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(54) Title: HUMAN CYTOKINE RECEPTOR

(57) Abstract: Cytokines and their receptors have proven usefulness in both basic research and as therapeutics. The present invention provides a new human cytokine receptor designated as "Zcytor18".

HUMAN CYTOKINE RECEPTOR

TECHNICAL FIELD

The present invention relates generally to a new protein expressed by human cells. In particular, the present invention relates to a novel gene that encodes a receptor, designated as "Zcytor18," and to nucleic acid molecules encoding Zcytor18 polypeptides.

BACKGROUND OF THE INVENTION

Cytokines are soluble, small proteins that mediate a variety of biological effects, including the regulation of the growth and differentiation of many cell types (see, for example, Arai *et al., Annu. Rev. Biochem. 59*:783 (1990); Mosmann, *Curr. Opin. Immunol. 3*:311 (1991); Paul and Seder, *Cell 76*:241 (1994)). Proteins that constitute the cytokine group include interleukins, interferons, colony stimulating factors, tumor necrosis factors, and other regulatory molecules. For example, human interleukin-17 is a cytokine which stimulates the expression of interleukin-6, intracellular adhesion molecule 1, interleukin-8, granulocyte macrophage colony-stimulating factor, and prostaglandin E2 expression, and plays a role in the preferential maturation of CD34+ hematopoietic precursors into neutrophils (Yao *et al., J. Immunol. 155*:5483 (1995); Fossiez *et al., J. Exp. Med. 183*:2593 (1996)).

Receptors that bind cytokines are typically composed of one or more integral membrane proteins that bind the cytokine with high affinity and transduce this binding event to the cell through the cytoplasmic portions of the certain receptor subunits. Cytokine receptors have been grouped into several classes on the basis of similarities in their extracellular ligand binding domains. For example, the receptor chains responsible for binding and/or transducing the effect of interferons are members of the type II cytokine receptor family, based upon a characteristic 200 residue extracellular domain.

The demonstrated *in vivo* activities of cytokines and their receptors illustrate the clinical potential of, and need for, other cytokines, cytokine receptors, cytokine agonists, and cytokine antagonists.

BRIEF SUMMARY OF THE INVENTION

The present invention provides a novel receptor, designated "Zcytor18." The present invention also provides Zcytor18 polypeptides and Zcytor18 fusion proteins, as well as nucleic acid molecules encoding such polypeptides and proteins, and methods for using these nucleic acid molecules and amino acid sequences.

DETAILED DESCRIPTION OF THE INVENTION

1. Overview

An illustrative nucleotide sequence that encodes Zcytor18 is provided by SEQ ID NO:1. The encoded polypeptide has the following amino acid sequence: MAPWLQLCSV FFTVNACLNG SQLAVAAGGS GRARGADTCG WRMKAAARPR LCVANEGVGP ASRNSGLYNI TFKYDNCTTY LNPVGKHVIA DAQNITISQY ACHDQVAVTI LWSPGALGIE FLKGFRVILE ELKSEGRQCQ QLILKDPKQL NSSFKRTGME SQPFLNMKFE TDYFVKVVPF PSIKNESNYH PFFFRTRACD LLLQPDNLAC KPFWKPRNLN ISQHGSDMQV SFDHAPHNFG FRFFYLHYKL KHEGPFKRKT CKQEQTTETT SCLLQNVSPG DYIIELVDDT NTTRKVMHYA LKPVHSPWAG PIRAVAITVP LVVISAFATL FTVMCRKKQQ ENIYSHLDEE SSESSTYTAA LPRERLRPRP KVFLCYSSKD GQNHMNVVQC FAYFLQDFCG CEVALDLWED FSLCREGQRE WVIQKIHESQ FIIVVCSKGM KYFVDKKNYK HKGGGRGSGK GELFLVAVSA IAEKLRQAKQ SSSAALSKFI AVYFDYSCEG DVPGILDLST KYRLMDNLPQ LCSHLHSRDH GLQEPGQHTR QGSRRNYFRS KSGRSLYVAI CNMHQFIDEE PDWFEKQFVP FHPPPLRYRE PVLEKFDSGL VLNDVMCKPG PESDFCLKVE AAVLGATGPA DSQHESQHGG LDQDGEARPA LDGSAALQPL LHTVKAGSPS DMPRDSGIYD SSVPSSELSL PLMEGLSTDQ TETSSLTESV SSSSGLGEEE PPALPSKLLS SGSCKADLGC RSYTDELHAV APL (SEQ ID NO:2).

Features of the Zcytor18 polypeptide include an extracellular domain at amino acid residues 1 to 313 of SEQ ID NO:2, a putative signal sequence at amino acid residues 1 to 35 of SEQ ID NO:2, a transmembrane domain at amino acid residues 314 to 335 of SEQ ID NO:2, and an intracellular domain at amino acid residues 336 to 753 of SEQ ID NO:2. The *Zcytor18* gene resides in human chromosome 3p14.3.

Northern analysis revealed that the *Zcytor18* gene is strongly expressed in testicular, ovarian, and uterine tissue, and moderately expressed in fetal heart, fetal kidney, fetal skin, and adult brain. In contrast, little expression was detected in muscle, bladder, adult kidney, adult lung, fetal small intestine, salivary gland, or adrenal gland,

and expression was not detectable in spleen, thymus, peripheral blood leukocytes, pancreas, liver, placenta, thyroid, lymph node, or bone marrow. Studies also indicate that *Zcytor18* gene expression is higher in breast tissue than in normal breast tissue. Thus, *Zcytor18* nucleotide and amino acid sequences can be used to differentiate tissues.

One variant form of Zcytor18 is characterized by the following amino acid substitutions in the amino acid sequence of SEQ ID NO:2: Thr^{269} to Met^{269}, and Val^{750} to Ala^{750}. Additional variants of human Zcytor18 can be identified by comparison with the murine sequence (SEQ ID NO:12), such as Leu^{246} to Val^{246}, and Lys^{257} to Arg^{257}. Nucleotide, amino acid, and degenerate sequences of the human variant form are provided as SEQ ID NOs:4, 5, and 6, respectively.

A splice variant of Zcytor18 lacks amino acid residues 43 to 56 of SEQ ID NO:2. Nucleotide, amino acid, and degenerate sequences of the variant form are provided as SEQ ID NOs:7, 8, and 9, respectively. Features of this Zcytor18 splice variant include an extracellular domain at amino acid residues 1 to 299 of SEQ ID NO:8, a putative signal sequence at amino acid residues 1 to 35 of SEQ ID NO:8, a transmembrane domain at amino acid residues 300 to 321 of SEQ ID NO:8, and an intracellular domain at amino acid residues 322 to 739 of SEQ ID NO:8.

As described below, the present invention provides isolated polypeptides comprising an amino acid sequence that is at least 70%, at least 80%, or at least 90% identical to a reference amino acid sequence selected from the group consisting of: (a) an amino acid sequence comprising amino acid residues 36 to 189 of SEQ ID NO:2, (b) amino acid residues 1 to 189 of SEQ ID NO:2, (c) amino acid residues 36 to 313 of SEQ ID NO:2, (d) amino acid residues 336 to 753 of SEQ ID NO:2, (e) amino acid residues 36 to 753 of SEQ ID NO:2, (f) amino acid residues 1 to 753 of SEQ ID NO:2, (g) amino acid resides 1 to 299 of SEQ ID NO:8, (h) amino acid residues 36 to 299 of SEQ ID NO:8, (i) amino acid residues 36 to 175 of SEQ ID NO:8, (j) amino acid residues 1 to 300 of SEQ ID NO:12, (k) amino acid residues 36 to 300 of SEQ ID NO:12, and (l) 1 to 739 of SEQ ID NO:12. Certain of these polypeptides can specifically bind with an antibody that specifically binds with a polypeptide consisting of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:8, or SEQ ID NO:12. Illustrative polypeptides include polypeptides comprising, or consisting of, amino acid residues 36 to 189 of SEQ ID NO:2, amino acid residues 36 to 753 of SEQ ID NO:2, 36 to 299 of SEQ ID NO:8, amino acid resides 36 to 175 of SEQ ID NO:8, or amino acid residues 36 to 300 of SEQ ID NO:12.

The present invention also provides isolated polypeptides comprising at least 15 contiguous amino acid residues of an amino acid sequence selected from the group consisting of: (a) amino acid residues 1 to 203 of SEQ ID NO:2, (b) amino acid

residues 36 to 203 of SEQ ID NO:2, (c) amino acid residues 36 to 313 of SEQ ID NO:2, (d) amino acid residues 1 to 753 of SEQ ID NO:2, (e) amino acid residues 1 to 189 of SEQ ID NO:8, (f) amino acid residues 36 to 189 of SEQ ID NO:8, (g) amino acid residues 36 to 299 of SEQ ID NO:8, (h) amino acid residues 1 to 739 of SEQ ID NO:8, (i) amino acid residues 1 to 300 of SEQ ID NO:12, (j) amino acid residues 36 to 300 of SEQ ID NO:12, and (k) 1 to 739 of SEQ ID NO:12. The present invention further provides isolated polypeptides comprising at least 30 contiguous amino acid residues of an amino acid sequence selected from the group consisting of: (l) amino acid residues 1 to 218 of SEQ ID NO:2, (m) amino acid residues 36 to 218 of SEQ ID NO:2, (n) amino acid residues 36 to 313 of SEQ ID NO:2, (o) amino acid residues 1 to 753 of SEQ ID NO:2, (p) amino acid residues 1 to 204 of SEQ ID NO:8, (q) amino acid residues 36 to 204 of SEQ ID NO:8, (r) amino acid residues 36 to 299 of SEQ ID NO:8, (s) amino acid residues 1 to 739 of SEQ ID NO:8, (t) amino acid residues 1 to 300 of SEQ ID NO:12, (u) amino acid residues 36 to 300 of SEQ ID NO:12, and (v) 1 to 739 of SEQ ID NO:12. Illustrative polypeptides include polypeptides that either comprise, or consist of, amino acid residues (a) to (v).

The present invention also includes variant Zcytor18 polypeptides, wherein the amino acid sequence of the variant polypeptide shares an identity with amino acid residues 36 to 189 of SEQ ID NO:2, amino acid residues 36 to 175 of SEQ ID NO:8, or amino acid residues 36 to 300 of SEQ ID NO:12, selected from the group consisting of at least 70% identity, at least 80% identity, at least 90% identity, at least 95% identity, or greater than 95% identity, and wherein any difference between the amino acid sequence of the variant polypeptide and the corresponding amino acid sequence of SEQ ID NO:2, SEQ ID NO:8, or SEQ ID NO:12, is due to one or more conservative amino acid substitutions. In addition, the present invention includes variant Zcytor18 polypeptides, characterized by the following amino acid substitutions in the amino acid sequence of SEQ ID NO:2: Thr^{269} to Met^{269}, and Val^{750} to Ala^{750}.

The polypeptides described herein can further comprise an affinity tag.

The present invention further provides antibodies and antibody fragments that specifically bind with such polypeptides. Exemplary antibodies include polyclonal antibodies, murine monoclonal antibodies, humanized antibodies derived from murine monoclonal antibodies, and human monoclonal antibodies. Illustrative antibody fragments include $F(ab')_2$, $F(ab)_2$, Fab', Fab, Fv, scFv, and minimal recognition units. The present invention further includes compositions comprising a carrier and a peptide, polypeptide, or antibody described herein.

The present invention also provides isolated nucleic acid molecules that encode a Zcytor18 polypeptide, wherein the nucleic acid molecule is selected from the

group consisting of: (a) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:3 or the nucleotide sequence of SEQ ID NO:9, (b) a nucleic acid molecule encoding an amino acid sequence that comprises amino acid residues 36 to 189 of SEQ ID NO:2, amino acid residues 36 to 313 of SEQ ID NO:2, amino acid residues 36 to 175 of SEQ ID NO:8, or amino acid residues 36 to 299 of SEQ ID NO:8, and (c) a nucleic acid molecule that remains hybridized following stringent wash conditions to a nucleic acid molecule consisting of the nucleotide sequence of nucleotides 192 to 652 of SEQ ID NO:1, the nucleotide sequence of nucleotides 192 to 610 of SEQ ID NO:7, the complement of the nucleotide sequence of nucleotides 192 to 652 of SEQ ID NO:1, or the complement of the nucleotide sequence of nucleotides 192 to 610 of SEQ ID NO:7. Illustrative nucleic acid molecules include those in which any difference between the amino acid sequence encoded by nucleic acid molecule (c) and the corresponding amino acid sequence of SEQ ID NO:2, or SEQ ID NO:8, is due to a conservative amino acid substitution. The present invention further contemplates isolated nucleic acid molecules that comprise nucleotides 192 to 652 of SEQ ID NO:1 or nucleotides 192 to 610 of SEQ ID NO:7, as well as nucleic acid molecules that comprise nucleotides 206 to 1000 of SEQ ID NO:11.

The present invention also includes vectors and expression vectors comprising such nucleic acid molecules. Such expression vectors may comprise a transcription promoter, and a transcription terminator, wherein the promoter is operably linked with the nucleic acid molecule, and wherein the nucleic acid molecule is operably linked with the transcription terminator. The present invention further includes recombinant host cells and recombinant viruses comprising these vectors and expression vectors. Illustrative host cells include avian, bacterial, yeast, fungal, insect, mammalian, and plant cells. Recombinant host cells comprising such expression vectors can be used to produce Zcytor18 polypeptides by culturing such recombinant host cells that comprise the expression vector and that produce the Zcytor18 protein, and, optionally, isolating the Zcytor18 protein from the cultured recombinant host cells. The present invention includes the protein products of such processes.

In addition, the present invention provides pharmaceutical compositions comprising a pharmaceutically acceptable carrier and at least one of such an expression vector or recombinant virus comprising such expression vectors. The present invention further includes pharmaceutical compositions, comprising a pharmaceutically acceptable carrier and a polypeptide described herein.

The present invention also contemplates methods for detecting the presence of *Zcytor18* RNA in a biological sample, comprising the steps of (a) contacting a *Zcytor18* nucleic acid probe under hybridizing conditions with either (i) test RNA

molecules isolated from the biological sample, or (ii) nucleic acid molecules synthesized from the isolated RNA molecules, wherein the probe has a nucleotide sequence comprising a portion of the nucleotide sequence of SEQ ID NO:1, or its complement, and (b) detecting the formation of hybrids of the nucleic acid probe and either the test RNA molecules or the synthesized nucleic acid molecules, wherein the presence of the hybrids indicates the presence of *Zcytor18* RNA in the biological sample. For example, suitable probes consist of the following nucleotide sequences: nucleotides 86 to 652 of SEQ ID NO:1, nucleotides 192 to 652 of SEQ ID NO:1, nucleotides 86 to 1024 of SEQ ID NO:1, nucleotides 192 to 1024 of SEQ ID NO:1, nucleotides 86 to 610 of SEQ ID NO:7, nucleotides 192 to 610 of SEQ ID NO:7, nucleotides 86 to 982 of SEQ ID NO:7, and nucleotides 192 to 982 of SEQ ID NO:7. Other suitable probes consist of the complement of these nucleotide sequences, or a portion of the nucleotide sequences or their complements. An example of a biological sample is a human biological sample, such as a biopsy or autopsy specimen.

The present invention further provides methods for detecting the presence of Zcytor18 polypeptide in a biological sample, comprising the steps of: (a) contacting the biological sample with an antibody or an antibody fragment that specifically binds with a polypeptide consisting of the amino acid sequence of SEQ ID NO:2, or SEQ ID NO:8, wherein the contacting is performed under conditions that allow the binding of the antibody or antibody fragment to the biological sample, and (b) detecting any of the bound antibody or bound antibody fragment. Such an antibody or antibody fragment may further comprise a detectable label selected from the group consisting of radioisotope, fluorescent label, chemiluminescent label, enzyme label, bioluminescent label, and colloidal gold. An example of a biological sample is a human biological sample, such as a biopsy or autopsy specimen.

The present invention also provides kits for performing these detection methods. For example, a kit for detection of *Zcytor18* gene expression may comprise a container that comprises a nucleic acid molecule, wherein the nucleic acid molecule is selected from the group consisting of (a) a nucleic acid molecule comprising the nucleotide sequence of nucleotides 86 to 652 of SEQ ID NO:1, (b) a nucleic acid molecule comprising the complement of nucleotides 192 to 652 of the nucleotide sequence of SEQ ID NO:1, (c) a nucleic acid molecule comprising the nucleotide sequence of nucleotides 86 to 610 of SEQ ID NO:7, (d) a nucleic acid molecule comprising the complement of nucleotides 192 to 610 of the nucleotide sequence of SEQ ID NO:7, and (e) a nucleic acid molecule that is a fragment of (a)-(d) consisting of at least eight nucleotides. Such a kit may also comprise a second container that comprises one or more reagents capable of indicating the presence of the nucleic acid molecule. On

the other hand, a kit for detection of Zcytor18 protein may comprise a container that comprises an antibody, or an antibody fragment, that specifically binds with a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:8.

The present invention also contemplates anti-idiotype antibodies, or anti-idiotype antibody fragments, that specifically bind an antibody or antibody fragment that specifically binds a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:8. An exemplary anti-idiotype antibody binds with an antibody that specifically binds a polypeptide consisting of amino acid residues 36 to 313 of SEQ ID NO:2, amino acid residues 36 to 189 of SEQ ID NO:2, amino acid residues 36 to 299 of SEQ ID NO:8, or amino acid residues 36 to 175 of SEQ ID NO:8.

The present invention also provides isolated nucleic acid molecules comprising a nucleotide sequence that encodes a Zcytor18 secretion signal sequence and a nucleotide sequence that encodes a biologically active polypeptide, wherein the Zcytor18 secretion signal sequence comprises an amino acid sequence of residues 1 to 35 of SEQ ID NO:2. Illustrative biologically active polypeptides include Factor VIIa, proinsulin, insulin, follicle stimulating hormone, tissue type plasminogen activator, tumor necrosis factor, interleukin, colony stimulating factor, interferon, erythropoietin, and thrombopoietin. Moreover, the present invention provides fusion proteins comprising a Zcytor18 secretion signal sequence and a polypeptide, wherein the Zcytor18 secretion signal sequence comprises an amino acid sequence of residues 1 to 35 of SEQ ID NO:2.

The present invention also provides fusion proteins, comprising a Zcytor18 polypeptide and an immunoglobulin moiety. In such fusion proteins, the immunoglobulin moiety may be an immunoglobulin heavy chain constant region, such as a human F_C fragment. The present invention further includes isolated nucleic acid molecules that encode such fusion proteins.

These and other aspects of the invention will become evident upon reference to the following detailed description. In addition, various references are identified below.

2. Definitions

In the description that follows, a number of terms are used extensively. The following definitions are provided to facilitate understanding of the invention.

As used herein, "nucleic acid" or "nucleic acid molecule" refers to polynucleotides, such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA),

oligonucleotides, fragments generated by the polymerase chain reaction (PCR), and fragments generated by any of ligation, scission, endonuclease action, and exonuclease action. Nucleic acid molecules can be composed of monomers that are naturally-occurring nucleotides (such as DNA and RNA), or analogs of naturally-occurring nucleotides (*e.g.*, α-enantiomeric forms of naturally-occurring nucleotides), or a combination of both. Modified nucleotides can have alterations in sugar moieties and/or in pyrimidine or purine base moieties. Sugar modifications include, for example, replacement of one or more hydroxyl groups with halogens, alkyl groups, amines, and azido groups, or sugars can be functionalized as ethers or esters. Moreover, the entire sugar moiety can be replaced with sterically and electronically similar structures, such as aza-sugars and carbocyclic sugar analogs. Examples of modifications in a base moiety include alkylated purines and pyrimidines, acylated purines or pyrimidines, or other well-known heterocyclic substitutes. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Analogs of phosphodiester linkages include phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilidate, phosphoramidate, and the like. The term "nucleic acid molecule" also includes so-called "peptide nucleic acids," which comprise naturally-occurring or modified nucleic acid bases attached to a polyamide backbone. Nucleic acids can be either single stranded or double stranded.

The term "complement of a nucleic acid molecule" refers to a nucleic acid molecule having a complementary nucleotide sequence and reverse orientation as compared to a reference nucleotide sequence. For example, the sequence 5' ATGCACGGG 3' is complementary to 5' CCCGTGCAT 3'.

The term "contig" denotes a nucleic acid molecule that has a contiguous stretch of identical or complementary sequence to another nucleic acid molecule. Contiguous sequences are said to "overlap" a given stretch of a nucleic acid molecule either in their entirety or along a partial stretch of the nucleic acid molecule.

The term "degenerate nucleotide sequence" denotes a sequence of nucleotides that includes one or more degenerate codons as compared to a reference nucleic acid molecule that encodes a polypeptide. Degenerate codons contain different triplets of nucleotides, but encode the same amino acid residue (*i.e.*, GAU and GAC triplets each encode Asp).

The term "structural gene" refers to a nucleic acid molecule that is transcribed into messenger RNA (mRNA), which is then translated into a sequence of amino acids characteristic of a specific polypeptide.

An "isolated nucleic acid molecule" is a nucleic acid molecule that is not integrated in the genomic DNA of an organism. For example, a DNA molecule that

encodes a growth factor that has been separated from the genomic DNA of a cell is an isolated DNA molecule. Another example of an isolated nucleic acid molecule is a chemically-synthesized nucleic acid molecule that is not integrated in the genome of an organism. A nucleic acid molecule that has been isolated from a particular species is smaller than the complete DNA molecule of a chromosome from that species.

A "nucleic acid molecule construct" is a nucleic acid molecule, either single- or double-stranded, that has been modified through human intervention to contain segments of nucleic acid combined and juxtaposed in an arrangement not existing in nature.

"Linear DNA" denotes non-circular DNA molecules having free 5' and 3' ends. Linear DNA can be prepared from closed circular DNA molecules, such as plasmids, by enzymatic digestion or physical disruption.

"Complementary DNA (cDNA)" is a single-stranded DNA molecule that is formed from an mRNA template by the enzyme reverse transcriptase. Typically, a primer complementary to portions of mRNA is employed for the initiation of reverse transcription. Those skilled in the art also use the term "cDNA" to refer to a double-stranded DNA molecule consisting of such a single-stranded DNA molecule and its complementary DNA strand. The term "cDNA" also refers to a clone of a cDNA molecule synthesized from an RNA template.

A "promoter" is a nucleotide sequence that directs the transcription of a structural gene. Typically, a promoter is located in the 5' non-coding region of a gene, proximal to the transcriptional start site of a structural gene. Sequence elements within promoters that function in the initiation of transcription are often characterized by consensus nucleotide sequences. These promoter elements include RNA polymerase binding sites, TATA sequences, CAAT sequences, differentiation-specific elements (DSEs; McGehee *et al.*, *Mol. Endocrinol. 7*:551 (1993)), cyclic AMP response elements (CREs), serum response elements (SREs; Treisman, *Seminars in Cancer Biol. 1*:47 (1990)), glucocorticoid response elements (GREs), and binding sites for other transcription factors, such as CRE/ATF (O'Reilly *et al., J. Biol. Chem. 267*:19938 (1992)), AP2 (Ye *et al., J. Biol. Chem. 269*:25728 (1994)), SP1, cAMP response element binding protein (CREB; Loeken, *Gene Expr. 3*:253 (1993)) and octamer factors (see, in general, Watson *et al.,* eds., *Molecular Biology of the Gene*, 4th ed. (The Benjamin/Cummings Publishing Company, Inc. 1987), and Lemaigre and Rousseau, *Biochem. J. 303*:1 (1994)). If a promoter is an inducible promoter, then the rate of transcription increases in response to an inducing agent. In contrast, the rate of transcription is not regulated by an inducing agent if the promoter is a constitutive promoter. Repressible promoters are also known.

A "core promoter" contains essential nucleotide sequences for promoter function, including the TATA box and start of transcription. By this definition, a core promoter may or may not have detectable activity in the absence of specific sequences that may enhance the activity or confer tissue specific activity.

A "regulatory element" is a nucleotide sequence that modulates the activity of a core promoter. For example, a regulatory element may contain a nucleotide sequence that binds with cellular factors enabling transcription exclusively or preferentially in particular cells, tissues, or organelles. These types of regulatory elements are normally associated with genes that are expressed in a "cell-specific," "tissue-specific," or "organelle-specific" manner.

An "enhancer" is a type of regulatory element that can increase the efficiency of transcription, regardless of the distance or orientation of the enhancer relative to the start site of transcription.

"Heterologous DNA" refers to a DNA molecule, or a population of DNA molecules, that does not exist naturally within a given host cell. DNA molecules heterologous to a particular host cell may contain DNA derived from the host cell species (*i.e.,* endogenous DNA) so long as that host DNA is combined with non-host DNA (*i.e.,* exogenous DNA). For example, a DNA molecule containing a non-host DNA segment encoding a polypeptide operably linked to a host DNA segment comprising a transcription promoter is considered to be a heterologous DNA molecule. Conversely, a heterologous DNA molecule can comprise an endogenous gene operably linked with an exogenous promoter. As another illustration, a DNA molecule comprising a gene derived from a wild-type cell is considered to be heterologous DNA if that DNA molecule is introduced into a mutant cell that lacks the wild-type gene.

A "polypeptide" is a polymer of amino acid residues joined by peptide bonds, whether produced naturally or synthetically. Polypeptides of less than about 10 amino acid residues are commonly referred to as "peptides."

A "protein" is a macromolecule comprising one or more polypeptide chains. A protein may also comprise non-peptidic components, such as carbohydrate groups. Carbohydrates and other non-peptidic substituents may be added to a protein by the cell in which the protein is produced, and will vary with the type of cell. Proteins are defined herein in terms of their amino acid backbone structures; substituents such as carbohydrate groups are generally not specified, but may be present nonetheless.

A peptide or polypeptide encoded by a non-host DNA molecule is a "heterologous" peptide or polypeptide.

An "integrated genetic element" is a segment of DNA that has been incorporated into a chromosome of a host cell after that element is introduced into the

cell through human manipulation. Within the present invention, integrated genetic elements are most commonly derived from linearized plasmids that are introduced into the cells by electroporation or other techniques. Integrated genetic elements are passed from the original host cell to its progeny.

A "cloning vector" is a nucleic acid molecule, such as a plasmid, cosmid, or bacteriophage, which has the capability of replicating autonomously in a host cell. Cloning vectors typically contain one or a small number of restriction endonuclease recognition sites that allow insertion of a nucleic acid molecule in a determinable fashion without loss of an essential biological function of the vector, as well as nucleotide sequences encoding a marker gene that is suitable for use in the identification and selection of cells transformed with the cloning vector. Marker genes typically include genes that provide tetracycline resistance or ampicillin resistance.

An "expression vector" is a nucleic acid molecule encoding a gene that is expressed in a host cell. Typically, an expression vector comprises a transcription promoter, a gene, and a transcription terminator. Gene expression is usually placed under the control of a promoter, and such a gene is said to be "operably linked to" the promoter. Similarly, a regulatory element and a core promoter are operably linked if the regulatory element modulates the activity of the core promoter.

A "recombinant host" is a cell that contains a heterologous nucleic acid molecule, such as a cloning vector or expression vector. In the present context, an example of a recombinant host is a cell that produces Zcytor18 from an expression vector. In contrast, Zcytor18 can be produced by a cell that is a "natural source" of Zcytor18, and that lacks an expression vector.

"Integrative transformants" are recombinant host cells, in which heterologous DNA has become integrated into the genomic DNA of the cells.

A "fusion protein" is a hybrid protein expressed by a nucleic acid molecule comprising nucleotide sequences of at least two genes. For example, a fusion protein can comprise at least part of a Zcytor18 polypeptide fused with a polypeptide that binds an affinity matrix. Such a fusion protein provides a means to isolate large quantities of Zcytor18 using affinity chromatography.

The term "receptor" denotes a cell-associated protein that binds to a bioactive molecule termed a "ligand." This interaction mediates the effect of the ligand on the cell. Receptors can be membrane bound, cytosolic or nuclear; monomeric (*e.g.*, thyroid stimulating hormone receptor, beta-adrenergic receptor) or multimeric (*e.g.*, PDGF receptor, growth hormone receptor, IL-3 receptor, GM-CSF receptor, G-CSF receptor, erythropoietin receptor and IL-6 receptor). Membrane-bound receptors are characterized by a multi-domain structure comprising an extracellular ligand-binding

domain and an intracellular effector domain that is typically involved in signal transduction. In certain membrane-bound receptors, the extracellular ligand-binding domain and the intracellular effector domain are located in separate polypeptides that comprise the complete functional receptor.

In general, the binding of ligand to receptor results in a conformational change in the receptor that causes an interaction between the effector domain and other molecule(s) in the cell, which in turn leads to an alteration in the metabolism of the cell. Metabolic events that are often linked to receptor-ligand interactions include gene transcription, phosphorylation, dephosphorylation, increases in cyclic AMP production, mobilization of cellular calcium, mobilization of membrane lipids, cell adhesion, hydrolysis of inositol lipids and hydrolysis of phospholipids.

The term "secretory signal sequence" denotes a DNA sequence that encodes a peptide (a "secretory peptide") that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway of a cell in which it is synthesized. The larger polypeptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

An "isolated polypeptide" is a polypeptide that is essentially free from contaminating cellular components, such as carbohydrate, lipid, or other proteinaceous impurities associated with the polypeptide in nature. Typically, a preparation of isolated polypeptide contains the polypeptide in a highly purified form, *i.e.,* at least about 80% pure, at least about 90% pure, at least about 95% pure, greater than 95% pure, or greater than 99% pure. One way to show that a particular protein preparation contains an isolated polypeptide is by the appearance of a single band following sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of the protein preparation and Coomassie Brilliant Blue staining of the gel. However, the term "isolated" does not exclude the presence of the same polypeptide in alternative physical forms, such as dimers or alternatively glycosylated or derivatized forms.

The terms "amino-terminal" and "carboxyl-terminal" are used herein to denote positions within polypeptides. Where the context allows, these terms are used with reference to a particular sequence or portion of a polypeptide to denote proximity or relative position. For example, a certain sequence positioned carboxyl-terminal to a reference sequence within a polypeptide is located proximal to the carboxyl terminus of the reference sequence, but is not necessarily at the carboxyl terminus of the complete polypeptide.

The term "expression" refers to the biosynthesis of a gene product. For example, in the case of a structural gene, expression involves transcription of the structural gene into mRNA and the translation of mRNA into one or more polypeptides.

The term "splice variant" is used herein to denote alternative forms of RNA transcribed from a gene. Splice variation arises naturally through use of alternative splicing sites within a transcribed RNA molecule, or less commonly between separately transcribed RNA molecules, and may result in several mRNAs transcribed from the same gene. Splice variants may encode polypeptides having altered amino acid sequence. The term splice variant is also used herein to denote a polypeptide encoded by a splice variant of an mRNA transcribed from a gene.

As used herein, the term "immunomodulator" includes cytokines, stem cell growth factors, lymphotoxins, co-stimulatory molecules, hematopoietic factors, and synthetic analogs of these molecules.

The term "complement/anti-complement pair" denotes non-identical moieties that form a non-covalently associated, stable pair under appropriate conditions. For instance, biotin and avidin (or streptavidin) are prototypical members of a complement/anti-complement pair. Other exemplary complement/anti-complement pairs include receptor/ligand pairs, antibody/antigen (or hapten or epitope) pairs, sense/antisense polynucleotide pairs, and the like. Where subsequent dissociation of the complement/anti-complement pair is desirable, the complement/anti-complement pair preferably has a binding affinity of less than 10^9 M^{-1}.

An "anti-idiotype antibody" is an antibody that binds with the variable region domain of an immunoglobulin. In the present context, an anti-idiotype antibody binds with the variable region of an anti-Zcytor18 antibody, and thus, an anti-idiotype antibody mimics an epitope of Zcytor18.

An "antibody fragment" is a portion of an antibody such as $F(ab')_2$, $F(ab)_2$, Fab', Fab, and the like. Regardless of structure, an antibody fragment binds with the same antigen that is recognized by the intact antibody. For example, an anti-Zcytor18 monoclonal antibody fragment binds with an epitope of Zcytor18.

The term "antibody fragment" also includes a synthetic or a genetically engineered polypeptide that binds to a specific antigen, such as polypeptides consisting of the light chain variable region, "Fv" fragments consisting of the variable regions of the heavy and light chains, recombinant single chain polypeptide molecules in which light and heavy variable regions are connected by a peptide linker ("scFv proteins"), and minimal recognition units consisting of the amino acid residues that mimic the hypervariable region.

A "chimeric antibody" is a recombinant protein that contains the variable domains and complementary determining regions derived from a rodent antibody, while the remainder of the antibody molecule is derived from a human antibody.

"Humanized antibodies" are recombinant proteins in which murine complementarity determining regions of a monoclonal antibody have been transferred from

heavy and light variable chains of the murine immunoglobulin into a human variable domain.

As used herein, a "therapeutic agent" is a molecule or atom, which is conjugated to an antibody moiety to produce a conjugate, which is useful for therapy. Examples of therapeutic agents include drugs, toxins, immunomodulators, chelators, boron compounds, photoactive agents or dyes, and radioisotopes.

A "detectable label" is a molecule or atom, which can be conjugated to an antibody moiety to produce a molecule useful for diagnosis. Examples of detectable labels include chelators, photoactive agents, radioisotopes, fluorescent agents, paramagnetic ions, or other marker moieties.

The term "affinity tag" is used herein to denote a polypeptide segment that can be attached to a second polypeptide to provide for purification or detection of the second polypeptide or provide sites for attachment of the second polypeptide to a substrate. In principal, any peptide or protein for which an antibody or other specific binding agent is available can be used as an affinity tag. Affinity tags include a poly-histidine tract, protein A (Nilsson *et al., EMBO J. 4*:1075 (1985); Nilsson *et al., Methods Enzymol. 198*:3 (1991)), glutathione S transferase (Smith and Johnson, *Gene 67*:31 (1988)), Glu-Glu affinity tag (Grussenmeyer *et al., Proc. Natl. Acad. Sci. USA 82*:7952 (1985)), substance P, FLAG peptide (Hopp *et al., Biotechnology 6*:1204 (1988)), streptavidin binding peptide, or other antigenic epitope or binding domain. See, in general, Ford *et al., Protein Expression and Purification 2*:95 (1991). DNA molecules encoding affinity tags are available from commercial suppliers (*e.g.,* Pharmacia Biotech, Piscataway, NJ).

A "naked antibody" is an entire antibody, as opposed to an antibody fragment, which is not conjugated with a therapeutic agent. Naked antibodies include both polyclonal and monoclonal antibodies, as well as certain recombinant antibodies, such as chimeric and humanized antibodies.

As used herein, the term "antibody component" includes both an entire antibody and an antibody fragment.

An "immunoconjugate" is a conjugate of an antibody component with a therapeutic agent or a detectable label.

As used herein, the term "antibody fusion protein" refers to a recombinant molecule that comprises an antibody component and a Zcytor18 polypeptide component. Examples of an antibody fusion protein include a protein that comprises a Zcytor18 extracellular domain, and either an Fc domain or an antigen-biding region.

A "target polypeptide" or a "target peptide" is an amino acid sequence that comprises at least one epitope, and that is expressed on a target cell, such as a tumor

cell, or a cell that carries an infectious agent antigen. T cells recognize peptide epitopes presented by a major histocompatibility complex molecule to a target polypeptide or target peptide and typically lyse the target cell or recruit other immune cells to the site of the target cell, thereby killing the target cell.

An "antigenic peptide" is a peptide, which will bind a major histocompatibility complex molecule to form an MHC-peptide complex, which is recognized by a T cell, thereby inducing a cytotoxic lymphocyte response upon presentation to the T cell. Thus, antigenic peptides are capable of binding to an appropriate major histocompatibility complex molecule and inducing a cytotoxic T cells response, such as cell lysis or specific cytokine release against the target cell, which binds or expresses the antigen. The antigenic peptide can be bound in the context of a class I or class II major histocompatibility complex molecule, on an antigen presenting cell or on a target cell.

In eukaryotes, RNA polymerase II catalyzes the transcription of a structural gene to produce mRNA. A nucleic acid molecule can be designed to contain an RNA polymerase II template in which the RNA transcript has a sequence that is complementary to that of a specific mRNA. The RNA transcript is termed an "anti-sense RNA" and a nucleic acid molecule that encodes the anti-sense RNA is termed an "anti-sense gene." Anti-sense RNA molecules are capable of binding to mRNA molecules, resulting in an inhibition of mRNA translation.

An "anti-sense oligonucleotide specific for Zcytor18" or a "Zcytor18 anti-sense oligonucleotide" is an oligonucleotide having a sequence (a) capable of forming a stable triplex with a portion of the *Zcytor18* gene, or (b) capable of forming a stable duplex with a portion of an mRNA transcript of the *Zcytor18* gene.

A "ribozyme" is a nucleic acid molecule that contains a catalytic center. The term includes RNA enzymes, self-splicing RNAs, self-cleaving RNAs, and nucleic acid molecules that perform these catalytic functions. A nucleic acid molecule that encodes a ribozyme is termed a "ribozyme gene."

An "external guide sequence" is a nucleic acid molecule that directs the endogenous ribozyme, RNase P, to a particular species of intracellular mRNA, resulting in the cleavage of the mRNA by RNase P. A nucleic acid molecule that encodes an external guide sequence is termed an "external guide sequence gene."

The term "variant *Zcytor18* gene" refers to nucleic acid molecules that encode a polypeptide having an amino acid sequence that is a modification of SEQ ID NO:2. Such variants include naturally-occurring polymorphisms of *Zcytor18* genes, as well as synthetic genes that contain conservative amino acid substitutions of the amino acid sequence of SEQ ID NO:2. Additional variant forms of *Zcytor18* genes are nucleic

acid molecules that contain insertions or deletions of the nucleotide sequences described herein. A variant *Zcytor18* gene can be identified, for example, by determining whether the gene hybridizes with a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, or its complement, under stringent conditions.

Alternatively, variant *Zcytor18* genes can be identified by sequence comparison. Two amino acid sequences have "100% amino acid sequence identity" if the amino acid residues of the two amino acid sequences are the same when aligned for maximal correspondence. Similarly, two nucleotide sequences have "100% nucleotide sequence identity" if the nucleotide residues of the two nucleotide sequences are the same when aligned for maximal correspondence. Sequence comparisons can be performed using standard software programs such as those included in the LASERGENE bioinformatics computing suite, which is produced by DNASTAR (Madison, Wisconsin). Other methods for comparing two nucleotide or amino acid sequences by determining optimal alignment are well-known to those of skill in the art (see, for example, Peruski and Peruski, *The Internet and the New Biology: Tools for Genomic and Molecular Research* (ASM Press, Inc. 1997), Wu *et al.* (eds.), "Information Superhighway and Computer Databases of Nucleic Acids and Proteins," in *Methods in Gene Biotechnology*, pages 123-151 (CRC Press, Inc. 1997), and Bishop (ed.), *Guide to Human Genome Computing*, 2nd Edition (Academic Press, Inc. 1998)). Particular methods for determining sequence identity are described below.

Regardless of the particular method used to identify a variant *Zcytor18* gene or variant Zcytor18 polypeptide, a variant gene or polypeptide encoded by a variant gene may be functionally characterized the ability to bind specifically to an anti-Zcytor18 antibody.

The term "allelic variant" is used herein to denote any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and may result in phenotypic polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequence. The term allelic variant is also used herein to denote a protein encoded by an allelic variant of a gene.

The term "ortholog" denotes a polypeptide or protein obtained from one species that is the functional counterpart of a polypeptide or protein from a different species. Sequence differences among orthologs are the result of speciation.

"Paralogs" are distinct but structurally related proteins made by an organism. Paralogs are believed to arise through gene duplication. For example, α-globin, β-globin, and myoglobin are paralogs of each other.

The present invention includes functional fragments of *Zcytor18* genes. Within the context of this invention, a "functional fragment" of a *Zcytor18* gene refers to a nucleic acid molecule that encodes a portion of a Zcytor18 polypeptide, which is a domain described herein or at least specifically binds with an anti-Zcytor18 antibody.

Due to the imprecision of standard analytical methods, molecular weights and lengths of polymers are understood to be approximate values. When such a value is expressed as "about" X or "approximately" X, the stated value of X will be understood to be accurate to ±10%.

3. Production of Nucleic Acid Molecules Encoding Zcytor18

Nucleic acid molecules encoding a human Zcytor18 can be obtained by screening a human cDNA or genomic library using polynucleotide probes based upon SEQ ID NO:1. These techniques are standard and well-established.

As an illustration, a nucleic acid molecule that encodes a human Zcytor18 can be isolated from a cDNA library. In this case, the first step would be to prepare the cDNA library by isolating RNA from a tissue, such as testicular tissue, using methods well-known to those of skill in the art. In general, RNA isolation techniques must provide a method for breaking cells, a means of inhibiting RNase-directed degradation of RNA, and a method of separating RNA from DNA, protein, and polysaccharide contaminants. For example, total RNA can be isolated by freezing tissue in liquid nitrogen, grinding the frozen tissue with a mortar and pestle to lyse the cells, extracting the ground tissue with a solution of phenol/chloroform to remove proteins, and separating RNA from the remaining impurities by selective precipitation with lithium chloride (see, for example, Ausubel *et al.* (eds.), *Short Protocols in Molecular Biology, 3rd Edition*, pages 4-1 to 4-6 (John Wiley & Sons 1995) ["Ausubel (1995)"]; Wu *et al.*, *Methods in Gene Biotechnology*, pages 33-41 (CRC Press, Inc. 1997) ["Wu (1997)"]).

Alternatively, total RNA can be isolated by extracting ground tissue with guanidinium isothiocyanate, extracting with organic solvents, and separating RNA from contaminants using differential centrifugation (see, for example, Chirgwin *et al.*, *Biochemistry 18*:52 (1979); Ausubel (1995) at pages 4-1 to 4-6; Wu (1997) at pages 33-41).

In order to construct a cDNA library, poly(A)$^+$ RNA must be isolated from a total RNA preparation. Poly(A)$^+$ RNA can be isolated from total RNA using the standard technique of oligo(dT)-cellulose chromatography (see, for example, Aviv and Leder, *Proc. Nat'l Acad. Sci. USA 69:*1408 (1972); Ausubel (1995) at pages 4-11 to 4-12).

Double-stranded cDNA molecules are synthesized from poly(A)$^+$ RNA using techniques well-known to those in the art. (see, for example, Wu (1997) at pages 41-46). Moreover, commercially available kits can be used to synthesize double-stranded cDNA molecules. For example, such kits are available from Life Technologies, Inc. (Gaithersburg, MD), CLONTECH Laboratories, Inc. (Palo Alto, CA), Promega Corporation (Madison, WI) and STRATAGENE (La Jolla, CA).

Various cloning vectors are appropriate for the construction of a cDNA library. For example, a cDNA library can be prepared in a vector derived from bacteriophage, such as a λgt10 vector. See, for example, Huynh *et al.*, "Constructing and Screening cDNA Libraries in λgt10 and λgt11," in *DNA Cloning: A Practical Approach Vol. I*, Glover (ed.), page 49 (IRL Press, 1985); Wu (1997) at pages 47-52.

Alternatively, double-stranded cDNA molecules can be inserted into a plasmid vector, such as a PBLUESCRIPT vector (STRATAGENE; La Jolla, CA), a LAMDAGEM-4 (Promega Corp.) or other commercially available vectors. Suitable cloning vectors also can be obtained from the American Type Culture Collection (Manassas, VA).

To amplify the cloned cDNA molecules, the cDNA library is inserted into a prokaryotic host, using standard techniques. For example, a cDNA library can be introduced into competent *E. coli* DH5 cells, which can be obtained, for example, from Life Technologies, Inc. (Gaithersburg, MD).

A human genomic library can be prepared by means well-known in the art (see, for example, Ausubel (1995) at pages 5-1 to 5-6; Wu (1997) at pages 307-327). Genomic DNA can be isolated by lysing tissue with the detergent Sarkosyl, digesting the lysate with proteinase K, clearing insoluble debris from the lysate by centrifugation, precipitating nucleic acid from the lysate using isopropanol, and purifying resuspended DNA on a cesium chloride density gradient.

DNA fragments that are suitable for the production of a genomic library can be obtained by the random shearing of genomic DNA or by the partial digestion of genomic DNA with restriction endonucleases. Genomic DNA fragments can be inserted into a vector, such as a bacteriophage or cosmid vector, in accordance with conventional techniques, such as the use of restriction enzyme digestion to provide appropriate termini, the use of alkaline phosphatase treatment to avoid undesirable joining of DNA molecules, and ligation with appropriate ligases. Techniques for such manipulation are well-known in the art (see, for example, Ausubel (1995) at pages 5-1 to 5-6; Wu (1997) at pages 307-327).

Alternatively, human genomic libraries can be obtained from commercial sources such as Research Genetics (Huntsville, AL) and the American Type Culture Collection (Manassas, VA).

A library containing cDNA or genomic clones can be screened with one or more polynucleotide probes based upon SEQ ID NO:1, using standard methods (see, for example, Ausubel (1995) at pages 6-1 to 6-11).

Nucleic acid molecules that encode a human *Zcytor18* gene can also be obtained using the polymerase chain reaction (PCR) with oligonucleotide primers having nucleotide sequences that are based upon the nucleotide sequences of the *Zcytor18* gene, as described herein. General methods for screening libraries with PCR are provided by, for example, Yu *et al.*, "Use of the Polymerase Chain Reaction to Screen Phage Libraries," in *Methods in Molecular Biology, Vol. 15: PCR Protocols: Current Methods and Applications*, White (ed.), pages 211-215 (Humana Press, Inc. 1993). Moreover, techniques for using PCR to isolate related genes are described by, for example, Preston, "Use of Degenerate Oligonucleotide Primers and the Polymerase Chain Reaction to Clone Gene Family Members," in *Methods in Molecular Biology, Vol. 15: PCR Protocols: Current Methods and Applications*, White (ed.), pages 317-337 (Humana Press, Inc. 1993).

Anti-Zcytor18 antibodies, produced as described below, can also be used to isolate DNA sequences that encode human *Zcytor18* genes from cDNA libraries. For example, the antibodies can be used to screen λgt11 expression libraries, or the antibodies can be used for immunoscreening following hybrid selection and translation (see, for example, Ausubel (1995) at pages 6-12 to 6-16; Margolis *et al.*, "Screening λ expression libraries with antibody and protein probes," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), pages 1-14 (Oxford University Press 1995)).

As an alternative, a *Zcytor18* gene can be obtained by synthesizing nucleic acid molecules using mutually priming long oligonucleotides and the nucleotide sequences described herein (see, for example, Ausubel (1995) at pages 8-8 to 8-9). Established techniques using the polymerase chain reaction provide the ability to synthesize DNA molecules at least two kilobases in length (Adang *et al.*, *Plant Molec. Biol. 21*:1131 (1993), Bambot *et al.*, *PCR Methods and Applications 2*:266 (1993), Dillon *et al.*, "Use of the Polymerase Chain Reaction for the Rapid Construction of Synthetic Genes," in *Methods in Molecular Biology, Vol. 15: PCR Protocols: Current Methods and Applications*, White (ed.), pages 263-268, (Humana Press, Inc. 1993), and Holowachuk *et al., PCR Methods Appl. 4*:299 (1995)).

The nucleic acid molecules of the present invention can also be synthesized with "gene machines" using protocols such as the phosphoramidite method. If chemically-synthesized double stranded DNA is required for an application such as the synthesis of a gene or a gene fragment, then each complementary strand is made separately. The production of short genes (60 to 80 base pairs) is technically

straightforward and can be accomplished by synthesizing the complementary strands and then annealing them. For the production of longer genes (>300 base pairs), however, special strategies may be required, because the coupling efficiency of each cycle during chemical DNA synthesis is seldom 100%. To overcome this problem, synthetic genes (double-stranded) are assembled in modular form from single-stranded fragments that are from 20 to 100 nucleotides in length. For reviews on polynucleotide synthesis, see, for example, Glick and Pasternak, *Molecular Biotechnology, Principles and Applications of Recombinant DNA* (ASM Press 1994), Itakura *et al., Annu. Rev. Biochem. 53*:323 (1984), and Climie *et al., Proc. Nat'l Acad. Sci. USA 87*:633 (1990).

The sequence of a *Zcytor18* cDNA or *Zcytor18* genomic fragment can be determined using standard methods. Zcytor18 polynucleotide sequences disclosed herein can also be used as probes or primers to clone 5' non-coding regions of a *Zcytor18* gene. Promoter elements from a *Zcytor18* gene can be used to direct the expression of heterologous genes in, for example, transgenic animals or patients treated with gene therapy. The identification of genomic fragments containing a *Zcytor18* promoter or regulatory element can be achieved using well-established techniques, such as deletion analysis (see, generally, Ausubel (1995)).

Cloning of 5' flanking sequences also facilitates production of Zcytor18 proteins by "gene activation," as disclosed in U.S. Patent No. 5,641,670. Briefly, expression of an endogenous *Zcytor18* gene in a cell is altered by introducing into the *Zcytor18* locus a DNA construct comprising at least a targeting sequence, a regulatory sequence, an exon, and an unpaired splice donor site. The targeting sequence is a *Zcytor18* 5' non-coding sequence that permits homologous recombination of the construct with the endogenous *Zcytor18* locus, whereby the sequences within the construct become operably linked with the endogenous *Zcytor18* coding sequence. In this way, an endogenous *Zcytor18* promoter can be replaced or supplemented with other regulatory sequences to provide enhanced, tissue-specific, or otherwise regulated expression.

4. *Production of Zcytor18 Variants*

The present invention provides a variety of nucleic acid molecules, including DNA and RNA molecules, which encode the Zcytor18 polypeptides disclosed herein. Those skilled in the art will readily recognize that, in view of the degeneracy of the genetic code, considerable sequence variation is possible among these polynucleotide molecules. SEQ ID NO:3 is a degenerate nucleotide sequence that encompasses all nucleic acid molecules that encode the Zcytor18 polypeptide of SEQ ID NO:2. Those

skilled in the art will recognize that the degenerate sequence of SEQ ID NO:3 also provides all RNA sequences encoding SEQ ID NO:2, by substituting U for T. Thus, the present invention contemplates Zcytor18 polypeptide-encoding nucleic acid molecules comprising nucleotide 86 to nucleotide 2344 of SEQ ID NO:1, and their RNA equivalents. Similarly, the present invention contemplates Zcytor18 polypeptide-encoding nucleic acid molecules comprising nucleotide 86 to nucleotide 2302 of SEQ ID NO:7, and their RNA equivalents.

Table 1 sets forth the one-letter codes used within SEQ ID NO:3 to denote degenerate nucleotide positions. "Resolutions" are the nucleotides denoted by a code letter. "Complement" indicates the code for the complementary nucleotide(s). For example, the code Y denotes either C or T, and its complement R denotes A or G, A being complementary to T, and G being complementary to C.

Table 1

Nucleotide	Resolution	Complement	Resolution
A	A	T	T
C	C	G	G
G	G	C	C
T	T	A	A
R	A|G	Y	C|T
Y	C|T	R	A|G
M	A|C	K	G|T
K	G|T	M	A|C
S	C|G	S	C|G
W	A|T	W	A|T
H	A|C|T	D	A|G|T
B	C|G|T	V	A|C|G
V	A|C|G	B	C|G|T
D	A|G|T	H	A|C|T
N	A|C|G|T	N	A|C|G|T

The degenerate codons used in SEQ ID NO:3, encompassing all possible codons for a given amino acid, are set forth in Table 2.

Table 2

Amino Acid	One Letter Code	Codons	Degenerate Codon
Cys	C	TGC TGT	TGY
Ser	S	AGC AGT TCA TCC TCG TCT	WSN
Thr	T	ACA ACC ACG ACT	ACN
Pro	P	CCA CCC CCG CCT	CCN
Ala	A	GCA GCC GCG GCT	GCN
Gly	G	GGA GGC GGG GGT	GGN
Asn	N	AAC AAT	AAY
Asp	D	GAC GAT	GAY
Glu	E	GAA GAG	GAR
Gln	Q	CAA CAG	CAR
His	H	CAC CAT	CAY
Arg	R	AGA AGG CGA CGC CGG CGT	MGN
Lys	K	AAA AAG	AAR
Met	M	ATG	ATG
Ile	I	ATA ATC ATT	ATH
Leu	L	CTA CTC CTG CTT TTA TTG	YTN
Val	V	GTA GTC GTG GTT	GTN
Phe	F	TTC TTT	TTY
Tyr	Y	TAC TAT	TAY
Trp	W	TGG	TGG
Ter	.	TAA TAG TGA	TRR
Asn|Asp	B		RAY
Glu|Gln	Z		SAR
Any	X		NNN

One of ordinary skill in the art will appreciate that some ambiguity is introduced in determining a degenerate codon, representative of all possible codons encoding an amino acid. For example, the degenerate codon for serine (WSN) can, in some circumstances, encode arginine (AGR), and the degenerate codon for arginine (MGN) can, in some circumstances, encode serine (AGY). A similar relationship exists between codons encoding phenylalanine and leucine. Thus, some polynucleotides encompassed by the degenerate sequence may encode variant amino acid sequences, but one of ordinary skill in the art can easily identify such variant sequences by reference to the amino acid sequences of SEQ ID NO:2. Variant sequences can be readily tested for functionality as described herein.

Different species can exhibit "preferential codon usage." In general, see, Grantham *et al., Nucl. Acids Res. 8*:1893 (1980), Haas *et al. Curr. Biol. 6*:315 (1996), Wain-Hobson *et al., Gene 13*:355 (1981), Grosjean and Fiers, *Gene 18*:199 (1982), Holm, *Nuc. Acids Res. 14*:3075 (1986), Ikemura, *J. Mol. Biol. 158*:573 (1982), Sharp and Matassi, *Curr. Opin. Genet. Dev. 4*:851 (1994), Kane, *Curr. Opin. Biotechnol. 6*:494 (1995), and Makrides, *Microbiol. Rev. 60*:512 (1996). As used herein, the term "preferential codon usage" or "preferential codons" is a term of art referring to protein translation codons that are most frequently used in cells of a certain species, thus favoring one or a few representatives of the possible codons encoding each amino acid (See Table 2). For example, the amino acid threonine (thr) may be encoded by ACA, ACC, ACG, or ACT, but in mammalian cells ACC is the most commonly used codon; in other species, for example, insect cells, yeast, viruses or bacteria, different threonine codons may be preferential. Preferential codons for a particular species can be introduced into the polynucleotides of the present invention by a variety of methods known in the art. Introduction of preferential codon sequences into recombinant DNA can, for example, enhance production of the protein by making protein translation more efficient within a particular cell type or species. Therefore, the degenerate codon sequences disclosed herein serve as a template for optimizing expression of polynucleotides in various cell types and species commonly used in the art and disclosed herein. Sequences containing preferential codons can be tested and optimized for expression in various species, and tested for functionality as disclosed herein.

The present invention further provides variant polypeptides and nucleic acid molecules that represent counterparts from other species (orthologs). These species include, but are not limited to mammalian, avian, amphibian, reptile, fish, insect and other vertebrate and invertebrate species. As an illustration, SEQ ID NO:11, SEQ ID NO:12, and SEQ ID NO:13 provide the nucleotide, amino acid, and degenerate nucleotide sequences, respectively, of murine Zcytor18. Features of the murine Zcytor18

polypeptide include an extracellular domain at amino acid residues 1 to 300 of SEQ ID NO:12, a putative signal sequence at amino acid residues 1 to 35 of SEQ ID NO:12, a transmembrane domain at amino acid residues 301 to 322 of SEQ ID NO:12, and an intracellular domain at amino acid residues 323 to 739 of SEQ ID NO:12. Murine *Zcytor18* gene expression has been detected in brain, kidney, lung, skin, testis, and uterus tissues of the mouse, while little or no expression was detectable in heart, liver, pancreas, and spleen tissues.

Zcytor18 polypeptides from other mammalian species, including mouse, porcine, ovine, bovine, canine, feline, equine, and other primate polypeptides, are also of interest. Orthologs of human Zcytor18 can be cloned using information and compositions provided by the present invention in combination with conventional cloning techniques. For example, a *Zcytor18* cDNA can be cloned using mRNA obtained from a tissue or cell type that expresses *Zcytor18* as disclosed herein. Suitable sources of mRNA can be identified by probing northern blots with probes designed from the sequences disclosed herein. A library is then prepared from mRNA of a positive tissue or cell line.

A *Zcytor18*-encoding cDNA can be isolated by a variety of methods, such as by probing with a complete or partial human cDNA or with one or more sets of degenerate probes based on the disclosed sequences. A cDNA can also be cloned using the polymerase chain reaction with primers designed from the representative human *Zcytor18* sequences disclosed herein. In addition, a cDNA library can be used to transform or transfect host cells, and expression of the cDNA of interest can be detected with an antibody to Zcytor18 polypeptide.

Those skilled in the art will recognize that the sequence disclosed in SEQ ID NO:1 represents a single allele of human *Zcytor18*, and that allelic variation and alternative splicing are expected to occur. Allelic variants of this sequence can be cloned by probing cDNA or genomic libraries from different individuals according to standard procedures. Allelic variants of the nucleotide sequences disclosed herein, including those containing silent mutations and those in which mutations result in amino acid sequence changes, are within the scope of the present invention, as are proteins which are allelic variants of the amino acid sequences disclosed herein. cDNA molecules generated from alternatively spliced mRNAs, which retain the properties of the Zcytor18 polypeptide are included within the scope of the present invention, as are polypeptides encoded by such cDNAs and mRNAs. Allelic variants and splice variants of these sequences can be cloned by probing cDNA or genomic libraries from different individuals or tissues according to standard procedures known in the art.

Within certain embodiments of the invention, the isolated nucleic acid molecules can hybridize under stringent conditions to nucleic acid molecules comprising nucleotide sequences disclosed herein. For example, such nucleic acid molecules can hybridize under stringent conditions to nucleic acid molecules comprising the nucleotide sequence of SEQ ID NO:1, to nucleic acid molecules consisting of the nucleotide sequence of nucleotides 192 to 652 of SEQ ID NO:1, nucleotide sequence of nucleotides 192 to 2344 of SEQ ID NO:1, or to nucleic acid molecules comprising a nucleotide sequence complementary to SEQ ID NO:1, the nucleotide sequence of nucleotides 192 to 652 of SEQ ID NO:1, or nucleotides 192 to 2344 of SEQ ID NO:1. In general, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe.

A pair of nucleic acid molecules, such as DNA-DNA, RNA-RNA and DNA-RNA, can hybridize if the nucleotide sequences have some degree of complementarity. Hybrids can tolerate mismatched base pairs in the double helix, but the stability of the hybrid is influenced by the degree of mismatch. The T_m of the mismatched hybrid decreases by 1°C for every 1-1.5% base pair mismatch. Varying the stringency of the hybridization conditions allows control over the degree of mismatch that will be present in the hybrid. The degree of stringency increases as the hybridization temperature increases and the ionic strength of the hybridization buffer decreases. Stringent hybridization conditions encompass temperatures of about 5-25°C below the T_m of the hybrid and a hybridization buffer having up to 1 M Na^+. Higher degrees of stringency at lower temperatures can be achieved with the addition of formamide which reduces the T_m of the hybrid about 1°C for each 1% formamide in the buffer solution. Generally, such stringent conditions include temperatures of 20-70°C and a hybridization buffer containing up to 6x SSC and 0-50% formamide. A higher degree of stringency can be achieved at temperatures of from 40-70°C with a hybridization buffer having up to 4x SSC and from 0-50% formamide. Highly stringent conditions typically encompass temperatures of 42-70°C with a hybridization buffer having up to 1x SSC and 0-50% formamide. Different degrees of stringency can be used during hybridization and washing to achieve maximum specific binding to the target sequence. Typically, the washes following hybridization are performed at increasing degrees of stringency to remove non-hybridized polynucleotide probes from hybridized complexes.

The above conditions are meant to serve as a guide and it is well within the abilities of one skilled in the art to adapt these conditions for use with a particular polypeptide hybrid. The T_m for a specific target sequence is the temperature (under

defined conditions) at which 50% of the target sequence will hybridize to a perfectly matched probe sequence. Conditions that influence the T_m include, the size and base pair content of the polynucleotide probe, the ionic strength of the hybridization solution, and the presence of destabilizing agents in the hybridization solution. Numerous equations for calculating T_m are known in the art, and are specific for DNA, RNA and DNA-RNA hybrids and polynucleotide probe sequences of varying length (see, for example, Sambrook *et al., Molecular Cloning: A Laboratory Manual*, Second Edition (Cold Spring Harbor Press 1989); Ausubel *et al.,* (eds.), *Current Protocols in Molecular Biology* (John Wiley and Sons, Inc. 1987); Berger and Kimmel (eds.), *Guide to Molecular Cloning Techniques*, (Academic Press, Inc. 1987); and Wetmur, *Crit. Rev. Biochem. Mol. Biol. 26*:227 (1990)). Sequence analysis software such as OLIGO 6.0 (LSR; Long Lake, MN) and *Primer Premier 4.0* (Premier Biosoft International; Palo Alto, CA), as well as sites on the Internet, are available tools for analyzing a given sequence and calculating T_m based on user defined criteria. Such programs can also analyze a given sequence under defined conditions and identify suitable probe sequences. Typically, hybridization of longer polynucleotide sequences, >50 base pairs, is performed at temperatures of about 20-25°C below the calculated T_m. For smaller probes, <50 base pairs, hybridization is typically carried out at the T_m or 5-10°C below. This allows for the maximum rate of hybridization for DNA-DNA and DNA-RNA hybrids.

The length of the polynucleotide sequence influences the rate and stability of hybrid formation. Smaller probe sequences, <50 base pairs, reach equilibrium with complementary sequences rapidly, but may form less stable hybrids. Incubation times of anywhere from minutes to hours can be used to achieve hybrid formation. Longer probe sequences come to equilibrium more slowly, but form more stable complexes even at lower temperatures. Incubations are allowed to proceed overnight or longer. Generally, incubations are carried out for a period equal to three times the calculated Cot time. Cot time, the time it takes for the polynucleotide sequences to reassociate, can be calculated for a particular sequence by methods known in the art.

The base pair composition of polynucleotide sequence will effect the thermal stability of the hybrid complex, thereby influencing the choice of hybridization temperature and the ionic strength of the hybridization buffer. A-T pairs are less stable than G-C pairs in aqueous solutions containing sodium chloride. Therefore, the higher the G-C content, the more stable the hybrid. Even distribution of G and C residues within the sequence also contribute positively to hybrid stability. In addition, the base pair composition can be manipulated to alter the T_m of a given sequence. For example, 5-methyldeoxycytidine can be substituted for deoxycytidine and 5-bromodeoxuridine can

be substituted for thymidine to increase the T_m, whereas 7-deazz-2'-deoxyguanosine can be substituted for guanosine to reduce dependence on T_m.

The ionic concentration of the hybridization buffer also affects the stability of the hybrid. Hybridization buffers generally contain blocking agents such as Denhardt's solution (Sigma Chemical Co., St. Louis, Mo.), denatured salmon sperm DNA, tRNA, milk powders (BLOTTO), heparin or SDS, and a Na^+ source, such as SSC (1x SSC: 0.15 M sodium chloride, 15 mM sodium citrate) or SSPE (1x SSPE: 1.8 M NaCl, 10 mM NaH_2PO_4, 1 mM EDTA, pH 7.7). Typically, hybridization buffers contain from between 10 mM - 1 M Na^+. The addition of destabilizing or denaturing agents such as formamide, tetralkylammonium salts, guanidinium cations or thiocyanate cations to the hybridization solution will alter the T_m of a hybrid. Typically, formamide is used at a concentration of up to 50% to allow incubations to be carried out at more convenient and lower temperatures. Formamide also acts to reduce non-specific background when using RNA probes.

As an illustration, a nucleic acid molecule encoding a variant Zcytor18 polypeptide can be hybridized with a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1 (or its complement) at 42°C overnight in a solution comprising 50% formamide, 5x SSC, 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution (100x Denhardt's solution: 2% (w/v) Ficoll 400, 2% (w/v) polyvinylpyrrolidone, and 2% (w/v) bovine serum albumin), 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA. One of skill in the art can devise variations of these hybridization conditions. For example, the hybridization mixture can be incubated at a higher temperature, such as about 65°C, in a solution that does not contain formamide. Moreover, premixed hybridization solutions are available (*e.g.*, EXPRESSHYB Hybridization Solution from CLONTECH Laboratories, Inc.), and hybridization can be performed according to the manufacturer's instructions.

Following hybridization, the nucleic acid molecules can be washed to remove non-hybridized nucleic acid molecules under stringent conditions, or under highly stringent conditions. Typical stringent washing conditions include washing in a solution of 0.5x - 2x SSC with 0.1% sodium dodecyl sulfate (SDS) at 55 - 65°C. As an illustration, nucleic acid molecules encoding a variant Zcytor18 polypeptide remain hybridized with a nucleic acid molecule comprising the nucleotide sequence of nucleotides 192 to 652 of SEQ ID NO:1 (or its complement) under stringent washing conditions, in which the wash stringency is equivalent to 0.5x - 2x SSC with 0.1% SDS at 55 - 65°C, including 0.5x SSC with 0.1% SDS at 55°C, or 2xSSC with 0.1% SDS at 65°C. One of skill in the art can readily devise equivalent conditions, for example, by substituting SSPE for SSC in the wash solution.

Typical highly stringent washing conditions include washing in a solution of 0.1x - 0.2x SSC with 0.1% sodium dodecyl sulfate (SDS) at 50 - 65°C. For example, nucleic acid molecules encoding a variant Zcytor18 polypeptide remain hybridized with a nucleic acid molecule comprising the nucleotide sequence of nucleotides 192 to 652 of SEQ ID NO:1 (or its complement) under highly stringent washing conditions, in which the wash stringency is equivalent to 0.1x - 0.2x SSC with 0.1% SDS at 50 - 65°C, including 0.1x SSC with 0.1% SDS at 50°C, or 0.2xSSC with 0.1% SDS at 65°C.

The present invention also provides isolated Zcytor18 polypeptides that have a substantially similar sequence identity to the polypeptides of SEQ ID NO:2, SEQ ID NO:8, or their orthologs. The term "substantially similar sequence identity" is used herein to denote polypeptides having at least 70%, at least 80%, at least 90%, at least 95% or greater than 95% sequence identity to the sequences shown in SEQ ID NO:2, SEQ ID NO:8, or their orthologs.

The present invention also contemplates *Zcytor18* variant nucleic acid molecules that can be identified using two criteria: a determination of the similarity between the encoded polypeptide with the amino acid sequence of SEQ ID NO:2, and a hybridization assay, as described above. Such *Zcytor18* variants include nucleic acid molecules (1) that remain hybridized with a nucleic acid molecule comprising the nucleotide sequence of nucleotides 192 to 652 of SEQ ID NO:1 (or its complement) under stringent washing conditions, in which the wash stringency is equivalent to 0.5x - 2x SSC with 0.1% SDS at 55 - 65°C, and (2) that encode a polypeptide having at least 70%, at least 80%, at least 90%, at least 95% or greater than 95% sequence identity to the amino acid sequence of SEQ ID NO:2. Alternatively, *Zcytor18* variants can be characterized as nucleic acid molecules (1) that remain hybridized with a nucleic acid molecule comprising the nucleotide sequence of nucleotides 192 to 652 of SEQ ID NO:1 (or its complement) under highly stringent washing conditions, in which the wash stringency is equivalent to 0.1x - 0.2x SSC with 0.1% SDS at 50 - 65°C, and (2) that encode a polypeptide having at least 70%, at least 80%, at least 90%, at least 95% or greater than 95% sequence identity to the amino acid sequence of SEQ ID NO:2.

Percent sequence identity is determined by conventional methods. See, for example, Altschul *et al., Bull. Math. Bio. 48*:603 (1986), and Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA 89*:10915 (1992). Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the "BLOSUM62" scoring matrix of Henikoff and Henikoff (*ibid.*) as shown in Table 3 (amino acids are indicated by the standard one-letter codes). The percent identity is then calculated as: ([Total number of identical matches]/ [length

of the longer sequence plus the number of gaps introduced into the longer sequence in order to align the two sequences])(100).

Table 3

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4																			
R	-1	5																		
N	-2	0	6																	
D	-2	-2	1	6																
C	0	-3	-3	-3	9															
Q	-1	1	0	0	-3	5														
E	-1	0	0	2	-4	2	5													
G	0	-2	0	-1	-3	-2	-2	6												
H	-2	0	1	-1	-3	0	0	-2	8											
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4										
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4									
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5								
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5							
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6						
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7					
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4				
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5			
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11		
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

Those skilled in the art appreciate that there are many established algorithms available to align two amino acid sequences. The "FASTA" similarity search algorithm of Pearson and Lipman is a suitable protein alignment method for examining the level of identity shared by an amino acid sequence disclosed herein and the amino acid sequence of a putative Zcytor18 variant. The FASTA algorithm is described by Pearson and Lipman, *Proc. Nat'l Acad. Sci. USA 85*:2444 (1988), and by Pearson, *Meth. Enzymol. 183*:63 (1990). Briefly, FASTA first characterizes sequence similarity by identifying regions shared by the query sequence (*e.g.,* SEQ ID NO:2) and a test sequence that have either the highest density of identities (if the ktup variable is 1) or pairs of identities (if ktup=2), without considering conservative amino acid substitutions, insertions, or deletions. The ten regions with the highest density of identities are then rescored by comparing the similarity of all paired amino acids using an amino acid substitution matrix, and the ends of the regions are "trimmed" to include only those residues that contribute to the highest score. If there are several regions with scores greater than the "cutoff" value (calculated by a predetermined formula based upon the length of the sequence and the ktup value), then the trimmed initial regions are examined to determine whether the regions can be joined to form an approximate alignment with gaps. Finally, the highest scoring regions of the two amino acid sequences are aligned using a modification of the Needleman-Wunsch-Sellers algorithm (Needleman and Wunsch, *J. Mol. Biol. 48*:444 (1970); Sellers, *SIAM J. Appl. Math. 26*:787 (1974)), which allows for amino acid insertions and deletions. Illustrative parameters for FASTA analysis are: ktup=1, gap opening penalty=10, gap extension penalty=1, and substitution matrix=BLOSUM62. These parameters can be introduced into a FASTA program by modifying the scoring matrix file ("SMATRIX"), as explained in Appendix 2 of Pearson, *Meth. Enzymol. 183*:63 (1990).

FASTA can also be used to determine the sequence identity of nucleic acid molecules using a ratio as disclosed above. For nucleotide sequence comparisons, the ktup value can range between one to six, preferably from three to six, most preferably three, with other parameters set as described above.

The present invention includes nucleic acid molecules that encode a polypeptide having a conservative amino acid change, compared with an amino acid sequence disclosed herein. For example, variants can be obtained that contain one or more amino acid substitutions of SEQ ID NO:2, or SEQ ID NO:8, in which an alkyl amino acid is substituted for an alkyl amino acid in a Zcytor18 amino acid sequence, an aromatic amino acid is substituted for an aromatic amino acid in a Zcytor18 amino acid sequence, a sulfur-containing amino acid is substituted for a sulfur-containing amino acid in a Zcytor18 amino acid sequence, a hydroxy-containing amino acid is substituted

for a hydroxy-containing amino acid in a Zcytor18 amino acid sequence, an acidic amino acid is substituted for an acidic amino acid in a Zcytor18 amino acid sequence, a basic amino acid is substituted for a basic amino acid in a Zcytor18 amino acid sequence, or a dibasic monocarboxylic amino acid is substituted for a dibasic monocarboxylic amino acid in a Zcytor18 amino acid sequence. Among the common amino acids, for example, a "conservative amino acid substitution" is illustrated by a substitution among amino acids within each of the following groups: (1) glycine, alanine, valine, leucine, and isoleucine, (2) phenylalanine, tyrosine, and tryptophan, (3) serine and threonine, (4) aspartate and glutamate, (5) glutamine and asparagine, and (6) lysine, arginine and histidine.

The BLOSUM62 table is an amino acid substitution matrix derived from about 2,000 local multiple alignments of protein sequence segments, representing highly conserved regions of more than 500 groups of related proteins (Henikoff and Henikoff, *Proc. Nat'l Acad. Sci. USA 89*:10915 (1992)). Accordingly, the BLOSUM62 substitution frequencies can be used to define conservative amino acid substitutions that may be introduced into the amino acid sequences of the present invention. Although it is possible to design amino acid substitutions based solely upon chemical properties (as discussed above), the language "conservative amino acid substitution" preferably refers to a substitution represented by a BLOSUM62 value of greater than -1. For example, an amino acid substitution is conservative if the substitution is characterized by a BLOSUM62 value of 0, 1, 2, or 3. According to this system, preferred conservative amino acid substitutions are characterized by a BLOSUM62 value of at least 1 (*e.g.*, 1, 2 or 3), while more preferred conservative amino acid substitutions are characterized by a BLOSUM62 value of at least 2 (*e.g.*, 2 or 3).

Particular variants of Zcytor18 are characterized by having at least 70%, at least 80%, at least 90%, at least 95% or greater than 95% sequence identity to the corresponding amino acid sequence (*e.g.*, SEQ ID NO:2 or SEQ ID NO:8), wherein the variation in amino acid sequence is due to one or more conservative amino acid substitutions.

Conservative amino acid changes in a *Zcytor18* gene can be introduced, for example, by substituting nucleotides for the nucleotides recited in SEQ ID NO:1. Such "conservative amino acid" variants can be obtained by oligonucleotide-directed mutagenesis, linker-scanning mutagenesis, mutagenesis using the polymerase chain reaction, and the like (see Ausubel (1995) at pages 8-10 to 8-22; and McPherson (ed.), *Directed Mutagenesis: A Practical Approach* (IRL Press 1991)). A variant Zcytor18 polypeptide can be identified by the ability to specifically bind anti-Zcytor18 antibodies.

The proteins of the present invention can also comprise non-naturally occurring amino acid residues. Non-naturally occurring amino acids include, without limitation, *trans*-3-methylproline, 2,4-methanoproline, *cis*-4-hydroxyproline, *trans*-4-hydroxyproline, *N*-methylglycine, *allo*-threonine, methylthreonine, hydroxyethylcysteine, hydroxyethylhomocysteine, nitroglutamine, homoglutamine, pipecolic acid, thiazolidine carboxylic acid, dehydroproline, 3- and 4-methylproline, 3,3-dimethylproline, *tert*-leucine, norvaline, 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, and 4-fluorophenylalanine. Several methods are known in the art for incorporating non-naturally occurring amino acid residues into proteins. For example, an *in vitro* system can be employed wherein nonsense mutations are suppressed using chemically aminoacylated suppressor tRNAs. Methods for synthesizing amino acids and aminoacylating tRNA are known in the art. Transcription and translation of plasmids containing nonsense mutations is typically carried out in a cell-free system comprising an *E. coli* S30 extract and commercially available enzymes and other reagents. Proteins are purified by chromatography. See, for example, Robertson *et al., J. Am. Chem. Soc. 113*:2722 (1991), Ellman *et al., Methods Enzymol. 202*:301 (1991), Chung *et al., Science 259*:806 (1993), and Chung *et al., Proc. Nat'l Acad. Sci. USA 90*:10145 (1993).

In a second method, translation is carried out in *Xenopus* oocytes by microinjection of mutated mRNA and chemically aminoacylated suppressor tRNAs (Turcatti *et al., J. Biol. Chem. 271*:19991 (1996)). Within a third method, *E. coli* cells are cultured in the absence of a natural amino acid that is to be replaced (*e.g.*, phenylalanine) and in the presence of the desired non-naturally occurring amino acid(s) (*e.g.*, 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, or 4-fluorophenylalanine). The non-naturally occurring amino acid is incorporated into the protein in place of its natural counterpart. See, Koide *et al., Biochem. 33*:7470 (1994). Naturally occurring amino acid residues can be converted to non-naturally occurring species by *in vitro* chemical modification. Chemical modification can be combined with site-directed mutagenesis to further expand the range of substitutions (Wynn and Richards, *Protein Sci. 2*:395 (1993)).

A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, non-naturally occurring amino acids, and unnatural amino acids may be substituted for Zcytor18 amino acid residues.

Essential amino acids in the polypeptides of the present invention can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science 244*:1081 (1989), Bass *et al., Proc. Nat'l Acad. Sci. USA 88*:4498 (1991), Coombs and Corey, "Site-Directed Mutagenesis and Protein Engineering," in *Proteins: Analysis and Design*, Angeletti (ed.),

pages 259-311 (Academic Press, Inc. 1998)). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for biological activity to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton *et al., J. Biol. Chem. 271*:4699 (1996).

Although sequence analysis can be used to further define the Zcytor18 ligand binding region, amino acids that play a role in Zcytor18 binding activity can also be determined by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids. See, for example, de Vos *et al., Science 255*:306 (1992), Smith *et al., J. Mol. Biol. 224*:899 (1992), and Wlodaver *et al., FEBS Lett. 309*:59 (1992).

Multiple amino acid substitutions can be made and tested using known methods of mutagenesis and screening, such as those disclosed by Reidhaar-Olson and Sauer (*Science 241*:53 (1988)) or Bowie and Sauer (*Proc. Nat'l Acad. Sci. USA 86:*2152 (1989)). Briefly, these authors disclose methods for simultaneously randomizing two or more positions in a polypeptide, selecting for functional polypeptide, and then sequencing the mutagenized polypeptides to determine the spectrum of allowable substitutions at each position. Other methods that can be used include phage display (*e.g.,* Lowman *et al., Biochem. 30*:10832 (1991), Ladner *et al.,* U.S. Patent No. 5,223,409, Huse, international publication No. WO 92/06204, and region-directed mutagenesis (Derbyshire *et al., Gene 46*:145 (1986), and Ner *et al., DNA 7*:127, (1988)). Moreover, Zcytor18 labeled with biotin or FITC can be used for expression cloning of Zcytor18 ligands.

Variants of the disclosed Zcytor18 nucleotide and polypeptide sequences can also be generated through DNA shuffling as disclosed by Stemmer, *Nature 370*:389 (1994), Stemmer, *Proc. Nat'l Acad. Sci. USA 91*:10747 (1994), and international publication No. WO 97/20078. Briefly, variant DNA molecules are generated by *in vitro* homologous recombination by random fragmentation of a parent DNA followed by reassembly using PCR, resulting in randomly introduced point mutations. This technique can be modified by using a family of parent DNA molecules, such as allelic variants or DNA molecules from different species, to introduce additional variability into the process. Selection or screening for the desired activity, followed by additional iterations of mutagenesis and assay provides for rapid "evolution" of sequences by selecting for desirable mutations while simultaneously selecting against detrimental changes.

Mutagenesis methods as disclosed herein can be combined with high-throughput, automated screening methods to detect activity of cloned, mutagenized polypeptides in host cells. Mutagenized DNA molecules that encode biologically active polypeptides, or polypeptides that bind with anti-Zcytor18 antibodies, can be recovered from the host cells and rapidly sequenced using modern equipment. These methods allow the rapid determination of the importance of individual amino acid residues in a polypeptide of interest, and can be applied to polypeptides of unknown structure.

The present invention also includes "functional fragments" of Zcytor18 polypeptides and nucleic acid molecules encoding such functional fragments. Routine deletion analyses of nucleic acid molecules can be performed to obtain functional fragments of a nucleic acid molecule that encodes a Zcytor18 polypeptide. As an illustration, DNA molecules comprising the nucleotide sequence of nucleotides 192 to 2347 of SEQ ID NO:1 can be digested with *Bal*31 nuclease to obtain a series of nested deletions. The fragments are then inserted into expression vectors in proper reading frame, and the expressed polypeptides are isolated and tested for the ability to bind anti-Zcytor18 antibodies. One alternative to exonuclease digestion is to use oligonucleotide-directed mutagenesis to introduce deletions or stop codons to specify production of a desired fragment. Alternatively, particular fragments of a *Zcytor18* gene can be synthesized using the polymerase chain reaction. An example of a functional fragment is the extracellular domain of Zcytor18 (*i.e.,* amino acid residues 36 to 313 of SEQ ID NO:2 or SEQ ID NO:5, or amino acid residues 36 to 299 of SEQ ID NO:8).

This general approach is exemplified by studies on the truncation at either or both termini of interferons have been summarized by Horisberger and Di Marco, *Pharmac. Ther. 66*:507 (1995). Moreover, standard techniques for functional analysis of proteins are described by, for example, Treuter *et al., Molec. Gen. Genet. 240*:113 (1993), Content *et al.*, "Expression and preliminary deletion analysis of the 42 kDa 2-5A synthetase induced by human interferon," in *Biological Interferon Systems, Proceedings of ISIR-TNO Meeting on Interferon Systems*, Cantell (ed.), pages 65-72 (Nijhoff 1987), Herschman, "The EGF Receptor," in *Control of Animal Cell Proliferation, Vol. 1*, Boynton *et al.*, (eds.) pages 169-199 (Academic Press 1985), Coumailleau *et al., J. Biol. Chem. 270*:29270 (1995); Fukunaga *et al., J. Biol. Chem. 270*:25291 (1995); Yamaguchi *et al., Biochem. Pharmacol. 50*:1295 (1995), and Meisel *et al., Plant Molec. Biol. 30*:1 (1996).

The present invention also contemplates functional fragments of a *Zcytor18* gene that have amino acid changes, compared with an amino acid sequence disclosed herein. A variant *Zcytor18* gene can be identified on the basis of structure by determining the level of identity with disclosed nucleotide and amino acid sequences, as

discussed above. An alternative approach to identifying a variant gene on the basis of structure is to determine whether a nucleic acid molecule encoding a potential variant *Zcytor18* gene can hybridize to a nucleic acid molecule comprising a nucleotide sequence, such as SEQ ID NO:1.

The present invention also provides polypeptide fragments or peptides comprising an epitope-bearing portion of a Zcytor18 polypeptide described herein. Such fragments or peptides may comprise an "immunogenic epitope," which is a part of a protein that elicits an antibody response when the entire protein is used as an immunogen. Immunogenic epitope-bearing peptides can be identified using standard methods (see, for example, Geysen *et al., Proc. Nat'l Acad. Sci. USA 81*:3998 (1983)).

In contrast, polypeptide fragments or peptides may comprise an "antigenic epitope," which is a region of a protein molecule to which an antibody can specifically bind. Certain epitopes consist of a linear or contiguous stretch of amino acids, and the antigenicity of such an epitope is not disrupted by denaturing agents. It is known in the art that relatively short synthetic peptides that can mimic epitopes of a protein can be used to stimulate the production of antibodies against the protein (see, for example, Sutcliffe *et al., Science 219*:660 (1983)).)). Antibodies that recognize short linear epitopes are particularly useful in analytic and diagnostic applications that use denatured protein, such as Western analysis, or in the analysis of fixed cells or tissue samples. Antibodies to linear epitopes are also useful for detecting fragments of Zcytor18, such as might occur in body fluids or culture media. Accordingly, antigenic epitope-bearing peptides and polypeptides of the present invention are useful to raise antibodies that bind with the polypeptides described herein.

Antigenic epitope-bearing peptides and polypeptides can contain at least four to ten amino acids, at least ten to fifteen amino acids, or about 15 to about 30 amino acids of an amino acid sequence disclosed herein. Such epitope-bearing peptides and polypeptides can be produced by fragmenting a Zcytor18 polypeptide, or by chemical peptide synthesis, as described herein. Moreover, epitopes can be selected by phage display of random peptide libraries (see, for example, Lane and Stephen, *Curr. Opin. Immunol. 5*:268 (1993), and Cortese *et al., Curr. Opin. Biotechnol. 7*:616 (1996)). Standard methods for identifying epitopes and producing antibodies from small peptides that comprise an epitope are described, for example, by Mole, "Epitope Mapping," in *Methods in Molecular Biology, Vol. 10*, Manson (ed.), pages 105-116 (The Humana Press, Inc. 1992), Price, "Production and Characterization of Synthetic Peptide-Derived Antibodies," in *Monoclonal Antibodies: Production, Engineering, and Clinical Application*, Ritter and Ladyman (eds.), pages 60-84 (Cambridge University Press 1995),

and Coligan *et al.* (eds.), *Current Protocols in Immunology*, pages 9.3.1 - 9.3.5 and pages 9.4.1 - 9.4.11 (John Wiley & Sons 1997).

For any Zcytor18 polypeptide, including variants and fusion proteins, one of ordinary skill in the art can readily generate a fully degenerate polynucleotide sequence encoding that variant using the information set forth in Tables 1 and 2 above. Moreover, those of skill in the art can use standard software to devise Zcytor18 variants based upon the nucleotide and amino acid sequences described herein. Accordingly, the present invention includes a computer-readable medium encoded with a data structure that provides at least one of the following sequences: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9. Suitable forms of computer-readable media include magnetic media and optically-readable media. Examples of magnetic media include a hard or fixed drive, a random access memory (RAM) chip, a floppy disk, digital linear tape (DLT), a disk cache, and a ZIP disk. Optically readable media are exemplified by compact discs (*e.g.*, CD-read only memory (ROM), CD-rewritable (RW), and CD-recordable), and digital versatile/video discs (DVD) (*e.g.*, DVD-ROM, DVD-RAM, and DVD+RW).

5. Production of Zcytor18 Polypeptides

The polypeptides of the present invention, including full-length polypeptides, functional fragments, and fusion proteins, can be produced in recombinant host cells following conventional techniques. To express a *Zcytor18* gene, a nucleic acid molecule encoding the polypeptide must be operably linked to regulatory sequences that control transcriptional expression in an expression vector and then, introduced into a host cell. In addition to transcriptional regulatory sequences, such as promoters and enhancers, expression vectors can include translational regulatory sequences and a marker gene which is suitable for selection of cells that carry the expression vector.

Expression vectors that are suitable for production of a foreign protein in eukaryotic cells typically contain (1) prokaryotic DNA elements coding for a bacterial replication origin and an antibiotic resistance marker to provide for the growth and selection of the expression vector in a bacterial host; (2) eukaryotic DNA elements that control initiation of transcription, such as a promoter; and (3) DNA elements that control the processing of transcripts, such as a transcription termination/polyadenylation sequence. As discussed above, expression vectors can also include nucleotide sequences encoding a secretory sequence that directs the heterologous polypeptide into the secretory pathway of a host cell. For example, a *Zcytor18* expression vector may comprise a *Zcytor18* gene and a secretory sequence derived from any secreted gene.

Zcytor18 proteins of the present invention may be expressed in mammalian cells. Examples of suitable mammalian host cells include African green monkey kidney cells (Vero; ATCC CRL 1587), human embryonic kidney cells (293-HEK; ATCC CRL 1573), baby hamster kidney cells (BHK-21, BHK-570; ATCC CRL 8544, ATCC CRL 10314), canine kidney cells (MDCK; ATCC CCL 34), Chinese hamster ovary cells (CHO-K1; ATCC CCL61; CHO DG44 (Chasin *et al., Som. Cell. Molec. Genet. 12*:555, 1986)), rat pituitary cells (GH1; ATCC CCL82), HeLa S3 cells (ATCC CCL2.2), rat hepatoma cells (H-4-II-E; ATCC CRL 1548) SV40-transformed monkey kidney cells (COS-1; ATCC CRL 1650) and murine embryonic cells (NIH-3T3; ATCC CRL 1658).

For a mammalian host, the transcriptional and translational regulatory signals may be derived from viral sources, such as adenovirus, bovine papilloma virus, simian virus, or the like, in which the regulatory signals are associated with a particular gene which has a high level of expression. Suitable transcriptional and translational regulatory sequences also can be obtained from mammalian genes, such as actin, collagen, myosin, and metallothionein genes.

Transcriptional regulatory sequences include a promoter region sufficient to direct the initiation of RNA synthesis. Suitable eukaryotic promoters include the promoter of the mouse *metallothionein I* gene (Hamer *et al., J. Molec. Appl. Genet. 1*:273 (1982)), the *TK* promoter of *Herpes* virus (McKnight, *Cell 31*:355 (1982)), the *SV40* early promoter (Benoist *et al., Nature 290*:304 (1981)), the *Rous* sarcoma virus promoter (Gorman *et al., Proc. Nat'l Acad. Sci. USA 79*:6777 (1982)), the cytomegalovirus promoter (Foecking *et al., Gene 45*:101 (1980)), and the mouse mammary tumor virus promoter (see, generally, Etcheverry, "Expression of Engineered Proteins in Mammalian Cell Culture," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), pages 163-181 (John Wiley & Sons, Inc. 1996)).

Alternatively, a prokaryotic promoter, such as the bacteriophage T3 RNA polymerase promoter, can be used to control *Zcytor18* gene expression in mammalian cells if the prokaryotic promoter is regulated by a eukaryotic promoter (Zhou *et al., Mol. Cell. Biol. 10*:4529 (1990), and Kaufman *et al., Nucl. Acids Res. 19*:4485 (1991)).

An expression vector can be introduced into host cells using a variety of standard techniques including calcium phosphate transfection, liposome-mediated transfection, microprojectile-mediated delivery, electroporation, and the like. The transfected cells can be selected and propagated to provide recombinant host cells that comprise the expression vector stably integrated in the host cell genome. Techniques for introducing vectors into eukaryotic cells and techniques for selecting such stable transformants using a dominant selectable marker are described, for example, by Ausubel

(1995) and by Murray (ed.), *Gene Transfer and Expression Protocols* (Humana Press 1991).

For example, one suitable selectable marker is a gene that provides resistance to the antibiotic neomycin. In this case, selection is carried out in the presence of a neomycin-type drug, such as G-418 or the like. Selection systems can also be used to increase the expression level of the gene of interest, a process referred to as "amplification." Amplification is carried out by culturing transfectants in the presence of a low level of the selective agent and then increasing the amount of selective agent to select for cells that produce high levels of the products of the introduced genes. A suitable amplifiable selectable marker is dihydrofolate reductase, which confers resistance to methotrexate. Other drug resistance genes (*e.g.*, hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can also be used. Alternatively, markers that introduce an altered phenotype, such as green fluorescent protein, or cell surface proteins such as CD4, CD8, Class I MHC, placental alkaline phosphatase may be used to sort transfected cells from untransfected cells by such means as FACS sorting or magnetic bead separation technology.

Zcytor18 polypeptides can also be produced by cultured mammalian cells using a viral delivery system. Exemplary viruses for this purpose include adenovirus, herpesvirus, vaccinia virus and adeno-associated virus (AAV). Adenovirus, a double-stranded DNA virus, is currently the best studied gene transfer vector for delivery of heterologous nucleic acid (for a review, see Becker *et al., Meth. Cell Biol. 43*:161 (1994), and Douglas and Curiel, *Science & Medicine 4*:44 (1997)). Advantages of the adenovirus system include the accommodation of relatively large DNA inserts, the ability to grow to high-titer, the ability to infect a broad range of mammalian cell types, and flexibility that allows use with a large number of available vectors containing different promoters.

By deleting portions of the adenovirus genome, larger inserts (up to 7 kb) of heterologous DNA can be accommodated. These inserts can be incorporated into the viral DNA by direct ligation or by homologous recombination with a co-transfected plasmid. An option is to delete the essential *E1* gene from the viral vector, which results in the inability to replicate unless the *E1* gene is provided by the host cell. Adenovirus vector-infected human 293 cells (ATCC Nos. CRL-1573, 45504, 45505), for example, can be grown as adherent cells or in suspension culture at relatively high cell density to produce significant amounts of protein (see Garnier *et al., Cytotechnol. 15*:145 (1994)).

Zcytor18 can also be expressed in other higher eukaryotic cells, such as avian, fungal, insect, yeast, or plant cells. The baculovirus system provides an efficient means to introduce cloned *Zcytor18* genes into insect cells. Suitable expression vectors

are based upon the *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV), and contain well-known promoters such as *Drosophila heat shock protein* (*hsp*) 70 promoter, *Autographa californica nuclear polyhedrosis virus immediate-early* gene promoter (*ie-1*) and the *delayed early 39K* promoter, baculovirus *p10* promoter, and the *Drosophila metallothionein* promoter. A second method of making recombinant baculovirus utilizes a transposon-based system described by Luckow (Luckow, *et al., J. Virol. 67*:4566 (1993)). This system, which utilizes transfer vectors, is sold in the BAC-to-BAC kit (Life Technologies, Rockville, MD). This system utilizes a transfer vector, PFASTBAC (Life Technologies) containing a Tn7 transposon to move the DNA encoding the Zcytor18 polypeptide into a baculovirus genome maintained in *E. coli* as a large plasmid called a "bacmid." See, Hill-Perkins and Possee, *J. Gen. Virol. 71*:971 (1990), Bonning, *et al., J. Gen. Virol. 75*:1551 (1994), and Chazenbalk, and Rapoport, *J. Biol. Chem. 270*:1543 (1995). In addition, transfer vectors can include an in-frame fusion with DNA encoding an epitope tag at the C- or N-terminus of the expressed Zcytor18 polypeptide, for example, a Glu-Glu epitope tag (Grussenmeyer *et al., Proc. Nat'l Acad. Sci. 82*:7952 (1985)). Using a technique known in the art, a transfer vector containing a *Zcytor18* gene is transformed into *E. coli*, and screened for bacmids, which contain an interrupted *lacZ* gene indicative of recombinant baculovirus. The bacmid DNA containing the recombinant baculovirus genome is then isolated using common techniques.

The illustrative PFASTBAC vector can be modified to a considerable degree. For example, the polyhedrin promoter can be removed and substituted with the baculovirus basic protein promoter (also known as P*cor*, p6.9 or MP promoter) which is expressed earlier in the baculovirus infection, and has been shown to be advantageous for expressing secreted proteins (see, for example, Hill-Perkins and Possee, *J. Gen. Virol. 71*:971 (1990), Bonning, *et al., J. Gen. Virol. 75*:1551 (1994), and Chazenbalk and Rapoport, *J. Biol. Chem. 270*:1543 (1995). In such transfer vector constructs, a short or long version of the basic protein promoter can be used. Moreover, transfer vectors can be constructed, which replace the native Zcytor18 secretory signal sequences with secretory signal sequences derived from insect proteins. For example, a secretory signal sequence from Ecdysteroid Glucosyltransferase (EGT), honey bee Melittin (Invitrogen Corporation; Carlsbad, CA), or baculovirus gp67 (PharMingen: San Diego, CA) can be used in constructs to replace the native Zcytor18 secretory signal sequence.

The recombinant virus or bacmid is used to transfect host cells. Suitable insect host cells include cell lines derived from IPLB-*Sf*-21, a *Spodoptera frugiperda* pupal ovarian cell line, such as *Sf*9 (ATCC CRL 1711), *Sf*21AE, and *Sf*21 (Invitrogen Corporation; San Diego, CA), as well as *Drosophila* Schneider-2 cells, and the HIGH

FIVEO cell line (Invitrogen) derived from *Trichoplusia ni* (U.S. Patent No. 5,300,435). Commercially available serum-free media can be used to grow and to maintain the cells. Suitable media are Sf900 II™ (Life Technologies) or ESF 921™ (Expression Systems) for the Sf9 cells; and Ex-cellO405™ (JRH Biosciences, Lenexa, KS) or Express FiveO™ (Life Technologies) for the *T. ni* cells. When recombinant virus is used, the cells are typically grown up from an inoculation density of approximately 2-5 x 10^5 cells to a density of 1-2 x 10^6 cells at which time a recombinant viral stock is added at a multiplicity of infection (MOI) of 0.1 to 10, more typically near 3.

Established techniques for producing recombinant proteins in baculovirus systems are provided by Bailey *et al.*, "Manipulation of Baculovirus Vectors," in *Methods in Molecular Biology, Volume 7: Gene Transfer and Expression Protocols*, Murray (ed.), pages 147-168 (The Humana Press, Inc. 1991), by Patel *et al.*, "The baculovirus expression system," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), pages 205-244 (Oxford University Press 1995), by Ausubel (1995) at pages 16-37 to 16-57, by Richardson (ed.), *Baculovirus Expression Protocols* (The Humana Press, Inc. 1995), and by Lucknow, "Insect Cell Expression Technology," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), pages 183-218 (John Wiley & Sons, Inc. 1996).

Fungal cells, including yeast cells, can also be used to express the genes described herein. Yeast species of particular interest in this regard include *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Pichia methanolica*. Suitable promoters for expression in yeast include promoters from *GAL1* (galactose), *PGK* (phosphoglycerate kinase), *ADH* (alcohol dehydrogenase), *AOX1* (alcohol oxidase), HIS4 (histidinol dehydrogenase), and the like. Many yeast cloning vectors have been designed and are readily available. These vectors include YIp-based vectors, such as YIp5, YRp vectors, such as YRp17, YEp vectors such as YEp13 and YCp vectors, such as YCp19. Methods for transforming *S. cerevisiae* cells with exogenous DNA and producing recombinant polypeptides therefrom are disclosed by, for example, Kawasaki, U.S. Patent No. 4,599,311, Kawasaki *et al.*, U.S. Patent No. 4,931,373, Brake, U.S. Patent No. 4,870,008, Welch *et al.*, U.S. Patent No. 5,037,743, and Murray *et al.*, U.S. Patent No. 4,845,075. Transformed cells are selected by phenotype determined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (*e.g.*, leucine). A suitable vector system for use in *Saccharomyces cerevisiae* is the *POT1* vector system disclosed by Kawasaki *et al.* (U.S. Patent No. 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. Additional suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (see, *e.g.*, Kawasaki, U.S. Patent No. 4,599,311,

Kingsman *et al.*, U.S. Patent No. 4,615,974, and Bitter, U.S. Patent No. 4,977,092) and alcohol dehydrogenase genes. See also U.S. Patents Nos. 4,990,446, 5,063,154, 5,139,936, and 4,661,454.

Transformation systems for other yeasts, including *Hansenula polymorpha, Schizosaccharomyces pombe, Kluyveromyces lactis, Kluyveromyces fragilis, Ustilago maydis, Pichia pastoris, Pichia methanolica, Pichia guillermondii* and *Candida maltosa* are known in the art. See, for example, Gleeson *et al., J. Gen. Microbiol. 132*:3459 (1986), and Cregg, U.S. Patent No. 4,882,279. *Aspergillus* cells may be utilized according to the methods of McKnight *et al.*, U.S. Patent No. 4,935,349. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino *et al.*, U.S. Patent No. 5,162,228. Methods for transforming *Neurospora* are disclosed by Lambowitz, U.S. Patent No. 4,486,533.

For example, the use of *Pichia methanolica* as host for the production of recombinant proteins is disclosed by Raymond, U.S. Patent No. 5,716,808, Raymond, U.S. Patent No. 5,736,383, Raymond *et al., Yeast 14*:11-23 (1998), and in international publication Nos. WO 97/17450, WO 97/17451, WO 98/02536, and WO 98/02565. DNA molecules for use in transforming *P. methanolica* will commonly be prepared as double-stranded, circular plasmids, which can be linearized prior to transformation. For polypeptide production in *P. methanolica*, the promoter and terminator in the plasmid can be that of a *P. methanolica* gene, such as a *P. methanolica* alcohol utilization gene (*AUG1* or *AUG2*). Other useful promoters include those of the dihydroxyacetone synthase (DHAS), formate dehydrogenase (FMD), and catalase (CAT) genes. To facilitate integration of the DNA into the host chromosome, the entire expression segment of the plasmid can be flanked at both ends by host DNA sequences. A suitable selectable marker for use in *Pichia methanolica* is a *P. methanolica ADE2* gene, which encodes phosphoribosyl-5-aminoimidazole carboxylase (AIRC; EC 4.1.1.21), and which allows *ade2* host cells to grow in the absence of adenine. For large-scale, industrial processes where it is desirable to minimize the use of methanol, host cells can be used in which both methanol utilization genes (*AUG1* and *AUG2*) are deleted. For production of secreted proteins, host cells can be deficient in vacuolar protease genes (*PEP4* and *PRB1*). Electroporation is used to facilitate the introduction of a plasmid containing DNA encoding a polypeptide of interest into *P. methanolica* cells. *P. methanolica* cells can be transformed by electroporation using an exponentially decaying, pulsed electric field having a field strength of from 2.5 to 4.5 kV/cm, preferably about 3.75 kV/cm, and a time constant (t) of from 1 to 40 milliseconds, most preferably about 20 milliseconds.

Expression vectors can also be introduced into plant protoplasts, intact plant tissues, or isolated plant cells. Methods for introducing expression vectors into plant tissue

include the direct infection or co-cultivation of plant tissue with *Agrobacterium tumefaciens*, microprojectile-mediated delivery, DNA injection, electroporation, and the like. See, for example, Horsch *et al., Science 227*:1229 (1985), Klein *et al., Biotechnology 10*:268 (1992), and Miki *et al.,* "Procedures for Introducing Foreign DNA into Plants," in *Methods in Plant Molecular Biology and Biotechnology*, Glick *et al.* (eds.), pages 67-88 (CRC Press, 1993).

Alternatively, *Zcytor18* genes can be expressed in prokaryotic host cells. Suitable promoters that can be used to express Zcytor18 polypeptides in a prokaryotic host are well-known to those of skill in the art and include promoters capable of recognizing the T4, T3, Sp6 and T7 polymerases, the P_R and P_L promoters of bacteriophage lambda, the *trp*, *recA*, heat shock, *lacUV5*, *tac*, *lpp-lacSpr*, *phoA*, and *lacZ* promoters of *E. coli,* promoters of *B. subtilis*, the promoters of the bacteriophages of *Bacillus*, *Streptomyces* promoters, the *int* promoter of bacteriophage lambda, the *bla* promoter of pBR322, and the CAT promoter of the chloramphenicol acetyl transferase gene. Prokaryotic promoters have been reviewed by Glick, *J. Ind. Microbiol. 1*:277 (1987), Watson *et al., Molecular Biology of the Gene, 4th Ed.* (Benjamin Cummins 1987), and by Ausubel *et al.* (1995).

Suitable prokaryotic hosts include *E. coli* and *Bacillus subtilus*. Suitable strains of *E. coli* include BL21(DE3), BL21(DE3)pLysS, BL21(DE3)pLysE, DH1, DH4I, DH5, DH5I, DH5IF', DH5IMCR, DH10B, DH10B/p3, DH11S, C600, HB101, JM101, JM105, JM109, JM110, K38, RR1, Y1088, Y1089, CSH18, ER1451, and ER1647 (see, for example, Brown (ed.), *Molecular Biology Labfax* (Academic Press 1991)). Suitable strains of *Bacillus subtilus* include BR151, YB886, MI119, MI120, and B170 (see, for example, Hardy, "Bacillus Cloning Methods," in *DNA Cloning: A Practical Approach*, Glover (ed.) (IRL Press 1985)).

When expressing a Zcytor18 polypeptide in bacteria such as *E. coli*, the polypeptide may be retained in the cytoplasm, typically as insoluble granules, or may be directed to the periplasmic space by a bacterial secretion sequence. In the former case, the cells are lysed, and the granules are recovered and denatured using, for example, guanidine isothiocyanate or urea. The denatured polypeptide can then be refolded and dimerized by diluting the denaturant, such as by dialysis against a solution of urea and a combination of reduced and oxidized glutathione, followed by dialysis against a buffered saline solution. In the latter case, the polypeptide can be recovered from the periplasmic space in a soluble and functional form by disrupting the cells (by, for example, sonication or osmotic shock) to release the contents of the periplasmic space and recovering the protein, thereby obviating the need for denaturation and refolding.

Methods for expressing proteins in prokaryotic hosts are well-known to those of skill in the art (see, for example, Williams *et al.*, "Expression of foreign proteins in *E. coli* using plasmid vectors and purification of specific polyclonal antibodies," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), page 15 (Oxford University Press 1995), Ward *et al.*, "Genetic Manipulation and Expression of Antibodies," in *Monoclonal Antibodies: Principles and Applications*, page 137 (Wiley-Liss, Inc. 1995), and Georgiou, "Expression of Proteins in Bacteria," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), page 101 (John Wiley & Sons, Inc. 1996)).

Standard methods for introducing expression vectors into bacterial, yeast, insect, and plant cells are provided, for example, by Ausubel (1995).

General methods for expressing and recovering foreign protein produced by a mammalian cell system are provided by, for example, Etcheverry, "Expression of Engineered Proteins in Mammalian Cell Culture," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), pages 163 (Wiley-Liss, Inc. 1996). Standard techniques for recovering protein produced by a bacterial system is provided by, for example, Grisshammer *et al.*, "Purification of over-produced proteins from *E. coli* cells," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), pages 59-92 (Oxford University Press 1995). Established methods for isolating recombinant proteins from a baculovirus system are described by Richardson (ed.), *Baculovirus Expression Protocols* (The Humana Press, Inc. 1995).

As an alternative, polypeptides of the present invention can be synthesized by exclusive solid phase synthesis, partial solid phase methods, fragment condensation or classical solution synthesis. These synthesis methods are well-known to those of skill in the art (see, for example, Merrifield, *J. Am. Chem. Soc. 85*:2149 (1963), Stewart *et al.*, "Solid Phase Peptide Synthesis" (2nd Edition), (Pierce Chemical Co. 1984), Bayer and Rapp, *Chem. Pept. Prot. 3*:3 (1986), Atherton *et al., Solid Phase Peptide Synthesis: A Practical Approach* (IRL Press 1989), Fields and Colowick, "Solid-Phase Peptide Synthesis," *Methods in Enzymology Volume 289* (Academic Press 1997), and Lloyd-Williams *et al., Chemical Approaches to the Synthesis of Peptides and Proteins* (CRC Press, Inc. 1997)). Variations in total chemical synthesis strategies, such as "native chemical ligation" and "expressed protein ligation" are also standard (see, for example, Dawson *et al., Science 266*:776 (1994), Hackeng *et al., Proc. Nat'l Acad. Sci. USA 94*:7845 (1997), Dawson, *Methods Enzymol. 287*: 34 (1997), Muir *et al, Proc. Nat'l Acad. Sci. USA 95*:6705 (1998), and Severinov and Muir, *J. Biol. Chem. 273*:16205 (1998)).

Peptides and polypeptides of the present invention comprise at least six, at least nine, or at least 15 contiguous amino acid residues of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:8. As an illustration, polypeptides can comprise at least six, at least nine, or at least 15 contiguous amino acid residues of any of the following amino acid sequences: (a) amino acid residues 1 to 203 of SEQ ID NO:2, (b) amino acid residues 36 to 203 of SEQ ID NO:2, (c) amino acid residues 36 to 313 of SEQ ID NO:2, (d) amino acid residues 1 to 753 of SEQ ID NO:2, (e) amino acid residues 1 to 189 of SEQ ID NO:8, (f) amino acid residues 36 to 189 of SEQ ID NO:8, (g) amino acid residues 36 to 299 of SEQ ID NO:8, and (h) amino acid residues 1 to 739 of SEQ ID NO:8. Within certain embodiments of the invention, the polypeptides comprise 20, 30, 40, 50, 100, or more contiguous residues of these amino acid sequences. For example, polypeptides can comprise at least 30 contiguous amino acid residues of an amino acid sequence selected from the group consisting of: (a) amino acid residues 1 to 218 of SEQ ID NO:2, (b) amino acid residues 36 to 218 of SEQ ID NO:2, (c) amino acid residues 36 to 313 of SEQ ID NO:2, (d) amino acid residues 1 to 753 of SEQ ID NO:2, (e) amino acid residues 1 to 204 of SEQ ID NO:8, (f) amino acid residues 36 to 204 of SEQ ID NO:8, (g) amino acid residues 36 to 299 of SEQ ID NO:8, and (h) amino acid residues 1 to 739 of SEQ ID NO:8. Nucleic acid molecules encoding such peptides and polypeptides are useful as polymerase chain reaction primers and probes.

6. *Production of Zcytor18 Fusion Proteins and Conjugates*

One general class of Zcytor18 analogs are variants having an amino acid sequence that is a mutation of the amino acid sequence disclosed herein. Another general class of Zcytor18 analogs is provided by anti-idiotype antibodies, and fragments thereof, as described below. Moreover, recombinant antibodies comprising anti-idiotype variable domains can be used as analogs (see, for example, Monfardini *et al., Proc. Assoc. Am. Physicians 108*:420 (1996)). Since the variable domains of anti-idiotype Zcytor18 antibodies mimic Zcytor18, these domains can provide Zcytor18 binding activity. Methods of producing anti-idiotypic catalytic antibodies are known to those of skill in the art (see, for example, Joron *et al., Ann. N Y Acad. Sci. 672*:216 (1992), Friboulet *et al., Appl. Biochem. Biotechnol. 47*:229 (1994), and Avalle *et al., Ann. N Y Acad. Sci. 864*:118 (1998)).

Another approach to identifying Zcytor18 analogs is provided by the use of combinatorial libraries. Methods for constructing and screening phage display and other combinatorial libraries are provided, for example, by Kay *et al., Phage Display of*

Peptides and Proteins (Academic Press 1996), Verdine, U.S. Patent No. 5,783,384, Kay, *et. al.,* U.S. Patent No. 5,747,334, and Kauffman *et al.,* U.S. Patent No. 5,723,323.

Zcytor18 polypeptides have both *in vivo* and *in vitro* uses. As an illustration, a soluble form of Zcytor18 can be added to cell culture medium to inhibit the effects of the Zcytor18 ligand produced by the cultured cells.

Fusion proteins of Zcytor18 can be used to express *Zcytor18* in a recombinant host, and to isolate the produced Zcytor18. As described below, particular Zcytor18 fusion proteins also have uses in diagnosis and therapy. One type of fusion protein comprises a peptide that guides a Zcytor18 polypeptide from a recombinant host cell. To direct a Zcytor18 polypeptide into the secretory pathway of a eukaryotic host cell, a secretory signal sequence (also known as a signal peptide, a leader sequence, prepro sequence or pre sequence) is provided in the *Zcytor18* expression vector. While the secretory signal sequence may be derived from Zcytor18, a suitable signal sequence may also be derived from another secreted protein or synthesized *de novo.* The secretory signal sequence is operably linked to a *Zcytor18*-encoding sequence such that the two sequences are joined in the correct reading frame and positioned to direct the newly synthesized polypeptide into the secretory pathway of the host cell. Secretory signal sequences are commonly positioned 5' to the nucleotide sequence encoding the polypeptide of interest, although certain secretory signal sequences may be positioned elsewhere in the nucleotide sequence of interest (see, *e.g.,* Welch *et al.,* U.S. Patent No. 5,037,743; Holland *et al.,* U.S. Patent No. 5,143,830).

Although the secretory signal sequence of Zcytor18 or another protein produced by mammalian cells (*e.g.,* tissue-type plasminogen activator signal sequence, as described, for example, in U.S. Patent No. 5,641,655) is useful for expression of Zcytor18 in recombinant mammalian hosts, a yeast signal sequence is preferred for expression in yeast cells. Examples of suitable yeast signal sequences are those derived from yeast mating phermone α-factor (encoded by the *MFα1* gene), invertase (encoded by the *SUC2* gene), or acid phosphatase (encoded by the *PHO5* gene). See, for example, Romanos *et al.*, "Expression of Cloned Genes in Yeast," in *DNA Cloning 2: A Practical Approach,* 2nd Edition, Glover and Hames (eds.), pages 123-167 (Oxford University Press 1995).

In bacterial cells, it is often desirable to express a heterologous protein as a fusion protein to decrease toxicity, increase stability, and to enhance recovery of the expressed protein. For example, *Zcytor18* can be expressed as a fusion protein comprising a glutathione S-transferase polypeptide. Glutathione S-transferease fusion proteins are typically soluble, and easily purifiable from *E. coli* lysates on immobilized glutathione columns. In similar approaches, a Zcytor18 fusion protein comprising a

maltose binding protein polypeptide can be isolated with an amylose resin column, while a fusion protein comprising the C-terminal end of a truncated Protein A gene can be purified using IgG-Sepharose. Established techniques for expressing a heterologous polypeptide as a fusion protein in a bacterial cell are described, for example, by Williams *et al.*, "Expression of Foreign Proteins in *E. coli* Using Plasmid Vectors and Purification of Specific Polyclonal Antibodies," in *DNA Cloning 2: A Practical Approach,* 2nd Edition, Glover and Hames (Eds.), pages 15-58 (Oxford University Press 1995). In addition, commercially available expression systems are available. For example, the PINPOINT Xa protein purification system (Promega Corporation; Madison, WI) provides a method for isolating a fusion protein comprising a polypeptide that becomes biotinylated during expression with a resin that comprises avidin.

Peptide tags that are useful for isolating heterologous polypeptides expressed by either prokaryotic or eukaryotic cells include polyHistidine tags (which have an affinity for nickel-chelating resin), *c-myc* tags, calmodulin binding protein (isolated with calmodulin affinity chromatography), substance P, the RYIRS tag (which binds with anti-RYIRS antibodies), the Glu-Glu tag, and the FLAG tag (which binds with anti-FLAG antibodies). See, for example, Luo *et al., Arch. Biochem. Biophys. 329*:215 (1996), Morganti *et al., Biotechnol. Appl. Biochem. 23*:67 (1996), and Zheng *et al., Gene 186*:55 (1997). Nucleic acid molecules encoding such peptide tags are available, for example, from Sigma-Aldrich Corporation (St. Louis, MO).

The present invention also contemplates that the use of the secretory signal sequence contained in the Zcytor18 polypeptides of the present invention to direct other polypeptides into the secretory pathway. A signal fusion polypeptide can be made wherein a secretory signal sequence derived from amino acid residues 1 to 35 of SEQ ID NO:2 is operably linked to another polypeptide using methods known in the art and disclosed herein. The secretory signal sequence contained in the fusion polypeptides of the present invention is preferably fused amino-terminally to an additional peptide to direct the additional peptide into the secretory pathway. Such constructs have numerous applications known in the art. For example, these novel secretory signal sequence fusion constructs can direct the secretion of an active component of a normally non-secreted protein, such as a receptor. Such fusions may be used in a transgenic animal or in a cultured recombinant host to direct peptides through the secretory pathway. With regard to the latter, exemplary polypeptides include pharmaceutically active molecules such as Factor VIIa, proinsulin, insulin, follicle stimulating hormone, tissue type plasminogen activator, tumor necrosis factor, interleukins (*e.g.*, interleukin-1 (IL-1), IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, and IL-21), colony stimulating factors (*e.g.*, granulocyte-colony stimulating

factor (G-CSF) and granulocyte macrophage-colony stimulating factor (GM-CSF)), interferons (*e.g.*, interferons-α, -β, -γ, -ω, -δ, -ε, and -τ), the stem cell growth factor designated "S1 factor," erythropoietin, and thrombopoietin. The Zcytor18 secretory signal sequence contained in the fusion polypeptides of the present invention is preferably fused amino-terminally to an additional peptide to direct the additional peptide into the secretory pathway. Fusion proteins comprising a Zcytor18 secretory signal sequence can be constructed using standard techniques.

Another form of fusion protein comprises a Zcytor18 polypeptide and an immunoglobulin heavy chain constant region, typically an F_c fragment, which contains two or three constant region domains and a hinge region but lacks the variable region. As an illustration, Chang *et al.*, U.S. Patent No. 5,723,125, describe a fusion protein comprising a human interferon and a human immunoglobulin Fc fragment. The C-terminal of the interferon is linked to the N-terminal of the Fc fragment by a peptide linker moiety. An example of a peptide linker is a peptide comprising primarily a T cell inert sequence, which is immunologically inert. An exemplary peptide linker has the amino acid sequence: GGSGG SGGGG SGGGG S (SEQ ID NO:10). In this fusion protein, an illustrative Fc moiety is a human γ4 chain, which is stable in solution and has little or no complement activating activity. Accordingly, the present invention contemplates a Zcytor18 fusion protein that comprises a Zcytor18 moiety and a human Fc fragment, wherein the C-terminus of the Zcytor18 moiety is attached to the N-terminus of the Fc fragment via a peptide linker, such as a peptide consisting of the amino acid sequence of SEQ ID NO:10. The Zcytor18 moiety can be a Zcytor18 molecule or a fragment thereof. For example, a fusion protein can comprise an Fc fragment (*e.g.*, a human Fc fragment), and amino acid residues 36 to 313 of SEQ ID NO:2, amino acid residues 36 to 189 of SEQ ID NO:2, amino acid residues 36 to 299 of SEQ ID NO:8, or amino acid residues 36 to 175 of SEQ ID NO:8.

In another variation, a Zcytor18 fusion protein comprises an IgG sequence, a Zcytor18 moiety covalently joined to the aminoterminal end of the IgG sequence, and a signal peptide that is covalently joined to the aminoterminal of the Zcytor18 moiety, wherein the IgG sequence consists of the following elements in the following order: a hinge region, a CH_2 domain, and a CH_3 domain. Accordingly, the IgG sequence lacks a CH_1 domain. The Zcytor18 moiety displays a Zcytor18 activity, as described herein, such as the ability to bind with a Zcytor18 ligand. This general approach to producing fusion proteins that comprise both antibody and nonantibody portions has been described by LaRochelle *et al.*, EP 742830 (WO 95/21258).

Fusion proteins comprising a Zcytor18 moiety and an Fc moiety can be used, for example, as an *in vitro* assay tool. For example, the presence of a Zcytor18

ligand in a biological sample can be detected using a Zcytor18-immunoglobulin fusion protein, in which the Zcytor18 moiety is used to bind the ligand, and a macromolecule, such as Protein A or anti-Fc antibody, is used to bind the fusion protein to a solid support. Such systems can be used to identify agonists and antagonists that interfere with the binding of a Zcytor18 ligand to its receptor.

Other examples of antibody fusion proteins include polypeptides that comprise an antigen-binding domain and a Zcytor18 fragment that contains a Zcytor18 extracellular domain. Such molecules can be used to target particular tissues for the benefit of Zcytor18 binding activity.

The present invention further provides a variety of other polypeptide fusions. For example, part or all of a domain(s) conferring a biological function can be swapped between Zcytor18 of the present invention with the functionally equivalent domain(s) from another member of the cytokine receptor family. Polypeptide fusions can be expressed in recombinant host cells to produce a variety of Zcytor18 fusion analogs. A Zcytor18 polypeptide can be fused to two or more moieties or domains, such as an affinity tag for purification and a targeting domain. Polypeptide fusions can also comprise one or more cleavage sites, particularly between domains. See, for example, Tuan *et al., Connective Tissue Research 34*:1 (1996).

Fusion proteins can be prepared by methods known to those skilled in the art by preparing each component of the fusion protein and chemically conjugating them. Alternatively, a polynucleotide encoding both components of the fusion protein in the proper reading frame can be generated using known techniques and expressed by the methods described herein. General methods for enzymatic and chemical cleavage of fusion proteins are described, for example, by Ausubel (1995) at pages 16-19 to 16-25.

Zcytor18 polypeptides can be used to identify and to isolate Zcytor18 ligands. For example, proteins and peptides of the present invention can be immobilized on a column and used to bind ligands from a biological sample that is run over the column (Hermanson *et al.* (eds.), *Immobilized Affinity Ligand Techniques*, pages 195-202 (Academic Press 1992)).

The activity of a Zcytor18 polypeptide can be observed by a silicon-based biosensor microphysiometer, which measures the extracellular acidification rate or proton excretion associated with receptor binding and subsequent physiologic cellular responses. An exemplary device is the CYTOSENSOR Microphysiometer manufactured by Molecular Devices, Sunnyvale, CA. A variety of cellular responses, such as cell proliferation, ion transport, energy production, inflammatory response, regulatory and receptor activation, and the like, can be measured by this method (see, for example, McConnell *et al., Science 257*:1906 (1992), Pitchford *et al., Meth. Enzymol. 228*:84

(1997), Arimilli *et al., J. Immunol. Meth. 212*:49 (1998), Van Liefde *et al., Eur. J. Pharmacol. 346*:87 (1998)). The microphysiometer can be used for assaying eukaryotic, prokaryotic, adherent, or non-adherent cells. By measuring extracellular acidification changes in cell media over time, the microphysiometer directly measures cellular responses to various stimuli, including agonists, ligands, or antagonists of Zcytor18.

For example, the microphysiometer is used to measure responses of an Zcytor18-expressing eukaryotic cell, compared to a control eukaryotic cell that does not express Zcytor18 polypeptide. Suitable cells responsive to Zcytor18-modulating stimuli include recombinant host cells comprising a Zcytor18 expression vector, and cells that naturally express Zcytor18. Extracellular acidification provides one measure for a Zcytor18-modulated cellular response. In addition, this approach can be used to identify ligands, agonists, and antagonists of Zcytor18 ligand. For example, a molecule can be identified as an agonist of Zcytor18 ligand by providing cells that express a Zcytor18 polypeptide, culturing a first portion of the cells in the absence of the test compound, culturing a second portion of the cells in the presence of the test compound, and determining whether the second portion exhibits a cellular response, in comparison with the first portion.

Alternatively, a solid phase system can be used to identify a Zcytor18 ligand, or an agonist or antagonist of a Zcytor18 ligand. For example, a Zcytor18 polypeptide or Zcytor18 fusion protein can be immobilized onto the surface of a receptor chip of a commercially available biosensor instrument (BIACORE, Biacore AB; Uppsala, Sweden). The use of this instrument is disclosed, for example, by Karlsson, *Immunol. Methods 145*:229 (1991), and Cunningham and Wells, *J. Mol. Biol. 234*:554 (1993).

In brief, a Zcytor18 polypeptide or fusion protein is covalently attached, using amine or sulfhydryl chemistry, to dextran fibers that are attached to gold film within a flow cell. A test sample is then passed through the cell. If a ligand is present in the sample, it will bind to the immobilized polypeptide or fusion protein, causing a change in the refractive index of the medium, which is detected as a change in surface plasmon resonance of the gold film. This system allows the determination of on- and off-rates, from which binding affinity can be calculated, and assessment of stoichiometry of binding. This system can also be used to examine antibody-antigen interactions, and the interactions of other complement/anti-complement pairs.

Zcytor18 binding domains can be further characterized by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids of Zcytor18 ligand agonists. See, for

example, de Vos *et al., Science 255*:306 (1992), Smith *et al., J. Mol. Biol. 224*:899 (1992), and Wlodaver *et al., FEBS Lett. 309*:59 (1992).

The present invention also contemplates chemically modified Zcytor18 compositions, in which a Zcytor18 polypeptide is linked with a polymer. Illustrative Zcytor18 polypeptides are soluble polypeptides that lack a functional transmembrane domain, such as a polypeptide consisting of amino acid residues 36 to 313 of SEQ ID NO:2, amino acid residues 36 to 189 of SEQ ID NO:2, amino acid residues 36 to 299 of SEQ ID NO:8, or amino acid residues 36 to 175 of SEQ ID NO:8. Typically, the polymer is water-soluble so that the Zcytor18 conjugate does not precipitate in an aqueous environment, such as a physiological environment. An example of a suitable polymer is one that has been modified to have a single reactive group, such as an active ester for acylation, or an aldehyde for alkylation, In this way, the degree of polymerization can be controlled. An example of a reactive aldehyde is polyethylene glycol propionaldehyde, or mono-(C1-C10) alkoxy, or aryloxy derivatives thereof (see, for example, Harris, *et al.*, U.S. Patent No. 5,252,714). The polymer may be branched or unbranched. Moreover, a mixture of polymers can be used to produce Zcytor18 conjugates.

Zcytor18 conjugates used for therapy can comprise pharmaceutically acceptable water-soluble polymer moieties. Suitable water-soluble polymers include polyethylene glycol (PEG), monomethoxy-PEG, mono-(C1-C10)alkoxy-PEG, aryloxy-PEG, poly-(N-vinyl pyrrolidone)PEG, tresyl monomethoxy PEG, PEG propionaldehyde, *bis*-succinimidyl carbonate PEG, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polyoxyethylated polyols (*e.g.,* glycerol), polyvinyl alcohol, dextran, cellulose, or other carbohydrate-based polymers. Suitable PEG may have a molecular weight from about 600 to about 60,000, including, for example, 5,000, 12,000, 20,000 and 25,000. A Zcytor18 conjugate can also comprise a mixture of such water-soluble polymers.

One example of a Zcytor18 conjugate comprises a Zcytor18 moiety and a polyalkyl oxide moiety attached to the *N*-terminus of the Zcytor18 moiety. PEG is one suitable polyalkyl oxide. As an illustration, Zcytor18 can be modified with PEG, a process known as "PEGylation." PEGylation of Zcytor18 can be carried out by any of the PEGylation reactions known in the art (see, for example, EP 0 154 316, Delgado *et al., Critical Reviews in Therapeutic Drug Carrier Systems 9*:249 (1992), Duncan and Spreafico, *Clin. Pharmacokinet. 27*:290 (1994), and Francis *et al., Int J Hematol 68*:1 (1998)). For example, PEGylation can be performed by an acylation reaction or by an alkylation reaction with a reactive polyethylene glycol molecule. In an alternative approach, Zcytor18 conjugates are formed by condensing activated PEG, in which a

terminal hydroxy or amino group of PEG has been replaced by an activated linker (see, for example, Karasiewicz *et al.*, U.S. Patent No. 5,382,657).

PEGylation by acylation typically requires reacting an active ester derivative of PEG with a Zcytor18 polypeptide. An example of an activated PEG ester is PEG esterified to *N*-hydroxysuccinimide. As used herein, the term "acylation" includes the following types of linkages between Zcytor18 and a water-soluble polymer: amide, carbamate, urethane, and the like. Methods for preparing PEGylated Zcytor18 by acylation will typically comprise the steps of (a) reacting a Zcytor18 polypeptide with PEG (such as a reactive ester of an aldehyde derivative of PEG) under conditions whereby one or more PEG groups attach to Zcytor18, and (b) obtaining the reaction product(s). Generally, the optimal reaction conditions for acylation reactions will be determined based upon known parameters and desired results. For example, the larger the ratio of PEG:Zcytor18, the greater the percentage of polyPEGylated Zcytor18 product.

The product of PEGylation by acylation is typically a polyPEGylated Zcytor18 product, wherein the lysine ε-amino groups are PEGylated via an acyl linking group. An example of a connecting linkage is an amide. Typically, the resulting Zcytor18 will be at least 95% mono-, di-, or tri-pegylated, although some species with higher degrees of PEGylation may be formed depending upon the reaction conditions. PEGylated species can be separated from unconjugated Zcytor18 polypeptides using standard purification methods, such as dialysis, ultrafiltration, ion exchange chromatography, affinity chromatography, and the like.

PEGylation by alkylation generally involves reacting a terminal aldehyde derivative of PEG with Zcytor18 in the presence of a reducing agent. PEG groups can be attached to the polypeptide via a $-CH_2-NH$ group.

Derivatization via reductive alkylation to produce a monoPEGylated product takes advantage of the differential reactivity of different types of primary amino groups available for derivatization. Typically, the reaction is performed at a pH that allows one to take advantage of the pKa differences between the ε-amino groups of the lysine residues and the α-amino group of the *N*-terminal residue of the protein. By such selective derivatization, attachment of a water-soluble polymer that contains a reactive group such as an aldehyde, to a protein is controlled. The conjugation with the polymer occurs predominantly at the *N*-terminus of the protein without significant modification of other reactive groups such as the lysine side chain amino groups. The present invention provides a substantially homogenous preparation of Zcytor18 monopolymer conjugates.

Reductive alkylation to produce a substantially homogenous population of monopolymer Zcytor18 conjugate molecule can comprise the steps of: (a) reacting a

Zcytor18 polypeptide with a reactive PEG under reductive alkylation conditions at a pH suitable to permit selective modification of the α-amino group at the amino terminus of the Zcytor18, and (b) obtaining the reaction product(s). The reducing agent used for reductive alkylation should be stable in aqueous solution and able to reduce only the Schiff base formed in the initial process of reductive alkylation. Illustrative reducing agents include sodium borohydride, sodium cyanoborohydride, dimethylamine borane, trimethylamine borane, and pyridine borane.

For a substantially homogenous population of monopolymer Zcytor18 conjugates, the reductive alkylation reaction conditions are those which permit the selective attachment of the water soluble polymer moiety to the *N*-terminus of Zcytor18. Such reaction conditions generally provide for pKa differences between the lysine amino groups and the α-amino group at the *N*-terminus. The pH also affects the ratio of polymer to protein to be used. In general, if the pH is lower, a larger excess of polymer to protein will be desired because the less reactive the *N*-terminal α-group, the more polymer is needed to achieve optimal conditions. If the pH is higher, the polymer:Zcytor18 need not be as large because more reactive groups are available. Typically, the pH will fall within the range of 3 to 9, or 3 to 6.

Another factor to consider is the molecular weight of the water-soluble polymer. Generally, the higher the molecular weight of the polymer, the fewer number of polymer molecules which may be attached to the protein. For PEGylation reactions, the typical molecular weight is about 2 kDa to about 100 kDa, about 5 kDa to about 50 kDa, or about 12 kDa to about 25 kDa. The molar ratio of water-soluble polymer to Zcytor18 will generally be in the range of 1:1 to 100:1. Typically, the molar ratio of water-soluble polymer to Zcytor18 will be 1:1 to 20:1 for polyPEGylation, and 1:1 to 5:1 for monoPEGylation.

General methods for producing conjugates comprising a polypeptide and water-soluble polymer moieties are known in the art. See, for example, Karasiewicz *et al.*, U.S. Patent No. 5,382,657, Greenwald *et al.*, U.S. Patent No. 5,738, 846, Nieforth *et al., Clin. Pharmacol. Ther. 59*:636 (1996), Monkarsh *et al., Anal. Biochem. 247*:434 (1997)).

The present invention contemplates compositions comprising a peptide or polypeptide described herein. Such compositions can further comprise a carrier. The carrier can be a conventional organic or inorganic carrier. Examples of carriers include water, buffer solution, alcohol, propylene glycol, macrogol, sesame oil, corn oil, and the like.

7. *Isolation of Zcytor18 Polypeptides*

The polypeptides of the present invention can be purified to at least about 80% purity, to at least about 90% purity, to at least about 95% purity, or greater than 95% purity with respect to contaminating macromolecules, particularly other proteins and nucleic acids, and free of infectious and pyrogenic agents. The polypeptides of the present invention may also be purified to a pharmaceutically pure state, which is greater than 99.9% pure. In certain preparations, purified polypeptide is substantially free of other polypeptides, particularly other polypeptides of animal origin.

Fractionation and/or conventional purification methods can be used to obtain preparations of Zcytor18 purified from natural sources (*e.g.,* testis tissue), synthetic Zcytor18 polypeptides, and recombinant Zcytor18 polypeptides and fusion Zcytor18 polypeptides purified from recombinant host cells. In general, ammonium sulfate precipitation and acid or chaotrope extraction may be used for fractionation of samples. Exemplary purification steps may include hydroxyapatite, size exclusion, FPLC and reverse-phase high performance liquid chromatography. Suitable chromatographic media include derivatized dextrans, agarose, cellulose, polyacrylamide, specialty silicas, and the like. PEI, DEAE, QAE and Q derivatives are suitable. Exemplary chromatographic media include those media derivatized with phenyl, butyl, or octyl groups, such as Phenyl-Sepharose FF (Pharmacia), Toyopearl butyl 650 (Toso Haas, Montgomeryville, PA), Octyl-Sepharose (Pharmacia) and the like; or polyacrylic resins, such as Amberchrom CG 71 (Toso Haas) and the like. Suitable solid supports include glass beads, silica-based resins, cellulosic resins, agarose beads, cross-linked agarose beads, polystyrene beads, cross-linked polyacrylamide resins and the like that are insoluble under the conditions in which they are to be used. These supports may be modified with reactive groups that allow attachment of proteins by amino groups, carboxyl groups, sulfhydryl groups, hydroxyl groups and/or carbohydrate moieties.

Examples of coupling chemistries include cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, hydrazide activation, and carboxyl and amino derivatives for carbodiimide coupling chemistries. These and other solid media are well known and widely used in the art, and are available from commercial suppliers. Selection of a particular method for polypeptide isolation and purification is a matter of routine design and is determined in part by the properties of the chosen support. See, for example, *Affinity Chromatography: Principles & Methods* (Pharmacia LKB Biotechnology 1988), and Doonan, *Protein Purification Protocols* (The Humana Press 1996).

Additional variations in Zcytor18 isolation and purification can be devised by those of skill in the art. For example, anti-Zcytor18 antibodies, obtained as

described below, can be used to isolate large quantities of protein by immunoaffinity purification.

The polypeptides of the present invention can also be isolated by exploitation of particular properties. For example, immobilized metal ion adsorption (IMAC) chromatography can be used to purify histidine-rich proteins, including those comprising polyhistidine tags. Briefly, a gel is first charged with divalent metal ions to form a chelate (Sulkowski, *Trends in Biochem. 3*:1 (1985)). Histidine-rich proteins will be adsorbed to this matrix with differing affinities, depending upon the metal ion used, and will be eluted by competitive elution, lowering the pH, or use of strong chelating agents. Other methods of purification include purification of glycosylated proteins by lectin affinity chromatography and ion exchange chromatography (M. Deutscher, (ed.), *Meth. Enzymol. 182*:529 (1990)). Within additional embodiments of the invention, a fusion of the polypeptide of interest and an affinity tag (*e.g.,* maltose-binding protein, an immunoglobulin domain) may be constructed to facilitate purification.

Zcytor18 polypeptides or fragments thereof may also be prepared through chemical synthesis, as described above. Zcytor18 polypeptides may be monomers or multimers; glycosylated or non-glycosylated; PEGylated or non-PEGylated; and may or may not include an initial methionine amino acid residue.

8. Production of Antibodies to Zcytor18 Proteins

Antibodies to Zcytor18 can be obtained, for example, using the product of a Zcytor18 expression vector or Zcytor18 isolated from a natural source as an antigen. Particularly useful anti-Zcytor18 antibodies "bind specifically" with Zcytor18. Antibodies are considered to be specifically binding if the antibodies exhibit at least one of the following two properties: (1) antibodies bind to Zcytor18 with a threshold level of binding activity, and (2) antibodies do not significantly cross-react with polypeptides related to Zcytor18.

With regard to the first characteristic, antibodies specifically bind if they bind to a Zcytor18 polypeptide, peptide or epitope with a binding affinity (K_a) of $10^6\ M^{-1}$ or greater, preferably $10^7\ M^{-1}$ or greater, more preferably $10^8\ M^{-1}$ or greater, and most preferably $10^9\ M^{-1}$ or greater. The binding affinity of an antibody can be readily determined by one of ordinary skill in the art, for example, by Scatchard analysis (Scatchard, *Ann. NY Acad. Sci. 51*:660 (1949)). With regard to the second characteristic, antibodies do not significantly cross-react with related polypeptide molecules, for example, if they detect Zcytor18, but not presently known polypeptides using a standard

Western blot analysis. Examples of known related polypeptides include known cytokine receptors.

Anti-Zcytor18 antibodies can be produced using antigenic Zcytor18 epitope-bearing peptides and polypeptides. Antigenic epitope-bearing peptides and polypeptides of the present invention contain a sequence of at least nine, or between 15 to about 30 amino acids contained within SEQ ID NO:2 or another amino acid sequence disclosed herein. However, peptides or polypeptides comprising a larger portion of an amino acid sequence of the invention, containing from 30 to 50 amino acids, or any length up to and including the entire amino acid sequence of a polypeptide of the invention, also are useful for inducing antibodies that bind with Zcytor18. It is desirable that the amino acid sequence of the epitope-bearing peptide is selected to provide substantial solubility in aqueous solvents (*i.e.*, the sequence includes relatively hydrophilic residues, while hydrophobic residues are typically avoided). Moreover, amino acid sequences containing proline residues may be also be desirable for antibody production.

As an illustration, potential antigenic sites in Zcytor18 were identified using the Jameson-Wolf method, Jameson and Wolf, *CABIOS 4*:181, (1988), as implemented by the PROTEAN program (version 3.14) of LASERGENE (DNASTAR; Madison, WI). Default parameters were used in this analysis.

The Jameson-Wolf method predicts potential antigenic determinants by combining six major subroutines for protein structural prediction. Briefly, the Hopp-Woods method, Hopp *et al., Proc. Nat'l Acad. Sci. USA 78*:3824 (1981), was first used to identify amino acid sequences representing areas of greatest local hydrophilicity (parameter: seven residues averaged). In the second step, Emini's method, Emini *et al., J. Virology 55*:836 (1985), was used to calculate surface probabilities (parameter: surface decision threshold (0.6) = 1). Third, the Karplus-Schultz method, Karplus and Schultz, *Naturwissenschaften 72*:212 (1985), was used to predict backbone chain flexibility (parameter: flexibility threshold (0.2) = 1). In the fourth and fifth steps of the analysis, secondary structure predictions were applied to the data using the methods of Chou-Fasman, Chou, "Prediction of Protein Structural Classes from Amino Acid Composition," in *Prediction of Protein Structure and the Principles of Protein Conformation*, Fasman (ed.), pages 549-586 (Plenum Press 1990), and Garnier-Robson, Garnier *et al., J. Mol. Biol. 120*:97 (1978) (Chou-Fasman parameters: conformation table = 64 proteins; α region threshold = 103; β region threshold = 105; Garnier-Robson parameters: α and β decision constants = 0). In the sixth subroutine, flexibility parameters and hydropathy/solvent accessibility factors were combined to determine a surface contour value, designated as the "antigenic index." Finally, a peak broadening

function was applied to the antigenic index, which broadens major surface peaks by adding 20, 40, 60, or 80% of the respective peak value to account for additional free energy derived from the mobility of surface regions relative to interior regions. This calculation was not applied, however, to any major peak that resides in a helical region, since helical regions tend to be less flexible.

The results of this analysis indicated that the following amino acid sequences of SEQ ID NO:2 would provide suitable antigenic molecules: amino acids 29 to 41, amino acids 56 to 67, 131 to 139, amino acids 145 to 161, amino acids 214 to 227, amino acids 251 to 271, amino acids 347 to 357, amino acids 362 to 371, amino acids 445 to 462, amino acids 526 to 555, amino acids 628 to 654, amino acids 665 to 688, and amino acids 695 to 735, while amino acids 45 to 53 of SEQ ID NO:8 would provide a suitable antigenic molecule. The present invention contemplates the use of any one of these antigenic amino acid sequences to generate antibodies to Zcytor18. The present invention also contemplates polypeptides comprising at least one of these antigenic molecules.

Polyclonal antibodies to recombinant Zcytor18 protein or to Zcytor18 isolated from natural sources can be prepared using methods well-known to those of skill in the art. See, for example, Green *et al.*, "Production of Polyclonal Antisera," in *Immunochemical Protocols* (Manson, ed.), pages 1-5 (Humana Press 1992), and Williams *et al.*, "Expression of foreign proteins in *E. coli* using plasmid vectors and purification of specific polyclonal antibodies," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), page 15 (Oxford University Press 1995). The immunogenicity of a Zcytor18 polypeptide can be increased through the use of an adjuvant, such as alum (aluminum hydroxide) or Freund's complete or incomplete adjuvant. Polypeptides useful for immunization also include fusion polypeptides, such as fusions of Zcytor18 or a portion thereof with an immunoglobulin polypeptide or with maltose binding protein. The polypeptide immunogen may be a full-length molecule or a portion thereof. If the polypeptide portion is "hapten-like," such portion may be advantageously joined or linked to a macromolecular carrier (such as keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or tetanus toxoid) for immunization.

Although polyclonal antibodies are typically raised in animals such as horses, cows, dogs, chicken, rats, mice, rabbits, guinea pigs, goats, or sheep, an anti-Zcytor18 antibody of the present invention may also be derived from a subhuman primate antibody. General techniques for raising diagnostically and therapeutically useful antibodies in baboons may be found, for example, in Goldenberg *et al.*, international patent publication No. WO 91/11465, and in Losman *et al., Int. J. Cancer 46*:310 (1990).

Alternatively, monoclonal anti-Zcytor18 antibodies can be generated. Rodent monoclonal antibodies to specific antigens may be obtained by methods known to those skilled in the art (see, for example, Kohler *et al., Nature 256*:495 (1975), Coligan *et al.* (eds.), *Current Protocols in Immunology, Vol. 1*, pages 2.5.1-2.6.7 (John Wiley & Sons 1991) ["Coligan"], Picksley *et al.*, "Production of monoclonal antibodies against proteins expressed in *E. coli*," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), page 93 (Oxford University Press 1995)).

Briefly, monoclonal antibodies can be obtained by injecting mice with a composition comprising a *Zcytor18* gene product, verifying the presence of antibody production by removing a serum sample, removing the spleen to obtain B-lymphocytes, fusing the B-lymphocytes with myeloma cells to produce hybridomas, cloning the hybridomas, selecting positive clones which produce antibodies to the antigen, culturing the clones that produce antibodies to the antigen, and isolating the antibodies from the hybridoma cultures.

In addition, an anti-Zcytor18 antibody of the present invention may be derived from a human monoclonal antibody. Human monoclonal antibodies are obtained from transgenic mice that have been engineered to produce specific human antibodies in response to antigenic challenge. In this technique, elements of the human heavy and light chain locus are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy chain and light chain loci. The transgenic mice can synthesize human antibodies specific for human antigens, and the mice can be used to produce human antibody-secreting hybridomas. Methods for obtaining human antibodies from transgenic mice are described, for example, by Green *et al., Nature Genet. 7*:13 (1994), Lonberg *et al., Nature 368*:856 (1994), and Taylor *et al., Int. Immun. 6*:579 (1994).

Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography (see, for example, Coligan at pages 2.7.1-2.7.12 and pages 2.9.1-2.9.3; Baines *et al.*, "Purification of Immunoglobulin G (IgG)," in *Methods in Molecular Biology, Vol. 10*, pages 79-104 (The Humana Press, Inc. 1992)).

For particular uses, it may be desirable to prepare fragments of anti-Zcytor18 antibodies. Such antibody fragments can be obtained, for example, by proteolytic hydrolysis of the antibody. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods. As an illustration, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted $F(ab')_2$. This fragment can be further cleaved using a

thiol reducing agent to produce 3.5S Fab' monovalent fragments. Optionally, the cleavage reaction can be performed using a blocking group for the sulfhydryl groups that result from cleavage of disulfide linkages. As an alternative, an enzymatic cleavage using pepsin produces two monovalent Fab fragments and an Fc fragment directly. These methods are described, for example, by Goldenberg, U.S. patent No. 4,331,647, Nisonoff *et al., Arch Biochem. Biophys. 89*:230 (1960), Porter, *Biochem. J. 73*:119 (1959), Edelman *et al.*, in *Methods in Enzymology Vol. 1*, page 422 (Academic Press 1967), and by Coligan at pages 2.8.1-2.8.10 and 2.10.-2.10.4.

Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

For example, Fv fragments comprise an association of V_H and V_L chains. This association can be noncovalent, as described by Inbar *et al., Proc. Nat'l Acad. Sci. USA 69*:2659 (1972). Alternatively, the variable chains can be linked by an intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde (see, for example, Sandhu, *Crit. Rev. Biotech. 12*:437 (1992)).

The Fv fragments may comprise V_H and V_L chains, which are connected by a peptide linker. These single-chain antigen binding proteins (scFv) are prepared by constructing a structural gene comprising DNA sequences encoding the V_H and V_L domains which are connected by an oligonucleotide. The structural gene is inserted into an expression vector, which is subsequently introduced into a host cell, such as *E. coli.* The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing scFvs are described, for example, by Whitlow *et al., Methods: A Companion to Methods in Enzymology 2*:97 (1991) (also see, Bird *et al., Science 242*:423 (1988), Ladner *et al.*, U.S. Patent No. 4,946,778, Pack *et al., Bio/Technology 11*:1271 (1993), and Sandhu, *supra*).

As an illustration, a scFV can be obtained by exposing lymphocytes to Zcytor18 polypeptide *in vitro*, and selecting antibody display libraries in phage or similar vectors (for instance, through use of immobilized or labeled Zcytor18 protein or peptide). Genes encoding polypeptides having potential Zcytor18 polypeptide binding domains can be obtained by screening random peptide libraries displayed on phage (phage display) or on bacteria, such as *E. coli.* Nucleotide sequences encoding the polypeptides can be obtained in a number of ways, such as through random mutagenesis and random polynucleotide synthesis. These random peptide display libraries can be used to screen for peptides, which interact with a known target which can be a protein or polypeptide, such as a ligand or receptor, a biological or synthetic macromolecule, or organic or

inorganic substances. Techniques for creating and screening such random peptide display libraries are known in the art (Ladner *et al.*, U.S. Patent No. 5,223,409, Ladner *et al.*, U.S. Patent No. 4,946,778, Ladner *et al.*, U.S. Patent No. 5,403,484, Ladner *et al.*, U.S. Patent No. 5,571,698, and Kay *et al.*, *Phage Display of Peptides and Proteins* (Academic Press, Inc. 1996)) and random peptide display libraries and kits for screening such libraries are available commercially, for instance from CLONTECH Laboratories, Inc. (Palo Alto, CA), Invitrogen Inc. (San Diego, CA), New England Biolabs, Inc. (Beverly, MA), and Pharmacia LKB Biotechnology Inc. (Piscataway, NJ). Random peptide display libraries can be screened using the Zcytor18 sequences disclosed herein to identify proteins which bind to Zcytor18.

Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides ("minimal recognition units") can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells (see, for example, Larrick *et al., Methods: A Companion to Methods in Enzymology* 2:106 (1991), Courtenay-Luck, "Genetic Manipulation of Monoclonal Antibodies," in *Monoclonal Antibodies: Production, Engineering and Clinical Application*, Ritter *et al.* (eds.), page 166 (Cambridge University Press 1995), and Ward *et al.*, "Genetic Manipulation and Expression of Antibodies," in *Monoclonal Antibodies: Principles and Applications*, Birch *et al.*, (eds.), page 137 (Wiley-Liss, Inc. 1995)).

Alternatively, an anti-Zcytor18 antibody may be derived from a "humanized" monoclonal antibody. Humanized monoclonal antibodies are produced by transferring mouse complementary determining regions from heavy and light variable chains of the mouse immunoglobulin into a human variable domain. Typical residues of human antibodies are then substituted in the framework regions of the murine counterparts. The use of antibody components derived from humanized monoclonal antibodies obviates potential problems associated with the immunogenicity of murine constant regions. General techniques for cloning murine immunoglobulin variable domains are described, for example, by Orlandi *et al., Proc. Nat'l Acad. Sci. USA* 86:3833 (1989). Techniques for producing humanized monoclonal antibodies are described, for example, by Jones *et al., Nature* 321:522 (1986), Carter *et al., Proc. Nat'l Acad. Sci. USA* 89:4285 (1992), Sandhu, *Crit. Rev. Biotech.* 12:437 (1992), Singer *et al.*, *J. Immun.* 150:2844 (1993), Sudhir (ed.), *Antibody Engineering Protocols* (Humana Press, Inc. 1995), Kelley, "Engineering Therapeutic Antibodies," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), pages 399-434 (John Wiley & Sons, Inc. 1996), and by Queen *et al.*, U.S. Patent No. 5,693,762 (1997).

Polyclonal anti-idiotype antibodies can be prepared by immunizing animals with anti-Zcytor18 antibodies or antibody fragments, using standard techniques. See, for example, Green *et al.*, "Production of Polyclonal Antisera," in *Methods In Molecular Biology: Immunochemical Protocols*, Manson (ed.), pages 1-12 (Humana Press 1992). Also, see Coligan at pages 2.4.1-2.4.7. Alternatively, monoclonal anti-idiotype antibodies can be prepared using anti-Zcytor18 antibodies or antibody fragments as immunogens with the techniques, described above. As another alternative, humanized anti-idiotype antibodies or subhuman primate anti-idiotype antibodies can be prepared using the above-described techniques. Methods for producing anti-idiotype antibodies are described, for example, by Irie, U.S. Patent No. 5,208,146, Greene, *et. al.*, U.S. Patent No. 5,637,677, and Varthakavi and Minocha, *J. Gen. Virol.* 77:1875 (1996).

9. Use of Zcytor18 Nucleotide Sequences to Detect Gene Expression and Gene Structure

Nucleic acid molecules can be used to detect the expression of a *Zcytor18* gene in a biological sample. Suitable probe molecules include double-stranded nucleic acid molecules comprising the nucleotide sequence of SEQ ID NO:1, or a portion thereof, as well as single-stranded nucleic acid molecules having the complement of the nucleotide sequence of SEQ ID NO:1, or a portion thereof. Probe molecules may be DNA, RNA, oligonucleotides, and the like. As used herein, the term "portion" refers to at least eight nucleotides to at least 20 or more nucleotides. Illustrative probes bind with regions of the *Zcytor18* gene that have a low sequence similarity to comparable regions in other cytokine receptor genes.

In a basic assay, a single-stranded probe molecule is incubated with RNA, isolated from a biological sample, under conditions of temperature and ionic strength that promote base pairing between the probe and target *Zcytor18* RNA species. After separating unbound probe from hybridized molecules, the amount of hybrids is detected.

Well-established hybridization methods of RNA detection include northern analysis and dot/slot blot hybridization (see, for example, Ausubel (1995) at pages 4-1 to 4-27, and Wu *et al.* (eds.), "Analysis of Gene Expression at the RNA Level," in *Methods in Gene Biotechnology*, pages 225-239 (CRC Press, Inc. 1997)). Nucleic acid probes can be detectably labeled with radioisotopes such as ^{32}P or ^{35}S. Alternatively, Zcytor18 RNA can be detected with a nonradioactive hybridization method (see, for example, Isaac (ed.), *Protocols for Nucleic Acid Analysis by Nonradioactive Probes* (Humana Press, Inc. 1993)). Typically, nonradioactive detection is achieved by

enzymatic conversion of chromogenic or chemiluminescent substrates. Illustrative nonradioactive moieties include biotin, fluorescein, and digoxigenin.

Zcytor18 oligonucleotide probes are also useful for *in vivo* diagnosis. As an illustration, ^{18}F-labeled oligonucleotides can be administered to a subject and visualized by positron emission tomography (Tavitian *et al., Nature Medicine 4*:467 (1998)).

Numerous diagnostic procedures take advantage of the polymerase chain reaction (PCR) to increase sensitivity of detection methods. Standard techniques for performing PCR are well-known (see, generally, Mathew (ed.), *Protocols in Human Molecular Genetics* (Humana Press, Inc. 1991), White (ed.), *PCR Protocols: Current Methods and Applications* (Humana Press, Inc. 1993), Cotter (ed.), *Molecular Diagnosis of Cancer* (Humana Press, Inc. 1996), Hanausek and Walaszek (eds.), *Tumor Marker Protocols* (Humana Press, Inc. 1998), Lo (ed.), *Clinical Applications of PCR* (Humana Press, Inc. 1998), and Meltzer (ed.), *PCR in Bioanalysis* (Humana Press, Inc. 1998)).

PCR primers can be designed to amplify a portion of the *Zcytor18* gene that has a low sequence similarity to a comparable region in other proteins, such as other cytokine receptor proteins.

One variation of PCR for diagnostic assays is reverse transcriptase-PCR (RT-PCR). In the RT-PCR technique, RNA is isolated from a biological sample, reverse transcribed to cDNA, and the cDNA is incubated with *Zcytor18* primers (see, for example, Wu *et al.* (eds.), "Rapid Isolation of Specific cDNAs or Genes by PCR," in *Methods in Gene Biotechnology*, pages 15-28 (CRC Press, Inc. 1997)). PCR is then performed and the products are analyzed using standard techniques.

As an illustration, RNA is isolated from biological sample using, for example, the guanidinium-thiocyanate cell lysis procedure described above. Alternatively, a solid-phase technique can be used to isolate mRNA from a cell lysate. A reverse transcription reaction can be primed with the isolated RNA using random oligonucleotides, short homopolymers of dT, or *Zcytor18* anti-sense oligomers. Oligo-dT primers offer the advantage that various mRNA nucleotide sequences are amplified that can provide control target sequences. Zcytor18 sequences are amplified by the polymerase chain reaction using two flanking oligonucleotide primers that are typically 20 bases in length.

PCR amplification products can be detected using a variety of approaches. For example, PCR products can be fractionated by gel electrophoresis, and visualized by ethidium bromide staining. Alternatively, fractionated PCR products can be transferred to a membrane, hybridized with a detectably-labeled Zcytor18 probe, and examined by autoradiography. Additional alternative approaches include the use of

digoxigenin-labeled deoxyribonucleic acid triphosphates to provide chemiluminescence detection, and the C-TRAK colorimetric assay.

Another approach for detection of *Zcytor18* expression is cycling probe technology, in which a single-stranded DNA target binds with an excess of DNA-RNA-DNA chimeric probe to form a complex, the RNA portion is cleaved with RNAase H, and the presence of cleaved chimeric probe is detected (see, for example, Beggs *et al., J. Clin. Microbiol. 34*:2985 (1996), Bekkaoui *et al., Biotechniques 20*:240 (1996)). Alternative methods for detection of *Zcytor18* sequences can utilize approaches such as nucleic acid sequence-based amplification, cooperative amplification of templates by cross-hybridization, and the ligase chain reaction (see, for example, Marshall *et al.*, U.S. Patent No. 5,686,272 (1997), Dyer *et al., J. Virol. Methods 60*:161 (1996), Ehricht *et al., Eur. J. Biochem. 243*:358 (1997), and Chadwick *et al., J. Virol. Methods 70*:59 (1998)). Other standard methods are known to those of skill in the art.

Zcytor18 probes and primers can also be used to detect and to localize *Zcytor18* gene expression in tissue samples. Methods for such *in situ* hybridization are well-known to those of skill in the art (see, for example, Choo (ed.), *In Situ Hybridization Protocols* (Humana Press, Inc. 1994), Wu *et al.* (eds.), "Analysis of Cellular DNA or Abundance of mRNA by Radioactive *In Situ* Hybridization (RISH)," in *Methods in Gene Biotechnology*, pages 259-278 (CRC Press, Inc. 1997), and Wu *et al.* (eds.), "Localization of DNA or Abundance of mRNA by Fluorescence *In Situ* Hybridization (RISH)," in *Methods in Gene Biotechnology*, pages 279-289 (CRC Press, Inc. 1997)). Various additional diagnostic approaches are well-known to those of skill in the art (see, for example, Mathew (ed.), *Protocols in Human Molecular Genetics* (Humana Press, Inc. 1991), Coleman and Tsongalis, *Molecular Diagnostics* (Humana Press, Inc. 1996), and Elles, *Molecular Diagnosis of Genetic Diseases* (Humana Press, Inc., 1996)). Suitable test samples include blood, urine, saliva, tissue biopsy, and autopsy material.

The *Zcytor18* gene resides in chromosome 3. Thus, nucleic acid probes that encode Zcytor18, or a fragment thereof, can be used to detect gross aberrations in chromosome 3. As an example, monosomy 3, trisomy 3, and other structural aberrations of chromosome 3 are associated with acute myelocytic leukemia, chronic leukemia, neuroendocrine cancer, polyclonal B-cell lymphocytosis, and renal cell carcinoma (see, for example, Callet-Bauchu *et al., Genes, Chromosomes, Cancer 26*:221 (1999); Hughson *et al., Mod. Pathol. 12*:301 (1999); Russo *et al., Br. J. Haematol. 105*:989 (1999); Lindquist *et al., Leukemia 14*:112 (2000)). Moreover, the *Zcytor18* gene resides in chromosome 3p14.3, a region that is associated with various diseases and disorders, including Wernicke-Korsakoff Syndrome, and Bardet-Biedl Syndrome 3.

In addition, mutations of cytokine receptors are associated with particular diseases. For example, polymorphisms of cytokine receptors are associated with pulmonary alveolar proteinosis, familial periodic fever, and erythroleukemia. Thus, *Zcytor18* nucleotide sequences can be used in linkage-based testing for various diseases, and to determine whether a subject's chromosomes contain a mutation in the *Zcytor18* gene. Detectable chromosomal aberrations at the *Zcytor18* gene locus include, but are not limited to, aneuploidy, gene copy number changes, insertions, deletions, restriction site changes and rearrangements. Of particular interest are genetic alterations that inactivate a *Zcytor18* gene.

Aberrations associated with the *Zcytor18* locus can be detected using nucleic acid molecules of the present invention by employing molecular genetic techniques, such as restriction fragment length polymorphism analysis, short tandem repeat analysis employing PCR techniques, amplification-refractory mutation system analysis, single-strand conformation polymorphism detection, RNase cleavage methods, denaturing gradient gel electrophoresis, fluorescence-assisted mismatch analysis, and other genetic analysis techniques known in the art (see, for example, Mathew (ed.), *Protocols in Human Molecular Genetics* (Humana Press, Inc. 1991), Marian, *Chest 108*:255 (1995), Coleman and Tsongalis, *Molecular Diagnostics* (Human Press, Inc. 1996), Elles (ed.) *Molecular Diagnosis of Genetic Diseases* (Humana Press, Inc. 1996), Landegren (ed.), *Laboratory Protocols for Mutation Detection* (Oxford University Press 1996), Birren *et al.* (eds.), *Genome Analysis, Vol. 2: Detecting Genes* (Cold Spring Harbor Laboratory Press 1998), Dracopoli *et al.* (eds.), *Current Protocols in Human Genetics* (John Wiley & Sons 1998), and Richards and Ward, "Molecular Diagnostic Testing," in *Principles of Molecular Medicine*, pages 83-88 (Humana Press, Inc. 1998)).

The protein truncation test is also useful for detecting the inactivation of a gene in which translation-terminating mutations produce only portions of the encoded protein (see, for example, Stoppa-Lyonnet *et al., Blood 91*:3920 (1998)). According to this approach, RNA is isolated from a biological sample, and used to synthesize cDNA. PCR is then used to amplify the *Zcytor18* target sequence and to introduce an RNA polymerase promoter, a translation initiation sequence, and an in-frame ATG triplet. PCR products are transcribed using an RNA polymerase, and the transcripts are translated *in vitro* with a T7-coupled reticulocyte lysate system. The translation products are then fractionated by SDS-PAGE to determine the lengths of the translation products. The protein truncation test is described, for example, by Dracopoli *et al.* (eds.), *Current Protocols in Human Genetics,* pages 9.11.1 - 9.11.18 (John Wiley & Sons 1998).

The present invention also contemplates kits for performing a diagnostic assay for *Zcytor18* gene expression or to detect mutations in the *Zcytor18* gene. Such kits

comprise nucleic acid probes, such as double-stranded nucleic acid molecules comprising the nucleotide sequence of nucleotides 192 to 652 of SEQ ID NO:1, the nucleotide sequence of SEQ ID NO:1, or a portion thereof, as well as single-stranded nucleic acid molecules having the complement of the nucleotide sequence of SEQ ID NO:1, or a portion thereof. Probe molecules may be DNA, RNA, oligonucleotides, and the like. Kits may comprise nucleic acid primers for performing PCR.

Such kits can contain all the necessary elements to perform a nucleic acid diagnostic assay described above. A kit will comprise at least one container comprising a *Zcytor18* probe or primer. The kit may also comprise a second container comprising one or more reagents capable of indicating the presence of *Zcytor18* sequences. Examples of such indicator reagents include detectable labels such as radioactive labels, fluorochromes, chemiluminescent agents, and the like. A kit may also comprise a means for conveying to the user that the *Zcytor18* probes and primers are used to detect *Zcytor18* gene expression. For example, written instructions may state that the enclosed nucleic acid molecules can be used to detect either a nucleic acid molecule that encodes Zcytor18, or a nucleic acid molecule having a nucleotide sequence that is complementary to a *Zcytor18*-encoding nucleotide sequence. The written material can be applied directly to a container, or the written material can be provided in the form of a packaging insert.

10. Use of Anti-Zcytor18 Antibodies to Detect Zcytor18

The present invention contemplates the use of anti-Zcytor18 antibodies to screen biological samples *in vitro* for the presence of Zcytor18. In one type of *in vitro* assay, anti-Zcytor18 antibodies are used in liquid phase. For example, the presence of Zcytor18 in a biological sample can be tested by mixing the biological sample with a trace amount of labeled Zcytor18 and an anti-Zcytor18 antibody under conditions that promote binding between Zcytor18 and its antibody. Complexes of Zcytor18 and anti-Zcytor18 in the sample can be separated from the reaction mixture by contacting the complex with an immobilized protein which binds with the antibody, such as an Fc antibody or *Staphylococcus* protein A. The concentration of Zcytor18 in the biological sample will be inversely proportional to the amount of labeled Zcytor18 bound to the antibody and directly related to the amount of free-labeled Zcytor18. Illustrative biological samples include blood, urine, saliva, tissue biopsy, and autopsy material.

Alternatively, *in vitro* assays can be performed in which anti-Zcytor18 antibody is bound to a solid-phase carrier. For example, antibody can be attached to a polymer, such as aminodextran, in order to link the antibody to an insoluble support such

as a polymer-coated bead, a plate or a tube. Other suitable *in vitro* assays will be readily apparent to those of skill in the art.

In another approach, anti-Zcytor18 antibodies can be used to detect Zcytor18 in tissue sections prepared from a biopsy specimen. Such immunochemical detection can be used to determine the relative abundance of Zcytor18 and to determine the distribution of Zcytor18 in the examined tissue. General immunochemistry techniques are well established (see, for example, Ponder, "Cell Marking Techniques and Their Application," in *Mammalian Development: A Practical Approach*, Monk (ed.), pages 115-38 (IRL Press 1987), Coligan at pages 5.8.1-5.8.8, Ausubel (1995) at pages 14.6.1 to 14.6.13 (Wiley Interscience 1990), and Manson (ed.), *Methods In Molecular Biology, Vol. 10: Immunochemical Protocols* (The Humana Press, Inc. 1992)).

Immunochemical detection can be performed by contacting a biological sample with an anti-Zcytor18 antibody, and then contacting the biological sample with a detectably labeled molecule, which binds to the antibody. For example, the detectably labeled molecule can comprise an antibody moiety that binds to anti-Zcytor18 antibody. Alternatively, the anti-Zcytor18 antibody can be conjugated with avidin/streptavidin (or biotin) and the detectably labeled molecule can comprise biotin (or avidin/streptavidin). Numerous variations of this basic technique are well-known to those of skill in the art.

Alternatively, an anti-Zcytor18 antibody can be conjugated with a detectable label to form an anti-Zcytor18 immunoconjugate. Suitable detectable labels include, for example, a radioisotope, a fluorescent label, a chemiluminescent label, an enzyme label, a bioluminescent label or colloidal gold. Methods of making and detecting such detectably-labeled immunoconjugates are well-known to those of ordinary skill in the art, and are described in more detail below.

The detectable label can be a radioisotope that is detected by autoradiography. Isotopes that are particularly useful for the purpose of the present invention are ^{3}H, ^{125}I, ^{131}I, ^{35}S and ^{14}C.

Anti-Zcytor18 immunoconjugates can also be labeled with a fluorescent compound. The presence of a fluorescently-labeled antibody is determined by exposing the immunoconjugate to light of the proper wavelength and detecting the resultant fluorescence. Fluorescent labeling compounds include fluorescein isothiocyanate, rhodamine, phycoerytherin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

Alternatively, anti-Zcytor18 immunoconjugates can be detectably labeled by coupling an antibody component to a chemiluminescent compound. The presence of the chemiluminescent-tagged immunoconjugate is determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of chemi-

luminescent labeling compounds include luminol, isoluminol, an aromatic acridinium ester, an imidazole, an acridinium salt and an oxalate ester.

Similarly, a bioluminescent compound can be used to label anti-Zcytor18 immunoconjugates of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Bioluminescent compounds that are useful for labeling include luciferin, luciferase and aequorin.

Alternatively, anti-Zcytor18 immunoconjugates can be detectably labeled by linking an anti-Zcytor18 antibody component to an enzyme. When the anti-Zcytor18-enzyme conjugate is incubated in the presence of the appropriate substrate, the enzyme moiety reacts with the substrate to produce a chemical moiety, which can be detected, for example, by spectrophotometric, fluorometric or visual means. Examples of enzymes that can be used to detectably label polyspecific immunoconjugates include β-galactosidase, glucose oxidase, peroxidase and alkaline phosphatase.

Those of skill in the art will know of other suitable labels, which can be employed in accordance with the present invention. The binding of marker moieties to anti-Zcytor18 antibodies can be accomplished using standard techniques known to the art. Typical methodology in this regard is described by Kennedy *et al., Clin. Chim. Acta 70*:1 (1976), Schurs *et al., Clin. Chim. Acta 81*:1 (1977), Shih *et al., Int'l J. Cancer 46*:1101 (1990), Stein *et al., Cancer Res. 50:*1330 (1990), and Coligan, *supra.*

Moreover, the convenience and versatility of immunochemical detection can be enhanced by using anti-Zcytor18 antibodies that have been conjugated with avidin, streptavidin, and biotin (see, for example, Wilchek *et al.* (eds.), "Avidin-Biotin Technology," *Methods In Enzymology, Vol. 184* (Academic Press 1990), and Bayer *et al.*, "Immunochemical Applications of Avidin-Biotin Technology," in *Methods In Molecular Biology, Vol. 10*, Manson (ed.), pages 149-162 (The Humana Press, Inc. 1992).

Methods for performing immunoassays are well-established. See, for example, Cook and Self, "Monoclonal Antibodies in Diagnostic Immunoassays," in *Monoclonal Antibodies: Production, Engineering, and Clinical Application*, Ritter and Ladyman (eds.), pages 180-208, (Cambridge University Press, 1995), Perry, "The Role of Monoclonal Antibodies in the Advancement of Immunoassay Technology," in *Monoclonal Antibodies: Principles and Applications*, Birch and Lennox (eds.), pages 107-120 (Wiley-Liss, Inc. 1995), and Diamandis, *Immunoassay* (Academic Press, Inc. 1996).

The present invention also contemplates kits for performing an immunological diagnostic assay for *Zcytor18* gene expression. Such kits comprise at least one container comprising an anti-Zcytor18 antibody, or antibody fragment. A kit may

also comprise a second container comprising one or more reagents capable of indicating the presence of Zcytor18 antibody or antibody fragments. Examples of such indicator reagents include detectable labels such as a radioactive label, a fluorescent label, a chemiluminescent label, an enzyme label, a bioluminescent label, colloidal gold, and the like. A kit may also comprise a means for conveying to the user that Zcytor18 antibodies or antibody fragments are used to detect Zcytor18 protein. For example, written instructions may state that the enclosed antibody or antibody fragment can be used to detect Zcytor18. The written material can be applied directly to a container, or the written material can be provided in the form of a packaging insert.

11. Therapeutic Uses of Polypeptides Having Zcytor18 Activity

Amino acid sequences having Zcytor18 activity can be used to modulate the immune system by binding Zcytor18 ligand, and thus, preventing the binding of Zcytor18 ligand with endogenous Zcytor18 receptor. As an illustration, polypeptides having Zcytor18 activity can be used to inhibit cell proliferation associated with, for example, psoriasis or the growth of a tumor (*e.g.*, a melanoma). Zcytor18 antagonists, such as anti-Zcytor18 antibodies, can also be used to modulate the immune system by inhibiting the binding of Zcytor18 ligand with the endogenous Zcytor18 receptor.

Accordingly, the present invention includes the use of proteins, polypeptides, and peptides having Zcytor18 activity (such as Zcytor18 polypeptides, Zcytor18 analogs (*e.g.*, anti-Zcytor18 anti-idiotype antibodies), and Zcytor18 fusion proteins) to a subject which lacks an adequate amount of Zcytor18 polypeptide, or which produces an excess of Zcytor18 ligand. Zcytor18 antagonists (*e.g.*, anti-Zcytor18 antibodies) can be also used to treat a subject, which produces an excess of either Zcytor18 ligand or Zcytor18. These molecules can be administered to any subject in need of treatment, and the present invention contemplates both veterinary and human therapeutic uses. Illustrative subjects include mammalian subjects, such as farm animals, domestic animals, and human patients. Human or murine Zcytor18 polypeptides can be used for these applications.

Generally, the dosage of administered Zcytor18 (or Zcytor18 analog or fusion protein) will vary depending upon such factors as the subject's age, weight, height, sex, general medical condition and previous medical history. Typically, it is desirable to provide the recipient with a dosage of Zcytor18 polypeptide, which is in the range of from about 1 pg/kg to 10 mg/kg (amount of agent/body weight of subject), although a lower or higher dosage also may be administered as circumstances dictate.

Administration of a Zcytor18 polypeptide to a subject can be intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, intrapleural, intrathecal, by perfusion through a regional catheter, or by direct intralesional injection. When administering therapeutic proteins by injection, the administration may be by continuous infusion or by single or multiple boluses.

Additional routes of administration include oral, mucosal-membrane, pulmonary, and transcutaneous. Oral delivery is suitable for polyester microspheres, zein microspheres, proteinoid microspheres, polycyanoacrylate microspheres, and lipid-based systems (see, for example, DiBase and Morrel, "Oral Delivery of Microencapsulated Proteins," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 255-288 (Plenum Press 1997)). The feasibility of an intranasal delivery is exemplified by such a mode of insulin administration (see, for example, Hinchcliffe and Illum, *Adv. Drug Deliv. Rev. 35*:199 (1999)). Dry or liquid particles comprising Zcytor18 can be prepared and inhaled with the aid of dry-powder dispersers, liquid aerosol generators, or nebulizers (*e.g.,* Pettit and Gombotz, *TIBTECH 16*:343 (1998); Patton *et al., Adv. Drug Deliv. Rev. 35*:235 (1999)). This approach is illustrated by the AERX diabetes management system, which is a hand-held electronic inhaler that delivers aerosolized insulin into the lungs. Studies have shown that proteins as large as 48,000 kDa have been delivered across skin at therapeutic concentrations with the aid of low-frequency ultrasound, which illustrates the feasibility of trascutaneous administration (Mitragotri *et al., Science 269*:850 (1995)). Transdermal delivery using electroporation provides another means to administer a molecule having Zcytor18 binding activity (Potts *et al., Pharm. Biotechnol. 10*:213 (1997)).

A pharmaceutical composition comprising a protein, polypeptide, or peptide having Zcytor18 binding activity can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the therapeutic proteins are combined in a mixture with a pharmaceutically acceptable carrier. A composition is said to be a "pharmaceutically acceptable carrier" if its administration can be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Other suitable carriers are well-known to those in the art. See, for example, Gennaro (ed.), *Remington's Pharmaceutical Sciences*, 19th Edition (Mack Publishing Company 1995).

For purposes of therapy, molecules having Zcytor18 binding activity and a pharmaceutically acceptable carrier are administered to a patient in a therapeutically effective amount. A combination of a protein, polypeptide, or peptide having Zcytor18 binding activity and a pharmaceutically acceptable carrier is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically

significant. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient. For example, an agent used to treat inflammation is physiologically significant if its presence alleviates the inflammatory response. As another example, an agent used to inhibit the growth of tumor cells is physiologically significant if the administration of the agent results in a decrease in the number of tumor cells, decreased metastasis, a decrease in the size of a solid tumor, or increased necrosis of a tumor.

A pharmaceutical composition comprising Zcytor18 (or Zcytor18 analog or fusion protein) can be furnished in liquid form, in an aerosol, or in solid form. Liquid forms, are illustrated by injectable solutions and oral suspensions. Exemplary solid forms include capsules, tablets, and controlled-release forms. The latter form is illustrated by miniosmotic pumps and implants (Bremer *et al., Pharm. Biotechnol. 10*:239 (1997); Ranade, "Implants in Drug Delivery," in *Drug Delivery Systems*, Ranade and Hollinger (eds.), pages 95-123 (CRC Press 1995); Bremer *et al.*, "Protein Delivery with Infusion Pumps," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 239-254 (Plenum Press 1997); Yewey *et al.*, "Delivery of Proteins from a Controlled Release Injectable Implant," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 93-117 (Plenum Press 1997)).

Liposomes provide one means to deliver therapeutic polypeptides to a subject intravenously, intraperitoneally, intrathecally, intramuscularly, subcutaneously, or via oral administration, inhalation, or intranasal administration. Liposomes are microscopic vesicles that consist of one or more lipid bilayers surrounding aqueous compartments (see, generally, Bakker-Woudenberg *et al., Eur. J. Clin. Microbiol. Infect. Dis. 12 (Suppl. 1)*:S61 (1993), Kim, *Drugs 46*:618 (1993), and Ranade, "Site-Specific Drug Delivery Using Liposomes as Carriers," in *Drug Delivery Systems*, Ranade and Hollinger (eds.), pages 3-24 (CRC Press 1995)). Liposomes are similar in composition to cellular membranes and as a result, liposomes can be administered safely and are biodegradable. Depending on the method of preparation, liposomes may be unilamellar or multilamellar, and liposomes can vary in size with diameters ranging from 0.02 μm to greater than 10 μm. A variety of agents can be encapsulated in liposomes: hydrophobic agents partition in the bilayers and hydrophilic agents partition within the inner aqueous space(s) (see, for example, Machy *et al., Liposomes In Cell Biology And Pharmacology* (John Libbey 1987), and Ostro *et al., American J. Hosp. Pharm. 46*:1576 (1989)). Moreover, it is possible to control the therapeutic availability of the encapsulated agent by varying liposome size, the number of bilayers, lipid composition, as well as the charge and surface characteristics of the liposomes.

Liposomes can adsorb to virtually any type of cell and then slowly release the encapsulated agent. Alternatively, an absorbed liposome may be endocytosed by cells that are phagocytic. Endocytosis is followed by intralysosomal degradation of liposomal lipids and release of the encapsulated agents (Scherphof *et al., Ann. N.Y. Acad. Sci. 446*:368 (1985)). After intravenous administration, small liposomes (0.1 to 1.0 μm) are typically taken up by cells of the reticuloendothelial system, located principally in the liver and spleen, whereas liposomes larger than 3.0 μm are deposited in the lung. This preferential uptake of smaller liposomes by the cells of the reticuloendothelial system has been used to deliver chemotherapeutic agents to macrophages and to tumors of the liver.

The reticuloendothelial system can be circumvented by several methods including saturation with large doses of liposome particles, or selective macrophage inactivation by pharmacological means (Claassen *et al., Biochim. Biophys. Acta 802*:428 (1984)). In addition, incorporation of glycolipid- or polyethelene glycol-derivatized phospholipids into liposome membranes has been shown to result in a significantly reduced uptake by the reticuloendothelial system (Allen *et al., Biochim. Biophys. Acta 1068*:133 (1991); Allen *et al., Biochim. Biophys. Acta 1150*:9 (1993)).

Liposomes can also be prepared to target particular cells or organs by varying phospholipid composition or by inserting receptors or ligands into the liposomes. For example, liposomes, prepared with a high content of a nonionic surfactant, have been used to target the liver (Hayakawa *et al.*, Japanese Patent 04-244,018; Kato *et al., Biol. Pharm. Bull. 16*:960 (1993)). These formulations were prepared by mixing soybean phospatidylcholine, α-tocopherol, and ethoxylated hydrogenated castor oil (HCO-60) in methanol, concentrating the mixture under vacuum, and then reconstituting the mixture with water. A liposomal formulation of dipalmitoylphosphatidylcholine (DPPC) with a soybean-derived sterylglucoside mixture (SG) and cholesterol (Ch) has also been shown to target the liver (Shimizu *et al., Biol. Pharm. Bull. 20*:881 (1997)).

Alternatively, various targeting ligands can be bound to the surface of the liposome, such as antibodies, antibody fragments, carbohydrates, vitamins, and transport proteins. For example, liposomes can be modified with branched type galactosyllipid derivatives to target asialoglycoprotein (galactose) receptors, which are exclusively expressed on the surface of liver cells (Kato and Sugiyama, *Crit. Rev. Ther. Drug Carrier Syst. 14*:287 (1997); Murahashi *et al., Biol. Pharm. Bull.20*:259 (1997)). Similarly, Wu *et al., Hepatology 27*:772 (1998), have shown that labeling liposomes with asialofetuin led to a shortened liposome plasma half-life and greatly enhanced uptake of asialofetuin-labeled liposome by hepatocytes. On the other hand, hepatic accumulation of liposomes comprising branched type galactosyllipid derivatives can be

inhibited by preinjection of asialofetuin (Murahashi *et al., Biol. Pharm. Bull.20*:259 (1997)). Polyaconitylated human serum albumin liposomes provide another approach for targeting liposomes to liver cells (Kamps *et al., Proc. Nat'l Acad. Sci. USA 94*:11681 (1997)). Moreover, Geho, *et al.* U.S. Patent No. 4,603,044, describe a hepatocyte-directed liposome vesicle delivery system, which has specificity for hepatobiliary receptors associated with the specialized metabolic cells of the liver.

In a more general approach to tissue targeting, target cells are prelabeled with biotinylated antibodies specific for a ligand expressed by the target cell (Harasym *et al., Adv. Drug Deliv. Rev. 32*:99 (1998)). After plasma elimination of free antibody, streptavidin-conjugated liposomes are administered. In another approach, targeting antibodies are directly attached to liposomes (Harasym *et al., Adv. Drug Deliv. Rev. 32*:99 (1998)).

Polypeptides having Zcytor18 binding activity can be encapsulated within liposomes using standard techniques of protein microencapsulation (see, for example, Anderson *et al., Infect. Immun. 31*:1099 (1981), Anderson *et al., Cancer Res. 50*:1853 (1990), and Cohen *et al., Biochim. Biophys. Acta 1063*:95 (1991), Alving *et al.* "Preparation and Use of Liposomes in Immunological Studies," in *Liposome Technology,* 2nd Edition, Vol. III, Gregoriadis (ed.), page 317 (CRC Press 1993), Wassef *et al., Meth. Enzymol. 149*:124 (1987)). As noted above, therapeutically useful liposomes may contain a variety of components. For example, liposomes may comprise lipid derivatives of poly(ethylene glycol) (Allen *et al., Biochim. Biophys. Acta 1150*:9 (1993)).

Degradable polymer microspheres have been designed to maintain high systemic levels of therapeutic proteins. Microspheres are prepared from degradable polymers such as poly(lactide-co-glycolide) (PLG), polyanhydrides, poly (ortho esters), nonbiodegradable ethylvinyl acetate polymers, in which proteins are entrapped in the polymer (Gombotz and Pettit, *Bioconjugate Chem. 6*:332 (1995); Ranade, "Role of Polymers in Drug Delivery," in *Drug Delivery Systems*, Ranade and Hollinger (eds.), pages 51-93 (CRC Press 1995); Roskos and Maskiewicz, "Degradable Controlled Release Systems Useful for Protein Delivery," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 45-92 (Plenum Press 1997); Bartus *et al., Science 281*:1161 (1998); Putney and Burke, *Nature Biotechnology 16*:153 (1998); Putney, *Curr. Opin. Chem. Biol. 2*:548 (1998)). Polyethylene glycol (PEG)-coated nanospheres can also provide carriers for intravenous administration of therapeutic proteins (see, for example, Gref *et al., Pharm. Biotechnol. 10*:167 (1997)).

The present invention also contemplates chemically modified polypeptides having binding Zcytor18 activity and Zcytor18 antagonists, in which a polypeptide is linked with a polymer, as discussed above.

Other dosage forms can be devised by those skilled in the art, as shown, for example, by Ansel and Popovich, *Pharmaceutical Dosage Forms and Drug Delivery Systems,* 5th Edition (Lea & Febiger 1990), Gennaro (ed.), *Remington's Pharmaceutical Sciences*, 19th Edition (Mack Publishing Company 1995), and by Ranade and Hollinger, *Drug Delivery Systems* (CRC Press 1996).

As an illustration, pharmaceutical compositions may be supplied as a kit comprising a container that comprises a polypeptide with a Zcytor18 extracellular domain or a Zcytor18 antagonist (*e.g.*, an antibody or antibody fragment that binds a Zcytor18 polypeptide). Therapeutic polypeptides can be provided in the form of an injectable solution for single or multiple doses, or as a sterile powder that will be reconstituted before injection. Alternatively, such a kit can include a dry-powder disperser, liquid aerosol generator, or nebulizer for administration of a therapeutic polypeptide. Such a kit may further comprise written information on indications and usage of the pharmaceutical composition. Moreover, such information may include a statement that the Zcytor18 composition is contraindicated in patients with known hypersensitivity to Zcytor18.

12. Therapeutic Uses of Zcytor18 Nucleotide Sequences

The present invention includes the use of *Zcytor18* nucleotide sequences to provide Zcytor18 to a subject in need of such treatment. In addition, a therapeutic expression vector can be provided that inhibits *Zcytor18* gene expression, such as an anti-sense molecule, a ribozyme, or an external guide sequence molecule.

There are numerous approaches to introduce a *Zcytor18* gene to a subject, including the use of recombinant host cells that express *Zcytor18*, delivery of naked nucleic acid encoding Zcytor18, use of a cationic lipid carrier with a nucleic acid molecule that encodes Zcytor18, and the use of viruses that express *Zcytor18*, such as recombinant retroviruses, recombinant adeno-associated viruses, recombinant adenoviruses, and recombinant Herpes simplex viruses (see, for example, Mulligan, *Science 260*:926 (1993), Rosenberg *et al., Science 242*:1575 (1988), LaSalle *et al., Science 259*:988 (1993), Wolff *et al., Science 247*:1465 (1990), Breakfield and Deluca, *The New Biologist 3*:203 (1991)). In an *ex vivo* approach, for example, cells are isolated from a subject, transfected with a vector that expresses a *Zcytor18* gene, and then transplanted into the subject.

In order to effect expression of a *Zcytor18* gene, an expression vector is constructed in which a nucleotide sequence encoding a *Zcytor18* gene is operably linked to

a core promoter, and optionally a regulatory element, to control gene transcription. The general requirements of an expression vector are described above.

Alternatively, a *Zcytor18* gene can be delivered using recombinant viral vectors, including for example, adenoviral vectors (*e.g.*, Kass-Eisler *et al., Proc. Nat'l Acad. Sci. USA 90*:11498 (1993), Kolls *et al., Proc. Nat'l Acad. Sci. USA 91*:215 (1994), Li *et al., Hum. Gene Ther. 4*:403 (1993), Vincent *et al., Nat. Genet. 5*:130 (1993), and Zabner *et al., Cell 75*:207 (1993)), adenovirus-associated viral vectors (Flotte *et al., Proc. Nat'l Acad. Sci. USA 90*:10613 (1993)), alphaviruses such as Semliki Forest Virus and Sindbis Virus (Hertz and Huang, *J. Vir. 66*:857 (1992), Raju and Huang, *J. Vir. 65*:2501 (1991), and Xiong *et al., Science* 243:1188 (1989)), herpes viral vectors (*e.g.*, U.S. Patent Nos. 4,769,331, 4,859,587, 5,288,641 and 5,328,688), parvovirus vectors (Koering *et al., Hum. Gene Therap. 5*:457 (1994)), pox virus vectors (Ozaki *et al., Biochem. Biophys. Res. Comm. 193*:653 (1993), Panicali and Paoletti, *Proc. Nat'l Acad. Sci. USA 79*:4927 (1982)), pox viruses, such as canary pox virus or vaccinia virus (Fisher-Hoch *et al., Proc. Nat'l Acad. Sci. USA 86*:317 (1989), and Flexner *et al., Ann. N.Y. Acad. Sci. 569*:86 (1989)), and retroviruses (*e.g.*, Baba *et al., J. Neurosurg 79*:729 (1993), Ram *et al., Cancer Res. 53*:83 (1993), Takamiya *et al., J. Neurosci. Res 33*:493 (1992), Vile and Hart, *Cancer Res. 53*:962 (1993), Vile and Hart, *Cancer Res. 53*:3860 (1993), and Anderson *et al.*, U.S. Patent No. 5,399,346). Within various embodiments, either the viral vector itself, or a viral particle which contains the viral vector may be utilized in the methods and compositions described below.

As an illustration of one system, adenovirus, a double-stranded DNA virus, is a well-characterized gene transfer vector for delivery of a heterologous nucleic acid molecule (for a review, see Becker *et al., Meth. Cell Biol. 43*:161 (1994); Douglas and Curiel, *Science & Medicine 4*:44 (1997)). The adenovirus system offers several advantages including: (i) the ability to accommodate relatively large DNA inserts, (ii) the ability to be grown to high-titer, (iii) the ability to infect a broad range of mammalian cell types, and (iv) the ability to be used with many different promoters including ubiquitous, tissue specific, and regulatable promoters. In addition, adenoviruses can be administered by intravenous injection, because the viruses are stable in the bloodstream.

Using adenovirus vectors where portions of the adenovirus genome are deleted, inserts are incorporated into the viral DNA by direct ligation or by homologous recombination with a co-transfected plasmid. In an exemplary system, the essential E1 gene is deleted from the viral vector, and the virus will not replicate unless the E1 gene is provided by the host cell. When intravenously administered to intact animals, adenovirus primarily targets the liver. Although an adenoviral delivery system with an E1 gene deletion cannot replicate in the host cells, the host's tissue will express and

process an encoded heterologous protein. Host cells will also secrete the heterologous protein if the corresponding gene includes a secretory signal sequence. Secreted proteins will enter the circulation from tissue that expresses the heterologous gene (*e.g.*, the highly vascularized liver).

Moreover, adenoviral vectors containing various deletions of viral genes can be used to reduce or eliminate immune responses to the vector. Such adenoviruses are E1-deleted, and in addition, contain deletions of E2A or E4 (Lusky *et al., J. Virol. 72*:2022 (1998); Raper *et al., Human Gene Therapy 9*:671 (1998)). The deletion of E2b has also been reported to reduce immune responses (Amalfitano *et al., J. Virol. 72*:926 (1998)). By deleting the entire adenovirus genome, very large inserts of heterologous DNA can be accommodated. The generation of so called "gutless" adenoviruses, where all viral genes are deleted, is particularly advantageous for insertion of large inserts of heterologous DNA (for a review, see Yeh. and Perricaudet, *FASEB J. 11*:615 (1997)).

High titer stocks of recombinant viruses capable of expressing a therapeutic gene can be obtained from infected mammalian cells using standard methods. For example, recombinant herpes simplex virus can be prepared in Vero cells, as described by Brandt *et al., J. Gen. Virol. 72*:2043 (1991), Herold *et al., J. Gen. Virol. 75*:1211 (1994), Visalli and Brandt, *Virology 185*:419 (1991), Grau *et al., Invest. Ophthalmol. Vis. Sci. 30*:2474 (1989), Brandt *et al., J. Virol. Meth. 36*:209 (1992), and by Brown and MacLean (eds.), *HSV Virus Protocols* (Humana Press 1997).

Alternatively, an expression vector comprising a *Zcytor18* gene can be introduced into a subject's cells by lipofection *in vivo* using liposomes. Synthetic cationic lipids can be used to prepare liposomes for *in vivo* transfection of a gene encoding a marker (Felgner *et al., Proc. Nat'l Acad. Sci. USA 84*:7413 (1987); Mackey *et al., Proc. Nat'l Acad. Sci. USA 85*:8027 (1988)). The use of lipofection to introduce exogenous genes into specific organs *in vivo* has certain practical advantages. Liposomes can be used to direct transfection to particular cell types, which is particularly advantageous in a tissue with cellular heterogeneity, such as the pancreas, liver, kidney, and brain. Lipids may be chemically coupled to other molecules for the purpose of targeting. Targeted peptides (*e.g.*, hormones or neurotransmitters), proteins such as antibodies, or non-peptide molecules can be coupled to liposomes chemically.

Electroporation is another alternative mode of administration. For example, Aihara and Miyazaki, *Nature Biotechnology 16*:867 (1998), have demonstrated the use of *in vivo* electroporation for gene transfer into muscle.

In an alternative approach to gene therapy, a therapeutic gene may encode a *Zcytor18* anti-sense RNA that inhibits the expression of *Zcytor18*. Suitable sequences

for anti-sense molecules can be derived from the nucleotide sequences of *Zcytor18* disclosed herein.

Alternatively, an expression vector can be constructed in which a regulatory element is operably linked to a nucleotide sequence that encodes a ribozyme. Ribozymes can be designed to express endonuclease activity that is directed to a certain target sequence in an mRNA molecule (see, for example, Draper and Macejak, U.S. Patent No. 5,496,698, McSwiggen, U.S. Patent No. 5,525,468, Chowrira and McSwiggen, U.S. Patent No. 5,631,359, and Robertson and Goldberg, U.S. Patent No. 5,225,337). In the context of the present invention, ribozymes include nucleotide sequences that bind with *Zcytor18* mRNA.

In another approach, expression vectors can be constructed in which a regulatory element directs the production of RNA transcripts capable of promoting RNase P-mediated cleavage of mRNA molecules that encode a *Zcytor18* gene. According to this approach, an external guide sequence can be constructed for directing the endogenous ribozyme, RNase P, to a particular species of intracellular mRNA, which is subsequently cleaved by the cellular ribozyme (see, for example, Altman *et al.,* U.S. Patent No. 5,168,053, Yuan *et al., Science 263*:1269 (1994), Pace *et al.,* international publication No. WO 96/18733, George *et al.,* international publication No. WO 96/21731, and Werner *et al.,* international publication No. WO 97/33991). For example, the external guide sequence can comprise a ten to fifteen nucleotide sequence complementary to *Zcytor18* mRNA, and a 3'-NCCA nucleotide sequence, wherein N is preferably a purine. The external guide sequence transcripts bind to the targeted mRNA species by the formation of base pairs between the mRNA and the complementary external guide sequences, thus promoting cleavage of mRNA by RNase P at the nucleotide located at the 5'-side of the base-paired region.

In general, the dosage of a composition comprising a therapeutic vector having a *Zcytor18* nucleotide sequence, such as a recombinant virus, will vary depending upon such factors as the subject's age, weight, height, sex, general medical condition and previous medical history. Suitable routes of administration of therapeutic vectors include intravenous injection, intraarterial injection, intraperitoneal injection, intramuscular injection, intratumoral injection, and injection into a cavity that contains a tumor. As an illustration, Horton *et al., Proc. Nat'l Acad. Sci. USA 96*:1553 (1999), demonstrated that intramuscular injection of plasmid DNA encoding interferon-α produces potent antitumor effects on primary and metastatic tumors in a murine model.

A composition comprising viral vectors, non-viral vectors, or a combination of viral and non-viral vectors of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby

vectors or viruses are combined in a mixture with a pharmaceutically acceptable carrier. As noted above, a composition, such as phosphate-buffered saline is said to be a "pharmaceutically acceptable carrier" if its administration can be tolerated by a recipient subject. Other suitable carriers are well-known to those in the art (see, for example, *Remington's Pharmaceutical Sciences, 19th Ed.* (Mack Publishing Co. 1995), and *Gilman's the Pharmacological Basis of Therapeutics, 7th Ed.* (MacMillan Publishing Co. 1985)).

For purposes of therapy, a therapeutic gene expression vector, or a recombinant virus comprising such a vector, and a pharmaceutically acceptable carrier are administered to a subject in a therapeutically effective amount. A combination of an expression vector (or virus) and a pharmaceutically acceptable carrier is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient subject. For example, an agent used to treat inflammation is physiologically significant if its presence alleviates the inflammatory response.

When the subject treated with a therapeutic gene expression vector or a recombinant virus is a human, then the therapy is preferably somatic cell gene therapy. That is, the preferred treatment of a human with a therapeutic gene expression vector or a recombinant virus does not entail introducing into cells a nucleic acid molecule that can form part of a human germ line and be passed onto successive generations (*i.e.,* human germ line gene therapy).

13. Production of Transgenic Mice

Transgenic mice can be engineered to over-express the *Zcytor18* gene in all tissues or under the control of a tissue-specific or tissue-preferred regulatory element. These over-producers of Zcytor18 can be used to characterize the phenotype that results from over-expression, and the transgenic animals can serve as models for human disease caused by excess Zcytor18. Transgenic mice that over-express Zcytor18 also provide model bioreactors for production of Zcytor18, such as soluble Zcytor18, in the milk or blood of larger animals. Methods for producing transgenic mice are well-known to those of skill in the art (see, for example, Jacob, "Expression and Knockout of Interferons in Transgenic Mice," in *Overexpression and Knockout of Cytokines in Transgenic Mice*, Jacob (ed.), pages 111-124 (Academic Press, Ltd. 1994), Monastersky and Robl (eds.), *Strategies in Transgenic Animal Science* (ASM Press 1995), and Abbud and Nilson, "Recombinant Protein Expression in Transgenic Mice," in *Gene Expression Systems:*

Using Nature for the Art of Expression, Fernandez and Hoeffler (eds.), pages 367-397 (Academic Press, Inc. 1999)).

For example, a method for producing a transgenic mouse that expresses a *Zcytor18* gene can begin with adult, fertile males (studs) (B6C3f1, 2-8 months of age (Taconic Farms, Germantown, NY)), vasectomized males (duds) (B6D2f1, 2-8 months, (Taconic Farms)), prepubescent fertile females (donors) (B6C3f1, 4-5 weeks, (Taconic Farms)) and adult fertile females (recipients) (B6D2f1, 2-4 months, (Taconic Farms)). The donors are acclimated for one week and then injected with approximately 8 IU/mouse of Pregnant Mare's Serum gonadotrophin (Sigma Chemical Company; St. Louis, MO) I.P., and 46-47 hours later, 8 IU/mouse of human Chorionic Gonadotropin (hCG (Sigma)) I.P. to induce superovulation. Donors are mated with studs subsequent to hormone injections. Ovulation generally occurs within 13 hours of hCG injection. Copulation is confirmed by the presence of a vaginal plug the morning following mating.

Fertilized eggs are collected under a surgical scope. The oviducts are collected and eggs are released into urinanalysis slides containing hyaluronidase (Sigma). Eggs are washed once in hyaluronidase, and twice in Whitten's W640 medium (described, for example, by Menino and O'Claray, *Biol. Reprod. 77*:159 (1986), and Dienhart and Downs, *Zygote 4*:129 (1996)) that has been incubated with 5% CO_2, 5% O_2, and 90% N_2 at 37°C. The eggs are then stored in a 37°C/5% CO_2 incubator until microinjection.

Ten to twenty micrograms of plasmid DNA containing a *Zcytor18* encoding sequence is linearized, gel-purified, and resuspended in 10 mM Tris-HCl (pH 7.4), 0.25 mM EDTA (pH 8.0), at a final concentration of 5-10 nanograms per microliter for microinjection. For example, the *Zcytor18* encoding sequences can encode a polypeptide comprising amino acid residues 36 to 313 of SEQ ID NO:2, amino acid residues 36 to 299 of SEQ ID NO:8, or amino acid residues 36 to 300 of SEQ ID NO:12.

Plasmid DNA is microinjected into harvested eggs contained in a drop of W640 medium overlaid by warm, CO_2-equilibrated mineral oil. The DNA is drawn into an injection needle (pulled from a 0.75mm ID, 1mm OD borosilicate glass capillary), and injected into individual eggs. Each egg is penetrated with the injection needle, into one or both of the haploid pronuclei.

Picoliters of DNA are injected into the pronuclei, and the injection needle withdrawn without coming into contact with the nucleoli. The procedure is repeated until all the eggs are injected. Successfully microinjected eggs are transferred into an organ tissue-culture dish with pre-gassed W640 medium for storage overnight in a 37°C/5% CO_2 incubator.

The following day, two-cell embryos are transferred into pseudopregnant recipients. The recipients are identified by the presence of copulation plugs, after copulating with vasectomized duds. Recipients are anesthetized and shaved on the dorsal left side and transferred to a surgical microscope. A small incision is made in the skin and through the muscle wall in the middle of the abdominal area outlined by the ribcage, the saddle, and the hind leg, midway between knee and spleen. The reproductive organs are exteriorized onto a small surgical drape. The fat pad is stretched out over the surgical drape, and a baby serrefine (Roboz, Rockville, MD) is attached to the fat pad and left hanging over the back of the mouse, preventing the organs from sliding back in.

With a fine transfer pipette containing mineral oil followed by alternating W640 and air bubbles, 12-17 healthy two-cell embryos from the previous day's injection are transferred into the recipient. The swollen ampulla is located and holding the oviduct between the ampulla and the bursa, a nick in the oviduct is made with a 28 g needle close to the bursa, making sure not to tear the ampulla or the bursa.

The pipette is transferred into the nick in the oviduct, and the embryos are blown in, allowing the first air bubble to escape the pipette. The fat pad is gently pushed into the peritoneum, and the reproductive organs allowed to slide in. The peritoneal wall is closed with one suture and the skin closed with a wound clip. The mice recuperate on a 37°C slide warmer for a minimum of four hours.

The recipients are returned to cages in pairs, and allowed 19-21 days gestation. After birth, 19-21 days postpartum is allowed before weaning. The weanlings are sexed and placed into separate sex cages, and a 0.5 cm biopsy (used for genotyping) is snipped off the tail with clean scissors.

Genomic DNA is prepared from the tail snips using, for example, a QIAGEN DNEASY kit following the manufacturer's instructions. Genomic DNA is analyzed by PCR using primers designed to amplify a *Zcytor18* gene or a selectable marker gene that was introduced in the same plasmid. After animals are confirmed to be transgenic, they are back-crossed into an inbred strain by placing a transgenic female with a wild-type male, or a transgenic male with one or two wild-type female(s). As pups are born and weaned, the sexes are separated, and their tails snipped for genotyping.

To check for expression of a transgene in a live animal, a partial hepatectomy is performed. A surgical prep is made of the upper abdomen directly below the zyphoid process. Using sterile technique, a small 1.5-2 cm incision is made below the sternum and the left lateral lobe of the liver exteriorized. Using 4-0 silk, a tie is made around the lower lobe securing it outside the body cavity. An atraumatic clamp is used to hold the tie while a second loop of absorbable Dexon (American Cyanamid; Wayne, N.J.) is placed proximal to the first tie. A distal cut is made from the Dexon tie and

approximately 100 mg of the excised liver tissue is placed in a sterile petri dish. The excised liver section is transferred to a 14 ml polypropylene round bottom tube and snap frozen in liquid nitrogen and then stored on dry ice. The surgical site is closed with suture and wound clips, and the animal's cage placed on a 37°C heating pad for 24 hours post operatively. The animal is checked daily post operatively and the wound clips removed 7-10 days after surgery. The expression level of Zcytor18 mRNA is examined for each transgenic mouse using an RNA solution hybridization assay or polymerase chain reaction.

In addition to producing transgenic mice that over-express Zcytor18, it is useful to engineer transgenic mice with either abnormally low or no expression of the gene. Such transgenic mice provide useful models for diseases associated with a lack of Zcytor18. As discussed above, *Zcytor18* gene expression can be inhibited using anti-sense genes, ribozyme genes, or external guide sequence genes. To produce transgenic mice that under-express the *Zcytor18* gene, such inhibitory sequences are targeted to *Zcytor18* mRNA. Methods for producing transgenic mice that have abnormally low expression of a particular gene are known to those in the art (see, for example, Wu *et al.*, "Gene Underexpression in Cultured Cells and Animals by Antisense DNA and RNA Strategies," in *Methods in Gene Biotechnology*, pages 205-224 (CRC Press 1997)).

An alternative approach to producing transgenic mice that have little or no *Zcytor18* gene expression is to generate mice having at least one normal *Zcytor18* allele replaced by a nonfunctional *Zcytor18* gene. One method of designing a nonfunctional *Zcytor18* gene is to insert another gene, such as a selectable marker gene, within a nucleic acid molecule that encodes Zcytor18. Standard methods for producing these so-called "knockout mice" are known to those skilled in the art (see, for example, Jacob, "Expression and Knockout of Interferons in Transgenic Mice," in *Overexpression and Knockout of Cytokines in Transgenic Mice*, Jacob (ed.), pages 111-124 (Academic Press, Ltd. 1994), and Wu *et al.*, "New Strategies for Gene Knockout," in *Methods in Gene Biotechnology*, pages 339-365 (CRC Press 1997)).

CLAIMS

What is claimed is:

1. An isolated polypeptide, comprising an amino acid sequence selected from the group consisting of: (a) an amino acid sequence comprising amino acid residues 36 to 189 of SEQ ID NO:2, (b) amino acid residues 1 to 189 of SEQ ID NO:2, (c) amino acid residues 36 to 313 of SEQ ID NO:2, (d) amino acid residues 336 to 753 of SEQ ID NO:2, (e) amino acid residues 36 to 753 of SEQ ID NO:2, (f) amino acid residues 1 to 753 of SEQ ID NO:2, (g) amino acid resides 1 to 299 of SEQ ID NO:8, (h) amino acid residues 36 to 299 of SEQ ID NO:8, (i) amino acid residues 36 to 175 of SEQ ID NO:8, (j) amino acid residues 1 to 300 of SEQ ID NO:12, and (k) amino acid residues 36 to 300 of SEQ ID NO:12.

2. The isolated polypeptide of claim 1, wherein the polypeptide comprises amino acid residues 1 to 189 of SEQ ID NO:2.

3. The isolated polypeptide of claim 1, wherein the polypeptide comprises amino acid residues 36 to 313 of SEQ ID NO:2.

4. An isolated nucleic acid molecule encoding a polypeptide that comprises an amino acid sequence selected from the group consisting of: (a) an amino acid sequence comprising amino acid residues 36 to 189 of SEQ ID NO:2, (b) amino acid residues 1 to 189 of SEQ ID NO:2, (c) amino acid residues 36 to 313 of SEQ ID NO:2, (d) amino acid residues 336 to 753 of SEQ ID NO:2, (e) amino acid residues 36 to 753 of SEQ ID NO:2, (f) amino acid residues 1 to 753 of SEQ ID NO:2, (g) amino acid resides 1 to 299 of SEQ ID NO:8, (h) amino acid residues 36 to 299 of SEQ ID NO:8, (i) amino acid residues 36 to 175 of SEQ ID NO:8, (j) amino acid residues 1 to 300 of SEQ ID NO:12, and (k) amino acid residues 36 to 300 of SEQ ID NO:12.

5. The isolated nucleic acid molecule of claim 4, comprising the nucleotide sequence of nucleotides 192 to 1024 of SEQ ID NO:1.

6. The isolated nucleic acid molecule of claim 4, comprising the nucleotide sequence of nucleotides 192 to 982 of SEQ ID NO:8.

7. The isolated nucleic acid molecule of claim 4, comprising the nucleotide sequence of nucleotides 206 to 1000 of SEQ ID NO:11.

8. A vector, comprising the isolated nucleic acid molecule of claim 4.

9. An expression vector, comprising a nucleic acid molecule that encodes amino acid residues 36 to 313 of SEQ ID NO:2, a transcription promoter, and a transcription terminator, wherein the promoter is operably linked with the nucleic acid molecule, and wherein the nucleic acid molecule is operably linked with the transcription terminator.

10. A recombinant host cell comprising the expression vector of claim 9, wherein the host cell is selected from the group consisting of bacterium, avian cell, yeast cell, fungal cell, insect cell, mammalian cell, and plant cell.

11. A method of using the expression vector of claim 9 to produce a polypeptide that comprises amino acid residues 36 to 313 of SEQ ID NO:2, comprising culturing recombinant host cells that comprise the expression vector and that produce the polypeptide.

12. The method of claim 11, further comprising isolating the polypeptide from the cultured recombinant host cells.

13. An antibody or antibody fragment that specifically binds with a polypeptide that has an amino acid sequence consisting of amino acid residues 1 to 189 of SEQ ID NO:2.

14. An anti-idiotype antibody that specifically binds with the antibody of claim 13.

15. A fusion protein, comprising the polypeptide of claim 1.

16. The fusion protein of claim 15, wherein the fusion protein further comprises an immunoglobulin moiety.

17. A composition, comprising the polypeptide of claim 1 and a carrier.

SEQUENCE LISTING

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Kuestner, Rolf E.
Gao, Zeren

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     75                  80                  85

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Ala Asp Ala Gln Asn Ile Thr Ile Ser Gln Tyr Ala Cys His Asp Gln
 90                  95                 100                 105

gtg gca gtc acc att ctt tgg tcc cca ggg gcc ctc ggc atc gaa ttc      448
Val Ala Val Thr Ile Leu Trp Ser Pro Gly Ala Leu Gly Ile Glu Phe
                110                 115                 120

ctg aaa gga ttt cgg gta ata ctg gag gag ctg aag tcg gag gga aga      496
Leu Lys Gly Phe Arg Val Ile Leu Glu Glu Leu Lys Ser Glu Gly Arg
            125                 130                 135

cag tgc caa caa ctg att cta aag gat ccg aag cag ctc aac agt agc      544
Gln Cys Gln Gln Leu Ile Leu Lys Asp Pro Lys Gln Leu Asn Ser Ser
        140                 145                 150

ttc aaa aga act gga atg gaa tct caa cct ttc ctg aat atg aaa ttt      592
Phe Lys Arg Thr Gly Met Glu Ser Gln Pro Phe Leu Asn Met Lys Phe
    155                 160                 165

gaa acg gat tat ttc gta aag gtt gtc cct ttt cct tcc att aaa aac      640
Glu Thr Asp Tyr Phe Val Lys Val Val Pro Phe Pro Ser Ile Lys Asn
170                 175                 180                 185

gaa agc aat tac cac cct ttc ttc ttt aga acc cga gcc tgt gac ctg      688
Glu Ser Asn Tyr His Pro Phe Phe Phe Arg Thr Arg Ala Cys Asp Leu
                190                 195                 200

ttg tta cag ccg gac aat cta gct tgt aaa ccc ttc tgg aag cct cgg      736
Leu Leu Gln Pro Asp Asn Leu Ala Cys Lys Pro Phe Trp Lys Pro Arg
            205                 210                 215

aac ctg aac atc agc cag cat ggc tcg gac atg cag gtg tcc ttc gac      784
Asn Leu Asn Ile Ser Gln His Gly Ser Asp Met Gln Val Ser Phe Asp
        220                 225                 230
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```
cat gca ccg cac aac ttc ggc ttc cgt ttc ttc tat ctt cac tac aag      832
His Ala Pro His Asn Phe Gly Phe Arg Phe Phe Tyr Leu His Tyr Lys
    235                 240                 245

ctc aag cac gaa gga cct ttc aag cga aag acc tgt aag cag gag caa      880
Leu Lys His Glu Gly Pro Phe Lys Arg Lys Thr Cys Lys Gln Glu Gln
250                 255                 260                 265

act aca gag acg acc agc tgc ctc ctt caa aat gtt tct cca ggg gat      928
Thr Thr Glu Thr Thr Ser Cys Leu Leu Gln Asn Val Ser Pro Gly Asp
                270                 275                 280

tat ata att gag ctg gtg gat gac act aac aca aca aga aaa gtg atg      976
Tyr Ile Ile Glu Leu Val Asp Asp Thr Asn Thr Thr Arg Lys Val Met
            285                 290                 295

cat tat gcc tta aag cca gtg cac tcc ccg tgg gcc ggg ccc atc aga     1024
His Tyr Ala Leu Lys Pro Val His Ser Pro Trp Ala Gly Pro Ile Arg
        300                 305                 310

gcc gtg gcc atc aca gtg cca ctg gta gtc ata tcg gca ttc gcg acg     1072
Ala Val Ala Ile Thr Val Pro Leu Val Val Ile Ser Ala Phe Ala Thr
    315                 320                 325

ctc ttc act gtg atg tgc cgc aag aag caa caa gaa aat ata tat tca     1120
Leu Phe Thr Val Met Cys Arg Lys Lys Gln Gln Glu Asn Ile Tyr Ser
330                 335                 340                 345

cat tta gat gaa gag agc tct gag tct tcc aca tac act gca gca ctc     1168
His Leu Asp Glu Glu Ser Ser Glu Ser Ser Thr Tyr Thr Ala Ala Leu
                350                 355                 360

cca aga gag agg ctc cgg ccg cgg ccg aag gtc ttt ctc tgc tat tcc     1216
Pro Arg Glu Arg Leu Arg Pro Arg Pro Lys Val Phe Leu Cys Tyr Ser
            365                 370                 375

agt aaa gat ggc cag aat cac atg aat gtc gtc cag tgt ttc gcc tac     1264
Ser Lys Asp Gly Gln Asn His Met Asn Val Val Gln Cys Phe Ala Tyr
        380                 385                 390

ttc ctc cag gac ttc tgt ggc tgt gag gtg gct ctg gac ctg tgg gaa     1312
Phe Leu Gln Asp Phe Cys Gly Cys Glu Val Ala Leu Asp Leu Trp Glu
    395                 400                 405
```

```
gac ttc agc ctc tgt aga gaa ggg cag aga gaa tgg gtc atc cag aag     1360
Asp Phe Ser Leu Cys Arg Glu Gly Gln Arg Glu Trp Val Ile Gln Lys
410                 415                 420                 425

atc cac gag tcc cag ttc atc att gtg gtt tgt tcc aaa ggt atg aag     1408
Ile His Glu Ser Gln Phe Ile Ile Val Val Cys Ser Lys Gly Met Lys
                430                 435                 440

tac ttt gtg gac aag aag aac tac aaa cac aaa gga ggt ggc cga ggc     1456
Tyr Phe Val Asp Lys Lys Asn Tyr Lys His Lys Gly Gly Gly Arg Gly
            445                 450                 455

tcg ggg aaa gga gag ctc ttc ctg gtg gcg gtg tca gcc att gcc gaa     1504
Ser Gly Lys Gly Glu Leu Phe Leu Val Ala Val Ser Ala Ile Ala Glu
        460                 465                 470

aag ctc cgc cag gcc aag cag agt tcg tcc gcg gcg ctc agc aag ttt     1552
Lys Leu Arg Gln Ala Lys Gln Ser Ser Ser Ala Ala Leu Ser Lys Phe
    475                 480                 485

atc gcc gtc tac ttt gat tat tcc tgc gag gga gac gtc ccc ggt atc     1600
Ile Ala Val Tyr Phe Asp Tyr Ser Cys Glu Gly Asp Val Pro Gly Ile
490                 495                 500                 505

cta gac ctg agt acc aag tac aga ctc atg gac aat ctt cct cag ctc     1648
Leu Asp Leu Ser Thr Lys Tyr Arg Leu Met Asp Asn Leu Pro Gln Leu
                510                 515                 520

tgt tcc cac ttg cac tcc cga gac cac ggc ctc cag gag ccg ggg cag     1696
Cys Ser His Leu His Ser Arg Asp His Gly Leu Gln Glu Pro Gly Gln
            525                 530                 535

cac acg cga cag ggc agc aga agg aac tac ttc cgg agc aag tca ggc     1744
His Thr Arg Gln Gly Ser Arg Arg Asn Tyr Phe Arg Ser Lys Ser Gly
        540                 545                 550

cgg tcc cta tac gtc gcc att tgc aac atg cac cag ttt att gac gag     1792
Arg Ser Leu Tyr Val Ala Ile Cys Asn Met His Gln Phe Ile Asp Glu
    555                 560                 565

gag ccc gac tgg ttc gaa aag cag ttc gtt ccc ttc cat cct cct cca     1840
```

```
Glu Pro Asp Trp Phe Glu Lys Gln Phe Val Pro Phe His Pro Pro Pro
570                 575                 580                 585

ctg cgc tac cgg gag cca gtc ttg gag aaa ttt gat tcg ggc ttg gtt     1888
Leu Arg Tyr Arg Glu Pro Val Leu Glu Lys Phe Asp Ser Gly Leu Val
                590                 595                 600

tta aat gat gtc atg tgc aaa cca ggg cct gag agt gac ttc tgc cta     1936
Leu Asn Asp Val Met Cys Lys Pro Gly Pro Glu Ser Asp Phe Cys Leu
            605                 610                 615

aag gta gag gcg gct gtt ctt ggg gca acc gga cca gcc gac tcc cag     1984
Lys Val Glu Ala Ala Val Leu Gly Ala Thr Gly Pro Ala Asp Ser Gln
        620                 625                 630

cac gag agt cag cat ggg ggc ctg gac caa gac ggg gag gcc cgg cct     2032
His Glu Ser Gln His Gly Gly Leu Asp Gln Asp Gly Glu Ala Arg Pro
    635                 640                 645

gcc ctt gac ggt agc gcc gcc ctg caa ccc ctg ctg cac acg gtg aaa     2080
Ala Leu Asp Gly Ser Ala Ala Leu Gln Pro Leu Leu His Thr Val Lys
650                 655                 660                 665

gcc ggc agc ccc tcg gac atg ccg cgg gac tca ggc atc tat gac tcg     2128
Ala Gly Ser Pro Ser Asp Met Pro Arg Asp Ser Gly Ile Tyr Asp Ser
                670                 675                 680

tct gtg ccc tca tcc gag ctg tct ctg cca ctg atg gaa gga ctc tcg     2176
Ser Val Pro Ser Ser Glu Leu Ser Leu Pro Leu Met Glu Gly Leu Ser
            685                 690                 695

acg gac cag aca gaa acg tct tcc ctg acg gag agc gtg tcc tcc tct     2224
Thr Asp Gln Thr Glu Thr Ser Ser Leu Thr Glu Ser Val Ser Ser Ser
        700                 705                 710

tca ggc ctg ggt gag gag gaa cct cct gcc ctt cct tcc aag ctc ctc     2272
Ser Gly Leu Gly Glu Glu Glu Pro Pro Ala Leu Pro Ser Lys Leu Leu
    715                 720                 725

tct tct ggg tca tgc aaa gca gat ctt ggt tgc cgc agc tac act gat     2320
Ser Ser Gly Ser Cys Lys Ala Asp Leu Gly Cys Arg Ser Tyr Thr Asp
730                 735                 740                 745
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gaa ctc cac gcg gtc gcc cct ttg taacaaaacg aaagagtcta agcattgcca 2374
Glu Leu His Ala Val Ala Pro Leu
750

ctttagctg 2383

<210> 2
<211> 753
<212> PRT
<213> Homo sapiens

<400> 2

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

```
Phe Arg Phe Phe Tyr Leu His Tyr Lys Leu Lys His Glu Gly Pro Phe
                245                 250                 255
Lys Arg Lys Thr Cys Lys Gln Glu Gln Thr Thr Glu Thr Thr Ser Cys
            260                 265                 270
Leu Leu Gln Asn Val Ser Pro Gly Asp Tyr Ile Ile Glu Leu Val Asp
        275                 280                 285
Asp Thr Asn Thr Thr Arg Lys Val Met His Tyr Ala Leu Lys Pro Val
    290                 295                 300
His Ser Pro Trp Ala Gly Pro Ile Arg Ala Val Ala Ile Thr Val Pro
305                 310                 315                 320
Leu Val Val Ile Ser Ala Phe Ala Thr Leu Phe Thr Val Met Cys Arg
                325                 330                 335
Lys Lys Gln Gln Glu Asn Ile Tyr Ser His Leu Asp Glu Glu Ser Ser
            340                 345                 350
Glu Ser Ser Thr Tyr Thr Ala Ala Leu Pro Arg Glu Arg Leu Arg Pro
        355                 360                 365
Arg Pro Lys Val Phe Leu Cys Tyr Ser Ser Lys Asp Gly Gln Asn His
    370                 375                 380
Met Asn Val Val Gln Cys Phe Ala Tyr Phe Leu Gln Asp Phe Cys Gly
385                 390                 395                 400
Cys Glu Val Ala Leu Asp Leu Trp Glu Asp Phe Ser Leu Cys Arg Glu
                405                 410                 415
Gly Gln Arg Glu Trp Val Ile Gln Lys Ile His Glu Ser Gln Phe Ile
            420                 425                 430
Ile Val Val Cys Ser Lys Gly Met Lys Tyr Phe Val Asp Lys Lys Asn
        435                 440                 445
Tyr Lys His Lys Gly Gly Gly Arg Gly Ser Gly Lys Gly Glu Leu Phe
    450                 455                 460
Leu Val Ala Val Ser Ala Ile Ala Glu Lys Leu Arg Gln Ala Lys Gln
465                 470                 475                 480
Ser Ser Ser Ala Ala Leu Ser Lys Phe Ile Ala Val Tyr Phe Asp Tyr
                485                 490                 495
Ser Cys Glu Gly Asp Val Pro Gly Ile Leu Asp Leu Ser Thr Lys Tyr
            500                 505                 510
Arg Leu Met Asp Asn Leu Pro Gln Leu Cys Ser His Leu His Ser Arg
        515                 520                 525
Asp His Gly Leu Gln Glu Pro Gly Gln His Thr Arg Gln Gly Ser Arg
    530                 535                 540
Arg Asn Tyr Phe Arg Ser Lys Ser Gly Arg Ser Leu Tyr Val Ala Ile
545                 550                 555                 560
Cys Asn Met His Gln Phe Ile Asp Glu Glu Pro Asp Trp Phe Glu Lys
                565                 570                 575
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```
Gln Phe Val Pro Phe His Pro Pro Pro Leu Arg Tyr Arg Glu Pro Val
            580                 585                 590
Leu Glu Lys Phe Asp Ser Gly Leu Val Leu Asn Asp Val Met Cys Lys
        595                 600                 605
Pro Gly Pro Glu Ser Asp Phe Cys Leu Lys Val Glu Ala Ala Val Leu
    610                 615                 620
Gly Ala Thr Gly Pro Ala Asp Ser Gln His Glu Ser Gln His Gly Gly
625                 630                 635                 640
Leu Asp Gln Asp Gly Glu Ala Arg Pro Ala Leu Asp Gly Ser Ala Ala
                645                 650                 655
Leu Gln Pro Leu Leu His Thr Val Lys Ala Gly Ser Pro Ser Asp Met
            660                 665                 670
Pro Arg Asp Ser Gly Ile Tyr Asp Ser Ser Val Pro Ser Ser Glu Leu
        675                 680                 685
Ser Leu Pro Leu Met Glu Gly Leu Ser Thr Asp Gln Thr Glu Thr Ser
    690                 695                 700
Ser Leu Thr Glu Ser Val Ser Ser Ser Ser Gly Leu Gly Glu Glu Glu
705                 710                 715                 720
Pro Pro Ala Leu Pro Ser Lys Leu Leu Ser Ser Gly Ser Cys Lys Ala
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Asp Leu Gly Cys Arg Ser Tyr Thr Asp Glu Leu His Ala Val Ala Pro
            740                 745                 750
Leu
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<210> 3
<211> 2259
<212> DNA
<213> Artificial Sequence

<220>
<223> This degenerate nucleotide sequence encodes the amino acid sequence of SEQ ID NO:2.

<221> misc_feature
<222> (1)...(2259)
<223> n = A,T,C or G

<400> 3

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tggmgnatga argcngcngc nmgnccnmgn ytntgygtng cnaaygargg ngtnggnccn     180
gcnwsnmgna aywsnggnyt ntayaayath acnttyaart aygayaaytg yacnacntay     240
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ytnaayccng tnggnaarca ygtnathgcn gaygcncara ayathacnat hwsncartay 300
gcntgycayg aycargtngc ngtnacnath ytntggwsnc cnggngcnyt nggnathgar 360
ttyytnaarg gnttymgngt nathytngar garytnaarw sngarggnmg ncartgycar 420
carytnathy tnaargaycc naarcarytn aaywsnwsnt tyaarmgnac nggnatggar 480
wsncarccnt tyytnaayat gaarttygar acngaytayt tygtnaargt ngtnccntty 540
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ytnytnytnc arccngayaa yytngcntgy aarccnttyt ggaarccnmg naayytnaay 660
athwsncarc ayggnwsnga yatgcargtn wsnttygayc aygcnccnca yaayttyggn 720
ttymgnttyt tytayytnca ytayaarytn aarcaygarg gnccnttyaa rmgnaaracn 780
tgyaarcarg arcaracnac ngaracnacn wsntgyytny tncaraaygt nwsnccnggn 840
gaytayatha thgarytngt ngaygayacn aayacnacnm gnaargtnat gcaytaygcn 900
ytnaarccng tncaywsncc ntgggcnggn ccnathmgng cngtngcnat hacngtnccn 960
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garaayatht aywsncayyt ngaygargar wsnwsngarw snwsnacnta yacngcngcn 1080
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aartayttyg tngayaaraa raaytayaar cayaarggng gnggnmgngg nwsnggnaar 1380
ggngarytnt tyytngtngc ngtnwsngcn athgcngara arytnmgnca rgcnaarcar 1440
wsnwsnwsng cngcnytnws naarttyath gcngtntayt tygaytayws ntgygarggn 1500
gaygtnccng gnathytnga yytnwsnacn aartaymgny tnatggayaa yytnccncar 1560
ytntgywsnc ayytncayws nmgngaycay ggnytncarg arccnggnca rcayacnmgn 1620
carggnwsnm gnmgnaayta yttymgnwsn aarwsnggnm gnwsnytnta ygtngcnath 1680
tgyaayatgc aycarttyat hgaygargar ccngaytggt tygaraarca rttygtnccn 1740
ttycayccnc cnccnytnmg ntaymgngar ccngtnytng araarttyga ywsnggnytn 1800
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gcngcngtny tnggngcnac nggnccngcn gaywsncarc aygarwsnca rcayggnggn 1920
ytngaycarg ayggngargc nmgnccngcn ytngayggnw sngcngcnyt ncarccnytn 1980
ytncayacng tnaargcngg nwsnccnwsn gayatgccnm gngaywsngg nathtaygay 2040
wsnwsngtnc cnwsnwsnga rytnwsnytn ccnytnatgg arggnytnws nacngaycar 2100
acngaracnw snwsnytnac ngarwsngtn wsnwsnwsnw snggnytngg ngargargar 2160
ccnccngcny tnccnwsnaa rytnytnwsn wsnggnwsnt gyaargcnga yytnggntgy 2220
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<212> DNA
<213> Homo sapiens

<220>
<221> CDS
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<400> 4

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                             Met Ala Pro Trp Leu Gln Leu Cys Ser
                              1               5

gtc ttc ttt acg gtc aac gcc tgc ctc aac ggc tcg cag ctg gct gtg        160
Val Phe Phe Thr Val Asn Ala Cys Leu Asn Gly Ser Gln Leu Ala Val
 10                  15                  20                  25

gcc gct ggc ggg tcc ggc cgc gcg cgg ggc gcc gac acc tgt ggc tgg        208
Ala Ala Gly Gly Ser Gly Arg Ala Arg Gly Ala Asp Thr Cys Gly Trp
                 30                  35                  40

agg atg aaa gcg gct gcc cga ccc cgg ctt tgt gtt gct aat gag gga        256
Arg Met Lys Ala Ala Ala Arg Pro Arg Leu Cys Val Ala Asn Glu Gly
             45                  50                  55

gtg ggg cca gcc agc aga aac agt ggg ctg tac aac atc acc ttc aaa        304
Val Gly Pro Ala Ser Arg Asn Ser Gly Leu Tyr Asn Ile Thr Phe Lys
         60                  65                  70

tat gac aat tgt acc acc tac ttg aat cca gtg ggg aag cat gtg att        352
Tyr Asp Asn Cys Thr Thr Tyr Leu Asn Pro Val Gly Lys His Val Ile
     75                  80                  85

gct gac gcc cag aat atc acc atc agc cag tat gct tgc cat gac caa        400
Ala Asp Ala Gln Asn Ile Thr Ile Ser Gln Tyr Ala Cys His Asp Gln
 90                  95                 100                 105

gtg gca gtc acc att ctt tgg tcc cca ggg gcc ctc ggc atc gaa ttc        448
Val Ala Val Thr Ile Leu Trp Ser Pro Gly Ala Leu Gly Ile Glu Phe
                110                 115                 120

ctg aaa gga ttt cgg gta ata ctg gag gag ctg aag tcg gag gga aga        496
Leu Lys Gly Phe Arg Val Ile Leu Glu Glu Leu Lys Ser Glu Gly Arg
            125                 130                 135

cag tgc caa caa ctg att cta aag gat ccg aag cag ctc aac agt agc        544
Gln Cys Gln Gln Leu Ile Leu Lys Asp Pro Lys Gln Leu Asn Ser Ser
        140                 145                 150
```

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ttc aaa aga act gga atg gaa tct caa cct ttc ctg aat atg aaa ttt      592
Phe Lys Arg Thr Gly Met Glu Ser Gln Pro Phe Leu Asn Met Lys Phe
    155                 160                 165

gaa acg gat tat ttc gta aag gtt gtc cct ttt cct tcc att aaa aac      640
Glu Thr Asp Tyr Phe Val Lys Val Val Pro Phe Pro Ser Ile Lys Asn
170                 175                 180                 185

gaa agc aat tac cac cct ttc ttc ttt aga acc cga gcc tgt gac ctg      688
Glu Ser Asn Tyr His Pro Phe Phe Phe Arg Thr Arg Ala Cys Asp Leu
                190                 195                 200

ttg tta cag ccg gac aat cta gct tgt aaa ccc ttc tgg aag cct cgg      736
Leu Leu Gln Pro Asp Asn Leu Ala Cys Lys Pro Phe Trp Lys Pro Arg
            205                 210                 215

aac ctg aac atc agc cag cat ggc tcg gac atg cag gtg tcc ttc gac      784
Asn Leu Asn Ile Ser Gln His Gly Ser Asp Met Gln Val Ser Phe Asp
        220                 225                 230

cac gca ccg cac aac ttc ggc ttc cgt ttc ttc tat ctt cac tac aag      832
His Ala Pro His Asn Phe Gly Phe Arg Phe Phe Tyr Leu His Tyr Lys
    235                 240                 245

ctc aag cac gaa gga cct ttc aag cga aag acc tgt aag cag gag caa      880
Leu Lys His Glu Gly Pro Phe Lys Arg Lys Thr Cys Lys Gln Glu Gln
250                 255                 260                 265

act aca gag atg acc agc tgc ctc ctt caa aat gtt tct cca ggg gat      928
Thr Thr Glu Met Thr Ser Cys Leu Leu Gln Asn Val Ser Pro Gly Asp
                270                 275                 280

tat ata att gag ctg gtg gat gac act aac aca aca aga aaa gtg atg      976
Tyr Ile Ile Glu Leu Val Asp Asp Thr Asn Thr Thr Arg Lys Val Met
            285                 290                 295

cat tat gcc tta aag cca gtg cac tcc ccg tgg gcc ggg ccc atc aga     1024
His Tyr Ala Leu Lys Pro Val His Ser Pro Trp Ala Gly Pro Ile Arg
        300                 305                 310

gcc gtg gcc atc aca gtg cca ctg gta gtc ata tcg gca ttc gcg acg     1072
Ala Val Ala Ile Thr Val Pro Leu Val Val Ile Ser Ala Phe Ala Thr
    315                 320                 325
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ctc ttc act gtg atg tgc cgc aag aag caa caa gaa aat ata tat tca     1120
Leu Phe Thr Val Met Cys Arg Lys Lys Gln Gln Glu Asn Ile Tyr Ser
330                 335                 340                 345

cat tta gat gaa gag agc tct gag tct tcc aca tac act gca gca ctc     1168
His Leu Asp Glu Glu Ser Ser Glu Ser Ser Thr Tyr Thr Ala Ala Leu
                350                 355                 360

cca aga gag agg ctc cgg ccg cgg ccg aag gtc ttt ctc tgc tat tcc     1216
Pro Arg Glu Arg Leu Arg Pro Arg Pro Lys Val Phe Leu Cys Tyr Ser
            365                 370                 375

agt aaa gat ggc cag aat cac atg aat gtc gtc cag tgt ttc gcc tac     1264
Ser Lys Asp Gly Gln Asn His Met Asn Val Val Gln Cys Phe Ala Tyr
        380                 385                 390

ttc ctc cag gac ttc tgt ggc tgt gag gtg gct ctg gac ctg tgg gaa     1312
Phe Leu Gln Asp Phe Cys Gly Cys Glu Val Ala Leu Asp Leu Trp Glu
    395                 400                 405

gac ttc agc ctc tgt aga gaa ggg cag aga gaa tgg gtc atc cag aag     1360
Asp Phe Ser Leu Cys Arg Glu Gly Gln Arg Glu Trp Val Ile Gln Lys
410                 415                 420                 425

atc cac gag tcc cag ttc atc att gtg gtt tgt tcc aaa ggt atg aag     1408
Ile His Glu Ser Gln Phe Ile Ile Val Val Cys Ser Lys Gly Met Lys
                430                 435                 440

tac ttt gtg gac aag aag aac tac aaa cac aaa gga ggt ggc cga ggc     1456
Tyr Phe Val Asp Lys Lys Asn Tyr Lys His Lys Gly Gly Gly Arg Gly
            445                 450                 455

tcg ggg aaa gga gag ctc ttc ctg gtg gcg gtg tca gcc att gcc gaa     1504
Ser Gly Lys Gly Glu Leu Phe Leu Val Ala Val Ser Ala Ile Ala Glu
        460                 465                 470

aag ctc cgc cag gcc aag cag agt tcg tcc gcg gcg ctc agc aag ttt     1552
Lys Leu Arg Gln Ala Lys Gln Ser Ser Ser Ala Ala Leu Ser Lys Phe
    475                 480                 485

atc gcc gtc tac ttt gat tat tcc tgc gag gga gac gtc ccc ggt atc     1600
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Ile Ala Val Tyr Phe Asp Tyr Ser Cys Glu Gly Asp Val Pro Gly Ile
490                 495                 500                 505

cta gac ctg agt acc aag tac aga ctc atg gac aat ctt cct cag ctc     1648
Leu Asp Leu Ser Thr Lys Tyr Arg Leu Met Asp Asn Leu Pro Gln Leu
                510                 515                 520

tgt tcc cac ctg cac tcc cga gac cac ggc ctc cag gag ccg ggg cag     1696
Cys Ser His Leu His Ser Arg Asp His Gly Leu Gln Glu Pro Gly Gln
            525                 530                 535

cac acg cga cag ggc agc aga agg aac tac ttc cgg agc aag tca ggc     1744
His Thr Arg Gln Gly Ser Arg Arg Asn Tyr Phe Arg Ser Lys Ser Gly
        540                 545                 550

cgg tcc cta tac gtc gcc att tgc aac atg cac cag ttt att gac gag     1792
Arg Ser Leu Tyr Val Ala Ile Cys Asn Met His Gln Phe Ile Asp Glu
    555                 560                 565

gag ccc gac tgg ttc gaa aag cag ttc gtt ccc ttc cat cct cct cca     1840
Glu Pro Asp Trp Phe Glu Lys Gln Phe Val Pro Phe His Pro Pro Pro
570                 575                 580                 585

ctg cgc tac cgg gag cca gtc ttg gag aaa ttt gat tcg ggc ttg gtt     1888
Leu Arg Tyr Arg Glu Pro Val Leu Glu Lys Phe Asp Ser Gly Leu Val
                590                 595                 600

tta aat gat gtc atg tgc aaa cca ggg cct gag agt gac ttc tgc cta     1936
Leu Asn Asp Val Met Cys Lys Pro Gly Pro Glu Ser Asp Phe Cys Leu
            605                 610                 615

aag gta gag gcg gct gtt ctt ggg gca acc gga cca gcc gac tcc cag     1984
Lys Val Glu Ala Ala Val Leu Gly Ala Thr Gly Pro Ala Asp Ser Gln
        620                 625                 630

cac gag agt cag cat ggg ggc ctg gac caa gac ggg gag gcc cgg cct     2032
His Glu Ser Gln His Gly Gly Leu Asp Gln Asp Gly Glu Ala Arg Pro
    635                 640                 645

gcc ctt gac ggt agc gcc gcc ctg caa ccc ctg ctg cac acg gtg aaa     2080
Ala Leu Asp Gly Ser Ala Ala Leu Gln Pro Leu Leu His Thr Val Lys
650                 655                 660                 665
```

```
gcc ggc agc ccc tcg gac atg ccg cgg gac tca ggc atc tat gac tcg     2128
Ala Gly Ser Pro Ser Asp Met Pro Arg Asp Ser Gly Ile Tyr Asp Ser
                670                 675                 680

tct gtg ccc tca tcc gag ctg tct ctg cca ctg atg gaa gga ctc tcg     2176
Ser Val Pro Ser Ser Glu Leu Ser Leu Pro Leu Met Glu Gly Leu Ser
            685                 690                 695

acg gac cag aca gaa acg tct tcc ctg acg gag agc gtg tcc tcc tct     2224
Thr Asp Gln Thr Glu Thr Ser Ser Leu Thr Glu Ser Val Ser Ser Ser
        700                 705                 710

tca ggc ctg ggt gag gag gaa cct cct gcc ctt cct tcc aag ctc ctc     2272
Ser Gly Leu Gly Glu Glu Glu Pro Pro Ala Leu Pro Ser Lys Leu Leu
    715                 720                 725

tct tct ggg tca tgc aaa gca gat ctt ggt tgc cgc agc tac act gat     2320
Ser Ser Gly Ser Cys Lys Ala Asp Leu Gly Cys Arg Ser Tyr Thr Asp
730                 735                 740                 745

gaa ctc cac gcg gcc gcc cct ttg taacaaaacg aaagagtcta agcattgcca     2374
Glu Leu His Ala Ala Ala Pro Leu
                750

ctttagctg                                                           2383
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<210> 5
<211> 753
<212> PRT
<213> Homo sapiens

<400> 5

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Met Ala Pro Trp Leu Gln Leu Cys Ser Val Phe Phe Thr Val Asn Ala
 1               5                  10                  15
Cys Leu Asn Gly Ser Gln Leu Ala Val Ala Ala Gly Gly Ser Gly Arg
            20                  25                  30
Ala Arg Gly Ala Asp Thr Cys Gly Trp Arg Met Lys Ala Ala Ala Arg
        35                  40                  45
Pro Arg Leu Cys Val Ala Asn Glu Gly Val Gly Pro Ala Ser Arg Asn
    50                  55                  60
Ser Gly Leu Tyr Asn Ile Thr Phe Lys Tyr Asp Asn Cys Thr Thr Tyr
65                  70                  75                  80
```

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

Gly Gln Arg Glu Trp Val Ile Gln Lys Ile His Glu Ser Gln Phe Ile
420 425 430
Ile Val Val Cys Ser Lys Gly Met Lys Tyr Phe Val Asp Lys Lys Asn
435 440 445
Tyr Lys His Lys Gly Gly Gly Arg Gly Ser Gly Lys Gly Glu Leu Phe
450 455 460
Leu Val Ala Val Ser Ala Ile Ala Glu Lys Leu Arg Gln Ala Lys Gln
465 470 475 480
Ser Ser Ser Ala Ala Leu Ser Lys Phe Ile Ala Val Tyr Phe Asp Tyr
485 490 495
Ser Cys Glu Gly Asp Val Pro Gly Ile Leu Asp Leu Ser Thr Lys Tyr
500 505 510
Arg Leu Met Asp Asn Leu Pro Gln Leu Cys Ser His Leu His Ser Arg
515 520 525
Asp His Gly Leu Gln Glu Pro Gly Gln His Thr Arg Gln Gly Ser Arg
530 535 540
Arg Asn Tyr Phe Arg Ser Lys Ser Gly Arg Ser Leu Tyr Val Ala Ile
545 550 555 560
Cys Asn Met His Gln Phe Ile Asp Glu Glu Pro Asp Trp Phe Glu Lys
565 570 575
Gln Phe Val Pro Phe His Pro Pro Pro Leu Arg Tyr Arg Glu Pro Val
580 585 590
Leu Glu Lys Phe Asp Ser Gly Leu Val Leu Asn Asp Val Met Cys Lys
595 600 605
Pro Gly Pro Glu Ser Asp Phe Cys Leu Lys Val Glu Ala Ala Val Leu
610 615 620
Gly Ala Thr Gly Pro Ala Asp Ser Gln His Glu Ser Gln His Gly Gly
625 630 635 640
Leu Asp Gln Asp Gly Glu Ala Arg Pro Ala Leu Asp Gly Ser Ala Ala
645 650 655
Leu Gln Pro Leu Leu His Thr Val Lys Ala Gly Ser Pro Ser Asp Met
660 665 670
Pro Arg Asp Ser Gly Ile Tyr Asp Ser Ser Val Pro Ser Ser Glu Leu
675 680 685
Ser Leu Pro Leu Met Glu Gly Leu Ser Thr Asp Gln Thr Glu Thr Ser
690 695 700
Ser Leu Thr Glu Ser Val Ser Ser Ser Ser Gly Leu Gly Glu Glu Glu
705 710 715 720
Pro Pro Ala Leu Pro Ser Lys Leu Leu Ser Ser Gly Ser Cys Lys Ala
725 730 735
Asp Leu Gly Cys Arg Ser Tyr Thr Asp Glu Leu His Ala Ala Ala Pro
740 745 750

Leu

<210> 6
<211> 2259
<212> DNA
<213> Artificial Sequence

<220>
<223> This degenerate nucleotide sequence encodes the amino acid sequence of SEQ ID NO:5.

<221> misc_feature
<222> (1)...(2259)
<223> n = A,T,C or G

<400> 6

atggcnccnt ggytncaryt ntgywsngtn ttyttyacng tnaaygcntg yytnaayggn 60
wsncarytng cngtngcngc nggnggnwsn ggnmgngcnm gnggngcnga yacntgyggn 120
tggmgnatga argcngcngc nmgnccnmgn ytntgygtng cnaaygargg ngtnggnccn 180
gcnwsnmgna aywsnggnyt ntayaayath acnttyaart aygayaaytg yacnacntay 240
ytnaayccng tnggnaarca ygtnathgcn gaygcncara ayathacnat hwsncartay 300
gcntgycayg aycargtngc ngtnacnath ytntggwsnc cnggngcnyt nggnathgar 360
ttyytnaarg gnttymgngt nathytngar garytnaarw sngarggnmg ncartgycar 420
carytnathy tnaargaycc naarcarytn aaywsnwsnt tyaarmgnac nggnatggar 480
wsncarccnt tyytnaayat gaarttygar acngaytayt tygtnaargt ngtnccntty 540
ccnwsnatha araaygarws naaytaycay ccnttyttyt tymgnacnmg ngcntgygay 600
ytnytnytnc arccngayaa yytngcntgy aarccnttyt ggaarccnmg naayytnaay 660
athwsncarc ayggnwsnga yatgcargtn wsnttygayc aygcnccnca yaayttyggn 720
ttymgnttyt tytayytnca ytayaarytn aarcaygarg gnccnttyaa rmgnaaracn 780
tgyaarcarg arcaracnac ngaratgacn wsntgyytny tncaraaygt nwsnccnggn 840
gaytayatha thgarytngt ngaygayacn aayacnacnm gnaargtnat gcaytaygcn 900
ytnaarccng tncaywsncc ntgggcnggn ccnathmgng cngtngcnat hacngtnccn 960
ytngtngtna thwsngcntt ygcnacnytn ttyacngtna tgtgymgnaa raarcarcar 1020
garaayatht aywsncayyt ngaygargar wsnwsngarw snwsnacnta yacngcngcn 1080
ytnccnmgng armgnytnmg nccnmgnccn aargtnttyy tntgytayws nwsnaargay 1140
ggncaraayc ayatgaaygt ngtncartgy ttygcntayt tyytncarga yttytgyggn 1200
tgygargtng cnytngayyt ntgggargay ttywsnytnt gymgngargg ncarmgngar 1260
tgggtnathc araarathca ygarwsncar ttyathathg tngtntgyws naarggnatg 1320
aartayttyg tngayaaraa raaytayaar cayaarggng gnggnmgngg nwsnggnaar 1380
ggngarytnt tyytngtngc ngtnwsngcn athgcngara arytnmgnca rgcnaarcar 1440
wsnwsnwsng cngcnytnws naarttyath gcngtntayt tygaytayws ntgygarggn 1500
gaygtnccng gnathytnga yytnwsnacn aartaymgny tnatggayaa yytnccncar 1560
ytntgywsnc ayytncayws nmgngaycay ggnytncarg arccnggnca rcayacnmgn 1620

```
carggnwsnm gnmgnaayta yttymgnwsn aarwsnggnm gnwsnytnta ygtngcnath    1680
tgyaayatgc aycarttyat hgaygargar ccngaytggt tygaraarca rttygtnccn    1740
ttycayccnc cnccnytnmg ntaymgngar ccngtnytng araarttyga ywsnggnytn    1800
gtnytnaayg aygtnatgtg yaarccnggn ccngarwsng ayttytgyyt naargtngar    1860
gcngcngtny tnggngcnac nggnccngcn gaywsncarc aygarwsnca rcayggnggn    1920
ytngaycarg ayggngargc nmgnccngcn ytngayggnw sngcngcnyt ncarccnytn    1980
ytncayacng tnaargcngg nwsnccnwsn gayatgccnm gngaywsngg nathtaygay    2040
wsnwsngtnc cnwsnwsnga rytnwsnytn ccnytnatgg arggnytnws nacngaycar    2100
acngaracnw snwsnytnac ngarwsngtn wsnwsnwsnw snggnytngg ngargargar    2160
ccnccngcny tnccnwsnaa rytnytnwsn wsnggnwsnt gyaargcnga yytnggntgy    2220
mgnwsntaya cngaygaryt ncaygcngcn gcnccnytn                           2259
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<210> 7
<211> 2341
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (86)...(2302)

<400> 7

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ggcctggctg ggcgagcgca cggcc atg gcc ccg tgg ctg cag ctc tgc tcc      112
                           Met Ala Pro Trp Leu Gln Leu Cys Ser
                            1               5

gtc ttc ttt acg gtc aac gcc tgc ctc aac ggc tcg cag ctg gct gtg      160
Val Phe Phe Thr Val Asn Ala Cys Leu Asn Gly Ser Gln Leu Ala Val
 10                  15                  20                  25

gcc gct ggc ggg tcc ggc cgc gcg cgg ggc gcc gac acc tgt ggc tgg      208
Ala Ala Gly Gly Ser Gly Arg Ala Arg Gly Ala Asp Thr Cys Gly Trp
                 30                  35                  40

agg gga gtg ggg cca gcc agc aga aac agt ggg ctg tac aac atc acc      256
Arg Gly Val Gly Pro Ala Ser Arg Asn Ser Gly Leu Tyr Asn Ile Thr
             45                  50                  55

ttc aaa tat gac aat tgt acc acc tac ttg aat cca gtg ggg aag cat      304
Phe Lys Tyr Asp Asn Cys Thr Thr Tyr Leu Asn Pro Val Gly Lys His
         60                  65                  70
```

```
gtg att gct gac gcc cag aat atc acc atc agc cag tat gct tgc cat      352
Val Ile Ala Asp Ala Gln Asn Ile Thr Ile Ser Gln Tyr Ala Cys His
     75                  80                  85

gac caa gtg gca gtc acc att ctt tgg tcc cca ggg gcc ctc ggc atc      400
Asp Gln Val Ala Val Thr Ile Leu Trp Ser Pro Gly Ala Leu Gly Ile
 90                  95                 100                 105

gaa ttc ctg aaa gga ttt cgg gta ata ctg gag gag ctg aag tcg gag      448
Glu Phe Leu Lys Gly Phe Arg Val Ile Leu Glu Glu Leu Lys Ser Glu
                110                 115                 120

gga aga cag tgc caa caa ctg att cta aag gat ccg aag cag ctc aac      496
Gly Arg Gln Cys Gln Gln Leu Ile Leu Lys Asp Pro Lys Gln Leu Asn
            125                 130                 135

agt agc ttc aaa aga act gga atg gaa tct caa cct ttc ctg aat atg      544
Ser Ser Phe Lys Arg Thr Gly Met Glu Ser Gln Pro Phe Leu Asn Met
        140                 145                 150

aaa ttt gaa acg gat tat ttc gta aag gtt gtc cct ttt cct tcc att      592
Lys Phe Glu Thr Asp Tyr Phe Val Lys Val Val Pro Phe Pro Ser Ile
    155                 160                 165

aaa aac gaa agc aat tac cac cct ttc ttc ttt aga acc cga gcc tgt      640
Lys Asn Glu Ser Asn Tyr His Pro Phe Phe Phe Arg Thr Arg Ala Cys
170                 175                 180                 185

gac ctg ttg tta cag ccg gac aat cta gct tgt aaa ccc ttc tgg aag      688
Asp Leu Leu Leu Gln Pro Asp Asn Leu Ala Cys Lys Pro Phe Trp Lys
                190                 195                 200

cct cgg aac ctg aac atc agc cag cat ggc tcg gac atg cag gtg tcc      736
Pro Arg Asn Leu Asn Ile Ser Gln His Gly Ser Asp Met Gln Val Ser
            205                 210                 215

ttc gac cat gca ccg cac aac ttc ggc ttc cgt ttc ttc tat ctt cac      784
Phe Asp His Ala Pro His Asn Phe Gly Phe Arg Phe Phe Tyr Leu His
        220                 225                 230

tac aag ctc aag cac gaa gga cct ttc aag cga aag acc tgt aag cag      832
Tyr Lys Leu Lys His Glu Gly Pro Phe Lys Arg Lys Thr Cys Lys Gln
    235                 240                 245
```

```
gag caa act aca gag acg acc agc tgc ctc ctt caa aat gtt tct cca      880
Glu Gln Thr Thr Glu Thr Thr Ser Cys Leu Leu Gln Asn Val Ser Pro
250                 255                 260                 265

ggg gat tat ata att gag ctg gtg gat gac act aac aca aca aga aaa      928
Gly Asp Tyr Ile Ile Glu Leu Val Asp Asp Thr Asn Thr Thr Arg Lys
                270                 275                 280

gtg atg cat tat gcc tta aag cca gtg cac tcc ccg tgg gcc ggg ccc      976
Val Met His Tyr Ala Leu Lys Pro Val His Ser Pro Trp Ala Gly Pro
            285                 290                 295

atc aga gcc gtg gcc atc aca gtg cca ctg gta gtc ata tcg gca ttc     1024
Ile Arg Ala Val Ala Ile Thr Val Pro Leu Val Val Ile Ser Ala Phe
        300                 305                 310

gcg acg ctc ttc act gtg atg tgc cgc aag aag caa caa gaa aat ata     1072
Ala Thr Leu Phe Thr Val Met Cys Arg Lys Lys Gln Gln Glu Asn Ile
    315                 320                 325

tat tca cat tta gat gaa gag agc tct gag tct tcc aca tac act gca     1120
Tyr Ser His Leu Asp Glu Glu Ser Ser Glu Ser Ser Thr Tyr Thr Ala
330                 335                 340                 345

gca ctc cca aga gag agg ctc cgg ccg cgg ccg aag gtc ttt ctc tgc     1168
Ala Leu Pro Arg Glu Arg Leu Arg Pro Arg Pro Lys Val Phe Leu Cys
                350                 355                 360

tat tcc agt aaa gat ggc cag aat cac atg aat gtc gtc cag tgt ttc     1216
Tyr Ser Ser Lys Asp Gly Gln Asn His Met Asn Val Val Gln Cys Phe
            365                 370                 375

gcc tac ttc ctc cag gac ttc tgt ggc tgt gag gtg gct ctg gac ctg     1264
Ala Tyr Phe Leu Gln Asp Phe Cys Gly Cys Glu Val Ala Leu Asp Leu
        380                 385                 390

tgg gaa gac ttc agc ctc tgt aga gaa ggg cag aga gaa tgg gtc atc     1312
Trp Glu Asp Phe Ser Leu Cys Arg Glu Gly Gln Arg Glu Trp Val Ile
    395                 400                 405

cag aag atc cac gag tcc cag ttc atc att gtg gtt tgt tcc aaa ggt     1360
```

```
Gln Lys Ile His Glu Ser Gln Phe Ile Ile Val Val Cys Ser Lys Gly
410                 415                 420                 425

atg aag tac ttt gtg gac aag aag aac tac aaa cac aaa gga ggt ggc    1408
Met Lys Tyr Phe Val Asp Lys Lys Asn Tyr Lys His Lys Gly Gly Gly
                430                 435                 440

cga ggc tcg ggg aaa gga gag ctc ttc ctg gtg gcg gtg tca gcc att    1456
Arg Gly Ser Gly Lys Gly Glu Leu Phe Leu Val Ala Val Ser Ala Ile
            445                 450                 455

gcc gaa aag ctc cgc cag gcc aag cag agt tcg tcc gcg gcg ctc agc    1504
Ala Glu Lys Leu Arg Gln Ala Lys Gln Ser Ser Ser Ala Ala Leu Ser
        460                 465                 470

aag ttt atc gcc gtc tac ttt gat tat tcc tgc gag gga gac gtc ccc    1552
Lys Phe Ile Ala Val Tyr Phe Asp Tyr Ser Cys Glu Gly Asp Val Pro
    475                 480                 485

ggt atc cta gac ctg agt acc aag tac aga ctc atg gac aat ctt cct    1600
Gly Ile Leu Asp Leu Ser Thr Lys Tyr Arg Leu Met Asp Asn Leu Pro
490                 495                 500                 505

cag ctc tgt tcc cac ttg cac tcc cga gac cac ggc ctc cag gag ccg    1648
Gln Leu Cys Ser His Leu His Ser Arg Asp His Gly Leu Gln Glu Pro
                510                 515                 520

ggg cag cac acg cga cag ggc agc aga agg aac tac ttc cgg agc aag    1696
Gly Gln His Thr Arg Gln Gly Ser Arg Arg Asn Tyr Phe Arg Ser Lys
            525                 530                 535

tca ggc cgg tcc cta tac gtc gcc att tgc aac atg cac cag ttt att    1744
Ser Gly Arg Ser Leu Tyr Val Ala Ile Cys Asn Met His Gln Phe Ile
        540                 545                 550

gac gag gag ccc gac tgg ttc gaa aag cag ttc gtt ccc ttc cat cct    1792
Asp Glu Glu Pro Asp Trp Phe Glu Lys Gln Phe Val Pro Phe His Pro
    555                 560                 565

cct cca ctg cgc tac cgg gag cca gtc ttg gag aaa ttt gat tcg ggc    1840
Pro Pro Leu Arg Tyr Arg Glu Pro Val Leu Glu Lys Phe Asp Ser Gly
570                 575                 580                 585
```

```
ttg gtt tta aat gat gtc atg tgc aaa cca ggg cct gag agt gac ttc     1888
Leu Val Leu Asn Asp Val Met Cys Lys Pro Gly Pro Glu Ser Asp Phe
                590                 595                 600

tgc cta aag gta gag gcg gct gtt ctt ggg gca acc gga cca gcc gac     1936
Cys Leu Lys Val Glu Ala Ala Val Leu Gly Ala Thr Gly Pro Ala Asp
            605                 610                 615

tcc cag cac gag agt cag cat ggg ggc ctg gac caa gac ggg gag gcc     1984
Ser Gln His Glu Ser Gln His Gly Gly Leu Asp Gln Asp Gly Glu Ala
        620                 625                 630

cgg cct gcc ctt gac ggt agc gcc gcc ctg caa ccc ctg ctg cac acg     2032
Arg Pro Ala Leu Asp Gly Ser Ala Ala Leu Gln Pro Leu Leu His Thr
    635                 640                 645

gtg aaa gcc ggc agc ccc tcg gac atg ccg cgg gac tca ggc atc tat     2080
Val Lys Ala Gly Ser Pro Ser Asp Met Pro Arg Asp Ser Gly Ile Tyr
650                 655                 660                 665

gac tcg tct gtg ccc tca tcc gag ctg tct ctg cca ctg atg gaa gga     2128
Asp Ser Ser Val Pro Ser Ser Glu Leu Ser Leu Pro Leu Met Glu Gly
                670                 675                 680

ctc tcg acg gac cag aca gaa acg tct tcc ctg acg gag agc gtg tcc     2176
Leu Ser Thr Asp Gln Thr Glu Thr Ser Ser Leu Thr Glu Ser Val Ser
            685                 690                 695

tcc tct tca ggc ctg ggt gag gag gaa cct cct gcc ctt cct tcc aag     2224
Ser Ser Ser Gly Leu Gly Glu Glu Glu Pro Pro Ala Leu Pro Ser Lys
        700                 705                 710

ctc ctc tct tct ggg tca tgc aaa gca gat ctt ggt tgc cgc agc tac     2272
Leu Leu Ser Ser Gly Ser Cys Lys Ala Asp Leu Gly Cys Arg Ser Tyr
    715                 720                 725

act gat gaa ctc cac gcg gtc gcc cct ttg taacaaaacg aaagagtcta      2322
Thr Asp Glu Leu His Ala Val Ala Pro Leu
730                 735

agcattgcca ctttagctg                                               2341
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<210> 8
<211> 739
<212> PRT
<213> Homo sapiens

<400> 8

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Met Ala Pro Trp Leu Gln Leu Cys Ser Val Phe Phe Thr Val Asn Ala
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Cys Leu Asn Gly Ser Gln Leu Ala Val Ala Ala Gly Gly Ser Gly Arg
            20                  25                  30
Ala Arg Gly Ala Asp Thr Cys Gly Trp Arg Gly Val Gly Pro Ala Ser
        35                  40                  45
Arg Asn Ser Gly Leu Tyr Asn Ile Thr Phe Lys Tyr Asp Asn Cys Thr
    50                  55                  60
Thr Tyr Leu Asn Pro Val Gly Lys His Val Ile Ala Asp Ala Gln Asn
65                  70                  75                  80
Ile Thr Ile Ser Gln Tyr Ala Cys His Asp Gln Val Ala Val Thr Ile
                85                  90                  95
Leu Trp Ser Pro Gly Ala Leu Gly Ile Glu Phe Leu Lys Gly Phe Arg
            100                 105                 110
Val Ile Leu Glu Glu Leu Lys Ser Glu Gly Arg Gln Cys Gln Gln Leu
        115                 120                 125
Ile Leu Lys Asp Pro Lys Gln Leu Asn Ser Ser Phe Lys Arg Thr Gly
    130                 135                 140
Met Glu Ser Gln Pro Phe Leu Asn Met Lys Phe Glu Thr Asp Tyr Phe
145                 150                 155                 160
Val Lys Val Val Pro Phe Pro Ser Ile Lys Asn Glu Ser Asn Tyr His
                165                 170                 175
Pro Phe Phe Phe Arg Thr Arg Ala Cys Asp Leu Leu Leu Gln Pro Asp
            180                 185                 190
Asn Leu Ala Cys Lys Pro Phe Trp Lys Pro Arg Asn Leu Asn Ile Ser
        195                 200                 205
Gln His Gly Ser Asp Met Gln Val Ser Phe Asp His Ala Pro His Asn
    210                 215                 220
Phe Gly Phe Arg Phe Phe Tyr Leu His Tyr Lys Leu Lys His Glu Gly
225                 230                 235                 240
Pro Phe Lys Arg Lys Thr Cys Lys Gln Glu Gln Thr Thr Glu Thr Thr
                245                 250                 255
Ser Cys Leu Leu Gln Asn Val Ser Pro Gly Asp Tyr Ile Ile Glu Leu
            260                 265                 270
Val Asp Asp Thr Asn Thr Thr Arg Lys Val Met His Tyr Ala Leu Lys
        275                 280                 285
```

```
Pro Val His Ser Pro Trp Ala Gly Pro Ile Arg Ala Val Ala Ile Thr
    290                 295                 300
Val Pro Leu Val Val Ile Ser Ala Phe Ala Thr Leu Phe Thr Val Met
305                 310                 315                 320
Cys Arg Lys Lys Gln Gln Glu Asn Ile Tyr Ser His Leu Asp Glu Glu
                325                 330                 335
Ser Ser Glu Ser Ser Thr Tyr Thr Ala Ala Leu Pro Arg Glu Arg Leu
            340                 345                 350
Arg Pro Arg Pro Lys Val Phe Leu Cys Tyr Ser Ser Lys Asp Gly Gln
        355                 360                 365
Asn His Met Asn Val Val Gln Cys Phe Ala Tyr Phe Leu Gln Asp Phe
    370                 375                 380
Cys Gly Cys Glu Val Ala Leu Asp Leu Trp Glu Asp Phe Ser Leu Cys
385                 390                 395                 400
Arg Glu Gly Gln Arg Glu Trp Val Ile Gln Lys Ile His Glu Ser Gln
                405                 410                 415
Phe Ile Ile Val Val Cys Ser Lys Gly Met Lys Tyr Phe Val Asp Lys
            420                 425                 430
Lys Asn Tyr Lys His Lys Gly Gly Gly Arg Gly Ser Gly Lys Gly Glu
        435                 440                 445
Leu Phe Leu Val Ala Val Ser Ala Ile Ala Glu Lys Leu Arg Gln Ala
    450                 455                 460
Lys Gln Ser Ser Ser Ala Ala Leu Ser Lys Phe Ile Ala Val Tyr Phe
465                 470                 475                 480
Asp Tyr Ser Cys Glu Gly Asp Val Pro Gly Ile Leu Asp Leu Ser Thr
                485                 490                 495
Lys Tyr Arg Leu Met Asp Asn Leu Pro Gln Leu Cys Ser His Leu His
            500                 505                 510
Ser Arg Asp His Gly Leu Gln Glu Pro Gly Gln His Thr Arg Gln Gly
        515                 520                 525
Ser Arg Arg Asn Tyr Phe Arg Ser Lys Ser Gly Arg Ser Leu Tyr Val
    530                 535                 540
Ala Ile Cys Asn Met His Gln Phe Ile Asp Glu Glu Pro Asp Trp Phe
545                 550                 555                 560
Glu Lys Gln Phe Val Pro Phe His Pro Pro Pro Leu Arg Tyr Arg Glu
                565                 570                 575
Pro Val Leu Glu Lys Phe Asp Ser Gly Leu Val Leu Asn Asp Val Met
            580                 585                 590
Cys Lys Pro Gly Pro Glu Ser Asp Phe Cys Leu Lys Val Glu Ala Ala
        595                 600                 605
Val Leu Gly Ala Thr Gly Pro Ala Asp Ser Gln His Glu Ser Gln His
    610                 615                 620
```

```
Gly Gly Leu Asp Gln Asp Gly Glu Ala Arg Pro Ala Leu Asp Gly Ser
625                 630                 635                 640
Ala Ala Leu Gln Pro Leu Leu His Thr Val Lys Ala Gly Ser Pro Ser
                645                 650                 655
Asp Met Pro Arg Asp Ser Gly Ile Tyr Asp Ser Ser Val Pro Ser Ser
            660                 665                 670
Glu Leu Ser Leu Pro Leu Met Glu Gly Leu Ser Thr Asp Gln Thr Glu
        675                 680                 685
Thr Ser Ser Leu Thr Glu Ser Val Ser Ser Ser Ser Gly Leu Gly Glu
    690                 695                 700
Glu Glu Pro Pro Ala Leu Pro Ser Lys Leu Leu Ser Ser Gly Ser Cys
705                 710                 715                 720
Lys Ala Asp Leu Gly Cys Arg Ser Tyr Thr Asp Glu Leu His Ala Val
                725                 730                 735
Ala Pro Leu
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<210> 9
<211> 2217
<212> DNA
<213> Artificial Sequence

<220>
<223> This degenerate nucleotide sequence encodes the amino acid sequence of SEQ ID NO:8.

<221> misc_feature
<222> (1)...(2217)
<223> n = A,T,C or G

<400> 9

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wsncarytng cngtngcngc nggnggnwsn ggnmgngcnm gnggngcnga yacntgyggn    120
tggmgnggng tnggnccngc nwsnmgnaay wsnggnytnt ayaayathac nttyaartay    180
gayaaytgya cnacntayyt naayccngtn ggnaarcayg tnathgcnga ygcncaraay    240
athacnathw sncartaygc ntgycaygay cargtngcng tnacnathyt ntggwsnccn    300
ggngcnytng gnathgartt yytnaarggn ttymgngtna thytngarga rytnaarwsn    360
garggnmgnc artgycarca rytnathytn aargayccna arcarytnaa ywsnwsntty    420
aarmgnacng gnatggarws ncarccntty ytnaayatga arttygarac ngaytaytty    480
gtnaargtng tnccnttycc nwsnathaar aaygarwsna aytaycaycc nttyttytty    540
mgnacnmgng cntgygayyt nytnytncar ccngayaayy tngcntgyaa rccnttytgg    600
aarccnmgna ayytnaayat hwsncarcay ggnwsngaya tgcargtnws nttygaycay    660
gcnccncaya ayttyggntt ymgnttytty tayytncayt ayaarytnaa rcaygarggn    720
ccnttyaarm gnaaracntg yaarcargar caracnacng aracnacnws ntgyytnytn    780
```

caraaygtnw snccnggnga ytayathath garytngtng aygayacnaa yacnacnmgn 840
aargtnatgc aytaygcnyt naarccngtn caywsnccnt gggcnggncc nathmgngcn 900
gtngcnatha cngtnccnyt ngtngtnath wsngcnttyg cnacnytntt yacngtnatg 960
tgymgnaara arcarcarga raayathtay wsncayytng aygargarws nwsngarwsn 1020
wsnacntaya cngcngcnyt nccnmgngar mgnytnmgnc cnmgnccnaa rgtnttyytn 1080
tgytaywsnw snaargaygg ncaraaycay atgaaygtng tncartgytt ygcntaytty 1140
ytncargayt tytgyggntg ygargtngcn ytngayytnt gggargaytt ywsnytntgy 1200
mgngarggnc armgngartg ggtnathcar aarathcayg arwsncartt yathathgtn 1260
gtntgywsna arggnatgaa rtayttygtn gayaaraara aytayaarca yaarggnggn 1320
ggnmgnggnw snggnaargg ngarytntty ytngtngcng tnwsngcnat hgcngaraar 1380
ytnmgncarg cnaarcarws nwsnwsngcn gcnytnwsna arttyathgc ngtntaytty 1440
gaytaywsnt gygarggnga ygtnccnggn athytngayy tnwsnacnaa rtaymgnytn 1500
atggayaayy tnccncaryt ntgywsncay ytncaywsnm gngaycaygg nytncargar 1560
ccnggncarc ayacnmgnca rggnwsnmgn mgnaaytayt tymgnwsnaa rwsnggnmgn 1620
wsnytntayg tngcnathtg yaayatgcay carttyathg aygargarcc ngaytggtty 1680
garaarcart tygtnccntt ycayccnccn ccnytnmgnt aymgngarcc ngtnytngar 1740
aarttygayw snggnytngt nytnaaygay gtnatgtgya arccnggncc ngarwsngay 1800
ttytgyytna argtngargc ngcngtnytn ggngcnacng gnccngcnga ywsncarcay 1860
garwsncarc ayggnggnyt ngaycargay ggngargcnm gnccngcnyt ngayggnwsn 1920
gcngcnytnc arccnytnyt ncayacngtn aargcnggnw snccnwsnga yatgccnmgn 1980
gaywsnggna thtaygayws nwsngtnccn wsnwsngary tnwsnytncc nytnatggar 2040
ggnytnwsna cngaycarac ngaracnwsn wsnytnacng arwsngtnws nwsnwsnwsn 2100
ggnytnggng argargarcc nccngcnytn ccnwsnaary tnytnwsnws nggnwsntgy 2160
aargcngayy tnggntgymg nwsntayacn gaygarytnc aygcngtngc nccnytn 2217

<210> 10
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Peptide linker.

<400> 10
Gly Gly Ser Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15

<210> 11
<211> 2443
<212> DNA
<213> Mouse

<220>

<221> CDS
<222> (101)...(2317)

<400> 11

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aacctagcgg agaccggccc aactgggcga gcgtacggcc atg gcc ccg tgg ctg      115
                                               Met Ala Pro Trp Leu
                                                1               5

cag ctc tgc tcc ttc ttc ttc act gtc aac gcc tgt ctc aac ggc tcg      163
Gln Leu Cys Ser Phe Phe Phe Thr Val Asn Ala Cys Leu Asn Gly Ser
                10                  15                  20

cag ctg gca gtg gcc gcg ggc ggc tcc ggc cgc gcg agg ggc gcg gac      211
Gln Leu Ala Val Ala Ala Gly Gly Ser Gly Arg Ala Arg Gly Ala Asp
            25                  30                  35

acc tgt ggc tgg agg gga gtg ggg ccg gcc agc agg aac agc gga ctg      259
Thr Cys Gly Trp Arg Gly Val Gly Pro Ala Ser Arg Asn Ser Gly Leu
        40                  45                  50

cac aac atc acc ttc aga tac gac aac tgt acc acc tac ttg aat ccc      307
His Asn Ile Thr Phe Arg Tyr Asp Asn Cys Thr Thr Tyr Leu Asn Pro
    55                  60                  65

ggc ggc ggg aag cat gcg att gct gat gct cag aac atc acc atc agc      355
Gly Gly Gly Lys His Ala Ile Ala Asp Ala Gln Asn Ile Thr Ile Ser
70                  75                  80                  85

cag tac gct tgc cac gac cag gtg gca gtc acc att ctt tgg tcc cca      403
Gln Tyr Ala Cys His Asp Gln Val Ala Val Thr Ile Leu Trp Ser Pro
                90                  95                  100

ggg gcc ctt ggc att gaa ttc cta aaa gga ttc cga gtc atc ctg gag      451
Gly Ala Leu Gly Ile Glu Phe Leu Lys Gly Phe Arg Val Ile Leu Glu
            105                 110                 115

gag ctg aag tcg gag ggc aga cag tgc caa cag ctg att cta aag gac      499
Glu Leu Lys Ser Glu Gly Arg Gln Cys Gln Gln Leu Ile Leu Lys Asp
        120                 125                 130

ccc aaa cag ctc aac agc agc ttc aga agg act gga atg gaa tct cag      547
```

```
Pro Lys Gln Leu Asn Ser Ser Phe Arg Arg Thr Gly Met Glu Ser Gln
    135                 140                 145

cct ttc ctg aat atg aaa ttt gag acg gat tac ttt gta aag att gtc      595
Pro Phe Leu Asn Met Lys Phe Glu Thr Asp Tyr Phe Val Lys Ile Val
150                 155                 160                 165

cct ttc cct tcc att aaa aat gaa agc aat tac cat ccc ttc ttc ttc      643
Pro Phe Pro Ser Ile Lys Asn Glu Ser Asn Tyr His Pro Phe Phe Phe
                170                 175                 180

aga aca cgg gcc tgt gac ctg ttg tta caa cct gac aac ttg gcc tgt      691
Arg Thr Arg Ala Cys Asp Leu Leu Leu Gln Pro Asp Asn Leu Ala Cys
            185                 190                 195

aag cct ttc tgg aag cct cga aac ctg aat atc agc cag cat ggt tct      739
Lys Pro Phe Trp Lys Pro Arg Asn Leu Asn Ile Ser Gln His Gly Ser
        200                 205                 210

gac atg cac gtg tcc ttc gac cat gcc ccg cag aac ttc ggc ttc cgt      787
Asp Met His Val Ser Phe Asp His Ala Pro Gln Asn Phe Gly Phe Arg
    215                 220                 225

ggc ttc cat gtt ctc tat aag ctc aag cac gaa ggc ccc ttc agg cgg      835
Gly Phe His Val Leu Tyr Lys Leu Lys His Glu Gly Pro Phe Arg Arg
230                 235                 240                 245

agg act tgc agg cag gac cag aat aca gag aca acc agc tgc ctc ctc      883
Arg Thr Cys Arg Gln Asp Gln Asn Thr Glu Thr Thr Ser Cys Leu Leu
                250                 255                 260

caa aac gtt tct cca ggg gac tat atc att gag ctg gtg gat gac agc      931
Gln Asn Val Ser Pro Gly Asp Tyr Ile Ile Glu Leu Val Asp Asp Ser
            265                 270                 275

aac acc acc agg aaa gct gct cag tat gtg gtg aag tca gtg cag tct      979
Asn Thr Thr Arg Lys Ala Ala Gln Tyr Val Val Lys Ser Val Gln Ser
        280                 285                 290

ccc tgg gct gga ccc atc aga gct gtg gcc atc act gtg cct ctg gtt     1027
Pro Trp Ala Gly Pro Ile Arg Ala Val Ala Ile Thr Val Pro Leu Val
    295                 300                 305
```

```
gtc ata tct gcg ttc gca acc ctg ttc act gtg atg tgc aga aag aag     1075
Val Ile Ser Ala Phe Ala Thr Leu Phe Thr Val Met Cys Arg Lys Lys
310                 315                 320                 325

caa caa gaa aat ata tat tca cat tta gat gaa gaa agc ccg gag tcg     1123
Gln Gln Glu Asn Ile Tyr Ser His Leu Asp Glu Glu Ser Pro Glu Ser
                330                 335                 340

tcc aca tac gct gcg gct ctc ccc aga gac agg ctc cgg cct cag ccc     1171
Ser Thr Tyr Ala Ala Ala Leu Pro Arg Asp Arg Leu Arg Pro Gln Pro
            345                 350                 355

aag gtc ttc ctc tgc tac tcc aat aaa gat ggc cag aat cac atg aac     1219
Lys Val Phe Leu Cys Tyr Ser Asn Lys Asp Gly Gln Asn His Met Asn
        360                 365                 370

gtg gtc cag tgt ttc gcc tat ttc ctg caa gat ttc tgt ggc tgt gag     1267
Val Val Gln Cys Phe Ala Tyr Phe Leu Gln Asp Phe Cys Gly Cys Glu
    375                 380                 385

gtg gct ctg gac ttg tgg gaa gat ttc agc ctc tgc aga gag ggg cag     1315
Val Ala Leu Asp Leu Trp Glu Asp Phe Ser Leu Cys Arg Glu Gly Gln
390                 395                 400                 405

aga gaa tgg gcc att cag aag atc cac gag tcc cag ttc atc att gtc     1363
Arg Glu Trp Ala Ile Gln Lys Ile His Glu Ser Gln Phe Ile Ile Val
                410                 415                 420

gtg tgc tcc aaa ggc atg aag tac ttt gta gat aag aag aac ttc aga     1411
Val Cys Ser Lys Gly Met Lys Tyr Phe Val Asp Lys Lys Asn Phe Arg
            425                 430                 435

cac aaa gga ggc agc cgc ggc gag gcg caa ggc gag ttc ttc ctg gtg     1459
His Lys Gly Gly Ser Arg Gly Glu Ala Gln Gly Glu Phe Phe Leu Val
        440                 445                 450

gcc gtg gca gcc att gct gag aag ctc cgt cag gcc aag cag agc tca     1507
Ala Val Ala Ala Ile Ala Glu Lys Leu Arg Gln Ala Lys Gln Ser Ser
    455                 460                 465

tct gcc gca ctg cgc aag ttc atc gcc gtc tac ttc gat tat tcc tgt     1555
```

```
Ser Ala Ala Leu Arg Lys Phe Ile Ala Val Tyr Phe Asp Tyr Ser Cys
470                 475                 480                 485

gaa ggg gat gta ccc tgc agc ctg gac ctg agc acc aag tac aag ctc     1603
Glu Gly Asp Val Pro Cys Ser Leu Asp Leu Ser Thr Lys Tyr Lys Leu
                490                 495                 500

atg gac cac ctt cct gag ctc tgt gcc cat ctg cac tca gga gag cag     1651
Met Asp His Leu Pro Glu Leu Cys Ala His Leu His Ser Gly Glu Gln
            505                 510                 515

gag gtg ctg ggt cag cac cca ggc cac agc agc aga agg aac tac ttc     1699
Glu Val Leu Gly Gln His Pro Gly His Ser Ser Arg Arg Asn Tyr Phe
        520                 525                 530

cgg agc aaa tcg ggc cgc tcc ctg tat gtt gcc att tgc aac atg cac     1747
Arg Ser Lys Ser Gly Arg Ser Leu Tyr Val Ala Ile Cys Asn Met His
    535                 540                 545

cag ttt att gat gag gag cct gac tgg ttt gag aag cag ttt ata ccc     1795
Gln Phe Ile Asp Glu Glu Pro Asp Trp Phe Glu Lys Gln Phe Ile Pro
550                 555                 560                 565

ttc caa cat ccc cct gtg cgc tac cag gag cca gtc ctg gag aaa ttt     1843
Phe Gln His Pro Pro Val Arg Tyr Gln Glu Pro Val Leu Glu Lys Phe
                570                 575                 580

gac tca ggc ttg gtt tta aat gat gtc ata agc aaa cca ggg cca gag     1891
Asp Ser Gly Leu Val Leu Asn Asp Val Ile Ser Lys Pro Gly Pro Glu
            585                 590                 595

agt gac ttc tgt cgg aaa gtc gag gct tgt gta ctt ggg gcc gct ggg     1939
Ser Asp Phe Cys Arg Lys Val Glu Ala Cys Val Leu Gly Ala Ala Gly
        600                 605                 610

cca gcc gac tct tat tca tac ctg gag agt cag cat gta ggc ctg gac     1987
Pro Ala Asp Ser Tyr Ser Tyr Leu Glu Ser Gln His Val Gly Leu Asp
    615                 620                 625

caa gac act gag gcc cag ccc tcc tgt gat agt gcc cct gcc ttg cag     2035
Gln Asp Thr Glu Ala Gln Pro Ser Cys Asp Ser Ala Pro Ala Leu Gln
630                 635                 640                 645
```

```
ccc ctg tta cac gca gtg aaa gct ggc agt ccc tca gag atg cca cgg      2083
Pro Leu Leu His Ala Val Lys Ala Gly Ser Pro Ser Glu Met Pro Arg
                650                 655                 660

gac tca ggc ata tat gat tct tct gta ccc tca tca gag ctc tct ctg      2131
Asp Ser Gly Ile Tyr Asp Ser Ser Val Pro Ser Ser Glu Leu Ser Leu
            665                 670                 675

cct ctg atg gag gga ctc tcc ccg gat cag ata gaa aca tct tct ctg      2179
Pro Leu Met Glu Gly Leu Ser Pro Asp Gln Ile Glu Thr Ser Ser Leu
        680                 685                 690

acc gag agt gta tct tcc tcc tct ggc cta ggt gag gag gac ccc cct      2227
Thr Glu Ser Val Ser Ser Ser Ser Gly Leu Gly Glu Glu Asp Pro Pro
    695                 700                 705

acc ctc cct tcc aag ctc ttt gcc tct ggg gtg tcc aga gaa cat ggt      2275
Thr Leu Pro Ser Lys Leu Phe Ala Ser Gly Val Ser Arg Glu His Gly
710                 715                 720                 725

tgc cac agc cac act gac gaa ctg caa gcg ctt gct cct ttg              2317
Cys His Ser His Thr Asp Glu Leu Gln Ala Leu Ala Pro Leu
                730                 735

taaggactcg gaagagtcta agcatcgcca ctttagctgc tgatctctct ggctccccag    2377
ttcacctctg tggttgtgca gcctacttgg agctgaaggc gcacacgggg atatctggaa    2437
tgaaat                                                              2443
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<210> 12
<211> 739
<212> PRT
<213> Mouse

<400> 12

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Cys Leu Asn Gly Ser Gln Leu Ala Val Ala Ala Gly Gly Ser Gly Arg
            20                  25                  30
Ala Arg Gly Ala Asp Thr Cys Gly Trp Arg Gly Val Gly Pro Ala Ser
        35                  40                  45
Arg Asn Ser Gly Leu His Asn Ile Thr Phe Arg Tyr Asp Asn Cys Thr
    50                  55                  60
```

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

Cys Arg Glu Gly Gln Arg Glu Trp Ala Ile Gln Lys Ile His Glu Ser
405 410 415
Gln Phe Ile Ile Val Val Cys Ser Lys Gly Met Lys Tyr Phe Val Asp
420 425 430
Lys Lys Asn Phe Arg His Lys Gly Gly Ser Arg Gly Glu Ala Gln Gly
435 440 445
Glu Phe Phe Leu Val Ala Val Ala Ala Ile Ala Glu Lys Leu Arg Gln
450 455 460
Ala Lys Gln Ser Ser Ser Ala Ala Leu Arg Lys Phe Ile Ala Val Tyr
465 470 475 480
Phe Asp Tyr Ser Cys Glu Gly Asp Val Pro Cys Ser Leu Asp Leu Ser
485 490 495
Thr Lys Tyr Lys Leu Met Asp His Leu Pro Glu Leu Cys Ala His Leu
500 505 510
His Ser Gly Glu Gln Glu Val Leu Gly Gln His Pro Gly His Ser Ser
515 520 525
Arg Arg Asn Tyr Phe Arg Ser Lys Ser Gly Arg Ser Leu Tyr Val Ala
530 535 540
Ile Cys Asn Met His Gln Phe Ile Asp Glu Glu Pro Asp Trp Phe Glu
545 550 555 560
Lys Gln Phe Ile Pro Phe Gln His Pro Pro Val Arg Tyr Gln Glu Pro
565 570 575
Val Leu Glu Lys Phe Asp Ser Gly Leu Val Leu Asn Asp Val Ile Ser
580 585 590
Lys Pro Gly Pro Glu Ser Asp Phe Cys Arg Lys Val Glu Ala Cys Val
595 600 605
Leu Gly Ala Ala Gly Pro Ala Asp Ser Tyr Ser Tyr Leu Glu Ser Gln
610 615 620
His Val Gly Leu Asp Gln Asp Thr Glu Ala Gln Pro Ser Cys Asp Ser
625 630 635 640
Ala Pro Ala Leu Gln Pro Leu Leu His Ala Val Lys Ala Gly Ser Pro
645 650 655
Ser Glu Met Pro Arg Asp Ser Gly Ile Tyr Asp Ser Ser Val Pro Ser
660 665 670
Ser Glu Leu Ser Leu Pro Leu Met Glu Gly Leu Ser Pro Asp Gln Ile
675 680 685
Glu Thr Ser Ser Leu Thr Glu Ser Val Ser Ser Ser Ser Gly Leu Gly
690 695 700
Glu Glu Asp Pro Pro Thr Leu Pro Ser Lys Leu Phe Ala Ser Gly Val
705 710 715 720
Ser Arg Glu His Gly Cys His Ser His Thr Asp Glu Leu Gln Ala Leu
725 730 735

Ala Pro Leu

<210> 13
<211> 2217
<212> DNA
<213> Artificial Sequence

<220>
<223> This degenerate nucleotide sequence encodes the amino acid sequence of SEQ ID NO:12.

<221> misc_feature
<222> (1)...(2217)
<223> n = A, T, C or G

<400> 13

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wsncarytng cngtngcngc nggnggnwsn ggnmgngcnm gnggngcnga yacntgyggn 120
tggmgnggng tnggnccngc nwsnmgnaay wsnggnytnc ayaayathac nttymgntay 180
gayaaytgya cnacntayyt naayccnggn ggnggnaarc aygcnathgc ngaygcncar 240
aayathacna thwsncarta ygcntgycay gaycargtng cngtnacnat hytntggwsn 300
ccnggngcny tnggnathga rttyytnaar ggnttymgng tnathytnga rgarytnaar 360
wsngarggnm gncartgyca rcarytnath ytnaargayc cnaarcaryt naaywsnwsn 420
ttymgnmgna cnggnatgga rwsncarccn ttyytnaaya tgaarttyga racngaytay 480
ttygtnaara thgtnccntt yccnwsnath aaraaygarw snaaytayca yccnttytty 540
ttymgnacnm gngcntgyga yytnytnytn carccngaya ayytngcntg yaarccntty 600
tggaarccnm gnaayytnaa yathwsncar cayggnwsng ayatgcaygt nwsnttygay 660
caygcnccnc araayttygg nttymgnggn ttycaygtny tntayaaryt naarcaygar 720
ggnccnttym gnmgnmgnac ntgymgncar gaycaraaya cngaracnac nwsntgyytn 780
ytncaraayg tnwsnccngg ngaytayath athgarytng tngaygayws naayacnacn 840
mgnaargcng cncartaygt ngtnaarwsn gtncarwsnc cntgggcngg nccnathmgn 900
gcngtngcna thacngtncc nytngtngtn athwsngcnt tygcnacnyt nttyacngtn 960
atgtgymgna araarcarca rgaraayath taywsncayy tngaygarga rwsnccngar 1020
wsnwsnacnt aygcngcngc nytnccnmgn gaymgnytnm gnccncarcc naargtntty 1080
ytntgytayw snaayaarga yggncaraay cayatgaayg tngtncartg yttygcntay 1140
ttyytncarg ayttytgygg ntgygargtn gcnytngayy tntgggarga yttywsnytn 1200
tgymgngarg gncarmgnga rtgggcnath caraarathc aygarwsnca rttyathath 1260
gtngtntgyw snaarggnat gaartaytty gtngayaara araayttymg ncayaarggn 1320
ggnwsnmgng gngargcnca rggngartty ttyytngtng cngtngcngc nathgcngar 1380
aarytnmgnc argcnaarca rwsnwsnwsn gcngcnytnm gnaarttyat hgcngtntay 1440
ttygaytayw sntgygargg ngaygtnccn tgywsnytng ayytnwsnac naartayaar 1500
ytnatggayc ayytnccnga rytntgygcn cayytncayw snggngarca rgargtnytn 1560
ggncarcayc cnggncayws nwsnmgnmgn aaytayttym gnwsnaarws nggnmgnwsn 1620

ytntaygtng cnathtgyaa yatgcaycar ttyathgayg argarccnga ytggttygar 1680
aarcarttya thccnttyca rcayccnccn gtnmgntayc argarccngt nytngaraar 1740
ttygaywsng gnytngtnyt naaygaygtn athwsnaarc cnggnccnga rwsngaytty 1800
tgymgnaarg tngargcntg ygtnytnggn gcngcnggnc cngcngayws ntaywsntay 1860
ytngarwsnc arcaygtngg nytngaycar gayacngarg cncarccnws ntgygaywsn 1920
gcnccngcny tncarccnyt nytncaygcn gtnaargcng gnwsnccnws ngaratgccn 1980
mgngaywsng gnathtayga ywsnwsngtn ccnwsnwsng arytnwsnyt nccnytnatg 2040
garggnytnw snccngayca rathgaracn wsnwsnytna cngarwsngt nwsnwsnwsn 2100
wsnggnytng gngargarga yccnccnacn ytnccnwsna arytnttygc nwsnggngtn 2160
wsnmgngarc ayggntgyca ywsncayacn gaygarytnc argcnytngc nccnytn 2217