereof, or of a naturally occurring allelic variant or mutant of SEQ ID NO:1, or the
coding region thereof. In an exemplary embodiment, a nucleic acid molecule of the present
invention comprises a nucleotide sequence which is at least 350, 400, 450, 500, 550, 600,
650, 700, 750, 800, 850, 900, 950, 1000, 1500, 2000, 2500, 3000, 3500, 4000, or 4500
nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic
acid molecule of SEQ ID NO:1, or the coding region thereof.

Probes based on the nucleotide sequences can be used to detect transcripts or
 genomic sequences encoding the same or homologous proteins. In preferred embodiments,
the probe further comprises a label group attached thereto, e.g., the label group can be a
radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes
can be used as a part of a diagnostic test kit for identifying cells or tissues which
misexpress a 14790 protein, such as by measuring a level of a kinase-encoding nucleic acid
in a sample of cells from a subject e.g., detecting kinase mRNA levels or determining
whether a genomic kinase gene has been mutated or deleted.

A nucleic acid fragment encoding a "biologically active portion of a 14790 protein"
can be prepared by isolating a portion of the nucleotide sequence of SEQ ID NO:1, or the
coding region thereof, which encodes a polypeptide having a kinase biological activity (the
biological activities of the 14790 proteins are described herein), expressing the encoded
portion of the 14790 protein (e.g., by recombinant expression \textit{in vitro}) and assessing the
activity of the encoded portion of the 14790 protein.

The invention further encompasses nucleic acid molecules that differ from the
nucleotide sequence shown in SEQ ID NO:1, or the coding region thereof, due to the
degeneracy of the genetic code and, thus, encode the same 14790 proteins as those encoded
by the nucleotide sequence shown in SEQ ID NO:1, or the coding region thereof. In
another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide
sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:2.
In addition to the kinase nucleotide sequences shown in SEQ ID NO:1, or the coding region thereof, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the 14790 proteins may exist within a population (e.g., the human population). Such genetic polymorphism in the kinase genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which include an open reading frame encoding a 14790 protein, preferably a mammalian 14790 protein, and can further include non-coding regulatory sequences, and introns. Such natural allelic variations include both functional and non-functional 14790 proteins and can typically result in 1-5% variance in the nucleotide sequence of a kinase gene. Any and all such nucleotide variations and resulting amino acid polymorphisms in genes that are the result of natural allelic variation and that do not alter the functional activity of a protein are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding other kinase family members and, thus, which have a nucleotide sequence which differs from the kinase sequences of SEQ ID NO:1, or the coding region thereof, are intended to be within the scope of the invention. For example, another kinase cDNA can be identified based on the nucleotide sequence of human kinase. Moreover, nucleic acid molecules encoding 14790 proteins from different species, and thus which have a nucleotide sequence which differs from the kinase sequences of SEQ ID NO:1, or the coding region thereof, are intended to be within the scope of the invention. For example, a mouse kinase cDNA can be identified based on the nucleotide sequence of a human kinase.

Nucleic acid molecules corresponding to natural allelic variants and homologues of the kinase cDNAs of the invention can be isolated based on their homology to the kinase nucleic acids disclosed herein using the cDNAs disclosed herein, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 15, 20, 25, 30 or more nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of
SEQ ID NO:1, or the coding region thereof. In other embodiment, the nucleic acid is at least 30, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1500, 2000, 2500, 3000, 3500, 4000, or 4500 nucleotides in length. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 30%, 40%, 50%, or 60% homologous to each other typically remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% homologous to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-limiting example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65°C. A more preferred example of stringent hybridization conditions is hybridization in 0.5M sodium phosphate, 7% SDS at 65°C, followed by one or more washes in 0.2 X SSC at 65°C. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1, or the coding region thereof, corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

In addition to naturally-occurring allelic variants of the kinase sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of SEQ ID NO:1, or the coding region thereof, thereby leading to changes in the amino acid sequence of the encoded 14790 proteins, without altering the functional ability of the 14790 proteins. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NO:1, or the coding region thereof. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of kinase (e.g., the sequence of SEQ ID NO:2) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the 14790 proteins of the present
invention, are predicted to be particularly unamenable to alteration. Furthermore, additional amino acid residues that are conserved between the 14790 proteins of the present invention and other kinase family members are not likely to be amenable to alteration.

Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding 14790 proteins that contain changes in amino acid residues that are not essential for activity. Such 14790 proteins differ in amino acid sequence from SEQ ID NO:2, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 41%, 42%, 45%, 50%, 55%, 59%, 60%, 65%, 70%, 75%, 80%, 81%, 85%, 90%, 95%, 98% or more homologous to the amino acid sequence of SEQ ID NO:2 (e.g., the entire amino acid sequence of SEQ ID NO:2).

An isolated nucleic acid molecule encoding a 14790 protein homologous to the protein of SEQ ID NO:2 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1 such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced into SEQ ID NO:1, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a 14790 protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a kinase coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for kinase biological activity to identify mutants that retain activity. Following mutagenesis of
SEQ ID NO:1, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

In a preferred embodiment, a mutant 14790 protein can be assayed for the ability to: 1) regulate transmission of signals from cellular receptors, e.g., cardiac cell growth factor receptors; 2) control entry of cells, e.g., cardiac cells, into mitosis; 3) modulate cellular differentiation; 4) modulate cell death; 5) regulate cytoskeleton function, e.g., actin bundling; or being able to phosphorylate the core protein of HBV.

In addition to the nucleic acid molecules encoding 14790 proteins described above, another aspect of the invention pertains to isolated nucleic acid molecules which are antisense thereto. An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire kinase coding strand, or only to a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding kinase. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues (e.g., the coding region of human kinase corresponds to 1-4947 of SEQ ID NO:1. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding kinase. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (i.e., also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding kinase disclosed herein (e.g., nucleic acids 1-4947 of SEQ ID NO:1) antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of kinase mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of kinase mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of kinase mRNA. An
antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl)uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydropyrimidine, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylxynosine, 2,2-dimethylguanine, 2-methyl adenine, 2-methylguanine, 3-methylecytosine, 5-methylecytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxy-carboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutosine, pseudouracil, queosine, 2-thiacytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid malester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated in situ such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a 14790 protein to thereby inhibit expression of the protein, e.g., by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of
administration of antisense nucleic acid molecules of the invention include direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gaultier et al. (1987) Nucleic Acids. Res. 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribo nucleotide (Inoue et al. (1987) Nucleic Acids Res. 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue et al. (1987) FEBS Lett. 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as a kinase mRNA, to which they have a complementary region. Thus, ribozymes (e.g., hammerhead ribozymes (described in Haselhoff and Gerlach (1988) Nature 334:585-591)) can be used to catalytically cleave kinase mRNA transcripts to thereby inhibit translation of kinase mRNA. A ribozyme having specificity for a kinase-encoding nucleic acid can be designed based upon the nucleotide sequence of a kinase cDNA disclosed herein (i.e., SEQ ID NO:1, or the coding region thereof). For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a kinase-encoding mRNA. See, e.g., Cech et al. U.S. Patent No. 4,987,071; and Cech et al. U.S. Patent No. 5,116,742. Alternatively, kinase mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a


In yet another embodiment, the kinase nucleic acid molecules of the present invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. et al. (1996) *Bioorganic & Medicinal Chemistry* 4 (1): 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, e.g., DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. et al. (1996) *supra*; Perry-O'Keefe et al. Proc. Natl. Acad. Sci. 93: 14670-675.

PNAs of kinase nucleic acid molecules can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of kinase nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (e.g., by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (e.g., S1 nucleases (Hyrup B. (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. et al. (1996) *supra*; Perry-O'Keefe *supra*).

In another embodiment, PNAs of kinase can be modified, (e.g., to enhance their stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by the
formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of kinase nucleic acid molecules can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, (e.g., RNase H and DNA polymerases), to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup B. (1996) *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup B. (1996) *supra* and Finn P.J. et al. (1996) *Nucleic Acids Res.* 24 (17): 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs, e.g., 5’-(4-methoxytrityl)amino-5’-deoxy-thymidine phosphoramidite, can be used as a between the PNA and the 5’ end of DNA (Mag, M. et al. (1989) *Nucleic Acid Res.* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5’ PNA segment and a 3’ DNA segment (Finn P.J. et al. (1996) *supra*). Alternatively, chimeric molecules can be synthesized with a 5’ DNA segment and a 3’ PNA segment (Peterser, K.H. et al. (1975) *Bioorganic Med. Chem. Lett.* 5: 1119-11124).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al. (1989) *Proc. Natl. Acad. Sci. US* 86:6553-6556; Lemaitre et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, e.g., PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, e.g., Krol et al. (1988) *Bio-Techniques* 6:958-976) or intercalating agents. (See, e.g., Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (e.g., a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

II. Isolated 14790 Proteins and Anti-Kinase Antibodies

One aspect of the invention pertains to isolated 14790 proteins, and biologically active portions thereof, as well as polypeptide fragments suitable for use as immunogens to
raise anti-kinase antibodies. In one embodiment, native 14790 proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, 14790 proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a 14790 protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the 14790 protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of 14790 protein in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of 14790 protein having less than about 30% (by dry weight) of non-14790 protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-14790 protein, still more preferably less than about 10% of non-14790 protein, and most preferably less than about 5% non-14790 protein. When the 14790 protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of 14790 protein in which the protein is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of 14790 protein having less than about 30% (by dry weight) of chemical precursors or non-kinase chemicals, more preferably less than about 20% chemical precursors or non-kinase chemicals, still more preferably less than about 10% chemical precursors or non-kinase chemicals, and most preferably less than about 5% chemical precursors or non-kinase chemicals.
Biologically active portions of a 14790 protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the 14790 protein, e.g., the amino acid sequence shown in SEQ ID NO:2, which include less amino acids than the full length 14790 proteins, and exhibit at least one activity of a 14790 protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the 14790 protein. A biologically active portion of a 14790 protein can be a polypeptide which is, for example, at least 10, 25, 50, 100 or more amino acids in length.

In a preferred embodiment, the 14790 protein has an amino acid sequence shown in SEQ ID NO:2. In other embodiments, the 14790 protein is substantially homologous to SEQ ID NO:2, and retains the functional activity of the protein of SEQ ID NO:2, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail in subsection I above. Accordingly, in another embodiment, the 14790 protein is a protein which comprises an amino acid sequence at least about 55%, 59%, 60%, 65%, 70%, 75%, 80%, 81%, 85%, 90%, 95%, 98% or more homologous to the amino acid sequence of SEQ ID NO:2 (e.g., the entire amino acid sequence of SEQ ID NO:2).

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence (e.g., when aligning a second sequence to the kinase amino acid sequence of SEQ ID NO:2 having 1649 amino acid residues, about at least 49, preferably at least 660, more preferably at least 825, even more preferably at least 990, and even more preferably at least 1155, 1320 or 1486 amino acid residues are aligned). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity
between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blossom 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at http://www.gcg.com), using a NWGappdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A more preferred embodiment, the percent identity between two nucleotide or amino acid sequences is determined using a Blossum 62 scoring matrix with a gap open penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

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The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the
NBLAST and XBLAST programs (version 2.0) of Altschul, et al. (1990) J. Mol. Biol. 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) Nucleic Acids Res. 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov.

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The invention also provides kinase chimeric or fusion proteins. As used herein, a kinase "chimeric protein" or "fusion protein" comprises a 14790 polypeptide operatively linked to a non-14790 polypeptide. A kinase "polypeptide" refers to a polypeptide having an amino acid sequence corresponding to kinase, whereas a "non-14790 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the 14790 protein, e.g., a protein which is different from the 14790 protein and which is derived from the same or a different organism. Within a kinase fusion protein the 14790 polypeptide can correspond to all or a portion of a protein. In a
preferred embodiment, a kinase fusion protein comprises at least one biologically active
portion of a 14790 protein. In another preferred embodiment, a kinase fusion protein
comprises at least two biologically active portions of a 14790 protein. Within the fusion
protein, the term "operatively linked" is intended to indicate that the 14790 polypeptide and
the non-14790 polypeptide are fused in-frame to each other. The non-14790 polypeptide
can be fused to the N-terminus or C-terminus of the 14790 polypeptide.

For example, in one embodiment, the fusion protein is a GST-kinase fusion protein
in which the kinase sequences are fused to the C-terminus of the GST sequences. Such
fusion proteins can facilitate the purification of recombinant kinase.

In another embodiment, the fusion protein is a 14790 protein containing a
heterologous signal sequence at its N-terminus. In certain host cells (e.g., mammalian host
cells), expression and/or secretion of kinase can be increased through use of a heterologous
signal sequence.

The kinase fusion proteins of the invention can be incorporated into pharmaceutical
compositions and administered to a subject in vivo. The kinase fusion proteins can be used
to affect the bioavailability of a kinase substrate. Use of kinase fusion proteins may be
useful therapeutically for the treatment of viral infections, e.g., infection of liver cells with
HBV. Moreover, the kinase-fusion proteins of the invention can be used as immunogens to
produce anti-kinase antibodies in a subject, to purify kinase ligands and in screening
assays to identify 14790 molecules which inhibit the interaction of with a kinase substrate.

Preferably, a kinase chimeric or fusion protein of the invention is produced by
standard recombinant DNA techniques. For example, DNA fragments coding for the
different polypeptide sequences are ligated together in-frame in accordance with
conventional techniques, for example by employing blunt-ended or stagger-ended termini
for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of
cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining,
and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by
conventional techniques including automated DNA synthesizers. Alternatively, PCR
amplification of gene fragments can be carried out using anchor primers which give rise to
complementary overhangs between two consecutive gene fragments which can
subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, Current Protocols in Molecular Biology, eds. Ausubel et al. John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). A kinase-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the 14790 protein.

The present invention also pertains to variants of the 14790 proteins which function as either kinase agonists (mimetics) or as kinase antagonists. Variants of the 14790 proteins can be generated by mutagenesis, e.g., discrete point mutation or truncation of a 14790 protein. An agonist of the 14790 proteins can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of a 14790 protein. An antagonist of a 14790 protein can inhibit one or more of the activities of the naturally occurring form of the 14790 kinase protein. An antagonist of the 14790 protein can inhibit one or more of the activities of the naturally occurring form of the kinase protein by, for example, by competitively modulating the ability of 14790 protein to phosphorylate the core protein of HBV. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the 14790 protein.

In one embodiment, variants of a 14790 protein which function as either kinase agonists (mimetics) or as kinase antagonists can be identified by screening combinatorial libraries of mutants, e.g., truncation mutants, of a 14790 protein for 14790 protein agonist or antagonist activity. In one embodiment, a variegated library of kinase variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of kinase variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential kinase sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display) containing the set of kinase sequences therein. There are a variety of methods which can be used to produce libraries of potential kinase variants from a degenerate

In addition, libraries of fragments of a 14790 protein coding sequence can be used to generate a variegated population of kinase fragments for screening and subsequent selection of variants of a 14790 protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of a kinase coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal, C-terminal and internal fragments of various sizes of the 14790 protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of 14790 proteins. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify kinase variants (Arkin and Yourvan (1992) Proc. Natl. Acad. Sci. USA 89:7811-7815; Delgrame et al. (1993) Protein Engineering 6(3):327-331).
In one embodiment, cell based assays can be exploited to analyze a variegated kinase library. For example, a library of expression vectors can be transfected into a cell line which ordinarily synthesizes and secretes kinase. The transfected cells are then cultured such that kinase and a particular mutant are secreted and the effect of expression of the mutant on activity in cell supernatants can be detected, e.g., by any of a number of enzymatic assays. Plasmid DNA can then be recovered from the cells which score for inhibition, or alternatively, potentiation of kinase activity, and the individual clones further characterized.

An isolated 14790 protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind kinase using standard techniques for polyclonal and monoclonal antibody preparation. A full-length 14790 protein can be used or, alternatively, the invention provides antigenic peptide fragments of kinase for use as immunogens. The antigenic peptide of kinase comprises at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2 and encompasses an epitope of kinase such that an antibody raised against the peptide forms a specific immune complex with kinase. Preferably, the antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

Preferred epitopes encompassed by the antigenic peptide are regions of kinase that are located on the surface of the protein, e.g., hydrophilic regions.

A kinase immunogen typically is used to prepare antibodies by immunizing a suitable subject, (e.g., rabbit, goat, mouse or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed 14790 protein or a chemically synthesized 14790 polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic kinase preparation induces a polyclonal anti-kinase antibody response.

Accordingly, another aspect of the invention pertains to anti-kinase antibodies. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain
an antigen binding site which specifically binds (immunoreacts with) an antigen, such as kinase. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')2 fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind kinase. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of kinase. A monoclonal antibody composition thus typically displays a single binding affinity for a particular 14790 protein with which it immunoreacts.

Polyclonal anti-kinase antibodies can be prepared as described above by immunizing a suitable subject with a kinase immunogen. The anti-kinase antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized kinase. If desired, the antibody molecules directed against kinase can be isolated from the mammal (e.g., from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, e.g., when the anti-kinase antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) Nature 256:495-497 (see also, Brown et al. (1981) J. Immunol. 127:539-46; Brown et al. (1980) J. Biol. Chem. 255:4980-83; Yeh et al. (1976) Proc. Natl. Acad. Sci. USA 76:2927-31; and Yeh et al. (1982) Int. J. Cancer 29:269-75), the more recent human B cell hybridoma technique (Kozbor et al. (1983) Immunol Today 4:72), the EBV-hybridoma technique (Cole et al. (1985), Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally R. H. Kenneth, in Monoclonal Antibodies: A New Dimension In Biological Analyses, Plenum Publishing Corp., New York, New York (1980); E. A. Lerner (1981) Yale J. Biol. Med., 54:387-402; M. L. Gelfer et al. (1977) Somatic Cell Genet. 3:231-36). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a kinase immunogen as described
above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds kinase.

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-kinase monoclonal antibody (see, e.g., G. Galfre et al. (1977) *Nature* 266:55052; Gefter et al. *Somatic Cell Genet.*, cited supra; Lerner, *Yale J. Biol. Med.*, cited supra; Kenneth, *Monoclonal Antibodies*, cited supra). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (e.g., a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, e.g., the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind kinase, e.g., using a standard ELISA assay.

Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-kinase antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with kinase to thereby isolate immunoglobulin library members that bind kinase. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display

An anti-kinase antibody (e.g., monoclonal antibody) can be used to isolate kinase by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-kinase antibody can facilitate the purification of natural kinase from cells and of recombinantly produced kinase expressed in host cells. Moreover, an anti-kinase antibody can be used to detect 14790 protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the 14790 protein. Anti-kinase antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include $^{125}\text{I}$, $^{131}\text{I}$, $^{35}\text{S}$ or $^{3}\text{H}$.

III. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding a 14790 protein (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors
are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in an in vitro transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990).

Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cell and those which direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., 14790 proteins, mutant forms of 14790 proteins, fusion proteins, and the like).

The recombinant expression vectors of the invention can be designed for expression of 14790 proteins in prokaryotic or eukaryotic cells. For example, 14790 proteins can be expressed in bacterial cells such as E. coli, insect cells (using baculovirus expression
vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated in vitro, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Purified fusion proteins can be utilized in kinase activity assays, (e.g., direct assays or competitive assays described in detail below), or to generate antibodies specific for 14790 proteins, for example. In a preferred embodiment, a kinase fusion protein expressed in a retroviral expression vector of the present invention can be utilized to infect bone marrow cells which are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (e.g., six (6) weeks).

60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV5 promoter.

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada et al., (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.


In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman et al. (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert et al. (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji et al. (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund et al. (1985) *Science* 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the α-fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to kinase mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, a 14790 protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, et al. *(Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989)*, and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell
on the same vector as that encoding a 14790 protein or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (i.e., express) a 14790 protein. Accordingly, the invention further provides methods for producing a 14790 protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding a 14790 protein has been introduced) in a suitable medium such that a 14790 protein is produced. In another embodiment, the method further comprises isolating a 14790 protein from the medium or the host cell.

The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which kinase-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous kinase sequences have been introduced into their genome or homologous recombinant animals in which endogenous kinase sequences have been altered. Such animals are useful for studying the function and/or activity of a kinase and for identifying and/or evaluating modulators of kinase activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, and the like. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, e.g., an embryonic cell of the animal, prior to development of the animal.
A transgenic animal of the invention can be created by introducing a kinase-encoding nucleic acid into the male pronuclei of a fertilized oocyte, e.g., by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The kinase cDNA sequence corresponding to SEQ ID NO:1 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of a human kinase gene, such as a mouse or rat kinase gene, can be used as a transgene. Alternatively, a kinase gene homologue, such as another kinase family member, can be isolated based on hybridization to the kinase cDNA sequences of SEQ ID NO:1, or the coding region thereof, (described further in subsection I above) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to a kinase transgene to direct expression of a 14790 protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, both by Leder et al., U.S. Patent No. 4,873,191 by Wagner et al. and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of a kinase transgene in its genome and/or expression of kinase mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding a 14790 protein can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a kinase gene into which a deletion, addition or substitution has been introduced to thereby alter, e.g., functionally disrupt, the kinase gene. The kinase gene can be a human gene (e.g., the SEQ ID NO:1), but more preferably, is a non-human homologue of a human kinase gene (e.g., a cDNA isolated by stringent hybridization with the nucleotide sequence of SEQ ID NO:1). For example, a mouse kinase gene can be used to construct a homologous recombination vector suitable for altering an endogenous kinase gene in the mouse genome. In a preferred embodiment, the vector is designed such that,
upon homologous recombination, the endogenous kinase gene is functionally disrupted (i.e., no longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous kinase gene is mutated or otherwise altered but still encodes a functional protein (e.g., the upstream regulatory region can be altered to thereby alter the expression of the endogenous 14790 protein). In the homologous recombination vector, the altered portion of the kinase gene is flanked at its 5' and 3' ends by additional nucleic acid sequence of the gene to allow for homologous recombination to occur between the exogenous kinase gene carried by the vector and an endogenous kinase gene in an embryonic stem cell. The additional flanking kinase nucleic acid sequence is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector (see e.g., Thomas, K.R. and Capecchi, M. R. (1987) *Cell* 51:503 for a description of homologous recombination vectors). The vector is introduced into an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced kinase gene has homologously recombined with the endogenous kinase gene are selected (see, e.g., Li, E. et al. (1992) *Cell* 69:915). The selected cells are then injected into a blastocyst of an animal (e.g., a mouse) to form aggregation chimeras (see e.g., Bradley, A. in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley, A. (1991) *Current Opinion in Biotechnology* 2:823-829 and in PCT International Publication Nos.: WO 90/11354 by Le Mouellec et al.; WO 91/01140 by Smithies et al.; WO 92/0968 by Zijlstra et al.; and WO 93/04169 by Berns et al.

In another embodiment, transgenic non-humans animals can be produced which contain selected systems which allow for regulated expression of the transgene. One example of such a system is the cre/loxP recombinase system of bacteriophage P1. For a
description of the cre/loxP recombinase system, see, e.g., Lakso et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman et al. (1991) *Science* 251:1351-1355. If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, e.g., by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmot, I. et al. (1997) *Nature* 385:810-813 and PCT International Publication Nos. WO 97/07668 and WO 97/07669. In brief, a cell, e.g., a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G0 phase. The quiescent cell can then be fused, e.g., through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyst and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, e.g., the somatic cell, is isolated.

**IV. Pharmaceutical Compositions**

The kinase nucleic acid molecules, 14790 proteins, and anti-kinase antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.
A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable
compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a 14790 protein or anti-kinase antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and
include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically
effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see e.g., Chen et al. (1994) Proc. Natl. Acad. Sci. USA 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.
V. **Uses and Methods of the Invention**

The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: a) screening assays; b) predictive medicine (e.g., diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenetics); and c) methods of treatment (e.g., therapeutic and prophylactic).

The isolated nucleic acid molecules of the invention can be used, for example, to express 14790 protein (e.g., via a recombinant expression vector in a host cell in gene therapy applications), to detect kinase mRNA (e.g., in a biological sample) or a genetic alteration in a kinase gene, and to modulate kinase activity, as described further below. The 14790 proteins can be used to treat disorders characterized by insufficient or excessive production of a kinase substrate or production of kinase inhibitors. In addition, the 14790 proteins can be used to screen for naturally occurring kinase substrates, to screen for drugs or compounds which modulate kinase activity, as well as to treat disorders characterized by insufficient or excessive production of 14790 protein or production of 14790 protein forms which have decreased or aberrant activity compared to kinase wild type protein. Moreover, the anti-kinase antibodies of the invention can be used to detect and isolate 14790 proteins, regulate the bioavailability of 14790 proteins, and modulate kinase activity.

A. **Screening Assays**

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, i.e., candidate or test compounds or agents (e.g., peptides, peptidomimetics, small molecules or other drugs) which bind to 14790 proteins, have a stimulatory or inhibitory effect on, for example, kinase expression or kinase activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of a kinase substrate.

In one embodiment, the invention provides assays for screening candidate or test compounds which are substrates of a 14790 protein or polypeptide or biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a 14790 protein or polypeptide or biologically active portion thereof, e.g., modulate the ability of kinase to interact with its cognate ligand. The test compounds of the present invention can be
obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).


In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a kinase target molecule (e.g., a kinase phosphorylation substrate) with a test compound and determining the ability of the test compound to modulate (e.g. stimulate or inhibit) the activity of the kinase target molecule. Determining the ability of the test compound to modulate the activity of a kinase target molecule can be accomplished, for example, by determining the ability of the 14790 protein to bind to or interact with the target molecule, or by determining the ability of the protein to phosphorylate the kinase target molecule.

The ability of the protein to phosphorylate a kinase target molecule can be determined by, for example, an *in vitro* kinase assay. Briefly, a kinase target molecule,
e.g., an immunoprecipitated kinase target molecule from a cell line expressing such a molecule, can be incubated with the 14790 protein and radioactive ATP, e.g., [γ-32P] ATP, in a buffer containing MgCl₂ and MnCl₂, e.g., 10 mM MgCl₂ and 5 mM MnCl₂.

Following the incubation, the immunoprecipitated kinase target molecule can be separated by SDS-polyacrylamide gel electrophoresis under reducing conditions, transferred to a membrane, e.g., a PVDF membrane, and autoradiographed. The appearance of detectable bands on the autoradiograph indicates that the kinase substrate has been phosphorylated. Phosphoaminoacid analysis of the phosphorylated substrate can also be performed in order to determine which residues on the substrate are phosphorylated. Briefly, the radiophosphorylated protein band can be excised from the SDS gel and subjected to partial acid hydrolysis. The products can then be separated by one-dimensional electrophoresis and analyzed on, for example, a phosphoimager and compared to ninhydrin-stained phosphoaminoacid standards.

Determining the ability of the 14790 protein to bind to or interact with a kinase target molecule can be accomplished by determining direct binding. Determining the ability of the 14790 protein to bind to or interact with a target molecule can be accomplished, for example, by coupling the 14790 protein with a radioisotope or enzymatic label such that binding of the protein to a kinase target molecule can be determined by detecting the labeled protein in a complex. For example, molecules, e.g., proteins, can be labeled with 125I, 35S, 14C, or 3H, either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, molecules can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

It is also within the scope of this invention to determine the ability of a compound to modulate the interaction between kinase and its target molecule, without the labeling of any of the interactants. For example, a microphysiometer can be used to detect the interaction of with its target molecule without the labeling of either kinase or the target molecule. McConnell, H. M. et al. (1992) Science 257:1906-1912. As used herein, a "microphysiometer" (e.g., Cytosensor) is an analytical instrument that measures the rate at
which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between compound and receptor.

In a preferred embodiment, determining the ability of the 14790 protein to bind to or interact with a kinase target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (e.g., intracellular Ca\(^{2+}\), diacylglycerol, IP\(_3\), etc.), detecting catalytic/enzymatic activity of the target an appropriate substrate, detecting the induction of a reporter gene (comprising a target-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, e.g., chloramphenicol acetyl transferase), or detecting a target-regulated cellular response.

In yet another embodiment, an assay of the present invention is a cell-free assay in which a protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the 14790 protein or biologically active portion thereof is determined. Binding of the test compound to the 14790 protein can be determined either directly or indirectly as described above. In a preferred embodiment, the assay includes contacting the 14790 protein or biologically active portion thereof with a known compound which binds kinase to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a 14790 protein, wherein determining the ability of the test compound to interact with a 14790 protein comprises determining the ability of the test compound to preferentially bind to kinase or biologically active portion thereof as compared to the known compound.

In another embodiment, the assay is a cell-free assay in which a 14790 protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate (e.g., stimulate or inhibit) the activity of the 14790 protein or biologically active portion thereof is determined. Determining the ability of the test compound to modulate the activity of a 14790 protein can be accomplished, for example, by determining the ability of the 14790 protein to bind to a kinase target molecule by one of the methods described above for determining direct binding. Determining the ability of the 14790 protein to bind to a kinase target molecule can also be accomplished using a

In an alternative embodiment, determining the ability of the test compound to modulate the activity of a 14790 protein can be accomplished by determining the ability of the 14790 protein to further modulate the activity of a kinase target molecule (e.g., a kinase mediated signal transduction pathway component). For example, the activity of the effector molecule on an appropriate target can be determined, or the binding of the effector to an appropriate target can be determined as previously described.

In yet another embodiment, the cell-free assay involves contacting a 14790 protein or biologically active portion thereof with a known compound which binds the 14790 protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the 14790 protein, wherein determining the ability of the test compound to interact with the 14790 protein comprises determining the ability of the 14790 protein to preferentially bind to or modulate the activity of a kinase target molecule.

The cell-free assays of the present invention are amenable to use of both soluble and/or membrane-bound forms of proteins (e.g., 14790 proteins or biologically active portions thereof, or receptors to which kinase binds). In the case of cell-free assays in which a membrane-bound form a 14790 protein is used (e.g., a cell surface kinase receptor) it may be desirable to utilize a solubilizing agent such that the membrane-bound form of the 14790 protein is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®, Isotridecypoly(ethylene glycol ether)$_{10}$, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-
cholamidopropyl]dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-
dodecyl=NN,N-dimethyl-3-ammonio-1-propane sulfonate.

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either kinase or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of kinase a test compound to a protein, or interaction of a 14790 protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and microcentrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/kinase fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtitre plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or 14790 protein, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtitre plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of kinase binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either a 14790 protein or a kinase target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated 14790 protein or target molecules can be prepared from biotin-NHS (N-hydroxysuccinimide) using techniques well known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with 14790 protein or target molecules but which do not interfere with binding of the 14790 protein to its target molecule can be derivatized to the wells of the plate, and unbound target or 14790 protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in
addition to those described above for the GST-immobilized complexes, include immunodeection of complexes using antibodies reactive with the 14790 protein or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the 14790 protein or target molecule.

In another embodiment, modulators of kinase expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of kinase mRNA or protein in the cell is determined. The level of expression of kinase mRNA or protein in the presence of the candidate compound is compared to the level of expression of kinase mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of kinase expression based on this comparison. For example, when expression of kinase mRNA or protein is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of kinase mRNA or protein expression. Alternatively, when expression of kinase mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of kinase mRNA or protein expression. The level of kinase mRNA or protein expression in the cells can be determined by methods described herein for detecting kinase mRNA or protein.

In yet another aspect of the invention, the 14790 proteins can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, e.g., U.S. Patent No. 5,283,317; Zervos et al. (1993) Cell 72:223-232; Madura et al. (1993) J. Biol. Chem. 268:12046-12054; Bartel et al. (1993) Biotechniques 14:920-924; Iwabuchi et al. (1993) Oncogene 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact with kinase ("kinase-binding proteins" or "kinase-bp") and are involved in kinase activity. Such kinase-binding proteins are also likely to be involved in the propagation of signals by the 14790 proteins or kinase targets as, for example, downstream elements of a kinase-mediated signaling pathway. Alternatively, such kinase-binding proteins are likely to be kinase inhibitors.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes
two different DNA constructs. In one construct, the gene that codes for a 14790 protein is fused to a gene encoding the DNA binding domain of a known transcription factor (e.g., GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, in vivo, forming a kinase dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (e.g., LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the 14790 protein which interacts with the protein.

This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein in an appropriate animal model. For example, an agent identified as described herein (e.g., a kinase modulating agent, an antisense kinase nucleic acid molecule, a kinase-specific antibody, or a kinase-binding partner) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

B. Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.
1. **Chromosome Mapping**

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the kinase nucleotide sequences, described herein, can be used to map the location of the kinase genes on a chromosome. The mapping of the kinase sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, kinase genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the kinase nucleotide sequences. Computer analysis of the kinase sequences can be used to predict primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the kinase sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (e.g., human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but human cells can, the one human chromosome that contains the gene encoding the needed enzyme, will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. (D'Eustachio P. et al. (1983) *Science* 220:919-924). Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the kinase nucleotide sequences to design oligonucleotide primers, sublocalization can be achieved with panels of fragments from
specific chromosomes. Other mapping strategies which can similarly be used to map a 90, 1p, or 1v kinase sequence to its chromosome include in situ hybridization (described in Fan, Y. et al. (1990) *Proc. Natl. Acad. Sci. USA*, 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries.

Fluorescence in situ hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical such as colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases will suffice to get good results at a reasonable amount of time. For a review of this technique, see Verma et al., *Human Chromosomes: A Manual of Basic Techniques* (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man, available on-line through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland, J. et al. (1987) *Nature*, 325:783-787.
Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

2. **Tissue Typing**

The kinase sequences of the present invention can also be used to identify individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

Furthermore, the sequences of the present invention can be used to provide an alternative technique which determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the kinase nucleotide sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the present invention can be used to obtain such identification sequences from individuals and from tissue. The kinase nucleotide sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences,
and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NO:1 can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers which each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as nucleotides 1-4947 in SEQ ID NO:1 are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

If a panel of reagents from nucleotide sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from extremely small tissue samples.

3. Use of Partial Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. Forensic biology is a scientific field employing genetic typing of biological evidence found at a crime scene as a means for positively identifying, for example, a perpetrator of a crime. To make such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, e.g., PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (i.e. another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated
fragments. Sequences targeted to noncoding regions of SEQ ID NO:1 are particularly appropriate for this use as greater numbers of polymorphisms occur in the noncoding regions, making it easier to differentiate individuals using this technique. Examples of polynucleotide reagents include the kinase nucleotide sequences or portions thereof, e.g., fragments derived from the noncoding regions of SEQ ID NO:1 having a length of at least 20 bases, preferably at least 30 bases.

The kinase nucleotide sequences described herein can further be used to provide polynucleotide reagents, e.g., labeled or labelable probes which can be used in, for example, an in situ hybridization technique, to identify a specific tissue, e.g., brain tissue. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such kinase probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, e.g., kinase primers or probes can be used to screen tissue culture for contamination (i.e. screen for the presence of a mixture of different types of cells in a culture).

C. Predictive Medicine:

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining 14790 protein and/or nucleic acid expression as well as kinase activity, in the context of a biological sample (e.g., blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant kinase expression or activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with 14790 protein, nucleic acid expression or activity. For example, mutations in a kinase gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby phophylactically treat an individual prior to the onset of a disorder characterized by or associated with 14790 protein, nucleic acid expression or activity.
Another aspect of the invention pertains to monitoring the influence of agents (e.g., drugs, compounds) on the expression or activity of kinase in clinical trials.

These and other agents are described in further detail in the following sections.

1. **Diagnostic Assays**

   An exemplary method for detecting the presence or absence of 14790 protein or nucleic acid in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting 14790 protein or nucleic acid (e.g., mRNA, genomic DNA) that encodes 14790 protein such that the presence of 14790 protein or nucleic acid is detected in the biological sample. A preferred agent for detecting mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to mRNA or genomic DNA. The nucleic acid probe can be, for example, a human nucleic acid, such as the nucleic acid of SEQ ID NO:1 or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to kinase mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

   A preferred agent for detecting protein is an antibody capable of binding to protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')2) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect kinase mRNA, protein, or genomic DNA in a biological sample in vitro as well as in vivo. For example, in vitro techniques for detection of kinase mRNA include Northern
hybridizations and in situ hybridizations. In vitro techniques for detection of 14790 protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. In vitro techniques for detection of kinase genomic DNA include Southern hybridizations. Furthermore, in vivo techniques for detection of 14790 protein include introducing into a subject a labeled anti-kinase antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains 14790 protein molecules from the test subject. Alternatively, the biological sample can contain kinase mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a serum sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting 14790 protein, mRNA, or genomic DNA, such that the presence of 14790 protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of 14790 protein, mRNA or genomic DNA in the control sample with the presence of 14790 protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of kinase in a biological sample. For example, the kit can comprise a labeled compound or agent capable of detecting 14790 protein or mRNA in a biological sample; means for determining the amount of kinase in the sample; and means for comparing the amount of kinase in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect 14790 protein or nucleic acid.

2. Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant kinase expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject
having or at risk of developing a disorder associated with 14790 protein, nucleic acid expression or activity. Thus, the present invention provides a method for identifying a disease or disorder associated with aberrant kinase expression or activity in which a test sample is obtained from a subject and 14790 protein or nucleic acid (e.g., mRNA, genomic DNA) is detected, wherein the presence of 14790 protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant kinase expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (e.g., serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant kinase expression or activity. Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant kinase expression or activity in which a test sample is obtained and 14790 protein or nucleic acid expression or activity is detected (e.g., wherein the abundance of 14790 protein or nucleic acid expression or activity is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant kinase expression or activity).

The methods of the invention can also be used to detect genetic alterations in a kinase gene, thereby determining if a subject with the altered gene is at risk for a disorder associated with the kinase gene. In preferred embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding a kinase-protein, or the mis-expression of the kinase gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from a kinase gene; 2) an addition of one or more nucleotides to a kinase gene; 3) a substitution of one or more nucleotides of a kinase gene; 4) a chromosomal rearrangement of a kinase gene; 5) an alteration in the level of a messenger RNA transcript of a kinase gene; 6) aberrant modification of a kinase gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing
pattern of a messenger RNA transcript of a kinase gene, 8) a non-wild type level of a kinase-protein, 9) allelic loss of a gene, and 10) inappropriate post-translational modification of a kinase-protein. As described herein, there are a large number of assay techniques known in the art which can be used for detecting alterations in a kinase gene. A preferred biological sample is a tissue or serum sample isolated by conventional means from a subject, e.g., a hepatic tissue sample.

In certain embodiments, detection of the alteration involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, e.g., U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, e.g., Landegran et al. (1988) Science 241:1077-1080; and Nakazawa et al. (1994) Proc. Natl. Acad. Sci. USA 91:360-364), the latter of which can be particularly useful for detecting point mutations in the kinase-gene (see Abravaya et al. (1995) Nucleic Acids Res. 23:675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (e.g., genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a kinase gene under conditions such that hybridization and amplification of the kinase-gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

In an alternative embodiment, mutations in a kinase gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in kinase can be identified by hybridizing a sample and control nucleic acids, e.g., DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin, M.T. et al. (1996) Human Mutation 7: 244-255; Kozal, M.J. et al. (1996) Nature Medicine 2: 753-759). For example, genetic mutations in kinase can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, M.T. et al. supra. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the kinase gene and detect mutations by comparing the sequence of the kinase sample with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxam and Gilbert ((1977) Proc. Natl. Acad. Sci. USA 74:560) or Sanger ((1977) Proc. Natl. Acad. Sci. USA 74:5463). It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) Biotechniques 19:448), including sequencing by mass spectrometry (see, e.g., PCT International Publication No. WO 94/16101; Cohen et al. (1996) Adv. Chromatogr. 36:127-162; and Griffin et al. (1993) Appl. Biochem. Biotechnol. 38:147-159).
Other methods for detecting mutations in the kinase gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers et al. (1985) Science 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes formed by hybridizing (labeled) RNA or DNA containing the wild-type kinase sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton et al. (1988) Proc. Natl Acad Sci USA 85:4397; Saleeba et al. (1992) Methods Enzymol. 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in kinase cDNAs obtained from samples of cells. For example, the mutY enzyme of E. coli cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu et al. (1994) Carcinogenesis 15:1657-1662). According to an exemplary embodiment, a probe based on kinase a sequence, e.g., a wild-type kinase sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in kinase genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and
wild type nucleic acids (Orita et al. (1989) Proc Natl. Acad. Sci USA: 86:2766, see also Cotton (1993) Mutat Res 285:125-144; and Hayashi (1992) Genet Anal Tech Appl 9:73-79). Single-stranded DNA fragments of sample and control kinase nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen et al. (1991) Trends Genet 7:5).

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers et al. (1985) Nature 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) Biophys Chem 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki et al. (1986) Nature 324:163); Saiki et al. (1989) Proc. Natl Acad. Sci USA 86:6230). Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides
used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs et al. (1989) Nucleic Acids Res. 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner et al. (1993) Tibtech 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini et al. (1992) Mol. Cell Probes 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) Proc. Natl. Acad. Sci USA 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5’ sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a kinase gene.

Furthermore, any cell type or tissue in which kinase is expressed may be utilized in the prognostic assays described herein.

3. Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (e.g., drugs or compounds) on the expression or activity of a 14790 protein can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase kinase gene expression, protein levels, or upregulate kinase activity, can be monitored in clinical trials of subjects exhibiting decreased kinase gene expression, protein levels, or downregulated kinase activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease kinase gene expression, protein levels, or downregulate kinase activity, can be monitored in clinical trials of subjects exhibiting increased kinase gene expression, protein levels, or upregulated activity. In such clinical trials, the expression or activity of a kinase gene, and preferably,
other genes that have been implicated in a disorder can be used as a "read out" or markers of the phenotype of a particular cell.

For example, and not by way of limitation, genes, including kinase, that are modulated in cells by treatment with an agent (e.g., compound, drug or small molecule) which modulates kinase activity (e.g., identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on a kinase associated disorder, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of kinase and other genes implicated in the kinase associated disorder, respectively. The levels of gene expression (i.e., a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of kinase or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during treatment of the individual with the agent.

In a preferred embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of a 14790 protein, mRNA, or genomic DNA in the pre-administration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the 14790 protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the 14790 protein, mRNA, or genomic DNA in the pre-administration sample with the 14790 protein, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of kinase to higher levels than detected, i.e., to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of kinase to lower levels than detected, i.e. to decrease the
effectiveness of the agent. According to such an embodiment, kinase expression or activity may be used as an indicator of the effectiveness of an agent, even in the absence of an observable phenotypic response.

4. Use of 14790 Molecules as Surrogate Markers

The 14790 molecules of the invention are also useful as markers of disorders or disease states, as markers for precursors of disease states, as markers for predisposition of disease states, as markers of drug activity, or as markers of the pharmacogenomic profile of a subject. Using the methods described herein, the presence, absence and/or quantity of the 14790 molecules of the invention may be detected, and may be correlated with one or more biological states in vivo. For example, the 14790 molecules of the invention may serve as surrogate markers for one or more disorders or disease states or for conditions leading up to disease states. As used herein, a “surrogate marker” is an objective biochemical marker which correlates with the absence or presence of a disease or disorder, or with the progression of a disease or disorder (e.g., with the presence or absence of a tumor). The presence or quantity of such markers is independent of the disease. Therefore, these markers may serve to indicate whether a particular course of treatment is effective in lessening a disease state or disorder. Surrogate markers are of particular use when the presence or extent of a disease state or disorder is difficult to assess through standard methodologies (e.g., early stage tumors), or when an assessment of disease progression is desired before a potentially dangerous clinical endpoint is reached (e.g., an assessment of cardiovascular disease may be made using cholesterol levels as a surrogate marker, and an analysis of HIV infection may be made using HIV RNA levels as a surrogate marker, well in advance of the undesirable clinical outcomes of myocardial infarction or fully-developed AIDS). Examples of the use of surrogate markers in the art include: Koomen et al. (2000) J. Mass. Spectrom. 35: 258-264; and James (1994) AIDS Treatment News Archive 209.

The 14790 molecules of the invention are also useful as pharmacodynamic markers. As used herein, a “pharmacodynamic marker” is an objective biochemical marker which correlates specifically with drug effects. The presence or quantity of a pharmacodynamic marker is not related to the disease state or disorder for which the drug is being administered; therefore, the presence or quantity of the marker is indicative of the presence
or activity of the drug in a subject. For example, a pharmacodynamic marker may be indicative of the concentration of the drug in a biological tissue, in that the marker is either expressed or transcribed or not expressed or transcribed in that tissue in relationship to the level of the drug. In this fashion, the distribution or uptake of the drug may be monitored by the pharmacodynamic marker. Similarly, the presence or quantity of the pharmacodynamic marker may be related to the presence or quantity of the metabolic product of a drug, such that the presence or quantity of the marker is indicative of the relative breakdown rate of the drug in vivo. Pharmacodynamic markers are of particular use in increasing the sensitivity of detection of drug effects, particularly when the drug is administered in low doses. Since even a small amount of a drug may be sufficient to activate multiple rounds of marker (e.g., a 14790 marker) transcription or expression, the amplified marker may be in a quantity which is more readily detectable than the drug itself. Also, the marker may be more easily detected due to the nature of the marker itself; for example, using the methods described herein, anti-14790 antibodies may be employed in an immune-based detection system for a 14790 protein marker, or 14790-specific radiolabeled probes may be used to detect a 14790 mRNA marker. Furthermore, the use of a pharmacodynamic marker may offer mechanism-based prediction of risk due to drug treatment beyond the range of possible direct observations. Examples of the use of pharmacodynamic markers in the art include: Matsuda et al. US 6,033,862; Hattis et al. (1991) Env. Health Perspect. 90: 229-238; Schentag (1999) Am. J. Health-Syst. Pharm. 56 Suppl. 3: S21-S24; and Nicolau (1999) Am. J. Health-Syst. Pharm. 56 Suppl. 3: S16-S20.

The 14790 molecules of the invention are also useful as pharmacogenomic markers. As used herein, a "pharmacogenomic marker" is an objective biochemical marker which correlates with a specific clinical drug response or susceptibility in a subject (see, e.g., McLeod et al. (1999) Eur. J. Cancer 35(12): 1650-1652). The presence or quantity of the pharmacogenomic marker is related to the predicted response of the subject to a specific drug or class of drugs prior to administration of the drug. By assessing the presence or quantity of one or more pharmacogenomic markers in a subject, a drug therapy which is most appropriate for the subject, or which is predicted to have a greater degree of success, may be selected. For example, based on the presence or quantity of RNA, or protein (e.g., 14790 protein or RNA) for specific tumor markers in a subject, a drug or course of
treatment may be selected that is optimized for the treatment of the specific tumor likely to be present in the subject. Similarly, the presence or absence of a specific sequence mutation in 14790 DNA may correlate 14790 drug response. The use of pharmacogenomic markers therefore permits the application of the most appropriate treatment for each subject without having to administer the therapy.

D. Methods of Treatment:

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant kinase expression or activity. With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. “Treatment”, as used herein, is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease, the symptoms of disease or the predisposition toward disease. A therapeutic agent includes, but is not limited to, small molecules, peptides, antibodies, ribozymes and antisense oligonucleotides. "Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers the study of how a patient's genes determine his or her response to a drug (e.g., a patient's "drug response phenotype", or "drug response genotype"). Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the 14790 molecules of the present invention or kinase modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

1. Prophylactic Methods

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In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant kinase expression or activity, by administering to the subject or an agent which modulates kinase expression or at least one kinase activity. Subjects at risk for a disease which is caused or contributed to by aberrant kinase expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of kinase aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of kinase aberrancy, for example, a kinase, kinase agonist or kinase antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

2. Therapeutic Methods

Another aspect of the invention pertains to methods of modulating kinase expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell with a kinase or agent that modulates one or more of the activities of 14790 protein activity associated with the cell. An agent that modulates 14790 protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring target molecule of a 14790 protein (e.g., a kinase phosphorylation substrate), a kinase antibody, a kinase agonist or antagonist, a peptidomimetic of a kinase agonist or antagonist, or other small molecule. In one embodiment, the agent stimulates one or more kinase activities. Examples of such stimulatory agents include active 14790 protein and a nucleic acid molecule encoding kinase that has been introduced into the cell. In another embodiment, the agent inhibits one or more kinase activities. Examples of such inhibitory agents include antisense kinase nucleic acid molecules, anti-kinase antibodies, and kinase inhibitors. These modulatory methods can be performed in vitro (e.g., by culturing the cell with the agent) or, alternatively, in vivo (e.g, by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of a 14790 protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates
(e.g., upregulates or downregulates) kinase expression or activity. In another embodiment, the method involves administering a 14790 protein or nucleic acid molecule as therapy to compensate for reduced or aberrant kinase expression or activity.

Stimulation of kinase activity is desirable in situations in which kinase is abnormally downregulated and/or in which increased kinase activity is likely to have a beneficial effect. For example, stimulation of kinase activity is desirable in situations in which a kinase is downregulated and/or in which increased kinase activity is likely to have a beneficial effect. Likewise, inhibition of kinase activity is desirable in situations in which kinase is abnormally upregulated and/or in which decreased kinase activity is likely to have a beneficial effect.

3. Pharmacogenomics

The 14790 molecules of the present invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on kinase activity (e.g., kinase gene expression) as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) disorders (e.g., infection with HBV) associated with aberrant kinase activity. In conjunction with such treatment, pharmacogenomics (i.e., the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer a 14790 molecule or kinase modulator as well as tailoring the dosage and/or therapeutic regimen of treatment with a 14790 molecule or kinase modulator.

conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (e.g., a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict a drug response. According to this method, if a gene that encodes a drug target is known (e.g., a 14790 protein or kinase receptor of the present invention), all common variants of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.
As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (e.g., N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so-called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Alternatively, a method termed the "gene expression profiling", can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (e.g., a 14790 molecule or kinase modulator of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a 14790 molecule or kinase modulator, such as a modulator identified by one of the exemplary screening assays described herein.
This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are incorporated herein by reference.

EXAMPLES

EXAMPLE 1 EXPRESSION AND TISSUE DISTRIBUTION OF 14790 OR 14790 mRNA

Northern blot hybridizations with various RNA samples can be performed under standard conditions and washed under stringent conditions, i.e., 0.2xSSC at 65°C. A DNA probe corresponding to all or a portion of the 14790 cDNA (SEQ ID NO:1) can be used. The DNA was radioactively labeled with $^{32}$P-dCTP using the Prime-It Kit (Stratagene, La Jolla, CA) according to the instructions of the supplier. Filters containing mRNA from mouse hematopoietic and endocrine tissues, and cancer cell lines (Clontech, Palo Alto, CA) can be probed in ExpressHyb hybridization solution (Clontech) and washed at high stringency according to manufacturer's recommendations.

TaqMan real-time quantitative RT-PCR was used to detect the presence of mRNA transcript corresponding to human 14790 in several tissues. It was found that the corresponding orthologs of 14790 are expressed in a variety of tissues. The results of this screening are shown in Figures 3-5. Tissue distribution of 14790 mRNA was also determined by in situ hybridization.

14790 mRNA was found to be expressed in human skeletal muscle, brain and liver. TaqMan RT-PCR analysis revealed that 14790 mRNA was found to be upregulated in liver cells which were infected with HBV as shown in Figure 3. Moreover, mRNA expression of 14790 was found to be restricted to hepatocytes of HBV infected livers as seen by in situ hybridization. 14790 mRNA was also found to be upregulated in HepG2.2.15 cells (HBV positive) compared to HepG2 parent cells (HBV negative) as shown in Figure 4. When HepG2.2.15 cells were treated with anti-HBV drug treatment, the upregulation of 14790 mRNA was eliminated. Thus indicating that a modulator of 14790 activity or mRNA may be used to treat infection by HBV.

EXAMPLE 2: EXPRESSION OF RECOMBINANT 14790 PROTEIN IN BACTERIAL CELLS
In this example, 14790 is expressed as a recombinant glutathione-S-transferase (GST) fusion polypeptide in *E. coli* and the fusion polypeptide is isolated and characterized. Specifically, 14790 is fused to GST and this fusion polypeptide is expressed in *E. coli*, e.g., strain PEB199. Expression of the GST-14790 fusion protein in PEB199 is induced with IPTG. The recombinant fusion polypeptide is purified from crude bacterial lysates of the induced PEB199 strain by affinity chromatography on glutathione beads. Using polyacrylamide gel electrophoretic analysis of the polypeptide purified from the bacterial lysates, the molecular weight of the resultant fusion polypeptide is determined.

**EXAMPLE 3: EXPRESSION OF RECOMBINANT 14790 PROTEIN IN COS CELLS**

To express the 14790 gene in COS cells, the pcDNA/Amp vector by Invitrogen Corporation (San Diego, CA) is used. This vector contains an SV40 origin of replication, an ampicillin resistance gene, an *E. coli* replication origin, a CMV promoter followed by a polylinker region, and an SV40 intron and polyadenylation site. A DNA fragment encoding the entire 14790 protein and an HA tag (Wilson *et al.* (1984) *Cell* 37:767) or a FLAG tag fused in-frame to its 3' end of the fragment is cloned into the polylinker region of the vector, thereby placing the expression of the recombinant protein under the control of the CMV promoter.

To construct the plasmid, the 14790 DNA sequence is amplified by PCR using two primers. The 5' primer contains the restriction site of interest followed by approximately twenty nucleotides of the 14790 coding sequence starting from the initiation codon; the 3' end sequence contains complementary sequences to the other restriction site of interest, a translation stop codon, the HA tag or FLAG tag and the last 20 nucleotides of the 14790 coding sequence. The PCR amplified fragment and the pcDNA/Amp vector are digested with the appropriate restriction enzymes and the vector is dephosphorylated using the CIAP enzyme (New England Biolabs, Beverly, MA). Preferably the two restriction sites chosen are different so that the 14790 gene is inserted in the correct orientation. The ligation mixture is transformed into *E. coli* cells (strains HB101, DH5α, SURE, available from Stratagene Cloning Systems, La Jolla, CA, can be used), the transformed culture is plated on ampicillin media plates, and resistant colonies are selected. Plasmid DNA is isolated
from transformants and examined by restriction analysis for the presence of the correct fragment.

COS cells are subsequently transfected with the 14790 -pcDNA/Amp plasmid DNA using the calcium phosphate or calcium chloride co-precipitation methods, DEAE-dextran-mediated transfection, lipofection, or electroporation. Other suitable methods for transfecting host cells can be found in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd ed., Cold Spring Harbor Laboratory*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989. The expression of the VR-3 or VR-5 polypeptide is detected by radiolabelling ($^{35}$S-methionine or $^{35}$S -cysteine available from NEN, Boston, MA, can be used) and immunoprecipitation (Harlow, E. and Lane, D. *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988) using an HA specific monoclonal antibody. Briefly, the cells are labelled for 8 hours with $^{35}$S-methionine (or $^{35}$S-cysteine). The culture media are then collected and the cells are lysed using detergents (RIPA buffer, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM Tris, pH 7.5). Both the cell lysate and the culture media are precipitated with an HA specific monoclonal antibody. Precipitated polypeptides are then analyzed by SDS-PAGE.

Alternatively, DNA containing the 14790 coding sequence is cloned directly into the polylinker of the pCDNA/Amp vector using the appropriate restriction sites. The resulting plasmid is transfected into COS cells in the manner described above, and the expression of the 14790 polypeptide is detected by radiolabelling and immunoprecipitation using a 14790 specific monoclonal antibody.

**EXAMPLE 4: ANALYSIS OF HUMAN 14790**

A BLASTN 1.4.9 search, using a score of 100 and a word length of 12 (Altschul et al. (1990) *J. Mol. Biol.* 215:403) of the nucleotide sequence of human kinase revealed that kinase is similar to the human protein kinase HPK-1 coding sequence (Accession No. V23831). This nucleic acid molecule is approximately 70% identical to kinase, over nucleotides 388-1214.

**EXAMPLE 5: ISOLATION OF HUMAN 14790 cDNA**

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The invention is based, at least in part, on the discovery of a human gene encoding a novel member of the kinase family. The human kinase family members were isolated from cDNA. A cDNA library was prepared therefrom using art known methods (described in, for example, Molecular Cloning A Laboratory Manual, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1989). Positive clones were isolated following comparison to homologs in public protein databases, including a comparison with known kinases and/or examination of the sequence for protein motifs of kinases.

The sequences of the positive clones were determined and found to contain open reading frames. The nucleotide sequence encoding the human 14790 protein is shown in Figure 1 and is set forth as SEQ ID NO:1. The protein encoded by this nucleic acid comprises about 1649 amino acids and has the amino acid sequence shown in Figure 1 and set forth as SEQ ID NO:2. The coding region (open reading frame) of SEQ ID NO:1 is shown in Figure 1 as SEQ ID NO:3, the portion of the nucleotide sequence corresponding to the amino acid sequence of SEQ ID NO:2. The clone comprising the entire coding region of human kinase was deposited with the American Type Culture Collection (ATCC®), 10801 University Boulevard, Manassas, VA 20110-2209, on ______, 1998, and assigned Accession No. ______.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.
What is claimed is:

1. An isolated 14790 nucleic acid molecule selected from the group consisting of:
   a) a nucleic acid molecule comprising a nucleotide sequence which is at least 60% identical to the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____;
   b) a nucleic acid molecule comprising a fragment of at least 300 nucleotides of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____;
   c) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____;
   d) a nucleic acid molecule which encodes a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____;
   e) a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, or a complement thereof, under stringent conditions;
f) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____; and

g) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____.

2. The isolated nucleic acid molecule of claim 1, which is the nucleotide sequence SEQ ID NO:1.

3. A host cell which contains the nucleic acid molecule of claim 1 or 2.

4. An isolated 14790 polypeptide selected from the group consisting of:

   a) a polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 60% identical to a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a complement thereof;

   b) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____, wherein the polypeptide is encoded by a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, or a complement thereof under stringent conditions;

   c) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2; and

   d) the amino acid sequence of SEQ ID NO:2.

5. An antibody which selectively binds to a polypeptide of claim 4.
6. A method for producing a polypeptide selected from the group consisting of:

   a) a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______;

   b) a polypeptide comprising a fragment of the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______;

   c) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number ______, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:1 or SEQ ID NO:3; and

   d) the amino acid sequence of SEQ ID NO:2; comprising culturing the host cell of claim 3 under conditions in which the nucleic acid molecule is expressed.

7. A method for detecting the presence of a nucleic acid molecule of claim 1 or a polypeptide encoded by the nucleic acid molecule in a sample, comprising:

   a) contacting the sample with a compound which selectively hybridizes to the nucleic acid molecule of claim 1 or binds to the polypeptide encoded by the nucleic acid molecule; and

   b) determining whether the compound hybridizes to the nucleic acid or binds to the polypeptide in the sample.

8. A kit comprising:
a) a compound which selectively hybridizes to a nucleic acid molecule of claim 1 or binds to a polypeptide encoded by the nucleic acid molecule; and

b) instructions for use.

9. A method for identifying a compound which binds to a polypeptide or modulates the activity of the polypeptide of claim 4 comprising the steps of:

   a) contacting a polypeptide, or a cell expressing a polypeptide of claim 4 with a test compound; and

   b) determining whether the polypeptide binds to the test compound or determining the effect of the test compound on the activity of the polypeptide.

10. A method for modulating the activity of a polypeptide of claim 4 comprising contacting the polypeptide or a cell expressing the polypeptide with a compound which binds to the polypeptide in a sufficient concentration to modulate the activity of the polypeptide.

11. A method of identifying a nucleic acid molecule associated with a hepatic associated disorder comprising:

   a) contacting a sample from a subject with or at risk of developing a hepatic associated disorder comprising nucleic acid molecules with a hybridization probe comprising at least 25 contiguous nucleotides of SEQ ID NO:1 defined in claim 2; and

   b) detecting the presence of a nucleic acid molecule in the sample that hybridizes to the probe, thereby identifying a nucleic acid molecule associated with a hepatic associated disorder.

12. A method of identifying a nucleic acid associated with a hepatic associated disorder comprising:

   a) contacting a sample from a subject having a hepatic associated disorder or at risk of developing a hepatic associated disorder comprising nucleic acid molecules
with a first and a second amplification primer, the first primer comprising at least 25 contiguous nucleotides of SEQ ID NO:1 defined in claim 2 and the second primer comprising at least 25 contiguous nucleotides from the complement of SEQ ID NO:1;

b) incubating the sample under conditions that allow nucleic acid amplification; and

c) detecting the presence of a nucleic acid molecule in the sample that is amplified, thereby identifying the nucleic acid molecule associated with a hepatic associated disorder.

13. A method of identifying a polypeptide associated with a hepatic associated disorder comprising:

a) contacting a sample comprising polypeptides with a 14790 binding partner of the 14790 polypeptide defined in claim 4; and

b) detecting the presence of a polypeptide in the sample that binds to the 14790 binding partner, thereby identifying the polypeptide associated with a hepatic associated disorder.

14. A method of identifying a subject having a hepatic associated disorder or at risk for developing a hepatic associated disorder comprising:

a) contacting a sample obtained from the subject comprising nucleic acid molecules with a hybridization probe comprising at least 25 contiguous nucleotides of SEQ ID NO:1 defined in claim 2; and

b) detecting the presence of a nucleic acid molecule in the sample that hybridizes to the probe, thereby identifying a subject having a hepatic associated disorder or at risk for developing a hepatic associated disorder.

15. A method of identifying a subject having a hepatic associated disorder or at risk for developing a hepatic associated disorder comprising:
a) contacting a sample obtained from the subject comprising nucleic acid molecules with a first and a second amplification primer, the first primer comprising at least 25 contiguous nucleotides of SEQ ID NO:1 defined in claim 2 and the second primer comprising at least 25 contiguous nucleotides from the complement of SEQ ID NO:1;

b) incubating the sample under conditions that allow nucleic acid amplification; and

c) detecting the presence of a nucleic acid molecule in the sample that is amplified, thereby identifying a subject having a hepatic associated disorder or at risk for developing a hepatic associated disorder.

16. A method of identifying a subject having a hepatic associated disorder or at risk for developing a hepatic associated disorder comprising:

a) contacting a sample obtained from the subject comprising polypeptides with a 14790 binding partner of the 14790 polypeptide defined in claim 4; and

b) detecting the presence of a polypeptide in the sample that binds to the 14790 binding partner, thereby identifying a subject having a hepatic associated disorder or at risk for developing a hepatic associated disorder.

17. A method for identifying a compound capable of treating a hepatic associated disorder characterized by aberrant 14790 nucleic acid expression or 14790 polypeptide activity comprising assaying the ability of the compound to modulate 14790 nucleic acid expression or 14790 polypeptide activity, thereby identifying a compound capable of treating a hepatic associated disorder characterized by aberrant 14790 nucleic acid expression or 14790 polypeptide activity.

18. The method of claim 17, wherein the hepatic associated disorder is liver fibrosis or infection with HBV.

19. A method for treating a subject having a hepatic associated disorder or at risk of developing a hepatic associated disorder comprising
administering to the subject a 14790 modulator of the nucleic acid molecule defined in claim 1 or the polypeptide encoded by the nucleic acid molecule or contacting a cell with a 14790 modulator.

20. The method of claim 19, wherein the a hepatic associated disorder is liver fibrosis or infection with HBV.

21. The method of claim 19, wherein the 14790 modulator is

i) a small molecule;

ii) peptide;

iii) phosphopeptide;

iv) anti-14790 antibody;

v) a 14790 polypeptide comprising the amino acid sequence of SEQ ID NO:2, or a fragment thereof;

vi) a 14790 polypeptide comprising an amino acid sequence which is at least 90 percent identical to the amino acid sequence of SEQ ID NO:2, wherein the percent identity is calculated using the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4; or

vii) an isolated naturally occurring allelic variant of a polypeptide consisting of the amino acid sequence of SEQ ID NO:2, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a complement of a nucleic acid molecule consisting of SEQ ID NO:1 at 6X SSC at 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 50-65°C.

22. The method of claim 19, wherein the 14790 modulator is

a) an antisense 14790 nucleic acid molecule;
b) is a ribozyme;

c) the nucleotide sequence of SEQ ID NO:1, or a fragment thereof;

d) a nucleic acid molecule encoding a polypeptide comprising an amino acid sequence which is at least 90 percent identical to the amino acid sequence of SEQ ID NO:2, wherein the percent identity is calculated using the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4;

e) a nucleic acid molecule encoding a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, wherein the nucleic acid molecule which hybridizes to a complement of a nucleic acid molecule consisting of SEQ ID NO:1 at 6X SSC at 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 50-65°C; or

f) a gene therapy vector.

23. A method for evaluating the efficacy of a treatment of a hepatic associated disorder, in a subject, comprising:

   a) treating a subject with a protocol under evaluation;

   b) assessing the expression level of a 14790 nucleic acid molecule defined in claim 1 or 14790 polypeptide encoded by the 14790 nucleic acid molecule,

   c) wherein a change in the expression level of 14790 nucleic acid or 14790 polypeptide after the treatment, relative to the level before the treatment, is indicative of the efficacy of the treatment of a hepatic associated disorder.

24. The method of claim 23, wherein the a hepatic associated disorder is liver fibrosis or infection with HBV.

25. A method of diagnosing a hepatic associated disorder in a subject, comprising:
evaluating the expression or activity of a 14790 nucleic acid molecule
defined in claim 1 or a 14790 polypeptide encoded by the 14790 polypeptide, such that a
difference in the level of 14790 nucleic acid or 14790 polypeptide relative to a normal
subject or a cohort of normal subjects is indicative of a hepatic associated disorder.

26. The method of claim 25, wherein the hepatic associated disorder is liver
fibrosis or infection with HBV.
TCGCCCAACGCGTCCGACCGCCGCCCCAGGCAAGGCAGGCTGCTTTGGG
SEQ.ID NO: 1

SEQ.ID NO: 2
M A G G R G A P
CGCAGCGCTGCC ATG GCT GGG GGC CGT GGG GCC CCC
8

SEQ.ID NO: 3
G R G R D E P P E S Y
GGG CGC GGC CGG GAC GAG CCT CCG GAG AGC TAC
19

P Q R Q D H E L Q A L
CCG CAA CGA CAG GAC CAC GAG CTA CAG GCC CTG
30

E A I Y G A D F Q D L
GAG GCC ATC TAC GGC GCG GAC TTC CAA GAC CTG
41

R P D A C G P V K E P
CGG CCG GAC GCT TGC GGA CCG GTC AAA GAG CCC
52

P E I N L V L Y P Q G
CCT GAA ATC AAT TTA GTT TTG TAC CCT CAA GGC
63

L T G E E V Y V K V D
CTA ACT GGT GAA GAA GTA TAT GTA AAA GTG GAT
74

L R V K C P P T Y P D
TTG AGG GTT AAA TGC CCA CCT ACC TAT CCA GAT
85

L V P E I E L K N A K
GTA GTT CCT GAA ATA GAG TTA AAA AAT GCC AAA
96

G L S N E S V N L L K
GGT CTA TCA AAT GAA AGT GTC AAT TTG TTA AAA
107

S R L E E L A K K H C
TCT CGC CTA GAA GAA CTG GCC AAG AAA CAC TGT
118

G E V M I F E L A Y H
GGG GAG GTG ATG ATC TTT GAA CTG GCT TAC CAC
129

V Q S F L S E H N K P
GTG CAG TCA TTT CTC AGC GAG CAT AAC AAG CCC
140

FIG. 1A

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P P K S F H E E M L E 151
CCT CCC AAG TCT TTT CAT GAA GAA ATG CTG GAA 453

R R A Q E E Q Q R L L 162
AGG CGG GCT CAG GAG GAG CAG CAG AGG CTG TTG 486

E A K R K E E Q E Q R 173
GAG GCC AAG CGG AAA GAA GAG CAG GAG CAA CGT 519

E I L H E I Q R R K E 184
GAA ATC CTG CAT GAG ATT CAG AGA AGG AAA GAA 552

E I K E E K K R K E M 195
GAG ATA AAA GAA GAG AAA AAA AGG AAA GAA ATG 585

A K Q E R L E I A S L 206
GCT AAG CAG GAA CGT TTG GAA ATT GCT AGT TTG 618

S N Q D H T S K K D P 217
TCA AAC CAA GAT CAT ACC TCT AAG AAG GAC CCA 651

G G H R T A A I L H G 228
GGA GGA CAC AGA ACG GCT GCC ATT CTA CAT GGA 684

G S P D F V G N G K H 239
GGC TCT CCT GAC TTT GTA GGA AAT GGT AAA CAT 717

R A N S S G R S R R E 250
CGG GCA AAC TCC TCA GGA AGG TCT AGG CGA GAA 750

R Q Y S V C N S E D S 261
CGT CAG TAT TCT GTA TGT AAT AGT GAA GAT TCT 783

P G S C E I L Y F N M 272
CCT GGC TCT TGT GAA ATT CTG TAT TTC AAT ATG 816

G S P D Q L M V H K G 283
GGG AGT CCT GAT CAG CTC ATG GTG CAC AAA GGG 849

FIG. 1B

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FIG. 1C

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S A S N V L V D A E G 437
AGT GCA TCT AAT GTC TTG GTG GAT GCA GAA GCC 1311

T V K I T D Y S I S K 448
ACC GTC AAG ATT ACG GAC TAT AGC ATT TCT AAG 1344

R L A D I C K E D V F 459
CGC CTC GCA GAC ATT TGC AAG GAG GAT GTG TTT 1377

E Q T R V R F S D N A 470
GAG CAA ACC CGA GTT CGT TTT AGT GAC AAT GCT 1410

L P Y K T G K K G D V 481
CTG CCT TAT AAA ACG GGG AAG AAA GGA GAT GTT 1443

W R L G L L L L S L S 492
TGG CGT CTG GGC CTT CTG CTG CTG TCC CTC AGC 1476

Q G Q E C G E Y P V T 503
CAA GGA CAG GAA TGT GGA GAG TAC CCT GTG ACC 1509

I P S D L P A D F Q D 514
ATC CCT AGT GAC TTA CCA GCT GAC TTT CAA GAT 1542

F L K K C V C L D D K 525
TTT CTA AAG AAA TGT GTG TGC TTG GAT GAC AAG 1575

E R W S P Q Q L L K H 536
GAA AGA TGG AGT CCC CAG CAG TTG TTG AAA CAC 1608

S F I N P Q P K M P L 547
AGC TTT ATA AAT CCC CAG CCA AAA ATG CCT CTA 1641

V E Q S P E D S G G Q 558
GTG GAA CAA AGT CCT GAA GAT TCT GGA GGA CAA 1674

D Y V E T V I P S N R 569
GAT TAT GTT GAG ACT GTT ATT CCT AGC AAC CCG 1707

L P S A A F F S E T Q 580
CTA CCC AGT GCT GCC TTC TTT AGT GAG ACA CAG 1740

FIG. 1D

SUBSTITUTE SHEET (RULE 26)
FIG. 1E

SUBSTITUTE SHEET (RULE 26)
FIG. 1F
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FGLA
TTTGCTTGGCGACA
GACCATCTAGCC
TTTCTT 2631

ADSKQDDQTG
GCTGACAGCAAA
CAGAGCCAGCT
ACAGGAAAGAC 2566

LIKSDPSGHLT
TTGATTAAATGCT
ACCTCATAGGT
CACCTTATAACT 2697

GMCVGTALYVS
GGGATGTTGGCA
CTGCTCTATGT
ATAGCCACTG 2730

EvQGSTKSAYN
GAGGTCCAAAGG
AGCACCAAAATC
TGCATACCAAC 2763

QKVDFSLGLITI
CAGAAAATGTGAT
CTCTTCACGCTG
GGATTATCATC 2796

FFEMSYHPMV
TTCTTTGAATGGCT
TCCATATCACCCC
AGTGCATGAC 2829

ASERIFVLNQ
GCTTCAAGGATCT
TTTGTCTACACCA
CTCCTCCCCT 2862

RDPTSPKFPE
AGAGATCCTACTGT
CCCTAAGTTCAT
CCAGAAGGAC 2895

FDGDGEHA
TTTGAGCTGAGGA
GCATGCAAAGC
AGGAAATCTCA 2928

VISSLNLNHDP
GCTATCTCTTGCTG
AACACGATCCCA
GCACTGGGAC 2961

KRPTATELK
AAACGGCACAACGCC
AAAGGCTGCTAGT
AAGAGT 2994

ELLPQPMEES
GAGCTGCTGCCCA
CCCCTACAGATGGAGGAGTCA 3027

ELHLHTEVLH
GAGCTGACACGAGCTGCAGTTCCACACGAC 3060

FIG. 1G

SUBSTITUTE SHEET (RULE 26)
N V D G K A Y R T M M 1031
AAC GTG GAT GGG AAG GCC TAC CGC ACC ATG ATG 3093

A Q I F S Q R I S P A 1042
GCC CAG ATC TTC TCG CAG CGC ATC TCC CCT GCC 3126

I D Y T Y D S D I L K 1053
ATC GAT TAC ACC TAT GAC AGC GAC ATA CTG AAG 3159

G N F S I R T A K M Q 1064
GGC AAC TTC TCA ATC CGT ACA GCC AAG ATG CAG 3192

Q H V C E T I I R I F 1075
CAG CAT GTG TGT GAA ACC ATC ATC CGC ATC TTT 3225

K R H G A V Q L C T P 1086
AAA AGA CAT GGA GCT GTT CAG TTG TGT ACT CCA 3258

L L P R N R Q I Y E 1097
CTA CTG CTT CCC CGA AAC AGA CAA ATA TAT GAG 3291

H N E A A L F M D H S 1108
CAC AAC GAA GCT GCC CTA TTC ATG GAC CAC AGC 3324

G M L V M L P F D L R 1119
GGG ATG CTG GTG ATG CTT CCT TTT GAC CTG CGG 3357

I P F A R Y V A R N N 1130
ATC CCT TTT GCA AGA TAT GTG GCA AGA AAT AAT 3390

I L N L K R Y C I E R 1141
ATA TTG AAT TTA AAA CGA TAC TGC ATA GAA CGT 3423

V F R P R K L D R F H 1152
GTG TTC AGG CCG CGC AAG TTA GAT CGA TTT CAT 3456

P K E L L E C A F D I 1163
CCC AAA GAA CTT CTG GAG TGT GCA TTT GAT ATT 3489

FIG. 1H
V T S T T N S F L P T 1174
GTC ACT TCT ACC ACC AAC AGC TTT CTG CCC ACT 3522

A E I I Y T I Y E I I 1185
GCT GAA ATT ATC TAC ACT ATC TAT GAA ATC ATC 3555

Q E F P A L Q E R N Y 1196
CAA GAG TTT CCA GCA CTT CAG GAA AGA AAT TAC 3588

S I Y L N H T M L L K 1207
AGT ATT TAT TTG AAC CAT ACC ATG TTA TTG AAA 3621

A I L L H C G I P E D 1218
GCA ATG CTC TTA CAC TGT GGG ATC CCA GAA GAT 3654

K L S Q V Y I I L Y D 1229
AAA CTC AGT CAA GTC TAC ATT ATT CTG TAT GAT 3687

A V T E K L T R R E V 1240
GCT GTG ACA GAG AAG CTG ACG AGG AGA GAA GTG 3720

E A K F C N L S L S S 1251
GAA GCT AAA TTT TGT AAT CTG TCT TTG TCT TCT 3753

N S L C R L Y K F I E 1262
AAT AGT CTG TGT CGA CTC TAC AAG TTT ATT GAA 3786

Q K G D L Q D L M P T 1273
CAG AAG GGA GAT TTG CCA GAT CTT ATG CCA ACA 3819

I N S L I K Q K T G I 1284
ATA AAT TCA TTA ATA AAA CAG AAA ACA GGT ATT 3852

A Q L V K Y G L K D L 1295
GCA CAG TTG GTG AAG TAT GGC TTA AAA GAC CTA 3885

E E V V G L L K K L G 1306
GAG GAG GTT GTT GGA CTG TTG AAG AAA CTC GGC 3918

FIG. 1I

SUBSTITUTE SHEET (RULE 26)
FIG. 1J

SUBSTITUTE SHEET (RULE 26)
FIG. 1K

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H V K V K S F E K E R 1471
CAT GTC AAG GTT AAG TCT TTC GAG AAG GAA AGG 4413

Q T E K R V L E T E L 1482
CAG ACA GAG AAG CGT GTG CTG GAG ACT GAA CTT 4446

V D H V L Q K L R T K 1493
GTG GAC CAT GTA CTG CAG AAA CTG ACG ACT AAA 4479

V T D E R N G R E A S 1504
GTC ACT GAT GAA AGG AAT GGC AGA GAA GCT TCC 4512

D N L A V Q N L K G S 1515
GAT AAT CTT GCA GTG CAA AAT CTG AAG GGG TCA 4545

F S N A S G L F E I H 1526
TTT TCT AAT GCT TCA GGT TTG TTT GAA ATC CAT 4578

G A T V V P I V S V L 1537
GGA GCA ACA GTG GTT CCC ATT GTG AGT GTG CTA 4611

A P E K L S A S T R R 1548
GCC CCG GAG AAG CTG TCA GCC AGC ACT AGG AGG 4644

R Y E T Q V Q T R L Q 1559
CGC TAT GAA ACT CAG GTA CAA ACT CGA CTT CAG 4677

T S L A N L H Q K S S 1570
ACC TCC CTT GCC AAC TTA CAT CAG AAA AGC AGT 4710

E I E I L A V D L P K 1581
GAA ATT GAA ATT CTG GCT GTG GAT CTA CCC AAA 4743

E T I L Q F L S L E W 1592
GAA ACA ATA TTA CAG TTT TTA TCA TTA GAG TGG 4776

D A D E Q A F N T T V 1603
GAT GCT GAT GAA CAG CTT AAT AAA ACA ACT GTG 4809

SUBSTITUTE SHEET (RULE 26)
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K Q L L S R L P K Q R 1614
AAG CAG CTG CTG TCA CGC CTG CCA AAG CAA AGA 4842

Y L K L V C D E I Y N 1625
TAC CTC AAA TTA GTC TGT GAT GAA ATT TAT AAC 4875

I K V E K K V S V L F 1636
ATC AAA GTA GAA AAA AAG GTG TCT GTG CTA TTT 4908

L Y S Y R D D Y Y R I 1647
CTG TAC AGC TAT AGA GAT GAC TAC TAC AGA ATC 4941

L F * 1650
TTA TTT TAA 4950 → SEQ.ID NO: 3

CCCTAAGAAGACTGTTCATTACAACCTCATTCAAACAGACAGAGGCTTATATCTG
GAATAATGGAAATGTTGTACATCTCATAATAATTAATTTCAAGA
AGAGGCTGGTGCAGTGCTCAGCCTACACTTTAAATCCGCAGCACTTTGGGAAAGC
CAAGGCAGGAGACTGCTTGAACCCAGAGGTTTGAGAACCAGCCTGAGAAGCA
CAAAGCAAGACACCACATCTCTATAAACACTAAAAAAATTAGTTGGGCATGG
TGCGACATGCTCTGTAGTCCAGCTACTCCAGAGGCTGAGATGGATGACTATCT
GAGCCTACAGGAGGCTGAGCTGAGCTGAGCTGACTGGACCTCAGGACT
CCAGTCTGGGACAACAGCAAGACACCTGTCTTTAAAAAAAAGAAAA
AAAAATTTTTTTTCTAAGAAGCTGTCTCATTACAAAGTTGAGCTTGGGTTAGTT
TTCTGTTGTAATATATATATATATATATATATATATATATATATATATATATATAT
TCATATACCTGCA

FIG. 1L

6065914_eIF2kinase_man 14790 MAGGRGAPGRRGDDEPEPSSYPQRSQDH
6066585_eIF2kinase_mouse MAGGRGASGRGRAEFQESYSSQQRDH

6065914_eIF2kinase_man 14790 ELQALEAIYGADFDQDLRPDACGPVK
6066585_eIF2kinase_mouse ELQALEAIYGSDFDQDLRPDARGVR

6065914_eIF2kinase_man 14790 EPPEINLVLPQGLTEEVYVKVDEL
6066585_eIF2kinase_mouse EPPEINLVLPQGLAGEEVYVQVEL

FIG. 2A

SUBSTITUTE SHEET (RULE 26)
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<th>Protein</th>
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<tr>
<td>6066585_eIF2kinase_mouse</td>
<td>QVKCPPTYPDVVPEIELKNAKGLSN</td>
</tr>
<tr>
<td>6065914_eIF2kinase_man</td>
<td>ESVNLLKSRLEELAKKHCGEMIFE</td>
</tr>
<tr>
<td>6066585_eIF2kinase_mouse</td>
<td>ESVNLLKSHLEELAKKQCGEMIFE</td>
</tr>
<tr>
<td>6065914_eIF2kinase_man</td>
<td>LAYHVQSFSLSEHNNPPKPSFHEMLE</td>
</tr>
<tr>
<td>6066585_eIF2kinase_mouse</td>
<td>LAHHVQSFSLSEHNNPPKPSFHEMLE</td>
</tr>
<tr>
<td>6065914_eIF2kinase_man</td>
<td>ERRAQEEQQLLEACKRKEEQREI</td>
</tr>
<tr>
<td>6066585_eIF2kinase_mouse</td>
<td>ERQAQEKKQLLEACKRKEEQREI</td>
</tr>
<tr>
<td>6065914_eIF2kinase_man</td>
<td>LHEIQRRKEEIKEKKRKEAKQER</td>
</tr>
<tr>
<td>6066585_eIF2kinase_mouse</td>
<td>LHEIQRRKEEIKEKKRKEAKQER</td>
</tr>
<tr>
<td>6065914_eIF2kinase_man</td>
<td>LEIASLSNQDHTSKKDPGGRATA</td>
</tr>
<tr>
<td>6066585_eIF2kinase_mouse</td>
<td>LEITSLTNQDYASKRDPAGHRAAAT</td>
</tr>
<tr>
<td>6065914_eIF2kinase_man</td>
<td>LGGSQPFVNGKHRANSSGRSRE</td>
</tr>
<tr>
<td>6066585_eIF2kinase_mouse</td>
<td>LGGSQPFVNGKARTYSSGRSRE</td>
</tr>
<tr>
<td>6065914_eIF2kinase_man</td>
<td>RQYSVCNSDSPSGSCELYFNGMSP</td>
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<tr>
<td>6066585_eIF2kinase_mouse</td>
<td>RQYSVCSGEPSPGCDILHFSVGSP</td>
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<td>6065914_eIF2kinase_man</td>
<td>DQLMVHKGKCIQSDEQLGKLVYNAL</td>
</tr>
<tr>
<td>6066585_eIF2kinase_mouse</td>
<td>DQLMVHKGRCVGSDEQLGKVYNAL</td>
</tr>
<tr>
<td>6065914_eIF2kinase_man</td>
<td>ETATTGFLYELLYEVLQWQQMKGPFL</td>
</tr>
<tr>
<td>6066585_eIF2kinase_mouse</td>
<td>ETATGSFVLLHEWVLQWQQ-MGPCL</td>
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</table>

**FIG. 2B**

SUBSTITUTE SHEET (RULE 26)
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6065914_eIF2kinase_man  -----------------------------------------
14790  TSQEKEKIDKKQIQGTETEFSNL
6066585_eIF2kinase_mouse  -----------------------------------------
14790  TSQEKEKIDKKRQIQGAETFSSL

6065914_eIF2kinase_man  -----------------------------------------
14790  VKLSPHNQVRLAMLKKEQDSSIVV
6066585_eIF2kinase_mouse  -----------------------------------------
14790  VKLSPNIVRYFAMNSREEEDSVI

6065914_eIF2kinase_man  -----------------------------------------
14790  DILVEHISGVSLEAIHSHSGPVPVH
6066585_eIF2kinase_mouse  -----------------------------------------
14790  DILAEHVGISLATHLSHSGPVPVAH

6065914_eIF2kinase_man  -----------------------------------------
14790  QLRRYTAQLLSGLDYLHHSNSVHVKK
6066585_eIF2kinase_mouse  -----------------------------------------
14790  QLRKYTAQLLAAGLDYLHHSNSVHVKK

6065914_eIF2kinase_man  -----------------------------------------
14790  LSASNVLVDAEGTVKIDYSISKRL
6066585_eIF2kinase_mouse  -----------------------------------------
14790  LSASSVLVDAEGTVKIDYSISKRL

6065914_eIF2kinase_man  -----------------------------------------
14790  ADICKEDVFQTRVRFSDNAALPYKT
6066585_eIF2kinase_mouse  -----------------------------------------
14790  ADICKEDVFQARVRFSDNAALPYKT

6065914_eIF2kinase_man  -----------------------------------------
14790  GKKGDVWRLGLLLLLSSLSSQGQEGCEY
6066585_eIF2kinase_mouse  -----------------------------------------
14790  GKKGDVWRLGLLLLLSSLSSQGQEGCEY

6065914_eIF2kinase_man  -----------------------------------------
14790  PVTIPSDLPAFDQDFLKKCVCLDDK
6066585_eIF2kinase_mouse  -----------------------------------------
14790  PVTIPSDLPAFDQDFLKKCVCLDDK

6065914_eIF2kinase_man  -----------------------------------------
14790  ERWSPQQLLKHSFINPQPKMPLVEQ
6066585_eIF2kinase_mouse  -----------------------------------------
14790  ERWSPQQLLKHSFINPQPKLPLVEQ

6065914_eIF2kinase_man  -----------------------------------------
14790  SPEDSGQQDYVETVIPNRLPSAAF

FIG. 2C

SUBSTITUTE SHEET (RULE 26)
6066585_eIF2kinase_mouse SPEDSGQDYIETVIPSNQLPSAAF
6065914_eIF2kinase_man  -----------------------------------
14790
6066585_eIF2kinase_mouse FSETQRFPSRYFIEFEELQLLGKGA
6065914_eIF2kinase_man  -----------------------------------
14790
6066585_eIF2kinase_mouse FGAIVKQNKLDCGCYAVKRIPINP
6065914_eIF2kinase_man  -----------------------------------
14790
6066585_eIF2kinase_mouse FGAIVKQNKLDCGCYAVKRIPINP
6065914_eIF2kinase_man  -----------------------------------
14790
6066585_eIF2kinase_mouse ASRFQFRRIKGEVTLRLSLRHHENIVR
6065914_eIF2kinase_man  -----------------------------------
14790
6066585_eIF2kinase_mouse ASRHFRRIKGEVTLRLSLRHHENIVR
6065914_eIF2kinase_man  -----------------------------------
14790
6066585_eIF2kinase_mouse YYNAWIERHERPAGTPOPPDSGPL
6065914_eIF2kinase_man  -----------------------------------
14790
6066585_eIF2kinase_mouse YYNAWIERHERPAVFPTPOPPDCTPQ
6065914_eIF2kinase_man  -----------------------------------
14790
6066585_eIF2kinase_mouse AQDSPATCGKTSGDTEELGSVEAAA
6065914_eIF2kinase_man  -----------------------------------
14790
6066585_eIF2kinase_mouse AQDSPATCGKTSGDTEELGSVEAAA
6065914_eIF2kinase_man  -----------------------------------
14790
6066585_eIF2kinase_man PPPILSSSVWSTGERSASARFPAG
6065914_eIF2kinase_man  -----------------------------------
14790
6066585_eIF2kinase_mouse PPPILSSSVWSTGERSASARFPAG
6065914_eIF2kinase_man  -----------------------------------
14790
6066585_eIF2kinase_mouse TPGSSSDDEEDDEDEDEHGGVFSQSFL
6065914_eIF2kinase_man  -----------------------------------
14790
6066585_eIF2kinase_mouse TPGSSSDDEED-EDERDGVFSQSFL
6065914_eIF2kinase_man  -----------------------------------
14790
6066585_eIF2kinase_man PASDSDDDIIFDNEDENKSKSQQDE
6065914_eIF2kinase_man  -----------------------------------
14790
6066585_eIF2kinase_man PASDSDDDIIFDNEDENKSKSQQDE
6065914_eIF2kinase_man  -----------------------------------
14790
6066585_eIF2kinase_man DCNEKNGCHESEPVTTEAVHYLYI

**FIG. 2D**

SUBSTITUTE SHEET (RULE 26)
6066585_eIF2kinase_mouse DCNQKDGSHEIEPSVTAAEVHYLYI

6065914_eIF2kinase_man ---------------------------
14790 QMEYCEKSTLRDTIDQGLRYRTVRL
6066585_eIF2kinase_mouse QMEYCEKSTLRDTIDQGLFRDTSRL

6065914_eIF2kinase_man ---------------------------
14790 WRLFREILDGLAYHEKGMHRDLK
6066585_eIF2kinase_mouse WRLFREILDGLAYHEKGMHRDLK

6065914_eIF2kinase_man ---------------------------
14790 PVNIFLDSDDHVKIGDFGLATDHLA
6066585_eIF2kinase_mouse PVNIFLDSDDHVKIGDFGLATDHLA

6065914_eIF2kinase_man ---------------------------
14790 FSADSKQDDQTDG-LIKSDDSGLT
6066585_eIF2kinase_mouse FTAEGKQDDQAGDGVIKSDPSGHLT

6065914_eIF2kinase_man ---------------------------
14790 GMVGTLAYVSPQVSSTKSAYNQKV
6066585_eIF2kinase_mouse GMVGTLAYVSPQVSSTKSAYNQKV

6065914_eIF2kinase_man ---------------------------
14790 DLFSGLIIFFFEMSYHPMVTAERIF
6066585_eIF2kinase_mouse DLFSGLIIFFFEMSYHPMVTAERIF

6065914_eIF2kinase_man ---------------------------
14790 VLNQLRDPTSKFPEDFDDGEHAKQ
6066585_eIF2kinase_mouse VLNQLRDPTSKFPEDFDDGEHTKQ

6065914_eIF2kinase_man ---------------------------
14790 KSVISWLLHDPAKRPTATELLKSE
6066585_eIF2kinase_mouse KSVISWLLHDPAKRPTAMELLKSE

FIG. 2E

SUBSTITUTE SHEET (RULE 26)
FIG. 2F
FIG. 2G

SUBSTITUTE SHEET (RULE 26)
**FIG. 2H**

SUBSTITUTE SHEET (RULE 26)
6065914_eIF2kinase_man
14790
6066585_eIF2kinase_mouse

LAVDLPKETILQFLSLEWDADQAEF
LAVDLPKETILQFLSLEWDADQAEF
***:**** * ****************

6065914_eIF2kinase_man
14790
6066585_eIF2kinase_mouse

NTTVKQLLSRLPKQRYLKLVCDEIY
NTTVKQLLSRLPKQRYLKLVCDEIY
**************************

6065914_eIF2kinase_man
14790
6066585_eIF2kinase_mouse

NIKVEKKVSVLFLYSYRDDYRRILF
NIKVEKKVSVLFLYSYRDDYRRILF
↑SEQ. ID NO: 4
↑SEQ. ID NO: 5
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**Fig. 2I**
Ser/thr Kinase 14790 Expression on HBV+ Liver

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<th>Liver-1</th>
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<th>Liver-3</th>
<th>HBV+Liver (MA101)</th>
<th>HBV+Liver (MA104)</th>
<th>HBV+Liver (Marra 511)</th>
<th>HApC+ Liver</th>
<th>HApC+ Liver</th>
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<tr>
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FIG. 3
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</table>

Relative Expression (Liver PT 260 used as Reference sample)
Gene 14790 Expression in Normal Human Tissue
Relative Expression (Thyroid as Reference)

FIG. 5
FIG. 6

- Alpha, Regions-Garnier-Robson
- Alpha, Regions-Chou-Fasman
- Beta, Regions-Garnier-Robson
- Beta, Regions-Chou-Fasman
- Turn, Regions-Garnier-Robson
- Turn, Regions-Chou-Fasman
- Coil, Regions-Garnier-Robson

- Hydrophilicity Plot-Kyte-Doolittle
- Alpha, Amphipathic Regions-Eisenberg
- Beta, Amphipathic Regions-Eisenberg
- Flexible Regions-Karplus-Schultz

- Antigenic Index-Jameson-wolf
- Surface Probability Plot-Emini
>14790
MAGGRGAPGGRGRDEPPESYPQRQDHHELQALEAIYGADFDQDLRPDACGPV
KEPPEINLVLPQLGTGEEVYVKVDRLVKCPPTYPDVPEIELEKNAKGL
SNESVNLLKSRLEELAKKHCHEVMIFELAYHVQSLSEHNKPPKSFHRE
EMLERRAQEEQQRLLAEAKRKEELEHQREILHEIQRRKEEIKKEEKKREMA
KQERLEIASLNSNQDHTSKKDPGHRTAAIIHLGGSPDFVNGNGHRANSSEG
RSRRERQYSVCNSEDSPGSCIEILYFNMGSPDQLMVHKGKCIGSDQQLGK
LVYNALETATGFFVLLYEWVLQWQKGMGPFLTQEQEKEKIDCKCKQIQGT
ETENSNLVKLSHPNVRVLAMNLKEQDSTVVDILVEHISGVSIAAHLSS
HSGPQPHQRLRRYTAQLLSGLDYLHNSNSVHVLSASNVLVDAGTVKICTDYSISKRLADICKCEDVEQTRVRFSDNALPYYYTGKGDVWRLGLLLLS
LSQGQECGEY

FIG. 7A
PSORT Prediction of Protein Localization

MITDISC: discrimination of mitochondrial targeting seq

R content: 3  Hyd Moment(75): 7.37
Hyd Moment(95): 6.02  G content: 5
D/E content: 2  S/7 content: 0

Gavel: prediction of cleavage sites for mitochondrial preseq
R-2 motif at 20  GRG:RD

MUCDISC: discrimination of nuclear localization signals
pat4: KKRK (5) at 190

FIG. 7B

SUBSTITUTE SHEET (RULE 26)
pat4: RPRK (4) at 1144
pat7: none
bipartite: RRAQEEQORLLEAKRKE at 152
bipartite: KRIIPINPASRQFRRIKG at 619
content of basic residues: 12:11
NLS Score: 1.08

ER Membrane Retention Signals:

XXRR-like motif in the N-terminus: AGGR

None

Final Results (k = 9/23):

56.5 %: nuclear
30.4 %: cytoplasmic
4.3 %: vacuolar
4.3 %: mitochondrial
4.3 %: vesicles of secretory system

prediction for 14790 is nuc (k=23)

<table>
<thead>
<tr>
<th>Start</th>
<th>End</th>
<th>Feature</th>
<th>Seq</th>
</tr>
</thead>
<tbody>
<tr>
<td>144</td>
<td>204</td>
<td>coiled coil</td>
<td>FHEEMLLERRA...AKQERLEIAS</td>
</tr>
</tbody>
</table>

Signal Peptide Predictions for 14790

<table>
<thead>
<tr>
<th>Method</th>
<th>Predict</th>
<th>Score</th>
<th>Mat@</th>
</tr>
</thead>
<tbody>
<tr>
<td>SignalP (eukaryote)</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: amino-terminal 70aa used for signal peptide prediction

Transmembrane Segments Predicted by MEMSAT

<table>
<thead>
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<th>Start</th>
<th>End</th>
<th>Orient</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1522</td>
<td>1538</td>
<td>ins--&gt;out</td>
<td>0.8</td>
</tr>
</tbody>
</table>

**FIG. 7C**
>14790
MAGGRLAPGRGLDEPEPESYPQRQDHRELQALETAYGADFDQLPDAACGPVK
EPPEINLVLQPGLTFGEVYVKVDLKVCPYPTPVDVPETELKNKAGLSN
ESVNLKSRLEELAKKHCQEVIFELAYHQFSFLSENKPKPVIFHHEML
ERRAQEEQQRRLEAKRRREEQREILHEIQRRREEERKREKMAQKER
LEIARSLNQDHSTSKDPGGHRATAAILHGGSPDFVNGKHRANSSGRSRRE
RQSYCVNSEDPSGSECILEYFMNGSPQDLMVHKGKCGSDPEQTLGKLVYNAL
ETATTGPFVLLYEWVLQWQKKMGFLTSQEKEKIDKCKQIQGTEEFNSL
VKLSHPNVRVLYAMNLKQDDILSVDVILVEISGVSLSAALHSHGPIPVH
QLRRRTAQLLSLQGLYLSNNVSVHKVLSASNLVDAEGTVKTIDYSISKRL
ADICKEQVFQTRVFSNALPQKTGKDVVRDLRLLSLSQGECGEY
PVTIPSDLPPADFQDFLKLCVCLDDKERWSPQQLKHSFNPQPKMALVEQ
SPEDSSGQYDVTVIPSNRLPSAAFFEQRSTRFYRFYIEEELQLLKGGA
FGAVIKVQNKLDDCYYAVKRIPINPSARQRFRFRKEGVTLLSLRHENIVR
YYNASTERHERPGTPPPPSGGLAKDDRAARGQPFASDGDGLSDVEAAA
PPPLSSVWEWSTSGERASARFPATGFGSSDDDEEDEHGGFVQSLFL
PASDSESIIIDEFIDEOSKSQLQDEDCNEKNGCHESEPSVTTFEEVHLYI
QMEYCEKSTLRDTIDQGLYRDFVTQNLRLFREILDSAYHEKGMHIDRLK
PNVIFLDSDDHVKIDFGGLATDHLAFSADSKQDDQTGDLSIDPSGHTLG
MVGTALYVPSQVEQSTKNSQVDLSSLGIMFFMSYMHTSASERIFLV
LNNQRLDPTSPKFPEDFDDGHEAKQKVSIVWLLLNDPAKRPTATELKLSEL
LPPQEQMESELMEVLTHTLVNGKAYRMTMAQQFSRSPAIDYTDYSD
ILKGFNSRTAKMQHVHCCETIIRIFKRHGAQVQLCTPLLLLPRNQYIEHNE
AAALFMDHSGMLVMPFDLRIPFRARYVARNNILNLRKCYIERFVFRPRKLD
FHPKELLECAFDIVGSTTNSFLPTAEIITYEIIEFQPALQERNSYEL
NHTMLLKAIIIGHCEPEDKLSQVYIYIDAVTEKLLREVEAKFCNLSLS
SNSTCRLYKFTEQKGDQLDMPTNSISLQKQTGIAVLKYGKLDKLEEVEVG
LLKGLIKLQVLINLGLVYKVQOHNGIIIFQFVAFIKRRQRAVPEIAAGG
RYDLLIPQFRGPFQALPGVPTAIQVSAIDKISAALVNEVSVITSSCDDLL
VSSVGQMSMSRANLNTQKLWTAGITAEMYDWSQSQEELQEYCHRHEITY
VALVSDKEGKSVFEKRTKVELETELVDHLQKLRTKVIDNERG
REASDNLAVQNLKGFNSASNGLFEIHGATVVFIVSVLAPEKLSASTRRRY
ETQVQRTLQTSLANLHQKSEISILAVDLPKETILQFSLWDADEQAFN
TTVKQLSRLPKQRYKLKCIDEYNIKVEKKSVLFLSYRDDYRILF

**FIG. 7D**

SUBSTITUTE SHEET (RULE 26)
Prosite Pattern Matches for 14790

Prosite version: Release 12.2 of February 1995

>PS00021|PD0C00001|ASN_GLYCOSYLATION N-glycosylation site.

Query: 100 NESV 103
Query: 242 NSSG 245
Query: 1055 NFSI 1058
Query: 1195 NYSI 1198
Query: 1201 NHTM 1204
Query: 1246 NLSL 1249
Query: 1414 NLTQ 1417
Query: 1518 NASG 1521
Query: 1600 NTTV 1603

>PS00004|PD0C00004|CAMP_PHOSPHO_SITE cAMP- and cGMP-dependent protein kinase phosphorylation site.

Query: 403 RRYT 406
Query: 988 KRPT 991
Query: 1630 KKVS 1633

>PS00005|PD0C00005|PKC_PHOSPHO_SITE protein kinase C phosphorylation site.

Query: 212 TSK 214
Query: 244 SGR 246
Query: 247 SRR 249
Query: 438 TVK 440
Query: 447 SKR 449
Query: 475 TGG 477
Query: 567 SNR 569
Query: 579 TQR 581
Query: 720 SAR 722
Query: 809 TLR 811
Query: 822 TVR 824
Query: 915 STK 917
Query: 945 SER 947
Query: 959 SPK 961

FIG. 7E
Query: 1036 SQR 1038
Query: 1057 SIR 1059
Query: 1060 TAK 1062
Query: 1232 TEK 1234
Query: 1236 TRR 1238
Query: 1416 TQK 1418
Query: 1455 SDK 1457
Query: 1473 TEK 1475
Query: 1545 STR 1547
Query: 1602 TVK 1604
Query: 1639 SYR 1641

>PS000006|PD000006|CK2_PHOSPHO_SITE Casein kinase II phosphorylation site.

Query: 65 TGEE 68
Query: 82 TYPD 85
Query: 108 SRLE 111
Query: 144 SFHE 147
Query: 207 SNQD 210
Query: 213 SKKD 216
Query: 247 SRRE 250
Query: 326 TSQE 329
Query: 343 TETE 346
Query: 411 SGLD 414
Query: 551 SPED 554
Query: 688 SDTD 691
Query: 707 SSVE 710
Query: 713 TSGE 716
Query: 730 SSDD 733
Query: 753 SDSE 756
Query: 809 TLRD 812
Query: 880 SKQD 883
Query: 943 TASE 946
Query: 991 TATE 994
Query: 1020 TNVD 1023
Query: 1180 TIYE 1183
Query: 1236 TRRE 1239

FIG. 7F

SUBSTITUTE SHEET (RULE 26)
Query: 1395 SSCD 1398
Query: 1435 SQEE 1438
Query: 1455 SDKE 1458
Query: 1570 SEIE 1573
Query: 1639 SYRD 1642

>PS00007|PD0C00007|TYR_PHOSPHO_SITE Tyrosine kinase phosphorylation site.

Query: 246 RSRRERQY 253
Query: 811 RDTIDQGLY 819
Query: 830 REILDGLAY 838
Query: 1444 RHHEITY 1450
Query: 1617 KLVCDEIY 1624

>PS00008|PD0C00008|MRYSTYL N-myristoylation site.

Query: 218 GGHRTA 223
Query: 384 GVSLAA 389
Query: 494 CQECGE 499
Query: 599 GAIFGAV 604
Query: 613 GCCYAV 618
Query: 684 GQPASD 689
Query: 742 GGVFSQ 747
Query: 782 GCHESE 787
Query: 900 GMVGT A 905
Query: 914 GSTKSA 919
Query: 1373 GVSI IA 1378
Query: 1514 GSFSNA 1519

>PS00009|PD0C00009|AMIDATION Amidation site.

Query: 475 TGKK 478

>PS00107|PD0C00100|PROTEIN_KINASE_ATP Protein kinases ATP-binding region signature.

**FIG. 7G**

SUBSTITUTE SHEET (RULE 26)
32/53

Query: 596 LGKGAFGAV 604

>PS00108|PD0C00100|PROTEIN_KINASE_ST
Serine/Threonine protein kinases active-site signature.
Query: 844 MIHRDLKPVNIFL 856

>PS00116|PD0C00107|DNA_POLYMERASE_B DNA polymerase family B signature.
Query: 687 ASDTDGLDS 695

Protein Family / Domain Matches, HMMer version 2

Searching for complete domains in PFAM
Hmmpfam search a single seq against HMM database
HMMER: 2.1.1 (Dec 1998)
Copyright (C) 1992-1998 Washington University
School of Medicine
HMMER is freely distributed under the GNU General
Public License (GPL).
-----------------------------------------------
HMM file: /prod/ddm/seqanal/PFAM/pfam4.4/Pfam
Sequence file: /prod/ddm/wspace/orfanal/oascript.16895.seq
-----------------------------------------------

Query: 14790

Scores for sequence family classification (score includes all domains):
Model Description
------- -------
pkinase Eukaryotic protein kinase domain
Ribosomal L23 Ribosomal protein L23
mRNA_cap_enzyme mRNA capping enzyme

FIG. 7H

SUBSTITUTE SHEET (RULE 26)
33/53

Score  E-value  N
-------  --------  ---
pkinase  282.0  7.8e-81  4
Ribosomal_L23  5.0  3.9  1
mRNA_capenzyme  -181.3  9.6  1

Parsed for domains:

Model  Domain  seq-f  seq-t  hmm-f  hmm-t
-------  ------  -----  ------  ------  ------
pkinase  1/4  332  443  .  30  134..
pkinase  2/4  501  539  ..  237  278.]  
pkinase  3/4  590  662  ..  1  66 [.  
pkinase  4/4  797  1001  ..  65  278.]  
Ribosomal_L23  1/1  1223  1244  ..  1  23 [.  
mRNA_capenzyme  1/1  1004  1309  ..  1  504 [.  

Score  E-value
-------  --------
pkinase  69.4  3.7e-18
pkinase  22.8  3.2e-05
pkinase  51.2  4.2e-13
pkinase  138.7  1.1e-37
Ribosomal_L23  5.0  3.9
mRNA_capenzyme  -181.3  9.6

Alignments of top-scoring domains:
Pkinase: domain 1 of 4, from 332 to 443: score 69.4, E = 3.7e-18

*-->ilk..kesls..lrEigilkrlsHpNIvrl1g
++++ k++++++ +E  l +lsHpN+vr+1
14790 332  KIDkcKKQIQgtETEFNSLVKLSHPNVVRYL 363

vfed...tddhlylvmEymeGdLfdylrrngpl5
++++  ++  E+  g +L+ +l+  gp++
14790 364  MNLKeqdDISVVDILVEHISGVSALLSHSGPIP 398

ekeakkialQilrGleYLHsngivHRDLKpeNILl
+++++++ Q+l+Gl+YLHsn++vH  L ++N+L+

FIG. 7I
34/53

14790 399 VHQLLRYTAKQLLSGLDYLHSNSVHVKSASNVLV 433
dengtvKiaD<-* SEQU. ID NO: 8
d +gtvKl+D
14790 434 DAEGTVKITT 443

pkinase: domain 2 of 4, from 501 to 539: score
22.8, E = 3.2e-5

*->rlplpsncSeelkdLlkclnkDPskRpGsatt
 + +ps ++ +++d+1kkc ++D ++R+ +
14790 501 PVTIPSDLPAFQDFLKKCVCLDDKERW---S 529

akeilnhpwf<-* SEQU. ID NO: 9
+++l+h ++
14790 530 PQQLLLKHSFI 539

pkinase: domain 3 of 4, from 590 to 662: score
51.2, E = 4.2e-13

*->yelleklvGsfGkVykakhk.tgkivAvKil
 +e 1+ 1G+G+fG V k+++k +g+ +AvK +
14790 590 FEELQQLLGKGAFGAVIKVQNKLDCYAVKRI 621

kkesls......lrEiqilkrlsHpNIvrllygve
 + s++ ++ E+ +l rl+H+Nlv++ ++
14790 622 PINPASrqfrriKGEVTLSSRLHHEIVRYNAWI 656

FIG. 7J

SUBSTITUTE SHEET (RULE 26)
dtddhl <* SEQ. ID NO: 10
++++++
14790 657 ERHERP 662

pkinase: domain 4 of 4, from 797 to 1001: score 138.7, E = 1.1e=37

*<-hlylvmEyemegGdLfdylrrngplsekeakki
+ly+ mEy+e+ L+d + + + + + + +
14790 797 YLYIQMEYCEKSTLRDTIDQGLYRDTVRLWRL 828

alQilrGleYLHsngivHRDLKpeNILldengtVvK
+++il GL+Y+H++g +HRDLKp NI+ld++ +vK
14790 829 FREILDGLAYIKEKMHIHRDLKPVNIFLDSDDHVK 863

iaDFGLARll....................ekl
i+DFGLA++ +++++++++++++++++++++++++
14790 864 IGDFGLATDHlafsadskqdtdqtdkdiksdpGHL 898

ttfvGTpwYmmAPEvileg...rgysskvDvWScG
+ + + + vGT Y+ +PEv +g++++ Y kvD SLG
14790 899 TGMVGTALYV-SPEV--QGstkSAYNQKVDLFSLG 930

viLyEllttggplfpagdpftgdevdqliifvl
+i++E+
14790 931 IIFFEMS----------------------- 937

klPfsdelpkrtdpleelfrikkr....rlplps
 P e++f +++ ++++ ++++p+

FIG. 7K

SUBSTITUTE SHEET (RULE 26)
Ribosomal_L23: domain 1 of 1, from 1223 to 1244: score 5.0, E = 3.9

RNA_cap_enzyme: domain 1 of 1, from 1004 to 1309: score -181.3, E = 9.6

FIG. 7L
elptyhrtgtllDGElvidinriaveqkTlrYvv
+ ++ l d ++ rYv
14790 1107 --------HSGMLVM-LPFD----LRIPF-ARYVA 1127
FDalaisGtqvgrd.lskrLgddefikavkKpf
++ ++ + i+r + L+ f
14790 1128 RNNILNLKRYCIERVfRPRKLDf-F-------HP 1153
defkkvmpdakilnqqkYNFpfkiglkhnsmly
+e+ d+++++ f++ + ++y
14790 1154 KELLECAFDIVTSTTT----NSFLPTEIIYTYI 1182
gqlklkaeskmvskadampkllHinDGILft
++ + a +
14790 1183 EIIQEFPA----------------L-------- 1191
cvrdtpyieGeiLVEPGNSyLdfnLkkWKPkee
+++++i+ + Ilk
14790 1192 QE-RNYSIYL----------NHTMLLKA----- 1208
nTvDFelilefeevndPeldekgfslyLdYda
+ + + + e++
14790 1209 ------ILLHCG--------IPEDK-------- 1219
mpGELfkslqVWqgGfnkrFevihtdqiffrv
+ ++++ + +++++ v
14790 1220 ------LSQVYIILY-DAVTEKLTRRE------V 1240
afqklgRlkhefaelsVsdkdykklkaleqpld
++ f +ls+s +l
14790 1241 EAK--------FCNLSLSSNSLCRLY-------- 1258
GrIVEcradieilIFQegrWeylrfRdDKqqa
+++E ++ + ++ + q+
14790 1259 -KFIEQKGDLQD--------LMPTINSLIKQK 1281
lKtgGYsgNhistskevlllsikDgvsieEelLkl
tg + +v kD +Ee+ l
14790 1282 --TG--------IAQLVKYGLKD----LEEVVGL 1301

FIG. 7M
Searching for complete domains in SMART
hmmppfam - search a single seq against HMM database
HMMER 2.1.1 (Dec 1998)
Copyright (c) 1992-1998 Washington University
School of Medicine
HMMER is freely distributed under the GNU General
Public License (GPL).

HMM file: /ddm/longo/robison/smart/smart/smart.all.hmmss
Sequence file: /prod/ddm/wspace/rof anal/oa-
script.16895.seq

Query: 14790

Scores for sequence family classification (score
includes all domains):

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<thead>
<tr>
<th>Model</th>
<th>Description</th>
<th>Score</th>
<th>E-value</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>serkin_6</td>
<td></td>
<td>184.2</td>
<td>2.2e-51</td>
<td>2</td>
</tr>
<tr>
<td>tyrkin_6</td>
<td></td>
<td>-40.3</td>
<td>2e-09</td>
<td>1</td>
</tr>
</tbody>
</table>

Parsed for domains:

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<tr>
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<th>Domain</th>
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<th>seq-t</th>
<th>hmm-f</th>
<th>hmm-t</th>
</tr>
</thead>
<tbody>
<tr>
<td>serkin_6</td>
<td>1/2</td>
<td>286</td>
<td>539..</td>
<td>1</td>
<td>231</td>
</tr>
<tr>
<td>serkin_6</td>
<td>2/2</td>
<td>590</td>
<td>1001..</td>
<td>1</td>
<td>231</td>
</tr>
<tr>
<td>tyrkin_6</td>
<td>1/1</td>
<td>590</td>
<td>1001..</td>
<td>1</td>
<td>280</td>
</tr>
</tbody>
</table>

<table>
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<th>Model</th>
<th>score</th>
<th>E-value</th>
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<tbody>
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<td>17.2</td>
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</tr>
<tr>
<td>serkin_6</td>
<td>166.8</td>
<td>3.5e-46</td>
</tr>
<tr>
<td>tyrkin_6</td>
<td>-40.3</td>
<td>2e-09</td>
</tr>
</tbody>
</table>
Alignments of top-scoring domains:

serkin_6: domain 1 of 2, from 286 to 539: score 17.2, E = 2.2e-11

*->YellkklGkGaFGkVyIardkktrglvAikvi
++lGk  Vy a ++ tg v  +
14790 286  IGSDEQLGK----LVYNALETATGGFV----LL 310

k.........................erilr
+  + +++ ++  +++++++ ++  +++ + ++
14790 311 YewvlqwqkkmgpfitsqekekidkckkqiQGTET 345

EikiIkk.dHPNIVkLydvf...dkly1VmE
E + L k HPN+V+++ +  +++++ +  + E
14790 346 EFNSLVKLSHPNVRYLAMNLKeqddsIVVDILVE 380

yceGdlGdlfdllkkkrgrgrlrvlsE.earfyfr
+++G  +L +l + g+  ++ ++ r+y++
14790 381 HISG--VSLAAHLSHSGP------IPVhQLRRYTA 407

QilsaLeYLYHsqgiIiHRDLKPeNiLLds..hvKla
Q+ls+L+YLHs+ ++H L +N+L+d  +++vK++
14790 408 QLLSGLDYLSNSVSVHKVLSASNVLVDAegTVKIT 442

DFGIArql..........ttfvGTpeYmAPEvl..
D  ++++l  +++  + t v  +++
14790 443 DYSISKRLadickedvfeQTRV----------RFsd 468

.....gYgkpavDiWSlGcilyElltGkpPFpqld
+  + + gk + D+W 1G +l 1 1 G+
14790 469 nalpyKRGK-KGDVWRLGLLLSLSQGQF----C 497

lifkkig....SpeakdLikklLvkdPekRlta.e
+  + +++  + d++kk+ + d  ++R++ ++
14790 498 GEYPVTrpsdlPAoFPQDFLKKCVDLDDKERWSPqQ 532

aLederdiakaHPFF<--* SEQ. ID NO: 14
+L+ H f+
14790 533 LLK------HSFTI 539

**FIG. 70**

SUBSTITUTE SHEET (RULE 26)
serkin_6: domain 2 of 2, from 590 to 1001: score 166.8, E = 3.5e-46

k..........................
+ ++ +++ ++ +++ + ++ ++++ ++
14790 590 Pinpasrqfrrikgevlllsrlhhenivryynawi 656

............... +++++++ ++++ +++ +++
14790 657 erherpapgptppdsqplakdddraargqpasdtdd 691

................
+ ++ + +++ +++ + ++++ + ++ +
14790 692 gldsveaappilsssvewstsgersasarfpat 726

................
+ ++ + +++ +++ + ++++ + ++ +
14790 727 gpgssddeddeddehggvqsqsflpasdsediiF 761

rilrEikiLkk....dHPNIVkLydvfed.....d
+ + E + ++++d+ ++++ + +++ +
14790 762 DNEDENSKSQnQdeDCNEKNGCHESEPSvteavH 796

klylVmeYeceGdlGdLfdllkkrgrgrglrvlsE.
+ly+ mEyce +L+d +++ + +
14790 797 HLlYlQMEYCEK--STLRDTIDQGLY------RDTv 823

earfyfrQilsaLeYlhsggIiHRDLKPeNiLLds
+++fr+il+++l+y+h++g iHRDLKP Ni+lLds
14790 824 RLWRLFREILDGLAYTHEKGMlHRDLKVNIFLds 858

..hvKlADFGlArql................
++hvK++DFGlA+ + ++++ + ++++
14790 859 ddHvKIGDFGLATDHlafsadskqddqtgdliksd 893

......ttfvGTpeYmAPEvl.....gYgpavDiW

FIG. 7P
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++++ t +vGT Y+++PEv +++++gY+ +vD
14790 894 psgh1TGMVGTALYVSPEVQgstksAYNQ-KVDLF 927

SLGcilyEltGkpPFp..qldlfkkig......
SLG+i++E+ + p ++ ++++++++
14790 928 SLGIIFFFEMSY-HPMVTasERIFVLNQLRdptsk 961

........SpeakdLikklLvkdPekRita.eaL
+++ ++++ k+ i+ lL+ dP+kR+ta+e+L
14790 962 fpedfddgeHAKQKSVISWLLNHDPAKRPTATELL 996

edeldikaHPff<-* SEQ. ID NO: 15
+ + + +
14790 997 K--------SELL 1001

tykin_6: domain 1 of 1, from 590 to 1001: score - 40.3, E = 2e-09

-->ltlgkk1GeGaGFeVykGtlk...ieVAVKtl
 + ++ LG GaFG V k + k ++ AVK +
14790 590 FEELQLLGKGAFGAVIKVQNKldgCCYAVKRI 621

keda....keeFlrEakiMkkLgkHpNiVkLlGv
 + +++ + + E ++++l +H+N1V+ + +
14790 622 PINPsrrqFRRIKGEVTLSSLR--HHENIVRYYNA 654

tceegrrFmevePlmivmEymegGdLldyLrknrp
 +e
14790 655 WIE-----------------------------RHERP 662

k..................................
++++++++++++ +++ +++ +++ +++ ++ +
14790 663 Agpgtpppdsgplakddraargqpasdtdgldsve 697

.................................
+++ +++ + + +++ +++ + + +++++++
14790 698 aaappplsssvwstsgersasarsfpatgpfgssd 732

.................................
+++++++ +++ + ++++++ +++++++

FIG. 7Q

SUBSTITUTE SHEET (RULE 26)
42/53

14790 733 dedddedehggvfsqsflpasd sesdiifd neden 767

............................
14790 768 sksqnqdedcneknngchesepsvteavhylyiqm 802

......................lslsdLlsfAlQIAkGMe
14790 803 eycekstlrdqidqglyRDTVRLWRLFREILDGLA 837

YLesknvfHRDLAARNCvgnkvvKlslsDFGLlsRd
14790 838 YIHEKGMlHRDLKPVNIFLDSDDHVlKlGDFGLATD 872

lyddDkkG...eskdyYrkkgkkgggtlllPir.
14790 873 HlAF-SADskqddqTqGDLIKSDPSGHTGMVTA1 906

WmAPEsl..kdkgFtskSDWVSFGVlLWEIfItlGe
14790 907 YVSPEVQgsTKSAYNQKVDLFSLGIIFFEM------ 936

qPYpgeiqqfmsnee...vleylkkGyRlpkPend
14790 937 -SYHPM------VTASErifVLNQLRDPFSTPFPEP-- 965

14790 966 ----FDGEHAKQKSISWLLNHDPAKRPTATEEL ---- 996

..verl<--* SEQ. ID NO: 16
14790 997 kSELL 1001

FIG. 7R

SUBSTITUTE SHEET (RULE 26)
## ProDom Matches

<table>
<thead>
<tr>
<th>ProdomId</th>
<th>Start</th>
<th>End</th>
<th>Description</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>View Prodom 40346</td>
<td>18</td>
<td>323</td>
<td>p99.2 (2)001712(1)// INITIATION FACTOR KINASE EUKARYOTIC-2 ALPHA EIF-2ALPHA</td>
<td>295</td>
</tr>
<tr>
<td>View Prodom 137719</td>
<td>19</td>
<td>1504</td>
<td>p99.2(1)074297_NEUCR// CPC3 PROTEIN</td>
<td>87</td>
</tr>
<tr>
<td>View Prodom 1</td>
<td>341</td>
<td>1017</td>
<td>p99.2(2773)CC2(14) KKIT (14) KPC(13)// KINASE PROTEIN TRANSFERASE ATP-</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BINDING SERIN/THREOINE-PROTEIN PHOSPHORYLATION RECEPTOR TYROSINE-PROTEIN</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PRECURSOR TRANSMEMBRANE</td>
<td></td>
</tr>
<tr>
<td>View Prodom 150228</td>
<td>1105</td>
<td>1206</td>
<td>p99.2(1)074297_NEUCR// CPC3 PROTEIN</td>
<td>90</td>
</tr>
<tr>
<td>View Prodom 2305</td>
<td>1106</td>
<td>1230</td>
<td>p99.2(23)SYH(12)// SYNTHETASE AMINOACYL-TRNA HISTIDYL-TRNA PROTEIN LIGASE</td>
<td>137</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ATP-BINDING BIOSYNTHESIS HISTIDINE--TRNA HISRS KINASE</td>
<td></td>
</tr>
<tr>
<td>View Prodom 42726</td>
<td>1274</td>
<td>1648</td>
<td>p99.2(2)0017121(1) 061651 (1)// INITIATION FACTOR KINASE EUKARYOTIC EIF-2</td>
<td>221</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ALPHA EIF-2ALPHA</td>
<td></td>
</tr>
</tbody>
</table>

**FIG. 7S**
View Prodom 40346
>40346 p99.2(2) 001712(1) 061651(1) //INITIATION
FACTOR KINASE EUKARYOTIC EIF-2 ALPHA EIF-2ALPHA
Length = 296

Score = 295(108.9 bits), Expect = 1.7e-28, Sum
P(2) = 1.7e-28 Identities = 58/144 (40%),
Positives = 95/144 (65%)

Query: 18 SYPQRQDHELQALEAIYGADFQDLRPDACGPVKEP 52
         S+ +RQ  EL+ +++I+G D +DLRP A + +P
Sbjct:  9 SFRERQAQELEVIKSIFGCVDLEDLRPQANPSLWK 43

Query: 53 PEINLVLQP--QGLTGEEVYVKVRLRVKCPPTYPD 85
         +I + LP  G E Y V  L V CP  YP
Sbjct:  44 TDIRIQLTPLRDSSNGLETYVCTKLHVTCPSKYPK 78

Query: 86 VVPEIELKNAKGLSNESVNLKSRLEELAKKHCGE 120
         + P+I L+ +KG+S++ + L+++L+ +++ GE
Sbjct:  79 LPPKISLEESKGMSDQLLEALRNQLQAQSOELRGE 113

**FIG. 7T**
45/53

Query: 121 VMIFELAYHVQSFSLSEHNKPPPKSFHEEMLRERAQ 154
VMI+ELA VQ+FL EHNKPP SF+++ML+ + +
Sbjct: 114 VMIFELAQTQAFLLLEHNKPKPGSFYDQMQLQDKQK 148

Query: 155 EEQQ 159
+Q+
Sbjct: 149 RDQE 152 SEQ. ID NO: 17

Score = 63 (27.2 bits), Expect = 1.7e-28, Sum P(2) = 1.7e = 28 Identities = 15/59 (25%), Positives = 30/59 (50%)

Query: 266 EILYFN-MGSPDQLMVHKGKCIISDEQLGKLVYNA 299
ELYF+ MG + +G C+G ++ G + Y
Sbjct: 230 ETLYFHKMGR----QIORQCCVGHSQQR-GCIIATG 259

Query: 300 LETATGFGVLLYEWVLQWQKKMG 323
++ G + + EW +++ + P
Sbjct: 260 IDMHCGQLLYITEW?IKYSQLEQP 283
↑SEQ. ID NO: 18

View Prodom 42726

>42726 p99.2(2) 001712(1) 061651(1) //INITIATION
FACTOR KINASE EUKARYOTIC EIF-2 ALPHA EIF-2ALPHA
Length =469

Score = 221 (82.9 bits), Expect = 7.5e-15, P = 7.5e-15 Identities = 92/341 (26%), Positives 160/341 (45%)

fig 7i

Query: 1274 INSLIKQRTGIAQLVKYGLKDEEEXXXXXXXX 1306
+ SL++ K A L + L++LE
Sbjct: 70 LKSLSMRGBGEAASLARGALRELETVVGLAYSLG 102

Query: 1307 XXXXXXXNLGLVYKVQQ--HNGIIFQFVAFIKR 1337
GL ++ GI++Q A +K
Sbjct: 103 VKCPIHIWAGLPIFDRASNGGIVWQMTADLKP 135

FIG. 7U
Query: 1338 RQRAVPEILAAGGRYDLLIPQF-RGPOALGFVP 1369
+ P +LA G RYD ++ +F + Q P
Sbjct: 136 NRSGHPSVLAIGERYDSMLHEFQKQAQKFNPAM 168
Query: 1370 TAIGV------SIAIDKISAAVNLMEESVTISSL 1396
A GV +++DK+ AAV +E + +
Sbjct: 169 PARGVLSAGALTFSLDKLVAAV-GVEYAKDCRA 200
Query: 1397 CDLLVSVQMSMSRAINTQKLT-WTAGITAEI 1428
D+ + G + + +L W+ GI I
Sbjct: 201 IDVGICVCGTRPPLKDVYIMRLLWSVGIRCGI 233
Query: 1429 MYDWSQSQEELQEQYCRHHEITYVALVSDEKSH 1461
+ S+ +E Q+ R + +V LV++ GS
Sbjct: 234 VEEAASELGDEAQDLARLGL-HVILVAEN-GS- 263
Query: 1462 VVKVSFEKEQRTQETKVETELVDHVLQKLRKV 1494
++V+SFE+ER E+ + TELV+ + + LR+
Sbjct: 264 LRVRSFERERFQERHTRLTEVEFIQKMLRS-- 294
Query: 1495 TDERNGREASDNLAVQNLKGSFSNAS------ 1520
D NG DN + + GS N S
Sbjct: 295 -DGLNGGTV-DNFHSALGSGDNRSSGKERE 325
Query: 1521 -----GLF-EIGHATV------VPIVSV--LAPE 1540
GL AT+ +P + V L +
Sbjct: 326 RGENGLSTSAASNATKNNYSQLPQLQVTFLTHD 358
Query: 1541 KLSASTRRRYETQVTQRLQTSLANLHQKSEIIE 1573
K +A+ +RR E QV ++ ++L+ +K + +
Sbjct: 359 KPTANYKRLDENQVAQQMSSTLSSLFKKETFV- 390
Query: 1574 ILAVDLPKETI 1584
+L V+LP +
Sbjct: 391 VLVVELPPAVV 401 SEQ. ID NO: 19

Score = 150 (57.9 bits), Except = 4.2e-07, P = 4.2e-07 Identities = 66/300 (22%), Positives = 137/300 (45%)
Query: 1368  VPTAIGVSIAIDKISAAVLMNEESVTISSCDLL 1400
       V + G++ ++DK+ AAV +E +  + D+
Sbjct:  173  VLSGAGLTFSLKLVAAV-GVEYAKDCRAIDVG  204

Query: 1401  VVSVGQMSMSRANLTQKL-WTATGTAEMYDW 1432
       + G  +  +L W+ GI  I+
Sbjct:  205  IVCVGTRPPLKDVTYIMRLLWSGVIRGIVEAA  237

Query: 1433  SQSSEQELQEYCRHHHEITYVALVSDKEGSHVVK 1465
       S+ +E Q+ R  +V LV++  V+ R
Sbjct:  238  SELGDEAQDLARLGAHLVILVAENGSLRVRSF  269

Query: 1466  SFEK--ERQTEKRLTELEVDHVLQK--LRTKV 1494
       E+ ER + L E + +L+ + L
Sbjct:  270  ERERFQERHLTRTEL-VEFIOQMLRSDGLNHTT  301

Query: 1495  TDERNGREA---SDNLAV------QNLKGSFSN 1518
       D + A DN +  G++
Sbjct:  302  VDNFSHLSALSGDNRSSGGERGENGRTS  334

Query: 1519  ASGLFEIHGATVVPIVSV--LAPLEKLSASTRRR 1549
       AS + +P + V L +K +A+ +RR
Sbjct:  335  ASNATIKNNSQLPNLQVTFLTHDKPTANYKRR  367

Query: 1550  YETQVQTRLQTSALNHQKSSEIEILAVDLPKE 1582
       E QV ++ ++L+ +K + + +L V+LP
Sbjct:  368  LENQVAAQOMSSTLSQFLKKETFV-VLVVELPPA  399

Query: 1583  TILQFSL--EWDADEQAFNTTVKQLLSRLPK-- 1612
       +  +  +  +  ++ + R K
Sbjct:  400  VVNAIVGAINPREIRKRETEPEINYVIERSKY  432

Query: 1613  QRYLKLVCDEIYNIKVKEKVKVSFLFLSYRDDY 1645
       +RY+ + +E+ +  + K ++ LYS D YY
Sbjct:  433  KRYISEINEEVDYLDSDAKPIVALYSISDSYY  465

Query: 1646  RIL  1648
       R++
Sbjct:  466  RVI  468 SEQ. ID NO: 20

FIG. 7W

SUBSTITUTE SHEET (RULE 26)
View Prodom 2305

>2305 p99.2 (23) SYH(12) // SYNTHETASE AMINOACYL-TRNA HISTIDYL-TRNA PROTEIN LIGASE ATP-BINDING BIOSYNTHESIS HISTIDINE--TRNA HISRS KINASE Length = 145

Score = 137 (53.3 bits), Expect = 3.9e-08, P = 3.9e-08 Identities = 41/131 (31%), Positives = 62/131 (47%)

Query: 1106 D H S G M L P F D L R I P F A R Y V A R N N I ---- L N L 1134
D G L + L + D L + P F A R Y V A N + L L

K R Y I + V + R + + R + E + C F D I +
Sbjct: 51 K R Y H I A K V Y R R D R P A M T R G R Y R E F Y Q C D F D I I - 82

Query: 1166 S T T N S F L P X X X X X X X X Q E F A L Q E R N Y S I 1198
++ P ++ + N + I
Sbjct: 83 G E Y D T M A P D A E I L K I L T E I L S Q L G I R E L G N F K I 115

Query: 1199 Y L N H T M L L K A I L L H C G I P E D K L S Q V Y I I L Y D A 1230
+ N H + L + L + L + P K Q Y + Y A
Sbjct: 116 K I N H R G I L D S L L Q ---- P W P K T L Q E Y L T Q Y K A 143

View Prodom 1

>1 p99.2 (2773) CC2(14) KK1T(14) KPC1(13) // KINASE PROTEIN TRANSFERASE ATP-BINDING SERINE/ThERONINE-PROTEIN PHOSPHORYLATION RECEPTOR TYROSINE-PROTEIN PRECURSOR TRANSMEMBRANE Length = 431

Score = 107 (42.7 bits), Expect = 0.0032, Sum P(2) = 0.0032 Identities = 36/106 (33%), Positives = 50/106 (47%)

FIG. 7X

SUBSTITUTE SHEET (RULE 26)
49/53

Query: 848 DLKPVNIFLDSDDH-------VK-IGDFGLATDHLA 875
DLKP NI LD + H +K I DFGLA +
Sbjct: 220 DLKPENILLDEESHENTPNMIKLIADFGHAKE---I 253

Query: 876 FSADSKQDDQQTGDLIKSDPSGHTGTMVGTALYS- 909
+S+ S ++ + + M GT YVS
Sbjct: 254 YSSSSTYEEMSSSQAVFGSHQTTSTMCGTPYYVSM 288

Query: 910 ----PEVQGSTKSA------YNQKVDLFSLGIIFFE 935
PE SA Y+ K D++S G+I +E
Sbjct: 289 KSMAPEYMAPESSATNYQKYSTKSDVWSFGVILYE 323

Query: 936 M 936
M
Sbjct: 323 M 323 SEQ.ID NO: 22

Score = 105 (42.0 bits), Expect = 3.5e-05 Sum
P(3)=3.5e-05 Identities = 35/102 (34%), Positives = 48/102 (47%)

Query: 824 RLWRLFREILDGLAYIHEK------GMIHR----DL 849
+L +I GL Y+H K G+IHR DL
Sbjct: 187 QLMHYVHQIAKGLEYLHSDKNQKHQGIIHRAKKVDL 221

Query: 850 KPVNIFLDSDDH-------VK-IGDFGLATDHLAFS 877
KP NI LD + H +K I DFGLA + +S
Sbjct: 222 KPNILLDEESHENTPNMIKLIADFGHAKE---IY 254

Query: 878 ADSKQDDQQTGDLIKSDPSGHTGTMVGTALYS 909
+S ++ + + M GT YVS
Sbjct: 255 SSSTYEEMSSSQAVFGSHQTTSTMCGTPYYVYS 286
↑SEQ.ID NO: 23

Score = 74 (31.1 bits), Expect = 0.91, Sum P(2) = 0.60 Identities = 30/127 (23%), Positives = 60/127 (47%)

Query: 341 QGTETEFSNLVKLHSPNVVRYLAMNLKEQDDSIVV 375
+G+ E+ ++ + ++ L++ ++ + ++
Sbjct: 134 EGSLVEYMEYMSGSSEDYMKKLSELETVMKIAMMIL 168

FIG. 7Y

SUBSTITUTE SHEET (RULE 26)
50/53

Query: 376 DIL-VEHISGVSLSAAHLSGPIPVHQLRRYTAQL 409
     + + H+S S + LSHSLQL Y Q+
Sbjct: 169 QFMQIMHMSSESES--LSHSS----QLMHYVHQI 195

Query: 410 LGSDLYLSNNS----VVKV----LSASNVLVDA 435
     GL+YLHS + ++H+ L N+L+D
Sbjct: 196 AKGLEYLHSKNNQKHQGIIHRRAKVLKDLKPNELLDE 230

Query: 436 EG------TVK-IDYSISKRL 450
     E +K I D+ ++K +
Sbjct: 231 ESHENTPMIKLIADFLAKEI 252
   ↑SEQ.ID NO: 24

Score = 65 (27.9 bits), Expect = 3.5e-05, Sum P(3) = 3.5e-05 Identities = 29/124 (23%), Positives = 50/124 (40%)

Query: 907 YVSPEVQGSTKSAYNQKVLDSLGSIFMM-SYHP 940
     Y++PE + Y+ K D++S G+I +EM + P
Sbjct: 294 YMAPESSATNYQKYSTKSDVWSFVGILYEMLTGKP 328

Query: 941 MVTSERIFVLNQLRDPTSPKFPEDFDGEGHKQK 975
     E +++ S KE + G +
Sbjct: 329 PFPFPGES--EVSEEEPYQSMKMNEMVLEMGPETIQ 361

Query: 976 SVISWLLNHDPAKRPT----------ATELLKS 998
     V+S ++ + P A +LLK
Sbjct: 362 KVMSKIVEKKGERMPQPSNSSNCPEVSQEAKDQLKK 396

Query: 999 ELLPPPQMEESELHEVLHH 1017
     L P+ E+L H
Sbjct: 397 CLQKDPEKRRPTFEEILQH 415 SEQ.ID NO: 25

Score = 55 (24.4 bits), Expect 3.5e-05, Sum P(3) = 3.5e-05 Identities = 12/23 (52%), Positives 18/23 (78%)

Query: 589 EFEELQ-LLGKGAFGAVIKVQNK 610
     ++E L+ LLGKG+FG V K ++K
Sbjct: 33 QYELLKKLLGKGSFGKVYKAKHK 55
   ↑SEQ.ID NO: 26

FIG. 7Z

SUBSTITUTE SHEET (RULE 26)
Score = 49 (22.3 bits), Expect 34., Sum P(2) = 1.0
Identities = 12/39 (30%), Positives = 24/39 (61%)

Query: 507 DLPADFQDFLKKCVCLD-DKERWSFQQLLKHS-FI 539
++ + +D LKKC+ D +K R + +++L+H F+
Sbjct: 385 EVSQEAKEKLKKCLQKDPEKRRTFLEEILQHPWFL 419

Query: 540 NPQP 543
P
Sbjct: 420 MRNP 423 SEQ.ID NO: 27

Score = 40 (19.1 bits), Expect = 0.0010, Sum P(3) = 0.0010 Identities = 8/11 (72%), Positives = 9/11 (81%)

Query: 596 LGKGAFFGAVIK 606
LG G+FGAV K
Sbjct: 2 LGTGSFGAVIK 12 SEQ.ID NO: 28

View Prodom 150228

>150228 p99.2 (1) O74297_NEUCR //CPC3 PROTEIN
Length = 108

Score = 90 (36.7 bits), Expect = 0.0039, P = 0.0039 Identities = 32/105 (30%), Positives = 43/105 (40%)

Query: 1105 MDHSGMLVMPLFDIRPFPARYVAR--NNILN 1133
+D +G ++ LPPDL + AR +AR N+ +
Sbjct: 3 LDQNGTVLQLPFDLMMGHASLRITNSPVV 33

Query: 1134 LKRYCIERVFRPRKLDRFHPKELLECAFDIVT 1165
K Y +FR R P E FDIVT
Sbjct: 35 QKSYSFGNIFRDRH-GGGQPDVYGEVDFDIVP 65

Query: 1166 STTNSF-LPXXXXXXXXXXQEFPPALQERNY 1196

FIG. 7A1

SUBSTITUTE SHEET (RULE 26)
View Prodom 13771

>137719 p99.2(1) 074297_NUCR //CPC3 PROTEIN Length = 304

Score = 87 (35.7 bits), Expect 0.61, Sum P(2) = (0.45) Identities=41/146 (26%), Positives = 64/156 (41%)

Query:
19 YPQRQDHELQALEAIYGADFDQDLRPDACGPVKEPP
   Y + Q+ E+ L + AIYG DF  K P

Sbjct:
44 YQEYQESSEVMVLQAIYGEDFTQHEAAHGAWQKSEP

Query:
54 EINLVLYPQGLTGEEVYVKVLRLVKCPPTYPDVVP
   ++ + P + +E+ V L V TYP P

Sbjct:
79 RFDIKIKPS--SDQEL--SVTGVMVATYPKTTP

Query:
89 EIELKNAKGLSNSVESSLKSRLLEELAKK---HCGE
   + +K+ L ES + E K +

Sbjct:
110 LLTIKDDHSL-RESTKFKIQQFVETQPKIYAQAEQ

Query:
121 VMIFELAHVQFSLSE--HNK------PP---PKS
   MI ++ ++ L E K P ++

Sbjct:
144 EMIDQIVEGIRDILEEAAQKKVQGLEIPSLEEERA

Query:
145 FHEEMLERRAQEEQQR 160
   HE L R AQ E++R

Sbjct:
179 AHEAEALRQAQSEKER 194 SEQ.ID NO: 30

Score = 49 (22.3 bits), Expect =0.61, Sum P(2) = 0.45 Identities = 13/48 (27%), Positives = 27/48 (56%)

FIG. 7A2

SUBSTITUTE SHEET (RULE 26)
Query: 1458 EGSHVKVKSFEKERQTEKRVLETELVDHVL 1487
   E ++ EKER+ K++ E++ + VL
Sbjct: 181 EAEARLAQSEKEEREERKKLEESKEEERVL 210

Query: 1488 QK-LRTKVTDERNGREAS 1504
   + L+ ++ +RN + S
Sbjct: 211 EDMLQEELKQRQRNKAQES 228 SEQ.ID NO: 31
Score = 49 (22.3 bits), Expect = 0.61, Sum P(2) = 0.45
Identities = 13/48 (27%), Positives = 27/48 (56%)

Query: 238 KHRANSSGRSRRERQYSVCNSEDSPGSC-EIL 268
   +++A S + R Q S + PG E L
Sbjct: 222 RNKAKESRKKNRSHQLSPDRAPQDPGETDETL 253

Query: 269 YFNMGSPDQLMVHKGKCIGSDEQLGKLVY 297
   F+ P ++ G + +GK V+
Sbjct: 254 MFDO--PCKITDGSNLFFQTVIGKTVF 280
   SEQ.ID NO: 32
Score = 47 (21.6 bits), Expect = 0.96, Sum P(2) = 0.62
Identities = 19/83 (22%), Positives = 33/83 (39%)

Query: 750 LPASDSESDIIIFDNEDENSKSQ-NQDEDCNEKNGC 783
   L S E ++ D E K Q N+ ++ +KN
Sbjct: 200 LEESKEEERVLEDMLQEELKQRQRNYAKESRKKNR 234

Query: 784 HESEPSVTTEAVHYLYIQMEYCEKSTLTDQGL 818
   H+ P + + ++ + D L
Sbjct: 235 HQLSPDRAPQDPGETDETMFDQPCCKITDGSNAL 269

Query: 819 YRDTVRLWRLFRE 831
   + TV +FRE
Sbjct: 270 FFQTVIGKTVFRE 282 SEQ.ID NO: 33

FIG. 7A3