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(54) Title: 20 HUMAN SECRETED PROTEINS

(57) Abstract

The present invention relates to 20 human secreted proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human secreted proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these human secreted proteins.
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20 Human Secreted Proteins

Field of the Invention

This invention relates to newly identified polynucleotides and the polypeptides encoded by these polynucleotides, uses of such polynucleotides and polypeptides, and their production.

Background of the Invention

Unlike bacteria, which exist as a single compartment surrounded by a membrane, human cells and other eucaryotes are subdivided by membranes into many functionally distinct compartments. Each membrane-bounded compartment, or organelle, contains different proteins essential for the function of the organelle. The cell uses “sorting signals,” which are amino acid motifs located within the protein, to target proteins to particular cellular organelles.

One type of sorting signal, called a signal sequence, a signal peptide, or a leader sequence, directs a class of proteins to an organelle called the endoplasmic reticulum (ER). The ER separates the membrane-bounded proteins from all other types of proteins. Once localized to the ER, both groups of proteins can be further directed to another organelle called the Golgi apparatus. Here, the Golgi distributes the proteins to vesicles, including secretory vesicles, the cell membrane, lysosomes, and the other organelles.

Proteins targeted to the ER by a signal sequence can be released into the extracellular space as a secreted protein. For example, vesicles containing secreted proteins can fuse with the cell membrane and release their contents into the extracellular space - a process called exocytosis. Exocytosis can occur constitutively or after receipt of a triggering signal. In the latter case, the proteins are stored in secretory vesicles (or secretory granules) until exocytosis is triggered. Similarly, proteins residing on the cell membrane can also be secreted into the extracellular space by proteolytic cleavage of a “linker” holding the protein to the membrane.

Despite the great progress made in recent years, only a small number of genes encoding human secreted proteins have been identified. These secreted proteins include the commercially valuable human insulin, interferon, Factor VIII, human growth hormone, tissue plasminogen activator, and erythropoietin. Thus, in light of the pervasive role of secreted proteins in human physiology, a need exists for identifying and characterizing novel human secreted proteins and the genes that encode them. This knowledge will allow one to detect, to treat, and to prevent medical disorders by using secreted proteins or the genes that encode them.
Summary of the Invention

The present invention relates to novel polynucleotides and the encoded polypeptides. Moreover, the present invention relates to vectors, host cells, antibodies, and recombinant methods for producing the polypeptides and polynucleotides. Also provided are diagnostic methods for detecting disorders related to the polypeptides, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying binding partners of the polypeptides.

Detailed Description

Definitions

The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide.

In the present invention, a "secreted" protein refers to those proteins capable of being directed to the ER, secretory vesicles, or the extracellular space as a result of a signal sequence, as well as those proteins released into the extracellular space without necessarily containing a signal sequence. If the secreted protein is released into the extracellular space, the secreted protein can undergo extracellular processing to produce a "mature" protein. Release into the extracellular space can occur by many mechanisms, including exocytosis and proteolytic cleavage.

As used herein, a "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:X or the cDNA contained within the clone deposited with the ATCC. For example, the polynucleotide can contain the nucleotide sequence of the full length cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, with or without the signal sequence, the secreted protein coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, a "polypeptide" refers to a molecule having the translated amino acid sequence generated from the polynucleotide as broadly defined.

In the present invention, the full length sequence identified as SEQ ID NO:X was often generated by overlapping sequences contained in multiple clones (contig
analysis). A representative clone containing all or most of the sequence for SEQ ID
NO:X was deposited with the American Type Culture Collection ("ATCC"). As
shown in Table 1, each clone is identified by a cDNA Clone ID (Identifier) and the
ATCC Deposit Number. The ATCC is located at 10801 University Boulevard,
Manassas, Virginia 20110-2209, USA. The ATCC deposit was made pursuant to the
terms of the Budapest Treaty on the international recognition of the deposit of
microorganisms for purposes of patent procedure.

A "polynucleotide" of the present invention also includes those polynucleotides
capable of hybridizing, under stringent hybridization conditions, to sequences contained
in SEQ ID NO:X, the complement thereof, or the cDNA within the clone deposited with
the ATCC. "Stringent hybridization conditions" refers to an overnight incubation at 42°C
in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM sodium
citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran
sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the
filters in 0.1x SSC at about 65°C.

Also contemplated are nucleic acid molecules that hybridize to the
polynucleotides of the present invention at lower stringency hybridization conditions.
Changes in the stringency of hybridization and signal detection are primarily
accomplished through the manipulation of formamide concentration (lower percentages
of formamide result in lowered stringency); salt conditions, or temperature. For
example, lower stringency conditions include an overnight incubation at 37°C in a
solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M NaH₂PO₄; 0.02M EDTA,
pH 7.4), 0.5% SDS, 30% formamide, 100 ug/ml salmon sperm blocking DNA;
followed by washes at 50°C with 1XSSPE, 0.1% SDS. In addition, to achieve even
lower stringency, washes performed following stringent hybridization can be done at
higher salt concentrations (e.g. 5X SSC).

Note that variations in the above conditions may be accomplished through the
inclusion and/or substitution of alternate blocking reagents used to suppress
background in hybridization experiments. Typical blocking reagents include
Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and
commercially available proprietary formulations. The inclusion of specific blocking
reagents may require modification of the hybridization conditions described above, due
to problems with compatibility.

Of course, a polynucleotide which hybridizes only to polyA+ sequences (such
as any 3' terminal polyA+ tract of a cDNA shown in the sequence listing), or to a
complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

The polynucleotide of the present invention can be composed of any polynucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotide can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

The polypeptide of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslational natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine,
formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI
anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation,
pegylation, proteolytic processing, phosphorylation, prenylation, racemization,
selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins
such as arginylation, and ubiquitination. (See, for instance, PROTEINS -
STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W.
H. Freeman and Company, New York (1993); POSTTRANSLATIONAL
COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic
Press, New York, pgs. 1-12 (1983); Seifter et al., Meth Enzymol 182:626-646 (1990);
Rattan et al., Ann NY Acad Sci 663:48-62 (1992).)

"SEQ ID NO:X" refers to a polynucleotide sequence while "SEQ ID NO:Y"
refers to a polypeptide sequence, both sequences identified by an integer specified in
Table 1.

"A polypeptide having biological activity" refers to polypeptides exhibiting
activity similar, but not necessarily identical to, an activity of a polypeptide of the
present invention, including mature forms, as measured in a particular biological assay,
with or without dose dependency. In the case where dose dependency does exist, it
need not be identical to that of the polypeptide, but rather substantially similar to the
dose-dependence in a given activity as compared to the polypeptide of the present
invention (i.e., the candidate polypeptide will exhibit greater activity or not more than
about 25-fold less and, preferably, not more than about tenfold less activity, and most
preferably, not more than about three-fold less activity relative to the polypeptide of the
present invention.)

25 Polynucleotides and Polypeptides of the Invention

FEATURES OF PROTEIN ENCODED BY GENE NO: 1

This gene is expressed primarily in brain and CD34 positive cells.

Therefore, polynucleotides and polypeptides of the invention are useful as
reagents for differential identification of the tissue(s) or cell type(s) present in a
biological sample and for diagnosis of diseases and conditions which include, but are
not limited to, central nervous system (CNS) and immune-system diseases. Similarly,
polypeptides and antibodies directed to these polypeptides are useful in providing
immunological probes for differential identification of the tissue(s) or cell type(s). For a
number of disorders of the above tissues or cells, particularly of the CNS and immune
system, expression of this gene at significantly higher or lower levels may be routinely
detected in certain tissues and cell types (e.g., brain and other tissue of the nervous
system, blood cells, and cells and tissue of the immune system, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred epitopes include those comprising a sequence shown in SEQ ID NO:31 as residues: Asp-44 to Gly-49, Val-84 to Lys-90.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosing and treating CNS and immune-system diseases.

**FEATURES OF PROTEIN ENCODED BY GENE NO: 2**

The translation product of this gene shares sequence homology with LIM domain proteins which are thought to be important in regulating cellular functions such as cell proliferation and differentiation. In particular, it is believed that this gene encodes the human ortholog of mouse testin. See, for example, Gene 156(2):283-286 (1995), which is incorporated herein by reference. LIM proteins are described in Proc. Natl. Acad. Sci. U.S.A. 90:4404-4408(1993), which is incorporated herein by reference. Based on the sequence similarity to other members of the LIM family, polypeptides encoded by this gene are expected to share certain biological activities with other LIM polypeptides, in particular mouse Testin. Preferred polypeptides encoded by this gene comprise the following amino acid sequence (LIM domain): CAGCDELIFSNEYTQAENQNWHLKHFCFDCDSIL (SEQ ID NO:51). Especially preferred polypeptides encoded by this gene comprise the following amino acid sequence: ARGFVCSTCHELLVDMIYFWKNEKLYCGRHYCD SEKPRCAGCDELIFSNEYTQAENQNWHLKHFCFDCDSILAGEIYVMVNDKPVC KPCYVKNHAVCQGCHNAIDPEVQRVTYNNFSWHASTECFLCSCSCLIG QKMFMPVEGMVFSVECKKRMS (SEQ ID NO:52). Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in testis and to a lesser extent in Hodgkin's lymphoma, T cell and adrenal gland tumor. Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune diseases, reproductive disorders and cancers. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing
immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive and immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., testis and other reproductive tissue, lymphoid tissue, tissue and cells of the immune system, blood cells, and adrenal gland, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution and homology to LIM proteins indicates that polynucleotides and polypeptides corresponding to this gene are useful for treating diseases of the immune system and male reproductive system.

**FEATURES OF PROTEIN ENCODED BY GENE NO: 3**

This gene is expressed primarily in infant brain, prostate, embryo and to a lesser extent in parathyroid, adrenal gland tumor, thymus.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune system and central nervous system (CNS) diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system and CNS, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., brain and other tissue of the nervous system, tissue and cells of the immune system, differentiating tissue, parathyroid, adrenal gland, and thymus, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for treating and diagnosis of immune system and CNS diseases.
FEATURES OF PROTEIN ENCODED BY GENE NO: 4

This gene maps to chromosome 4 (Chr.4, D4S395-D4S414) and therefore polynucleotides of the present invention can be used in linkage analysis as a marker for chromosome 4.

This gene is expressed primarily in infant brain, embryo, parathyroid tumor and melanocyte and to a lesser extent in testis, chondrosarcoma, epididyma, placenta, endothelial cells and many other cell types, tissues and organs.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, disorders of the nervous system, developmental related defects and abnormalities. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the nervous system and immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., brain and other tissue of the nervous system, embryonic or differentiating cells or tissue, parathyroid, melanocytes, chondrocytes, testis and other reproductive tissue, placenta, and endothelial cells, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred epitopes include those comprising a sequence shown in SEQ ID NO:34 as residues: Glu-28 to Thr-35.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of disorders of the nervous system, such as congenital malformations, degenerative diseases, trauma, inflammatory diseases, neoplasia, metabolic disorders, and immune diseases, particularly with T-cell involvement. The abundant expression in the parathyroid tumor indicates that protein products of this gene are useful in modulating calcium metabolism.

FEATURES OF PROTEIN ENCODED BY GENE NO: 5

The translation product of this gene shares sequence homology with ancient ubiquitous 46 kDa protein AUP46 precursor [Mus musculus] which is thought to be important in tissue and organ development.
This gene is expressed primarily in testes and placenta and to a lesser extent in fetal liver, brain, and activated T-cells.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, liver diseases and immunological disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and digestive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., testes and other reproductive tissue, placenta, liver, brain and other tissue of the nervous system, and T-cells and other blood cells, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred epitopes include those comprising a sequence shown in SEQ ID NO:35 as residues: Pro-4 to Pro-9, Asp-14 to Gly-20, Arg-78 to His-87, Glu-161 to Gly-170, Leu-252 to Arg-258, Lys-269 to Pro-293, Asp-344 to Thr-349, Ser-379 to Gln-391, Arg-399 to Asp-410.

The tissue distribution and homology to AUP46 indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of diseases in testes, placenta, liver, brain and activated T-cells, particularly diseases related to development of the organs associated with the foregoing tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO: 6

The translation product of this gene shares sequence homology with ATP7 region hypothetical protein which is thought to be important in development. This gene maps to chromosome 17 (D17S849-D17S796) and therefore polynucleotides of the present invention can be used in linkage analysis as a marker for chromosome 17.

This gene is expressed primarily in breast, brain and liver and to a lesser extent in prostate and thymus.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, breast cancer, brain tumor and liver cancer. Similarly, polypeptides and
antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and nerve system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues (e.g., developing tissue, mammary tissue, brain and other tissue of the nervous system, liver, prostate, and thymus, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

FEATURES OF PROTEIN ENCODED BY GENE NO: 7

This gene is expressed primarily in liver, spleen, bone marrow and to a lesser extent in amygdala.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, liver, spleen diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and digestive systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues (e.g., liver, spleen, bone marrow, cells and tissue of the immune system, and amygdala and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

FEATURES OF PROTEIN ENCODED BY GENE NO: 8

Preferred mature polypeptides encoded by this gene comprise the following amino acid sequence:

GVARGHRDGRQASRRWLQEGGQECEKDWFLRAPPKRFMTVSGL
PKKQCPDHFKGNVKTRHRHRKPNKHSRACQQFLKQCLRSLPALPL

(SEQ ID NO:53).
This gene is expressed primarily in lung and to a lesser extent in pancreatic carcinoma and gall bladder.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, lung diseases and pancreatic carcinoma. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune, pulmonary and digestive systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues (e.g., lung, pancreas, and gall bladder, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred epitopes include those comprising a sequence shown in SEQ ID NO:38 as residues: Gly-31 to Gln-37.

FEATURES OF PROTEIN ENCODED BY GENE NO: 9

This gene is expressed primarily in rhabdomyosarcoma and pituitary, and, to a lesser extent, in fetal lung and keratinocytes.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, rhabdomyosarcoma and disorders of the endocrine system or other endocrinopathies, including, but not limited to, endocrine polyglandular syndrome, endocrinoma, endocrine ophthalmopathy, and any of the great number of disease states and disorders which are caused by or relate to the abnormal secretion of factors originating in the endocrine gland. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and musculoskeletal system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., skeletal muscle, pituitary, endocrine glands, lung and keratinocytes, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e.,
the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred epitopes include those comprising a sequence shown in SEQ ID NO:39 as residues: Pro-34 to Phe-40.

5 FEATURES OF PROTEIN ENCODED BY GENE NO: 10

This gene is expressed primarily in endometrial tumor, osteoblasts, and smooth muscle, and, to a lesser extent, in osteoclastoma, heart, and lung.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, endometrial tumor, osteoclastoma, and other bone remodeling disorders, and heart and lung diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and bone systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues (e.g., endometrium, bone, heart and other cardiovascular tissue, lung and other pulmonary tissue, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred epitopes include those comprising a sequence shown in SEQ ID NO:40 as residues: Thr-33 to Arg-40.

25 FEATURES OF PROTEIN ENCODED BY GENE NO: 11

This gene is expressed primarily in meningima and dermatofibrosarcoma.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, fibrotic and neoplastic conditions of skin, connective tissue and other mesenchymal organs. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues (e.g., meninx, liver, skin, and vascular tissue, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal
fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred epitopes include those comprising a sequence shown in SEQ ID NO:41 as residues: Gln-50 to Met-56.

Tissue distribution of this gene indicates that it may be useful in the study, treatment, and diagnosis of fibrotic disorders and neoplasms of skin, liver, and other tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO: 12

This gene is expressed primarily in placenta, and colon cancer, and to a lesser extent in adult lung and brain frontal cortex.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system and neural system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues (e.g., placenta, colon, cells and tissue of the immune system, lung and other pulmonary tissue, brain and other tissue of the nervous system, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred epitopes include those comprising a sequence shown in SEQ ID NO:42 as residues: Met-1 to His-9, Arg-31 to Gly-38, Gly-102 to Trp-108.

The tissue distribution indicates that the protein product of this gene is useful for the treatment of neoplasia.

FEATURES OF PROTEIN ENCODED BY GENE NO: 13

This gene is expressed primarily in thymus, bone marrow, T-cells, macrophages, and, to a lesser extent, in breast and testes.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are
not limited to, cancer, autoimmune diseases, bone diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., thymus, bone, T-cells and other blood cells, mammary tissue, and testes and other reproductive tissue, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred epitopes include those comprising a sequence shown in SEQ ID NO:43 as residues: Pro-36 to Trp-42, Arg-48 to Trp-56, Ser-58 to Ser-67.

FEATURES OF PROTEIN ENCODED BY GENE NO: 14

This gene is expressed primarily in breast cancer, pituitary, and activated T-cells, and, to a lesser extent, in frontal cortex and breast.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, breast cancer, growth, and immune disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and neural system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., mammary tissue, pituitary, T-cells and other blood cells, cells and tissue of the immune system, brain and other tissue of the nervous system, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. The tissue distribution indicates that the protein product of this gene is useful for diagnosis or treatment of breast cancer and growth disorders.
FEATURES OF PROTEIN ENCODED BY GENE NO: 15

This gene is primarily expressed exclusively in neutrophils.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, lymphoma and bacterial infection. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, and more particularly, in neutrophils, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., lymphoid tissue, hematopoietic tissue, neutrophils and other blood cells, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that the protein product of this gene is useful for treatment of lymphomas and various hematopoietic disorders, as well as in the diagnosis and treatment of bacterial infections, sepsis, and in disorders which particularly involve neutrophils.

FEATURES OF PROTEIN ENCODED BY GENE NO: 16

In specific embodiments, polypeptides of the invention comprise the sequence: HTQVEFIPRMQC (SEQ ID NO:54), LKIRKPINVYHINRL (SEQ ID NO:55), RKMGIERNFHQSGKGI (SEQ ID NO:56), KVPTANIILNGERLNAFPIRT (SEQ ID NO:57), MYFLSSLLIHEHVISVIFSIL (SEQ ID NO:60), and/or IFSSVLHSFQYTPNPV PFFFRTFPSTLFF (SEQ ID NO:58). Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in neutrophils only.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, lymphomas. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or
lower levels may be routinely detected in certain tissues (e.g., lymphoid tissue, hematopoietic tissue, neutrophils and other blood cells, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that the protein product of this gene is useful for treatment of lymphomas and a variety of hematopoietic disorders.

**FEATURES OF PROTEIN ENCODED BY GENE NO: 17**

This gene maps to chromosome 19 and therefore polynucleotides of the present invention can be used in linkage analysis as a marker for chromosome 19.

This gene is expressed primarily in prostate cancer, adult lung and adult pulmonary and to a lesser extent in prostate and adrenal gland tumor.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, prostate cancer and endocrine disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues (e.g., prostate, lung and other pulmonary tissue, endocrine tissue, adrenal gland, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred epitopes include those comprising a sequence shown in SEQ ID NO:47 as residues: Gly-2 to Gly-9, Thr-39 to Arg-47.

The tissue distribution indicates that the protein product of this gene is useful for diagnosis or treatment of prostate cancer, prostate disorders and endocrine disorders.

**FEATURES OF PROTEIN ENCODED BY GENE NO: 18**

This sequence shares high degree homology with the UFO oncoprotein. This protein is a tyrosine kinase receptor. While the functions of this UFO receptor are unknown, it is known that the receptor plays a role in tumorigenesis.
This gene is expressed primarily in L8 cell line.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred epitopes include those comprising a sequence shown in SEQ ID NO:48 as residues: Ala-37 to Ser-49.

The tissue distribution and homology with the UFO oncoprotein indicates that the protein product of this gene is useful for the treatment of cancer.

FEATURES OF PROTEIN ENCODED BY GENE NO: 19

This gene is expressed primarily in L8 cell line.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer and the immune system. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that the protein product of this gene is useful for the treatment of cancer.
FEATURES OF PROTEIN ENCODED BY GENE NO: 20

In specific embodiments, polypeptides of the invention comprise the sequence EDGSAPREGETSAPRLPEVVRITSAGIC (SEQ ID NO:61). Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in A1 and A14 cell lines.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that the protein product of this gene is useful for the diagnosis and treatment of cancer.
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<td>HRLMD77</td>
<td>97955 03/13/97 209074 05/22/97</td>
<td>ZAP Express</td>
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<tr>
<td>Gene No.</td>
<td>cDNA Clone ID</td>
<td>ATCC Deposit Nr and Date</td>
<td>Vector</td>
<td>NT SEQ ID NO: X</td>
<td>Total NT Seq.</td>
<td>5' NT of Clone Seq.</td>
<td>3' NT of Clone Seq.</td>
<td>5' NT of Start Codon</td>
<td>5' NT of First AA of Signal Pep</td>
<td>AA SEQ ID NO: Y</td>
<td>First AA of Sig Pep</td>
<td>Last AA of Sig Pep</td>
<td>First AA of Secreted Portion</td>
<td>Last AA of ORF</td>
</tr>
<tr>
<td>----------</td>
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</table>
Table 1 summarizes the information corresponding to each “Gene No.” described above. The nucleotide sequence identified as “NT SEQ ID NO:X” was assembled from partially homologous (“overlapping”) sequences obtained from the “cDNA clone ID” identified in Table 1 and, in some cases, from additional related DNA clones. The overlapping sequences were assembled into a single contiguous sequence of high redundancy (usually three to five overlapping sequences at each nucleotide position), resulting in a final sequence identified as SEQ ID NO:X.

The cDNA Clone ID was deposited on the date and given the corresponding deposit number listed in “ATCC Deposit No:Z and Date.” Some of the deposits contain multiple different clones corresponding to the same gene. “Vector” refers to the type of vector contained in the cDNA Clone ID.

“Total NT Seq.” refers to the total number of nucleotides in the contig identified by “Gene No.” The deposited clone may contain all or most of these sequences, reflected by the nucleotide position indicated as “5’ NT of Clone Seq.” and the “3’ NT of Clone Seq.” of SEQ ID NO:X. The nucleotide position of SEQ ID NO:X of the putative start codon (methionine) is identified as “5’ NT of Start Codon.” Similarly, the nucleotide position of SEQ ID NO:X of the predicted signal sequence is identified as “5’ NT of First AA of Signal Pep.”

The translated amino acid sequence, beginning with the methionine, is identified as “AA SEQ ID NO:Y,” although other reading frames can also be easily translated using known molecular biology techniques. The polypeptides produced by these alternative open reading frames are specifically contemplated by the present invention.

The first and last amino acid position of SEQ ID NO:Y of the predicted signal peptide is identified as “First AA of Sig Pep” and “Last AA of Sig Pep.” The predicted first amino acid position of SEQ ID NO:Y of the secreted portion is identified as “Predicted First AA of Secreted Portion.” Finally, the amino acid position of SEQ ID NO:Y of the last amino acid in the open reading frame is identified as “Last AA of ORF.”

SEQ ID NO:X and the translated SEQ ID NO:Y are sufficiently accurate and otherwise suitable for a variety of uses well known in the art and described further below. For instance, SEQ ID NO:X is useful for designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID NO:X or the cDNA contained in the deposited clone. These probes will also hybridize to nucleic acid molecules in biological samples, thereby enabling a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID NO:Y may be used to generate antibodies which bind specifically to the secreted proteins encoded by the cDNA clones identified in Table 1.
Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides not only the generated nucleotide sequence identified as SEQ ID NO:X and the predicted translated amino acid sequence identified as SEQ ID NO:Y, but also a sample of plasmid DNA containing a human cDNA of the invention deposited with the ATCC, as set forth in Table 1. The nucleotide sequence of each deposited clone can readily be determined by sequencing the deposited clone in accordance with known methods. The predicted amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by a particular clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited human cDNA, collecting the protein, and determining its sequence.

The present invention also relates to the genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, or the deposited clone. The corresponding gene can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include preparing probes or primers from the disclosed sequence and identifying or amplifying the corresponding gene from appropriate sources of genomic material.

Also provided in the present invention are species homologs. Species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for the desired homologue.

The polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

The polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein (see below).
It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification, such as multiple histidine residues, or an additional sequence for stability during recombinant production.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a polypeptide, including the secreted polypeptide, can be substantially purified by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988). Polypeptides of the invention also can be purified from natural or recombinant sources using antibodies of the invention raised against the secreted protein in methods which are well known in the art.

**Signal Sequences**

Methods for predicting whether a protein has a signal sequence, as well as the cleavage point for that sequence, are available. For instance, the method of McGeoch, Virus Res. 3:271-286 (1985), uses the information from a short N-terminal charged region and a subsequent uncharged region of the complete (uncleaved) protein. The method of von Heinje, Nucleic Acids Res. 14:4683-4690 (1986) uses the information from the residues surrounding the cleavage site, typically residues -13 to +2, where +1 indicates the amino terminus of the secreted protein. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80%. (von Heinje, supra.) However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

In the present case, the deduced amino acid sequence of the secreted polypeptide was analyzed by a computer program called SignalP (Henrik Nielsen et al., Protein Engineering 10:1-6 (1997)), which predicts the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. The analysis of the amino acid sequences of the secreted proteins described herein by this program provided the results shown in Table 1.

As one of ordinary skill would appreciate, however, cleavage sites sometimes vary from organism to organism and cannot be predicted with absolute certainty. Accordingly, the present invention provides secreted polypeptides having a sequence shown in SEQ ID NO:Y which have an N-terminus beginning within 5 residues (i.e., + or - 5 residues) of the predicted cleavage point. Similarly, it is also recognized that in some cases, cleavage of the signal sequence from a secreted protein is not entirely
uniform, resulting in more than one secreted species. These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention. Moreover, the signal sequence identified by the above analysis may not necessarily predict the naturally occurring signal sequence. For example, the naturally occurring signal sequence may be further upstream from the predicted signal sequence. However, it is likely that the predicted signal sequence will be capable of directing the secreted protein to the ER. These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Polynucleotide and Polypeptide Variants

"Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide or polypeptide of the present invention, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the polynucleotide or polypeptide of the present invention.

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the polypeptide. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be an entire sequence shown in Table 1, the ORF (open reading frame), or any fragment specified as described herein.

As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the presence invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. (1990) 6:237-245). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are:

Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization
Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence because of 5’ or 3’ deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5’ and 3’ truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5’ or 3’ ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5’ and 3’ of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5’ and 3’ bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5’ end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5’ end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5’ and 3’ ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5’ or 3’ of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5’ and 3’ of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query
amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (indels) or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequences shown in Table 1 or to the amino acid sequence encoded by deposited DNA clone can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. (1990) 6:237-245). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity.

For subject sequences truncated at the N- and C-termini, relative to the the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence.
For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence.

This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to made for the purposes of the present invention.

The variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutitions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as E. coli).

Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985).) These allelic variants can vary at either the polynucleotide and/or polypeptide level.

Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides of the present invention. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the secreted protein without substantial loss of biological function. The authors of Ron et al., J. Biol. Chem. 268: 2984-2988 (1993), reported variant KGF proteins having heparin binding activity even after
deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., J. Biotechnology 7:199-216 (1988).)

Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol. Chem 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1α. They used random mutagenesis to generate over 3,500 individual IL-1α mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." (See, Abstract.) In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

Thus, the invention further includes polypeptide variants which show substantial biological activity. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie, J. U. et al., Science 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.
The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used. (Cunningham and Wells, Science 244:1081-1085 (1989).) The resulting mutant molecules can then be tested for biological activity.

As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

Besides conservative amino acid substitution, variants of the present invention include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate’s immunogenic activity. (Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).)

Polynucleotide and Polypeptide Fragments
In the present invention, a "polynucleotide fragment" refers to a short polynucleotide having a nucleic acid sequence contained in the deposited clone or shown in SEQ ID NO:X. The short nucleotide fragments are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from the cDNA sequence contained in the deposited clone or the nucleotide sequence shown in SEQ ID NO:X. These nucleotide fragments are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., 50, 150, 500, 600, 2000 nucleotides) are preferred.

Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments having a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700, or 701 to the end of SEQ ID NO:X or the cDNA contained in the deposited clone. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has biological activity.

In the present invention, a "polypeptide fragment" refers to a short amino acid sequence contained in SEQ ID NO:Y or encoded by the cDNA contained in the deposited clone. Protein fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 102-120, 121-140, 141-160, or 161 to the end of the coding region. Moreover, polypeptide fragments can be about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes.

Preferred polypeptide fragments include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred.
Similarly, polynucleotide fragments encoding these polypeptide fragments are also preferred.

Also preferred are polypeptide and polynucleotide fragments characterized by structural or functional domains, such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Polypeptide fragments of SEQ ID NO:Y falling within conserved domains are specifically contemplated by the present invention. Moreover, polynucleotide fragments encoding these domains are also contemplated.

Other preferred fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

**Epitopes & Antibodies**

In the present invention, "epitopes" refer to polypeptide fragments having antigenic or immunogenic activity in an animal, especially in a human. A preferred embodiment of the present invention relates to a polypeptide fragment comprising an epitope, as well as the polynucleotide encoding this fragment. A region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." In contrast, an "immunogenic epitope" is defined as a part of a protein that elicits an antibody response. (See, for instance, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998-4002 (1983).)


In the present invention, antigenic epitopes preferably contain a sequence of at least seven, more preferably at least nine, and most preferably between about 15 to about 30 amino acids. Antigenic epitopes are useful to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. (See, for instance, Wilson et al., Cell 37:767-778 (1984); Sutcliffe, J. G. et al., Science 219:660-666 (1983).)

Similarly, immunogenic epitopes can be used to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., supra; Wilson et al., supra; Chow, M. et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle, F. J. et al., J. Gen. Virol. 66:2347-2354 (1985).) A preferred immunogenic epitope includes
the secreted protein. The immunogenic epitopes may be presented together with a
carrier protein, such as an albumin, to an animal system (such as rabbit or mouse) or, if
it is long enough (at least about 25 amino acids), without a carrier. However,
immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be
sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a
denatured polypeptide (e.g., in Western blotting.)

As used herein, the term "antibody" (Ab) or "monoclonal antibody" (Mab) is
meant to include intact molecules as well as antibody fragments (such as, for example,
Fab and F(ab')2 fragments) which are capable of specifically binding to protein. Fab
and F(ab')2 fragments lack the Fc fragment of intact antibody, clear more rapidly from
the circulation, and may have less non-specific tissue binding than an intact antibody.
(Wahl et al., J. Nucl. Med. 24:316-325 (1983).) Thus, these fragments are preferred,
as well as the products of a FAB or other immunoglobulin expression library.
Moreover, antibodies of the present invention include chimeric, single chain, and
humanized antibodies.

Fusion Proteins

Any polypeptide of the present invention can be used to generate fusion
proteins. For example, the polypeptide of the present invention, when fused to a
second protein, can be used as an antigenic tag. Antibodies raised against the
polypeptide of the present invention can be used to indirectly detect the second protein
by binding to the polypeptide. Moreover, because secreted proteins target cellular
locations based on trafficking signals, the polypeptides of the present invention can be
used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to polypeptides of the present invention
include not only heterologous signal sequences, but also other heterologous functional
regions. The fusion does not necessarily need to be direct, but may occur through
linker sequences.

Moreover, fusion proteins may also be engineered to improve characteristics of
the polypeptide of the present invention. For instance, a region of additional amino
acids, particularly charged amino acids, may be added to the N-terminus of the
polypeptide to improve stability and persistence during purification from the host cell or
subsequent handling and storage. Also, peptide moieties may be added to the
polypeptide to facilitate purification. Such regions may be removed prior to final
preparation of the polypeptide. The addition of peptide moieties to facilitate handling of
polypeptides are familiar and routine techniques in the art.
Moreover, polypeptides of the present invention, including fragments, and specifically epitopes, can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP A 394,827; Traunecker et al., Nature 331:84-86 (1988).) Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995).)

Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP-A 0232 262.) Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, D. Bennett et al., J. Molecular Recognition 8:52-58 (1995); K. Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).)

Moreover, the polypeptides of the present invention can be fused to marker sequences, such as a peptide which facilitates purification of the fused polypeptide. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein. (Wilson et al., Cell 37:767 (1984).)

Thus, any of these above fusions can be engineered using the polynucleotides or the polypeptides of the present invention.
Vectors, Host Cells, and Protein Production

The present invention also relates to vectors containing the polynucleotide of the present invention, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the E. coli lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in E. coli and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as E. coli, Streptomyces and Salmonella typhimurium cells; fungal cells, such as yeast cells; insect cells such as Drosophila S2 and Spodoptera Si9 cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptk99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.
Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods in Molecular Biology (1986). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

A polypeptide of this invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapitate chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

Polypeptides of the present invention, and preferably the secreted form, can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

**Uses of the Polynucleotides**

Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

The polynucleotides of the present invention are useful for chromosome identification. There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat
polymorphisms), are presently available. Each polynucleotide of the present invention can be used as a chromosome marker.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the sequences shown in SEQ ID NO:X. Primers can be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the SEQ ID NO:X will yield an amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, and preselection by hybridization to construct chromosome specific-cDNA libraries.

Precise chromosomal location of the polynucleotides can also be achieved using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see Verma et al., "Human Chromosomes: a Manual of Basic Techniques," Pergamon Press, New York (1988).

For chromosome mapping, the polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes). Preferred polynucleotides correspond to the noncoding regions of the cDNAs because the coding sequences are more likely conserved within gene families, thus increasing the chance of cross hybridization during chromosomal mapping.

Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a particular disease. (Disease mapping data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library).) Assuming 1 megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

Thus, once coinheritance is established, differences in the polynucleotide and the corresponding gene between affected and unaffected individuals can be examined.
First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the polypeptide and the corresponding gene from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

Furthermore, increased or decreased expression of the gene in affected individuals as compared to unaffected individuals can be assessed using polynucleotides of the present invention. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

In addition to the foregoing, a polynucleotide can be used to control gene expression through triple helix formation or antisense DNA or RNA. Both methods rely on binding of the polynucleotide to DNA or RNA. For these techniques, preferred polynucleotides are usually 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988).) Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat disease.

Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the present invention offer a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell.

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

The polynucleotides of the present invention can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a unique set of DNA sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQA class II HLA gene, are used in forensic biology to identify individuals. (Erlich, H., PCR Technology, Freeman and Co. (1992).) Once these specific polymorphic loci are amplified, they are digested with one or more restriction enzymes, yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQA class II HLA gene. Similarly, polynucleotides of the present invention can be used as polymorphic markers for forensic purposes.

There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to particular tissue prepared from the sequences of the present invention. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

In the very least, the polynucleotides of the present invention can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.
Uses of the Polypeptides

Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

A polypeptide of the present invention can be used to assay protein levels in a biological sample using antibody-based techniques. For example, protein expression in tissues can be studied with classical immunohistological methods. (Jalkanen, M., et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, M., et al., J. Cell. Biol. 105:3087-3096 (1987).) Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (125I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (112In), and technetium (99mTc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying secreted protein levels in a biological sample, proteins can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, 131I, 112In, 99mTc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of 99mTc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).)
Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression of a polypeptide of the present invention in cells or body fluid of an individual; (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a disorder.

Moreover, polypeptides of the present invention can be used to treat disease. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B), to inhibit the activity of a polypeptide (e.g., an oncogene), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth).

Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat disease. For example, administration of an antibody directed to a polypeptide of the present invention can bind and reduce overproduction of the polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

At the very least, the polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Polypeptides can also be used to raise antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, the polypeptides of the present invention can be used to test the following biological activities.

**Biological Activities**

The polynucleotides and polypeptides of the present invention can be used in assays to test for one or more biological activities. If these polynucleotides and polypeptides do exhibit activity in a particular assay, it is likely that these molecules may be involved in the diseases associated with the biological activity. Thus, the polynucleotides and polypeptides could be used to treat the associated disease.

**Immune Activity**

A polypeptide or polynucleotide of the present invention may be useful in treating deficiencies or disorders of the immune system, by activating or inhibiting the
proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune deficiencies or disorders may be genetic, somatic, such as cancer or some autoimmune disorders, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, a polynucleotide or polypeptide of the present invention can be used as a marker or detector of a particular immune system disease or disorder.

A polynucleotide or polypeptide of the present invention may be useful in treating or detecting deficiencies or disorders of hematopoietic cells. A polypeptide or polynucleotide of the present invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat those disorders associated with a decrease in certain (or many) types hematopoietic cells. Examples of immunologic deficiency syndromes include, but are not limited to: blood protein disorders (e.g. agammaglobulinemia, dysgammaglobulinemia), ataxia telangiectasia, common variable immunodeficiency, Digeorge Syndrome, HIV infection, HTLV-BLV infection, leukocyte adhesion deficiency syndrome, lymphopenia, phagocyte bactericidal dysfunction, severe combined immunodeficiency (SCIDs), Wiskott-Aldrich Disorder, anemia, thrombocytopenia, or hemoglobinuria.

Moreover, a polypeptide or polynucleotide of the present invention could also be used to modulate hemostatic (the stopping of bleeding) or thrombolytic activity (clot formation). For example, by increasing hemostatic or thrombolytic activity, a polynucleotide or polypeptide of the present invention could be used to treat blood coagulation disorders (e.g., afibrinogenemia, factor deficiencies), blood platelet disorders (e.g. thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, a polynucleotide or polypeptide of the present invention that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting. These molecules could be important in the treatment of heart attacks (infarction), strokes, or scarring.

A polynucleotide or polypeptide of the present invention may also be useful in treating or detecting autoimmune disorders. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of a polypeptide or polynucleotide of the present invention that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune disorders.
Examples of autoimmune disorders that can be treated or detected by the present invention include, but are not limited to: Addison’s Disease, hemolytic anemia, antiphospholipid syndrome, rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture’s Syndrome, Graves’ Disease, Multiple Sclerosis, Myasthenia Gravis, Neuritis, Ophthalmia, Bullous Pemphigoid, Pemphigus, Polyendocrinopathies, Purpura, Reiter’s Disease, Stiff-Man Syndrome, Autoimmune Thyroiditis, Systemic Lupus Erythematosus, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye disease.

Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated by a polypeptide or polynucleotide of the present invention. Moreover, these molecules can be used to treat anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

A polynucleotide or polypeptide of the present invention may also be used to treat and/or prevent organ rejection or graft-versus-host disease (GVHD). Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. The administration of a polypeptide or polynucleotide of the present invention that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

Similarly, a polypeptide or polynucleotide of the present invention may also be used to modulate inflammation. For example, the polypeptide or polynucleotide may inhibit the proliferation and differentiation of cells involved in an inflammatory response. These molecules can be used to treat inflammatory conditions, both chronic and acute conditions, including inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn’s disease, or resulting from over production of cytokines (e.g., TNF or IL-1.)

**Hyperproliferative Disorders**

A polypeptide or polynucleotide can be used to treat or detect hyperproliferative disorders, including neoplasms. A polypeptide or polynucleotide of the present invention may inhibit the proliferation of the disorder through direct or indirect
interactions. Alternatively, a polypeptide or polynucleotide of the present invention may proliferate other cells which can inhibit the hyperproliferative disorder.

For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative disorders can be treated. This immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating hyperproliferative disorders, such as a chemotherapeutic agent.

Examples of hyperproliferative disorders that can be treated or detected by a polynucleotide or polypeptide of the present invention include, but are not limited to neoplasms located in the: abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

Similarly, other hyperproliferative disorders can also be treated or detected by a polynucleotide or polypeptide of the present invention. Examples of such hyperproliferative disorders include, but are not limited to: hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemia, purpura, sarcoidosis, Sezary Syndrome, Waldenström’s Macroglobulinemia, Gaucher’s Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

**Infectious Disease**

A polypeptide or polynucleotide of the present invention can be used to treat or detect infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, the polypeptide or polynucleotide of the present invention may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated or detected by a polynucleotide or polypeptide of the present invention. Examples of viruses, include, but are not limited to the following DNA and RNA viral families: Arbovirus, Adenoviridae, Arenaviridae, Arteriviridae, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes
Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza), Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including but not limited to: arthritis, bronchiolitis, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt’s Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi’s, warts), and viremia. A polypeptide or polynucleotide of the present invention can be used to treat or detect any of these symptoms or diseases.

Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated or detected by a polynucleotide or polypeptide of the present invention include, but not limited to, the following Gram-Negative and Gram-positive bacterial families and fungi: Actinomycetales (e.g., Corynebacterium, Mycobacterium, Norcardia), Aspergillus, Bacillales (e.g., Anthrax, Clostridium), Bacteroidaceae, Blastomycosis, Bordetella, Borrelia, Brucellosis, Candidiasis, Campylobacter, Coccidioidomycosis, Cryptococcus, Dermatococcus, Enterobacteriaceae (Klebsiella, Salmonella, Serratia, Yersinia), Erysipelothrix, Helicobacter, Legionellosis, Leptospirosis, Listeria, Mycoplasmatales, Neisseriaceae (e.g., Acinetobacter, Gonorrhea, Menigococcal), Pasteurellaceae Infections (e.g., Actinobacillus, Heamophilus, Pasteurella), Pseudomonas, Rickettsiae, Chlamydiaceae, Syphilis, and Staphylococcal. These bacterial or fungal families can cause the following diseases or symptoms, including but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter’s Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning, Typhoid, pneumonia, Gonorrhea, meningitis, Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocyeses), toxemia, urinary tract infections, wound infections.

A polypeptide or polynucleotide of the present invention can be used to treat or detect any of these symptoms or diseases.
Moreover, parasitic agents causing disease or symptoms that can be treated or detected by a polynucleotide or polypeptide of the present invention include, but not limited to, the following families: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas. These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), Malaria, pregnancy complications, and toxoplasmosis. A polypeptide or polynucleotide of the present invention can be used to treat or detect any of these symptoms or diseases.

Preferably, treatment using a polypeptide or polynucleotide of the present invention could either be by administering an effective amount of a polypeptide to the patient, or by removing cells from the patient, supplying the cells with a polynucleotide of the present invention, and returning the engineered cells to the patient (ex vivo therapy). Moreover, the polypeptide or polynucleotide of the present invention can be used as an antigen in a vaccine to raise an immune response against infectious disease.

**Regeneration**

A polynucleotide or polypeptide of the present invention can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues. (See, Science 276:59-87 (1997).) The regeneration of tissues could be used to repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteoarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vascular (including vascular endothelium), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

Moreover, a polynucleotide or polypeptide of the present invention may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. A polynucleotide or polypeptide of the present invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue
regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

Similarly, nerve and brain tissue could also be regenerated by using a polynucleotide or polypeptide of the present invention to proliferate and differentiate nerve cells. Diseases that could be treated using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic disorders (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stroke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated using the polynucleotide or polypeptide of the present invention.

**Chemotaxis**

A polynucleotide or polypeptide of the present invention may have chemotaxis activity. A chemotactic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

A polynucleotide or polypeptide of the present invention may increase chemotactic activity of particular cells. These chemotactic molecules can then be used to treat inflammation, infection, hyperproliferative disorders, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotactic molecules can be used to treat wounds and other trauma to tissues by attracting immune cells to the injured location. Chemotactic molecules of the present invention can also attract fibroblasts, which can be used to treat wounds.

It is also contemplated that a polynucleotide or polypeptide of the present invention may inhibit chemotactic activity. These molecules could also be used to treat disorders. Thus, a polynucleotide or polypeptide of the present invention could be used as an inhibitor of chemotaxis.

**Binding Activity**

A polypeptide of the present invention may be used to screen for molecules that bind to the polypeptide or for molecules to which the polypeptide binds. The binding of the polypeptide and the molecule may activate (agonist), increase, inhibit
(antagonist), or decrease activity of the polypeptide or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

Preferably, the molecule is closely related to the natural ligand of the polypeptide, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic. (See, Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991).) Similarly, the molecule can be closely related to the natural receptor to which the polypeptide binds, or at least, a fragment of the receptor capable of being bound by the polypeptide (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

Preferably, the screening for these molecules involves producing appropriate cells which express the polypeptide, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, Drosophila, or E. coli. Cells expressing the polypeptide (or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either the polypeptide or the molecule.

The assay may simply test binding of a candidate compound to the polypeptide, wherein binding is detected by a label, or in an assay involving competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to the polypeptide.

Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide, measuring polypeptide/molecule activity or binding, and comparing the polypeptide/molecule activity or binding to a standard.

Preferably, an ELISA assay can measure polypeptide level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can measure polypeptide level or activity by either binding, directly or indirectly, to the polypeptide or by competing with the polypeptide for a substrate.

All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the polypeptide/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of the polypeptide from suitably manipulated cells or tissues.
Therefore, the invention includes a method of identifying compounds which bind to a polypeptide of the invention comprising the steps of: (a) incubating a candidate binding compound with a polypeptide of the invention; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with a polypeptide of the invention, (b) assaying a biological activity, and (b) determining if a biological activity of the polypeptide has been altered.

**Other Activities**

A polypeptide or polynucleotide of the present invention may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

A polypeptide or polynucleotide of the present invention may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, a polypeptide or polynucleotide of the present invention may be used to modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

A polypeptide or polynucleotide of the present invention may be used to change a mammal’s mental state or physical state by influencing biorhythms, caricadic rhythms, depression (including depressive disorders), tendency for violence, tolerance for pain, reproductive capabilities (preferably by Activin or Inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

A polypeptide or polynucleotide of the present invention may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components.

**Other Preferred Embodiments**

Other preferred embodiments of the claimed invention include an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 50 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of
positions beginning with the nucleotide at about the position of the 5’ Nucleotide of the Clone Sequence and ending with the nucleotide at about the position of the 3’ Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5’ Nucleotide of the Start Codon and ending with the nucleotide at about the position of the 3’ Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Similarly preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5’ Nucleotide of the First Amino Acid of the Signal Peptide and ending with the nucleotide at about the position of the 3’ Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 150 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

Further preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 500 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

A further preferred embodiment is a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the nucleotide sequence of SEQ ID NO:X beginning with the nucleotide at about the position of the 5’ Nucleotide of the First Amino Acid of the Signal Peptide and ending with the nucleotide at about the position of the 3’ Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence of SEQ ID NO:X.

Also preferred is an isolated nucleic acid molecule which hybridizes under stringent hybridization conditions to a nucleic acid molecule, wherein said nucleic acid molecule which hybridizes does not hybridize under stringent hybridization conditions to a nucleic acid molecule having a nucleotide sequence consisting of only A residues or of only T residues.

Also preferred is a composition of matter comprising a DNA molecule which comprises a human cDNA clone identified by a cDNA Clone Identifier in Table 1, which DNA molecule is contained in the material deposited with the American Type
Culture Collection and given the ATCC Deposit Number shown in Table 1 for said cDNA Clone Identifier.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in the nucleotide sequence of a human cDNA clone identified by a cDNA Clone Identifier in Table 1, which DNA molecule is contained in the deposit given the ATCC Deposit Number shown in Table 1.

Also preferred is an isolated nucleic acid molecule, wherein said sequence of at least 50 contiguous nucleotides is included in the nucleotide sequence of the complete open reading frame sequence encoded by said human cDNA clone.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 150 contiguous nucleotides in the nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 500 contiguous nucleotides in the nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is a method for detecting in a biological sample a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1; which method comprises a step of comparing a nucleotide sequence of at least one nucleic acid molecule in said sample with a sequence selected from said group and determining whether the sequence of said nucleic acid molecule in said sample is at least 95% identical to said selected sequence.

Also preferred is the above method wherein said step of comparing sequences comprises determining the extent of nucleic acid hybridization between nucleic acid molecules in said sample and a nucleic acid molecule comprising said sequence selected from said group. Similarly, also preferred is the above method wherein said step of comparing sequences is performed by comparing the nucleotide sequence determined from a nucleic acid molecule in said sample with said sequence selected from said group. The nucleic acid molecules can comprise DNA molecules or RNA molecules.
A further preferred embodiment is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting nucleic acid molecules in said sample, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

The method for identifying the species, tissue or cell type of a biological sample can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a secreted protein identified in Table 1, which method comprises a step of detecting in a biological sample obtained from said subject nucleic acid molecules, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

The method for diagnosing a pathological condition can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a composition of matter comprising isolated nucleic acid molecules wherein the nucleotide sequences of said nucleic acid molecules comprise a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1. The nucleic acid molecules can comprise DNA molecules or RNA molecules.
Also preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1.

Also preferred is a polypeptide, wherein said sequence of contiguous amino acids is included in the amino acid sequence of SEQ ID NO:Y in the range of positions beginning with the residue at about the position of the First Amino Acid of the Secreted Portion and ending with the residue at about the Last Amino Acid of the Open Reading Frame as set forth for SEQ ID NO:Y in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the complete amino acid sequence of SEQ ID NO: Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is a polypeptide wherein said sequence of contiguous amino acids is included in the amino acid sequence of the secreted portion of the secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.
Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is an isolated antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is a method for detecting in a biological sample a polypeptide comprising an amino acid sequence which is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1; which method comprises a step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group and determining whether the sequence of said polypeptide molecule in said sample is at least 90% identical to said sequence of at least 10 contiguous amino acids.

Also preferred is the above method wherein said step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group comprises determining the extent of specific binding of polypeptides in said sample to an antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is the above method wherein said step of comparing sequences is performed by comparing the amino acid sequence determined from a polypeptide molecule in said sample with said sequence selected from said group.
Also preferred is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting polypeptide molecules in said sample, if any, comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is the above method for identifying the species, tissue or cell type of a biological sample, which method comprises a step of detecting polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the above group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a secreted protein identified in Table 1, which method comprises a step of detecting in a biological sample obtained from said subject polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

In any of these methods, the step of detecting said polypeptide molecules includes using an antibody.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a nucleotide sequence encoding a polypeptide wherein said polypeptide comprises an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.
Also preferred is an isolated nucleic acid molecule, wherein said nucleotide sequence encoding a polypeptide has been optimized for expression of said polypeptide in a prokaryotic host.

Also preferred is an isolated nucleic acid molecule, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is a method of making a recombinant vector comprising inserting any of the above isolated nucleic acid molecule into a vector. Also preferred is the recombinant vector produced by this method. Also preferred is a method of making a recombinant host cell comprising introducing the vector into a host cell, as well as the recombinant host cell produced by this method.

Also preferred is a method of making an isolated polypeptide comprising culturing this recombinant host cell under conditions such that said polypeptide is expressed and recovering said polypeptide. Also preferred is this method of making an isolated polypeptide, wherein said recombinant host cell is a eukaryotic cell and said polypeptide is a secreted portion of a human secreted protein comprising an amino acid sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y beginning with the residue at the position of the First Amino Acid of the Secreted Portion of SEQ ID NO:Y wherein Y is an integer set forth in Table 1 and said position of the First Amino Acid of the Secreted Portion of SEQ ID NO:Y is defined in Table 1; and an amino acid sequence of a secreted portion of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1. The isolated polypeptide produced by this method is also preferred.

Also preferred is a method of treatment of an individual in need of an increased level of a secreted protein activity, which method comprises administering to such an individual a pharmaceutical composition comprising an amount of an isolated polypeptide, polynucleotide, or antibody of the claimed invention effective to increase the level of said protein activity in said individual.

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

Example 1: Isolation of a Selected cDNA Clone From the Deposited Sample

Each cDNA clone in a cited ATCC deposit is contained in a plasmid vector. Table 1 identifies the vectors used to construct the cDNA library from which each clone was isolated. In many cases, the vector used to construct the library is a phage vector from which a plasmid has been excised. The table immediately below correlates the related plasmid for each phage vector used in constructing the cDNA library. For example, where a particular clone is identified in Table 1 as being isolated in the vector "Lambda Zap," the corresponding deposited clone is in "pBluescript."

<table>
<thead>
<tr>
<th>Vector Used to Construct Library</th>
<th>Corresponding Deposited Plasmid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lambda Zap</td>
<td>pBluescript (pbs)</td>
</tr>
<tr>
<td>Uni-Zap XR</td>
<td>pBluescript (pbs)</td>
</tr>
<tr>
<td>Zap Express</td>
<td>pBK</td>
</tr>
<tr>
<td>lamid BA</td>
<td>plamid BA</td>
</tr>
<tr>
<td>pSport1</td>
<td>pSport1</td>
</tr>
<tr>
<td>pCMV Sport 2.0</td>
<td>pCMV Sport 2.0</td>
</tr>
<tr>
<td>pCMV Sport 3.0</td>
<td>pCMV Sport 3.0</td>
</tr>
<tr>
<td>pCR®2.1</td>
<td>pCR®2.1</td>
</tr>
</tbody>
</table>


The S and K refers to the orientation of the polylinker to the T7 and T3 primer sequences which flank the polylinker region ("S" is for Sacl and "K" is for Kpnl which are the first sites on each respective end of the linker). "+" or "-" refer to the orientation of the f1 origin of replication ("ori"), such that in one orientation, single stranded rescue initiated from the f1 ori generates sense strand DNA and in the other, antisense.

Vectors pSport1, pCMV Sport 2.0 and pCMV Sport 3.0, were obtained from Life Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors contain an ampicillin resistance gene and may be transformed into E. coli strain...
DH10B, also available from Life Technologies. (See, for instance, Gruber, C. E., et al., Focus 15:59 (1993).) Vector lafmid BA (Bento Soares, Columbia University, NY) contains an ampicillin resistance gene and can be transformed into E. coli strain XL-1 Blue. Vector pCR®2.1, which is available from Invitrogen, 1600 Faraday Avenue, Carlsbad, CA 92028, contains an ampicillin resistance gene and may be transformed into E. coli strain DH10B, available from Life Technologies. (See, for instance, Clark, J. M., Nuc. Acids Res. 16:9677-9686 (1988) and Mead, D. et al., Bio/Technology 9: (1991).) Preferably, a polynucleotide of the present invention does not comprise the phage vector sequences identified for the particular clone in Table 1, as well as the corresponding plasmid vector sequences designated above.

The deposited material in the sample assigned the ATCC Deposit Number cited in Table 1 for any given cDNA clone also may contain one or more additional plasmids, each comprising a cDNA clone different from that given clone. Thus, deposits sharing the same ATCC Deposit Number contain at least a plasmid for each cDNA clone identified in Table 1. Typically, each ATCC deposit sample cited in Table 1 comprises a mixture of approximately equal amounts (by weight) of about 50 plasmid DNAs, each containing a different cDNA clone; but such a deposit sample may include plasmids for more or less than 50 cDNA clones, up to about 500 cDNA clones.

Two approaches can be used to isolate a particular clone from the deposited sample of plasmid DNAs cited for that clone in Table 1. First, a plasmid is directly isolated by screening the clones using a polynucleotide probe corresponding to SEQ ID NO:X.

Particularly, a specific polynucleotide with 30-40 nucleotides is synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported.

The oligonucleotide is labeled, for instance, with ³²P-γ-ATP using T4 polynucleotide kinase and purified according to routine methods. (E.g., Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring, NY (1982).) The plasmid mixture is transformed into a suitable host, as indicated above (such as XL-1 Blue (Stratagene)) using techniques known to those of skill in the art, such as those provided by the vector supplier or in related publications or patents cited above. The transformants are plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate. These plates are screened using Nylon membranes according to routine methods for bacterial colony screening (e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Edit., (1989), Cold Spring Harbor Laboratory Press, pages 1.93 to 1.104), or other techniques known to those of skill in the art.
Alternatively, two primers of 17-20 nucleotides derived from both ends of the SEQ ID NO:X (i.e., within the region of SEQ ID NO:X bounded by the 5' NT and the 3' NT of the clone defined in Table 1) are synthesized and used to amplify the desired cDNA using the deposited cDNA plasmid as a template. The polymerase chain reaction is carried out under routine conditions, for instance, in 25 μl of reaction mixture with 0.5 μg of the above cDNA template. A convenient reaction mixture is 1.5-5 mM MgCl₂, 0.01% (w/v) gelatin, 20 μM each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of PCR (denaturation at 94°C for 1 min; annealing at 55°C for 1 min; elongation at 72°C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the selected sequence by subcloning and sequencing the DNA product.

Several methods are available for the identification of the 5' or 3' non-coding portions of a gene which may not be present in the deposited clone. These methods include but are not limited to, filter probing, clone enrichment using specific probes, and protocols similar or identical to 5' and 3' "RACE" protocols which are well known in the art. For instance, a method similar to 5' RACE is available for generating the missing 5' end of a desired full-length transcript. (Fromont-Racine et al., Nucleic Acids Res. 21(7):1683-1684 (1993).)

Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcripts. A primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest is used to PCR amplify the 5' portion of the desired full-length gene. This amplified product may then be sequenced and used to generate the full length gene.

This above method starts with total RNA isolated from the desired source, although poly-A+ RNA can be used. The RNA preparation can then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase should then be inactivated and the RNA treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

This modified RNA preparation is used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction is
used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the desired gene.

Example 2: Isolation of Genomic Clones Corresponding to a Polynucleotide

A human genomic P1 library (Genomic Systems, Inc.) is screened by PCR using primers selected for the cDNA sequence corresponding to SEQ ID NO:X,, according to the method described in Example 1. (See also, Sambrook.)

Example 3: Tissue Distribution of Polypeptide

Tissue distribution of mRNA expression of polynucleotides of the present invention is determined using protocols for Northern blot analysis, described by, among others, Sambrook et al. For example, a cDNA probe produced by the method described in Example 1 is labeled with $^{32}$P using the rediprime™ DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe is purified using CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe is then used to examine various human tissues for mRNA expression.

Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) (Clontech) are examined with the labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70°C overnight, and the films developed according to standard procedures.

Example 4: Chromosomal Mapping of the Polynucleotides

An oligonucleotide primer set is designed according to the sequence at the 5' end of SEQ ID NO:X. This primer preferably spans about 100 nucleotides. This primer set is then used in a polymerase chain reaction under the following set of conditions: 30 seconds, 95°C; 1 minute, 56°C; 1 minute, 70°C. This cycle is repeated 32 times followed by one 5 minute cycle at 70°C. Human, mouse, and hamster DNA is used as template in addition to a somatic cell hybrid panel containing individual chromosomes or chromosome fragments (Bios, Inc). The reactions are analyzed on
either 8% polyacrylamide gels or 3.5% agarose gels. Chromosome mapping is
determined by the presence of an approximately 100 bp PCR fragment in the particular
somatic cell hybrid.

Example 5: Bacterial Expression of a Polypeptide

A polynucleotide encoding a polypeptide of the present invention is amplified
using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA
sequence, as outlined in Example 1, to synthesize insertion fragments. The primers
used to amplify the cDNA insert should preferably contain restriction sites, such as
BamHI and XbaI, at the 5' end of the primers in order to clone the amplified product
into the expression vector. For example, BamHI and XbaI correspond to the restriction
enzyme sites on the bacterial expression vector pQE-9. (Qiagen, Inc., Chatsworth,
CA). This plasmid vector encodes antibiotic resistance (Amp¹), a bacterial origin of
replication (ori), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site
(RBS), a 6-histidine tag (6-His), and restriction enzyme cloning sites.

The pQE-9 vector is digested with BamHI and XbaI and the amplified fragment
is ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial
RBS. The ligation mixture is then used to transform the E. coli strain M15/rep4
(Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which expresses
the lacI repressor and also confers kanamycin resistance (Kan¹). Transformants are
identified by their ability to grow on LB plates and ampicillin/kanamycin resistant
colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid
culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml).
The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The
cells are grown to an optical density 600 (O.D.⁶⁰⁰) of between 0.4 and 0.6. IPTG
(Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1 mM.
IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased
gene expression.

Cells are grown for an extra 3 to 4 hours. Cells are then harvested by
centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic
agent 6 Molar Guanidine HCl by stirring for 3-4 hours at 4°C. The cell debris is
removed by centrifugation, and the supernatant containing the polypeptide is loaded
onto a nickel-nitrido-tri-acetic acid ("Ni-NTA") affinity resin column (available from
QIAGEN, Inc., supra). Proteins with a 6 x His tag bind to the Ni-NTA resin with high
affinity and can be purified in a simple one-step procedure (for details see: The

Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8, the
column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed
with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is eluted with
6 M guanidine-HCl, pH 5.

The purified protein is then renatured by dialyzing it against phosphate-buffered
saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the
protein can be successfully refolded while immobilized on the Ni-NTA column. The
recommended conditions are as follows: renature using a linear 6M-1M urea gradient in
500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors.
The renaturation should be performed over a period of 1.5 hours or more. After
renaturation the proteins are eluted by the addition of 250 mM imidazole. Imidazole
is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer
plus 200 mM NaCl. The purified protein is stored at 4°C or frozen at -80°C.

In addition to the above expression vector, the present invention further includes
an expression vector comprising phage operator and promoter elements operatively
linked to a polynucleotide of the present invention, called pHE4a. (ATCC Accession
Number 209645, deposited on February 25, 1998.) This vector contains: 1) a
neomycinphosphotransferase gene as a selection marker, 2) an E. coli origin of
replication, 3) a T5 phage promoter sequence, 4) two lac operator sequences, 5) a
Shine-Delgarno sequence, and 6) the lactose operon repressor gene (lacIq). The origin
of replication (oriC) is derived from pUC19 (LTI, Gaithersburg, MD). The promoter
sequence and operator sequences are made synthetically.

DNA can be inserted into the pHEa by restricting the vector with NdeI and
XbaI, BamHI, XhoI, or Asp718, running the restricted product on a gel, and isolating
the larger fragment (the stuffer fragment should be about 310 base pairs). The DNA
insert is generated according to the PCR protocol described in Example 1, using PCR
primers having restriction sites for NdeI (5' primer) and XbaI, BamHI, XhoI, or
Asp718 (3' primer). The PCR insert is gel purified and restricted with compatible
enzymes. The insert and vector are ligated according to standard protocols.

The engineered vector could easily be substituted in the above protocol to
express protein in a bacterial system.
Example 6: Purification of a Polypeptide from an Inclusion Body

The following alternative method can be used to purify a polypeptide expressed in E. coli when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10°C.

5 Upon completion of the production phase of the E. coli fermentation, the cell culture is cooled to 4-10°C and the cells harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

The cells are then lysed by passing the solution through a microfluidizer (Microfluidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 xg centrifugation for 15 min., the pellet is discarded and the polypeptide containing supernatant is incubated at 4°C overnight to allow further GuHCl extraction.

Following high speed centrifugation (30,000 xg) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous stirring. The refolded diluted protein solution is kept at 4°C without mixing for 12 hours prior to further purification steps.

To clarify the refolded polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16 μm membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perceptive Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.
Fractions containing the polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant \( A_{280} \) monitoring of the effluent. Fractions containing the polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant polypeptide should exhibit greater than 95% purity after the above refolding and purification steps. No major contaminant bands should be observed from Comassie blue stained 16% SDS-PAGE gel when 5 \( \mu \)g of purified protein is loaded. The purified protein can also be tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

**Example 7: Cloning and Expression of a Polypeptide in a Baculovirus Expression System**

In this example, the plasmid shuttle vector pA2 is used to insert a polynucleotide into a baculovirus to express a polypeptide. This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as BamHI, Xba I and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak Drosophila promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned polynucleotide.

Many other baculovirus vectors can be used in place of the vector above, such as pAc373, pVL941, and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., Virology 170:31-39 (1989).
Specifically, the cDNA sequence contained in the deposited clone, including the AUG initiation codon and the naturally associated leader sequence identified in Table 1, is amplified using the PCR protocol described in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the pA2 vector does not need a second signal peptide. Alternatively, the vector can be modified (pA2 GP) to include a baculovirus leader sequence, using the standard methods described in Summers et al., "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures," Texas Agricultural Experimental Station Bulletin No. 1555 (1987).

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("GeneClean" BIO 101 Inc., La Jolla, Ca.).

The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. E. coli HB101 or other suitable E. coli hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria containing the plasmid are identified by digesting DNA from individual colonies and analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing.

Five μg of a plasmid containing the polynucleotide is co-transfected with 1.0 μg of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417 (1987). One μg of BaculoGold™ virus DNA and 5 μg of the plasmid are mixed in a sterile well of a microtiter plate containing 50 μl of serum-free Grace’s medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 μl Lipofectin plus 90 μl Grace’s medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace’s medium without serum. The plate is then incubated for 5 hours at 27° C. The transfection solution is then removed from the plate and 1 ml of Grace’s insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27° C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, supra. An agarose gel with "Blue Gal" (Life
Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of
gal-expressing clones, which produce blue-stained plaques. (A detailed description of a
"plaque assay" of this type can also be found in the user's guide for insect cell culture
and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10.)

After appropriate incubation, blue stained plaques are picked with the tip of a
micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then
resuspended in a microcentrifuge tube containing 200 μl of Grace's medium and the
suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in
35 mm dishes. Four days later the supernatants of these culture dishes are harvested
and then they are stored at 4°C.

To verify the expression of the polypeptide, Sf9 cells are grown in Grace's
medium supplemented with 10% heat-inactivated FBS. The cells are infected with the
recombinant baculovirus containing the polynucleotide at a multiplicity of infection
("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is
removed and is replaced with SF900 II medium minus methionine and cysteine
(available from Life Technologies Inc., Rockville, MD). After 42 hours, 5 μCi of 35S-
methionine and 5 μCi 35S-cysteine (available from Amersham) are added. The cells are
further incubated for 16 hours and then are harvested by centrifugation. The proteins in
the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE
followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified
protein may be used to determine the amino terminal sequence of the produced protein.

Example 8: Expression of a Polypeptide in Mammalian Cells

The polypeptide of the present invention can be expressed in a mammalian cell.

A typical mammalian expression vector contains a promoter element, which mediates
the initiation of transcription of mRNA, a protein coding sequence, and signals required
for the termination of transcription and polyadenylation of the transcript. Additional
elements include enhancers, Kozak sequences and intervening sequences flanked by
donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved
with the early and late promoters from SV40, the long terminal repeats (LTRs) from
Retroviruses, e.g., RSV, HTLV-I, HIV-I and the early promoter of the cytomegalovirus
(CMV). However, cellular elements can also be used (e.g., the human actin promoter).

Suitable expression vectors for use in practicing the present invention include,
for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden),
pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12MI (ATCC 67109),
pCMVSport 2.0, and pCMVSport 3.0. Mammalian host cells that could be used
include, human Hela, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the polypeptide can be expressed in stable cell lines containing the polynucleotide integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that carry several hundred or even several thousand copies of the gene of interest. (See, e.g., Alt, F. W., et al., J. Biol. Chem. 253:1357-1370 (1978); Hamlin, J. L. and Ma, C., Biochem. et Biophys. Acta, 1097:107-143 (1990); Page, M. J. and Sydenham, M. A., Biotechnology 9:64-68 (1991).) Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., Biochem J. 227:277-279 (1991); Bebbington et al., Bio/Technology 10:169-175 (1992).) Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

Derivatives of the plasmid pSV2-dhfr (ATCC Accession No. 37146), the expression vectors pC4 (ATCC Accession No. 209646) and pC6 (ATCC Accession No.209647) contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., Molecular and Cellular Biology, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart et al., Cell 41:521-530 (1985).) Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors also contain the 3’ intron, the polyadenylation and termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control of the SV40 early promoter.

Specifically, the plasmid pC6, for example, is digested with appropriate restriction enzymes and then dephosphorylated using calf intestinal phosphatases by procedures known in the art. The vector is then isolated from a 1% agarose gel.

A polynucleotide of the present invention is amplified according to the protocol outlined in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the vector does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)
The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The amplified fragment is then digested with the same restriction enzyme and purified on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC6 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene is used for transfection. Five µg of the expression plasmid pC6 is cotransfected with 0.5 µg of the plasmid pSV2-neo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the neo gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 ng/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 µM, 2 µM, 5 µM, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200 µM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

**Example 9: Protein Fusions**

The polypeptides of the present invention are preferably fused to other proteins. These fusion proteins can be used for a variety of applications. For example, fusion of the present polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification. (See Example 5; see also EP A 394,827; Traunecker, et al., *Nature* 331:84-86 (1988).) Similarly, fusion to IgG-1, IgG-3, and albumin increases the halflife time in vivo. Nuclear localization signals fused to the polypeptides of the present invention can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion
proteins described above can be made by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule, or the protocol described in Example 5.

Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5’ and 3’ ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

For example, if pC4 (Accession No. 209646) is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3’ BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the vector, and a polynucleotide of the present invention, isolated by the PCR protocol described in Example 1, is ligated into this BamHI site. Note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be produced.

If the naturally occurring signal sequence is used to produce the secreted protein, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g.,WO 96/34891.)

20 Human IgG Fc region:

GGGATTCGGAGCCCAATCTTTCTGACAAAAACTCACACATGCCCAATCGTCG
CAGCACCTGAGAATCGAGCTCATCTCTCTCTTTCCCTCTCCCTCTCCCTCT
CAAAGGACAATCTCAAGTCTGATCTCCGGGATCTGGTACATGCTGGTGG
GGACGTGAAGCCAGGACCCCTGTGAGTTGCTCAAGTTTACACTGTAGTGACG

25 GCCTGAGAGTGTCATATGGCAAGAAAGCAGCAGGGAGAGCAGTACAAAC
AGCAGCTACCGTGTGGTGCTACGCTCTCTGACGAGCTGACCACTGGGCTG
ATGCCAAGGAGTCAAGGCAAGGCTTCAGGATCAAAAGGCCCTCTCCCTCTCCCT
ACCTGAGAAACCATCTCTCTGAAAGGCAAGGAGGAGCAGGACCCAGACGCCG
GTACACCCTGCCCCCAATCCGGAATGAGCTGAGAAAGAAACAGGCTAGCCT

30 GACCTGCCTGTCAAAGGCTCTCTAAATCAGACATCGCCTGGAGTGGA
GAGCAATGGGCAAGGAGGAAGAACTACAAAGACACCGCTCTCGGCTGG
ACCTGGAGCGCTCTTCTCTCTGACTGAAGGCTACCCGTTGAGAAAGAGCA
GGTTGCAAGGGAAGCTCTTCTCTATGCCTCCAGTGGATGCAAGAGGCTGTC
ACAACACCCTCAGGAGAAGAGCCTCTCTGTGCTCGGGGTTAATGAGTG

35 GACGGCAGGACTCTAGAGGAT (SEQ ID NO:1)
Example 10: Production of an Antibody from a Polypeptide

The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) For example, cells expressing a polypeptide of the present invention is administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of the secreted protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or protein binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology. (Köhler et al., Nature 256:495 (1975); Köhler et al., Eur. J. Immunol. 6:511 (1976); Köhler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981).) In general, such procedures involve immunizing an animal (preferably a mouse) with polypeptide or, more preferably, with a secreted polypeptide-expressing cell. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56°C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µg/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981).) The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide.

Alternatively, additional antibodies capable of binding to the polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the protein-specific antibody can be blocked by the polypeptide.
Such antibodies comprise anti-idiotypic antibodies to the protein-specific antibody and can be used to immunize an animal to induce formation of further protein-specific antibodies.

It will be appreciated that Fab and F(ab')2 and other fragments of the antibodies of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments). Alternatively, secreted protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

For in vivo use of antibodies in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

**Example 11: Production Of Secreted Protein For High-Throughput Screening Assays**

The following protocol produces a supernatant containing a polypeptide to be tested. This supernatant can then be used in the Screening Assays described in Examples 13-20.

First, dilute Poly-D-Lysine (644 587 Boehringer-Mannheim) stock solution (1mg/ml in PBS) 1:20 in PBS (w/o calcium or magnesium 17-516F Biowhittaker) for a working solution of 50ug/ml. Add 200 ul of this solution to each well (24 well plates) and incubate at RT for 20 minutes. Be sure to distribute the solution over each well (note: a 12-channel pipetter may be used with tips on every other channel). Aspirate off the Poly-D-Lysine solution and rinse with 1ml PBS (Phosphate Buffered Saline). The PBS should remain in the well until just prior to plating the cells and plates may be poly-lysine coated in advance for up to two weeks.

Plate 293T cells (do not carry cells past P+20) at 2 x 10^5 cells/well in .5ml DMEM(Dulbecco’s Modified Eagle Medium)(with 4.5 G/L glucose and L-glutamine (12-604F Biowhittaker))/10% heat inactivated FBS(14-503F Biowhittaker)/1x Penstrep(17-602E Biowhittaker). Let the cells grow overnight.
The next day, mix together in a sterile solution basin: 300 ul Lipofectamine (18324-012 Gibco/BRL) and 5ml OptiMEM I (31985070 Gibco/BRL)/96-well plate. With a small volume multi-channel pipetter, aliquot approximately 2ug of an expression vector containing a polynucleotide insert, produced by the methods described in Examples 8 or 9, into an appropriately labeled 96-well round bottom plate. With a multi-channel pipetter, add 50ul of the Lipofectamine/OptiMEM I mixture to each well. Pipette up and down gently to mix. Incubate at RT 15-45 minutes. After about 20 minutes, use a multi-channel pipetter to add 150ul OptiMEM I to each well. As a control, one plate of vector DNA lacking an insert should be transfected with each set of transfections.

Preferably, the transfection should be performed by tag-teaming the following tasks. By tag-teaming, hands on time is cut in half, and the cells do not spend too much time on PBS. First, person A aspirates off the media from four 24-well plates of cells, and then person B rinses each well with .5-1ml PBS. Person A then aspirates off PBS rinse, and person B, using a12-channel pipetter with tips on every other channel, adds the 200ul of DNA/Lipofectamine/OptiMEM I complex to the odd wells first, then to the even wells, to each row on the 24-well plates. Incubate at 37°C for 6 hours.

While cells are incubating, prepare appropriate media, either 1%BSA in DMEM with 1x penistrep, or CHO-5 media (116.6 mg/L of CaCl2 (anhyd); 0.00130 mg/L CuSO4·5H2O; 0.050 mg/L of Fe(NO3)3·9H2O; 0.417 mg/L of FeSO4·7H2O; 311.80 mg/L of KCl; 28.64 mg/L of MgCl2; 48.84 mg/L of MgSO4; 6995.50 mg/L of NaCl; 2400.0 mg/L of NaHCO3; 62.50 mg/L of NaH2PO4·H2O; 71.02 mg/L of Na2HPO4; 0.4320 mg/L of ZnSO4·7H2O; .002 mg/L of Arachidonic Acid; 1.022 mg/L of Cholesterol; .070 mg/L of DL-alpha-Tocopherol-Acetate; 0.0520 mg/L of Linoleic Acid; 0.010 mg/L of Linolenic Acid; 0.010 mg/L of Myristic Acid; 0.010 mg/L of Oleic Acid; 0.010 mg/L of Palmitic Acid; 0.010 mg/L of Palmitic Acid; 100 mg/L of Pluronic F-68; 0.010 mg/L of Stearic Acid; 2.20 mg/L of Tween 80; 4551 mg/L of D-Glucose; 130.85 mg/ml of L-Alanine; 147.50 mg/ml of L-Arginine-HCL; 7.50 mg/ml of L-Asparagin-H2O; 6.65 mg/ml of L-Aspartic Acid; 29.56 mg/ml of L-Cystine-2HCL-H2O; 31.29 mg/ml of L-Cysteine-2HCL; 7.35 mg/ml of L-Glutamic Acid; 365.0 mg/ml of L-Glutamine; 18.75 mg/ml of Glycine; 52.48 mg/ml of L-Histidine-HCL-H2O; 106.97 mg/ml of L-Isoleucine; 111.45 mg/ml of L-Leucine; 163.75 mg/ml of L-Lysine HCL; 32.34 mg/ml of L-Methionine; 68.48 mg/ml of L-Phenylalainine; 40.0 mg/ml of L-Proline; 26.25 mg/ml of L-Serine; 101.05 mg/ml of L-Threonine; 19.22 mg/ml of L-Tryptophan; 91.79 mg/ml of L-Tyrosine-2Na-2H2O; 99.65 mg/ml of L-Valine; 0.0035 mg/L of Biotin; 3.24 mg/L of D-Ca Pantothenate; 11.78 mg/L of...
Choline Chloride; 4.65 mg/L of Folic Acid; 15.60 mg/L of i-Inositol; 3.02 mg/L of Niacinamide; 3.00 mg/L of Pyridoxal HCL; 0.031 mg/L of Pyridoxine HCL; 0.319 mg/L of Riboflavin; 3.17 mg/L of Thiamine HCL; 0.365 mg/L of Thymidine; and 0.680 mg/L of Vitamin B<sub>12</sub>; 25 mM of HEPES Buffer; 2.39 mg/L of Na Hypoxanthine; 0.105 mg/L of Lipoic Acid; 0.081 mg/L of Sodium Putrescine-2HCL; 55.0 mg/L of Sodium Pyruvate; 0.0067 mg/L of Sodium Selenite; 20uM of Ethanolamine; 0.122 mg/L of Ferric Citrate; 41.70 mg/L of Methyl-B-Cyclodextrin complexed with Linoleic Acid; 33.33 mg/L of Methyl-B-Cyclodextrin complexed with Oleic Acid; and 10 mg/L of Methyl-B-Cyclodextrin complexed with Retinal) with 2mm glutamine and 1x penstrep. (BSA (81-068-3 Bayer) 100gm dissolved in 1L DMEM for a 10% BSA stock solution). Filter the media and collect 50 ul for endotoxin assay in 15ml polystyrene conical.

The transfection reaction is terminated, preferably by tag-teaming, at the end of the incubation period. Person A aspirates off the transfection media, while person B adds 1.5ml appropriate media to each well. Incubate at 37°C for 45 or 72 hours depending on the media used: 1%BSA for 45 hours or CHO-5 for 72 hours.

On day four, using a 300ul multichannel pipetter, aliquot 600ul in one 1ml deep well plate and the remaining supernatant into a 2ml deep well. The supernatants from each well can then be used in the assays described in Examples 13-20.

It is specifically understood that when activity is obtained in any of the assays described below using a supernatant, the activity originates from either the polypeptide directly (e.g., as a secreted protein) or by the polypeptide inducing expression of other proteins, which are then secreted into the supernatant. Thus, the invention further provides a method of identifying the protein in the supernatant characterized by an activity in a particular assay.

**Example 12: Construction of GAS Reporter Construct**

One signal transduction pathway involved in the differentiation and proliferation of cells is called the Jaks-STATs pathway. Activated proteins in the Jaks-STATs pathway bind to gamma activation site “GAS” elements or interferon-sensitive responsive element (“ISRE”), located in the promoter of many genes. The binding of a protein to these elements alter the expression of the associated gene.

GAS and ISRE elements are recognized by a class of transcription factors called Signal Transducers and Activators of Transcription, or “STATs.” There are six members of the STATs family. Stat1 and Stat3 are present in many cell types, as is Stat2 (as response to IFN-alpha is widespread). Stat4 is more restricted and is not in
many cell types though it has been found in T helper class I, cells after treatment with IL-12. Stat5 was originally called mammary growth factor, but has been found at higher concentrations in other cells including myeloid cells. It can be activated in tissue culture cells by many cytokines.

The STATs are activated to translocate from the cytoplasm to the nucleus upon tyrosine phosphorylation by a set of kinases known as the Janus Kinase ("Jaks") family. Jaks represent a distinct family of soluble tyrosine kinases and include Tyk2, Jak1, Jak2, and Jak3. These kinases display significant sequence similarity and are generally catalytically inactive in resting cells.

The Jaks are activated by a wide range of receptors summarized in the Table below. (Adapted from review by Schidler and Darnell, Ann. Rev. Biochem. 64:621-51 (1995).) A cytokine receptor family, capable of activating Jaks, is divided into two groups: (a) Class 1 includes receptors for IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-11, IL-12, IL-15, Epo, PRL, GH, G-CSF, GM-CSF, LIF, CNTF, and thrombopoietin; and (b) Class 2 includes IFN-a, IFN-g, and IL-10. The Class 1 receptors share a conserved cysteine motif (a set of four conserved cysteines and one tryptophan) and a WSXWS motif (a membrane proxial region encoding Trp-Ser-Xxx-Trp-Ser (SEQ ID NO:2)).

Thus, on binding of a ligand to a receptor, Jaks are activated, which in turn activate STATs, which then translocate and bind to GAS elements. This entire process is encompassed in the Jaks-STATs signal transduction pathway.

Therefore, activation of the Jaks-STATs pathway, reflected by the binding of the GAS or the ISRE element, can be used to indicate proteins involved in the proliferation and differentiation of cells. For example, growth factors and cytokines are known to activate the Jaks-STATs pathway. (See Table below.) Thus, by using GAS elements linked to reporter molecules, activators of the Jaks-STATs pathway can be identified.
<table>
<thead>
<tr>
<th>Ligand</th>
<th>tyk2</th>
<th>JAKs</th>
<th>Jak1</th>
<th>Jak2</th>
<th>Jak3</th>
<th>STATS</th>
<th>GAS(elements) or ISRE</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN family</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN-a/B</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>1,2,3</td>
<td></td>
<td>ISRE</td>
</tr>
<tr>
<td>IFN-g</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td></td>
<td>GAS (IRF1&gt;Lys6&gt;IFP)</td>
</tr>
<tr>
<td>IL-10</td>
<td>+</td>
<td>?</td>
<td>?</td>
<td>-</td>
<td>1,3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>gp130 family</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 (Pleiotrophic)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>1,3</td>
<td></td>
<td>GAS (IRF1&gt;Lys6&gt;IFP)</td>
</tr>
<tr>
<td>IL-11(Pleiotrophic)</td>
<td>?</td>
<td>+</td>
<td>?</td>
<td>?</td>
<td>1,3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OnM(Pleiotrophic)</td>
<td>?</td>
<td>+</td>
<td>?</td>
<td>?</td>
<td>1,3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LIF(Pleiotrophic)</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>1,3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNTF(Pleiotrophic)</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>1,3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-CSF(Pleiotrophic)</td>
<td>?</td>
<td>+</td>
<td>?</td>
<td>?</td>
<td>1,3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-12(Pleiotrophic)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>1,3</td>
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<tr>
<td>g-c family</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-2 (lymphocytes)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>1,3,5</td>
<td></td>
<td>GAS</td>
</tr>
<tr>
<td>IL-4 (lymph/myeloid)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>6</td>
<td></td>
<td>GAS (IRF1 = IFP &gt;&gt; Ly6)(IgH)</td>
</tr>
<tr>
<td>IL-7 (lymphocytes)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>5</td>
<td></td>
<td>GAS</td>
</tr>
<tr>
<td>IL-9 (lymphocytes)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>5</td>
<td></td>
<td>GAS</td>
</tr>
<tr>
<td>IL-13 (lymphocyte)</td>
<td>-</td>
<td>+</td>
<td>?</td>
<td>?</td>
<td>6</td>
<td></td>
<td>GAS</td>
</tr>
<tr>
<td>IL-15</td>
<td>?</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>5</td>
<td></td>
<td>GAS</td>
</tr>
<tr>
<td>gp140 family</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-3 (myeloid)</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>5</td>
<td></td>
<td>GAS (IRF1&gt;IFP&gt;&gt;Ly6)</td>
</tr>
<tr>
<td>IL-5 (myeloid)</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>5</td>
<td></td>
<td>GAS</td>
</tr>
<tr>
<td>GM-CSF (myeloid)</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>5</td>
<td></td>
<td>GAS</td>
</tr>
<tr>
<td>Growth hormone family</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>GH</td>
<td>?</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>5</td>
<td></td>
<td>GAS</td>
</tr>
<tr>
<td>PRL</td>
<td>?</td>
<td>+/-</td>
<td>+</td>
<td>-</td>
<td>1,3,5</td>
<td></td>
<td>GAS (B-CAS&gt;IRF1=IFP&gt;&gt;Ly6)</td>
</tr>
<tr>
<td>EPO</td>
<td>?</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>5</td>
<td></td>
<td>GAS (B-CAS&gt;IRF1=IFP&gt;&gt;Ly6)</td>
</tr>
<tr>
<td>Receptor Tyrosine Kinases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGF</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>1,3</td>
<td></td>
<td>GAS (IRF1)</td>
</tr>
<tr>
<td>PDGF</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>1,3</td>
<td></td>
<td>GAS (not IRF1)</td>
</tr>
<tr>
<td>CSF-1</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>1,3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
To construct a synthetic GAS containing promoter element, which is used in the Biological Assays described in Examples 13-14, a PCR based strategy is employed to generate a GAS-SV40 promoter sequence. The 5’ primer contains four tandem copies of the GAS binding site found in the IRF1 promoter and previously demonstrated to bind STATs upon induction with a range of cytokines (Rothman et al., Immunity 1:457-468 (1994).), although other GAS or ISRE elements can be used instead. The 5’ primer also contains 18bp of sequence complementary to the SV40 early promoter sequence and is flanked with an XhoI site. The sequence of the 5’ primer is:

5’:GGCGCTCGAGATTTACCCTGGAAATCTAGATTTCGGAAATGATTTCCCCGAAATGATTTCCCGAAATATCCTGCACTCTCAATTAGTCAGCAACCATGTC

The downstream primer is complementary to the SV40 promoter and is flanked with a Hind III site: 5’:GGCGGCAAGCTTTTTGTCAAAGGCCTAGGC

PCR amplification is performed using the SV40 promoter template present in the B-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI/Hind III and subcloned into BLSK2-. (Stratagene.) Sequencing with forward and reverse primers confirms that the insert contains the following sequence:5’:CTCGAGATTTACCCTGGAAATCTAGATTTCGGAAATGATTTCCCCGAAATGATTTCCCGAAATATCCTGCACTCTCAATTAGTCAGCAACCATGTC

20 CCGCCCCTAATCTCCGCCATCCCGCACCCTAACCTCCGCCAGTTCGGCCCATTCTCGGCGCATGGCGATCTAAATTTTTTTATTTATGCAGAGCCGCAGGGCCGCCTCGGCCTCAGCTAGCTATCCGAGAAGTAGTGAGGGAGGGCTTTTTTTGGAGGCTCCTAGGCTTTTTGCCCGAAGCTT;3’ (SEQ ID NO:5)

With this GAS promoter element linked to the SV40 promoter, a GAS:SEAP2 reporter construct is next engineered. Here, the reporter molecule is a secreted alkaline phosphatase, or “SEAP.” Clearly, however, any reporter molecule can be instead of SEAP, in this or in any of the other Examples. Well known reporter molecules that can be used instead of SEAP include chloramphenicol acetyltransferase (CAT), luciferase, alkaline phosphatase, B-galactosidase, green fluorescent protein (GFP), or any protein detectable by an antibody.

The above sequence confirmed synthetic GAS-SV40 promoter element is subcloned into the pSEAP-Promoter vector obtained from Clontech using HindIII and XhoI, effectively replacing the SV40 promoter with the amplified GAS:SV40 promoter element, to create the GAS-SEAP vector. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.
Thus, in order to generate mammalian stable cell lines expressing the GAS-SEAP reporter, the GAS-SEAP cassette is removed from the GAS-SEAP vector using SalI and NotI, and inserted into a backbone vector containing the neomycin resistance gene, such as pGFP-1 (Clontech), using these restriction sites in the multiple cloning site, to create the GAS-SEAP/Neo vector. Once this vector is transfected into mammalian cells, this vector can then be used as a reporter molecule for GAS binding as described in Examples 13-14.

Other constructs can be made using the above description and replacing GAS with a different promoter sequence. For example, construction of reporter molecules containing NFkB and EGR promoter sequences are described in Examples 15 and 16. However, many other promoters can be substituted using the protocols described in these Examples. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be substituted, alone or in combination (e.g., GAS/NF-KB/EGR, GAS/NF-KB, IL-2/NFAT, or NF-KB/GAS). Similarly, other cell lines can be used to test reporter construct activity, such as HELA (epithelial), HUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), HUVAC (aortic), or Cardiomyocyte.

Example 13: High-Throughput Screening Assay for T-cell Activity.

The following protocol is used to assess T-cell activity by identifying factors, such as growth factors and cytokines, that may proliferate or differentiate T-cells. T-cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The T-cell used in this assay is Jurkat T-cells (ATCC Accession No. TIB-152), although Molt-3 cells (ATCC Accession No. CRL-1552) and Molt-4 cells (ATCC Accession No. CRL-1582) cells can also be used.

Jurkat T-cells are lymphoblastic CD4+ Th1 helper cells. In order to generate stable cell lines, approximately 2 million Jurkat cells are transfected with the GAS-SEAP/neo vector using DMRIE-C (Life Technologies)(transfection procedure described below). The transfected cells are seeded to a density of approximately 20,000 cells per well and transfectants resistant to 1 mg/ml genticin selected. Resistant colonies are expanded and then tested for their response to increasing concentrations of interferon gamma. The dose response of a selected clone is demonstrated.

Specifically, the following protocol will yield sufficient cells for 75 wells containing 200 ul of cells. Thus, it is either scaled up, or performed in multiple to generate sufficient cells for multiple 96 well plates. Jurkat cells are maintained in RPMI + 10% serum with 1%Pen-Strep. Combine 2.5 mls of OPTI-MEM (Life Technologies)
with 10 ug of plasmid DNA in a T25 flask. Add 2.5 ml OPTI-MEM containing 50 ul of DMRIE-C and incubate at room temperature for 15-45 mins.

During the incubation period, count cell concentration, spin down the required number of cells (10^9 per transfection), and resuspend in OPTI-MEM to a final concentration of 10^7 cells/ml. Then add 1ml of 1 x 10^7 cells in OPTI-MEM to T25 flask and incubate at 37°C for 6 hrs. After the incubation, add 10 ml of RPMI + 15% serum.

The Jurkat:GAS-SEAP stable reporter lines are maintained in RPMI + 10% serum, 1 mg/ml Genticin, and 1% Pen-Strep. These cells are treated with supernatants containing a polypeptide as produced by the protocol described in Example 11.

On the day of treatment with the supernatant, the cells should be washed and resuspended in fresh RPMI + 10% serum to a density of 500,000 cells per ml. The exact number of cells required will depend on the number of supernatants being screened. For one 96 well plate, approximately 10 million cells (for 10 plates, 100 million cells) are required.

Transfer the cells to a triangular reservoir boat, in order to dispense the cells into a 96 well dish, using a 12 channel pipette. Using a 12 channel pipette, transfer 200 ul of cells into each well (therefore adding 100,000 cells per well).

After all the plates have been seeded, 50 ul of the supernatants are transferred directly from the 96 well plate containing the supernatants into each well using a 12 channel pipette. In addition, a dose of exogenous interferon gamma (0.1, 1.0, 10 ng) is added to wells H9, H10, and H11 to serve as additional positive controls for the assay.

The 96 well dishes containing Jurkat cells treated with supernatants are placed in an incubator for 48 hrs (note: this time is variable between 48-72 hrs). 35 ul samples from each well are then transferred to an opaque 96 well plate using a 12 channel pipette. The opaque plates should be covered (using sellophene covers) and stored at -20°C until SEAP assays are performed according to Example 17. The plates containing the remaining treated cells are placed at 4°C and serve as a source of material for repeating the assay on a specific well if desired.

As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate Jurkat T cells. Over 30 fold induction is typically observed in the positive control wells.
**Example 14: High-Throughput Screening Assay Identifying Myeloid Activity**

The following protocol is used to assess myeloid activity by identifying factors, such as growth factors and cytokines, that may proliferate or differentiate myeloid cells.

Myeloid cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The myeloid cell used in this assay is U937, a pre-monocyte cell line, although TF-1, HL60, or KG1 can be used.

To transiently transfec{t} U937 cells with the GAS/SEAP/Neo construct produced in Example 12, a DEAE-Dextran method (Kharbanda et. al., 1994, Cell Growth & Differentiation, 5:259-265) is used. First, harvest $2 \times 10^6$ U937 cells and wash with PBS. The U937 cells are usually grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin.

Next, suspend the cells in 1 ml of 20 mM Tris-HCl (pH 7.4) buffer containing 0.5 mg/ml DEAE-Dextran, 8 ug GAS-SEAP2 plasmid DNA, 140 mM NaCl, 5 mM KCl, 375 uM Na$_2$HPO$_4.7$H$_2$O, 1 mM MgCl$_2$, and 675 uM CaCl$_2$. Incubate at 37°C for 45 min.

Wash the cells with RPMI 1640 medium containing 10% FBS and then resuspend in 10 ml complete medium and incubate at 37°C for 36 hr.

The GAS-SEAP/U937 stable cells are obtained by growing the cells in 400 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 400 ug/ml G418 for couple of passages.

These cells are tested by harvesting $1 \times 10^8$ cells (this is enough for ten 96-well plates assay) and wash with PBS. Suspend the cells in 200 ml above described growth medium, with a final density of $5 \times 10^5$ cells/ml. Plate 200 ul cells per well in the 96-well plate (or $1 \times 10^6$ cells/well).

Add 50 ul of the supernatant prepared by the protocol described in Example 11. Incubate at 37°C for 48 to 72 hr. As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate U937 cells. Over 30 fold induction is typically observed in the positive control wells. SEAP assay the supernatant according to the protocol described in Example 17.
Example 15: High-Throughput Screening Assay Identifying Neuronal Activity.

When cells undergo differentiation and proliferation, a group of genes are activated through many different signal transduction pathways. One of these genes, EGR1 (early growth response gene 1), is induced in various tissues and cell types upon activation. The promoter of EGR1 is responsible for such induction. Using the EGR1 promoter linked to reporter molecules, activation of cells can be assessed.

Particularly, the following protocol is used to assess neuronal activity in PC12 cell lines. PC12 cells (rat phenochromocytoma cells) are known to proliferate and/or differentiate by activation with a number of mitogens, such as TPA (tetradecanoyl phorbol acetate), NGF (nerve growth factor), and EGF (epidermal growth factor). The EGR1 gene expression is activated during this treatment. Thus, by stably transfecting PC12 cells with a construct containing an EGR promoter linked to SEAP reporter, activation of PC12 cells can be assessed.

The EGR/SEAP reporter construct can be assembled by the following protocol. The EGR-1 promoter sequence (-633 to +1)(Sakamoto K et al., Oncogene 6:867-871 (1991)) can be PCR amplified from human genomic DNA using the following primers:

5' GCGCTCGAGGGATGACAGCGTAGAACCCTGGG -3' (SEQ ID NO:6)
5' GCGAAGCTTCCGGACCTCCCCGCGATCCGCCTC-3' (SEQ ID NO:7)

Using the GAS:SEAP/Neo vector produced in Example 12, EGR1 amplified product can then be inserted into this vector. Linearize the GAS:SEAP/Neo vector using restriction enzymes XhoI/HindIII, removing the GAS/SV40 stuffer. Restrict the EGR1 amplified product with these same enzymes. Ligate the vector and the EGR1 promoter.

To prepare 96 well-plates for cell culture, two mls of a coating solution (1:30 dilution of collagen type I (Upstate Biotech Inc. Cat#08-115) in 30% ethanol (filter sterilized)) is added per one 10 cm plate or 50 ml per well of the 96-well plate, and allowed to air dry for 2 hr.

PC12 cells are routinely grown in RPMI-1640 medium (Bio Whittaker) containing 10% horse serum (JRH BIOSCIENCES, Cat. # 12449-78P), 5% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 ug/ml streptomycin on a precoated 10 cm tissue culture dish. One to four split is done every three to four days. Cells are removed from the plates by scraping and resuspended with pipetting up and down for more than 15 times.

Transfect the EGR/SEAP/Neo construct into PC12 using the Lipofectamine protocol described in Example 11. EGR-SEAP/PC12 stable cells are obtained by growing the cells in 300 ug/ml G418. The G418-free medium is used for routine
growth but every one to two months, the cells should be re-grown in 300 ug/ml G418 for couple of passages.

To assay for neuronal activity, a 10 cm plate with cells around 70 to 80% confluent is screened by removing the old medium. Wash the cells once with PBS (Phosphate buffered saline). Then starve the cells in low serum medium (RPMI-1640 containing 1% horse serum and 0.5% FBS with antibiotics) overnight.

The next morning, remove the medium and wash the cells with PBS. Scrape off the cells from the plate, suspend the cells well in 2 ml low serum medium. Count the cell number and add more low serum medium to reach final cell density as 5x10^5 cells/ml.

Add 200 ul of the cell suspension to each well of 96-well plate (equivalent to 1x10^5 cells/well). Add 50 ul supernatant produced by Example 11, 37°C for 48 to 72 hr. As a positive control, a growth factor known to activate PC12 cells through EGR can be used, such as 50 ng/ul of Neuronal Growth Factor (NGF). Over fifty-fold induction of SEAP is typically seen in the positive control wells. SEAP assay the supernatant according to Example 17.

Example 16: High-Throughput Screening Assay for T-cell Activity

NF-κB (Nuclear Factor κB) is a transcription factor activated by a wide variety of agents including the inflammatory cytokines IL-1 and TNF, CD30 and CD40, lymphotxin-alpha and lymphotxin-beta, by exposure to LPS or thrombin, and by expression of certain viral gene products. As a transcription factor, NF-κB regulates the expression of genes involved in immune cell activation, control of apoptosis (NF-κB appears to shield cells from apoptosis), B and T-cell development, anti-viral and antimicrobial responses, and multiple stress responses.

In non-stimulated conditions, NF-κB is retained in the cytoplasm with I-κB (Inhibitor κB). However, upon stimulation, I-κB is phosphorylated and degraded, causing NF-κB to shuttle to the nucleus, thereby activating transcription of target genes. Target genes activated by NF-κB include IL-2, IL-6, GM-CSF, ICAM-1 and class 1 MHC.

Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the NF-κB promoter element are used to screen the supernatants produced in Example 11. Activators or inhibitors of NF-κB would be useful in treating
diseases. For example, inhibitors of NF-κB could be used to treat those diseases related to the acute or chronic activation of NF-κB, such as rheumatoid arthritis.

To construct a vector containing the NF-κB promoter element, a PCR based strategy is employed. The upstream primer contains four tandem copies of the NF-κB binding site (GGGGGACTTTCCC) (SEQ ID NO:8), 18 bp of sequence complementary to the 5’ end of the SV40 early promoter sequence, and is flanked with an XhoI site: 5’:GCGGACCTCGAGGGACATTTCGCCGAGGACTTTCCGGGTTCCCATCCTGCCCATCTCAATTAGC:3’ (SEQ ID NO:9)

The downstream primer is complementary to the 3’ end of the SV40 promoter and is flanked with a Hind III site: 5’:GCGGCAAGCTTCTTGCAAGAGCTAGGC:3’ (SEQ ID NO:4)

PCR amplification is performed using the SV40 promoter template present in the pB-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with Xhol and Hind III and subcloned into BLSK2. (Stratagene)

Sequencing with the T7 and T3 primers confirms the insert contains the following sequence: 5’:CTCGAGGGGACTTTCCGGGACTTTCCGGGACATTTCGCCGAGCCTTTCCATCTGCCCATTAGTCAGCAACCATAGTCGCCCGCCCTAATCCGCCATCCGCCCCTAAGTCCGCCCATTTCCTCCGGCCCTAGGCTAGCATTCCAGGATGAGGGGCTTCTTTTGAGAGGCTAGGTTTTTGCAAAAAGCTT:3’ (SEQ ID NO:10)

Next, replace the SV40 minimal promoter element present in the pSEAP2-promoter plasmid (Clontech) with this NF-κB/SV40 fragment using XhoI and HindIII.

However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

In order to generate stable mammalian cell lines, the NF-κB/SV40/SEAP cassette is removed from the above NF-κB/SEAP vector using restriction enzymes SalI and NotI, and inserted into a vector containing neomycin resistance. Particularly, the NF-κB/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the GFP gene, after restricting pGFP-1 with SalI and NotI.

Once NF-κB/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are created and maintained according to the protocol described in Example 13. Similarly,
the method for assaying supernatants with these stable Jurkat T-cells is also described in Example 13. As a positive control, exogenous TNF alpha (0.1, 1, 10 ng) is added to wells H9, H10, and H11, with a 5-10 fold activation typically observed.

5 Example 17: Assay for SEAP Activity

As a reporter molecule for the assays described in Examples 13-16, SEAP activity is assayed using the Tropix Phospho-light Kit (Cat. BP-400) according to the following general procedure. The Tropix Phospho-light Kit supplies the Dilution, Assay, and Reaction Buffers used below.

Prime a dispenser with the 2.5x Dilution Buffer and dispense 15 µl of 2.5x dilution buffer into Optiplates containing 35 µl of a supernatant. Seal the plates with a plastic sealer and incubate at 65°C for 30 min. Separate the Optiplates to avoid uneven heating.

Cool the samples to room temperature for 15 minutes. Empty the dispenser and prime with the Assay Buffer. Add 50 µl Assay Buffer and incubate at room temperature 5 min. Empty the dispenser and prime with the Reaction Buffer (see the table below). Add 50 µl Reaction Buffer and incubate at room temperature for 20 minutes. Since the intensity of the chemiluminescent signal is time dependent, and it takes about 10 minutes to read 5 plates on luminometer, one should treat 5 plates at each time and start the second set 10 minutes later.

Read the relative light unit in the luminometer. Set H12 as blank, and print the results. An increase in chemiluminescence indicates reporter activity.

<table>
<thead>
<tr>
<th>Reaction Buffer Formulation:</th>
</tr>
</thead>
<tbody>
<tr>
<td># of plates</td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>11</td>
</tr>
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**Example 18: High-Throughput Screening Assay Identifying Changes in Small Molecule Concentration and Membrane Permeability**

Binding of a ligand to a receptor is known to alter intracellular levels of small molecules, such as calcium, potassium, sodium, and pH, as well as alter membrane potential. These alterations can be measured in an assay to identify supernatants which bind to receptors of a particular cell. Although the following protocol describes an assay for calcium, this protocol can easily be modified to detect changes in potassium, sodium, pH, membrane potential, or any other small molecule which is detectable by a fluorescent probe.

The following assay uses Fluorometric Imaging Plate Reader ("FLIPR") to measure changes in fluorescent molecules (Molecular Probes) that bind small molecules. Clearly, any fluorescent molecule detecting a small molecule can be used instead of the calcium fluorescent molecule, fluo-3, used here.

For adherent cells, seed the cells at 10,000 -20,000 cells/well in a Co-star black 96-well plate with clear bottom. The plate is incubated in a CO₂ incubator for 20 hours. The adherent cells are washed two times in Biotek washer with 200 ul of HBSS (Hank’s Balanced Salt Solution) leaving 100 ul of buffer after the final wash.

A stock solution of 1 mg/ml fluo-3 is made in 10% pluronic acid DMSO. To load the cells with fluo-3, 50 ul of 12 ug/ml fluo-3 is added to each well. The plate is
incubated at 37°C in a CO₂ incubator for 60 min. The plate is washed four times in the Biotek washer with HBSS leaving 100 ul of buffer.

For non-adherent cells, the cells are spun down from culture media. Cells are re-suspended to 2-5x10⁶ cells/ml with HBSS in a 50-ml conical tube. 4 ul of 1 mg/ml fluo-3 solution in 10% pluronic acid DMSO is added to each ml of cell suspension. The tube is then placed in a 37°C water bath for 30-60 min. The cells are washed twice with HBSS, resuspended to 1x10⁶ cells/ml, and dispensed into a microplate, 100 ul/well. The plate is centrifuged at 1000 rpm for 5 min. The plate is then washed once in Denley CellWash with 200 ul, followed by an aspiration step to 100 ul final volume.

For a non-cell based assay, each well contains a fluorescent molecule, such as fluo-3. The supernatant is added to the well, and a change in fluorescence is detected.

To measure the fluorescence of intracellular calcium, the FLIPR is set for the following parameters: (1) System gain is 300-800 mW; (2) Exposure time is 0.4 second; (3) Camera F/stop is F/2; (4) Excitation is 488 nm; (5) Emission is 530 nm; and (6) Sample addition is 50 ul. Increased emission at 530 nm indicates an extracellular signaling event which has resulted in an increase in the intracellular Ca²⁺ concentration.

**Example 19: High-Throughput Screening Assay Identifying Tyrosine Kinase Activity**

The Protein Tyrosine Kinases (PTK) represent a diverse group of transmembrane and cytoplasmic kinases. Within the Receptor Protein Tyrosine Kinase (RPTK) group are receptors for a range of mitogenic and metabolic growth factors including the PDGF, FGF, EGF, NGF, HGF and Insulin receptor subfamilies. In addition there are a large family of RPTKs for which the corresponding ligand is unknown. Ligands for RPTKs include mainly secreted small proteins, but also membrane-bound and extracellular matrix proteins.

Activation of RPTK by ligands involves ligand-mediated receptor dimerization, resulting in transphosphorylation of the receptor subunits and activation of the cytoplasmic tyrosine kinases. The cytoplasmic tyrosine kinases include receptor associated tyrosine kinases of the src-family (e.g., src, yes, lck, lyn, fyn) and non-receptor linked and cytosolic protein tyrosine kinases, such as the Jak family, members of which mediate signal transduction triggered by the cytokine superfamily of receptors (e.g., the Interleukins, Interferons, GM-CSF, and Leptin).

Because of the wide range of known factors capable of stimulating tyrosine kinase activity, the identification of novel human secreted proteins capable of activating
tyrosine kinase signal transduction pathways are of interest. Therefore, the following
protocol is designed to identify those novel human secreted proteins capable of
activating the tyrosine kinase signal transduction pathways.

Seed target cells (e.g., primary keratinocytes) at a density of approximately
25,000 cells per well in a 96 well Loprodyne Silent Screen Plates purchased from
Nalge Nunc (Naperville, IL). The plates are sterilized with two 30 minute rinses with
100% ethanol, rinsed with water and dried overnight. Some plates are coated for 2 hr
with 100 ml of cell culture grade type I collagen (50 mg/ml), gelatin (2%) or polylysine
(50 mg/ml), all of which can be purchased from Sigma Chemicals (St. Louis, MO) or
10% Matrigel purchased from Becton Dickinson (Bedford,MA), or calf serum, rinsed
with PBS and stored at 4°C. Cell growth on these plates is assayed by seeding 5,000
cells/well in growth medium and indirect quantitation of cell number through use of
alamarBlue as described by the manufacturer Alamar Biosciences, Inc. (Sacramento,
CA) after 48 hr. Falcon plate covers #3071 from Becton Dickinson (Bedford,MA) are
used to cover the Loprodyne Silent Screen Plates. Falcon Microtest III cell culture
plates can also be used in some proliferation experiments.

To prepare extracts, A431 cells are seeded onto the nylon membranes of
Loprodyne plates (20,000/200ml/well) and cultured overnight in complete medium.
Cells are quiesced by incubation in serum-free basal medium for 24 hr. After 5-20
minutes treatment with EGF (60ng/ml) or 50 ul of the supernatant produced in Example
11, the medium was removed and 100 ml of extraction buffer ((20 mM HEPES pH
7.5, 0.15 M NaCl, 1% Triton X-100, 0.1% SDS, 2 mM Na3VO4, 2 mM Na4P2O7
and a cocktail of protease inhibitors (# 1836170) obtained from Boeheringer Mannheim
(Indianapolis, IN) is added to each well and the plate is shaken on a rotating shaker for
5 minutes at 4°C. The plate is then placed in a vacuum transfer manifold and the extract
filtered through the 0.45 mm membrane bottoms of each well using house vacuum.
Extracts are collected in a 96-well catch/assay plate in the bottom of the vacuum
manifold and immediately placed on ice. To obtain extracts clarified by centrifugation,
the content of each well, after detergent solubilization for 5 minutes, is removed and
30 centrifuged for 15 minutes at 4°C at 16,000 x g.

Test the filtered extracts for levels of tyrosine kinase activity. Although many
methods of detecting tyrosine kinase activity are known, one method is described here.

Generally, the tyrosine kinase activity of a supernatant is evaluated by
determining its ability to phosphorylate a tyrosine residue on a specific substrate (a
biotinylated peptide). Biotinylated peptides that can be used for this purpose include
PSK 1 (corresponding to amino acids 6-20 of the cell division kinase cdc2-p34) and
PSK2 (corresponding to amino acids 1-17 of gastrin). Both peptides are substrates for a range of tyrosine kinases and are available from Boehringer Mannheim.

The tyrosine kinase reaction is set up by adding the following components in order. First, add 10ul of 5uM Biotinylated Peptide, then 10ul ATP/Mg$_{2+}$ (5mM ATP/50mM MgCl$_2$), then 10ul of 5x Assay Buffer (40mM imidazole hydrochloride, pH7.3, 40 mM beta-glycerophosphate, 1mM EGTA, 100mM MgCl$_2$, 5 mM MnCl$_2$, 0.5 mg/ml BSA), then 5ul of Sodium Vanadate(1mM), and then 5ul of water. Mix the components gently and preincubate the reaction mix at 30°C for 2 min. Initial the reaction by adding 10ul of the control enzyme or the filtered supernatant.

The tyrosine kinase assay reaction is then terminated by adding 10 ul of 120mm EDTA and place the reactions on ice.

Tyrosine kinase activity is determined by transferring 50 ul aliquot of reaction mixture to a microtiter plate (MTP) module and incubating at 37°C for 20 min. This allows the streptavidin coated 96 well plate to associate with the biotinylated peptide. Wash the MTP module with 300ul/well of PBS four times. Next add 75 ul of antiphosphotyrosine antibody conjugated to horse radish peroxidase(anti-P-Tyr-POD(0.5u/ml)) to each well and incubate at 37°C for one hour. Wash the well as above.

Next add 100ul of peroxidase substrate solution (Boehringer Mannheim) and incubate at room temperature for at least 5 mins (up to 30 min). Measure the absorbance of the sample at 405 nm by using ELISA reader. The level of bound peroxidase activity is quantitated using an ELISA reader and reflects the level of tyrosine kinase activity.

**Example 20: High-Throughput Screening Assay Identifying Phosphorylation Activity**

As a potential alternative and/or compliment to the assay of protein tyrosine kinase activity described in Example 19, an assay which detects activation (phosphorylation) of major intracellular signal transduction intermediates can also be used. For example, as described below one particular assay can detect tyrosine phosphorylation of the Erk-1 and Erk-2 kinases. However, phosphorylation of other molecules, such as Raf, JNK, p38 MAP, Map kinase kinase (MEK), MEK kinase, Src, Muscle specific kinase (MuSK), IRAK, Tec, and Janus, as well as any other phosphoserine, phosphotyrosine, or phosphothreonine molecule, can be detected by substituting these molecules for Erk-1 or Erk-2 in the following assay.
Specifically, assay plates are made by coating the wells of a 96-well ELISA plate with 0.1ml of protein G (1ug/ml) for 2 hr at room temp, (RT). The plates are then rinsed with PBS and blocked with 3% BSA/PBS for 1 hr at RT. The protein G plates are then treated with 2 commercial monoclonal antibodies (100ng/well) against Erk-1 and Erk-2 (1 hr at RT) (Santa Cruz Biotechnology). (To detect other molecules, this step can easily be modified by substituting a monoclonal antibody detecting any of the above described molecules.) After 3-5 rinses with PBS, the plates are stored at 4°C until use.

A431 cells are seeded at 20,000/well in a 96-well Lopodyne filterplate and cultured overnight in growth medium. The cells are then starved for 48 hr in basal medium (DMEM) and then treated with EGF (6ng/well) or 50 ul of the supernatants obtained in Example 11 for 5-20 minutes. The cells are then solubilized and extracts filtered directly into the assay plate.

After incubation with the extract for 1 hr at RT, the wells are again rinsed. As a positive control, a commercial preparation of MAP kinase (10ng/well) is used in place of A431 extract. Plates are then treated with a commercial polyclonal (rabbit) antibody (1ug/ml) which specifically recognizes the phosphorylated epitope of the Erk-1 and Erk-2 kinases (1 hr at RT). This antibody is biotinylated by standard procedures. The bound polyclonal antibody is then quantitated by successive incubations with Europium-streptavidin and Europium fluorescence enhancing reagent in the Wallac DELFIA instrument (time-resolved fluorescence). An increased fluorescent signal over background indicates a phosphorylation.

**Example 21: Method of Determining Alterations in a Gene**

**Corresponding to a Polynucleotide**

RNA isolated from entire families or individual patients presenting with a phenotype of interest (such as a disease) is be isolated. cDNA is then generated from these RNA samples using protocols known in the art. (See, Sambrook.) The cDNA is then used as a template for PCR, employing primers surrounding regions of interest in


PCR products are then sequenced using primers labeled at their 5' end with T4 polynucleotide kinase, employing SequiTherm Polymerase. (Epicentre Technologies).

The intron-exon borders of selected exons is also determined and genomic PCR
products analyzed to confirm the results. PCR products harboring suspected mutations is then cloned and sequenced to validate the results of the direct sequencing.

PCR products is cloned into T-tailed vectors as described in Holton, T.A. and Graham, M.W., Nucleic Acids Research, 19:1156 (1991) and sequenced with T7 polymerase (United States Biochemical). Affected individuals are identified by mutations not present in unaffected individuals.

Genomic rearrangements are also observed as a method of determining alterations in a gene corresponding to a polynucleotide. Genomic clones isolated according to Example 2 are nick-translated with digoxigenin deoxy-uridine 5'-triphosphate (Boehringer Manheim), and FISH performed as described in Johnson, Cg. et al., Methods Cell Biol. 35:73-99 (1991). Hybridization with the labeled probe is carried out using a vast excess of human cot-1 DNA for specific hybridization to the corresponding genomic locus.

Chromosomes are counterstained with 4,6-diamino-2-phenylidole and propidium iodide, producing a combination of C- and R-bands. Aligned images for precise mapping are obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination with a cooled charge-coupled device camera (Photometrics, Tucson, AZ) and variable excitation wavelength filters. (Johnson, Cv. et al., Genet. Anal. Tech. Appl., 8:75 (1991).) Image collection, analysis and chromosomal fractional length measurements are performed using the ISee Graphical Program System. (Inovision Corporation, Durham, NC.) Chromosome alterations of the genomic region hybridized by the probe are identified as insertions, deletions, and translocations. These alterations are used as a diagnostic marker for an associated disease.

Example 22: Method of Detecting Abnormal Levels of a Polypeptide in a Biological Sample

A polypeptide of the present invention can be detected in a biological sample, and if an increased or decreased level of the polypeptide is detected, this polypeptide is a marker for a particular phenotype. Methods of detection are numerous, and thus, it is understood that one skilled in the art can modify the following assay to fit their particular needs.

For example, antibody-sandwich ELISAs are used to detect polypeptides in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies, at a final concentration of 0.2 to 10 ug/ml. The antibodies are either monoclonal or polyclonal and are produced by the method described in Example 10.
The wells are blocked so that non-specific binding of the polypeptide to the well is reduced.

The coated wells are then incubated for > 2 hours at RT with a sample containing the polypeptide. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove unbounded polypeptide.

Next, 50 ul of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature. The plates are again washed three times with deionized or distilled water to remove unbounded conjugate.

Add 75 ul of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard curve, using serial dilutions of a control sample, and plot polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale). Interpolate the concentration of the polypeptide in the sample using the standard curve.

**Example 23: Formulating a Polypeptide**

The secreted polypeptide composition will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the secreted polypeptide alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmacologically effective amount of secreted polypeptide administered parenterally per dose will be in the range of about 1 \( \mu g/kg/day \) to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the secreted polypeptide is typically administered at a dose rate of about 1 \( \mu g/kg/hour \) to about 50 \( \mu g/kg/hour \), either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Pharmaceutical compositions containing the secreted protein of the invention are administered orally, rectally, parenterally, intracistemally, intravaginally,
intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

The secreted polypeptide is also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules.


For parenteral administration, in one embodiment, the secreted polypeptide is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

Generally, the formulations are prepared by contacting the polypeptide uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.
The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The secreted polypeptide is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

Any polypeptide to be used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic polypeptide compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Polypeptides ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous polypeptide solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized polypeptide using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides of the present invention may be employed in conjunction with other therapeutic compounds.
Example 24: Method of Treating Decreased Levels of the Polypeptide

It will be appreciated that conditions caused by a decrease in the standard or normal expression level of a secreted protein in an individual can be treated by administering the polypeptide of the present invention, preferably in the secreted form. Thus, the invention also provides a method of treatment of an individual in need of an increased level of the polypeptide comprising administering to such an individual a pharmaceutical composition comprising an amount of the polypeptide to increase the activity level of the polypeptide in such an individual.

For example, a patient with decreased levels of a polypeptide receives a daily dose 0.1-100 ug/kg of the polypeptide for six consecutive days. Preferably, the polypeptide is in the secreted form. The exact details of the dosing scheme, based on administration and formulation, are provided in Example 23.

Example 25: Method of Treating Increased Levels of the Polypeptide

Antisense technology is used to inhibit production of a polypeptide of the present invention. This technology is one example of a method of decreasing levels of a polypeptide, preferably a secreted form, due to a variety of etiologies, such as cancer.

For example, a patient diagnosed with abnormally increased levels of a polypeptide is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the treatment was well tolerated. The formulation of the antisense polynucleotide is provided in Example 23.

Example 26: Method of Treatment Using Gene Therapy

One method of gene therapy transplants fibroblasts, which are capable of expressing a polypeptide, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37°C for approximately one week.
At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks.

pMV-7 (Kirschmeier, P.T. et al., DNA, 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

The cDNA encoding a polypeptide of the present invention can be amplified using PCR primers which correspond to the 5' and 3' end sequences respectively as set forth in Example 1. Preferably, the 5' primer contains an EcoRI site and the 3' primer includes a HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform bacteria HB101, which are then plated onto agar containing kanamycin for the purpose of confirming that the vector has the gene of interest properly inserted.

The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the gene is then added to the media and the packaging cells transduced with the vector. The packaging cells now produce infectious viral particles containing the gene (the packaging cells are now referred to as producer cells).

Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his. Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether protein is produced.

The engineered fibroblasts are then transplanted onto the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads.
It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other disclosures) in the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference.
SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANTS: Human Genome Sciences, Inc. et al.

(ii) TITLE OF INVENTION: 20 Human Secreted Proteins

(iii) NUMBER OF SEQUENCES: 61

(iv) CORRESPONDENCE ADDRESS:
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette, 3.50 inch, 1.4Mb storage
(B) COMPUTER: HP Vectra 486/33
(C) OPERATING SYSTEM: MSDOS version 6.2
(D) SOFTWARE: ASCII Text

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: April 07, 1998
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
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(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: A. Anders Brookes
(B) REGISTRATION NUMBER: 36,373
(C) REFERENCE/DOCKET NUMBER: P2005PCT

(vi) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (301) 309-8504
(B) TELEFAX: (301) 309-8439

(2) INFORMATION FOR SEQ ID NO: 1:

(1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 733 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:
GGGATCCGGAA GCCCAATACT TCTGACAAAA CTCACACATG CCCACCGTGCC CACCCACCTG
AATTCGAGG TGCCCGCTCA GTCTTCTCTT TCCCCCCAAA ACCCAAGGAC ACCTTCATGA 120
TCCCGGGAC TCCGTGACGT CAATGCGTGC TCTGTTGACGT AACGCAAGAA GACCTTTGAG
180
TCAAGTTCGAA CTCGAGCTGG GACGCGGGTG AGTGTCAATAA TGCCCAAGACA AAGCCTCGGG
240
AGAAGCAGTA CAAACAGGAC TACCGTGCTG TCAAGCTCTCT CACGGCTCCTG CACCGGACT
300
GGCTGATGTC ACGAGAGTAC AAGTCCAGAG TTCTCCACCAA AGCCTCCCA ACAACCCCATG
360
AGAAAACCCT CTCCAAAGCC AAAAGCAGAC CCGAGAAGCC ACAGGTGTAAC ACCTTGCCCC
420
CATCCTGCGG TAAGGCTAGC AAGAACCAGG TCAGCCTGCA CTGCCTGCTC AAAGCCTCTCT
480
ATCCAGACGA CATCCTCGCTG GAGTGGAGAA GCAATGGGCA GCCGAGGAAC AACTACAGAG
540
CCACGCCTCC CGTGGCTGAC TCAGGACGCT CCTGTCTTTCT CTACAGCAAG CTACGGTGCG
600
ACAAGAGCCA CTGCGAGCAG GGGAAACCTG TCTCATCGTC COTGATGCTG GAAGCCCTGC
660
ACAAACCCTA CACGGAGAAG AGGCTCTCCC TGCTCTCGGG TAAAATGAGTG CGACGGCGGC
720
GACTCTAGAG GAT
733

(2) INFORMATION FOR SEQ ID NO: 2:

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(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 5 amino acids
   (B) TYPE: amino acid
   (D) TOPOLOGY: linear

35
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Trp Ser Xaa Trp Ser
1  5

40

(2) INFORMATION FOR SEQ ID NO: 3:

45
(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 86 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: double
   (D) TOPOLOGY: linear

50
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GCCGCTGAG ATTTCGCCGA AATCTAGATT TCCCGAAAT GATTTCCCG AAATGATTTC
60

55
CCCGAAATAT CTGCATATCT AAATAG
86

(2) INFORMATION FOR SEQ ID NO: 4:
(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 27 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: double
   (D) TOPOLOGY: linear

(xii) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

   GCGCAGCT TTTGCAAAG CCTAGGC

(2) INFORMATION FOR SEQ ID NO: 5:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 271 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: double
      (D) TOPOLOGY: linear

   (xii) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

   CTCGAGATTT CCCCAGAAATC TAGATTCCC CGAAATGATT TCCCGAAAT GATTTTCCCG
   AAATATCTGC CATCTCAATT AGTCGCAAC CATAGTCCCC CCCCTAACTC GCCCCCATCCC
   GCCCCAATCT CCCCAGGATT CCCGCCCCAT TCGGCCCCAT GGCTGACTAA TTTTTTTTTAT
   TTATGCAAG GCCGAGGCGG CCCCAGGCTC TGAAGCTATTC CAGAAGTACT GAGGAGGCTT
   TTTTGAGGCT CTAAGGTCAT GCCAAAGAC T

(2) INFORMATION FOR SEQ ID NO: 6:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 32 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: double
      (D) TOPOLOGY: linear

   (xii) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

   GCGCTCGAGG GATGACAGCCG ATAGAACCCT GCG

(2) INFORMATION FOR SEQ ID NO: 7:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 31 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: double
      (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```plaintext
GGGAAGCTTC GGGACTCCCC GGATCCGCT C
```

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 12 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: double
   (D) TOPOLOGY: linear

(xii) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```plaintext
GGGACTTTTC CC
```

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 73 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: double
   (D) TOPOLOGY: linear

(xxi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```plaintext
GGGCCCCGGA GGGGACTTTTC GGGGGACTT TCCGGGACT TCCATCGTG
CCATCTCAAT TAG
```

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 256 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: double
   (D) TOPOLOGY: linear

(xiv) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

```plaintext
CTCGAGGGGAA CTTTCCCCGG GACTTTCGAG GACTTTCCG GACCTTTCCA TCTGCCATCT
CAATTAGTCA GCAAATTGAGG TCCGCCCCC AACTCCGCCC ATCCCCCCCC TAACTCGCC
CAGTTCCGCC CATTCCCGGC CCCATCCGC CACTATTTT TTTATTTTTG CAGAAGCCGGA
GGCGGGCTCG GGCTCTGAGC TATTCAGAAA GTAGTGAGGA GCTTTTTTGG GAGGCTTAGG
```

```plaintext
CTTTTCGAAA AAGCTT
```
(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 919 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

AATTCGCGAC AGGTCCCAATG CAGTTTTTCA GCCAGTGCA NCTTGATACC AAAACCAATA 60
AAAAAAACAA ACACAAACAA AACATAAACG TATAGACCAA AGCTCTCATG ATTAGATGC 120
AAAAACTCTAA AATTGAAAAA AAAAGTCTAG TGATATCCAT AAACTGATAC ATCAGCAAGA 180
GATGTTTATT AGGCAATCA AAAGATGATT TATATTTTT TAAAAATCA ATGTTGCCCC 240
CCCTCCCTCT TTCTCTTTGT TCCTCCCTTT GAGTTTTATG TGCTCTCTTT TGCCCTCCCT 300
TCCCGAGTGT CAGGAGTATG ATCCGCAATG TGGGATGAGA GAGGTTGCG GATGTGCTCT 360
CTGGCAACCA AGAAGGCTGA GGTTGATGAG TGGAAGGGAC AGGCGGAGGA ATGGGCACGC 420
ATTGTAAGA GATGGTACAC ACTGACAAAA TGCCTGAGA ATGAGTTGAG CAGGTTCTCT 480
CCCTGGTGGAGA GAGCTATTGT AGAAGAATAGG AGATGGAGG AGCTAGAAGA CCGTGACTCT 540
GGGATTAGA TGAAACTCAG ATCTTTAAAA TACATAGGAA CANTAGAGAA ATGTCTGGCT 600
GTCGCCCATAT ACATATATTT TGCGATTCAT TCACCGAGA GACATAAAAT GCAGTCACAG 660
CTCGAACAAG TAAAACAAAC CAACCTCCCA GTTATATTAT TTCAAAATCT ATCCACAAAA 720
AAAGGCAACA GGGATGATTCT CAGTCGGGAG ATGGGAGGAA AGAGAGATGG AGCCTGAACT 780
ATTCTAATGG CTTAACAGAAA AGAAAAACCT CTGGCTGCGG TCAGTGCCCT ATGTTTTCAAT 840
TTCTAGCATG TTAGGAGCTG TARGGCGGT GTTGTCTGGA GCCCGAGGTT TTTAGCAATC 900
CCAGGCAAC ATGCAATA 919

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1026 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GGCCAGGAAGTT TTTGCTGCA GCACCTGCAA TGAACTCCTG GTTGACATGA TTTATTTTTG 60
GAAGAAGAG AAGCTATT ACT GTGCGAGACA TTACTTGAGC AGCCAGGAAAG ACCCCTGTCG
120
TGGCGGAGA CAGCTGTATG TAGGCACTTAG ACTGCTCACAG GCAGAAGAAC AGAATGAGCA
180
CCTGAAACAC TCTGCTGCTGT TGGACGTGCA TACCTATCTA TCTGGGAAGA TATACGATAT
240
GCTAAGTGAC AGAGCCGGTG GCAAGCCCTG CTATGTGAAG AATCAGCTGTG TGGTGATCA
300
AGGAGACCCAG ATCCGGCTCG ACCCAGAAGT GCAAGGCTGTG ACCATTAACA ATTTGAGCTG
360
GCAAGAGTCC AGACAGTCTT CTGCTGCTGCT TGGCTGACAC AATGCTCTCA TGCTGCAAGA
420
GTTCTAGCCA GTGAAAGGGA AGTGTTCTCT TGCTAGGGAA TGTGAAGAGA GAGTGCTTTA
480
GGGGAGAGGC ACCCAGAAGT ATCGAGCCAT AGCTATCCAA AGTGGCTCAG ATTCTACTTG
540
AAAATGCAAA TGCTGAAAGA AAAAAAGCTA AAAAAGAGAA CTGTTAAGAGA AACCAAAGGA
600
TTGGTTGAA AATAGTTTGC CATTTTTTCT TCACTATTTT TTTTACTGTC TCACTTTTTA
660
AACCTGTGTT TAGGGATGG ACCGACCCCAT AGGAAGAATAT CTGATCTTTC CATAGCTTTT
720
CAATAGGGAA ATTCTTTTTT GAAGCTGGCA TCTCATTTAA CTCCATGCTT CTATTCCATT
780
TGCGGGCAC ACCTAAAAGT TAGTACCTACG AATGGGAAGGA TGACAGTTCC TAGCTCTACA
840
CTCTTTTTT CCCCTCCATTG TGGAAAAAGA AAGAAAAGCT AAATGTGCCC TAAATCCAGA
900
GCGCTGCTTT TAGATCCCTCG TCTTTATTCAC TGACCTTTTG TAGAATAACA CAGGAAATTG
960
TTTTTTGACA AAGNGGGATG CGGTTGGGTA TGCGAGCTCG CTGCTTTTTAA AGCCCATGCG
1020
ATTNAC
1026

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2067 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

AATCCGCGTAC QAACCTTTTAA TGGGAAATG AACAGAATG AAGGGAGAGG
60
AAGATGACCT TCTCCATCCT CCCCTCCCTC CTCCCTGATAG TGGGAATCCAA CTATATGA
120
CTCCTCTTT TGGGAGTGGC TCTTTTTTGG ACCCTTGGGA ATTAGGGTTT TCTCTGGTTA
180
TATTTTATCTG GCGGAGTTARCA GTATATAGT GATGGGGAAAG GTGGAGAAGG AAGAGGATAC
240
TGAATCTCAG AAGACCCACT CTAGAGTGGT GCTTGGACAG ATGGCTGTTT CTTCCTTCAC
300
CTGCGAGTTT GCCGAGGAGA TGGCTACCAT CTGGGAGACA TCCACTGGAG GCAGATCTCA
360
CMCCCAAGCA GTTAAACCTAG TGGAAAAAGC ATCTGGCTCC ATGGAAATGC AGGGGGTTCG
(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1341 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xii) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GCCACGAGCT GGCCTGACG AACGGGAAAC CGGGAGCTCG GCCACGCGTG GCCGGGCTGC
GCTGAGGGCT GCTTCCCGGC TCCCGAGACCA TGGCTAGGAG AGCGGCTGCG AGGCTGCGCT
CAGCGCTGCG GCGGAACCGC GCGCGGCGGT CGCGGGCGGC CGGIAAGTCC TGCAGCCGTC
GGGCTGCGCG CTTTGGCAAC CGGGGCGAGA AGACTGAGGA GCCCGCGGAG GACCTGGATC
CGGGCGCTCT GGGTAGTTGT CCAGGCCGC TCTCGGCAAGA GGGCGCTCAG TTAGAAGCAT
CAAAACACCA ATTGAGTAAAT GAAGAGTTGC GAAATAGCTTA TCCAACTATT GATGGGATCC
CTAAATATGAT ACCACAGGCA GCTAGGATGA CACOTCAAGA TAAAGGACCA GAAGAATGG
AGCAAGCGTCT GTTCAATAAT TAAAAAAATT TAAAAACGC AACAGCCCAAC TTTCCTTAAT
ACCATATACCT TTTTAAAAAC CAGTOCGAGG TAAAGGTTGG AAGAAAGAAA TGTTTCTGTC
TCTTCTACG TGACTGTCG TTATCCACT GGGTTCTTTA GCGAGACGTG TCTACTCGGC
CTCTGGGAA GAAACCTTCC CACAGGGCGT CACTAGCACA GCCAGGCTTT GCTTTTACAG
CCTCCTCCTG CCTATTTACA TACCATGTTA TGTTTTCTCT CACCTTGGAAG CTGGGATGGG
TAATRAACCT TCCAGGCATA AGTGGACCA CTAGACCAAA TACGTCUPAT ATATTAANGA
TACGTCTGG GCTACGCTT CGAAGAAGCT CAAATGAGTG AATTGAGTPT CGGGGAAANA
GGCTTTGGCT TGCGGATATC AGGGTAGAGG CTTGAGGAGG CTATAGGCGT AGACTCTCTCT
TTTATATGC TCTTGTCTGC CCTTGGTTTT TTGAAGGCTC TGACTTATTAA CTTGCGTATG
AGAAGAACA TTGGAGCATG GTCGCGGTTG GAGATGAACA TCCCTAATTG ACAATGATGT
ACTATTTCTT ATICCCATTCA TCTAGAGGTC ATGGAAATT TGTGTGTGTT GATGTTTGAAG
CTTCAAAGGC TTGCTAAAG TACGATGGTA AGGATGACTG AAAATATCTC AAAAGAAGTA
ATGTGGAATA GTCCCGCTTA AGGGAGGAA TGCAATTGGA GAAATGTGAT ATAAAAACAC
ATATACCAAT AGAACATTTA TGACTATCA AAAACTGAGT TGTAAAATAT ACCTTCTATT
CTGTCCCCATT AATTGCTTAT ATTACCAGTA AAACTGTTGA CACCTTCCCA TAAAAAAAAT

(2) INFORMATION FOR SEQ ID NO: 15:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1443 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

10  GGGCGGGGCG TCCGTAGGAC ACGAGTATGG AGCTTCCCTC AGGGCCGGGG CGGGACGGGC
    TCTTTGACTC GCGCCGGGCTT CGGGGCAGCT GGCTCTCTACT CTGTGCTGCTG CTCCTATAGG
    CGCCAGGGGCTG CTGGCCCTGC GCCTCTCTCT CCAGATGCGG CTGTCTGCTG
    TCAAGCTGCC CTGAGCAACAC AGGGCTCTTC CGCAGATGCTG AGGGCGAACG ATGGTGCGGG
    TGCTAGGGCT CGGGCGCCGG CAGGAGAAGCTCGCAGCTCG CCAGCACAGT GTCAGGAGGTC

20  TCATTCTCAA CCCTTGACAC CTTTTCGACC ACAACAGATG CAAATTGCTT ACCACCTGTA
    GCAACCCTCTT AATCAAGAAG CCCCCAGACT TGGGTGTGTG GTTGGGCGGC TTCATGGAAG
    TGAATGGCGG GGGCGAGTGG GTGGAGTACG TCAAGAGATT CTGGAGCTCC ACGAGGCTGTC
    CCCCCACTCC TCGCCGCTTA TTCCCTGAGG AAGAGCCAC CAGGGCGGG GAGGGCTCC
    TGCCCTTCAG TTCCCTGCCA TTTTCTACT AAGATGTGGT ACAACCTCC TTTCCGCAAG

30  TCTCGAGACC CTTGCTCTCT GTGAGGCGGT CAGAGGCTCC TGAGGCTCCA GAATGGCTCTG
    GTCACTTTTT CGGCCCTTTTC AGGGCGATTC AAGTAAGGCTG GTTGGTGTCT GTTCACTGGCC
    AACAGGGGGA AGCCGAGTGG GAGGCGCAGC TCCGCTGACG AAGAGCCGGA GGCAAGAAAT
    TGGCCAGAC AGGGCAGCGC TGGCAAGCAG CTTGACAGGG ACAGAGCAAT AACGACAAAA
    GACACCCAG ATGGGGCAGG CAGTGCAAGCC AGTCTCTCTTT CCCCTCCCTT CTTGGTGCTTT

40  CTCTTGTGAC GCAAGCTGGCA ACTCTGGGCTC AGGAAGTGCAA GGAAGTTTGG CCCCCATGTCG
    CATTGGGGCT CATCCGAGAA GACCTGGGCA AGACTGCTCTG TTGAGAATGG AATATTACAA
    ATGGCGCTGA GGGCGCGGTA GCTTTTAGAG CTTGCAAGAC ATCAAAGGGA ACTCACTCCC
    TACCCAGAGC CTGGCGCTCC AAGTGTCCCC GCCTTGCCGC GTGACCCCTC CACCCAAACAG
    CCCCTGGGTT TGGCCAGCCT TCGCCGGCGG GGCGAGGAGG CCTCCAGAGG CGCCCGAACG

50  CAGTAGATGA TACAGCAGAG AGGGAGTCTGA CAGAGAGACG AGGCCAGGGG GCTGAGCTAG
    CTTACAAGAA CGAACGGGCA CCCCCGGCGG CAGAGGGCGC AGTGGGGGCA GCCCTCAGCC
    AAGCTGCAAC AGGGGAGATG GGTGGGTGTT AAAAAGGGGA GGTGGCGCT CCCCCAATGT
    CACTCTAATT TCCAGGGTTT CACTCAGAA AAAAAAAA AAGCTGCGGGG GGGGGGGGGG
    CGT

60
(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 654 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:
GGCAATTCAT TTCCGACTTC AGGAGGACAA AAGGCCGCC ACAATTGCTA TCCACCCAC
15
CAGTCCACAC ATGGTTGACG TGACACACAG ACACACATGAA GAAGACTTCT TCCTCTACAT
TGCCTCATG AGCAAAAGTG TCTACGCTCT GTGAAAGCTG TC GCCCTTGAG CTGAGGGGGG
20
CTCCATTCT ACAAGAAGAG AGGTGCGCCCC CTTTTCTGGA CTCCTCTTCT CTTCAAGCTC
AAAACACGAC TTCTTTATTC GCCACCCCTG TGGAGACTTC TGGAGGCTCA GTCTGTGCTA
CAGGCTGGTA TGTGGTGGTG TTCTGTGCCC TTTCTGACTG CCAAGGGGCG TCAGAACCCC
AGCAATCCTC TCTTCTTAT CACTTCTTTT TGCTGCTGCTG TGGAGGGGAG CTGAAATGCT
GGGAGGAGG TAGAGGGCAG ACAAATAGAG AGGAACCCAC CAAGCTGGAA AAAAAAAAA
AAAAACCTGGA GGGGCGGCC GGGCCCTAAGG GGGGGNTTA NANT
35

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 749 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:
AAAGATGCTT GCCCCAGGTA ATTTGACCAA AGGCCACAGT GAACTCCGCG GTGCGCTGAGG
50
AAAGGAGGAG CACCCACAGG CGTCTGGGAG GAGACATAA GCCTCAAAAT G3AATTCAT
AAATCCTAATA ATAGAGAAAA CATAACAATTT GTGAGATATAT CCAGAATAAT TAACCGCTTT
CCAGAAGCAG AGGAGTACTC ACTGAAAAAT GGTACGGTTT ATGTGGGATT AAATGCTGCT
CCTTTGCGGC TCCATCGCGA CACCTTTTTT CGACACATCT TGAATGTGGC AAAGGCCGCGC
ATAGCGGTGG GTCTTCAAT CTGAGGATA CTTTTTCTTTA CAACAGACTT AACTTACAGA
TTTTTGTTAA GTTTCTCTTT ATATCAGAGT GNTTGTGATT GTGAAACCGT TACCAATAACA
60
CGAGCTGGAC TGACCTGGCT TGTTATTGGT GGTCTATACC CTGTTTTCTT GGTATACCT

GTAAGGCTTG CTCTAGCACG CAGGTAATCA TCAAGCTCTGT TACCCACAAA AGGCCAACATC

TACAGTTACT GAGATTAGAC TGTAGAGGCT GGTCTTAAAA AAGATGTTATT TCTCATTTTG

CTCCAGACTA TGTTTTACAG ATACCTTGGG TCAGAAACAT ATAAAACACT TTTAAAAAGCC

CTTCAGTAT CTGAACTCAG CAAAGAAAAT CACTGATTTT AAAACATATA GAAAAACAAA

ATAAAGTGGT AAAACARARAA AAAAAAAA

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 511 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

AATTCGGAAC GAGCCAGATT CCCATAAACG ACRAGGCCTA ATCTGTTACG TACACGCAAG

ACAGGCTCAC CTCACTCTTT CTCGCCCTCC AAATTGGAAACG CTGGCCCTGGG ACTAAAGCAGAT

AGACCCACAG CGGTAGTATAC CGAACCTCAG TCAGCCCCAG GGATCGAGGG CCTCCAGCAAG

GGAACCTTCC ATMATTTTCT TCAAGCAACT TACAGCTGCA CCGACAGTTG CGATGAAAAGT

TCTAAATCTT TTCCCCTTCTTC TGTTGCTGCC ACTAACCTGTG ATGECATGSG TCTCTAGCAG

 CCTGAATCCA GGCTGGCCCA GAGGGCAGCAG GAGCCGAGGCG CAGGCTTCTA GGAAGATGCT

CCAGGAAGGC GGCCAAAGAT GTRAGTGCAG AGAAATGTTGTC CTGAGACGCC GAAGAGAAA

TTCACTACAG TTCTGCCGCT GCAAAAGAAC CARTGGCCTT GTGATCTTTT CAAAGCCATG

TGAAGAAAAC AAAGCACCAA AGGACACCACA G

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 689 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

GGCCAGAGGA GGGATGCCC CAAGGGGCTG CTAGCCCTGC TCCGGGGCCT TCTGACCTGC

GGCGCGCTGC AAAGCCGAGG CACTGGGCAG GATGSGCTGC TCTTTGACGA TGATCGGCGGCG
CGCTTCCTCA CCATCCCTCG GCTGTTCTTC GCCTGCCAGG GCCTCTGTCT GCCTCCCATG
180
GCTGSGAGCG CGCTGGCCCG GCCCGCGGTT GCCGGGAGGC TCTGCTGATGC TGAAGGTCCC
240
AATCTGGCAGC CCTCCGACCT GCCTCCGGCG YCTGTGGGCTT NACGGTGCTG CGGTGCGCCG
300
ggGAGGCCATC GGAACGCTCTG TACTGCGCTG TGCTCTGCTC TCTCCTCTCG GGCTCGGCGG
360
CTCAGTGGTGT CTCGAGGCGT GAGGCCAGCA GGGAGACCTC ACCACTCAGT CCCCTTTGCG
420
CTGGGGGACC CATTTCAAGAG TCTCTTGAAG GAGGGTAATCT TGGATGGGCCC ACCCGGCTGA
480
AGTCCTCCTG ATGGCTACCC TTCAAAKKCAA AGGGCGACGC TCTCTATTTT TCTGGGACAA
540
AACTGAGAC CTGCCCCCTAC ACAAAAACCTT TGACAATAC TGGGCTGCTC TACCGAGCCT
600
TGGAAAGAAAA TGCCCTCAAG TCACCTCACT TCACCAAGAG AGGATAAAAA CTTGCAACCTYG
660
GGAGCCAGG TGCTTTGCGT CACACCTGT
720

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 1147 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: double
   (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

TCGACCCACG CGTCCGGCGG GCCTCTTTTC CGGGAGGCGG GTGGCGCGTG AGGGCGAGAA
60
CGGGGTGCG GGGCCGACCT TGGGCCGCGT CCCTGGTCTA GACGTGGGAT
120
GCGGCAAGCT GGGAAGGAGG AAGGGCAGCT GTGGCGAGCT GCCCTGCGGC ATGACTCTGCT
180
ATGCCTGCTG GTGGCGCTCT TACCTGGCTCA TCAGGCGAGG ATATATTTAT GATGTATATG
240
TGGAACCTCC AAGGCGCGGT TCATGTAGCT ATGAAACTGCG GCATCGACGG CGCAGAGCTT
300
CTTGGTGAAT GAAAGGAAAT GGAACATATA TGATGAAAAG ATCTGATACCC AGCTCTCATAT
360
TTGACAATTGG AGTTTATGG TGCATGATCC TGAGCCGATC GAATCGACCA AAAATCCCAG
420
AACCTCAATG ATTCCTGCTT GTGTGGCTTG GATGGGCTTG TGCGCTATGT AGGTTTTTCA
480
TCGGCGATGT ATGGCATGAG ATGAAACTCG CGGGCTATCCT GATGGGTAG ATGGCGTCGG
540
AGAGAAATAC ATGGCGACTG GAGTTGGCTC CTGTCAATGG ATGGTTAAG GCTGCTACAA
600
TTCTCTATAT TGAAATTGGA AAAAGATAAG AAGCGACGAG TAAAGAAGAT ATCTAGTGAA
660
AAAAGAGAAA GCGTATGGGA GCTGGGACTA GAAATTTCCT TTGTTATGAA AGAGAAAGAT
720
TTTACAGAG ATTTCCTCCTG CTGCTGCTTG GATGGGCTTG TGCGCTATGT AGGTTTTTCA
780
(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 532 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CTTTGGTTCC TTTCTCTCTAC ACCAGGAGTT TTTATTGGCC TGGTTCCGAG AGTAATGAGG
GCCATGAGGT CAGCCCACTT CCTCTGGGCT CTGCYYPITCA TGGAGTCCTT GGGGCTCTAA
CTGACATGAGT GAGCCACTCG AGCTCTACTAC CTGCCATCC GGGATGTCGA GTGGAACTAT
GCTCCACCAG GAAGAAATGT CATCAGCAAC CAGCGCTCTGG AAGCTGACAT GAGAGTTTAA
TTTCTGATGG TTTTTGAAGG GAGTTTATGG GAGCAGCTTGT GAAGTCAAGA AGTACCCCTCT
TGAGGCCCTT TCCCGACGGT CTGGTACAGG CAGCGCTCTTG ATGCCCGCA GCTGGACATA
CTCAGTGAGCA GATTTCTCGG GAAGACCTGA GTGGTCGCA AACACCACTC TCCATCCTGA
GTTGCGCTGA ACGATTTTGG AGCTCTAAG ARAATATCTT CATATGCTTT TCCATACCC
ATACCCACCA TGCTACTTAT TTAAATACAA CTTTTGGACT TTAAAAAAA AA

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2743 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

TGAACCCGCCC CTCCTCCGAC GAGAGACTGG CCAGGCCCAGA AGAGAAAAA
ACACAAGCTA AAAAGTCCTG AGTACGCTTG TTVGGCCGCA AACCTGAACC CACCTTTTGC
ACCACCGGGG ACCCGCACTT CTTCGGCCA CCCACCCCTG AGAGGGTGCG CGGCGGACCC
5
CAGTAGTAAG AAAACACTGT CACCTCGTCT CAGGAGGCGT CGAAGGCGCA CGGAGCGCTT
CCTTTAGAAC GCTGCAAGCA CAGAGCAACT TCTGACGCCC ACTTCTCAAAAT GGCGTACTCC
240
AAACTGACAC TCCCGAGCTC CAAGCTGGA CCCCAGACGG CGGAAAAACC TGGAACCCAG
10
CGCCCGGCGA TGCCAGAGCC GGGGTTGCTT GGGGGTGTCG CTCCCTGCAG ACCGGGCGCG
CGGGGCTGCG GGGGGTGTGC CCGATGCGG ACTGAGGGCG GTGGATGAGGA CGGCCAATGC
480
AAAAATGTG TCGATTCTCTA GAGGCGCTGA GACGGCTCAGA ATGCACTGA TGCGGGGAAA
20
ACGCAGATAC TTGGTCCGTT AGTGGTCTGC GTGGAGCTTAT TTGTACAGGC TGCGGTATTGA
720
TCAGAGAATG ACGTATTAT TATCCACAGA TGAAAGATTT CAACAGTACG TACCATGCTT
TGGGTTTTAT GGTGTTTTGT GACCCCGTCA TTGGGATCTAT GTGGGTTTTT TTGGGAGGTT
30
ATGGGCTAAA AGAAAAAGAC ATAGTAACAC CGGAGTGGGCCTTGTTTTTTT TGGGCTTTCC
1020
TCATTTTTTT CGGGGCTGTT CGTTTTAAGGT TGGCCCCAC TGAAACTTAA TGCGAGTGAA
1080
CACATCTGAT TTGCCACAGC ACAAGACCCC TGCGGGGTTT TTGTTTTTTT TGGATCTGTC
1140
ACTCCCAACC TTGGTGAATG CCAATTTCTA AACCTATTCC GGAGAGAGCT CTCAGCTTTA
1200
AGTTGATGAA TACTAAAAGC AGCAAGAACG CTAAACACAC ACCAAAATT AACCTAGTTCA
1260
TGACTTGTAT TAACTTATAA AATGGCTAGA GAAACCTTCA CATGATTTAT TTTTGCCAAA
1320
TTTATCAAT GTAAATTTG TAAAAATGA AAGAAATTAC AAAAAGAAAT ATGGATTGTT
1380
CAAGTGAAGT AATGTTGAGT TCTGAGGGTC AAAACACACA TGAAAGGGCT CGGAAGATTT
1440
AATGTTGTTA TGCAAAAGTG GTCTCTCTGT TCATCAATTTG TAAATGAAAT ATAAACACAT
1500
TTTAGTTTTT AAAATTTTCC GTGGCCAAGA TTTCTCTCTCA CATGAAATTTT TATTTTCTTT
1560
TACAAAAAT TCTGGAAGA TTTAATGPA AA TTGGCTTGG AAGGTCTCCC AAGGGAGTGA
1620
TGACCTGGTT GGAAGAGAGG AGCAACTAGT GCCGCACACC AGGGCTTCTGT TGCCCCCTCC
1680
ATGGTAAAGT CTGCCGCTTTG CCGCGATTTT CCAAGGGCAG GAGGATGTGA CTCCAGCATG
1740
ACAGTGGGCT CGGTGGGAT GCACAGTGCAG TCAGACATCCA CCAATGGAAG AAAGAAAAA
1800
AGGGCGAGAA CTGCCAGATCT CGATCTTTAGA CAGGGGACAA TTTCTTTTGA GTGTGCTC
1860
TAATAAACTG GCTTCCATGT TGCAATTTGC ATGCTGACTG AACCACAGGC CTAATAGACC 1920
AGCAGGCTCA ACTAGAAATGG CAAACACTAG CTACCTGGGAA CTCTTGAGCT CTCTTCCCTTCT 1980
TCAGAAGCTT CTGCCCCCACA GCTGCCCCCAC CTCTGCAAGA TGAGATACAC TCTCTACGGC 2040
CAACAGTTGA AATGGTTCTT ATGAAAATGTG CTACAATTAG CCTTCCGGAAT ATTTGTGCGG 2100
TGATTTAAAT GCTGGGCATTT CATTTCGATG TGAGCCATGG TCACCTGACATA GTCTTCACRC 2160
TGTGAATATT GCTGGGGGAT CAAGACTGTC CTCAAAGAAA TGACTGCTGT TCACCCCAAG 2220
TGACCTCGTG AGTTTTTTTTC TACAAAGTG CATAAAATGT GAAAAACTGGA GAAGACTTCT 2280
GAGGTGGAGG CCCACCTCTCT CTTCCTCAGAT AGGAGGGGAC AGACCAATCGA AATTTAAAGG 2340
ACCTCCCTCC AGCTCAGAGGA CTAGTGGTTTT AATCCTCAGG CAGTGCATTCC CCCACCCCTA 2400
CAACAGGACA CAACCTCTTT CCCCACAGTG CAATTCGAAAT TATGCTCAAG GAATGCCAGC 2460
CACTGGTAA AACTGTCGAG AGAAAAGCATG CATTTCCACA AGGACTAGAT ATCAGTGATT 2520
TGATATTTTTG CTTTTTTTGA TTATCAGTCT TGCTGAATGA GCAAGAGAGT ATCCTAGTAG 2580
CAGGCTTTAT TACAGATAGA AAGAATCTT CAGAAAGTGTC AATGAAATTA ACCAAAACCT 2640
TTATAATGCT GCTGTAACC AAGAAATATG TGAAGACATC ATCTTTAAGTA AACTAAAACAT 2700
TTATTATTTAAA CTTATTTATG TGACTCTTTAA ACTAAAAAAA AAA 2743

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 820 base pairs
(B) TYPE: nucleic acid
(C) STRAND: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

AACTCCGACC GACATCAACAG CTTTACACTGG CTTCCTCAGAA CAAATCAAT CCACTACCTTCT 45
CTCGAAAGGC CTCTAATACCT CTCTCAAGCT TGACAAGTAG CCAACCTTCC AGCATGGAGCAG 120
AGACCTCACG TACCTCTTAA AGCAATAAGTA CACCTGGCAG TCTCTATAGGA GAGTACCTCAA 180
GGAGAAGCTAC ATCTATGCTGT TGAACGGGAAA CTGACCCTTCTG TCACATTGGCA AGGTTACCTT 240
CGTGCTCTCC CGACACCTCA CAYGTGGGGAG GCTGCTCACA GAACTACCAT GCATTAGGAC 300
ATCCTCACAG AAGTGGTTCTT CAGCAGCATG GTCTACATTCC TCAACATTAC CACACCACCAC 360
ACCATTCTCC CAACCTGAGAC CGGGCGCCGCG CCACCATTAG GGACACCAGC CGTGTCGAGC 420
CTATCAGCTGC GCGCAGGCTT ACCAAGTGCCT TGCGCAAGAG CGTGTTCACAG GACAGTGGCA 480
CCAGGTGCG CAGGGAATTC ATGGCCGAGA CGAGCGAGATC CATCATCAGCC AATGGAAAGA 540
(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 995 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

ATTATGGAATT CCGACGAGA CGGACAGCTG GGAGCTGGCC TGGGCCAGGA CGGACCGTG 60
CCCTGGAGAT GGAAAAGGAG CCCTGGCACG GAGCGTCCAG CCAAAGCGCT CACCTCCTGT 120
TTCTCCTACT GCTCTGCGCC TGGCTGGCAA GGCACTCCGC TCCCTGGAAC 180
GCTGGGGGCT CCCACCCCTC TTACCACCCC CGGAGGAAAC GCGAGGCCG CGGGGTGTGT 240
TTCCCTGCGT GGGTGCCGGT TGGAGCGGTT GATCAACTGAT CAGAAAGAGA ATGACCCTTA 300
GATGCGCGA CTTGCGGTG TGTGTTTTTTA AATTTTTTTA AACCAAGAAG TATTTCTCTCT 360
GCTCTGCGG TCTCACCATC TCCCGACGAG GAGTCGAACGC CACACTCTGC AGGAACCCTT 420
TGGACACTTC AGGCTCAGAG TGGAAATCTTT TAAAGACAGG ACCCTAGGTT CAGGAAAAGG 480
GAAAAGAAA CTGGGCAACT GATAGCGCAG ACAAGAAAAG CAATAATATA TAAATAAATAT 540
AATAATAAAG AGAAATTAAA TAAATAATA AAAACATAA GACAGCCCTT TGTTGAGGTC 600
AGGAGGGGAG AGGGCTCCGC CGGAGTGGG TCTTGGCGTG GATTTGCCA CAGCAACTTC 660
CTGAGGAGAG CACTTGATAT GAACCGGCG CCCTCGGTTG TCAAGGCGCG AGGTTTTATT 720
CTGATAGCTG CGAGGCGACA CACAAAGATG CAACCCCTCTA ATAAACATGA TGGGCCAGTC 780
CCACCTCCGT CCGCGCGCTG NCCCTATGCC CCCCGAGGCC TGGGATCTGC AGGGTGCGTT 840
GGGCGCGGG GCCCTGCCGC TCTTGGCGTT GATTTGCCTC CCGTGCGCC AGCGGGTTCC 900
AGGCTCAGGA AGTACAGTCG TGGGCGACT GACCTCGGCT GCTCTGGGCC CTGGGGGCTA 960
TTTGACAAAT ATTTGCCCAA GCGCTCCCAA GCNCA
(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 649 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: double
   (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

GAATTCCGCA CGACCACCTT TAAAGCATT CATTTTCCCT AATTTYTCCT 60
AAATATCCAT TGTTTCTCTT CACCTCCTTG TCTGACGATG TCTCCCATGT 120
CACCACAGGG
GAAGCTGTYA TGTCACAAGA TGTTGTTACT GATGACCTTG GAATTWTTCC 180
CCTGCCCCCA
GCTCGGGACT AGTGAATTA TTAATTCAT GTGCTTTTTT TTAAGCCATA GCAAAGCAAT 240
TTATCTTCAA CATATATCAT TTTAAACATC TTTAATATAT TACAAATTTA 300
CTGCTYCTYC
TGGCACAAAT CAGCTATCCAA GTAACATTIA TTAATTAAGT TATGWCACA TACCAAGAT 360
TTTACAGGCT TTTAATAAGT CAGGCCATTY CAAGATTTC TCTCTGGTTA 420
RAAGAAATTG
TGGGAAGAA GTTGAATAAAT CACTAAATTTA CATCTATATC AAGACACCTT 480
TTCTCGASGT
AAATTAGCT ACTGTTGTCTT TTTTTAATTT ACTAAGNAAA TATCATGCCA
540
TACCTGTCTT ATATGCTATAT TATCTCCTCC TTTCCAGGAC CACACTYCTC 600
GTTCAGAAGG
GGAGACTGTG CTCTAAGCGA GTCAGAGAGA TGTTTTCAGT APATTTTNTA 649

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 979 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: double
   (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

AGTTTCTTTG CTCCTCTCTG TAAATTTAG AATAACCTTG TCTATATCTA CAGATCTTGC 60
TGGAATTITG ATATAAGATG CATTGACTCT TTTAACAAT TGGAGGGAGA TTTGATACCT 120
TTTCTGCGG TCTTTGTAATC TGAACCATG TTTATATCTG TATATTTGAC ATTTTGGATT 180
TTTCCTCAGT GTGCTACTCT TTTGAATATA CAAATCTCTG ACCCTTTTTT TTTAGAATTA
240
CACCTGTATC TTTATTTTTT GAACTAGTG AATGGAATTT GATTTTTTAA TTTACATTCC 300
CATTTTGTCC ATTGCTATTA TACTGAAAAA ARAATTGGCTT TTTGAGTTTT ATCTGGTATC
360
CCACAATCTT CCGAACTCTM CTGTTTCTAA RACTTTTTGT ARAATTACGGG GAATTTCYAC 420
ATACACAGTT AGCACATCTG CAAATGGGA TAGTTTTGCT TTTCCTTA ACAAAGCTAT
ACTTTTTTTA TGGTTTCTT GCTTTTACCC GAGAATTCT AGAGCCTGTG TGAGAAATAG
TGGATATCTT TGGCTCTGTC AGTCTCTAT AGGAAAGGCA TTCAGTCTT CACCAATTTAG
TATAAGCCTA GCGTCAGGA CTCTTTAGAT CCGTCTACCA GATGGATGAA AGATACCTTC
TATTCCTCAT TTCTCTGAGA GCTTTTTAAA AAAAGAATGCA ATGGCTGCTC GATTTTGACA
AAATGCTTTC TGTCTGAGTT GCTATGAACA AGTGTTTTTC TTATCCAGCC TGTTAAATAG
GTGAGTTACA TTGATGCGCT TTCTGACCTT GACACAGAT TGATCCCTGG GATAAAACTC
CACCTGGGCT TGAGAANAAG ATTCITTTTT TTTTTTTTTTTTTTTTTTTTTTGGATG TGAGCTTCGC
TCGTCACCC AGCCCGAGGT GCACGGGGGT GATGGGGGCT CAGGGCAAGT TCCCTCCTCT
GCTTCCCAT CATTCTCAA

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 905 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

CGACGCTTTT ACCTTGTTAG CCAGCTCGGT TTGTGCGGCC AAATTGCTCT AAAATGCTGT
CAAAAAAGG AATTGAGCTG ATTTCTCCTA ATAGGCGACG GCTAAGCTCT ATATTGCTGT
GIGAGTACAG AAACGCTTTCCTGACCATTTA CTCTTGAGCT TCTCGAGCT CTCTGTTTAC
ATACAGTTTT TCGAGATCCT AACTCCAGGT AGACCGCAGG AGAGCTCCCT CAGGATTCTC
TCAAAAAGCG AAAAATATAG GCAAGATTTT GTTTCTCCTA AAAAATAAGC ACCATTTTACA
TGAGATGTTT TGGGCGGTGA GTTCTTAGT GAAAGGCTCT TTGGGGCGCT AAAACTGATG
TTTTGCGAG TTACCTCCAG GAAACCGAGG TTGTTGAGT TTACAGACAC ATTAACCAA
AGGGCGTGGG AAAACCTCCCT TCCAGCTCTA GGGATGCTGT CAGGACCC CACTTACCAG
TGCGCTCTCTT CTCAAACTCCT ACCTTTAGGA GTCTTGTGGAT ATTAAAGATG GCCATTTTGG
ATGGGAATTT GCAATGACGA CAGGGTTTGTG AGTCTGCTGT TTTTTTTTTT TCTTCTTCTT
TCGGCGGAC TGGGGGACCT CTCCTGCAAGTT CAGATTTAA CTTTTTTTTT TCTTACTTCA
TTTTAAAATA GAATAAACG GTGAAATGTT GTCTCAATGT TAAAGGGATA ATCTCTGCTAC
CGCTCCCTCC CTGATGTTTC TGAATTAACAC TACTGGAAGA GCTCTGGGCTG GCCTCTTTCTA
TCGCTGATGT GCTTTTCCCT TGGAGCAAAT CTTGAGTGTG CAGTGTCAAA AGATGTACTC
TTCTCAACAA GAAAACCTTA AATCCGCTGT GCCCAAAAAA AAAAAAAA AAAAAAAA
CTCGA

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 299 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

GAATTCCTTT TTCTTTTTTT TTCTTTTTTGAC AGAGCTCCTAC TGCTCTCCGC AGCTCTGGAGT 60
GGAGAGCTAC ATCTCGGGCT TTCTGACAAC TCTCTCCITCC AGCTTCAAGC GATCTCTCATG
CCCTCGGCCC ACAACTAGGC GGGATGACAG GCAATGCATCA CCAGACCTCG CTAATTITTTG 120
TTATTTTTGT AGAGACGGCG TTCTCACCATC TTGGCCAGAC TGCTCTCCTA CTCTTGCCCCT 180
GAGTGAATTC GTCTGCTTCA GCTCTCCAAA AAAAAAAA AAAAAAAA AGAGATTTCC 240

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 338 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

GAATTCCTCT TTCTTTTTTT TTCTTTTTTT GAGACAAGGG TTCTCCTCTG TGTCCTGGGC 60
TAAAGTGGAG TGGAGCAATC ATAGCTCAGT GTGGCCCTCA CTCTCCGGTC TGCAATGATC
ATCTGCCCTC AGCTCCCTAA GTACCTAGGA GTCTGCACCC AACAGCCCAA GCTAAGTATT
TTATTTGAG TAGAGCTGATGT TTGGCCAGCG CTACATCCAA ATCTGCGGCC 180
TCAAACGACT CCCTGCGCTTT TTCTTTTTAA ANNNAAAA 320

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 500 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

```
GAATCTCTTTT TTTTTTTTTT TTTCCTCAGT ACCCActACA GCATTATCTT TGTCCTCAGG
TTGAGGAGT CATCCCTCTCC CAGGAGGGCC GAGACAAGTG CACCTAGGTT GCCGGAAGTT
GTGACGATCA CTCTGCTCTG TATCTGTGTA CCCAACCAG GCCACGTGGGA CTCCTCCAGA
TGGGAGGAC GATGGAAGAG AAAAGAAGAAA GGCTGAGGAG AAGGATGAGAC CACTCACCAC
TTGCTGGGAAT AAACAAATCT GCAGCTGTGT TTTTAACACT TCATTITCAT CATGCCCACAC
TGTCGCTAG TGAAATACGG CCAACCACAG AGGCAGGGCC TTATCTTTCG AGACCAGAAA
TGCTTTTGGC AGATGGGGAGT GAAACAGGTT CTTGTYGAAGAA GGGATCAAGT TAGGATCTTT
GAGNTGCGAA GAATTTCTGT GCATGYCTCTG CTAGACACTA AAAAAAAA AAAAAAAA
GAATCTAAAA AGCTTCTCGA
```

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 94 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

```
Met Ile Tyr Tyr Phe Leu Lys Asn Gln Cys Gly Leu Pro Phe Leu Phe
1 5 10 15
Leu Leu Ile Pro Leu Phe Glu Phe Leu Cys Val Ser Phe Ala Phe Pro
20 25 30
Ser Gln Ser Gly Gly Val Arg Pro Ala Leu Trp Asp Glu Arg Ser Cys
35 40 45
Gly Tyr Val Ser Ala Gly Thr Lys Arg Ala Glu Gly Glu Val Trp Lys
50 55 60
Gly Gln Gly Glu Glu Met Gly Ser Ile Val Lys Arg Leu Val Pro Leu
65 70 75 80
Ser Lys Tyr Val Glu Asn Asp Asp Gly Lys Val Ser Pro Cys
85 90
```

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

Met His Pro Gln Ser Ala Phe Cys Ala Leu Ala Ala Ala Asn Ala Ser
1  5  10  15

Leu Gly Arg Ser Ser Cys Gln
20

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

Met His Cys Phe Phe Leu Trp Leu Leu Leu Phe Gly Leu Leu Gly Ile
1  5  10  15

Ser Gly Phe Leu Gly Tyr Ile Ser Val Ala Gly Xaa Ser Ile Tyr Val
20  25  30

25 Met Trp Lys Val Glu Lys Glu Met Asn Thr
35  40

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

Met Phe Leu Ser Leu Pro Thr Leu Thr Val Leu Ile Pro Leu Val Ser
1  5  10  15

Leu Ala Gly Leu Phe Tyr Ser Ala Ser Val Glu Glu Asn Phe Pro Gln
20  25  30

45 Gly Cys Thr Ser Thr Ala Ser Leu Cys Phe Tyr Ser Leu Leu Leu Pro
35  40  45

Ile Thr Ile Pro Val Tyr Val Phe Phe His Leu Trp Thr Trp Met Gly
50  55  60

50 Ile Lys Leu Phe Arg His Asn
65  70

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 410 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

Met Glu Leu Pro Ser Gly Pro Gly Pro Glu Arg Leu Phe Asp Ser His
1     5       10          15

Arg Leu Pro Gly Asp Cys Phe Leu Leu Leu Leu Leu Leu Tyr Ala
20    25       30

Pro Val Gly Phe Cys Leu Leu Leu Leu Arg Leu Phe Gly Ile His
35    40       45

Val Phe Leu Val Ser Cys Ala Leu Pro Asp Ser Val Leu Arg Arg Phe
50    55       60

Val Val Arg Thr Met Cys Ala Val Leu Gly Leu Val Ala Arg Gin Glu
65    70       75          80

Asp Ser Gly Leu Arg Asp His Ser Val Arg Val Leu Ile Ser Asn His
85    90       95

Val Thr Pro Phe Asp His Asn Ile Val Asn Leu Leu Thr Thr Cys Ser
100   105     110

Thr Pro Leu Leu Asn Ser Pro Pro Ser Phe Val Cys Trp Ser Arg Gly
115   120     125

Phe Met Glu Met Asn Gly Arg Gly Leu Val Glu Ser Lys Arg
130   135     140

Phe Cys Ala Ser Thr Arg Leu Pro Thr Pro Leu Leu Leu Phe Pro
145   150     155          160

Glu Glu Glu Ala Thr Asn Gly Arg Gly Leu Arg Asp Ser Ser
165   170     175

Trp Pro Phe Ser Ile Gin Asp Val Val Gin Pro Leu Thr Leu Gin Val
180   185     190

Gln Arg Pro Leu Val Ser Val Thr Val Ser Asp Ala Ser Trp Val Ser
195   200     205

Glu Leu Leu Trp Ser Leu Phe Val Pro Phe Thr Val Tyr Gln Val Arg
210   215     220

Trp Leu Arg Pro Val His Arg Gln Leu Gly Glu Ala Asn Glu Glu Phe
225   230     235          240

Ala Leu Arg Val Gln Gin Leu Val Ala Lys Glu Leu Gly Gln Thr Gly
245   250     255

Thr Arg Leu Thr Pro Ala Asp Lys Ala Glu His Met Lys Arg Gin Arg
260   265     270

His Pro Arg Leu Arg Pro Gln Ser Ala Gln Ser Ser Phe Pro Pro Ser
275   280     285

Pro Gly Pro Ser Pro Asp Val Gln Leu Ala Thr Leu Ala Gln Arg Val
290   295     300
(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

Met Val Glu Leu Ala Ser Cys Asn Ser Pro Phe Ser Phe Leu Pro Leu
1 5 10 15
Ser Leu Pro Ala Phe Pro Ile Leu Leu
20 25

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

Met Leu Phe Pro Ile Leu Leu Gln Thr Met Phe Ser Ala Tyr Leu Gly
1 5 10 15
Ser Glu Gln Tyr Lys Leu Leu Ile Lys Ala Leu Gln Leu Ser Glu Pro
20 25 30
Gly Lys Glu Ile His
35

(2) INFORMATION FOR SEQ ID NO: 38:
(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 64 amino acids
   (B) TYPE: amino acid
   (D) TOPOLOGY: linear

(xii) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

   Met Lys Val Leu Ile Ser Ser Leu Leu Leu Leu Leu Pro Leu Met Leu
   1  5   10  15

   Met Ser Met Val Ser Ser Ser Ser Leu Asn Pro Gly Val Ala Arg Gly His
   20  25  30

   Arg Asp Arg Gly Gln Xaa Ser Arg Arg Trp Leu Gln Glu Gly Gln
   35  40  45

   Glu Cys Xaa Cys Lys Asp Trp Phe Leu Arg Ala Arg Glu Glu Asn Ser
   50  55  60

(2) INFORMATION FOR SEQ ID NO: 39:

   (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 155 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

   (xii) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

   Met Cys Cys Ser Leu Ser Met Ile Gly Ala Ala Ser Ser Ser Pro Ser Ser
   1  5  10  15

   Gly Cys Ser Ala Arg Ala Arg Ala Ser Gly Leu Pro Trp Leu Trp
   20  25  30

   Gln Pro Cys Pro Gly Pro Arg Phe Arg Cys Ser Leu Trp Met Arg Arg
   35  40  45

   Ser Gln Ile Val Ala Pro Ser Thr Cys Ala Pro Arg Ser Gly Ala Xaa
   50  55  60

   Gly Leu Ala Val Gly Cys Gly Ala Ile Gly Ala Leu Val Leu Gly Ala
   65  70  75  80

   Gly Leu Leu Phe Ser Leu Arg Ser Val Arg Ser Val Leu Arg Ala
   85  90  95

   Gly Gly Gln Gin Val Thr Leu Thr Thr His Ala Pro Phe Gly Leu Gly
   100 105 110

   Ala His Phe Thr Val Pro Leu Lys Gin Val Ser Cys Met Ala His Arg
   115 120 125

   Gly Glu Val Pro Ala Met Leu Pro Leu Lys Xaa Lys Gly Arg Arg Phe
   130 135 140

   Tyr Phe Leu Leu Asp Lys Thr Gly His Phe Pro
   145 150 155
(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

Met Thr Val Tyr Ala Leu Val Val Val Ser Tyr Phe Leu Ile Thr Gly
1  5  10  15

Gly Ile Ile Tyr Asp Val Ile Val Glu Pro Pro Ser Val Gly Ser Met
20  25  30

Thr Asp Glu His Gly His Gln Arg Pro Val Ala Phe Leu Ala Tyr Arg
35  40  45

Val Asn Gly Gln Tyr Ile Met Glu Gly Leu Ala Ser Ser Phe Leu Phe
50  55  60

Thr Met Gly Gly Leu Gly Phe Ile Leu Asp Arg Ser Asn Ala Pro
65  70  75  80

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

Cys Val Leu Leu Ser Phe Phe Met Ala Arg Val Phe Met Arg Met Lys
100 105 110

Leu Pro Gly Tyr Leu Met Gly
115

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 56 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

Met Glu Ser Gly His Leu Leu Trp Ala Leu Leu Phe Met Gln Ser Leu
1  5  10  15

Trp Pro Gln Leu Thr Asp Gly Ala Thr Arg Val Tyr Tyr Leu Gly Ile
20  25  30

Arg Asp Val Gln Trp Asn Tyr Ala Pro Lys Gly Arg Asn Val Ile Thr
35  40  45

Asn Gln Pro Leu Asp Ser Asp Met
50  55

(2) INFORMATION FOR SEQ ID NO: 42:
(i) **SEQUENCE CHARACTERISTICS:**
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) **SEQUENCE DESCRIPTION:** SEQ ID NO: 42:

Met Lys Asp Phe Asn His Ser Tyr His Ala Cys Gly Val Ile Ala Thr
1  5  10  15

Ile Ala Phe Leu Met Ile Asn Ala Val Ser Asn Gly Glu Val Arg Gly
20 25 30

Asp Ser Tyr Ser Glu Gly Cys Leu Gly Gln Thr Gly Ala Arg Ile Trp
35 40 45

Leu Phe Val Gly Phe Met Leu Ala Phe Gly Ser Leu Ile Ala Ser Met
50 55 60

Trp Ile Leu Phe Gly Gly Tyr Val Ala Lys Glu Asp Ile Val Tyr
65 70 75 80

Pro Gly Ile Ala Val Phe Phe Glu Ala Asp Ala Phe Phe Gly Gly
85 90 95

Leu Val Phe Lys Phe Gly Arg Thr Glu Asp Leu Trp Gln
100 105

(2) **INFORMATION FOR SEQ ID NO: 43:**

(i) **SEQUENCE CHARACTERISTICS:**
(A) LENGTH: 69 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) **SEQUENCE DESCRIPTION:** SEQ ID NO: 43:

Met Val Thr Ile Phe Asn Ile Ile Thr Thr Thr Thr Ile Leu Pro Thr
1  5 10  15

Gln Thr Ala Ala Ala Pro Pro Ser Trp Thr Pro Ala Val Cys Ser Leu
20 25 30

Ser Ser Trp Pro Gly Ser Pro Arg Ser Trp Ala Gly Pro Val Leu Arg
35 40 45

Asp Ser Ala Arg Arg Cys Ala Trp Asn Ser Trp Thr Thr Arg Ala Asp
50 55 60

Pro Ser Ser Ala Met
65

(2) **INFORMATION FOR SEQ ID NO: 44:**

(i) **SEQUENCE CHARACTERISTICS:**
(A) LENGTH: 67 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

Met Gly Lys Cys Leu Cys Arg Gly Ala Glu Leu Ser Leu Cys Phe Ser
  1  5  10  15
Phe Phe Pro Leu Leu Leu Pro Leu His Thr Pro Val Ala Gly Arg Asn
  20  25  30
Leu Gly Phe Pro Glu Ser Leu Gly Val Pro Pro Phe Leu Pro His Pro
  35  40  45
Gly Gly Thr Pro Arg Ala Pro Gly Leu Phe Leu Leu Leu Leu Phe Ser Phe
  50  55  60
Trp Ala Val
  65

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 41 amino acids
     (B) TYPE: amino acid
     (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

Met Leu Leu Leu Met Ile Leu Glu Xaa Phe Pro Cys Pro Gln Pro Gly
  1  5  10  15
Thr Asn Val Ile Ile Ile Ser Met Cys Phe Phe Leu Ser His Ser Asn
  20  25  30
Ala Ile Tyr Leu Gln His Tyr His Phe
  35  40

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 9 amino acids
     (B) TYPE: amino acid
     (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

Met Leu Tyr Leu Leu Tyr Leu Ala Phe
  1  5

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 47 amino acids
     (B) TYPE: amino acid
     (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:
Met Gly Asn Cys His Asp His Arg Gly Leu Glu Phe Cys Phe Phe Phe Phe Phe Phe Phe Gly Gly Leu Gly Asp Ser Ser Gln Asp His Ile 1 5 10 15
Leu Ala Ser Phe Ser Pro Thr Pro Phe Arg Lys Ile Ser Asn Arg 20 25 30 35 40 45

(2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

Met His His Thr Leu Leu Ile Phe Val Phe Leu Val Glu Thr Ala 1 5 10 15
Phe His Leu Gly Gln Thr Gly Leu Lys Leu Ala Ser Ser Asp 20 25 30
Ser Ser Ala Ser Ala Ser Gln Lys Lys Lys Lys Lys Lys Lys Asn 35 40 45
Ser

(2) INFORMATION FOR SEQ ID NO: 49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

Met His Gln His Ala Gln Leu Ser Ile Leu Phe Leu Val Glu Ile Arg 1 5 10 15
Ser Cys Cys Val Ala Gln Ala Ser Leu Lys Phe Leu Ala Ser Ser Asn 20 25 30
Pro Ser Ala Leu Ala Ser Gln Ile Val Gly Phe Xaa Arg His 35 40 45

(2) INFORMATION FOR SEQ ID NO: 50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:
Met Asp His Ser Pro Phe Ala Gly Ile Asn Asn Thr Ala Val Leu Val
  1     5     10      15
Leu Thr Leu His Phe His His Ala Thr Leu Ser Val Thr Glu
  20    25     30

(2) INFORMATION FOR SEQ ID NO: 51:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 35 amino acids
    (B) TYPE: amino acid
    (D) TOPOLOGY: linear

(xii) SEQUENCE DESCRIPTION: SEQ ID NO: 51:
Cys Ala Gly Cys Asp Glu Leu Ile Phe Ser Asn Glu Tyr Thr Gln Ala
  1     5     10      15
Glu Asn Gln Asn Trp His Leu Lys His Phe Cys Cys Phe Asp Cys Asp
  20    25     30
Ser Ile Leu
  35

(2) INFORMATION FOR SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 159 amino acids
    (B) TYPE: amino acid
    (D) TOPOLOGY: linear

(xii) SEQUENCE DESCRIPTION: SEQ ID NO: 52:
Ala Arg Gly Phe Val Cys Ser Thr Cys His Glu Leu Leu Val Asp Met
  1     5     10      15
Ile Tyr Phe Trp Lys Asn Glu Lys Leu Tyr Cys Gly Arg His Tyr Cys
  20    25     30
Asp Ser Glu Lys Pro Arg Cys Ala Gly Cys Asp Glu Leu Ile Phe Ser
  35    40     45
Asn Glu Tyr Thr Gln Ala Glu Asn Gln Asn Trp His Leu Lys His Phe
  50    55     60
Cys Cys Phe Asp Cys Asp Ser Ile Leu Ala Gly Glu Ile Tyr Val Met
  65    70     75      80
Val Asn Lys Pro Val Cys Lys Pro Cys Tyr Val Lys Asn His Ala
  85    90     95
Val Val Cys Gln Gly Cys His Asn Ala Ile Asp Pro Glu Val Gln Arg
 100   105    110
Val Thr Tyr Asn Phe Ser Trp His Ala Ser Thr Glu Cys Phe Leu
115   120   125
Cys Ser Cys Cys Ser Lys Cys Leu Ile Gly Glu Lys Phe Met Pro Val
130 Glu Gly Met Val Phe Cys Ser Val Glu Cys Lys Lys Arg Met Ser 145 150 155

(2) INFORMATION FOR SEQ ID NO: 53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 93 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

Gly Val Ala Arg Gly His Arg Asp Arg Gly Gin Ala Ser Arg Arg Trp 1 5 10 15
Leu Gln Glu Gly Gly Gin Glu Cys Gin Lys Asp Trp Phe Leu Arg 20 25 30
Ala Pro Arg Arg Lys Phe Met Thr Val Ser Gly Leu Pro Lys Gin 35 40 45
Cys Pro Cys Asp His Phe Lys Gly Asn Val Lys Thr Arg His Gin 50 55 60
Arg His His Arg Lys Pro Asn Lys His Ser Arg Ala Cys Gin Gin Phe 65 70 75 80
Leu Lys Gin Cys Gin Leu Arg Ser Phe Ala Leu Pro Leu 85 90

(2) INFORMATION FOR SEQ ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

His Thr Gln Val Glu Phe Ile Pro Arg Met Gin Cys 1 5 10

(2) INFORMATION FOR SEQ ID NO: 55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

Leu Lys Ile Arg Lys Pro Ile Asn Val Ile Tyr His Ile Asn Arg Leu 1 5 10 15
(2) INFORMATION FOR SEQ ID NO: 56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xii) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

Arg Lys Met Gly Ile Glu Arg Asn Phe His Gln Ser Gly Lys Gly Ile
1  5  10  15

(2) INFORMATION FOR SEQ ID NO: 57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xii) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

Lys Val Pro Thr Ala Asn Ile Ile Leu Asn Gly Glu Arg Leu Asn Ala
1  5  10  15
Phe Pro Ile Arg Thr
20

(2) INFORMATION FOR SEQ ID NO: 58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xii) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

Ile Phe Ser Ser Val Leu His Ser Phe Gln Tyr Thr Asn Pro Val Pro
1  5  10  15
Phe Phe Phe Arg Phe Thr Pro Ser Thr Leu Phe Phe
20  25

(2) INFORMATION FOR SEQ ID NO: 59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xii) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

Lys Val Pro Thr Ala Asn Ile Ile Leu Asn Gly Glu Arg Leu Asn Ala
1  5  10  15
Phe Pro Ile Arg Thr
60
(2) INFORMATION FOR SEQ ID NO: 60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:

Met Tyr Phe Leu Ser Ser Leu Leu Ile His Glu His Val Ile Ser Val
1      5      10     15

Ile Phe Ser Ile Leu
20

(2) INFORMATION FOR SEQ ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

Glu Asp Gly Ser Ala Pro Arg Glu Gly Glu Thr Ser Ala Pro Arg Leu
1      5      10     15

Pro Glu Val Val Arg Ile Thr Ser Ala Gly Ile Cys
20     25
INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description
   on page 19, line N/A

B. IDENTIFICATION OF DEPOSIT

   Further deposits are identified on an additional sheet □
   Name of depository institution
   American Type Culture Collection
   Address of depository institution (including postal code and country)
   American Type Culture Collections
   10801 University Blvd.
   Manassas, VA 20110-2209
   United States of America
   Date of deposit March 13, 1997
   Accession Number 97955

C. ADDITIONAL INDICATIONS (leave blank if not applicable)
   This information is continued on an additional sheet □

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")

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Form PCT/R0/134 (July 1992)
INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

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Name of depositary institution: American Type Culture Collection

Address of depositary institution (including postal code and country):
American Type Culture Collections
10801 University Blvd.
Manassas, VA 20110-2209
United States of America

Date of deposit: May 22, 1997
Accession Number: 209074

C. ADDITIONAL INDICATIONS (leave blank if not applicable)

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D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

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Form PCT/RO/134 (July 1992)
What Is Claimed Is:

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:
   (a) a polynucleotide fragment of SEQ ID NO:X or a polynucleotide fragment of the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
   (b) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:Y or a polypeptide fragment encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
   (c) a polynucleotide encoding a polypeptide domain of SEQ ID NO:Y or a polypeptide domain encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
   (d) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:Y or a polypeptide epitope encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
   (e) a polynucleotide encoding a polypeptide of SEQ ID NO:Y or the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X, having biological activity;
   (f) a polynucleotide which is a variant of SEQ ID NO:X;
   (g) a polynucleotide which is an allelic variant of SEQ ID NO:X;
   (h) a polynucleotide which encodes a species homologue of the SEQ ID NO:Y;
   (i) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(h), wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A residues or of only T residues.

2. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding a secreted protein.

3. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:Y or the polypeptide encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X.
4. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:X or the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X.

5. The isolated nucleic acid molecule of claim 2, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

6. The isolated nucleic acid molecule of claim 3, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

7. A recombinant vector comprising the isolated nucleic acid molecule of claim 1.

8. A method of making a recombinant host cell comprising the isolated nucleic acid molecule of claim 1.


10. The recombinant host cell of claim 9 comprising vector sequences.

11. An isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

   (a) a polypeptide fragment of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;
   (b) a polypeptide fragment of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z, having biological activity;
   (c) a polypeptide domain of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;
   (d) a polypeptide epitope of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;
   (e) a secreted form of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;
   (f) a full length protein of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;
(g) a variant of SEQ ID NO:Y;
(h) an allelic variant of SEQ ID NO:Y; or
(i) a species homologue of the SEQ ID NO:Y.

12. The isolated polypeptide of claim 11, wherein the secreted form or the full length protein comprises sequential amino acid deletions from either the C-terminus or the N-terminus.

13. An isolated antibody that binds specifically to the isolated polypeptide of claim 11.

14. A recombinant host cell that expresses the isolated polypeptide of claim 11.

15. A method of making an isolated polypeptide comprising:
   (a) culturing the recombinant host cell of claim 14 under conditions such that said polypeptide is expressed; and
   (b) recovering said polypeptide.

16. The polypeptide produced by claim 15.

17. A method for preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of the polypeptide of claim 11 or the polynucleotide of claim 1.

18. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:
   (a) determining the presence or absence of a mutation in the polynucleotide of claim 1; and
   (b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or absence of said mutation.

19. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:
   (a) determining the presence or amount of expression of the polypeptide of claim 11 in a biological sample; and
   (b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.
20. A method for identifying a binding partner to the polypeptide of claim 11 comprising:
   (a) contacting the polypeptide of claim 11 with a binding partner; and
   (b) determining whether the binding partner effects an activity of the polypeptide.

21. The gene corresponding to the cDNA sequence of SEQ ID NO:Y.

22. A method of identifying an activity in a biological assay, wherein the method comprises:
   (a) expressing SEQ ID NO:X in a cell;
   (b) isolating the supernatant;
   (c) detecting an activity in a biological assay; and
   (d) identifying the protein in the supernatant having the activity.

23. The product produced by the method of claim 22.
INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

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B. IDENTIFICATION OF DEPOSIT

Further deposits are identified on an additional sheet ☐

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American Type Culture Collections
10801 University Blvd.
Manassas, VA 20110-2209
United States of America

Date of deposit: March 13, 1997
Accession Number: 97955

C. ADDITIONAL INDICATIONS (leave blank if not applicable)
This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4)EPC).

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")

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CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.
DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant’s Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.
INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

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B. IDENTIFICATION OF DEPOSIT

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Manassas, VA 20110-2209
United States of America

Date of deposit: May 22, 1997
Accession Number: 209074

C. ADDITIONAL INDICATIONS (leave blank if not applicable)

This information is continued on an additional sheet □

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

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E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

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Form PCT/RO/134 (July 1992)
CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

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