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(54) Title: COMPOSITIONS AND METHODS FOR THE TREATMENT OF NATURAL KILLER CELL RELATED DISEASES

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Abstract: The present invention relates to compositions containing novel proteins and methods of using those compositions for the diagnosis and treatment of immune related diseases.

COMPOSITIONS AND METHODS FOR THE TREATMENT OF NATURAL KILLER CELL
RELATED DISEASES

Field of the Invention

5 The present invention relates to compositions and methods useful for
the diagnosis and treatment of immune related diseases.

Background of the Invention

10 It is to be understood that, if any prior art publication is referred
to herein, such reference does not constitute an admission that the
publication forms a part of the common general knowledge in the art,
in Australia or any other country.

15 Immune related and inflammatory diseases are the manifestation or
consequence of fairly complex, often multiple interconnected
biological pathways which in normal physiology are critical to respond
to insult or injury, initiate repair from insult or injury, and mount
innate and acquired defense against foreign organisms. Disease or
pathology occurs when these normal physiological pathways cause
20 additional insult or injury either as directly related to the
intensity of the response, as a consequence of abnormal regulation or
excessive stimulation, as a reaction to self, or as a combination of
these.

25 Though the genesis of these diseases often involves multistep pathways
and often multiple different biological systems/pathways, intervention
at critical points in one or more of these pathways can have an
ameliorative or therapeutic effect. Therapeutic intervention can occur
by either antagonism of a detrimental process/pathway or stimulation
30 of a beneficial process/pathway.

35 Many immune related diseases are known and have been extensively
studied. Such diseases include immune-mediated inflammatory diseases,
non-immune-mediated inflammatory diseases, infectious diseases,
immunodeficiency diseases, neoplasia, etc.

Immune related diseases could be treated by suppressing the immune

response. Using neutralizing antibodies that inhibit molecules having immune stimulatory activity would be beneficial in the treatment of immune-mediated and inflammatory diseases. Molecules which inhibit the immune response can be utilized (proteins directly or via the use of 5 antibody agonists) to inhibit the immune response and thus ameliorate immune related disease.

Natural killer (NK) cells are an important effector cell of the innate immune system. They are specialized to effect killing against host 10 cells that have either been infected by viruses, parasites or that have become cancerous. Phenotypically, NK cells are large granular lymphocytes that constitute ~2 % of the circulating lymphocyte population. They are commonly identified by cell surface expression of CD56 and CD16. NK cells mature in the bone marrow from a CD34+ 15 precursor cell that they share with T cells. The mature NK cell, shares expression of CD8, cytolytic machinery, and some KIRs, with T cells, but remains distinct from T cells by the lack of CD3 and the T cell receptors. Like cytotoxic T cells, they contain granules filled with pore forming protein, cytotoxins, serine esterases and 20 proteoglycans that mediate lysis of target cells. Both cytotoxic T cells and NK cells kill on contact by binding to their targets and delivering their lethal burst of chemicals that produces holes in the target cell's membrane. Unlike cytotoxic T cells, NK cells do not need to recognize a specific antigen before initiating lysis. Rather, NK 25 cell activation can be mediated by growth factors and cytokines such as, IL-2, IL-12 and IL-15 have been shown to mediate proliferative and cytotoxic activities or by a delicate balance between two classes of NK cell receptors, one that activates the cells, and another that inhibits. Killer Ig-like receptors (KIRs) are NK cell receptors that 30 transmit an inhibitory signal if they encounter class I MHC molecules on a cell surface. This is important for killing of both cancerous cells and virally infected cells. Because viruses often suppress class I MHC expression in cells they infect, the virus-infected cell becomes susceptible to killing by NK cells. Likewise, cancer cells have 35 reduced or no class I MHC expression also become susceptible to killing by NK cells.

Natural cytotoxicity receptors (NCRs) constitute a family of activating receptors on NK cells. In some effector-target systems, the surface density of NCRs correlates with the cytolytic activity of the NK cells, while in other systems killing requires cooperation between 5 NCR, another activating receptor NKG2D and its adaptor polypeptide DAP10. Additionally, the strength of the stimulatory signals can be influenced by engagement of co-receptors such as 2B4 and NTB-A. The ligands for NCRs and NKG2D, hemoglutinins and MICA, MICB respectively are not expressed by most normal cells, but are induced in most tumor 10 cell lines. Expression of the ligands by tumor cells triggers a dramatic immune response resulting in tumor cell rejection.

Activation of NK cells with IL-15 or IL-12 have been shown to induce both cytotoxic and proliferative effects. Junctional adhesion molecule 15 2 (JAM2) has been shown to bind to NK cells and has been hypothesized to play a role in lymphocyte extravasation to sites of inflammation. Therefore, a DNA microarray experiment comparing differential 20 expression of genes from these three modes of activation versus resting NK cells has the potential to reveal novel genes or novel gene associations with NK cell activity. Therapeutic antibodies, peptides or small molecules could be developed to target specific genes revealed by these microarrays for the treatment of immune mediated inflammatory diseases and malignancies.

25 Despite the above research in NK cells, there is a great need for additional diagnostic and therapeutic agents capable of detecting the presence of NK cell mediated disorders in a mammal and for effectively reducing these disorders. Accordingly, it is an objective of the present invention to identify polypeptides that are differentially 30 expressed in activated NK cells as compared to resting NK cells, and to use those polypeptides, and their encoding nucleic acids, to produce compositions of matter useful in the therapeutic treatment and diagnostic detection of NK cell mediated disorders in mammals.

35 In the claims which follow and in the preceding description of the invention, except where the context requires otherwise due to express

22 Mar 2010
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- 3a -

language or necessary implication, the word "comprise" or variations such as "comprises" or "comprising" is used in an inclusive sense, i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various 5 embodiments of the invention.

Summary of the Invention

A. Embodiments

The present invention concerns compositions and methods useful for the 10 diagnosis and treatment of immune related disease in mammals, including humans. The present invention is based on the identification of proteins (including agonist and antagonist antibodies) which are a result of stimulation of the immune response in mammals. Immune related diseases can be treated by suppressing or enhancing the immune 15 response. Molecules that enhance the immune response stimulate or potentiate the immune response to an antigen. Molecules which stimulate the immune response can be used therapeutically where enhancement of the immune response would be beneficial. Alternatively, molecules that suppress the immune response attenuate or reduce the 20 immune response to an antigen (e.g., neutralizing antibodies) can be used therapeutically where attenuation of the immune response would be beneficial (e.g., inflammation).

In a first aspect, the present invention provides a method of 25 diagnosing an immune related disease in a mammal, said method comprising detecting the level of expression of a gene encoding a PRO polypeptide of the invention as described in Figure 1400 (SEQ ID NO: 1400), (a) in a test sample of tissue cells obtained from the mammal, and (b) in a control sample of known normal tissue cells of the same 30 cell type, wherein a higher or lower level of expression of said gene in the test sample as compared to the control sample is indicative of the presence of an immune related disease in the mammal from which the test tissue cells were obtained, wherein the immune related disease is systemic lupus erythematosis, an 35 idiopathic inflammatory myopathy, Sjögren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, thyroiditis, diabetes mellitus, immune-mediated

2224679_1 (GHHmeters)

2003295401 22 Mar 2010

- 3b -

renal disease, a demyelinating disease of the central or peripheral nervous system, idiopathic demyelinating polyneuropathy, Guillain-Barré syndrome, a chronic inflammatory demyelinating polyneuropathy, a hepatobiliary disease, infectious or autoimmune chronic active hepatitis, primary biliary cirrhosis, granulomatous hepatitis, sclerosing cholangitis, inflammatory bowel disease, gluten-sensitive enteropathy, Whipple's disease, an autoimmune or immune-mediated skin disease, a bullous skin disease, erythema multiforme, contact dermatitis, psoriasis, an allergic disease, asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity, urticaria, an immunologic disease of the lung, eosinophilic pneumonias, idiopathic pulmonary fibrosis, hypersensitivity pneumonitis, a transplantation associated disease, graft rejection or graft-versus-host-disease.

In a second aspect, the present invention provides a method of diagnosing an immune related disease in a mammal, said method comprising detecting the level of expression of a gene as described in Figure 1399 (SEQ ID NO: 1399), (a) in a test sample of tissue cells obtained from the mammal, and (b) in a control sample of known normal tissue cells of the same cell type, wherein a higher or lower level of expression of said gene in the test sample as compared to the control sample is indicative of the presence of an immune related disease in the mammal from which the test tissue cells were obtained, wherein the immune related disease is systemic lupus erythematosus, an idiopathic inflammatory myopathy, Sjögren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, thyroiditis, diabetes mellitus, immune-mediated renal disease, a demyelinating disease of the central or peripheral nervous system, idiopathic demyelinating polyneuropathy, Guillain-Barré syndrome, a chronic inflammatory demyelinating polyneuropathy, a hepatobiliary disease, infectious or autoimmune chronic active hepatitis, primary biliary cirrhosis, granulomatous hepatitis, sclerosing cholangitis, inflammatory bowel disease, gluten-sensitive enteropathy, Whipple's disease, an autoimmune or immune-mediated skin disease, a bullous skin disease, erythema multiforme, contact dermatitis, psoriasis, an allergic disease, asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity, urticaria, an immunologic

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- 3c -

disease of the lung, eosinophilic pneumonias, idiopathic pulmonary fibrosis, hypersensitivity pneumonitis, a transplantation associated disease, graft rejection or graft-versus-host-disease.

5 In a third aspect, the present invention provides a method of diagnosing an immune related disease in a mammal, said method comprising:
(a) contacting an anti-PRO antibody with a test sample of tissue cells obtained from said mammal and
10 (b) detecting the formation of a complex between the antibody and a PRO polypeptide as described in Figure 1400 (SEQ ID NO:1400) in the test sample, wherein formation of said complex is indicative of the presence of an immune related disease in the mammal from which the test tissue cells were obtained,
15 wherein the immune related disease is systemic lupus erythematosis, an idiopathic inflammatory myopathy, Sjögren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, thyroiditis, diabetes mellitus, immune-mediated renal disease, a demyelinating disease of the central or peripheral
20 nervous system, idiopathic demyelinating polyneuropathy, Guillain-Barré syndrome, a chronic inflammatory demyelinating polyneuropathy, a hepatobiliary disease, infectious or autoimmune chronic active hepatitis, primary biliary cirrhosis, granulomatous hepatitis, sclerosing cholangitis, inflammatory bowel disease, gluten-sensitive
25 enteropathy, Whipple's disease, an autoimmune or immune-mediated skin disease, a bullous skin disease, erythema multiforme, contact dermatitis, psoriasis, an allergic disease, asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity, urticaria, an immunologic disease of the lung, eosinophilic pneumonias, idiopathic pulmonary
30 fibrosis, hypersensitivity pneumonitis, a transplantation associated disease, graft rejection or graft-versus-host-disease.

In a fourth aspect, the present invention provides a method of diagnosing an inflammatory immune response in a mammal, said method comprising detecting the level of expression of a gene encoding a PRO polypeptide of the invention as described in Figure 1400 (SEQ ID NO: 1400), (a) in a test sample of tissue cells obtained from the mammal,

- 3d -

and (b) in a control sample of known normal tissue cells of the same cell type, wherein a higher or lower level of expression of said gene in the test sample as compared to the control sample is indicative of the presence of an inflammatory immune response in the mammal from which the test tissue cells were obtained,
5 wherein the inflammatory immune response is systemic lupus erythematosis, an idiopathic inflammatory myopathy, Sjögren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, thyroiditis, diabetes mellitus, immune-mediated renal disease, a demyelinating disease of the central or peripheral nervous system, idiopathic demyelinating polyneuropathy, Guillain-Barré syndrome, a chronic inflammatory demyelinating polyneuropathy, a hepatobiliary disease, infectious or autoimmune chronic active hepatitis, primary biliary cirrhosis, granulomatous hepatitis, sclerosing cholangitis, inflammatory bowel disease, gluten-sensitive enteropathy, Whipple's disease, an autoimmune or immune-mediated skin disease, a bullous skin disease, erythema multiforme, contact dermatitis, psoriasis, an allergic disease, asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity, urticaria, an immunologic disease of the lung, eosinophilic pneumonias, idiopathic pulmonary fibrosis, hypersensitivity pneumonitis, a transplantation associated disease, graft rejection or graft-versus-host-disease.

In a fifth aspect, the present invention provides a method of
25 diagnosing an inflammatory immune response in a mammal, said method comprising detecting the level of expression of a gene as described in Figure 1399 (SEQ ID NO: 1399), (a) in a test sample of tissue cells obtained from the mammal, and (b) in a control sample of known normal tissue cells of the same cell type, wherein a higher or lower level of
30 expression of said gene in the test sample as compared to the control sample is indicative of the presence of an inflammatory immune response in the mammal from which the test tissue cells were obtained, wherein the inflammatory immune response is systemic lupus erythematosis, an idiopathic inflammatory myopathy, Sjögren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, thyroiditis, diabetes mellitus, immune-mediated renal disease, a demyelinating disease of the central

- 3e -

or peripheral nervous system, idiopathic demyelinating polyneuropathy, Guillain-Barré syndrome, a chronic inflammatory demyelinating polyneuropathy, a hepatobiliary disease, infectious or autoimmune chronic active hepatitis, primary biliary cirrhosis,
5 granulomatous hepatitis, sclerosing cholangitis, inflammatory bowel disease, gluten-sensitive enteropathy, Whipple's disease, an autoimmune or immune-mediated skin disease, a bullous skin disease, erythema multiforme, contact dermatitis, psoriasis, an allergic disease, asthma, allergic rhinitis, atopic dermatitis, food
10 hypersensitivity, urticaria, an immunologic disease of the lung, eosinophilic pneumonias, idiopathic pulmonary fibrosis, hypersensitivity pneumonitis, a transplantation associated disease, graft rejection or graft-versus-host-disease.

15 Accordingly, the PRO polypeptides, agonists and antagonists thereof are also useful to prepare medicines and medicaments for the treatment of immune-related and inflammatory diseases. In a specific aspect, such medicines and medicaments comprise a therapeutically effective amount of a PRO polypeptide, agonist or antagonist thereof with a
20 pharmaceutically acceptable carrier. Preferably, the admixture is sterile.

In a further embodiment, the invention concerns a method of identifying agonists or antagonists to a PRO polypeptide which
25 comprises contacting the PRO polypeptide with a candidate molecule and monitoring a biological activity mediated by said PRO polypeptide. Preferably, the PRO polypeptide is a native sequence PRO polypeptide. In a specific aspect, the PRO agonist or antagonist is an anti-PRO antibody.

30 In another embodiment, the invention concerns a composition of matter comprising a PRO polypeptide or an agonist or antagonist antibody which binds the polypeptide in admixture with a carrier or excipient. In one aspect, the composition comprises a therapeutically effective
35 amount of the polypeptide or antibody. In another aspect, when the composition comprises an immune stimulating molecule, the composition is useful for: (a) increasing infiltration of inflammatory cells into

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- 3f -

a tissue of a mammal in need thereof, (b) stimulating or enhancing an immune response in a mammal in need thereof, (c) increasing the proliferation of NK cells in a mammal in need thereof in response to an antigen, (d) stimulating the activity of NK cells or (e) increasing the vascular permeability. In a further aspect, when the composition comprises an immune inhibiting molecule, the composition is useful for: (a) decreasing infiltration of inflammatory cells into a tissue of a mammal in need thereof, (b) inhibiting or reducing an immune response in a mammal in need thereof, (c) decreasing the activity of NK cells or (d) decreasing the proliferation of NK cells in a mammal in need thereof in response to an antigen. In another aspect, the composition comprises a further active ingredient, which may, for example, be a further antibody or a cytotoxic or chemotherapeutic agent.

15

Preferably, the composition is sterile.

In another embodiment, the invention concerns a method of treating an immune related disorder in a mammal in need thereof, comprising administering to the mammal an effective amount of a PRO polypeptide, an agonist thereof, or an antagonist thereto. In a preferred aspect, the immune related disorder is selected from the group consisting of : systemic lupus erythematosus, rheumatoid arthritis, osteoarthritis, juvenile chronic arthritis, spondyloarthropathies, systemic sclerosis, idiopathic inflammatory myopathies, Sjogren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, thyroiditis, diabetes mellitus, immune-mediated renal disease, demyelinating diseases of the central and peripheral nervous systems such as multiple sclerosis, idiopathic demyelinating polyneuropathy or Guillain-Barre syndrome, and chronic inflammatory demyelinating polyneuropathy, hepatobiliary diseases such as infectious, autoimmune chronic active hepatitis, primary biliary cirrhosis, granulomatous hepatitis, and sclerosing cholangitis, inflammatory bowel disease, gluten-sensitive enteropathy, and Whipple's disease, autoimmune or immune-mediated skin diseases including bullous skin diseases, erythema multiforme and contact dermatitis, psoriasis, allergic diseases such as asthma, allergic

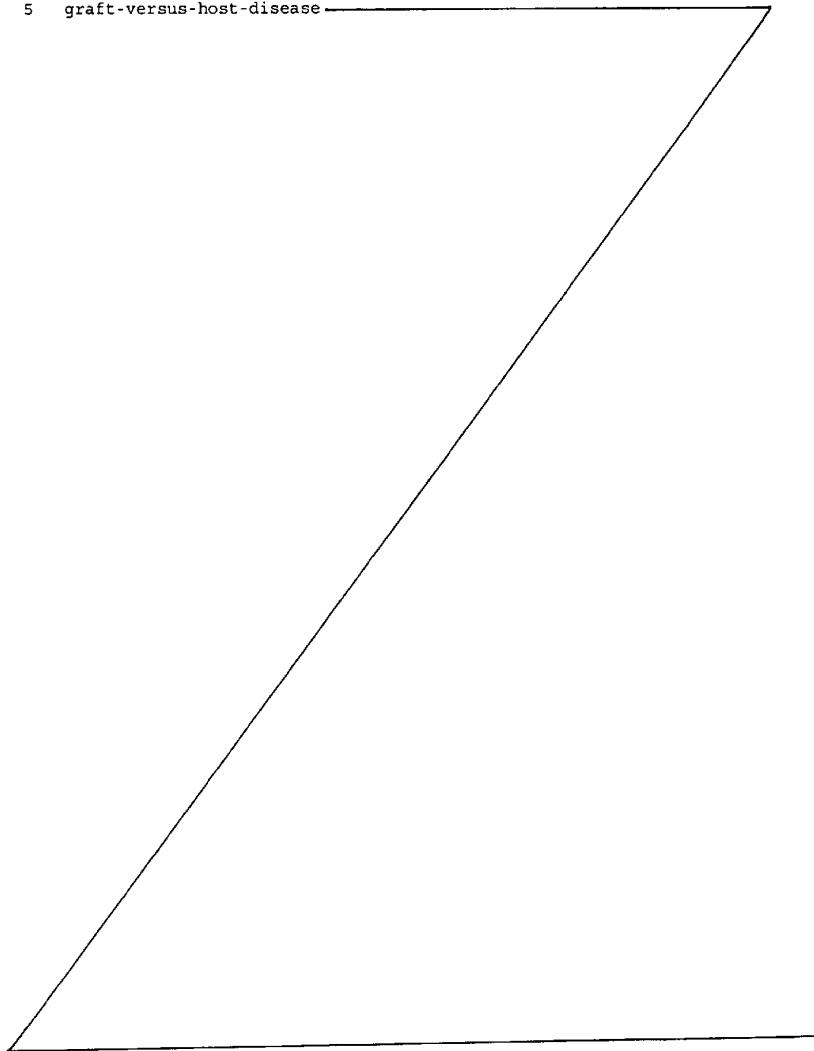
2224679_1 (GHMatters)

2003295401 22 Mar 2010

- 3g -

rhinitis, atopic dermatitis, food hypersensitivity and urticaria,
immunologic diseases of the lung such as eosinophilic pneumonias,
idiopathic pulmonary fibrosis and hypersensitivity pneumonitis,
transplantation associated diseases including graft rejection and

5 graft-versus-host-disease



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In another embodiment, the invention provides an antibody which specifically binds to any of the above or below described polypeptides. Optionally, the antibody is a monoclonal antibody, humanized antibody, antibody fragment or single-chain antibody. In one aspect, the present invention concerns an isolated antibody which binds a PRO polypeptide. In another aspect, the antibody mimics the activity of a 5 PRO polypeptide (an agonist antibody) or conversely the antibody inhibits or neutralizes the activity of a PRO polypeptide (an antagonist antibody). In another aspect, the antibody is a monoclonal antibody, which preferably has nonhuman complementarity determining region (CDR) residues and human framework region (FR) residues. The antibody may be labeled and may be immobilized on a solid support. In a further aspect, the antibody is an antibody fragment, a monoclonal antibody, a single-chain antibody, or an anti-idiotypic 10 antibody.

In yet another embodiment, the present invention provides a composition comprising an anti-PRO antibody in admixture with a pharmaceutically acceptable carrier. In one aspect, the composition comprises a therapeutically effective amount of the antibody. Preferably, the composition is sterile. The composition may be administered in the form of a liquid pharmaceutical formulation, which may be preserved to achieve 15 extended storage stability. Alternatively, the antibody is a monoclonal antibody, an antibody fragment, a humanized antibody, or a single-chain antibody.

In a further embodiment, the invention concerns an article of manufacture, comprising:
(a) a composition of matter comprising a PRO polypeptide or agonist or antagonist thereof;
(b) a container containing said composition; and
20 (c) a label affixed to said container, or a package insert included in said container referring to the use of said PRO polypeptide or agonist or antagonist thereof in the treatment of an immune related disease. The composition may comprise a therapeutically effective amount of the PRO polypeptide or the agonist or antagonist thereof.

In yet another embodiment, the present invention concerns a method of diagnosing an immune 25 related disease in a mammal, comprising detecting the level of expression of a gene encoding a PRO polypeptide (a) in a test sample of tissue cells obtained from the mammal, and (b) in a control sample of known normal tissue cells of the same cell type, wherein a higher or lower expression level in the test sample as compared to the control sample indicates the presence of immune related disease in the mammal from which the test tissue cells were obtained.

30 In another embodiment, the present invention concerns a method of diagnosing an immune disease in a mammal, comprising (a) contacting an anti-PRO antibody with a test sample of tissue cells obtained from the mammal, and (b) detecting the formation of a complex between the antibody and a PRO polypeptide, in the test sample; wherein the formation of said complex is indicative of the presence or absence of said disease. The detection may be qualitative or quantitative, and may be performed in 35 comparison with monitoring the complex formation in a control sample of known normal tissue cells of the same cell type. A larger quantity of complexes formed in the test sample indicates the presence or absence of an immune disease in the mammal from which the test tissue cells were obtained. The antibody preferably carries a detectable label. Complex formation can be monitored, for example, by light microscopy, flow cytometry, fluorimetry, or other techniques known in the art. The test sample is usually 40 obtained from an individual suspected of having a deficiency or abnormality of the immune system.

In another embodiment, the invention provides a method for determining the presence of a PRO polypeptide in a sample comprising exposing a test sample of cells suspected of containing the PRO polypeptide to an anti-PRO antibody and determining the binding of said antibody to said cell sample. In a specific aspect, the sample comprises a cell suspected of containing the PRO polypeptide and the antibody binds to the cell. The antibody is preferably detectably labeled and/or bound to a solid support.

In another embodiment, the present invention concerns an immune-related disease diagnostic kit, comprising an anti-PRO antibody and a carrier in suitable packaging. The kit preferably contains instructions for using the antibody to detect the presence of the PRO polypeptide. Preferably the carrier is pharmaceutically acceptable.

10 In another embodiment, the present invention concerns a diagnostic kit, containing an anti-PRO antibody in suitable packaging. The kit preferably contains instructions for using the antibody to detect the PRO polypeptide.

15 In another embodiment, the invention provides a method of diagnosing an immune-related disease in a mammal which comprises detecting the presence or absence of a PRO polypeptide in a test sample of tissue cells obtained from said mammal, wherein the presence or absence of the PRO polypeptide in said test sample is indicative of the presence of an immune-related disease in said mammal.

In another embodiment, the present invention concerns a method for identifying an agonist of a PRO polypeptide comprising:

20 (a) contacting cells and a test compound to be screened under conditions suitable for the induction of a cellular response normally induced by a PRO polypeptide; and
(b) determining the induction of said cellular response to determine if the test compound is an effective agonist, wherein the induction of said cellular response is indicative of said test compound being an effective agonist.

25 In another embodiment, the invention concerns a method for identifying a compound capable of inhibiting the activity of a PRO polypeptide comprising contacting a candidate compound with a PRO polypeptide under conditions and for a time sufficient to allow these two components to interact and determining whether the activity of the PRO polypeptide is inhibited. In a specific aspect, either the candidate compound or the PRO polypeptide is immobilized on a solid support. In another aspect, the non-immobilized component carries a detectable label. In a preferred aspect, this method comprises the steps of:

30 (a) contacting cells and a test compound to be screened in the presence of a PRO polypeptide under conditions suitable for the induction of a cellular response normally induced by a PRO polypeptide; and
(b) determining the induction of said cellular response to determine if the test compound is an effective antagonist.

35 In another embodiment, the invention provides a method for identifying a compound that inhibits the expression of a PRO polypeptide in cells that normally express the polypeptide, wherein the method comprises contacting the cells with a test compound and determining whether the expression of the PRO polypeptide is inhibited. In a preferred aspect, this method comprises the steps of:

(a) contacting cells and a test compound to be screened under conditions suitable for allowing expression of the PRO polypeptide; and
40 (b) determining the inhibition of expression of said polypeptide.

In yet another embodiment, the present invention concerns a method for treating an immune-related disorder in a mammal that suffers therefrom comprising administering to the mammal a nucleic acid molecule that codes for either (a) a PRO polypeptide, (b) an agonist of a PRO polypeptide or (c) an antagonist of a PRO polypeptide, wherein said agonist or antagonist may be an anti-PRO antibody. In a 5 preferred embodiment, the mammal is human. In another preferred embodiment, the nucleic acid is administered via *ex vivo* gene therapy. In a further preferred embodiment, the nucleic acid is comprised within a vector, more preferably an adenoviral, adeno-associated viral, lentiviral or retroviral vector.

In yet another aspect, the invention provides a recombinant viral particle comprising a viral vector 10 consisting essentially of a promoter, nucleic acid encoding (a) a PRO polypeptide, (b) an agonist polypeptide of a PRO polypeptide, or (c) an antagonist polypeptide of a PRO polypeptide, and a signal sequence for cellular secretion of the polypeptide, wherein the viral vector is in association with viral structural proteins. Preferably, the signal sequence is from a mammal, such as from a native PRO polypeptide.

In a still further embodiment, the invention concerns an *ex vivo* producer cell comprising a nucleic acid construct that expresses retroviral structural proteins and also comprises a retroviral vector consisting 15 essentially of a promoter, nucleic acid encoding (a) a PRO polypeptide, (b) an agonist polypeptide of a PRO polypeptide or (c) an antagonist polypeptide of a PRO polypeptide, and a signal sequence for cellular secretion of the polypeptide, wherein said producer cell packages the retroviral vector in association with the structural proteins to produce recombinant retroviral particles.

In a still further embodiment, the invention provides a method of increasing the activity of NK cells 20 in a mammal comprising administering to said mammal (a) a PRO polypeptide, (b) an agonist of a PRO polypeptide, or (c) an antagonist of a PRO polypeptide, wherein the activity of NK cells in the mammal is increased.

In a still further embodiment, the invention provides a method of decreasing the activity of NK cells 25 in a mammal comprising administering to said mammal (a) a PRO polypeptide, (b) an agonist of a PRO polypeptide, or (c) an antagonist of a PRO polypeptide, wherein the activity of NK cells in the mammal is decreased.

In a still further embodiment, the invention provides a method of increasing the proliferation of NK cells in a mammal comprising administering to said mammal (a) a PRO polypeptide, (b) an agonist of a PRO polypeptide, or (c) an antagonist of a PRO polypeptide, wherein the proliferation of NK cells in the mammal 30 is increased.

In a still further embodiment, the invention provides a method of decreasing the proliferation of NK cells in a mammal comprising administering to said mammal (a) a PRO polypeptide, (b) an agonist of a PRO polypeptide, or (c) an antagonist of a PRO polypeptide, wherein the proliferation of NK cells in the mammal is decreased.

35 B. Additional Embodiments

In other embodiments of the present invention, the invention provides vectors comprising DNA encoding any of the herein described polypeptides. Host cell comprising any such vector are also provided. By way of example, the host cells may be CHO cells, *E. coli*, or yeast. A process for producing any of the herein described polypeptides is further provided and comprises culturing host cells under conditions

suitable for expression of the desired polypeptide and recovering the desired polypeptide from the cell culture.

In other embodiments, the invention provides chimeric molecules comprising any of the herein described polypeptides fused to a heterologous polypeptide or amino acid sequence. Example of such 5 chimeric molecules comprise any of the herein described polypeptides fused to an epitope tag sequence or a Fc region of an immunoglobulin.

In another embodiment, the invention provides an antibody which specifically binds to any of the above or below described polypeptides. Optionally, the antibody is a monoclonal antibody, humanized antibody, antibody fragment or single-chain antibody.

10 In yet other embodiments, the invention provides oligonucleotide probes useful for isolating genomic and cDNA nucleotide sequences or as antisense probes, wherein those probes may be derived from any of the above or below described nucleotide sequences.

In other embodiments, the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence that encodes a PRO polypeptide.

15 In one aspect, the isolated nucleic acid molecule comprises a nucleotide sequence having at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity,

20 alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about

25 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity to (a) a DNA molecule encoding a PRO polypeptide having a full-length amino acid sequence as disclosed herein, an amino acid sequence lacking the signal peptide as disclosed herein, an extracellular domain of a transmembrane protein,

30 with or without the signal peptide, as disclosed herein or any other specifically defined fragment of the full-length amino acid sequence as disclosed herein, or (b) the complement of the DNA molecule of (a).

In other aspects, the isolated nucleic acid molecule comprises a nucleotide sequence having at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid 35 sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity,

40 alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid

sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity to (a) a DNA molecule 5 comprising the coding sequence of a full-length PRO polypeptide cDNA as disclosed herein, the coding sequence of a PRO polypeptide lacking the signal peptide as disclosed herein, the coding sequence of an extracellular domain of a transmembrane PRO polypeptide, with or without the signal peptide, as disclosed herein or the coding sequence of any other specifically defined fragment of the full-length amino acid sequence as disclosed herein, or (b) the complement of the DNA molecule of (a).

10 In a further aspect, the invention concerns an isolated nucleic acid molecule comprising a nucleotide sequence having at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid 15 sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity to (a) a DNA molecule that encodes the same mature polypeptide encoded by any of the human protein cDNAs as disclosed herein, or (b) the complement of the DNA molecule of (a).

20 25 Another aspect the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding a PRO polypeptide which is either transmembrane domain-deleted or transmembrane domain-inactivated, or is complementary to such encoding nucleotide sequence, wherein the transmembrane domain(s) of such polypeptide are disclosed herein. Therefore, soluble extracellular domains of the herein described PRO polypeptides are contemplated.

30 35 40 Another embodiment is directed to fragments of a PRO polypeptide coding sequence, or the complement thereof, that may find use as, for example, hybridization probes, for encoding fragments of a PRO polypeptide that may optionally encode a polypeptide comprising a binding site for an anti-PRO antibody or as antisense oligonucleotide probes. Such nucleic acid fragments are usually at least about 20 nucleotides in length, alternatively at least about 30 nucleotides in length, alternatively at least about 40 nucleotides in length, alternatively at least about 50 nucleotides in length, alternatively at least about 60 nucleotides in length, alternatively at least about 70 nucleotides in length, alternatively at least about 80 nucleotides in length, alternatively at least about 90 nucleotides in length, alternatively at least about 100 nucleotides in length, alternatively at least about 110 nucleotides in length, alternatively at least about 120 nucleotides in length, alternatively at least about 130 nucleotides in length, alternatively at least about 140 nucleotides in length, alternatively at least about 150 nucleotides in length, alternatively at least about 160

nucleotides in length, alternatively at least about 170 nucleotides in length, alternatively at least about 180 nucleotides in length, alternatively at least about 190 nucleotides in length, alternatively at least about 200 nucleotides in length, alternatively at least about 250 nucleotides in length, alternatively at least about 300 nucleotides in length, alternatively at least about 350 nucleotides in length, alternatively at least about 400

5 nucleotides in length, alternatively at least about 450 nucleotides in length, alternatively at least about 500 nucleotides in length, alternatively at least about 600 nucleotides in length, alternatively at least about 700 nucleotides in length, alternatively at least about 800 nucleotides in length, alternatively at least about 900 nucleotides in length and alternatively at least about 1000 nucleotides in length, wherein in this context the term "about" means the referenced nucleotide sequence length plus or minus 10% of that referenced length.

10 It is noted that novel fragments of a PRO polypeptide-encoding nucleotide sequence may be determined in a routine manner by aligning the PRO polypeptide-encoding nucleotide sequence with other known nucleotide sequences using any of a number of well known sequence alignment programs and determining which PRO polypeptide-encoding nucleotide sequence fragment(s) are novel. All of such PRO polypeptide-encoding nucleotide sequences are contemplated herein. Also contemplated are the PRO polypeptide fragments

15 encoded by these nucleotide molecule fragments, preferably those PRO polypeptide fragments that comprise a binding site for an anti-PRO antibody.

In another embodiment, the invention provides isolated PRO polypeptide encoded by any of the isolated nucleic acid sequences herein above identified.

In a certain aspect, the invention concerns an isolated PRO polypeptide, comprising an amino acid sequence having at least about 80% amino acid sequence identity, alternatively at least about 81% amino acid sequence identity, alternatively at least about 82% amino acid sequence identity, alternatively at least about 83% amino acid sequence identity, alternatively at least about 84% amino acid sequence identity, alternatively at least about 85% amino acid sequence identity, alternatively at least about 86% amino acid sequence identity, alternatively at least about 87% amino acid sequence identity, alternatively at least about

20 88% amino acid sequence identity, alternatively at least about 89% amino acid sequence identity, alternatively at least about 90% amino acid sequence identity, alternatively at least about 91% amino acid sequence identity, alternatively at least about 92% amino acid sequence identity, alternatively at least about 93% amino acid sequence identity, alternatively at least about 94% amino acid sequence identity, alternatively at least about 95% amino acid sequence identity, alternatively at least about 96% amino acid

25 sequence identity, alternatively at least about 97% amino acid sequence identity, alternatively at least about 98% amino acid sequence identity and alternatively at least about 99% amino acid sequence identity to a PRO polypeptide having a full-length amino acid sequence as disclosed herein, an amino acid sequence lacking the signal peptide as disclosed herein, an extracellular domain of a transmembrane protein, with or without the signal peptide, as disclosed herein or any other specifically defined fragment of the full-length

30 amino acid sequence as disclosed herein.

In a further aspect, the invention concerns an isolated PRO polypeptide comprising an amino acid sequence having at least about 80% amino acid sequence identity, alternatively at least about 81% amino acid sequence identity, alternatively at least about 82% amino acid sequence identity, alternatively at least about 83% amino acid sequence identity, alternatively at least about 84% amino acid sequence identity, alternatively at least about 85% amino acid sequence identity, alternatively at least about 86% amino acid

35

sequence identity, alternatively at least about 87% amino acid sequence identity, alternatively at least about 88% amino acid sequence identity, alternatively at least about 89% amino acid sequence identity, alternatively at least about 90% amino acid sequence identity, alternatively at least about 91% amino acid sequence identity, alternatively at least about 92% amino acid sequence identity, alternatively at least about 93% amino acid sequence identity, alternatively at least about 94% amino acid sequence identity, alternatively at least about 95% amino acid sequence identity, alternatively at least about 96% amino acid sequence identity, alternatively at least about 97% amino acid sequence identity, alternatively at least about 98% amino acid sequence identity and alternatively at least about 99% amino acid sequence identity to an amino acid sequence encoded by any of the human protein cDNAs as disclosed herein.

10 In a specific aspect, the invention provides an isolated PRO polypeptide without the N-terminal signal sequence and/or the initiating methionine and is encoded by a nucleotide sequence that encodes such an amino acid sequence as herein before described. Processes for producing the same are also herein described, wherein those processes comprise culturing a host cell comprising a vector which comprises the appropriate encoding nucleic acid molecule under conditions suitable for expression of the PRO polypeptide and recovering the PRO polypeptide from the cell culture.

15 Another aspect the invention provides an isolated PRO polypeptide which is either transmembrane domain-deleted or transmembrane domain-inactivated. Processes for producing the same are also herein described, wherein those processes comprise culturing a host cell comprising a vector which comprises the appropriate encoding nucleic acid molecule under conditions suitable for expression of the PRO polypeptide and recovering the PRO polypeptide from the cell culture.

20 In yet another embodiment, the invention concerns agonists and antagonists of a native PRO polypeptide as defined herein. In a particular embodiment, the agonist or antagonist is an anti-PRO antibody or a small molecule.

25 In a further embodiment, the invention concerns a method of identifying agonists or antagonists to a PRO polypeptide which comprise contacting the PRO polypeptide with a candidate molecule and monitoring a biological activity mediated by said PRO polypeptide. Preferably, the PRO polypeptide is a native PRO polypeptide.

30 In a still further embodiment, the invention concerns a composition of matter comprising a PRO polypeptide, or an agonist or antagonist of a PRO polypeptide as herein described, or an anti-PRO antibody, in combination with a carrier. Optionally, the carrier is a pharmaceutically acceptable carrier.

35 Another embodiment of the present invention is directed to the use of a PRO polypeptide, or an agonist or antagonist thereof as herein before described, or an anti-PRO antibody, for the preparation of a medicament useful in the treatment of a condition which is responsive to the PRO polypeptide, an agonist or antagonist thereof or an anti-PRO antibody.

35 BRIEF DESCRIPTION OF THE DRAWINGS

40 In the list of figures for the present application, specific cDNA sequences which are differentially expressed in activated Natural Killer (NK) cells as compared to normal resting NK cells are individually identified with a specific alphanumerical designation. These cDNA sequences are differentially expressed in NK cells that are specifically treated as described in Example 1 below. If start and/or stop

codons have been identified in a cDNA sequence shown in the attached figures, they are shown in bold and underlined font, and the encoded polypeptide is shown in the next consecutive figure.

The Figures 1-1477 show the nucleic acids of the invention and their encoded PRO polypeptides. Also included, for convenience is a List of Figures attached hereto as Appendix A, which 5 gives the figure number and the corresponding DNA or PRO number.

List of Figures

Figure 1: DNA329899, NP_002785.1, 200039.s.at
 Figure 2: PRO69614
 Figure 3: DNA304680, HSPCB, 200064.at
 Figure 4: PRO71106
 Figure 5: DNA88643, NP_000190.1, 35626.at
 Figure 6: PRO2455
 Figure 7: DNA331286, NP_006143.1, 35974.at
 Figure 8: PRO86388
 Figure 9: DNA331287, NP_036455.1, 36711.at
 Figure 10: PRO83282
 Figure 11: DNA254127, NP_008925.1, 38241.at
 Figure 12: PRO49242
 Figure 13A-B: DNA103216, BAA31595.1, 38671.at
 Figure 14: PRO4546
 Figure 15A-B: DNA329093, NP_006631.1, 41220.at
 Figure 16: PRO84745
 Figure 17: DNA326185, NP_073607.2, 45633.at
 Figure 18: PRO82602
 Figure 19: DNA329913, SSB-3, 46256.at
 Figure 20: PRO85228
 Figure 21: DNA331288, ARHGAP8, 47069.at
 Figure 22: PRO86389
 Figure 23: DNA328364, NP_068577.1, 52940.at
 Figure 24: PRO84223
 Figure 25: DNA324633, BC000478, 200691.s.at
 Figure 26: PRO81277
 Figure 27: DNA324633, NP_004125.2, 200692.s.at
 Figure 28: PRO81277
 Figure 29: DNA88350, NP_000168.1, 200696.s.at
 Figure 30: PRO2758
 Figure 31: DNA287207, NP_006316.1, 200750.s.at
 Figure 32: PRO39268
 Figure 33: DNA324135, BC001854, 200768.s.at
 Figure 34: PRO80837
 Figure 35: DNA324135, NP_005902.1, 200769.s.at
 Figure 36: PRO80837
 Figure 37: DNA324060, NP_002530.1, 200790.at
 Figure 38: PRO80773
 Figure 39: DNA287211, NP_002147.1, 200806.s.at
 Figure 40: PRO69492
 Figure 41: DNA287211, HSPD1, 200807.s.at
 Figure 42: PRO69492
 Figure 43A-B: DNA255281, NP_006380.1, 200825.s.at
 Figure 44: PRO50357
 Figure 45: DNA328380, HSHLAEHCM, 200904.at
 Figure 46: DNA272695, NP_001722.1, 200920.s.at
 Figure 47: PRO60817
 Figure 48: DNA327255, NP_002385.2, 200924.s.at
 Figure 49: PRO57298
 Figure 50: DNA225878, NP_004334.1, 200935.at
 Figure 51: PRO36341
 Figure 52A-B: DNA287217, NP_001750.1, 200951.s.at
 Figure 53: PRO36766
 Figure 54A-B: DNA287217, CCND2, 200952.s.at
 Figure 55: PRO36766
 Figure 56A-B: DNA226303, HUMRSC289, 200953.s.at
 Figure 57: PRO36766
 Figure 58A-B: DNA331289, ABLIM1, 200965.s.at
 Figure 59: PRO86390
 Figure 60: DNA328388, BC010273, 201013.s.at
 Figure 61: PRO84240
 Figure 62: DNA328388, NP_006443.1, 201014.s.at
 Figure 63: PRO84240
 Figure 64: DNA328391, NP_004408.1, 201041.s.at
 Figure 65: PRO84242
 Figure 66: DNA287198, NP_006073.1, 201090.x.at
 Figure 67: PRO69484
 Figure 68: DNA304719, NP_002296.1, 201105.at
 Figure 69: PRO71145
 Figure 70: DNA329931, AF053642, 201111.at
 Figure 71: DNA273865, NP_006221.1, 201115.at
 Figure 72: PRO61824
 Figure 73: DNA326273, NP_001961.1, 201123.s.at
 Figure 74: PRO82678
 Figure 75: DNA329103, NP_002112.2, 201137.s.at
 Figure 76: PRO84752
 Figure 77: DNA329104, NP_004085.1, 201144.s.at
 Figure 78: PRO69550
 Figure 79: DNA151802, NP_003661.1, 201169.s.at
 Figure 80: PRO12890
 Figure 81: DNA151802, BHLHB2, 201170.s.at
 Figure 82: PRO12890
 Figure 83A-B: DNA103453, HUME16GEN, 201195.s.at
 Figure 84: PRO4780
 Figure 85: DNA103488, NP_002583.1, 201202.at
 Figure 86: PRO4815
 Figure 87: DNA287173, ENO1, 201231.s.at
 Figure 88: PRO69463
 Figure 89: DNA287331, NP_002645.1, 201251.at
 Figure 90: PRO69595
 Figure 91: DNA270950, NP_003182.1, 201263.at
 Figure 92: PRO59281
 Figure 93: DNA328405, NP_112556.1, 201277.s.at
 Figure 94: PRO84252
 Figure 95: DNA328406, NP_001334.1, 201279.s.at
 Figure 96: PRO84253
 Figure 97: DNA328406, DAB2, 201280.s.at
 Figure 98: PRO84253
 Figure 99: DNA331290, NP_038474.1, 201285.at
 Figure 100: PRO86391
 Figure 101: DNA327546, HSTOP2A10, 201292.at
 Figure 102: DNA329106, NP_003013.1, 201311.s.at
 Figure 103: PRO83360
 Figure 104: DNA329002, NP_001753.1, 201327.s.at

Figure 105: PRO4912
 Figure 106A-B: DNA274141, AF205218, 201362_at
 Figure 107: PRO62077
 Figure 108A-B: DNA331291, AB020657, 201363_s_at
 Figure 109: PRO62077
 Figure 110: DNA329107, NP_008818.3, 201367_s_at
 Figure 111: PRO84754
 Figure 112A-B: DNA329108, 1383643.16, 201368_at
 Figure 113: PRO84755
 Figure 114: DNA329107, ZFP36L2, 201369_s_at
 Figure 115: PRO84754
 Figure 116: DNA329939, 1393503.1, 201417_at
 Figure 117: PRO85248
 Figure 118: DNA226600, NP_003371.1, 201426_s_at
 Figure 119: PRO37063
 Figure 120: DNA272286, NP_001743.1, 201432_at
 Figure 121: PRO60544
 Figure 122: DNA325704, NP_004981.2, 201475_x_at
 Figure 123: PRO82188
 Figure 124: DNA327551, NP_001024.1, 201477_s_at
 Figure 125: PRO59289
 Figure 126: DNA304459, BC005020, 201489_at
 Figure 127: PRO37073
 Figure 128: DNA304459, NP_005720.1, 201490_s_at
 Figure 129: PRO37073
 Figure 130: DNA323741, NP_003123.1, 201516_at
 Figure 131: PRO80498
 Figure 132: DNA331292, NP_002779.1, 201532_at
 Figure 133: PRO84262
 Figure 134: DNA272171, NP_002379.2, 201555_at
 Figure 135: PRO60438
 Figure 136: DNA227071, NP_000260.1, 201577_at
 Figure 137: PRO37534
 Figure 138A-B: DNA329944, AB032988, 201581_at
 Figure 139: DNA272904, NP_006784.1, 201619_at
 Figure 140: PRO60991
 Figure 141: DNA329115, NP_434702.1, 201631_s_at
 Figure 142: PRO84760
 Figure 143A-B: DNA331293, AB011155, 201681_s_at
 Figure 144: DNA270883, NP_001061.1, 201714_at
 Figure 145: PRO59218
 Figure 146: DNA327559, NP_058432.1, 201752_s_at
 Figure 147: PRO83589
 Figure 148: DNA331294, ADD3, 201753_s_at
 Figure 149: PRO86393
 Figure 150: DNA227035, NP_006730.1, 201755_at
 Figure 151: PRO37498
 Figure 152: DNA287167, NP_006627.1, 201761_at
 Figure 153: PRO59136
 Figure 154: DNA329952, NET1, 201830_s_at
 Figure 155: PRO85256
 Figure 156: DNA329118, NP_068660.1, 201853_s_at
 Figure 157: PRO83123
 Figure 158A-B: DNA331295, NP_002710.1, 201877_s_at
 Figure 159: PRO86394

Figure 160: DNA150805, NP_055703.1, 201889_at
 Figure 161: PRO11583
 Figure 162: DNA253582, DNA253582, 201890_at
 Figure 163: PRO49181
 Figure 164: DNA329956, NP_000875.1, 201892_s_at
 Figure 165: PRO85260
 Figure 166: DNA328431, NP_001817.1, 201897_s_at
 Figure 167: PRO45093
 Figure 168: DNA227112, NP_006397.1, 201923_at
 Figure 169: PRO37575
 Figure 170: DNA275240, NP_005906.2, 201930_at
 Figure 171: PRO62927
 Figure 172: DNA274167, NP_006422.1, 201946_s_at
 Figure 173: PRO62097
 Figure 174: DNA275214, NP_002473.1, 201970_s_at
 Figure 175: PRO62908
 Figure 176: DNA88666, NP_002334.1, 202018_s_at
 Figure 177: PRO2892
 Figure 178A-B: DNA225991, NP_000518.1, 202068_s_at
 Figure 179: PRO36454
 Figure 180: DNA327567, NP_005521.1, 202069_s_at
 Figure 181: PRO83596
 Figure 182A-B: DNA269686, NP_002994.1, 202083_s_at
 Figure 183: PRO58096
 Figure 184: DNA328440, NP_004517.1, 202107_s_at
 Figure 185: PRO84274
 Figure 186A-B: DNA304990, NP_006394.1, 202150_s_at
 Figure 187: PRO71288
 Figure 188: DNA328442, NP_006078.2, 202154_x_at
 Figure 189: PRO84275
 Figure 190: DNA328444, MGC14458, 202246_s_at
 Figure 191: PRO84277
 Figure 192: DNA304716, NP_510867.1, 202284_s_at
 Figure 193: PRO71142
 Figure 194: DNA270142, NP_005947.2, 202309_at
 Figure 195: PRO58531
 Figure 196: DNA328448, NP_000777.1, 202314_at
 Figure 197: PRO62362
 Figure 198: DNA331296, UNG, 202330_s_at
 Figure 199: PRO86395
 Figure 200: DNA325115, NP_001435.1, 202345_s_at
 Figure 201: PRO81689
 Figure 202: DNA331297, MXII, 202364_at
 Figure 203: PRO86396
 Figure 204A-B: DNA150955, NP_036420.1, 202369_s_at
 Figure 205: PRO12559
 Figure 206: DNA290234, NP_002914.1, 202388_at
 Figure 207: PRO70333
 Figure 208: DNA66487, NP_002458.1, 202431_s_at
 Figure 209: PRO1213
 Figure 210: DNA325532, NP_009104.1, 202458_at
 Figure 211: PRO307

Figure 212A-B: DNA269642, NP_004557.1, 202464.s.at
 Figure 213: PRO58054
 Figure 214: DNA227921, NP_003789.1, 202468.s.at
 Figure 215: PRO38384
 Figure 216: DNA329123, NP_002873.1, 202483.s.at
 Figure 217: PRO84765
 Figure 218A-B: DNA103449, NP_008862.1, 202497.x.at
 Figure 219: PRO4776
 Figure 220A-B: DNA103449, SLC2A3, 202499.s.at
 Figure 221: PRO4776
 Figure 222: DNA234442, NP_055551.1, 202503.s.at
 Figure 223: PRO38852
 Figure 224A-B: DNA277809, NP_055582.1, 202524.s.at
 Figure 225: PRO64556
 Figure 226A-B: DNA255105, NP_000850.1, 202539.s.at
 Figure 227: PRO50187
 Figure 228A-B: DNA255105, HMGCR, 202540.s.at
 Figure 229: PRO50187
 Figure 230: DNA275244, DNA275244, 202557.at
 Figure 231: DNA329979, NP_001062.1, 202589.at
 Figure 232: PRO82821
 Figure 233: DNA274881, NP_001896.1, 202613.at
 Figure 234: PRO62626
 Figure 235: DNA59763, NP_000192.1, 202637.s.at
 Figure 236: PRO160
 Figure 237: DNA59763, ICAM1, 202638.s.at
 Figure 238: PRO160
 Figure 239: DNA254129, NP_006001.1, 202655.at
 Figure 240: PRO49244
 Figure 241: DNA326896, NP_003672.1, 202671.s.at
 Figure 242: PRO69486
 Figure 243: DNA274690, NP_008869.1, 202690.s.at
 Figure 244: PRO62479
 Figure 245: DNA31298, NP_055271.2, 202730.s.at
 Figure 246: PRO81909
 Figure 247: DNA331299, PDCD4, 202731.at
 Figure 248: PRO86397
 Figure 249: DNA150713, NP_006570.1, 202735.at
 Figure 250: PRO12082
 Figure 251A-B: DNA328464, 977954.20, 202769.at
 Figure 252: PRO84290
 Figure 253: DNA226578, NP_004345.1, 202770.s.at
 Figure 254: PRO37041
 Figure 255: DNA273346, NP_055316.1, 202779.s.at
 Figure 256: PRO61349
 Figure 257A-B: DNA226364, NP_001612.1, 202820.at
 Figure 258: PRO36827
 Figure 259: DNA329988, NP_036460.1, 202843.at
 Figure 260: PRO1471
 Figure 261: DNA328483, NP_061163.1, 202911.at
 Figure 262: PRO84309
 Figure 263: DNA331300, BIN1, 202931.x.at
 Figure 264: PRO86398
 Figure 265A-B: DNA331301, NP_005424.1, 202932.at
 Figure 266: PRO12922
 Figure 267A-B: DNA331302, YES1, 202933.s.at
 Figure 268: PRO12922
 Figure 269: DNA329134, BC012085, 202951.at
 Figure 270: PRO59254
 Figure 271A-B: DNA328473, NP_006473.1, 202968.s.at
 Figure 272: PRO84299
 Figure 273A-B: DNA194837, NP_055714.1, 202975.s.at
 Figure 274: PRO24100
 Figure 275A-B: DNA194837, RHOBTB3, 202976.s.at
 Figure 276: PRO24100
 Figure 277: DNA329136, NP_057475.1, 203023.at
 Figure 278: PRO84772
 Figure 279A-B: DNA271865, NP_055566.1, 203037.s.at
 Figure 280: PRO60145
 Figure 281: DNA269918, NP_003633.1, 203138.at
 Figure 282: PRO58316
 Figure 283A-B: DNA331303, NP_003129.1, 203181.x.at
 Figure 284: PRO86399
 Figure 285: DNA331304, BC015747, 203221.at
 Figure 286: PRO84167
 Figure 287: DNA328294, NP_005068.2, 203222.s.at
 Figure 288: PRO84167
 Figure 289A-C: DNA274481, NP_000323.1, 203231.s.at
 Figure 290: PRO62384
 Figure 291A-C: DNA274481, SCA1, 203232.s.at
 Figure 292: PRO62384
 Figure 293: DNA76514, NP_000409.1, 203233.at
 Figure 294: PRO2540
 Figure 295: DNA327590, NP_003355.1, 203234.at
 Figure 296: PRO83608
 Figure 297: DNA325507, NP_005842.1, 203252.at
 Figure 298: PRO69461
 Figure 299: DNA302020, NP_005564.1, 203276.at
 Figure 300: PRO70993
 Figure 301A-B: DNA331305, NP_055716.1, 203286.at
 Figure 302: PRO86400
 Figure 303: DNA271959, NP_002885.1, 203344.s.at
 Figure 304: PRO60234
 Figure 305: DNA324514, NP_002349.1, 203362.s.at
 Figure 306: PRO81169
 Figure 307: DNA325749, NP_003868.1, 203372.s.at
 Figure 308: PRO12839
 Figure 309: DNA325749, STAT12, 203373.at
 Figure 310: PRO12839
 Figure 311: DNA329140, NP_476433.1, 203391.at
 Figure 312: PRO84775
 Figure 313: DNA323927, NP_005563.1, 203411.s.at
 Figure 314: PRO80660

Figure 315: DNA151037, NP_036461.1, 203414.at
 Figure 316: PRO12586
 Figure 317A-B: DNA256807, NP_057339.1, 203420.at
 Figure 318: PRO51738
 Figure 319A-B: DNA275186, DNA275186, 203432.at
 Figure 320A-B: DNA330010, NP_005721.2, 203445.s.at
 Figure 321: PRO85298
 Figure 322: DNA331306, NP_001715.1, 203502.at
 Figure 323: PRO58024
 Figure 324A-B: DNA331307, NP_003096.1, 203509.at
 Figure 325: PRO86401
 Figure 326A-B: DNA272399, NP_001197.1, 203542.s.at
 Figure 327: PRO60653
 Figure 328A-B: DNA272399, BTEB1, 203543.s.at
 Figure 329: PRO60653
 Figure 330: DNA287246, NP_004044.2, 203612.at
 Figure 331: PRO69521
 Figure 332A-B: DNA331308, BCL2, 203685.at
 Figure 333: PRO86402
 Figure 334: DNA324183, DPP4, 203716.s.at
 Figure 335: PRO80881
 Figure 336: DNA196562, HSPCHDP7, 203717.at
 Figure 337: PRO25042
 Figure 338: DNA150784, NP_001974.1, 203720.s.at
 Figure 339: PRO12800
 Figure 340A-B: DNA325369, NP_055877.2, 203737.s.at
 Figure 341: PRO81905
 Figure 342: DNA275339, NP_005685.1, 203880.at
 Figure 343: PRO63012
 Figure 344: DNA82376, NP_002407.1, 203915.at
 Figure 345: PRO1723
 Figure 346: DNA272338, NP_001245.1, 203967.at
 Figure 347: PRO60595
 Figure 348: DNA272338, CDC6, 203968.s.at
 Figure 349: PRO60595
 Figure 350: DNA271374, NP_005474.1, 203976.s.at
 Figure 351: PRO59673
 Figure 352: DNA329145, DUSP4, 204014.at
 Figure 353: PRO84780
 Figure 354: DNA330034, NP_002907.1, 204023.at
 Figure 355: PRO85319
 Figure 356: DNA328271, NP_008988.2, 204026.s.at
 Figure 357: PRO81868
 Figure 358: DNA226737, NP_004576.1, 204070.at
 Figure 359: PRO37200
 Figure 360A-C: DNA331309, NP_075463.1, 204072.s.at
 Figure 361: PRO86403
 Figure 362: DNA216689, NP_002975.1, 204103.at
 Figure 363: PRO34276
 Figure 364: DNA304489, NP_003495.1, 204126.s.at
 Figure 365: PRO71058
 Figure 366: DNA330037, BC000149, 204127.at
 Figure 367: PRO82290
 Figure 368: DNA325824, NP_002906.1, 204128.s.at
 Figure 369: PRO82290
 Figure 370: DNA272655, NP_001818.1, 204170.s.at
 Figure 371: PRO60781
 Figure 372: DNA226881, NP_002008.2, 204236.at
 Figure 373: PRO37344
 Figure 374A-B: DNA287273, NP_006435.1, 204240.s.at
 Figure 375: PRO69545
 Figure 376: DNA228132, NP_076995.1, 204256.at
 Figure 377: PRO38595
 Figure 378: DNA273802, NP_066950.1, 204285.s.at
 Figure 379: PRO61763
 Figure 380: DNA273802, PMAIP1, 204286.s.at
 Figure 381: PRO61763
 Figure 382: DNA331310, NP_000472.1, 204294.at
 Figure 383: PRO86404
 Figure 384: DNA150972, NP_005252.1, 204472.at
 Figure 385: PRO12162
 Figure 386A-B: DNA331311, NP_056054.1, 204500.s.at
 Figure 387: PRO86405
 Figure 388: DNA331312, NP_003600.2, 204504.s.at
 Figure 389: PRO82595
 Figure 390: DNA225993, NP_000646.1, 204563.at
 Figure 391: PRO36156
 Figure 392A-B: DNA330054, NP_004746.1, 204633.s.at
 Figure 393: PRO85334
 Figure 394: DNA103526, LRMP, 204674.at
 Figure 395: PRO4853
 Figure 396A-B: DNA331313, 481411.2, 204695.at
 Figure 397: PRO38036
 Figure 398A-B: DNA325192, NP_038203.1, 204744.s.at
 Figure 399: PRO81753
 Figure 400: DNA330060, NP_002443.2, 204766.s.at
 Figure 401: PRO85340
 Figure 402: DNA329154, BC000323, 204767.s.at
 Figure 403: PRO69568
 Figure 404: DNA325479, NP_004102.1, 204768.s.at
 Figure 405: PRO69568
 Figure 406: DNA330062, NP_006017.1, 204805.s.at
 Figure 407: PRO85342
 Figure 408: DNA254127, BTN3A3, 204821.at
 Figure 409: PRO49242
 Figure 410: DNA328544, NP_006673.1, 204834.at
 Figure 411: PRO84347
 Figure 412: DNA328546, NP_005249.1, 204867.at
 Figure 413: PRO84349
 Figure 414: DNA329157, NP_004271.1, 204905.s.at
 Figure 415: PRO62861
 Figure 416: DNA331095, NP_005216.1, 204947.at
 Figure 417: PRO86245
 Figure 418: DNA325061, NP_005208.1, 205033.s.at

Figure 419: PRO9980
 Figure 420: DNA328297, NP_477097.1, 205034.s.at
 Figure 421: PRO59418
 Figure 422: DNA324991, ASNS, 205047.s.at
 Figure 423: PRO81585
 Figure 424: DNA331314, NP_055366.1, 205086.s.at
 Figure 425: PRO86406
 Figure 426: DNA330074, HUMHFM145, 205098.s.at
 Figure 427: PRO85353
 Figure 428: DNA226177, NP_001286.1, 205099.s.at
 Figure 429: PRO36640
 Figure 430: DNA192060, NP_002974.1, 205114.s.at
 Figure 431: PRO21960
 Figure 432: DNA299899, NP_002148.1, 205133.s.at
 Figure 433: PRO62760
 Figure 434: DNA273535, NP_004217.1, 205214.s.at
 Figure 435: PRO61515
 Figure 436: DNA227081, NP_000390.2, 205249.s.at
 Figure 437: PRO37544
 Figure 438A-B: DNA188301, NP_002300.1, 205266.s.at
 Figure 439: PRO21834
 Figure 440A-B: DNA331315, LRP8, 205282.s.at
 Figure 441: PRO70090
 Figure 442: DNA227173, NP_001456.1, 205285.s.at
 Figure 443: PRO37636
 Figure 444A-B: DNA331316, 983055.1, 205296.s.at
 Figure 445: PRO86407
 Figure 446: DNA325568, NP_001265.1, 205393.s.at
 Figure 447: PRO12187
 Figure 448: DNA325568, CHEK1, 205394.s.at
 Figure 449: PRO12187
 Figure 450: DNA328566, NP_060446.1, 205510.s.at
 Figure 451: PRO84363
 Figure 452: DNA330085, D86324, 205518.s.at
 Figure 453: PRO85359
 Figure 454: DNA330086, NP_079184.1, 205519.s.at
 Figure 455: PRO85360
 Figure 456: DNA254810, NP_056536.1, 205527.s.at
 Figure 457: PRO49906
 Figure 458: DNA328567, NP_006797.2, 205548.s.at
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 Figure 460: DNA329013, NP_005649.1, 205599.s.at
 Figure 461: PRO20128
 Figure 462: DNA330088, NP_003087.1, 205644.s.at
 Figure 463: PRO61962
 Figure 464: DNA330089, NP_004200.2, 205691.s.at
 Figure 465: PRO12507
 Figure 466: DNA331317, NP_003636.1, 205768.s.at
 Figure 467: PRO51139
 Figure 468: DNA331318, SLC27A2, 205769.s.at
 Figure 469: PRO51139
 Figure 470: DNA76517, NP_002176.1, 205798.s.at
 Figure 471: PRO2541
 Figure 472: DNA194766, NP_079504.1, 205804.s.at
 Figure 473: PRO24046
 Figure 474A-B: DNA331319, NP_004749.1, 205839.s.at
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 Figure 476: DNA327651, NP_005612.1, 205863.s.at
 Figure 477: PRO83649
 Figure 478: DNA331320, HSU37122, 205882.x.s.at
 Figure 479: PRO86409
 Figure 480: DNA287318, NP_002683.1, 205909.s.at
 Figure 481: PRO69583
 Figure 482: DNA329166, NP_006518.1, 206052.s.at
 Figure 483: PRO84793
 Figure 484: DNA329168, CLC, 206207.s.at
 Figure 485: PRO84794
 Figure 486: DNA281446, NP_031394.1, 206220.s.at
 Figure 487: PRO66285
 Figure 488: DNA331321, NP_057473.1, 206245.s.at
 Figure 489: PRO86410
 Figure 490A-B: DNA331322, NP_055523.1, 206316.s.at
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 Figure 492: DNA218278, NP_000408.1, 206341.s.at
 Figure 493: PRO34330
 Figure 494: DNA329169, NP_002986.1, 206366.x.s.at
 Figure 495: PRO1610
 Figure 496A-B: DNA225567, NP_004659.1, 206522.s.at
 Figure 497: PRO36030
 Figure 498: DNA227540, NP_003036.1, 206566.s.at
 Figure 499: PRO38003
 Figure 500: DNA329171, NP_060246.1, 206583.s.at
 Figure 501: PRO84795
 Figure 502: DNA88374, NP_002095.1, 206666.s.at
 Figure 503: PRO2768
 Figure 504: DNA330105, HUMNCA, 206676.s.at
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 Figure 506: DNA328590, C6orf32, 206707.x.s.at
 Figure 507: PRO84375
 Figure 508: DNA325853, NP_075387.1, 206958.s.at
 Figure 509: PRO82315
 Figure 510: DNA35629, NP_000586.2, 206975.s.at
 Figure 511: PRO7
 Figure 512: DNA188346, NP_001450.1, 206980.s.at
 Figure 513: PRO21766
 Figure 514A-B: DNA227659, NP_000570.1, 206991.s.at
 Figure 515: PRO38122
 Figure 516A-B: DNA227750, NP_001550.1, 206999.s.at
 Figure 517: PRO38213
 Figure 518: DNA188289, NP_001548.1, 207008.s.at
 Figure 519: PRO21820
 Figure 520: DNA255987, NP_004685.1, 207038.s.at
 Figure 521: PRO51038
 Figure 522: DNA227481, VAMP1, 207100.s.at
 Figure 523: PRO37944
 Figure 524: DNA218655, NP_000585.1, 207113.s.at
 Figure 525: PRO34451
 Figure 526A-B: DNA327674, NP_002739.1, 207121.s.at

Figure 527: PRO83661
 Figure 528: DNA331323, NP_001250.1, 207143_at
 Figure 529: PRO86412
 Figure 530: DNA83048, NP_001916.1, 207269_at
 Figure 531: PRO2057
 Figure 532: DNA331324, LTB, 207339_s_at
 Figure 533: PRO11604
 Figure 534: DNA226396, NP_002180.1, 207375_s_at
 Figure 535: PRO36859
 Figure 536: DNA329178, BTN3A1, 207485_x_at
 Figure 537: PRO84801
 Figure 538: DNA304473, NP_001552.2, 207536_s_at
 Figure 539: PRO2023
 Figure 540A-B: DNA330120, FLJ10971, 207606_s_at
 Figure 541: PRO85382
 Figure 542: DNA227606, NP_001872.2, 207630_s_at
 Figure 543: PRO38069
 Figure 544: DNA196426, NP_037440.1, 207651_at
 Figure 545: PRO24924
 Figure 546: DNA256401, NP_004063.1, 207652_s_at
 Figure 547: PRO51442
 Figure 548: DNA328763, NP_001219.2, 207686_s_at
 Figure 549: PRO84511
 Figure 550: DNA325654, NP_054752.1, 207761_s_at
 Figure 551: PRO4348
 Figure 552: DNA329184, CITED2, 207980_s_at
 Figure 553: PRO84807
 Figure 554: DNA227224, NP_060877.1, 208029_s_at
 Figure 555: PRO37687
 Figure 556: DNA328610, NP_112601.2, 208146_s_at
 Figure 557: PRO84392
 Figure 558: DNA325329, NP_004719.1, 208152_s_at
 Figure 559: PRO81872
 Figure 560: DNA328611, RASGRP2, 208206_s_at
 Figure 561: PRO84393
 Figure 562: DNA331325, BC006443, 208433_s_at
 Figure 563: DNA103427, NP_005239.1, 208438_s_at
 Figure 564: PRO4755
 Figure 565A-C: DNA331326, ATM, 208442_s_at
 Figure 566: PRO86413
 Figure 567: DNA331327, NP_036382.2, 208456_s_at
 Figure 568: PRO86414
 Figure 569: DNA331328, NP_000690.1, 208498_s_at
 Figure 570: PRO2157
 Figure 571A-B: DNA273567, NP_004944.1, 208625_s_at
 Figure 572: PRO61545
 Figure 573: DNA329188, BC012142, 208638_at
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 Figure 575: DNA330139, AK022493, 208657_s_at
 Figure 576: PRO85398
 Figure 577: DNA304686, NP_002565.1, 208680_at
 Figure 578: PRO71112
 Figure 579: DNA287189, NP_002038.1, 208693_s_at
 Figure 580: PRO69475
 Figure 581: DNA330140, AF275798, 208696_at
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 Figure 583: DNA327696, AF228339, 208763_s_at
 Figure 584: PRO83679
 Figure 585: DNA238565, NP_005907.2, 208795_s_at
 Figure 586: PRO39210
 Figure 587: DNA330145, NP_002788.1, 208799_at
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 Figure 589: DNA273521, NP_002070.1, 208813_at
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 Figure 591: DNA227874, NP_003320.1, 208864_s_at
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 Figure 593: DNA328624, BC003562, 208891_at
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 Figure 595: DNA331329, DUSP6, 208892_s_at
 Figure 596: PRO84404
 Figure 597: DNA331330, BC005047, 208893_s_at
 Figure 598: PRO82215
 Figure 599: DNA327701, NP_001203.1, 208910_s_at
 Figure 600: PRO82667
 Figure 601: DNA226500, NP_005619.1, 208916_at
 Figure 602: PRO36963
 Figure 603: DNA329552, NP_063948.1, 208925_at
 Figure 604: PRO85097
 Figure 605: DNA328629, NP_006079.1, 208977_x_at
 Figure 606: PRO84407
 Figure 607: DNA330154, HUMPECAM27, 208981_at
 Figure 608: DNA330155, 7692317.2, 208982_at
 Figure 609: PRO85407
 Figure 610: DNA328631, AK027318, 209006_s_at
 Figure 611: PRO84409
 Figure 612: DNA328632, DJ465N24.2.1Homo, 209007_s_at
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 Figure 614: PRO84413
 Figure 615: DNA274202, NP_006804.1, 209034_at
 Figure 616: PRO62131
 Figure 617A-C: DNA328637, HSA7042, 209053_s_at
 Figure 618: PRO81109
 Figure 619: DNA327713, BC010653, 209146_at
 Figure 620: PRO37975
 Figure 621A-B: DNA328642, AF073310, 209184_s_at
 Figure 622: PRO84418
 Figure 623: DNA331331, AF161416, 209185_s_at
 Figure 624A-B: DNA328643, HUMHK1A, 209186_at
 Figure 625: PRO84419
 Figure 626: DNA103520, NP_002639.1, 209193_at
 Figure 627: PRO4847
 Figure 628: DNA226176, NP_003458.1, 209201_x_at
 Figure 629: PRO36639
 Figure 630: DNA326267, NP_004861.1, 209208_at
 Figure 631: PRO82674
 Figure 632: DNA328645, NP_009006.1, 209216_nt
 Figure 633: PRO84421
 Figure 634: DNA227483, NP_003120.1, 209218_at
 Figure 635: PRO37946
 Figure 636: DNA331332, BC007405, 209238_at

Figure 637: PRO86416
 Figure 638A-B: DNA331333, 371440.32, 209240.s.at
 Figure 639: PRO86417
 Figure 640: DNA328649, NP_116093.1, 209251.x.at
 Figure 641: PRO84424
 Figure 642: DNA25255, NP_071437.1, 209267.s.at
 Figure 643: PRO50332
 Figure 644: DNA269750, NP_002919.1, 209324.s.at
 Figure 645: PRO58159
 Figure 646: DNA269750, RGS16, 209325.s.at
 Figure 647: PRO58159
 Figure 648: DNA269630, NP_003281.1, 209344.at
 Figure 649: PRO58042
 Figure 650: DNA330170, AF109161, 209357.at
 Figure 651: PRO84807
 Figure 652: DNA253811, NP_004410.2, 209457.at
 Figure 653: PRO49214
 Figure 654: DNA324899, NP_002938.1, 209507.at
 Figure 655: PRO81503
 Figure 656: DNA328663, NP_057157.1, 209524.at
 Figure 657: PRO36183
 Figure 658: DNA328663, CGI-142, 209526.s.at
 Figure 659: PRO36183
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 Figure 661: PRO70011
 Figure 662: DNA328599, HSNFKBS, 209636.at
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 Figure 664: DNA330186, NP_004327.1, 209642.at
 Figure 665: PRO85434
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 Figure 667: PRO85439
 Figure 668: DNA329178, HSU09552, 209770.at
 Figure 669: PRO84801
 Figure 670: DNA329205, NP_001343.1, 209782.s.at
 Figure 671: PRO84821
 Figure 672: DNA226436, NP_001772.1, 209795.at
 Figure 673: PRO36899
 Figure 674A-B: DNA196499, AB002384, 209829.at
 Figure 675: PRO24988
 Figure 676: DNA330197, NP_112190.1, 209832.s.at
 Figure 677: PRO85445
 Figure 678: DNA331334, AF117233, 209845.at
 Figure 679: PRO86418
 Figure 680: DNA273915, NP_036215.1, 209864.at
 Figure 681: PRO61867
 Figure 682: DNA330198, AB014719, 209871.s.at
 Figure 683: PRO85446
 Figure 684: DNA154921, DNA154921, 209967.s.at
 Figure 685A-B: DNA227134, NP_000918.1, 209993.at
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 Figure 687: DNA226658, NP_003736.1, 209999.x.at
 Figure 688: PRO37121
 Figure 689: DNA226658, SSI-1, 210001.s.at
 Figure 690: PRO37121
 Figure 691A-B: DNA328685, NP_127497.1, 210113.s.at
 Figure 692: PRO34751
 Figure 693: DNA330203, NP_003755.1, 210190.at
 Figure 694: PRO85449
 Figure 695: DNA331335, AF070576, 210202.s.at
 Figure 696: DNA217253, NP_000749.1, 210229.s.at
 Figure 697: PRO34295
 Figure 698: DNA328690, NP_524145.1, 210240.s.at
 Figure 699: PRO59660
 Figure 700: DNA330206, NP_005801.2, 210288.at
 Figure 701: PRO85450
 Figure 702: DNA225528, NP_000610.1, 210354.at
 Figure 703: PRO35991
 Figure 704: DNA331336, TANK, 210458.s.at
 Figure 705: PRO86419
 Figure 706: DNA329217, BC003406, 210571.s.at
 Figure 707: PRO84828
 Figure 708: DNA330210, HSU03858, 210607.at
 Figure 709: PRO126
 Figure 710: DNA331337, TNFSF11, 210643.at
 Figure 711: PRO206
 Figure 712: DNA227446, NP_003551.1, 210647.x.at
 Figure 713: PRO37909
 Figure 714: DNA256521, NP_038459.1, 210690.at
 Figure 715: PRO51556
 Figure 716: DNA331338, AF188298, 210757.x.at
 Figure 717: PRO86420
 Figure 718: DNA237817, NP_001307.1, 210766.s.at
 Figure 719: PRO38923
 Figure 720A-B: DNA330216, NP_006445.1, 210778.s.at
 Figure 721: PRO85457
 Figure 722: DNA188234, NP_000630.1, 210865.at
 Figure 723: PRO21942
 Figure 724: DNA228132, LCE, 210868.s.at
 Figure 725: PRO38595
 Figure 726: DNA238565, MCM7, 210983.s.at
 Figure 727: PRO39210
 Figure 728: DNA326239, NP_006752.1, 210996.s.at
 Figure 729: PRO39530
 Figure 730: DNA325039, NP_004902.1, 211048.s.at
 Figure 731: PRO2733
 Figure 732: DNA288254, NP_006000.2, 211058.x.at
 Figure 733: PRO69536
 Figure 734: DNA288254, TUBA3, 211072.x.at
 Figure 735: PRO69536
 Figure 736: DNA188234, TNFSF6, 211333.s.at
 Figure 737: PRO21942
 Figure 738: DNA331339, B3GALT3, 211379.x.at
 Figure 739: PRO1074
 Figure 740A-B: DNA275066, NP_000170.1, 211450.s.at
 Figure 741: PRO62786
 Figure 742: DNA331340, HUMCD26A, 211478.s.at
 Figure 743: PRO80881
 Figure 744: DNA226578, CCNG2, 211559.s.at
 Figure 745: PRO37041

Figure 746: DNA331341, AB055881, 211583.x.at
 Figure 747: PRO86421
 Figure 748: DNA327760, NP_114430.1, 211685.s.at
 Figure 749: PRO83729
 Figure 750: DNA328706, BC021909, 211714.x.at
 Figure 751: PRO10347
 Figure 752: DNA329225, EVI2B, 211742.s.at
 Figure 753: PRO84833
 Figure 754: DNA328649, TUBA6, 211750.x.at
 Figure 755: PRO84424
 Figure 756: DNA254725, NP_002257.1, 211762.s.at
 Figure 757: PRO49824
 Figure 758: DNA330226, AF198052, 211794.at
 Figure 759: PRO85463
 Figure 760: DNA227173, FYB, 211795.s.at
 Figure 761: PRO37636
 Figure 762A-B: DNA331342, DEFCAP, 211822.s.at
 Figure 763: PRO86422
 Figure 764: DNA331343, AK026398, 211824.x.at
 Figure 765: PRO86423
 Figure 766: DNA226881, FLI1, 211825.s.at
 Figure 767: PRO37344
 Figure 768: DNA226176, CXCR4, 211919.s.at
 Figure 769: PRO36639
 Figure 770: DNA272286, CAT, 211922.s.at
 Figure 771: PRO060544
 Figure 772A-B: DNA325227, NP_005338.1, 211936.at
 Figure 773: PRO81785
 Figure 774A-B: DNA329227, HSANBP5, 211953.s.at
 Figure 775: PRO82307
 Figure 776A-C: DNA331344, 1390535.1, 211986.at
 Figure 777: PRO86424
 Figure 778: DNA287433, NP_006810.1, 212009.s.at
 Figure 779: PRO069690
 Figure 780: DNA330236, 228447.20, 212071.s.at
 Figure 781: PRO85472
 Figure 782A-B: DNA150956, BAA06685.1, 212110.at
 Figure 783: PRO12560
 Figure 784: DNA330240, CAA52801.1, 212141.at
 Figure 785: PRO85475
 Figure 786: DNA330240, HSP1CDC21, 212142.at
 Figure 787A-B: DNA150829, AB014568, 212144.at
 Figure 788: DNA324232, NP_005991.1, 212242.at
 Figure 789: PRO26228
 Figure 790: DNA329182, BC016852, 212259.s.at
 Figure 791: PRO84805
 Figure 792: DNA150980, HUMMAC30X, 212279.s.at
 Figure 793: DNA150980, DNA150980, 212281.s.at
 Figure 794: PRO12566
 Figure 795: DNA253017, DNA253017, 212282.at
 Figure 796: PRO48926
 Figure 797: DNA328719, BC012895, 212295.s.at
 Figure 798: PRO84475
 Figure 799: DNA271103, NP_005796.1, 212296.at
 Figure 800: PRO59425
 Figure 801A-B: DNA331345, NP_003596.1, 212307.s.at
 Figure 802: PRO59330
 Figure 803: DNA326808, BC019307, 212312.at
 Figure 804: PRO83141
 Figure 805: DNA331346, BC011685, 212330.at
 Figure 806: PRO62868
 Figure 807: DNA331347, 170995.1, 212334.at
 Figure 808: PRO86425
 Figure 809A-B: DNA330216, MAD4, 212346.s.at
 Figure 810: PRO85457
 Figure 811: DNA331348, AF040963, 212347.x.at
 Figure 812: PRO85457
 Figure 813A-B: DNA330250, NP_060727.1, 212406.s.at
 Figure 814: PRO85483
 Figure 815: DNA330251, NP_059965.1, 212430.at
 Figure 816: PRO85484
 Figure 817: DNA269630, TPM4, 212481.s.at
 Figure 818: PRO58042
 Figure 819A-B: DNA328731, 234169.5, 212500.at
 Figure 820: PRO84484
 Figure 821: DNA328732, NP_116193.1, 212502.at
 Figure 822: PRO84485
 Figure 823: DNA226041, NP_005555.1, 212531.at
 Figure 824: PRO36504
 Figure 825: DNA269882, HSWE1HU, 212533.at
 Figure 826: PRO58280
 Figure 827A-D: DNA328737, 148650.1, 212560.at
 Figure 828: PRO84490
 Figure 829: DNA275100, DNA275100, 212589.at
 Figure 830: DNA331349, BC013106, 212590.at
 Figure 831: PRO86426
 Figure 832: DNA327776, 1379302.1, 212593.s.at
 Figure 833: PRO83742
 Figure 834: DNA151487, DNA151487, 212594.at
 Figure 835: PRO11833
 Figure 836: DNA287198, K-ALPHA-1, 212639.x.at
 Figure 837: PRO69484
 Figure 838: DNA328744, AF318364, 212680.x.at
 Figure 839: PRO84496
 Figure 840A-B: DNA331350, NP_060903.2, 212689.s.at
 Figure 841: PRO86427
 Figure 842: DNA273193, DNA273193, 212811.x.at
 Figure 843: DNA331351, BC004298, 212823.s.at
 Figure 844: DNA254940, BAA91770.1, 213008.at
 Figure 845: PRO50030
 Figure 846A-B: DNA330275, BAA25487.1, 213045.at
 Figure 847: PRO85504
 Figure 848: DNA323879, NP_003991.1, 213060.s.at
 Figure 849: PRO80622
 Figure 850A-B: DNA331352, BAA76818.1, 213088.s.at
 Figure 851: PRO86429
 Figure 852A-B: DNA331353, AB023191, 213092.x.at

Figure 853A-C: DNA329244, 979567.11, 213106.s.at
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 Figure 855: DNA328567, BTG3, 213134.x.at
 Figure 856: PRO84364
 Figure 857: DNA330277, CAB45152.1, 213142.x.at
 Figure 858: PRO85506
 Figure 859A-B: DNA329248, BAA20816.1, 213302.s.at
 Figure 860: PRO84850
 Figure 861A-B: DNA331354, PPP2R5C, 213305.s.at
 Figure 862: PRO86430
 Figure 863: DNA155418, DNA155418, 213326.s.at
 Figure 864A-B: DNA331355, AAG24545.1, 213330.s.at
 Figure 865: PRO86431
 Figure 866: DNA331356, CASP8, 213373.s.at
 Figure 867: PRO86432
 Figure 868: DNA106195, DNA106195, 213454.s.at
 Figure 869: DNA327795, BC014226, 213457.s.at
 Figure 870: DNA328766, NP_006077.1, 213476.x.at
 Figure 871: PRO84514
 Figure 872: DNA227483, SQLE, 213562.s.at
 Figure 873: PRO37946
 Figure 874: DNA327800, 1251176.10, 213575.s.at
 Figure 875: PRO83763
 Figure 876: DNA159560, DNA159560, 213577.s.at
 Figure 877: DNA331357, BC010494, 213646.x.at
 Figure 878: PRO38556
 Figure 879: DNA330293, BC011922, 213666.s.at
 Figure 880: PRO85520
 Figure 881: DNA325704, MARS, 213671.s.at
 Figure 882: PRO82188
 Figure 883: DNA328629, TUBB2, 213726.x.at
 Figure 884: PRO84407
 Figure 885: DNA328771, HSMYOSIE, 213733.s.at
 Figure 886: DNA150713, EBP, 213787.s.at
 Figure 887: PRO12082
 Figure 888: DNA330295, NP_037515.1, 213951.s.at
 Figure 889: PRO85522
 Figure 890: DNA331358, NP_037445.1, 213996.s.at
 Figure 891: PRO86433
 Figure 892: DNA329136, HSPC111, 214011.s.at
 Figure 893: PRO84772
 Figure 894: DNA270687, DNA270687, 214032.s.at
 Figure 895: DNA227224, LC27, 214039.s.at
 Figure 896: PRO37687
 Figure 897: DNA330298, BC011911, 214095.s.at
 Figure 898: PRO83772
 Figure 899: DNA329254, BC004215, 214096.s.at
 Figure 900: PRO84854
 Figure 901A-B: DNA331359, 332730.12, 214155.s.at
 Figure 902: PRO86434
 Figure 903: DNA331360, AK022497, 214177.s.at
 Figure 904: PRO86435
 Figure 905: DNA331361, NP_003318.1, 214228.x.at
 Figure 906: PRO2398
 Figure 907: DNA331362, AF275719, 214359.s.at
 Figure 908: PRO86436
 Figure 909: DNA326089, NP_000508.1, 214414.x.at
 Figure 910: PRO3629
 Figure 911: DNA271374, CHAF1A, 214426.x.at
 Figure 912: PRO59673
 Figure 913: DNA327811, SHMT2, 214437.s.at
 Figure 914: PRO83772
 Figure 915: DNA331363, AF001383, 214439.x.at
 Figure 916: PRO86437
 Figure 917: DNA150971, NP_002249.1, 214470.s.at
 Figure 918: PRO12564
 Figure 919: DNA331364, CREM, 214508.x.at
 Figure 920: PRO86438
 Figure 921: DNA216515, NP_003166.1, 214567.s.at
 Figure 922: PRO34267
 Figure 923: DNA330303, BAA05499.1, 214662.s.at
 Figure 924: PRO85528
 Figure 925: DNA330308, 307914.1, 215029.s.at
 Figure 926: PRO85533
 Figure 927: DNA196372, HSCLXL, 215037.s.at
 Figure 928: PRO24874
 Figure 929: DNA270522, NP_006013.1, 215111.s.at
 Figure 930: PRO58899
 Figure 931: DNA330314, 026641.5, 215275.s.at
 Figure 932: PRO85538
 Figure 933: DNA328801, 407831.1, 215392.s.at
 Figure 934: PRO84543
 Figure 935A-B: DNA331134, NP_003381.1, 215711.s.at
 Figure 936: PRO86275
 Figure 937A-B: DNA256461, NP_009017.1, 216228.s.at
 Figure 938: PRO51498
 Figure 939: DNA329266, BC000142, 216237.s.at
 Figure 940: PRO12845
 Figure 941: DNA88296, NP_005733.1, 216640.s.at
 Figure 942: PRO2274
 Figure 943: DNA225720, AF151900, 216693.x.at
 Figure 944: PRO36183
 Figure 945A-B: DNA66475, NP_004439.1, 216836.s.at
 Figure 946: PRO1204
 Figure 947: DNA331365, NP_004168.1, 216985.s.at
 Figure 948: PRO86439
 Figure 949: DNA331366, HUMGPCR, 217028.s.at
 Figure 950: PRO4516
 Figure 951A-B: DNA331367, BAA34514.1, 217100.s.at
 Figure 952: PRO86440
 Figure 953: DNA331368, NP_112233.1, 217226.s.at
 Figure 954: PRO86441
 Figure 955: DNA331369, HSU88968, 217294.s.at
 Figure 956: DNA328819, NP_057145.1, 217783.s.at
 Figure 957: PRO84557
 Figure 958: DNA328303, NP_056525.1, 217807.s.at
 Figure 959: PRO84173

Figure 960: DNA227172, NP_066952.1, 217848.s.at
 Figure 961: PRO37635
 Figure 962: DNA330345, NP_055130.1, 217906.at
 Figure 963: PRO85566
 Figure 964: DNA227218, NP_003721.2, 217983.s.at
 Figure 965: PRO37681
 Figure 966: DNA227218, RNASE6PL, 217984.at
 Figure 967: PRO37681
 Figure 968: DNA328831, NP_057329.1, 217989.at
 Figure 969: PRO233
 Figure 970: DNA328833, BC018929, 217996.at
 Figure 971: PRO84569
 Figure 972: DNA328834, AF220656, 217997.at
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 Figure 974: PRO61079
 Figure 975: DNA328837, NP_057149.1, 218046.s.at
 Figure 976: PRO81876
 Figure 977: DNA329276, NP_077001.1, 218069.at
 Figure 978: PRO12104
 Figure 979: DNA330356, NP_006318.1, 218118.s.at
 Figure 980: PRO85572
 Figure 981: DNA327857, NP_057386.1, 218142.s.at
 Figure 982: PRO83799
 Figure 983: DNA327858, NP_036473.1, 218238.at
 Figure 984: PRO83800
 Figure 985: DNA328850, NP_057187.1, 218254.s.at
 Figure 986: PRO84581
 Figure 987A-B: DNA331370, NP_055101.2, 218266.s.at
 Figure 988: PRO11501
 Figure 989: DNA331371, NP_060401.1, 218272.at
 Figure 990: PRO86442
 Figure 991: DNA331372, FLJ20950, 218298.s.at
 Figure 992: PRO86443
 Figure 993: DNA327862, NP_060445.1, 218349.s.at
 Figure 994: PRO83803
 Figure 995: DNA328854, NP_056979.1, 218350.s.at
 Figure 996: PRO84585
 Figure 997: DNA304468, NP_077300.1, 218358.at
 Figure 998: PRO71044
 Figure 999: DNA328856, NP_068376.1, 218380.at
 Figure 1000: PRO84586
 Figure 1001: DNA327865, NP_079105.1, 218454.at
 Figure 1002: PRO83806
 Figure 1003: DNA329286, NP_005691.2, 218567.x.at
 Figure 1004: PRO69644
 Figure 1005A-B: DNA273435, NP_057532.1, 218585.s.at
 Figure 1006: PRO61430
 Figure 1007: DNA330377, NP_036577.1, 218638.s.at
 Figure 1008: PRO85590
 Figure 1009: DNA304835, NP_071327.1, 218681.s.at
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 Figure 1011: DNA329288, NP_061910.1, 218695.at
 Figure 1012: PRO84880
 Figure 1013: DNA326185, FLJ13912, 218719.s.at
 Figure 1014: PRO82602
 Figure 1015: DNA330381, NP_076958.1, 218741.at
 Figure 1016: PRO38668
 Figure 1017: DNA331373, NP_075526.1, 218769.s.at
 Figure 1018: PRO86444
 Figure 1019A-B: DNA327971, NP_006104.3, 218807.at
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 Figure 1021: DNA330388, NP_078905.1, 218883.s.at
 Figure 1022: PRO85597
 Figure 1023: DNA331092, NP_078918.2, 218885.s.at
 Figure 1024: PRO86243
 Figure 1025: DNA226633, NP_060376.1, 218886.at
 Figure 1026: PRO37096
 Figure 1027: DNA328881, NP_057706.2, 218890.x.at
 Figure 1028: PRO49469
 Figure 1029: DNA325622, NP_060518.1, 218894.s.at
 Figure 1030: PRO82113
 Figure 1031: DNA328364, SIGIRR, 218921.at
 Figure 1032: PRO84223
 Figure 1033: DNA329050, NP_057053.1, 218982.s.at
 Figure 1034: PRO84712
 Figure 1035: DNA330391, NP_076999.1, 219000.s.at
 Figure 1036: PRO34008
 Figure 1037: DNA227187, NP_057703.1, 219014.at
 Figure 1038: PRO37650
 Figure 1039: DNA329293, NP_057136.1, 219037.at
 Figure 1040: PRO84883
 Figure 1041: DNA331374, NP_009177.2, 219157.at
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 Figure 1043: DNA331375, AK000192, 219168.s.at
 Figure 1044: PRO86446
 Figure 1045: DNA329223, NP_037517.1, 219183.s.at
 Figure 1046: PRO84831
 Figure 1047: DNA329296, NP_060328.1, 219258.at
 Figure 1048: PRO84886
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 Figure 1053: DNA331377, NP_060753.1, 219347.at
 Figure 1054: PRO86448
 Figure 1055: DNA254518, NP_057354.1, 219371.s.at
 Figure 1056: PRO49625
 Figure 1057: DNA328902, NP_071750.1, 219452.at
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 Figure 1059: DNA327890, NP_079021.1, 219493.at
 Figure 1060: PRO83826
 Figure 1061A-B: DNA227179, NP_059120.1, 219505.at
 Figure 1062: PRO37642
 Figure 1063: DNA329299, NP_004660.1, 219529.at
 Figure 1064: PRO84888
 Figure 1065: DNA330410, NP_060925.1, 219555.s.at
 Figure 1066: PRO85618

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 Figure 1070: PRO86449
 Figure 1071: DNA255255, LOC64116, 219869.s.at
 Figure 1072: PRO50332
 Figure 1073: DNA256325, NP_005470.1, 219889.at
 Figure 1074: PRO51367
 Figure 1075: DNA328923, NP_075379.1, 219892.at
 Figure 1076: PRO84640
 Figure 1077: DNA330421, NP_057438.2, 219911.s.at
 Figure 1078: PRO85626
 Figure 1079: DNA330423, NP_037466.2, 219920.s.at
 Figure 1080: PRO85628
 Figure 1081: DNA328924, NP_057150.2, 219933.at
 Figure 1082: PRO84641
 Figure 1083: DNA330537, NP_060533.2, 220085.at
 Figure 1084: PRO81892
 Figure 1085: DNA227302, NP_037401.1, 220132.s.at
 Figure 1086: PRO37765
 Figure 1087A-B: DNA328930, NP_038465.1, 220253.s.at
 Figure 1088: PRO23525
 Figure 1089: DNA330436, NP_037394.1, 220319.s.at
 Figure 1090: PRO85639
 Figure 1091: DNA327904, NP_071419.2, 220330.s.at
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 Figure 1097: DNA255734, NP_057607.1, 220646.s.at
 Figure 1098: PRO50791
 Figure 1099A-B: DNA327908, MCM10, 220651.s.at
 Figure 1100: PRO83843
 Figure 1101: DNA323756, NP_057267.2, 220688.s.at
 Figure 1102: PRO80512
 Figure 1103: DNA331380, DKFZp566O084Homo, 220690.s.at
 Figure 1104: DNA288247, NP_478059.1, 220892.s.at
 Figure 1105: PRO70011
 Figure 1106: DNA331381, BA108L7.2, 220974.x.at
 Figure 1107: PRO86451
 Figure 1108: DNA287397, NP_112214.1, 220987.s.at
 Figure 1109: PRO69654
 Figure 1110: DNA328945, NP_079177.2, 221081.s.at
 Figure 1111: PRO84657
 Figure 1112: DNA331382, CISH, 221223.x.at
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 Figure 1115: PRO85652
 Figure 1116: DNA328948, NP_110437.1, 221253.s.at
 Figure 1117: PRO84659
 Figure 1118: DNA326507, NP_112490.2, 221267.s.at
 Figure 1119: PRO82879
 Figure 1120: DNA329471, NP_110387.1, 221417.x.at
 Figure 1121: PRO85036
 Figure 1122A-B: DNA329314, 1149046.7, 221478.at
 Figure 1123: PRO84902
 Figure 1124: DNA227303, NP_004322.1, 221479.s.at
 Figure 1125: PRO37766
 Figure 1126: DNA326221, NP_057179.1, 221521.s.at
 Figure 1127: PRO82634
 Figure 1128: DNA330457, NP_076944.1, 221559.s.at
 Figure 1129: PRO85658
 Figure 1130: DNA329318, IRO033793, 221564.at
 Figure 1131: DNA329319, BC006401, 221601.s.at
 Figure 1132: PRO1607
 Figure 1133: DNA329319, NP_005440.1, 221602.s.at
 Figure 1134: PRO1607
 Figure 1135: DNA330459, NP_060083.1, 221677.s.at
 Figure 1136: PRO50083
 Figure 1137: DNA328961, NP_443112.1, 221756.at
 Figure 1138: PRO84667
 Figure 1139: DNA328961, MGC17330, 221757.at
 Figure 1140: PRO84667
 Figure 1141: DNA331383, BC007588, 221769.at
 Figure 1142: DNA331384, AK026326, 221985.at
 Figure 1143: PRO86454
 Figure 1144: DNA330467, NP_060114.1, 221986.s.at
 Figure 1145: PRO85665
 Figure 1146: DNA254739, NP_068766.1, 221987.s.at
 Figure 1147: PRO49837
 Figure 1148: DNA257797, DNA257797, 222036.s.at
 Figure 1149: DNA257798, DNA257798, 222037.s.at
 Figure 1150: DNA325648, NP_037409.2, 222077.s.at
 Figure 1151: PRO82139
 Figure 1152A-B: DNA331385, AF27488984, 222088.s.at
 Figure 1153: DNA331386, HST000012, 222150.s.at
 Figure 1154A-B: DNA331387, NP_008919.2, 222162.s.at
 Figure 1155: PRO86456
 Figure 1156: DNA328977, NP_071344.1, 222216.s.at
 Figure 1157: PRO84678
 Figure 1158: DNA325821, BC014334, 222402.at
 Figure 1159: PRO82287
 Figure 1160A-B: DNA256489, NP_079110.1, 222464.s.at
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 Figure 1162: DNA304460, BC003048, 222500.at
 Figure 1163: PRO4984
 Figure 1164: DNA327942, NP_060596.1, 222642.s.at
 Figure 1165: PRO83870
 Figure 1166: DNA327943, NP_055399.1, 222646.s.at
 Figure 1167: PRO865
 Figure 1168A-B: DNA273435, RAMP, 222680.s.at
 Figure 1169: PRO61430
 Figure 1170: DNA330486, HSM802473, 222692.s.at
 Figure 1171: DNA331388, NP_068747.1, 222753.s.at
 Figure 1172: PRO3567
 Figure 1173: DNA329335, AK023411, 222843.at

Figure 1174: PRO84919
 Figure 1175: DNA331389, NP_071428.2, 222848.s.at
 Figure 1176: PRO81238
 Figure 1177: DNA287404, FLJ22833, 222872.x.at
 Figure 1178: PRO69661
 Figure 1179: DNA330500, AK022872, 222889.s.at
 Figure 1180: PRO85693
 Figure 1181A-B: DNA287236, AB024334, 222985.s.at
 Figure 1182: PRO10607
 Figure 1183: DNA329571, NP_057547.3, 222996.s.at
 Figure 1184: PRO51662
 Figure 1185: DNA189412, NP_057390.1, 223054.s.at
 Figure 1186: PRO25349
 Figure 1187: DNA288247, PSA, 223062.s.at
 Figure 1188: PRO70011
 Figure 1189: DNA331390, AAF28975.1, 223070.s.at
 Figure 1190: PRO86457
 Figure 1191: DNA324209, NP_057018.1, 223096.s.at
 Figure 1192: PRO80902
 Figure 1193: DNA330436, MIR, 223129.x.at
 Figure 1194: PRO85639
 Figure 1195: DNA330513, AF212221, 223130.s.at
 Figure 1196: PRO85703
 Figure 1197: DNA254276, NP_054896.1, 223180.s.at
 Figure 1198: PRO49387
 Figure 1199: DNA254416, NP_060915.1, 223209.s.at
 Figure 1200: PRO49526
 Figure 1201A-B: DNA257461, NP_113607.1, 223217.s.at
 Figure 1202: PRO52040
 Figure 1203A-B: DNA257461, MAIL, 223218.s.at
 Figure 1204: PRO52040
 Figure 1205: DNA326056, NP_072088.1, 223264.s.at
 Figure 1206: PRO82491
 Figure 1207: DNA330518, BC002493, 223274.s.at
 Figure 1208: PRO85708
 Figure 1209: DNA329355, NP_150596.1, 223299.s.at
 Figure 1210: PRO50434
 Figure 1211: DNA227125, AF132297, 223377.x.at
 Figure 1212: PRO37588
 Figure 1213A-B: DNA331391, NP_065947.1, 223464.s.at
 Figure 1214: PRO49998
 Figure 1215: DNA329456, NP_057126.1, 223490.s.at
 Figure 1216: PRO85023
 Figure 1217: DNA330536, NP_115666.1, 223542.s.at
 Figure 1218: PRO85722
 Figure 1219: DNA330537, AF155827, 223556.s.at
 Figure 1220: PRO81892
 Figure 1221: DNA287253, LOC85028, 223773.s.at
 Figure 1222: PRO69527
 Figure 1223: DNA327200, NP_114156.1, 223836.s.at
 Figure 1224: PRO1065
 Figure 1225: DNA331392, NP_004186.1, 223851.s.at
 Figure 1226: PRO364
 Figure 1227: DNA331393, D83532, 223961.s.at
 Figure 1228: PRO86458
 Figure 1229: DNA330552, BC001104, 223984.s.at
 Figure 1230: PRO85736
 Figure 1231: DNA330558, NP_057588.1, 224330.s.at
 Figure 1232: PRO84950
 Figure 1233: DNA328323, NP_114148.2, 224428.s.at
 Figure 1234: PRO69531
 Figure 1235: DNA331394, MGC11316, 224482.s.at
 Figure 1236: PRO86459
 Figure 1237: DNA327976, NP_116120.1, 224511.s.at
 Figure 1238: PRO69574
 Figure 1239: DNA329374, NP_115735.1, 224523.s.at
 Figure 1240: PRO84953
 Figure 1241: DNA331395, TNFRSF18, 224553.s.at
 Figure 1242: PRO86460
 Figure 1243: DNA331396, 1357555.1, 224603.s.at
 Figure 1244: PRO86461
 Figure 1245A-B: DNA327981, 344095.3, 224654.s.at
 Figure 1246: PRO83901
 Figure 1247A-B: DNA330574, AB033054, 224698.s.at
 Figure 1248: DNA324173, NP_115766.2, 224714.s.at
 Figure 1249: PRO80871
 Figure 1250: DNA257352, DNA257352, 224739.s.at
 Figure 1251: PRO51940
 Figure 1252A-B: DNA287330, BAA86479.1, 224799.s.at
 Figure 1253: PRO69594
 Figure 1254A-B: DNA287330, AB032991, 224801.s.at
 Figure 1255: DNA331397, AK001723, 224802.s.at
 Figure 1256: PRO23259
 Figure 1257A-C: DNA329379, 010205.2, 224847.s.at
 Figure 1258: PRO84957
 Figure 1259: DNA257789, NP_116219.1, 224903.s.at
 Figure 1260: PRO52338
 Figure 1261: DNA151170, DNA151170, 224989.s.at
 Figure 1262: PRO12626
 Figure 1263A-B: DNA327993, 898436.7, 225133.s.at
 Figure 1264: PRO81138
 Figure 1265: DNA327996, BC010181, 225195.s.at
 Figure 1266: PRO83915
 Figure 1267: DNA329394, BC010416, 225201.s.at
 Figure 1268: DNA329396, NP_060866.1, 225253.s.at
 Figure 1269: PRO84972
 Figure 1270: DNA304802, AAH00967.1, 225439.s.at
 Figure 1271: PRO71212
 Figure 1272A-B: DNA330617, 336147.2, 225447.s.at
 Figure 1273: PRO59923
 Figure 1274: DNA196561, DNA196561, 225470.s.at
 Figure 1275A-B: DNA331398, 234824.7, 225478.s.at
 Figure 1276: PRO86462
 Figure 1277: DNA329405, HSM800962, 225520.s.at
 Figure 1278A-B: DNA330620, CAB55950.1, 225533.s.at
 Figure 1279: PRO85794
 Figure 1280: DNA330621, AF116628, 225535.s.at
 Figure 1281: DNA328008, 240051.4, 225541.s.at

Figure 1282: PRO83926
 Figure 1283: DNA329406, 1503139.10, 225562.at
 Figure 1284: PRO84979
 Figure 1285: DNA304469, NP_149078.1, 225621.at
 Figure 1286: PRO71045
 Figure 1287: DNA31399, 994419.37, 225622.at
 Figure 1288: PRO86463
 Figure 1289A-B: DNA331400, NP_060910.2, 225626.at
 Figure 1290: PRO86464
 Figure 1291: DNA329940, NP_001805.1, 225647.s.at
 Figure 1292: PRO2679
 Figure 1293A-B: DNA288261, NP_037414.2, 225655.at
 Figure 1294: PRO70021
 Figure 1295A-B: DNA331401, 336865.4, 225700.at
 Figure 1296: PRO86465
 Figure 1297: DNA304821, BC011254, 225706.at
 Figure 1298: DNA254820, DNA254820, 225707.at
 Figure 1299: PRO49916
 Figure 1300: DNA330633, BC003515, 225723.at
 Figure 1301: DNA329417, 411336.1, 225842.at
 Figure 1302: PRO84989
 Figure 1303A-B: DNA331402, 197159.1, 225845.at
 Figure 1304: PRO86466
 Figure 1305: DNA287370, BAB14983.1, 225866.at
 Figure 1306: PRO69630
 Figure 1307A-B: DNA331403, TP53INP1, 225912.at
 Figure 1308: PRO86467
 Figure 1309A-B: DNA331405, 979005.2, 225956.at
 Figure 1310: PRO86468
 Figure 1311: DNA328021, BC004538, 226038.at
 Figure 1312A-B: DNA329428, 1446144.8, 226218.at
 Figure 1313: PRO84999
 Figure 1314: DNA193896, DNA193896, 226276.at
 Figure 1315: PRO23314
 Figure 1316: DNA328028, NP_005773.1, 226319.s.at
 Figure 1317: PRO83945
 Figure 1318: DNA328028, ALY, 226320.at
 Figure 1319: PRO83945
 Figure 1320A-B: DNA331406, 399773.27, 226334.s.at
 Figure 1321: PRO86470
 Figure 1322A-B: DNA331407, 198233.1, 226352.at
 Figure 1323: PRO86471
 Figure 1324A-B: DNA331409, AB051464, 226370.at
 Figure 1325A-B: DNA330675, 177663.2, 226372.at
 Figure 1326: PRO85847
 Figure 1327: DNA330677, 1384190.6, 226390.at
 Figure 1328: PRO85849
 Figure 1329: DNA331410, HSM802051, 226416.at
 Figure 1330: PRO86474
 Figure 1331: DNA330679, BC013040, 226456.at
 Figure 1332A-B: DNA330680, BC022792, 226481.at
 Figure 1333: PRO85852
 Figure 1334: DNA330684, 984114.1, 226548.at
 Figure 1335: PRO85855
 Figure 1336: DNA257914, DNA257914, 226743.at
 Figure 1337: PRO52447
 Figure 1338: DNA328038, 216863.2, 226811.at
 Figure 1339: PRO83953
 Figure 1340: DNA328044, 039170.3, 226936.at
 Figure 1341: PRO83958
 Figure 1342A-B: DNA330705, 198782.1, 227020.at
 Figure 1343: PRO85876
 Figure 1344A-B: DNA330706, AF445027, 227027.at
 Figure 1345: PRO85877
 Figure 1346: DNA331411, 232146.1, 227200.at
 Figure 1347: PRO86475
 Figure 1348: DNA330715, BC022374, 227212.s.at
 Figure 1349: PRO85886
 Figure 1350: DNA331412, 1378353.1, 227223.at
 Figure 1351: PRO86476
 Figure 1352A-B: DNA329442, AH007300S2, 227265.at
 Figure 1353: DNA329444, BC017821, 227278.at
 Figure 1354: PRO85012
 Figure 1355: DNA330718, 025465.3, 227295.at
 Figure 1356: PRO85889
 Figure 1357A-B: DNA330721, 198680.1, 227350.at
 Figure 1358: PRO85892
 Figure 1359: DNA226872, NP_001955.1, 227404.s.at
 Figure 1360: PRO37335
 Figure 1361: DNA329450, BC017226, 227726.at
 Figure 1362: PRO85018
 Figure 1363: DNA59606, DNA59606, 227803.at
 Figure 1364: PRO1107
 Figure 1365: DNA329456, RRP40, 227916.x.at
 Figure 1366: PRO85023
 Figure 1367: DNA330745, BC011716, 228069.at
 Figure 1368: PRO85913
 Figure 1369: DNA329460, BC017117, 228092.at
 Figure 1370: PRO85027
 Figure 1371: DNA330436, AF187016, 228098.s.at
 Figure 1372: PRO85639
 Figure 1373: DNA329461, BC016615, 228113.at
 Figure 1374: PRO85028
 Figure 1375: DNA331413, 286318.11, 228284.at
 Figure 1376: PRO86477
 Figure 1377: DNA331414, 1450017.11, 228559.at
 Figure 1378: PRO86478
 Figure 1379: DNA331415, 345279.19, 228788.at
 Figure 1380: PRO86479
 Figure 1381: DNA330780, 335374.1, 228955.at
 Figure 1382: PRO85944
 Figure 1383: DNA330784, 233595.21, 228990.at
 Figure 1384: PRO85948
 Figure 1385: DNA330787, 349981.7, 229041.s.at
 Figure 1386: PRO85951
 Figure 1387: DNA327307, AF442769, 229215.at
 Figure 1388: PRO83560
 Figure 1389: DNA287421, 234832.1, 229437.at

Figure 1390: PRO69678
 Figure 1391: DNA330799, 481875.1, 229551.s.at
 Figure 1392: PRO85963
 Figure 1393A-B: DNA330809, 336997.1, 229844.at
 Figure 1394: PRO85973
 Figure 1395: DNA329466, AF327346, 230069.at
 Figure 1396: PRO23814
 Figure 1397A-B: DNA331416, FREQ, 230146.s.at
 Figure 1398: PRO11501
 Figure 1399: DNA329468, BC011589, 230170.at
 Figure 1400: PRO88
 Figure 1401: DNA330818, 212282.1, 230304.at
 Figure 1402: PRO85982
 Figure 1403: DNA257756, DNA257756, 230405.at
 Figure 1404: DNA329471, EDG8, 230464.at
 Figure 1405: PRO85036
 Figure 1406: DNA331417, 333493.3, 230748.at
 Figure 1407: PRO86480
 Figure 1408: DNA331418, 7693630.2, 230917.at
 Figure 1409: PRO86481
 Figure 1410A-B: DNA287217, DNA287217, 231259.s.at
 Figure 1411: PRO36766
 Figure 1412: DNA330843, 201388.1, 231832.at
 Figure 1413: PRO86006
 Figure 1414: DNA331419, 085942.3, 232001.at
 Figure 1415: PRO86482
 Figure 1416: DNA331420, 029520.1, 232210.at
 Figure 1417: PRO86483
 Figure 1418: DNA331421, 1448461.1, 232614.at
 Figure 1419: PRO86484
 Figure 1420: DNA328194, 998827.1, 233068.at
 Figure 1421: PRO84097
 Figure 1422: DNA287404, AK026486, 233085.s.at
 Figure 1423: PRO69661
 Figure 1424: DNA331422, 077853.1, 233289.at
 Figure 1425: PRO86485
 Figure 1426: DNA331423, AF176071, 233467.s.at
 Figure 1427: DNA329571, HSPPC195, 233955.x.at
 Figure 1428: PRO51662
 Figure 1429: DNA331424, LOC112840, 235025.at
 Figure 1430: PRO86486
 Figure 1431: DNA330888, 7687712.2, 235088.at
 Figure 1432: PRO69581
 Figure 1433: DNA330891, AK027315, 235113.at
 Figure 1434: PRO86052
 Figure 1435A-C: DNA331425, 228001.3, 235116.at
 Figure 1436: PRO20128
 Figure 1437: DNA328146, BC019239, 235117.at
 Figure 1438: PRO84051
 Figure 1439: DNA330906, NP..116171.2, 235458.at
 Figure 1440: PRO86067
 Figure 1441: DNA194081, DNA194081, 235556.at
 Figure 1442: PRO23477
 Figure 1443: DNA330916, 234580.1, 235670.at
 Figure 1444: PRO86077
 Figure 1445: DNA330943, 1042935.2, 237009.at
 Figure 1446: PRO86104
 Figure 1447: DNA331426, 361450.1, 237542.at
 Figure 1448: PRO86487
 Figure 1449: DNA331427, AB052906, 238542.at
 Figure 1450: PRO791
 Figure 1451: DNA258952, DNA258952, 239901.at
 Figure 1452: DNA328206, 1384214.3, 240277.at
 Figure 1453: PRO84109
 Figure 1454: DNA331428, 7692702.1, 241803.s.at
 Figure 1455: PRO86488
 Figure 1456: DNA329506, NP..387510.1, 241937.s.at
 Figure 1457: PRO85067
 Figure 1458: DNA331429, NP..110403.1, 242020.s.at
 Figure 1459: PRO86489
 Figure 1460: DNA331030, 407930.2, 242648.at
 Figure 1461: PRO86188
 Figure 1462: DNA331037, 206873.1, 242890.at
 Figure 1463: PRO86195
 Figure 1464: DNA329507, 407430.1, 242943.at
 Figure 1465: PRO85068
 Figure 1466: DNA331043, 005042.1, 243134.at
 Figure 1467: PRO86201
 Figure 1468: DNA331053, 243689.1, 243509.at
 Figure 1469: PRO86211
 Figure 1470: DNA331430, 030957.1, 243808.at
 Figure 1471: PRO86490
 Figure 1472: DNA331431, 201839.1, 243840.at
 Figure 1473: PRO86491
 Figure 1474: DNA331432, 151634.1, 244035.at
 Figure 1475: PRO86492
 Figure 1476: DNA331433, 020071.1, 244434.at
 Figure 1477: PRO86493

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTSI. Definitions

The terms "PRO polypeptide" and "PRO" as used herein and when immediately followed by a numerical designation refer to various polypeptides, wherein the complete designation (i.e., PRO/number) refers to specific polypeptide sequences as described herein. The terms "PRO/number polypeptide" and "PRO/number" wherein the term "number" is provided as an actual numerical designation as used herein encompass native sequence polypeptides and polypeptide variants (which are further defined herein). The PRO polypeptides described herein may be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant or synthetic methods. The term "PRO polypeptide" refers to each individual PRO/number polypeptide disclosed herein. All disclosures in this specification which refer to the "PRO polypeptide" refer to each of the polypeptides individually as well as jointly. For example, descriptions of the preparation of, purification of, derivation of, formation of antibodies to or against, administration of, compositions containing, treatment of a disease with, etc., pertain to each polypeptide of the invention individually. The term "PRO polypeptide" also includes variants of the PRO/number polypeptides disclosed herein.

A "native sequence PRO polypeptide" comprises a polypeptide having the same amino acid sequence as the corresponding PRO polypeptide derived from nature. Such native sequence PRO polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. The term "native sequence PRO polypeptide" specifically encompasses naturally-occurring truncated or secreted forms of the specific PRO polypeptide (e.g., an extracellular domain sequence), naturally-occurring variant forms (e.g., alternatively spliced forms) and naturally-occurring allelic variants of the polypeptide. In various embodiments of the invention, the native sequence PRO polypeptides disclosed herein are mature or full-length native sequence polypeptides comprising the full-length amino acids sequences shown in the accompanying figures. Start and stop codons are shown in bold font and underlined in the figures. However, while the PRO polypeptide disclosed in the accompanying figures are shown to begin with methionine residues designated herein as amino acid position 1 in the figures, it is conceivable and possible that other methionine residues located either upstream or downstream from the amino acid position 1 in the figures may be employed as the starting amino acid residue for the PRO polypeptides.

The PRO polypeptide "extracellular domain" or "ECD" refers to a form of the PRO polypeptide which is essentially free of the transmembrane and cytoplasmic domains. Ordinarily, a PRO polypeptide ECD will have less than 1% of such transmembrane and/or cytoplasmic domains and preferably, will have less than 0.5% of such domains. It will be understood that any transmembrane domains identified for the PRO polypeptides of the present invention are identified pursuant to criteria routinely employed in the art for identifying that type of hydrophobic domain. The exact boundaries of a transmembrane domain may vary but most likely by no more than about 5 amino acids at either end of the domain as initially identified herein. Optionally, therefore, an extracellular domain of a PRO polypeptide may contain from about 5 or fewer amino acids on either side of the transmembrane domain/extracellular domain boundary as identified in the Examples or specification and such polypeptides, with or without the associated signal peptide, and nucleic acid encoding them, are contemplated by the present invention.

The approximate location of the "signal peptides" of the various PRO polypeptides disclosed herein are shown in the present specification and/or the accompanying figures. It is noted, however, that the C-terminal boundary of a signal peptide may vary, but most likely by no more than about 5 amino acids on either side of the signal peptide C-terminal boundary as initially identified herein, wherein the C-terminal

5 boundary of the signal peptide may be identified pursuant to criteria routinely employed in the art for identifying that type of amino acid sequence element (e.g., Nielsen et al., *Prot. Eng.* 10:1-6 (1997) and von Heinje et al., *Nucl. Acids. Res.* 14:4683-4690 (1986)). Moreover, it is also recognized that, in some cases, cleavage of a signal sequence from a secreted polypeptide is not entirely uniform, resulting in more than one secreted species. These mature polypeptides, where the signal peptide is cleaved within no more than about 10 5 amino acids on either side of the C-terminal boundary of the signal peptide as identified herein, and the polynucleotides encoding them, are contemplated by the present invention.

"PRO polypeptide variant" means an active PRO polypeptide as defined above or below having at least about 80% amino acid sequence identity with a full-length native sequence PRO polypeptide sequence as disclosed herein, a PRO polypeptide sequence lacking the signal peptide as disclosed herein, an 15 extracellular domain of a PRO polypeptide, with or without the signal peptide, as disclosed herein or any other fragment of a full-length PRO polypeptide sequence as disclosed herein. Such PRO polypeptide variants include, for instance, PRO polypeptides wherein one or more amino acid residues are added, or deleted, at the N- or C-terminus of the full-length native amino acid sequence. Ordinarily, a PRO polypeptide variant will have at least about 80% amino acid sequence identity, alternatively at least about 20 81% amino acid sequence identity, alternatively at least about 82% amino acid sequence identity, alternatively at least about 83% amino acid sequence identity, alternatively at least about 84% amino acid sequence identity, alternatively at least about 85% amino acid sequence identity, alternatively at least about 86% amino acid sequence identity, alternatively at least about 87% amino acid sequence identity, alternatively at least about 88% amino acid sequence identity, alternatively at least about 89% amino acid 25 sequence identity, alternatively at least about 90% amino acid sequence identity, alternatively at least about 91% amino acid sequence identity, alternatively at least about 92% amino acid sequence identity, alternatively at least about 93% amino acid sequence identity, alternatively at least about 94% amino acid sequence identity, alternatively at least about 95% amino acid sequence identity, alternatively at least about 96% amino acid sequence identity, alternatively at least about 97% amino acid sequence identity, 30 alternatively at least about 98% amino acid sequence identity and alternatively at least about 99% amino acid sequence identity to a full-length native sequence PRO polypeptide sequence as disclosed herein, a PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO polypeptide, with or without the signal peptide, as disclosed herein or any other specifically defined fragment of a full-length PRO polypeptide sequence as disclosed herein. Ordinarily, PRO variant 35 polypeptides are at least about 10 amino acids in length, alternatively at least about 20 amino acids in length, alternatively at least about 30 amino acids in length, alternatively at least about 40 amino acids in length, alternatively at least about 50 amino acids in length, alternatively at least about 60 amino acids in length, alternatively at least about 70 amino acids in length, alternatively at least about 80 amino acids in length, alternatively at least about 90 amino acids in length, alternatively at least about 100 amino acids in length.

alternatively at least about 150 amino acids in length, alternatively at least about 200 amino acids in length, alternatively at least about 300 amino acids in length, or more.

"Percent (%) amino acid sequence identity" with respect to the PRO polypeptide sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific PRO polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2, wherein the complete source code for the ALIGN-2 program is provided in Table 1 below. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc. and the source code shown in Table 1 below has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available through Genentech, Inc., South San Francisco, California or may be compiled from the source code provided in Table 1 below. The ALIGN-2 program should be compiled for use on a UNIX operating system, preferably digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. As examples of % amino acid sequence identity calculations using this method, Tables 2 and 3 demonstrate how to calculate the % amino acid sequence identity of the amino acid sequence designated "Comparison Protein" to the amino acid sequence designated "PRO", wherein "PRO" represents the amino acid sequence of a hypothetical PRO polypeptide of interest, "Comparison Protein" represents the amino acid sequence of a polypeptide against which the "PRO" polypeptide of interest is being compared, and "X", "Y" and "Z" each represent different hypothetical amino acid residues.

Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

40 However, % amino acid sequence identity values may also be obtained as described below by using the WU-

BLAST-2 computer program (Altschul et al., *Methods in Enzymology* 266:460-480 (1996)). Most of the WU-BLAST-2 search parameters are set to the default values. Those not set to default values, i.e., the adjustable parameters, are set with the following values: overlap span = 1, overlap fraction = 0.125, word threshold (I) = 11, and scoring matrix = BLOSUM62. When WU-BLAST-2 is employed, a % amino acid sequence identity value is determined by dividing (a) the number of matching identical amino acid residues between the amino acid sequence of the PRO polypeptide of interest having a sequence derived from the native PRO polypeptide and the comparison amino acid sequence of interest (i.e., the sequence against which the PRO polypeptide of interest is being compared which may be a PRO variant polypeptide) as determined by WU-BLAST-2 by (b) the total number of amino acid residues of the PRO polypeptide of interest. For example, in the statement "a polypeptide comprising an the amino acid sequence A which has or having at least 80% amino acid sequence identity to the amino acid sequence B", the amino acid sequence A is the comparison amino acid sequence of interest and the amino acid sequence B is the amino acid sequence of the PRO polypeptide of interest.

Percent amino acid sequence identity may also be determined using the sequence comparison program NCBI-BLAST2 (Altschul et al., *Nucleic Acids Res.* 25:3389-3402 (1997)). The NCBI-BLAST2 sequence comparison program may be downloaded from <http://www.ncbi.nlm.nih.gov> or otherwise obtained from the National Institute of Health, Bethesda, MD. NCBI-BLAST2 uses several search parameters, wherein all of those search parameters are set to default values including, for example, unmask = yes, strand = all, expected occurrences = 10, minimum low complexity length = 15/5, multi-pass e-value = 0.01, constant for multi-pass = 25, dropoff for final gapped alignment = 25 and scoring matrix = BLOSUM62.

In situations where NCBI-BLAST2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \text{ times the fraction } X/Y$$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program NCBI-BLAST2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A.

"PRO variant polynucleotide" or "PRO variant nucleic acid sequence" means a nucleic acid molecule which encodes an active PRO polypeptide as defined below and which has at least about 80% nucleic acid sequence identity with a nucleotide acid sequence encoding a full-length native sequence PRO polypeptide sequence as disclosed herein, a full-length native sequence PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO polypeptide, with or without the signal peptide, as disclosed herein or any other fragment of a full-length PRO polypeptide sequence as disclosed herein. Ordinarily, a PRO variant polynucleotide will have at least about 80% nucleic acid

sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity with a nucleic acid sequence encoding a full-length native sequence PRO polypeptide sequence as disclosed herein, a full-length native sequence PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO polypeptide, with or without the signal sequence, as disclosed herein or any other fragment of a full-length PRO polypeptide sequence as disclosed herein. Variants do not encompass the native nucleotide sequence.

Ordinarily, PRO variant polynucleotides are at least about 30 nucleotides in length, alternatively at least about 60 nucleotides in length, alternatively at least about 90 nucleotides in length, alternatively at least about 120 nucleotides in length, alternatively at least about 150 nucleotides in length, alternatively at least about 180 nucleotides in length, alternatively at least about 210 nucleotides in length, alternatively at least about 240 nucleotides in length, alternatively at least about 270 nucleotides in length, alternatively at least about 300 nucleotides in length, alternatively at least about 450 nucleotides in length, alternatively at least about 600 nucleotides in length, alternatively at least about 900 nucleotides in length, or more.

"Percent (%) nucleic acid sequence identity" with respect to PRO-encoding nucleic acid sequences identified herein is defined as the percentage of nucleotides in a candidate sequence that are identical with the nucleotides in the PRO nucleic acid sequence of interest, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. For purposes herein, however, % nucleic acid sequence identity values are generated using the sequence comparison computer program ALIGN-2, wherein the complete source code for the ALIGN-2 program is provided in Table 1 below. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc. and the source code shown in Table 1 below has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available through Genentech, Inc., South San Francisco, California or may be compiled from the source code provided in Table 1 below. The ALIGN-2 program should be compiled for use on a UNIX operating system, preferably digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for nucleic acid sequence comparisons, the % nucleic acid sequence identity of a given nucleic acid sequence C to, with, or against a given nucleic acid sequence

D (which can alternatively be phrased as a given nucleic acid sequence C that has or comprises a certain % nucleic acid sequence identity to, with, or against a given nucleic acid sequence D) is calculated as follows:

100 times the fraction W/Z

5

where W is the number of nucleotides scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of C and D, and where Z is the total number of nucleotides in D. It will be appreciated that where the length of nucleic acid sequence C is not equal to the length of nucleic acid sequence D, the % nucleic acid sequence identity of C to D will not equal the % nucleic acid sequence identity of D to C. As examples of % nucleic acid sequence identity calculations, Tables 4 and 5, demonstrate how to calculate the % nucleic acid sequence identity of the nucleic acid sequence designated "Comparison DNA" to the nucleic acid sequence designated "PRO-DNA", wherein "PRO-DNA" represents a hypothetical PRO-encoding nucleic acid sequence of interest, "Comparison DNA" represents the nucleotide sequence of a nucleic acid molecule against which the "PRO-DNA" nucleic acid molecule of interest is being compared, and "N", "L" and "V" each represent different hypothetical nucleotides.

Unless specifically stated otherwise, all % nucleic acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program. However, % nucleic acid sequence identity values may also be obtained as described below by using the WU-BLAST-2 computer program (Altschul et al., *Methods in Enzymology* 266:460-480 (1996)). Most of the WU-BLAST-2 search parameters are set to the default values. Those not set to default values, i.e., the adjustable parameters, are set with the following values: overlap span = 1, overlap fraction = 0.125, word threshold (T) = 11, and scoring matrix = BLOSUM62. When WU-BLAST-2 is employed, a % nucleic acid sequence identity value is determined by dividing (a) the number of matching identical nucleotides between the nucleic acid sequence of the PRO polypeptide-encoding nucleic acid molecule of interest having a sequence derived from the native sequence PRO polypeptide-encoding nucleic acid and the comparison nucleic acid molecule of interest (i.e., the sequence against which the PRO polypeptide-encoding nucleic acid molecule of interest is being compared which may be a variant PRO polynucleotide) as determined by WU-BLAST-2 by (b) the total number of nucleotides of the PRO polypeptide-encoding nucleic acid molecule of interest. For example, in the statement "an isolated nucleic acid molecule comprising a nucleic acid sequence A which has or having at least 80% nucleic acid sequence identity to the nucleic acid sequence B", the nucleic acid sequence A is the comparison nucleic acid molecule of interest and the nucleic acid sequence B is the nucleic acid sequence of the PRO polypeptide-encoding nucleic acid molecule of interest.

Percent nucleic acid sequence identity may also be determined using the sequence comparison program NCBI-BLAST2 (Altschul et al., *Nucleic Acids Res.* 25:3389-3402 (1997)). The NCBI-BLAST2 sequence comparison program may be downloaded from <http://www.ncbi.nlm.nih.gov> or otherwise obtained from the National Institute of Health, Bethesda, MD. NCBI-BLAST2 uses several search parameters, wherein all of those search parameters are set to default values including, for example, unmask = yes, strand = all, expected occurrences = 10, minimum low complexity length = 15/5, multi-pass e-value = 0.01, constant for multi-pass = 25, dropoff for final gapped alignment = 25 and scoring matrix = BLOSUM62.

In situations where NCBI-BLAST2 is employed for sequence comparisons, the % nucleic acid sequence identity of a given nucleic acid sequence C to, with, or against a given nucleic acid sequence D (which can alternatively be phrased as a given nucleic acid sequence C that has or comprises a certain % nucleic acid sequence identity to, with, or against a given nucleic acid sequence D) is calculated as follows:

5

100 times the fraction W/Z

where W is the number of nucleotides scored as identical matches by the sequence alignment program NCBI-BLAST2 in that program's alignment of C and D, and where Z is the total number of nucleotides in

10 D. It will be appreciated that where the length of nucleic acid sequence C is not equal to the length of nucleic acid sequence D, the % nucleic acid sequence identity of C to D will not equal the % nucleic acid sequence identity of D to C.

In other embodiments, PRO variant polynucleotides are nucleic acid molecules that encode an active PRO polypeptide and which are capable of hybridizing, preferably under stringent hybridization and 15 wash conditions, to nucleotide sequences encoding a full-length PRO polypeptide as disclosed herein. PRO variant polypeptides may be those that are encoded by a PRO variant polynucleotide.

"Isolated," when used to describe the various polypeptides disclosed herein, means polypeptide that has been identified and separated and/or recovered from a component of its natural environment.

Contaminant components of its natural environment are materials that would typically interfere with

20 diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated polypeptide includes polypeptide *in situ* within 25 recombinant cells, since at least one component of the PRO polypeptide natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by at least one purification step.

An "isolated" PRO polypeptide-encoding nucleic acid or other polypeptide-encoding nucleic acid is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the polypeptide-encoding nucleic acid. An

30 isolated polypeptide-encoding nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated polypeptide-encoding nucleic acid molecules therefore are distinguished from the specific polypeptide-encoding nucleic acid molecule as it exists in natural cells. However, an isolated polypeptide-encoding nucleic acid molecule includes polypeptide-encoding nucleic acid molecules contained in cells that ordinarily express the polypeptide where, for example, the nucleic acid molecule is in 35 a chromosomal location different from that of natural cells.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

10 The term "antibody" is used in the broadest sense and specifically covers, for example, single anti-PRO monoclonal antibodies (including agonist, antagonist, and neutralizing antibodies), anti-PRO antibody compositions with polyepitopic specificity, single chain anti-PRO antibodies, and fragments of anti-PRO antibodies (see below). The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the 15 population are identical except for possible naturally-occurring mutations that may be present in minor amounts.

"Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter 20 probes need lower temperatures. Hybridization generally depends on the ability of denatured DNA to reanneal when complementary strands are present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature which can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and 25 explanation of stringency of hybridization reactions, see Ausubel et al., Current Protocols in Molecular Biology, Wiley Interscience Publishers, (1995).

"Stringent conditions" or "high stringency conditions", as defined herein, may be identified by those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a 30 denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with 35 washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C.

"Moderately stringent conditions" may be identified as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, New York: Cold Spring Harbor Press, 1989, and include the use of washing 40 solution and hybridization conditions (e.g., temperature, ionic strength and %SDS) less stringent than those described above. An example of moderately stringent conditions is overnight incubation at 37°C in a

solution comprising: 20% formamide, 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5 x Denhardt's solution, 10% dextran sulfate, and 20 mg/ml denatured sheared salmon sperm DNA, followed by washing the filters in 1 x SSC at about 37-50°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

5 The term "epitope tagged" when used herein refers to a chimeric polypeptide comprising a PRO polypeptide fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with activity of the polypeptide to which it is fused. The tag polypeptide preferably also is fairly unique so that the antibody 10 does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 and 50 amino acid residues (preferably, between about 10 and 20 amino acid residues).

15 As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding specificity of a heterologous protein (an "adhesin") with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesins comprise a fusion of an amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site of an antibody (i.e., is "heterologous"), and an immunoglobulin constant domain sequence. The adhesin part of an immunoadhesin molecule typically is a contiguous amino acid sequence comprising at least the binding site of a receptor or a ligand. The immunoglobulin constant domain sequence in the immunoadhesin may be obtained from any 20 immunoglobulin, such as IgG-1, IgG-2, IgG-3, or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM.

25 "Active" or "activity" for the purposes herein refers to form(s) of a PRO polypeptide which retain a biological and/or an immunological activity of native or naturally-occurring PRO, wherein "biological" activity refers to a biological function (either inhibitory or stimulatory) caused by a native or naturally- occurring PRO other than the ability to induce the production of an antibody against an antigenic epitope possessed by a native or naturally-occurring PRO and an "immunological" activity refers to the ability to induce the production of an antibody against an antigenic epitope possessed by a native or naturally- occurring PRO.

30 The term "antagonist" is used in the broadest sense, and includes any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity of a native PRO polypeptide disclosed herein. In a similar manner, the term "agonist" is used in the broadest sense and includes any molecule that mimics a biological activity of a native PRO polypeptide disclosed herein. Suitable agonist or antagonist molecules specifically include agonist or antagonist antibodies or antibody fragments, fragments or amino acid sequence variants of native PRO polypeptides, peptides, antisense oligonucleotides, small organic 35 molecules, etc. Methods for identifying agonists or antagonists of a PRO polypeptide may comprise contacting a PRO polypeptide with a candidate agonist or antagonist molecule and measuring a detectable change in one or more biological activities normally associated with the PRO polypeptide.

36 "Treatment" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder. Those

in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented.

"Chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to an acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time.

5 "Intermittent" administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, cats, cattle, horses, sheep, pigs, goats, rabbits, etc. Preferably, the mammal is human.

10 Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

"Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of 15 physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating 20 agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

"Antibody fragments" comprise a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies (Zapata et al., *Protein Eng.* 8(10): 1057-1062 [1995]); single-chain 25 antibody molecules; and multispecific antibodies formed from antibody fragments.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. Pepsin treatment yields an F(ab')₂ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

30 "Fv" is the minimum antibody fragment which contains a complete antigen-recognition and - binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising 35 only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab fragments differ from Fab' fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge 40 region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains

bear a free thiol group. F(ab')₂ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their 5 constant domains.

Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2.

10 "Single-chain Fv" or "sFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the sFv to form the desired structure for antigen binding. For a review of sFv, see Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

15 The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) in the same polypeptide chain (V_H-V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 20 93/11161; and Hollinger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993).

20 An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody 25 will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the 30 antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

An antibody that "specifically binds to" or is "specific for" a particular polypeptide or an epitope on a particular polypeptide is one that binds to that particular polypeptide or epitope on a particular polypeptide without substantially binding to any other polypeptide or polypeptide epitope.

35 The word "label" when used herein refers to a detectable compound or composition which is conjugated directly or indirectly to the antibody so as to generate a "labeled" antibody. The label may be detectable by itself (e.g. radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

40 By "solid phase" is meant a non-aqueous matrix to which the antibody of the present invention can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass

(e.g., controlled porc glass), polysaccharides (e.g., agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in others it is a purification column (e.g., an affinity chromatography column). This term also includes a discontinuous solid phase of discrete particles, such as those described in U.S. Patent No.

5 4,275,149.

A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (such as a PRO polypeptide or antibody thereto) to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

10 A "small molecule" is defined herein to have a molecular weight below about 500 Daltons.

The term "immune related disease" means a disease in which a component of the immune system of a mammal causes, mediates or otherwise contributes to a morbidity in the mammal. Also included are diseases in which stimulation or intervention of the immune response has an ameliorative effect on progression of the disease. Included within this term are immune-mediated inflammatory diseases, non-immune-mediated inflammatory diseases, infectious diseases, immunodeficiency diseases, neoplasia, etc.

The term "Natural Killer cell mediated disease" means a disease in which NK cells directly or indirectly mediate or otherwise contribute to a morbidity in a mammal. The NK cell mediated disease may be associated with cell mediated effects, lymphokine mediated effects, etc., and even effects associated with other immune cells if the cells are involved.

20 Examples of immune-related and inflammatory diseases, some of which are immune mediated, which can be treated according to the invention include systemic lupus erythematosus, rheumatoid arthritis, juvenile chronic arthritis, spondyloarthropathies, systemic sclerosis (scleroderma), idiopathic inflammatory myopathies (dermatomyositis, polymyositis), Sjögren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia (immune pancytopenia, paroxysmal nocturnal hemoglobinuria), autoimmune

25 thrombocytopenia (idiopathic thrombocytopenic purpura, immune-mediated thrombocytopenia), thyroiditis (Grave's disease, Hashimoto's thyroiditis, juvenile lymphocytic thyroiditis, atrophic thyroiditis), diabetes mellitus, immune-mediated renal disease (glomerulonephritis, tubulointerstitial nephritis), demyelinating diseases of the central and peripheral nervous systems such as multiple sclerosis, idiopathic demyelinating polyneuropathy or Guillain-Barré syndrome, and chronic inflammatory demyelinating polyneuropathy,

30 hepatobiliary diseases such as infectious hepatitis (hepatitis A, B, C, D, E and other non-hepatotropic viruses), autoimmune chronic active hepatitis, primary biliary cirrhosis, granulomatous hepatitis, and sclerosing cholangitis, inflammatory bowel disease (ulcerative colitis: Crohn's disease), gluten-sensitive enteropathy, and Whipple's disease, autoimmune or immune-mediated skin diseases including bullous skin diseases, erythema multiforme and contact dermatitis, psoriasis, allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity and urticaria, immunologic diseases of the lung such as eosinophilic pneumonias, idiopathic pulmonary fibrosis and hypersensitivity pneumonitis, transplantation associated diseases including graft rejection and graft -versus-host-disease. Infectious diseases including viral diseases such as AIDS (HIV infection), hepatitis A, B, C, D, and E, herpes, etc., bacterial infections, fungal infections, protozoal infections and parasitic infections.

35

The term "effective amount" is a concentration or amount of a PRO polypeptide and/or agonist/antagonist which results in achieving a particular stated purpose. An "effective amount" of a PRO polypeptide or agonist or antagonist thereof may be determined empirically. Furthermore, a "therapeutically effective amount" is a concentration or amount of a PRO polypeptide and/or agonist/antagonist which is effective for achieving a stated therapeutic effect. This amount may also be determined empirically.

5 The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g., I¹³¹, I¹²⁵, Y⁹⁰ and Re¹⁸⁶), chemotherapeutic agents, and toxins such as enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof.

10 A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include adriamycin, doxorubicin, epirubicin, 5-fluorouracil, cytosine arabinoside ("Ara-C"), cyclophosphamide, thioguanine, busulfan, cytoxin, taxoids, e.g., paclitaxel (Taxol, Bristol-Myers Squibb Oncology, Princeton, NJ), and docetaxel (Taxotere, Rhône-Poulenc Rorer, Antony, France), taxotere, methotrexate, cisplatin, melphalan, vinblastine, bleomycin, etoposide, ifosfamide, mitomycin C, 15 mitoxantrone, vincristine, vinorelbine, carboplatin, teniposide, daunomycin, carminomycin, aminopterin, dactinomycin, mitomycins, esperamicins (see U.S. Pat. No. 4,675,187), melphalan and other related nitrogen mustards. Also included in this definition are hormonal agents that act to regulate or inhibit hormone action on tumors such as tamoxifen and onapristone.

20 A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell, especially cancer cell overexpressing any of the genes identified herein, either *in vitro* or *in vivo*. Thus, the growth inhibitory agent is one which significantly reduces the percentage of cells overexpressing such genes in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxol, and topo II inhibitors such 25 as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in *The Molecular Basis of Cancer*, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogens, and antineoplastic drugs" by Murakami *et al.* (WB Saunders: Philadelphia, 1995), especially p. 30 13.

30 The term "cytokine" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; 35 thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor- α and - β ; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF- β ; platelet-growth factor; transforming 40 growth factors (TGFs) such as TGF- α and TGF- β ; insulin-like growth factor-I and -II; erythropoietin (EPO);

osteoinductive factors; interferons such as interferon- α , - β , and - γ ; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1 α , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12, IL-15 and tumor necrosis factor such as TNF- α or TNF- β ; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding specificity of a heterologous protein (an "adhesin") with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesins comprise a fusion of an amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site of an antibody (*i.e.*, is "heterologous"), and an immunoglobulin constant domain sequence. The adhesin part of an immunoadhesin molecule typically is a contiguous amino acid sequence comprising at least the binding site of a receptor or a ligand. The immunoglobulin constant domain sequence in the immunoadhesin may be obtained from any immunoglobulin, such as IgG-1, IgG-2, IgG-3, or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM.

Table 1

Table 1 (cont')

```

/*
 */
#include <stdio.h>
#include <ctype.h>

5  #define MAXJMP 16      /* max jumps in a diag */
#define MAXGAP 24      /* don't continue to penalize gaps larger than this */
#define J MPS 1024     /* max jmps in a path */
#define MX 4          /* save if there's at least MX-1 bases since last jmp */

10 #define DMAT 3        /* value of matching bases */
#define DMIS 0        /* penalty for mismatched bases */
#define DINSO 8        /* penalty for a gap */
#define DINSI 1        /* penalty per base */
#define PINS0 8        /* penalty for a gap */
#define PINS1 4        /* penalty per residuc */

15 struct jmp {
    short n[MAXJMP];      /* size of jmp (neg for delay) */
    unsigned short x[MAXJMP]; /* base no. of jmp in seq x */
    /* limits seq to 2^16-1 */
};

20 struct diag {
    int score;      /* score at last jmp */
    long offset;    /* offset of prev block */
    short ijmp;     /* current jmp index */
    struct jmp *jp; /* list of jmps */
};

25 struct path {
    int spc;        /* number of leading spaces */
    short n[J MPS]; /* size of jmp (gap) */
    int x[J MPS];   /* loc of jmp (last elem before gap) */
};

30 };

35 char *ofile;      /* output file name */
char *namex[2];    /* seq names: getseqs() */
char *prog;        /* prog name for err msgs */
40 char *seqx[2];   /* seqs: getseqs() */
int dmax;          /* best diag: nw() */
int dmax0;         /* final diag */
int dna;           /* set if dna: main() */
int endgaps;       /* set if penalizing end gaps */
45 int gapx, gapy;  /* total gaps in seqs */
int len0, len1;    /* seq lens */
int ngapx, ngapy; /* total size of gaps */
int smax;          /* max score: nw() */
int *xbm;          /* bitmap for matching */
50 long offset;     /* current offset in jmp file */
struct diag *dx;   /* holds diagonals */
struct path *ppl[2]; /* holds path for seqs */

55 char *calloc(), *malloc(), *index(), *strcpy();
char *getseq(), *g_calloc();

```

Table 1 (cont')

```

/* Needleman-Wunsch alignment program
 *
5   * usage: progs file1 file2
 *   * where file1 and file2 are two dna or two protein sequences.
 *   * The sequences can be in upper- or lower-case and may contain ambiguity
 *   * Any lines beginning with ';' or '<' are ignored
 *   * Max file length is 65535 (limited by unsigned short x in the jmp struct)
 *   * A sequence with 1/3 or more of its elements ACGTU is assumed to be DNA
10  *   * Output is in the file "align.out"
 *
 * The program may create a tmp file in /tmp to hold info about traceback.
 * Original version developed under BSD 4.3 on a vax 8650
 */
15  #include "nw.h"
#include "day.h"

static _dbval[26] = {
20  1,14,2,13,0,0,4,11,0,0,12,0,3,15,0,0,0,5,6,8,8,7,9,0,10,0
};

static _pbval[26] = {
25  1,2(1<<('D'-'A'))(1<<('N'-'A')), 4, 8, 16, 32, 64,
128, 256, 0xFFFFFFFF, 1<<10, 1<<11, 1<<12, 1<<13, 1<<14,
1<<15, 1<<16, 1<<17, 1<<18, 1<<19, 1<<20, 1<<21, 1<<22,
1<<23, 1<<24, 1<<25|(1<<('E'-'A'))(1<<('Q'-'A'))
};

main(ac, av)
30  main
    int ac;
    char *av[ ];
{
    prog = av[0];
35  if (ac != 3) {
        fprintf(stderr, "usage: %s file1 file2\n", prog);
        fprintf(stderr, "where file1 and file2 are two dna or two protein sequences.\n");
        fprintf(stderr, "The sequences can be in upper- or lower-case\n");
        fprintf(stderr, "Any lines beginning with ';' or '<' are ignored\n");
40  fprintf(stderr, "Output is in the file \"align.out\"\n");
        exit(1);
    }
    namex[0] = av[1];
    namex[1] = av[2];
45  seqx[0] = getseq(namex[0], &len0);
    seqx[1] = getseq(namex[1], &len1);
    xbm = (dma)? _dbval : _pbval;

    endgaps = 0; /* 1 to penalize endgaps */
50  ofile = "align.out"; /* output file */

    nw0; /* fill in the matrix, get the possible jmps */
    readjmps(); /* get the actual jmps */
    print(); /* print stats, alignment */
55  cleanup(0); /* unlink any tmp files */
}

```

Table 1 (cont')

```

/* do the alignment, return best score: main()
 * dmat: values in Fitch and Smith, PNAS, 80, 1382-1386, 1983
 * pro: PAM 250 values
5  * When scores are equal, we prefer mismatches to any gap, prefer
 * a new gap to extending an ongoing gap, and prefer a gap in seqx
 * to a gap in seq y.
 */
nw()

10  nw
{
    char      *px, *py;      /* seqs and ptrs */
    int       *ndely, *dely;  /* keep track of dely */
    int       ndelx, delx;   /* keep track of delx */
15    int       *tmp;        /* for swapping row0, row1 */
    int       mis;          /* score for each type */
    int       ins0, ins1;   /* insertion penalties */
    register  id;          /* diagonal index */
    register  ij;          /* jmp index */
20    register  *col0, *col1; /* score for curr, last row */
    register  xx, yy;      /* index into seqs */

    dx = (struct diag *)g_malloc("to get diag", len0+len1+1, sizeof(struct diag));

25    ndely = (int *)g_malloc("to get ndely", len1+1, sizeof(int));
    dely = (int *)g_malloc("to get dely", len1+1, sizeof(int));
    col0 = (int *)g_malloc("to get col0", len1+1, sizeof(int));
    col1 = (int *)g_malloc("to get col1", len1+1, sizeof(int));
    ins0 = (dma)? DINS0 : PINS0;
    ins1 = (dma)? DINS1 : PINS1;

30    smax = -10000;
    if (endgaps) {
        for (col0[0] = dely[0] = -ins0, yy = 1; yy <= len1; yy++) {
            col0[yy] = dely[yy] = col0[yy-1] - ins1;
            ndely[yy] = yy;
        }
        col0[0] = 0;          /* Waterman Bull Math Biol 84 */
    }
40    else {
        for (yy = 1; yy <= len1; yy++) {
            dely[yy] = -ins0;
        }
    }

45    /* fill in match matrix
     */
    for (px = seqx[0], xx = 1; xx <= len0; px++, xx++) {
        /* initialize first entry in col
         */
        if (endgaps) {
            if (xx == 1)
                col1[0] = delx = -(ins0+ins1);
            else
                col1[0] = delx = col0[0] - ins1;
            ndelx = xx;
        }
55        }
        else {
            col1[0] = 0;
            delx = -ins0;
            ndelx = 0;
    }
60
}

```

Table 1 (cont')

```

...nw
5      for (py = seqx[1], yy = 1; yy <= len1; py++, yy++) {
          mis = col0[yy-1];
          if (dna)
              mis += (xbm[*px-'A']&xbm[*py-'A'])? DMAT : DMIS;
          else
              mis += _day[*px-'A'][*py-'A'];
10     /* update penalty for del in x seq;
          * favor new del over ongoing del
          * ignore MAXGAP if weighting endgaps
          */
15     if (endgaps || ndely[yy] < MAXGAP) {
          if (col0[yy] - ins0 >= dely[yy]) {
              dely[yy] = col0[yy] - (ins0+ins1);
              ndely[yy] = 1;
          } else {
              dely[yy] -= ins1;
              ndely[yy]++;
          }
      } else {
          if (col0[yy] - (ins0+ins1) >= dely[yy]) {
              dely[yy] = col0[yy] - (ins0+ins1);
              ndely[yy] = 1;
          } else
              ndely[yy]++;
      }
20
25
30     /* update penalty for del in y seq;
          * favor new del over ongoing del
          */
35     if (endgaps || ndelx < MAXGAP) {
          if (col1[yy-1] - ins0 >= delx) {
              delx = col1[yy-1] - (ins0+ins1);
              ndelx = 1;
          } else {
              delx -= ins1;
              ndelx++;
          }
      } else {
          if (col1[yy-1] - (ins0+ins1) >= delx) {
              delx = col1[yy-1] - (ins0+ins1);
              ndelx = 1;
          } else
              ndelx++;
      }
40
45
50     /* pick the maximum score; we're favoring
          * mis over any del and delx over dely
          */
55
60

```

Table 1 (cont')

...nw

```

5      id = xx - yy + len1 - 1;
      if (mis >= delx && mis >= dely[yy])
          col1[yy] = mis;
      else if (delx >= dely[yy]) {
          col1[yy] = delx;
          ij = dx[id].jmp;
          if (dx[id].jp.n[0] && (!dna || (ndelx >= MAXJMP
10      && xx > dx[id].jp.x[ij]+MX) || mis > dx[id].score+DINS0)) {
              dx[id].jmp+=;
              if (++ij >= MAXJMP) {
                  writejmps(id);
                  ij = dx[id].jmp = 0;
                  dx[id].offset = offset;
                  offset += sizeof(struct jmp) + sizeof(offset);
              }
          }
          dx[id].jp.n[ij] = ndelx;
          dx[id].jp.x[ij] = xx;
          dx[id].score = delx;
      }
      else {
          col1[yy] = dely[yy];
          ij = dx[id].jmp;
          if (dx[id].jp.n[0] && (!dna || (ndely[yy] >= MAXJMP
20      && xx > dx[id].jp.x[ij]+MX) || mis > dx[id].score+DINS0)) {
              dx[id].jmp+=;
              if (++ij >= MAXJMP) {
                  writejmps(id);
                  ij = dx[id].jmp = 0;
                  dx[id].offset = offset;
                  offset += sizeof(struct jmp) + sizeof(offset);
              }
          }
          dx[id].jp.n[ij] = -ndely[yy];
          dx[id].jp.x[ij] = xx;
          dx[id].score = dely[yy];
      }
      if (xx == len0 && yy < len1) {
          /* last col
          */
          if (endgaps)
              col1[yy] -= ins0+ins1*(len1-yy);
          if (col1[yy] > smax) {
              smax = col1[yy];
              dmax = id;
          }
      }
      if (endgaps && xx < len0)
          col1[yy-1] -= ins0+ins1*(len0-xx);
      if (col1[yy-1] > smax) {
          smax = col1[yy-1];
          dmax = id;
      }
      tmp = col0; col0 = col1; col1 = tmp;
  }
  (void) free((char *)ndely);
  (void) free((char *)dely);
  (void) free((char *)col0);
  (void) free((char *)col1);
}

```

Table 1 (cont')

```

/*
 *
 * print() -- only routine visible outside this module
 *
 * static:
 * getmat() -- trace back best path, count matches: print()
 * pr_align() -- print alignment of described in array p[ ]; print()
 * dumpblock() -- dump a block of lines with numbers, stars: pr_align()
 * nums() -- put out a number line: dumpblock()
 * putline() -- put out a line (name, [num], seq, [num]): dumpblock()
 * stars() -- put a line of stars: dumpblock()
 * stripname() -- strip any path and prefix from a seqname
 */
15  #include "nw.h"

#define SPC      3
#define P_LINE   256      /* maximum output line */
20  #define P_SPC   3      /* space between name or num and seq */

extern _day[26][26];
int   olen;           /* set output line length */
FILE  *fx;            /* output file */

25  print()
{
    print
    {
        int   lx, ly, firstgap, lastgap; /* overlap */

30  if ((fx = fopen(ofile, "w")) == 0) {
            fprintf(stderr, "%s: can't write %s\n", prog, ofile);
            cleanup();
        }
35  fprintf(fx, "<first sequence: %s (length = %d)\n", namex[0], len0);
    fprintf(fx, "<second sequence: %s (length = %d)\n", namex[1], len1);
    olen = 60;
    lx = len0;
    ly = len1;
40  firstgap = lastgap = 0; /* leading gap in x */
    if (dmax < len1 - 1) {
        pp[0].spc = firstgap = len1 - dmax - 1;
        ly -= pp[0].spc;
    }
45  else if (dmax > len1 - 1) { /* leading gap in y */
        pp[1].spc = firstgap = dmax - (len1 - 1);
        lx -= pp[1].spc;
    }
50  if (dmax0 < len0 - 1) { /* trailing gap in x */
        lastgap = len0 - dmax0 - 1;
        lx -= lastgap;
    }
55  else if (dmax0 > len0 - 1) { /* trailing gap in y */
        lastgap = dmax0 - (len0 - 1);
        ly -= lastgap;
    }
    getmat(lx, ly, firstgap, lastgap);
    pr_align();
}
60

```

Table 1 (cont?)

```

/*
 * trace back the best path, count matches
 */
5  static
  getmat(lx, ly, firstgap, lastgap)                                getmat
  {
    int      lx, ly;                                              /* "core" (minus endgaps) */
    int      firstgap, lastgap;                                     /* leading/trailing overlap */
    {
10   int      nm, i0, i1, siz0, siz1;
    char     outx[32];
    double   pct;
    register int      n0, n1;
    register char    *p0, *p1;
15   /* get total matches, score
    */
    i0 = i1 = siz0 = siz1 = 0;
    p0 = seqx[0] + pp[1].spc;
    p1 = seqx[1] + pp[0].spc;
20   n0 = pp[1].spc + 1;
    n1 = pp[0].spc + 1;

    nm = 0;
25   while (*p0 && *p1) {
      if (siz0) {
        p1++;
        n1++;
        siz0--;
30   }
      else if (siz1) {
        p0++;
        n0++;
        siz1--;
35   }
      else {
        if (xbm[*p0-'A']&xbm[*p1-'A'])
          nm++;
        if (n0++ == pp[0].x[i0])
          siz0 = pp[0].n[i0++];
        if (n1++ == pp[1].x[i1])
          siz1 = pp[1].n[i1++];
        p0++;
        p1++;
45   }
    }

    /* pct homology:
     * if penalizing endgaps, base is the shorter seq
     * else, knock off overhangs and take shorter core
     */
50   if (endgaps)
      lx = (len0 < len1)? len0 : len1;
    else
      lx = (lx < ly)? lx : ly;
    pct = 100.*(double)nm/(double)lx;
    fprintf(fx, "%n");
    fprintf(fx, "<%d match%s in an overlap of %d: %.2f percent similarity\n",
60   nm, (nm == 1)? "" : "es", lx, pct);
  }

```

Table 1 (cont?)

```

5      sprintf(fx, "<gaps in first sequence: %d", gapx);           ...getmat
if (gapx) {
  (void) sprintf(outx, " (%d %s%$s)",                               ...
    ngapx, (dna)? "base": "residue", (ngapx == 1)? "" : "s");
  sprintf(fx, "%s", outx);

10     sprintf(fx, ", gaps in second sequence: %d", gapy);
if (gapy) {
  (void) sprintf(outx, " (%d %s%$s)",                               ...
    ngapy, (dna)? "base": "residue", (ngapy == 1)? "" : "s");
  sprintf(fx, "%s", outx);
}
15     if (dna)
      sprintf(fx,
        "\n<score: %d (match = %d, mismatch = %d, gap penalty = %d + %d per base)\n",
        smax, DMAT, DMIS, DINSO, DINSI);
    else
      sprintf(fx,
        "\n<score: %d (Dayhoff PAM 250 matrix, gap penalty = %d + %d per residue)\n",
        smax, PINS0, PINS1);
if (endgaps)
  sprintf(fx,
25      "<endgaps penalized, left endgap: %d %s%, right endgap: %d %s%$u",
    firstgap, (dna)? "base" : "residue", (firstgap == 1)? "" : "s",
    lastgap, (dna)? "base" : "residue", (lastgap == 1)? "" : "s");
else
  sprintf(fx, "<endgaps not penalized\n");
30   }
static   nn;           /* matches in core -- for checking */
static   lmax;          /* lengths of stripped file names */
static   ij[2];          /* jmp index for a path */
static   nc[2];          /* number at start of current line */
35   static   ni[2];          /* current elem number -- for gapping */
static   siz[2];          /* */
static   *ps[2];          /* ptr to current element */
static   *po[2];          /* ptr to next output char slot */
static   out[2][P_LINE];  /* output line */
40   static   star[P_LINE]; /* set by stars() */

/*
 * print alignment of described in struct path pp[ ]
 */
45   static
pr_align()
{
  int      nn;           /* char count */
  int      more;
50   register i;
  for (i = 0, lmax = 0; i < 2; i++) {
    nn = stripname(namex[i]);
    if (nn > lmax)
      lmax = nn;
55   }
    nc[i] = 1;
    ni[i] = 1;
    siz[i] = ij[i] = 0;
    ps[i] = seqx[i];
    po[i] = out[i];
}
60

```

Table 1 (cont')

```

for (nn = nm = 0, more = 1; more; ) {
    for (i = more = 0; i < 2; i++) {
        /*
         * do we have more of this sequence?
         */
        if (*ps[i])
            continue;
    10   more++;
    15   if (pp[i].spc) { /* leading space */
        *po[i]++ = ' ';
        pp[i].spc--;
    }
    20   else if (siz[i]) { /* in a gap */
        *po[i]++ = ' ';
        siz[i]--;
    }
    25   else { /* we're putting a seq element
        */
        *po[i] = *ps[i];
        if (islower(*ps[i]))
            *ps[i] = toupper(*ps[i]);
        po[i]++;
        ps[i]++;
    }
    30   /*
     * are we at next gap for this seq?
     */
    35   if (ni[i] == pp[i].xij[i]) {
        /*
         * we need to merge all gaps
         * at this location
         */
        siz[i] = pp[i].nij[i]++;
        while (ni[i] == pp[i].xij[i])
            siz[i] += pp[i].nij[i]++;
    }
    40   ni[i]++;
    45   if (++nn == olen || !more && nn) {
        dumpblock();
        for (i = 0; i < 2; i++)
            po[i] = out[i];
        nn = 0;
    }
    50   }
    55   /*
     * dump a block of lines, including numbers, stars: pr_align()
     */
    static dumpblock()
    {
        register i;
        for (i = 0; i < 2; i++)
            *po[i] -= '0';
    60   }

```

Table 1 (cont')

```

5      (void)putc('\n', fx);
for (i = 0; i < 2; i++) {
    if (*out[i] && (*out[i] != ' ' || *(po[i]) != ' ')) {
        if (i == 0)
            nums(i);
        if (i == 0 && *out[1])
            stars();
        putline(i);
        if (i == 0 && *out[1])
            fprintf(fx, star);
        if (i == 1)
            nums(i);
    }
}
20  /*
 * put out a number line: dumpblock()
 */
static
nums(ix)
25  int      ix;      /* index in out[ ] holding seq line */
{
    char      nline[P_LINE];
    register  i, j;
    register char  *pn, *px, *py;
30
    for (pn = nline, i = 0; i < lmax+P_SPC; i++, pn++)
        *pn = ' ';
    for (j = nc[ix], py = out[ix]; *py; py++, pn++) {
        if (*py == ' ' || *py == '-')
            *pn = ' ';
        else {
            if (i%10 == 0 || (i == 1 && nc[ix] != 1)) {
                j = (i < 0)? -i : i;
                for (px = pn; j; j /= 10, px--)
                    *px = j%10 + '0';
                if (i < 0)
                    *px = ' ';
            }
            else
                *pn = ' ';
        }
        i++;
    }
    *pn = '\0';
50
    nc[ix] = i;
    for (pn = nline; *pn; pn++)
        (void)putc(*pn, fx);
    (void)putc('\n', fx);
}
55  /*
 * put out a line (name, [num], seq, [num]): dumpblock()
 */
static
putline(ix)
60  int      ix;
{

```

Table 1 (cont')

```

5           int          i;
register char      *px;
...putline

for (px = namex[ix], i = 0; *px && *px != ':'; px++, i++)
    (void) putc(*px, fx);
for (; i < lmax+P_SPC; i++)
    (void) putc(' ', fx);

10          /* these count from 1:
           * n[i] is current element (from 1)
           * nc[i] is number at start of current line
           */
15          for (px = out[ix]; *px; px++)
    (void) putc(*px&0x7F, fx);
    (void) putc('\n', fx);
}
20          /*

           * put a line of stars (seqs always in out[0], out[1]): dumpblock()
           */
25          static
stars()
stars
{
30          int          i;
register char      *p0, *p1, cx, *px;

if (!*out[0] || (*out[0] == ' ' && *(po[0]) == ')' ||
    !*out[1] || (*out[1] == ' ' && *(po[1]) == ')'))
    return;
35          px = star;
for (i = lmax+P_SPC; i; i--)
    *px++ = ' ';

for (p0 = out[0], p1 = out[1]; *p0 && *p1; p0++, p1++) {
40          if (isalpha(*p0) && isalpha(*p1)) {
                if (xbm[*p0-'A']&xbm[*p1-'A']) {
                    cx = '*';
                    nm++;
}
45          else if (ldna && _day[*p0-'A'][*p1-'A'] > 0)
                    cx = ':';
                else
                    cx = ',';
}
50          }
else
    cx = ':';
*p0++ = cx;
}
55          *px++ = '\n';
*px = '0';
}
}
60

```

Table 1 (cont')

```

/*
 * strip path or prefix from pn, return len: pr_align()
 */
5  static
  stripname(pn)
  {
10    register char *px, *py;
    py = 0;
    for (px = pn; *px; px++)
      if (*px == '\\')
15      py = px + 1;
    if (py)
      (void) strcpy(pn, py);
    return(strlen(pn));
20  }

```

25

30

35

40

45

50

55

60

52

Table 1 (cont')

```

/*
 * cleanup() -- cleanup any tmp file
 * getseq() -- read in seq, set dna, len, maxlen
 * g_malloc() -- malloc() with error checkin
 * readjmps() -- get the good jmps, from tmp file if necessary
 * writejmps() -- write a filled array of jmps to a tmp file: nw()
 */
5   #include "nw.h"
10  #include <sys/file.h>

15  char  *jname = "/tmp/hongXXXXXX";           /* tmp file for jmps */
FILE  *fj;

20  int   cleanup();                         /* cleanup tmp file */
long  lseek();                           cleanup

25  /*
 * remove any tmp file if we blow
 */
30  cleanup(i)
    int   i;
{
    if (i)
        (void) unlink(jname);
    exit(i);
}

35  /*
 * read, return ptr to seq, set dna, len, maxlen
 * skip lines starting with ',', '<', or '>'
 * seq in upper or lower case
 */
40  char  *
getseq(file, len)
    char  *file;    /* file name */
    int   *len;     /* seq len */
{
    char  line[1024], *pseq;
    register char  *px, *py;
    int   natgc, tlen;
    FILE  *fp;

45  if ((fp = fopen(file, "r")) == 0) {
        fprintf(stderr, "%s: can't read %s\n", prog, file);
        exit(1);
    }
    tlen = natgc = 0;
50  while (fgets(line, 1024, fp)) {
        if (*line == ';' || *line == '<' || *line == '>')
            continue;
        for (px = line; *px != '\n'; px++)
            if (isupper(*px) || islower(*px))
                tlen++;
    }
55  if ((pseq = malloc((unsigned)(tlen+6))) == 0) {
        fprintf(stderr, "%s: malloc() failed to get %d bytes for %s\n", prog, tlen+6, file);
        exit(1);
    }
60  pseq[0] = pseq[1] = pseq[2] = pseq[3] = '\0';

```

Table 1 (cont')

```

5           py = pseq + 4;
*len = tlen;
rewind(&fp);
10          while (fgets(line, 1024, fp)) {
if (*line == ';' || *line == '<' || *line == '>')
    continue;
for (px = line; *px != '\n'; px++) {
    if (isupper(*px))
        *py++ = *px;
    else if (islower(*px))
        *py++ = toupper(*px);
    if (index("ATGCCU", *(py-1)))
        natgc++;
}
*py++ = '\0';
*py = '\0';
(void) fclose(fp);
dna = natgc > (tlen/3);
return(pseq+4);
}
25          char * g_calloc(msg, nx, sz)
{
    char *msg;           /* program, calling routine */
    int  nx, sz;          /* number and size of elements */
30          {
    char *px, *calloc();
    if ((px = calloc((unsigned)nx, (unsigned)sz)) == 0) {
        if (*msg) {
35            fprintf(stderr, "%s: g_calloc() failed (n=%d, sz=%d)\n", prog, msg, nx, sz);
            exit(1);
        }
    }
    return(px);
}
40          /* * get final jmps from dx[] or tmp file, set pp[], reset dmax: main()
 */
45          readjmps()
readjmps()
{
    int fd = -1;
    int siz, i0, i1;
50          register i, j, xx;
    if (i0) {
        (void) fclose(&f);
        if ((fd = open(jname, O_RDONLY, 0)) < 0) {
55            fprintf(stderr, "%s: can't open() %s\n", prog, jname);
            cleanup(1);
        }
    }
    for (i = i0 = i1 = 0, dmax0 = dmax, xx = len0; ; i++) {
        while (1) {
60            for (j = dx[dmax].jmp; j >= 0 && dx[dmax].jp.x[j] >= xx; j--)
                ;
    }
}

```

Table 1 (cont')**...readjmps**

```

if (j < 0 && dx[dmax].offset && fj) {
    (void) lseek(fd, dx[dmax].offset, 0);
    (void) read(fd, (char *)&dx[dmax].jp, sizeof(struct jmp));
    (void) read(fd, (char *)&dx[dmax].offset, sizeof(dx[dmax].offset));
    dx[dmax].jmp = MAXIMP-1;
}
else
    break;
}
if (i >= JMPS) {
    fprintf(stderr, "%s: too many gaps in alignment\n", prog);
    cleanup(1);
}
if (j >= 0) {
    siz = dx[dmax].jp.n[j];
    xx = dx[dmax].jp.x[j];
    dmax += siz;
}
if (siz < 0) {
    /* gap in second seq */
    pp[1].n[i1] = -siz;
    xx += siz;
    /* id = xx - yy + len1 - 1
     */
    pp[1].x[i1] = xx - dmax + len1 - 1;
    gapy++;
    ngapx -= siz;
}
/* ignore MAXGAP when doing endgaps */
siz = (siz < MAXGAP || endgaps)? -siz : MAXGAP;
i1++;
}
else if (siz > 0) {
    /* gap in first seq */
    pp[0].n[i0] = siz;
    pp[0].x[i0] = xx;
    gapx++;
    ngapx += siz;
}
/* ignore MAXGAP when doing endgaps */
siz = (siz < MAXGAP || endgaps)? siz : MAXGAP;
i0++;
}
}
else
    break;
}
/* reverse the order of jmps
 */
for (j = 0, i0--; j < i0; j++, i0--) {
    i = pp[0].n[j]; pp[0].n[j] = pp[0].n[i0]; pp[0].n[i0] = i;
    i = pp[0].x[j]; pp[0].x[j] = pp[0].x[i0]; pp[0].x[i0] = i;
}
for (j = 0, i1--; j < i1; j++, i1--) {
    i = pp[1].n[j]; pp[1].n[j] = pp[1].n[i1]; pp[1].n[i1] = i;
    i = pp[1].x[j]; pp[1].x[j] = pp[1].x[i1]; pp[1].x[i1] = i;
}
if (fd >= 0)
    (void) close(fd);
if (fj) {
    (void) unlink(jname);
    fj = 0;
    offset = 0;
}
}

```

Table 1 (cont')

```

5      /*
6       * write a filled jmp struct offset of the prev one (if any): nw0
7       */
8      writejmps(ix)
9      {
10         writejmps
11         int     ix;
12         {
13             char   *mktemp();
14
15             if (fj) {
16                 if (mktemp(jname) < 0) {
17                     fprintf(stderr, "%s: can't mktemp() %s\n", prog, jname);
18                     cleanup(1);
19                 }
20                 if ((fj = fopen(jname, "w")) == 0) {
21                     fprintf(stderr, "%s: can't write %s\n", prog, jname);
22                     exit(1);
23                 }
24             }
25             (void) fwrite((char *)&dx[ix].jp, sizeof(struct jmp), 1, fj);
26             (void) fwrite((char *)&dx[ix].offset, sizeof(dx[ix].offset), 1, fj);
27         }
28     }

```

Table 2

5 PRO XXXXXXXXXXXXXXXXXX (Length = 15 amino acids)
 Comparison Protein XXXXXYYYYYYYY (Length = 12 amino acids)
 % amino acid sequence identity =
 (the number of identically matching amino acid residues between the two polypeptide sequences as
 10 determined by ALIGN-2) divided by (the total number of amino acid residues of the PRO polypeptide) =
 5 divided by 15 = 33.3%

Table 3

15 PRO XXXXXXXXXXXX (Length = 10 amino acids)
 Comparison Protein XXXXXYYYYYYZZZ (Length = 15 amino acids)
 % amino acid sequence identity =
 (the number of identically matching amino acid residues between the two polypeptide sequences as
 20 determined by ALIGN-2) divided by (the total number of amino acid residues of the PRO polypeptide) =
 5 divided by 10 = 50%

Table 4

25 PRO-DNA NNNNNNNNNNNNNNN (Length = 14 nucleotides)
 Comparison DNA NNNNNNNNLLLLLLLLL (Length = 16 nucleotides)
 % nucleic acid sequence identity =
 30 (the number of identically matching nucleotides between the two nucleic acid sequences as determined by
 ALIGN-2) divided by (the total number of nucleotides of the PRO-DNA nucleic acid sequence) =
 6 divided by 14 = 42.9%

Table 5

35 PRO-DNA NNNNNNNNNNNNN (Length = 12 nucleotides)
 Comparison DNA NNNNLLLVV (Length = 9 nucleotides)
 % nucleic acid sequence identity =
 40

(the number of identically matching nucleotides between the two nucleic acid sequences as determined by ALIGN-2) divided by (the total number of nucleotides of the PRO-DNA nucleic acid sequence) =
4 divided by 12 = 33.3%

5 II. Compositions and Methods of the Invention

A. Full-Length PRO Polypeptides

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO polypeptides. In particular, cDNAs encoding various PRO polypeptides have been identified and isolated, as disclosed in further detail in the Examples 10 below. However, for sake of simplicity, in the present specification the protein encoded by the full length native nucleic acid molecules disclosed herein as well as all further native homologues and variants included in the foregoing definition of PRO, will be referred to as "PRO/number", regardless of their origin or mode of preparation.

As disclosed in the Examples below, various cDNA clones have been disclosed. The predicted 15 amino acid sequence can be determined from the nucleotide sequence using routine skill. For the PRO polypeptides and encoding nucleic acids described herein, Applicants have identified what is believed to be the reading frame best identifiable with the sequence information available at the time.

B. PRO Polypeptide Variants

In addition to the full-length native sequence PRO polypeptides described herein, it is contemplated 20 that PRO variants can be prepared. PRO variants can be prepared by introducing appropriate nucleotide changes into the PRO DNA, and/or by synthesis of the desired PRO polypeptide. Those skilled in the art will appreciate that amino acid changes may alter post-translational processes of the PRO, such as changing the number or position of glycosylation sites or altering the membrane anchoring characteristics.

Variations in the native full-length sequence PRO or in various domains of the PRO described 25 herein, can be made, for example, using any of the techniques and guidelines for conservative and non-conservative mutations set forth, for instance, in U.S. Patent No. 5,364,934. Variations may be a substitution, deletion or insertion of one or more codons encoding the PRO that results in a change in the amino acid sequence of the PRO as compared with the native sequence PRO. Optionally, the variation is by substitution of at least one amino acid with any other amino acid in one or more of the domains of the PRO. 30 Guidance in determining which amino acid residue may be inserted, substituted or deleted without adversely affecting the desired activity may be found by comparing the sequence of the PRO with that of homologous known protein molecules and minimizing the number of amino acid sequence changes made in regions of high homology. Amino acid substitutions can be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, 35 i.e., conservative amino acid replacements. Insertions or deletions may optionally be in the range of about 1 to 5 amino acids. The variation allowed may be determined by systematically making insertions, deletions or substitutions of amino acids in the sequence and testing the resulting variants for activity exhibited by the full-length or mature native sequence.

PRO polypeptide fragments are provided herein. Such fragments may be truncated at the N- 40 terminus or C-terminus, or may lack internal residues, for example, when compared with a full length native

protein. Certain fragments lack amino acid residues that are not essential for a desired biological activity of the PRO polypeptide.

PRO fragments may be prepared by any of a number of conventional techniques. Desired peptide fragments may be chemically synthesized. An alternative approach involves generating PRO fragments by 5 enzymatic digestion, e.g., by treating the protein with an enzyme known to cleave proteins at sites defined by particular amino acid residues, or by digesting the DNA with suitable restriction enzymes and isolating the desired fragment. Yet another suitable technique involves isolating and amplifying a DNA fragment encoding a desired polypeptide fragment, by polymerase chain reaction (PCR). Oligonucleotides that define the desired termini of the DNA fragment are employed at the 5' and 3' primers in the PCR. Preferably, PRO 10 polypeptide fragments share at least one biological and/or immunological activity with the native PRO polypeptide disclosed herein.

In particular embodiments, conservative substitutions of interest are shown in Table 6 under the heading of preferred substitutions. If such substitutions result in a change in biological activity, then more substantial changes, denominated exemplary substitutions in Table 6, or as further described below in 15 reference to amino acid classes, are introduced and the products screened.

Table 6

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	val; leu; ile	val
Arg (R)	lys; gln; asn	lys
Asn (N)	gln; his; lys; arg	gln
Asp (D)	glu	glu
Cys (C)	ser	ser
Gln (Q)	asn	asn
Glu (E)	asp	asp
Gly (G)	pro; ala	ala
His (H)	asn; gln; lys; arg	arg
Ile (I)	leu; val; met; ala; phe; norleucine	leu
Leu (L)	norleucine; ile; val; met; ala; phe	ile
Lys (K)	arg; gln; asn	arg
Met (M)	leu; phe; ile	leu
Phe (F)	leu; val; ile; ala; tyr	leu
Pro (P)	ala	ala
Ser (S)	thr	thr
Thr (T)	ser	ser
Trp (W)	tyr; phe	tyr
Tyr (Y)	trp; pho; thr; scr	phe
Val (V)	ile; leu; met; phe; ala; norleucine	leu

30 Substantial modifications in function or immunological identity of the PRO polypeptide are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

35 (1) hydrophobic: norleucine, met, ala, val, leu, ile;
 (2) neutral hydrophilic: cys, ser, thr;
 (3) acidic: asp, glu;
 (4) basic: asn, gln, his, lys, arg;
 (5) residues that influence chain orientation: gly, pro; and
 40 (6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Such substituted residues also may be introduced into the conservative substitution sites or, more preferably, into the remaining (non-conserved) sites.

The variations can be made using methods known in the art such as oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis [Carter et al., *Nucl. Acids Res.*, **13**:4331 (1986); Zoller et al., *Nucl. Acids Res.*, **10**:6487 (1987)], cassette mutagenesis [Wells et al., *Gene*, **34**:315 (1985)], restriction selection mutagenesis [Wells et al., *Philos. Trans. R. Soc. London SerA*, **317**:415 (1986)] or other known techniques can be performed on the cloned DNA to produce the PRO variant DNA.

Scanning amino acid analysis can also be employed to identify one or more amino acids along a contiguous sequence. Among the preferred scanning amino acids are relatively small, neutral amino acids. Such amino acids include alanine, glycine, serine, and cysteine. Alanine is typically a preferred scanning amino acid among this group because it eliminates the side-chain beyond the beta-carbon and is less likely to alter the main-chain conformation of the variant [Cunningham and Wells, *Science*, 244: 1081-1085 (1989)]. Alanine is also typically preferred because it is the most common amino acid. Further, it is frequently found in both buried and exposed positions [Creighton, *The Proteins*, (W.H. Freeman & Co., N.Y.); Chothia, *J. Mol. Biol.*, 150:1 (1976)]. If alanine substitution does not yield adequate amounts of variant, an isoteric amino acid can be used.

10 C. Modifications of PRO

Covalent modifications of PRO are included within the scope of this invention. One type of covalent modification includes reacting targeted amino acid residues of a PRO polypeptide with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues of the PRO. Derivatization with bifunctional agents is useful, for instance, for crosslinking PRO to a water-insoluble support matrix or surface for use in the method for purifying anti-PRO antibodies, and vice-versa. Commonly used crosslinking agents include, e.g., 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), bifunctional maleimides such as bis-N-maleimido-1,8-octane and agents such as methyl-3-[(p-azidophenyl)dithio]propioimidate.

20 Other modifications include deamidation of glutamyl and asparaginyl residues to the corresponding glutamyl and aspartyl residues, respectively, hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α -amino groups of lysine, arginine, and histidine side chains [T.E. Creighton, *Proteins: Structure and Molecular Properties*, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)], acetylation of the N-terminal amine, and amidation 25 of any C-terminal carboxyl group.

Another type of covalent modification of the PRO polypeptide included within the scope of this invention comprises altering the native glycosylation pattern of the polypeptide. "Altering the native glycosylation pattern" is intended for purposes herein to mean deleting one or more carbohydrate moieties found in native sequence PRO (either by removing the underlying glycosylation site or by deleting the 30 glycosylation by chemical and/or enzymatic means), and/or adding one or more glycosylation sites that are not present in the native sequence PRO. In addition, the phrase includes qualitative changes in the glycosylation of the native proteins, involving a change in the nature and proportions of the various carbohydrate moieties present.

35 Addition of glycosylation sites to the PRO polypeptide may be accomplished by altering the amino acid sequence. The alteration may be made, for example, by the addition of, or substitution by, one or more serine or threonine residues to the native sequence PRO (for O-linked glycosylation sites). The PRO amino acid sequence may optionally be altered through changes at the DNA level, particularly by mutating the DNA encoding the PRO polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids.

Another means of increasing the number of carbohydrate moieties on the PRO polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. Such methods are described in the art, e.g., in WO 87/05330 published 11 September 1987, and in Aplin and Wriston, *CRC Crit. Rev. Biochem.*, pp. 259-306 (1981).

5 Removal of carbohydrate moieties present on the PRO polypeptide may be accomplished chemically or enzymatically or by mutational substitution of codons encoding for amino acid residues that serve as targets for glycosylation. Chemical deglycosylation techniques are known in the art and described, for instance, by Hakimuddin, et al., *Arch. Biochem. Biophys.*, 259:52 (1987) and by Edge et al., *Anal. Biochem.*, 118:131 (1981). Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved
10 by the use of a variety of endo- and exo-glycosidases as described by Thotakura et al., *Meth. Enzymol.*, 138:350 (1987).

Another type of covalent modification of PRO comprises linking the PRO polypeptide to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol (PEG), polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 15 4,791,192 or 4,179,337.

The PRO of the present invention may also be modified in a way to form a chimeric molecule comprising PRO fused to another, heterologous polypeptide or amino acid sequence.

In one embodiment, such a chimeric molecule comprises a fusion of the PRO with a tag polypeptide which provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag is generally placed at the amino- or carboxyl- terminus of the PRO. The presence of such epitope-tagged forms of the PRO can be detected using an antibody against the tag polypeptide. Also, provision of the epitope tag enables the PRO to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag. Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; the flu HA tag polypeptide and its antibody 12CA5 [Field et al., *Mol. Cell. Biol.*, 8:2159-2165 (1988)]; the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto [Evan et al., *Molecular and Cellular Biology*, 5:3610-3616 (1985)]; and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody [Paborsky et al., *Protein Engineering*, 3(6):547-553 (1990)]. Other tag polypeptides include the Flag-peptide [Hopp et al., *BioTechnology*, 6:1204-1210 (1988)]; the KT3 epitope peptide [Martin et al., *Science*, 255:192-194 (1992)]; an alpha-tubulin epitope peptide [Skinner et al., *J. Biol. Chem.*, 266:15163-15166 (1991)]; and the T7 gene 10 protein peptide tag [Lutz-Freyermuth et al., *Proc. Natl. Acad. Sci. USA*, 87:6393-6397 (1990)].

In an alternative embodiment, the chimeric molecule may comprise a fusion of the PRO with an immunoglobulin or a particular region of an immunoglobulin. For a bivalent form of the chimeric molecule (also referred to as an "immunoadhesin"), such a fusion could be to the Fc region of an IgG molecule. The Ig fusions preferably include the substitution of a soluble (transmembrane domain deleted or inactivated) form of a PRO polypeptide in place of at least one variable region within an Ig molecule. In a particularly preferred embodiment, the immunoglobulin fusion includes the hinge, CH2 and CH3, or the hinge, CH1, CH2 and CH3 regions of an IgG1 molecule. For the production of immunoglobulin fusions see also US 40 Patent No. 5,428,130 issued June 27, 1995.

5 D. Preparation of PRO

The description below relates primarily to production of PRO by culturing cells transformed or transfected with a vector containing PRO nucleic acid. It is, of course, contemplated that alternative methods, which are well known in the art, may be employed to prepare PRO. For instance, the PRO sequence, or portions thereof, may be produced by direct peptide synthesis using solid-phase techniques [see, e.g., Stewart et al., Solid-Phase Peptide Synthesis, W.H. Freeman Co., San Francisco, CA (1969); Merrifield, J. Am. Chem. Soc., **85**:2149-2154 (1963)]. *In vitro* protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be accomplished, for instance, using an Applied Biosystems Peptide Synthesizer (Foster City, CA) using manufacturer's instructions. Various portions of the PRO may be chemically synthesized separately and combined using chemical or enzymatic methods to produce the full-length PRO.

10 1. Isolation of DNA Encoding PRO

DNA encoding PRO may be obtained from a cDNA library prepared from tissue believed to possess the PRO mRNA and to express it at a detectable level. Accordingly, human PRO DNA can be conveniently obtained from a cDNA library prepared from human tissue, such as described in the Examples. The PRO-encoding gene may also be obtained from a genomic library or by known synthetic procedures (e.g., automated nucleic acid synthesis).

15 Libraries can be screened with probes (such as antibodies to the PRO or oligonucleotides of at least about 20-80 bases) designed to identify the gene of interest or the protein encoded by it. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures, such as described in Sambrook et al., Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1989). An alternative means to isolate the gene encoding PRO is to use PCR methodology [Sambrook et al., supra; Dieffenbach et al., PCR Primer: A Laboratory Manual (Cold Spring Harbor Laboratory Press, 1995)].

20 25 The Examples below describe techniques for screening a cDNA library. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized. The oligonucleotide is preferably labeled such that it can be detected upon hybridization to DNA in the library being screened. Methods of labeling are well known in the art, and include the use of radiolabels like ³²P-labeled ATP, biotinylation or enzyme labeling. Hybridization conditions, including 30 moderate stringency and high stringency, are provided in Sambrook et al., supra.

35 Sequences identified in such library screening methods can be compared and aligned to other known sequences deposited and available in public databases such as GenBank or other private sequence databases. Sequence identity (at either the amino acid or nucleotide level) within defined regions of the molecule or across the full-length sequence can be determined using methods known in the art and as described herein.

Nucleic acid having protein coding sequence may be obtained by screening selected cDNA or genomic libraries using the deduced amino acid sequence disclosed herein for the first time, and, if necessary, using conventional primer extension procedures as described in Sambrook et al., supra, to detect precursors and processing intermediates of mRNA that may not have been reverse-transcribed into cDNA.

2. Selection and Transformation of Host Cells

Host cells are transfected or transformed with expression or cloning vectors described herein for PRO production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. The culture 5 conditions, such as media, temperature, pH and the like, can be selected by the skilled artisan without undue experimentation. In general, principles, protocols, and practical techniques for maximizing the productivity of cell cultures can be found in Mammalian Cell Biotechnology: a Practical Approach, M. Butler, ed. (IRL Press, 1991) and Sambrook et al., supra.

Methods of eukaryotic cell transfection and prokaryotic cell transformation are known to the 10 ordinarily skilled artisan, for example, CaCl_2 , CaPO_4 , liposome-mediated and electroporation. Depending on the host cell used, transformation is performed using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in Sambrook et al., supra, or electroporation is generally used for prokaryotes. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw et al., Gene, 23:315 (1983) and WO 89/05859 published 29 June 15 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, Virology, 52:456-457 (1978) can be employed. General aspects of mammalian cell host system transfections have been described in U.S. Patent No. 4,399,216. Transformations into yeast are typically carried out according to the method of Van Solingen et al., J. Bact., 130:946 (1977) and Hsiao et al., Proc. Natl. Acad. Sci. (USA), 76:3829 (1979). However, other methods for introducing DNA into cells, 20 such as by nuclear microinjection, electroporation, bacterial protoplast fusion with intact cells, or polycations, e.g., polybrene, polyornithine, may also be used. For various techniques for transforming mammalian cells, see Keown et al., Methods in Enzymology, 185:527-537 (1990) and Mansour et al., Nature, 336:348-352 (1988).

Suitable host cells for cloning or expressing the DNA in the vectors herein include prokaryote, 25 yeast, or higher eukaryote cells. Suitable prokaryotes include but are not limited to eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *E. coli*. Various *E. coli* strains are publicly available, such as *E. coli* K12 strain MM294 (ATCC 31,446); *E. coli* X1776 (ATCC 31,537); *E. coli* strain W3110 (ATCC 27,325) and K5 772 (ATCC 53,635). Other suitable prokaryotic host 30 cells include Enterobacteriaceae such as *Escherichia*, e.g., *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, e.g., *Salmonella typhimurium*, *Serratia*, e.g., *Serratia marcescens*, and *Shigella*, as well as *Bacilli* such as *B. subtilis* and *B. licheniformis* (e.g., *B. licheniformis* 41P disclosed in DD 266,710 published 12 April 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. These examples are 35 illustrative rather than limiting. Strain W3110 is one particularly preferred host or parent host because it is a common host strain for recombinant DNA product fermentations. Preferably, the host cell secretes minimal amounts of proteolytic enzymes. For example, strain W3110 may be modified to effect a genetic mutation in the genes encoding proteins endogenous to the host, with examples of such hosts including *E. coli* W3110 strain 1A2, which has the complete genotype *tonA*; *E. coli* W3110 strain 9E4, which has the complete genotype *tonA ptr3*; *E. coli* W3110 strain 27C7 (ATCC 55,244), which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT kanr*; *E. coli* W3110 strain 37D6, which has the complete genotype 40 *tonA ptr3 phoA E15 (argF-lac)169 degP ompT rbs7 ilvG kanr*; *E. coli* W3110 strain 40B4, which is strain

37D6 with a non-kanamycin resistant *degP* deletion mutation; and an *E. coli* strain having mutant periplasmic protease disclosed in U.S. Patent No. 4,946,783 issued 7 August 1990. Alternatively, *in vitro* methods of cloning, e.g., PCR or other nucleic acid polymerase reactions, are suitable.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable 5 cloning or expression hosts for PRO-encoding vectors. *Saccharomyces cerevisiae* is a commonly used lower eukaryotic host microorganism. Others include *Schizosaccharomyces pombe* (Beach and Nurse, *Nature*, 290: 140 [1981]; EP 139,383 published 2 May 1985); *Kluyveromyces* hosts (U.S. Patent No. 4,943,529; Fleer et al., *Bio/Technology*, 9:968-975 (1991)) such as, e.g., *K. lactis* (MW98-8C, CBS683, CBS4574; Louvencourt et al., *J. Bacteriol.*, 154(2):737-742 [1983]), *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickeramii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilicola* (ATCC 36,906; Van den Berg et al., *Bio/Technology*, 8:135 (1990)), *K. thermotolerans*, and *K. marxianus*; *yearrowia* (EP 402,226); *Pichia pastoris* (EP 183,070; Sreekrishna et al., *J. Basic Microbiol.*, 28:265-278 [1988]); *Candida*; *Trichoderma reesia* (EP 244,234); *Neurospora crassa* (Case et al., *Proc. Natl. Acad. Sci. USA*, 76:5259-5263 [1979]); *Schwanniomyces* such as *Schwanniomyces occidentalis* (EP 394,538 published 31 October 10 1990); and filamentous fungi such as, e.g., *Neurospora*, *Penicillium*, *Tolypocladium* (WO 91/00357 published 10 January 1991), and *Aspergillus* hosts such as *A. nidulans* (Ballance et al., *Biochem. Biophys. Res. Commun.*, 112:284-289 [1983]; Tilburn et al., *Gene*, 26:205-221 [1983]; Yelton et al., *Proc. Natl. Acad. Sci. USA*, 81: 1470-1474 [1984]) and *A. niger* (Kelly and Hynes, *EMBO J.*, 4:475-479 [1985]). Methylotropic yeasts are suitable herein and include, but are not limited to, yeast capable of growth on 15 methanol selected from the genera consisting of *Hansenula*, *Candida*, *Kloeckera*, *Pichia*, *Saccharomyces*, *Torulopsis*, and *Rhodotorula*. A list of specific species that are exemplary of this class of yeasts may be found in C. Anthony, *The Biochemistry of Methylotrophs*, 269 (1982).

Suitable host cells for the expression of glycosylated PRO are derived from multicellular 20 organisms. Examples of invertebrate cells include insect cells such as *Drosophila S2* and *Spodoptera Sf9*, as well as plant cells. Examples of useful mammalian host cell lines include Chinese hamster ovary (CHO) and 25 COS cells. More specific examples include monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., *J. Gen. Virol.*, 36:59 (1977)); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA*, 77:4216 (1980)); mouse sertoli cells (TM4, Mather, *Biol. Reprod.*, 23:243-251 30 (1980)); human lung cells (WI38, ATCC CCL 75); human liver cells (Hep G2, HB 8065); and mouse mammary tumor (MMT 060562, ATCC CCL51). The selection of the appropriate host cell is deemed to be within the skill in the art.

3. Selection and Use of a Replicable Vector

The nucleic acid (e.g., cDNA or genomic DNA) encoding PRO may be inserted into a replicable 35 vector for cloning (amplification of the DNA) or for expression. Various vectors are publicly available. The vector may, for example, be in the form of a plasmid, cosmid, viral particle, or phage. The appropriate nucleic acid sequence may be inserted into the vector by a variety of procedures. In general, DNA is inserted into an appropriate restriction endonuclease site(s) using techniques known in the art. Vector components generally include, but are not limited to, one or more of a signal sequence, an origin of 40 replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination

sequence. Construction of suitable vectors containing one or more of these components employs standard ligation techniques which are known to the skilled artisan.

The PRO may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which may be a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the PRO-encoding DNA that is inserted into the vector. The signal sequence may be a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the signal sequence may be, e.g., the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces* α -factor leaders, the latter described in U.S. Patent No. 5,010,182), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression, mammalian signal sequences may be used to direct secretion of the protein, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders.

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2μ plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells.

Expression and cloning vectors will typically contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for *Bacilli*.

An example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the PRO-encoding nucleic acid, such as DHFR or thymidine kinase. An appropriate host cell when wild-type DHFR is employed is the CHO cell line deficient in DHFR activity, prepared and propagated as described by Urlaub et al., *Proc. Natl. Acad. Sci. USA*, 77:4216 (1980). A suitable selection gene for use in yeast is the *trp1* gene present in the yeast plasmid YRp7 [Stinchcomb et al., *Nature*, 282:39 (1979); Kingsman et al., *Gene*, 7:141 (1979); Tschemper et al., *Gene*, 10:157 (1980)]. The *trp1* gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1 [Jones, *Genetics*, 85:12 (1977)].

Expression and cloning vectors usually contain a promoter operably linked to the PRO-encoding nucleic acid sequence to direct mRNA synthesis. Promoters recognized by a variety of potential host cells are well known. Promoters suitable for use with prokaryotic hosts include the β -lactamase and lactose promoter systems [Chang et al., *Nature*, 275:615 (1978); Goeddel et al., *Nature*, 281:544 (1979)], alkaline phosphatase, a tryptophan (trp) promoter system [Goeddel, *Nucleic Acids Res.*, 8:4057 (1980); EP 36,776], and hybrid promoters such as the tac promoter [deBoer et al., *Proc. Natl. Acad. Sci. USA*, 80:21-25 (1983)]. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding PRO.

Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase [Hitzeman et al., *J. Biol. Chem.*, 255:2073 (1980)] or other glycolytic enzymes [Hess et al., *J. Adv. Enzyme Reg.*, 7:149 (1968); Holland, *Biochemistry*, 17:4900 (1978)], such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, 5 glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytchrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, 10 metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 73,657.

PRO transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 15 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g., the actin promoter or an immunoglobulin promoter, and from heat-shock promoters, provided such promoters are compatible with the host cell systems.

Transcription of a DNA encoding the PRO by higher eukaryotes may be increased by inserting an 20 enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α -fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the 25 late side of the replication origin, and adenovirus enhancers. The enhancer may be spliced into the vector at a position 5' or 3' to the PRO coding sequence, but is preferably located at a site 5' from the promoter.

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or 30 nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding PRO.

Still other methods, vectors, and host cells suitable for adaptation to the synthesis of PRO in recombinant vertebrate cell culture are described in Gething et al., *Nature*, 293:620-625 (1981); Mantei et 35 al., *Nature*, 281:40-46 (1979); EP 117,060; and EP 117,058.

4. Detecting Gene Amplification/Expression

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA [Thomas, *Proc. Natl. Acad. Sci. USA*, 77:5201-5205 (1980)], dot blotting (DNA analysis), or *in situ* hybridization, using an 40 appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be

employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

5 Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of cells or tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native sequence PRO polypeptide or against a
10 synthetic peptide based on the DNA sequences provided herein or against exogenous sequence fused to PRO DNA and encoding a specific antibody epitope.

5. Purification of Polypeptide

Forms of PRO may be recovered from culture medium or from host cell lysates. If membrane-bound, it can be released from the membrane using a suitable detergent solution (e.g. Triton-X 100) or by
15 enzymatic cleavage. Cells employed in expression of PRO can be disrupted by various physical or chemical means, such as freeze-thaw cycling, sonication, mechanical disruption, or cell lysing agents.

It may be desired to purify PRO from recombinant cell proteins or polypeptides. The following procedures are exemplary of suitable purification procedures: by fractionation on an ion-exchange column;
20 ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; protein A Sepharose columns to remove contaminants such as IgG; and metal chelating columns to bind epitope-tagged forms of the PRO. Various methods of protein purification may be employed and such methods are known in the art and described for example in Deutscher, Methods in Enzymology, 182 (1990); Scopes, Protein Purification: Principles and Practice, Springer-Verlag, New York
25 (1982). The purification step(s) selected will depend, for example, on the nature of the production process used and the particular PRO produced.

E. Tissue Distribution

The location of tissues expressing the PRO can be identified by determining mRNA expression in various human tissues. The location of such genes provides information about which tissues are most likely
30 to be affected by the stimulating and inhibiting activities of the PRO polypeptides. The location of a gene in a specific tissue also provides sample tissue for the activity blocking assays discussed below.

As noted before, gene expression in various tissues may be measured by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA (Thomas, *Proc. Natl. Acad. Sci. USA*, 77:5201-5205 [1980]), dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled
35 probe, based on the sequences provided herein. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes.

Gene expression in various tissues, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to
40 quantitate directly the expression of gene product. Antibodies useful for immunohistochemical staining

and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native sequence of a PRO polypeptide or against a synthetic peptide based on the DNA sequences encoding the PRO polypeptide or against an exogenous sequence fused to a DNA encoding a PRO polypeptide and encoding a specific antibody epitope. General 5 techniques for generating antibodies, and special protocols for Northern blotting and *in situ* hybridization are provided below.

5 F. Antibody Binding Studies

The activity of the PRO polypeptides can be further verified by antibody binding studies, in which 10 the ability of anti-PRO antibodies to inhibit the effect of the PRO polypeptides, respectively, on tissue cells is tested. Exemplary antibodies include polyclonal, monoclonal, humanized, bispecific, and heteroconjugate antibodies, the preparation of which will be described hereinbelow.

Antibody binding studies may be carried out in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Zola, *Monoclonal Antibodies: A Manual of Techniques*, pp.147-158 (CRC Press, Inc., 1987).

15 Competitive binding assays rely on the ability of a labeled standard to compete with the test sample analyte for binding with a limited amount of antibody. The amount of target protein in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies preferably are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies may conveniently 20 be separated from the standard and analyte which remain unbound.

Sandwich assays involve the use of two antibodies, each capable of binding to a different 25 immunogenic portion, or epitope, of the protein to be detected. In a sandwich assay, the test sample analyte is bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three-part complex. See, e.g., US Pat No. 4,376,110. The second antibody may itself be labeled with a detectable moiety (direct sandwich assays) or may be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assay). For example, one type of sandwich assay is an ELISA assay, in which case the detectable moiety is an enzyme.

For immunohistochemistry, the tissue sample may be fresh or frozen or may be embedded in paraffin and fixed with a preservative such as formalin, for example.

30 G. Cell-Based Assays

Cell-based assays and animal models for immune related diseases can be used to further understand the relationship between the genes and polypeptides identified herein and the development and pathogenesis of immune related disease.

In a different approach, cells of a cell type known to be involved in a particular immune related 35 disease are transfected with the cDNAs described herein, and the ability of these cDNAs to stimulate or inhibit immune function is analyzed. Suitable cells can be transfected with the desired gene, and monitored for immune function activity. Such transfected cell lines can then be used to test the ability of poly- or monoclonal antibodies or antibody compositions to inhibit or stimulate immune function, for example to modulate NK cell proliferation or inflammatory cell infiltration. Cells transfected with the coding sequences

of the genes identified herein can further be used to identify drug candidates for the treatment of immune related diseases.

In addition, primary cultures derived from transgenic animals (as described below) can be used in the cell-based assays herein, although stable cell lines are preferred. Techniques to derive continuous cell lines from transgenic animals are well known in the art (see, e.g., Small *et al.*, *Mol. Cell. Biol.* 5: 642-648 [1985]).

The use of an agonist stimulating compound has also been validated experimentally. Activation of 4-1BB by treatment with an agonist anti-4-1BB antibody enhances eradication of tumors. Hellstrom, I. and Hellstrom, K. E., *Crit. Rev. Immunol.* (1998) 18:1. Immunoadjuvant therapy for treatment of tumors, described in more detail below, is another example of the use of the stimulating compounds of the invention.

Alternatively, an immune stimulating or enhancing effect can also be achieved by administration of a PRO which has vascular permeability enhancing properties. Enhanced vascular permeability would be beneficial to disorders which can be attenuated by local infiltration of immune cells (e.g., NK cells, monocytes/macrophages, eosinophils, PMNs) and inflammation.

On the other hand, PRO polypeptides, as well as other compounds of the invention, which are direct inhibitors of NK cell proliferation/activation, lymphokine secretion, and/or vascular permeability can be directly used to suppress the immune response. These compounds are useful to reduce the degree of the immune response and to treat immune related diseases characterized by a hyperactive, superoptimal, or autoimmune response. The use of compound which suppress vascular permeability would be expected to reduce inflammation. Such uses would be beneficial in treating conditions associated with excessive inflammation.

Alternatively, compounds, e.g., antibodies, which bind to stimulating PRO polypeptides and block the stimulating effect of these molecules produce a net inhibitory effect and can be used to suppress the NK cell mediated immune response by inhibiting NK cell proliferation/activation and/or lymphokine secretion. Blocking the stimulating effect of the polypeptides suppresses the immune response of the mammal.

H. Animal Models

The results of the cell based *in vitro* assays can be further verified using *in vivo* animal models and assays for NK cell function. A variety of well known animal models can be used to further understand the role of the genes identified herein in the development and pathogenesis of immune related disease, and to test the efficacy of candidate therapeutic agents, including antibodies, and other antagonists of the native polypeptides, including small molecule antagonists. The *in vivo* nature of such models makes them predictive of responses in human patients. Animal models of immune related diseases include both non-recombinant and recombinant (transgenic) animals. Non-recombinant animal models include, for example, rodent, e.g., murine models. Such models can be generated by introducing cells into syngeneic mice using standard techniques, e.g., subcutaneous injection, tail vein injection, spleen implantation, intraperitoneal implantation, implantation under the renal capsule, etc.

Graft-versus-host disease occurs when immunocompetent cells are transplanted into immunosuppressed or tolerant patients. The donor cells recognize and respond to host antigens. The response can vary from life threatening severe inflammation to mild cases of diarrhea and weight loss.

Graft-versus-host disease models provide a means of assessing NK cell reactivity against MHC antigens and minor transplant antigens. A suitable procedure is described in detail in *Current Protocols in Immunology*, above, unit 4.3.

Contact hypersensitivity is a simple delayed type hypersensitivity *in vivo* assay of cell mediated immune function. In this procedure, cutaneous exposure to exogenous haptens which gives rise to a delayed type hypersensitivity reaction which is measured and quantitated. Contact sensitivity involves an initial sensitizing phase followed by an elicitation phase. The elicitation phase occurs when the T lymphocytes encounter an antigen to which they have had previous contact. Swelling and inflammation occur, making this an excellent model of human allergic contact dermatitis. A suitable procedure is described in detail in 10 *Current Protocols in Immunology*, Eds. J. E. Cologan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach and W. Strober, John Wiley & Sons, Inc., 1994, unit 4.2. See also Grabbe, S. and Schwarz, T, *Immun. Today* 19 (1): 37-44 (1998).

Recombinant (transgenic) animal models can be engineered by introducing the coding portion of the genes identified herein into the genome of animals of interest, using standard techniques for producing 15 transgenic animals. Animals that can serve as a target for transgenic manipulation include, without limitation, mice, rats, rabbits, guinea pigs, sheep, goats, pigs, and non-human primates, *e.g.*, baboons, chimpanzees and monkeys. Techniques known in the art to introduce a transgene into such animals include pronucleic microinjection (Hoppe and Wanger, U.S. Patent No. 4,873,191); retrovirus-mediated gene transfer into germ lines (*e.g.*, Van der Putten *et al.*, *Proc. Natl. Acad. Sci. USA* 82, 6148-615 [1985]); gene 20 targeting in embryonic stem cells (Thompson *et al.*, *Cell* 56, 313-321 [1989]); electroporation of embryos (*Lo, Mol. Cel. Biol.* 3, 1803-1814 [1983]); sperm-mediated gene transfer (Lavitano *et al.*, *Cell* 57, 717-73 [1989]). For review, see, for example, U.S. Patent No. 4,736,866.

For the purpose of the present invention, transgenic animals include those that carry the transgene only in part of their cells ("mosaic animals"). The transgene can be integrated either as a single transgene, or 25 in concatamers, *e.g.*, head-to-head or head-to-tail tandems. Selective introduction of a transgene into a particular cell type is also possible by following, for example, the technique of Lasko *et al.*, *Proc. Natl. Acad. Sci. USA* 89, 6232-636 (1992).

The expression of the transgene in transgenic animals can be monitored by standard techniques. For example, Southern blot analysis or PCR amplification can be used to verify the integration of the 30 transgene. The level of mRNA expression can then be analyzed using techniques such as *in situ* hybridization, Northern blot analysis, PCR, or immunocytochemistry.

The animals may be further examined for signs of immune disease pathology, for example by histological examination to determine infiltration of immune cells into specific tissues. Blocking experiments can also be performed in which the transgenic animals are treated with the compounds of the 35 invention to determine the extent of the NK cell proliferation, stimulation or inhibition of the compounds. In these experiments, blocking antibodies which bind to the PRO polypeptide, prepared as described above, are administered to the animal and the effect on immune function is determined.

Alternatively, "knock out" animals can be constructed which have a defective or altered gene encoding a polypeptide identified herein, as a result of homologous recombination between the endogenous 40 gene encoding the polypeptide and altered genomic DNA encoding the same polypeptide introduced into an

embryonic cell of the animal. For example, cDNA encoding a particular polypeptide can be used to clone genomic DNA encoding that polypeptide in accordance with established techniques. A portion of the genomic DNA encoding a particular polypeptide can be deleted or replaced with another gene, such as a gene encoding a selectable marker which can be used to monitor integration. Typically, several kilobases of 5 unaltered flanking DNA (both at the 5' and 3' ends) are included in the vector [see e.g., Thomas and Capecchi, *Cell*, **51**:503 (1987) for a description of homologous recombination vectors]. The vector is introduced into an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced DNA 10 has homologously recombined with the endogenous DNA are selected [see e.g., Li *et al.*, *Cell*, **69**:915 (1992)]. The selected cells are then injected into a blastocyst of an animal (e.g., a mouse or rat) to form aggregation chimeras [see e.g., Bradley, in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E. J. Robertson, ed. (IRL, Oxford, 1987), pp. 113-152]. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term to create a "knock out" animal. Progeny harboring the homologously recombined DNA in their germ cells can be 15 identified by standard techniques and used to breed animals in which all cells of the animal contain the homologously recombined DNA. Knockout animals can be characterized for instance, for their ability to defend against certain pathological conditions and for their development of pathological conditions due to absence of the polypeptide.

I. ImmunoAdjuvant Therapy

In one embodiment, the immunostimulating compounds of the invention can be used in 20 immunoadjuvant therapy for the treatment of tumors (cancer). It is now well established that NK cells recognize human tumor specific antigens. One group of tumor antigens, encoded by the MAGE, BAGE and GAGE families of genes, are silent in all adult normal tissues, but are expressed in significant amounts in tumors, such as melanomas, lung tumors, head and neck tumors, and bladder carcinomas. DeSmct, C. *et al.*, (1996) *Proc. Natl. Acad. Sci. USA*, **93**:7149. It has been shown that stimulation of immune cells induces 25 tumor regression and an antitumor response both *in vitro* and *in vivo*. Melero, I. *et al.*, *Nature Medicine* (1997) **3**:682; Kwon, E. D. *et al.*, *Proc. Natl. Acad. Sci. USA* (1997) **94**: 8099; Lynch, D. H. *et al.*, *Nature Medicine* (1997) **3**:625; Finn, O. J. and Lotze, M. T., *J. Immunol.* (1998) **21**:114. The stimulatory 30 compounds of the invention can be administered as adjuvants, alone or together with a growth regulating agent, cytotoxic agent or chemotherapeutic agent, to stimulate NK cell proliferation/activation and an antitumor response to tumor antigens. The growth regulating, cytotoxic, or chemotherapeutic agent may be administered in conventional amounts using known administration regimes. Immunostimulating activity by the compounds of the invention allows reduced amounts of the growth regulating, cytotoxic, or chemotherapeutic agents thereby potentially lowering the toxicity to the patient.

J. Screening Assays for Drug Candidates

35 Screening assays for drug candidates are designed to identify compounds that bind to or complex with the polypeptides encoded by the genes identified herein or a biologically active fragment thereof, or otherwise interfere with the interaction of the encoded polypeptides with other cellular proteins. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates. Small molecules contemplated 40 include synthetic organic or inorganic compounds, including peptides, preferably soluble peptides,

(poly)peptide-immunoglobulin fusions, and, in particular, antibodies including, without limitation, poly- and monoclonal antibodies and antibody fragments, single-chain antibodies, anti-idiotypic antibodies, and chimeric or humanized versions of such antibodies or fragments, as well as human antibodies and antibody fragments. The assays can be performed in a variety of formats, including protein-protein binding assays, 5 biochemical screening assays, immunoassays and cell based assays, which are well characterized in the art. All assays are common in that they call for contacting the drug candidate with a polypeptide encoded by a nucleic acid identified herein under conditions and for a time sufficient to allow these two components to interact.

In binding assays, the interaction is binding and the complex formed can be isolated or detected in 10 the reaction mixture. In a particular embodiment, the polypeptide encoded by the gene identified herein or the drug candidate is immobilized on a solid phase, e.g., on a microtiter plate, by covalent or non-covalent attachments. Non-covalent attachment generally is accomplished by coating the solid surface with a solution of the polypeptide and drying. Alternatively, an immobilized antibody, e.g., a monoclonal antibody, specific for the polypeptide to be immobilized can be used to anchor it to a solid surface. The assay is performed by 15 adding the non-immobilized component, which may be labeled by a detectable label, to the immobilized component, e.g., the coated surface containing the anchored component. When the reaction is complete, the non-reacted components are removed, e.g., by washing, and complexes anchored on the solid surface are detected. When the originally non-immobilized component carries a detectable label, the detection of label 20 immobilized on the surface indicates that complexing occurred. Where the originally non-immobilized component does not carry a label, complexing can be detected, for example, by using a labelled antibody specifically binding the immobilized complex.

If the candidate compound interacts with but does not bind to a particular protein encoded by a gene identified herein, its interaction with that protein can be assayed by methods well known for detecting 25 protein-protein interactions. Such assays include traditional approaches, such as, cross-linking, co-immunoprecipitation, and co-purification through gradients or chromatographic columns. In addition, protein-protein interactions can be monitored by using a yeast-based genetic system described by Fields and co-workers [Fields and Song, *Nature (London)* **340**, 245-246 (1989); Chien *et al.*, *Proc. Natl. Acad. Sci. USA* **88**, 9578-9582 (1991)] as disclosed by Chevray and Nathans, *Proc. Natl. Acad. Sci. USA* **89**, 5789-5793 (1991). Many transcriptional activators, such as yeast GAL4, consist of two physically discrete modular 30 domains, one acting as the DNA-binding domain, while the other one functioning as the transcription activation domain. The yeast expression system described in the foregoing publications (generally referred to as the "two-hybrid system") takes advantage of this property, and employs two hybrid proteins, one in which the target protein is fused to the DNA-binding domain of GAL4, and another, in which candidate activating proteins are fused to the activation domain. The expression of a GAL1-lacZ reporter gene under 35 control of a GAL4-activated promoter depends on reconstitution of GAL4 activity via protein-protein interaction. Colonies containing interacting polypeptides are detected with a chromogenic substrate for β -galactosidase. A complete kit (MATCHMAKERTM) for identifying protein-protein interactions between two specific proteins using the two-hybrid technique is commercially available from Clontech. This system can also be extended to map protein domains involved in specific protein interactions as well as to pinpoint 40 amino acid residues that are crucial for these interactions.

In order to find compounds that interfere with the interaction of a gene identified herein and other intra- or extracellular components can be tested, a reaction mixture is usually prepared containing the product of the gene and the intra- or extracellular component under conditions and for a time allowing for the interaction and binding of the two products. To test the ability of a test compound to inhibit binding, the 5 reaction is run in the absence and in the presence of the test compound. In addition, a placebo may be added to a third reaction mixture, to serve as positive control. The binding (complex formation) between the test compound and the intra- or extracellular component present in the mixture is monitored as described above. The formation of a complex in the control reaction(s) but not in the reaction mixture containing the test compound indicates that the test compound interferes with the interaction of the test compound and its 10 reaction partner.

K. Compositions and Methods for the Treatment of Immune Related Diseases

The compositions useful in the treatment of immune related diseases include, without limitation, proteins, antibodies, small organic molecules, peptides, phosphopeptides, antisense and ribozyme molecules, triple helix molecules, etc. that inhibit or stimulate immune function, for example, NK cell 15 proliferation/activation, lymphokine release, or immune cell infiltration.

For example, antisense RNA and RNA molecules act to directly block the translation of mRNA by hybridizing to targeted mRNA and preventing protein translation. When antisense DNA is used, oligodeoxynucleotides derived from the translation initiation site, e.g., between about -10 and +10 positions of the target gene nucleotide sequence, are preferred.

20 Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. Ribozymes act by sequence-specific hybridization to the complementary target RNA, followed by endonucleolytic cleavage. Specific ribozyme cleavage sites within a potential RNA target can be identified by known techniques. For further details see, e.g., Rossi, *Current Biology* 4, 469-471 (1994), and PCT publication No. WO 97/33551 (published September 18, 1997).

25 Nucleic acid molecules in triple helix formation used to inhibit transcription should be single-stranded and composed of deoxynucleotides. The base composition of these oligonucleotides is designed such that it promotes triple helix formation via Hoogsteen base pairing rules, which generally require sizeable stretches of purines or pyrimidines on one strand of a duplex. For further details see, e.g., PCT publication No. WO 97/33551, *supra*.

30 These molecules can be identified by any or any combination of the screening assays discussed above and/or by any other screening techniques well known for those skilled in the art.

L. Anti-PRO Antibodies

The present invention further provides anti-PRO antibodies. Exemplary antibodies include polyclonal, monoclonal, humanized, bispecific, and heteroconjugate antibodies.

35

1. Polyclonal Antibodies

The anti-PRO antibodies may comprise polyclonal antibodies. Methods of preparing polyclonal antibodies are known to the skilled artisan. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing 40 agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections.

The immunizing agent may include the PRO polypeptide or a fusion protein thereof. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. Examples of adjuvants which may be 5 employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

2. Monoclonal Antibodies

The anti-PRO antibodies may, alternatively, be monoclonal antibodies. Monoclonal antibodies 10 may be prepared using hybridoma methods, such as those described by Kohler and Milstein, Nature, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized *in vitro*.
15 The immunizing agent will typically include the PRO polypeptide or a fusion protein thereof. Generally, either peripheral blood lymphocytes ("PBLs") are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to 20 form a hybridoma cell [Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, (1986) pp. 59-103]. Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that preferably contains one or more substances that 25 inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from 30 the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies [Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, (1987) pp. 51-63].

35 The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against PRO. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be 40 determined by the Scatchard analysis of Munson and Pollard, Anal. Biochem., 107:220 (1980).

After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods [Goding, *supra*]. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells may be grown *in vivo* as ascites in a mammal.

5 The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese 15 hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences [U.S. Patent No. 4,816,567; Morrison et al., *supra*] or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non- 20 immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light 25 chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent crosslinking.

In vitro methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine techniques 30 known in the art.

3. Human and Humanized Antibodies

The anti-PRO antibodies of the invention may further comprise humanized antibodies or human antibodies. Humanized forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding 35 subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by 40 corresponding non-human residues. Humanized antibodies may also comprise residues which are found

neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The 5 humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin [Jones et al., *Nature*, 321:522-525 (1986); Riechmann et al., *Nature*, 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.*, 2:593-596 (1992)].

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These 10 non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones et al., *Nature*, 321:522-525 (1986); Riechmann et al., *Nature*, 332:323-327 (1988); Verhoeyen et al., *Science*, 239:1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric 15 antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be produced using various techniques known in the art, including phage 20 display libraries [Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks et al., *J. Mol. Biol.*, 222:581 (1991)]. The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies (Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985) and Boerner et al., *J. Immunol.*, 147(1):86-95 (1991)). Similarly, human antibodies can be made by introducing of human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous 25 immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks et al., *Bio/Technology* 10, 779-783 (1992); Lonberg et al., *Nature* 368 856-859 (1994); 30 Morrison, *Nature* 368, 812-13 (1994); Fishwild et al., *Nature Biotechnology* 14, 845-51 (1996); Neuberger, *Nature Biotechnology* 14, 826 (1996); Lonberg and Huszar, *Intern. Rev. Immunol.* 13 65-93 (1995).

The antibodies may also be affinity matured using known selection and/or mutagenesis methods as described above. Preferred affinity matured antibodies have an affinity which is five times, more preferably 10 times, even more preferably 20 or 30 times greater than the starting antibody (generally murine, 35 humanized or human) from which the matured antibody is prepared.

4. Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for the

PRO, the other one is for any other antigen, and preferably for a cell-surface protein or receptor or receptor subunit.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities [Milstein and Cuello, *Nature*, 305:537-539 (1983)]. Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker et al., *EMBO J.*, 10:3655-3659 (1991).

Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies see, for example, Suresh et al., *Methods in Enzymology*, 121:210 (1986).

According to another approach described in WO 96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the CH3 region of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g. F(ab')₂, bispecific antibodies). Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared can be prepared using chemical linkage. Brennan et al., *Science* 229:81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')₂ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Fab' fragments may be directly recovered from *E. coli* and chemically coupled to form bispecific antibodies. Shalaby et al., *J. Exp. Med.* 175:217-225 (1992) describe the production of a fully humanized bispecific antibody F(ab')₂ molecule. Each Fab' fragment was separately secreted from *E. coli* and

subjected to directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelnik *et al.*, *J. Immunol.* 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See, Gruber *et al.*, *J. Immunol.* 152:5368 (1994). Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt *et al.*, *J. Immunol.* 147:60 (1991).

Exemplary bispecific antibodies may bind to two different epitopes on a given PRO polypeptide herein. Alternatively, an anti-PRO polypeptide arm may be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (e.g. CD2, CD3, CD28, or B7), or Fc receptors for IgG (Fc_γR), such as Fc_γRI (CD64), Fc_γRII (CD32) and Fc_γRIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular PRO polypeptide. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express a particular PRO polypeptide. These antibodies possess a PRO-binding arm and an arm which binds a cytotoxic agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA. Another bispecific antibody of interest binds the PRO polypeptide and further binds tissue factor (TF).

5. Heteroconjugate Antibodies

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells [U.S. Patent No. 4,676,980], and for treatment of HIV infection [WO 91/00360; WO 92/200373; EP 03089]. It is contemplated that the antibodies may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptopbutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

6. Effector Function Engineering

It may be desirable to modify the antibody of the invention with respect to effector function, so as to enhance, *e.g.*, the effectiveness of the antibody in treating cancer. For example, cysteine residue(s) may

be introduced into the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron *et al.*, J. Exp Med., **176**: 1191-1195 (1992) and Shopes, J. Immunol., **148**: 2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff *et al.*, Cancer Research, **53**: 2560-2565 (1993). Alternatively, an antibody can be engineered that has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson *et al.*, Anti-Cancer Drug Design, **3**: 219-230 (1989).

7. Immunoconjugates

10 The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (*e.g.*, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (*i.e.*, a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, 15 nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, saponaria officinalis inhibitor, gelonin, mitogelin, restrictocin, phenomycin, enomycin, and the trichothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include 20 ^{212}Bi , ^{131}I , ^{131}In , ^{90}Y , and ^{186}Re .

Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridylthio)propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis-(p-25 azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al.*, Science, **238**: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylcnc triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to 30 the antibody. See WO94/11026.

In another embodiment, the antibody may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (*e.g.*, avidin) that is conjugated to a cytotoxic agent (*e.g.*, a radionucleotide).

35 8. Immunoliposomes

The antibodies disclosed herein may also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al.*, Proc. Natl. Acad. Sci. USA, **82**: 3688 (1985); Hwang *et al.*, Proc. Natl. Acad. Sci. USA, **77**: 4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent 40 No. 5,013,556.

Particularly useful liposomes can be generated by the reverse-phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin *et al.*, *J. Biol. Chem.*, **257**: 286-288 (1982) via a disulfide-interchange reaction. A chemotherapeutic agent (such as Doxorubicin) is optionally contained within the liposome. See Gabizon *et al.*, *J. National Cancer Inst.*, **81**(19): 1484 (1989).

M. Pharmaceutical Compositions

10 The active PRO molecules of the invention (e.g., PRO polypeptides, anti-PRO antibodies, and/or variants of each) as well as other molecules identified by the screening assays disclosed above, can be administered for the treatment of immune related diseases, in the form of pharmaceutical compositions.

15 Therapeutic formulations of the active PRO molecule, preferably a polypeptide or antibody of the invention, are prepared for storage by mixing the active molecule having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. [1980]), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecylmethylbenzyl ammonium chloride; 20 hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other 25 carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEENTM, PLURONICSTM or polyethylene glycol (PEG).

30 Compounds identified by the screening assays disclosed herein can be formulated in an analogous manner, using standard techniques well known in the art.

Lipofections or liposomes can also be used to deliver the PRO molecule into cells. Where antibody fragments are used, the smallest inhibitory fragment which specifically binds to the binding domain of the target protein is preferred. For example, based upon the variable region sequences of an antibody, peptide molecules can be designed which retain the ability to bind the target protein sequence. Such peptides can be 35 synthesized chemically and/or produced by recombinant DNA technology (see, e.g., Marasco *et al.*, *Proc. Natl. Acad. Sci. USA* **90**, 7889-7893 [1993]).

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition may comprise a cytotoxic agent, cytokine or

growth inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active PRO molecules may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-5 microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

10 The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Sustained-release preparations or the PRO molecules may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), 15 or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ -ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOTTM (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(*l*)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release 20 proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be 25 intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

N. Methods of Treatment

It is contemplated that the polypeptides, antibodies and other active compounds of the present invention may be used to treat various immune related diseases and conditions, such as NK cell diseases, 30 including those characterized by infiltration of inflammatory cells into a tissue, stimulation of NK cells, inhibition of NK cells, increased or decreased vascular permeability or the inhibition thereof.

Exemplary conditions or disorders to be treated with the polypeptides, antibodies and other compounds of the invention, include, but are not limited to systemic lupus erythematosus, rheumatoid arthritis, juvenile chronic arthritis, osteoarthritis, spondyloarthropathies, systemic sclerosis (scleroderma), 35 idiopathic inflammatory myopathies (dermatomyositis, polymyositis), Sjögren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia (immune pancytopenia, paroxysmal nocturnal hemoglobinuria), autoimmune thrombocytopenia (idiopathic thrombocytopenic purpura, immune-mediated thrombocytopenia), thyroiditis (Grave's disease, Hashimoto's thyroiditis, juvenile lymphocytic thyroiditis, atrophic thyroiditis), diabetes mellitus, immune-mediated renal disease (glomerulonephritis, tubulointerstitial 40 nephritis), demyelinating diseases of the central and peripheral nervous systems such as multiple sclerosis,

idiopathic demyelinating polyneuropathy or Guillain-Barré syndrome, and chronic inflammatory demyelinating polyneuropathy, hepatobiliary diseases such as infectious hepatitis (hepatitis A, B, C, D, E and other non-hepatotropic viruses), autoimmune chronic active hepatitis, primary biliary cirrhosis, granulomatous hepatitis, and sclerosing cholangitis, inflammatory bowel disease (ulcerative colitis: Crohn's disease), gluten-sensitive enteropathy, and Whipple's disease, autoimmune or immune-mediated skin diseases including bullous skin diseases, erythema multiforme and contact dermatitis, psoriasis, allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity and urticaria, immunologic diseases of the lung such as eosinophilic pneumonias, idiopathic pulmonary fibrosis and hypersensitivity pneumonitis, transplantation associated diseases including graft rejection and graft -versus-host-disease.

10 Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disease that mainly involves the synovial membrane of multiple joints with resultant injury to the articular cartilage. The pathogenesis is T lymphocyte dependent and is associated with the production of rheumatoid factors, auto-antibodies directed against self IgG, with the resultant formation of immune complexes that attain high levels in joint fluid and blood. These complexes in the joint may induce the marked infiltrate of 15 lymphocytes and NK cells into the synovium and subsequent marked synovial changes; the joint space/fluid if infiltrated by similar cells with the addition of numerous neutrophils. Tissues affected are primarily the joints, often in symmetrical pattern. However, extra-articular disease also occurs in two major forms. One form is the development of extra-articular lesions with ongoing progressive joint disease and typical lesions of pulmonary fibrosis, vasculitis, and cutaneous ulcers. The second form of extra-articular disease is the so 20 called Felty's syndrome which occurs late in the RA disease course, sometimes after joint disease has become quiescent, and involves the presence of neutropenia, thrombocytopenia and splenomegaly. This can be accompanied by vasculitis in multiple organs with formations of infarcts, skin ulcers and gangrene. Patients often also develop rheumatoid nodules in the subcutis tissue overlying affected joints; the nodules late stage have necrotic centers surrounded by a mixed inflammatory cell infiltrate. Other manifestations 25 which can occur in RA include: pericarditis, pleuritis, coronary arteritis, intestinal pneumonitis with pulmonary fibrosis, keratoconjunctivitis sicca, and rheumatoid nodules. As described above, Natural Killer cells are not believed to be involved in the early events of RA, but a study by Dalbeth et al., have found CD56 + NK cells in the synovial fluid of inflamed joints and these cells are believed to respond to IL-12 and IL-15 secreted by macrophages also found in synovial fluid from the inflamed joints (Dalbeth et al., 2002 30 Arthritis Rheum 46(7): 1763-72).

35 Juvenile chronic arthritis is a chronic idiopathic inflammatory disease which begins often at less than 16 years of age. Its phenotype has some similarities to RA; some patients which are rheumatoid factor positive are classified as juvenile rheumatoid arthritis. The disease is sub-classified into three major categories: pauciarticular, polyarticular, and systemic. The arthritis can be severe and is typically destructive and leads to joint ankylosis and retarded growth. Other manifestations can include chronic anterior uveitis and systemic amyloidosis.

40 Systemic sclerosis (scleroderma) has an unknown etiology. A hallmark of the disease is induration of the skin; likely this is induced by an active inflammatory process. Scleroderma can be localized or systemic; vascular lesions are common and endothelial cell injury in the microvasculature is an early and important event in the development of systemic sclerosis; the vascular injury may be immune mediated. An

immunologic basis is implied by the presence of mononuclear cell infiltrates in the cutaneous lesions and the presence of anti-nuclear antibodies in many patients. ICAM-1 is often upregulated on the cell surface of fibroblasts in skin lesions suggesting that T cell interaction with these cells may have a role in the pathogenesis of the disease. As well as T cells, NK cells are proposed to play a role in the progression of 5 scleroderma in patients where the disease has progressed late into the disease course, and in those patients who did not express anti-Scl 70 antibodies (Holcombe et al., 1995 Ann Rheum Dis 54(1): 69-72). Other organs involved include: the gastrointestinal tract: smooth muscle atrophy and fibrosis resulting in abnormal peristalsis/motility; kidney: concentric subendothelial intimal proliferation affecting small arcuate and interlobular arteries with resultant reduced renal cortical blood flow, results in proteinuria, azotemia and 10 hypertension; skeletal muscle: atrophy, interstitial fibrosis; inflammation; lung: interstitial pneumonitis and interstitial fibrosis; and heart: contraction band necrosis, scarring/fibrosis.

Idiopathic inflammatory myopathies including dermatomyositis, polymyositis and others are 15 disorders of chronic muscle inflammation of unknown etiology resulting in muscle weakness. Muscle injury/inflammation is often symmetric and progressive. Autoantibodies are associated with most forms. These myositis-specific autoantibodies are directed against and inhibit the function of components, proteins and RNA's, involved in protein synthesis.

Sarcoidosis is a condition of unknown etiology which is characterized by the presence of 20 epithelioid granulomas in nearly any tissue in the body; involvement of the lung is most common. The pathogenesis involves the persistence of activated macrophages and lymphoid cells at sites of the disease with subsequent chronic sequelae resultant from the release of locally and systemically active products released by these cell types.

Autoimmune hemolytic anemia including autoimmune hemolytic anemia, immune pancytopenia, and paroxysmal nocturnal hemoglobinuria is a result of production of antibodies that react with antigens expressed on the surface of red blood cells (and in some cases other blood cells including platelets as well) 25 and is a reflection of the removal of those antibody coated cells via complement mediated lysis and/or ADCC/Fc-receptor-mediated mechanisms.

Thyroiditis including Grave's disease, Hashimoto's thyroiditis, juvenile lymphocytic thyroiditis, and 30 atrophic thyroiditis, are the result of an autoimmune response against thyroid antigens with production of antibodies that react with proteins present in and often specific for the thyroid gland. Experimental models exist including spontaneous models: rats (BUF and BB rats) and chickens (obese chicken strain); inducible models: immunization of animals with either thyroglobulin, thyroid microsomal antigen (thyroid peroxidase). NK cell activity was specifically studied in patients with Graves' disease and Hashimoto's thyroiditis, and a significant reduction in NK cell activity was found in both patient populations (Wenzel et al., 1998 Thyroid 8(11):1019-1022).

Inflammatory and Fibrotic Lung Disease, including Eosinophilic Pneumonias; Idiopathic 35 Pulmonary Fibrosis, and Hypersensitivity Pneumonitis may involve a disregulated immune-inflammatory response. Inhibition of that response would be of therapeutic benefit.

Psoriasis is a T lymphocyte-mediated inflammatory disease. Lesions contain infiltrates of T lymphocytes, macrophages and antigen processing cells, and some neutrophils.

Other diseases in which intervention of the immune and/or inflammatory response have benefit are infectious disease including but not limited to viral infection (including but not limited to AIDS, hepatitis A, B, C, D, E and herpes) bacterial infection, fungal infections, and protozoal and parasitic infections. Molecules (or derivatives/agonists) which stimulate the immune reaction can be utilized therapeutically to 5 enhance the immune response to infectious agents, diseases of immunodeficiency (molecules/derivatives/agonists) which stimulate the immune reaction can be utilized therapeutically to enhance the immune response for conditions of inherited, acquired, infectious induced (as in HIV infection), or iatrogenic (*i.e.*, as from chemotherapy) immunodeficiency, and neoplasia.

It has been demonstrated that some human cancer patients develop an antibody and/or NK cell 10 response to antigens on neoplastic cells. It has also been shown in animal models of neoplasia that enhancement of the immune response can result in rejection or regression of that particular neoplasm. Molecules that enhance the NK cell response have utility *in vivo* in enhancing the immune response against neoplasia. Molecules which enhance the NK cell proliferative response (or small molecule agonists or antibodies that affected the same receptor in an agonistic fashion) can be used therapeutically to treat cancer. 15 Molecules that inhibit the NK cell response also function *in vivo* during neoplasia to suppress the immune response to a neoplasm; such molecules can either be expressed by the neoplastic cells themselves or their expression can be induced by the neoplasm in other cells. Antagonism of such inhibitory molecules (either with antibody, small molecule antagonists or other means) enhances immune-mediated tumor rejection.

Additionally, inhibition of molecules with proinflammatory properties may have therapeutic benefit 20 in reperfusion injury; stroke; myocardial infarction; atherosclerosis; acute lung injury; hemorrhagic shock; burn; sepsis/septic shock; acute tubular necrosis; endometriosis; degenerative joint disease and pancreatitis.

The compounds of the present invention, *e.g.*, polypeptides or antibodies, are administered to a 25 mammal, preferably a human, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation (intranasal, intrapulmonary) routes. Intravenous or inhaled administration of polypeptides and antibodies is preferred.

In immunoadjuvant therapy, other therapeutic regimens, such administration of an anti-cancer 30 agent, may be combined with the administration of the proteins, antibodies or compounds of the instant invention. For example, the patient to be treated with the immunoadjuvant of the invention may also receive an anti-cancer agent (chemotherapeutic agent) or radiation therapy. Preparation and dosing schedules for such chemotherapeutic agents may be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in *Chemotherapy Service* Ed., M.C. Perry, Williams & Wilkins, Baltimore, MD (1992). The chemotherapeutic agent may precede, or follow administration of the immunoadjuvant or may be given 35 simultaneously therewith. Additionally, an anti-estrogen compound such as tamoxifen or an anti-progesterone such as onapristone (see, EP 616812) may be given in dosages known for such molecules.

It may be desirable to also administer antibodies against other immune disease associated or tumor 40 associated antigens, such as antibodies which bind to CD20, CD11a, CD18, ErbB2, EGFR, ErbB3, ErbB4, or vascular endothelial factor (VEGF). Alternatively, or in addition, two or more antibodies binding the same or two or more different antigens disclosed herein may be coadministered to the patient. Sometimes, it

may be beneficial to also administer one or more cytokines to the patient. In one embodiment, the PRO polypeptides are coadministered with a growth inhibitory agent. For example, the growth inhibitory agent may be administered first, followed by a PRO polypeptide. However, simultaneous administration or administration first is also contemplated. Suitable dosages for the growth inhibitory agent are those 5 presently used and may be lowered due to the combined action (synergy) of the growth inhibitory agent and the PRO polypeptide.

For the treatment or reduction in the severity of immune related disease, the appropriate dosage of a compound of the invention will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the agent is administered for preventive or therapeutic purposes, previous 10 therapy, the patient's clinical history and response to the compound, and the discretion of the attending physician. The compound is suitably administered to the patient at one time or over a series of treatments.

For example, depending on the type and severity of the disease, about 1 μ g/kg to 15 mg/kg (e.g., 0.1-20 mg/kg) of polypeptide or antibody is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily 15 dosage might range from about 1 μ g/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

O. Articles of Manufacture

In another embodiment of the invention, an article of manufacture containing materials (e.g., comprising a PRO molecule) useful for the diagnosis or treatment of the disorders described above is provided. The article of manufacture comprises a container and an instruction. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for diagnosing or 20 treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The active agent in the composition is usually a polypeptide or an antibody of the invention. An instruction or label on, or associated with, the container indicates that the composition is used for diagnosing or treating the condition of choice. The article of manufacture may further comprise a second container comprising a 25 pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

P. Diagnosis and Prognosis of Immune Related Disease

Cell surface proteins, such as proteins which are overexpressed in certain immune related diseases, 35 are excellent targets for drug candidates or disease treatment. The same proteins along with secreted proteins encoded by the genes amplified in immune related disease states find additional use in the diagnosis and prognosis of these diseases. For example, antibodies directed against the protein products of genes amplified in multiple sclerosis, rheumatoid arthritis, or another immune related disease, can be used as diagnostics or prognostics.

For example, antibodies, including antibody fragments, can be used to qualitatively or quantitatively detect the expression of proteins encoded by amplified or overexpressed genes ("marker gene products"). The antibody preferably is equipped with a detectable, e.g., fluorescent label, and binding can be monitored by light microscopy, flow cytometry, fluorimetry, or other techniques known in the art. These 5 techniques are particularly suitable, if the overexpressed gene encodes a cell surface protein. Such binding assays are performed essentially as described above.

In situ detection of antibody binding to the marker gene products can be performed, for example, by immunofluorescence or immunoelectron microscopy. For this purpose, a histological specimen is removed from the patient, and a labeled antibody is applied to it, preferably by overlaying the antibody on a biological 10 sample. This procedure also allows for determining the distribution of the marker gene product in the tissue examined. It will be apparent for those skilled in the art that a wide variety of histological methods are readily available for *in situ* detection.

The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

15 All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

EXAMPLES

Commercially available reagents referred to in the examples were used according to manufacturer's instructions unless otherwise indicated. The source of those cells identified in the following examples, and 20 throughout the specification, by ATCC accession numbers is the American Type Culture Collection, Manassas, VA.

EXAMPLE 1: Microarray analysis of NK cells.

Nucleic acid microarrays, often containing thousands of gene sequences, are useful for identifying 25 differentially expressed genes in diseased tissues as compared to their normal counterparts. Using nucleic acid microarrays, test and control mRNA samples from test and control tissue samples are reverse transcribed and labeled to generate cDNA probes. The cDNA probes are then hybridized to an array of nucleic acids immobilized on a solid support. The array is configured such that the sequence and position of each member of the array is known. For example, a selection of genes known to be expressed in certain 30 disease states may be arrayed on a solid support. Hybridization of a labeled probe with a particular array member indicates that the sample from which the probe was derived expresses that gene. If the hybridization signal of a probe from a test (in this instance, activated NK cells) sample is greater than hybridization signal of a probe from a control (in this instance, resting NK cells) sample, the gene or genes 35 expressed in the test tissue are identified. The implication of this result is that an overexpressed protein in a test tissue is useful not only as a diagnostic marker for the presence of the disease condition, but also as a therapeutic target for treatment of the disease condition.

The methodology of hybridization of nucleic acids and microarray technology is well known in the art. In one example, the specific preparation of nucleic acids for hybridization and probes, slides, and hybridization conditions are all detailed in PCT Patent Application Serial No. PCT/US01/10482, filed on 40 March 30, 2001 and which is herein incorporated by reference.

For this experiment, peripheral blood NK cells were isolated from leukopacks by negative selection using the NK cell isolation kit with the Miltenyi MACS™ magnetic cell sorting system (Miltenyi Biotec). Cell purity was confirmed by staining with PE anti-CD56 for FACS analysis. Purity of cell preps ranged from 89% to 96%. Cell culture conditions were as follows: Set up in-vitro cultures in 6 well plates - 5 ml cultures/well using RPMI 1640, 10%heat inactivated FBS, 100 units/mL of Penicillin, 100 mg/mL of streptomycin, 2 mM L-glutamine, and 5.5 x 10-5 Beta-mercaptoethanol. The cells were cultured for 16 hours in the base media described above, but with the addition of IL-12 (10nM), IL-15(10nM) or JAM-1(10nM). An untreated control sample was also cultured for 16 hours (16 Hour Timepoint). Activation of NK cells was monitored by FACS for cell surface expression of CD56 and CD69. Uncultured, untreated CD56(+) cells, for use as a control (Time 0). RNA was isolated using the Qiagen Midi™ preps as per the instructions in the manual with the addition of an on-column DNase I digestion after the first RW1 wash step. RNA was eluted in RNase free water and subsequently concentrated by ethanol precipitation. Precipitated RNA was taken up in nuclease free water to a final minimum concentration of 0.5 micrograms per microliter.

15 The isolated mRNA was labeled and hybridized to Affimax™ (Affymetrix Inc. Santa Clara, CA) microarray chips and proprietary Genentech microarrays. The cells harvested at Time 0 timepoint, and the 16 Hour timepoint were subjected to the same analysis. Genes were compared whose expression was upregulated at the 16 Hour Timepoint as compared to untreated 16 Hour Timepoint and uncultured, untreated Time 0 timepoints.

20 Below are the results of these experiments, demonstrating that various PRO polypeptides of the present invention are differentially expressed in activated NK cells after 16 hours in culture with IL-12, IL-15 or JAM2 as compared to normal resting NK cells cultured for 16 hours or resting NK cells harvested at Time 0. Specifically, Figure 438A-B, Figure 511, Figure 687, Figure 697, Figure 703, Figure 1057, Figure 1084, Figure 1126 and Figure 1228 identify sequences that are highly overexpressed in activated NK cells as compared to normal resting NK cells.

25 As described above, these data demonstrate that the PRO polypeptides of the present invention are useful not only as diagnostic markers for the presence of one or more immune disorders, but also serve as therapeutic targets for the treatment of those immune disorders.

The Figures 1-1477 show the nucleic acids of the invention and their encoded PRO polypeptides.

30

EXAMPLE 2: Use of PRO as a hybridization probe

The following method describes use of a nucleotide sequence encoding PRO as a hybridization probe.

35 DNA comprising the coding sequence of full-length or mature PRO as disclosed herein is employed as a probe to screen for homologous DNAs (such as those encoding naturally-occurring variants of PRO) in human tissue cDNA libraries or human tissue genomic libraries.

Hybridization and washing of filters containing either library DNAs is performed under the following high stringency conditions. Hybridization of radiolabeled PRO-derived probe to the filters is performed in a solution of 50% formamide, 5x SSC, 0.1% SDS, 0.1% sodium pyrophosphate, 50 mM

sodium phosphate, pH 6.8, 2x Denhardt's solution, and 10% dextran sulfate at 42°C for 20 hours. Washing of the filters is performed in an aqueous solution of 0.1x SSC and 0.1% SDS at 42°C.

DNAs having a desired sequence identity with the DNA encoding full-length native sequence PRO can then be identified using standard techniques known in the art.

5

EXAMPLE 3: Expression of PRO in *E. coli*

This example illustrates preparation of an unglycosylated form of PRO by recombinant expression in *E. coli*.

The DNA sequence encoding PRO is initially amplified using selected PCR primers. The primers 10 should contain restriction enzyme sites which correspond to the restriction enzyme sites on the selected expression vector. A variety of expression vectors may be employed. An example of a suitable vector is pBR322 (derived from *E. coli*; see Bolivar et al., *Gene*, 2:95 (1977)) which contains genes for ampicillin and tetracycline resistance. The vector is digested with restriction enzyme and dephosphorylated. The PCR amplified sequences are then ligated into the vector. The vector will preferably include sequences which 15 encode for an antibiotic resistance gene, a trp promoter, a polyhis leader (including the first six STII codons, polyhis sequence, and enterokinase cleavage site), the PRO coding region, lambda transcriptional terminator, and an argU gene.

The ligation mixture is then used to transform a selected *E. coli* strain using the methods described 20 in Sambrook et al., *supra*. Transformants are identified by their ability to grow on LB plates and antibiotic resistant colonies are then selected. Plasmid DNA can be isolated and confirmed by restriction analysis and DNA sequencing.

Selected clones can be grown overnight in liquid culture medium such as LB broth supplemented with antibiotics. The overnight culture may subsequently be used to inoculate a larger scale culture. The cells are then grown to a desired optical density, during which the expression promoter is turned on.

25 After culturing the cells for several more hours, the cells can be harvested by centrifugation. The cell pellet obtained by the centrifugation can be solubilized using various agents known in the art, and the solubilized PRO protein can then be purified using a metal chelating column under conditions that allow tight binding of the protein.

PRO may be expressed in *E. coli* in a poly-His tagged form, using the following procedure. The 30 DNA encoding PRO is initially amplified using selected PCR primers. The primers will contain restriction enzyme sites which correspond to the restriction enzyme sites on the selected expression vector, and other useful sequences providing for efficient and reliable translation initiation, rapid purification on a metal chelation column, and proteolytic removal with enterokinase. The PCR-amplified, poly-His tagged sequences are then ligated into an expression vector, which is used to transform an *E. coli* host based on 35 strain 52 (W3110 fuhA(tonA) lon galE rpoHts(htpRts) clpP(lacIq)). Transformants are first grown in LB containing 50 mg/ml carbenicillin at 30°C with shaking until an O.D.600 of 3-5 is reached. Cultures are then diluted 50-100 fold into CRAP media (prepared by mixing 3.57 g (NH₄)₂SO₄, 0.71 g sodium citrate•2H₂O, 1.07 g KCl, 5.36 g Difco yeast extract, 5.36 g Sheffield hycase SF in 500 mL water, as well as 110 mM MPOS, pH 7.3, 0.55% (w/v) glucose and 7 mM MgSO₄) and grown for approximately 20-30 hours

at 30°C with shaking. Samples are removed to verify expression by SDS-PAGE analysis, and the bulk culture is centrifuged to pellet the cells. Cell pellets are frozen until purification and refolding.

E. coli paste from 0.5 to 1 L fermentations (6-10 g pellets) is resuspended in 10 volumes (w/v) in 7 M guanidine, 20 mM Tris, pH 8 buffer. Solid sodium sulfite and sodium tetrathionate is added to make final 5 concentrations of 0.1M and 0.02 M, respectively, and the solution is stirred overnight at 4°C. This step results in a denatured protein with all cysteine residues blocked by sulfitolization. The solution is centrifuged at 40,000 rpm in a Beckman Ultracentrifuge for 30 min. The supernatant is diluted with 3-5 volumes of metal chelate column buffer (6 M guanidine, 20 mM Tris, pH 7.4) and filtered through 0.22 micron filters to clarify. The clarified extract is loaded onto a 5 ml Qiagen Ni-NTA metal chelate column equilibrated in the 10 metal chelate column buffer. The column is washed with additional buffer containing 50 mM imidazole (Calbiochem, Ultrol grade), pH 7.4. The protein is eluted with buffer containing 250 mM imidazole. Fractions containing the desired protein are pooled and stored at 4°C. Protein concentration is estimated by its absorbance at 280 nm using the calculated extinction coefficient based on its amino acid sequence.

The proteins are refolded by diluting the sample slowly into freshly prepared refolding buffer 15 consisting of: 20 mM Tris, pH 8.6, 0.3 M NaCl, 2.5 M urea, 5 mM cysteine, 20 mM glycine and 1 mM EDTA. Refolding volumes are chosen so that the final protein concentration is between 50 to 100 micrograms/ml. The refolding solution is stirred gently at 4°C for 12-36 hours. The refolding reaction is quenched by the addition of TFA to a final concentration of 0.4% (pH of approximately 3). Before further 20 purification of the protein, the solution is filtered through a 0.22 micron filter and acetonitrile is added to 2-10% final concentration. The refolded protein is chromatographed on a Poros R1/H reversed phase column using a mobile buffer of 0.1% TFA with elution with a gradient of acetonitrile from 10 to 80%. Aliquots of fractions with A280 absorbance are analyzed on SDS polyacrylamide gels and fractions 25 containing homogeneous refolded protein are pooled. Generally, the properly refolded species of most proteins are eluted at the lowest concentrations of acetonitrile since those species are the most compact with their hydrophobic interiors shielded from interaction with the reversed phase resin. Aggregated species are usually eluted at higher acetonitrile concentrations. In addition to resolving misfolded forms of proteins from the desired form, the reversed phase step also removes endotoxin from the samples.

Fractions containing the desired folded PRO polypeptide are pooled and the acetonitrile removed 30 using a gentle stream of nitrogen directed at the solution. Proteins are formulated into 20 mM Hepes, pH 6.8 with 0.14 M sodium chloride and 4% mannitol by dialysis or by gel filtration using G25 Superfine (Pharmacia) resins equilibrated in the formulation buffer and sterile filtered.

Many of the PRO polypeptides disclosed herein were successfully expressed as described above.

EXAMPLE 4: Expression of PRO in mammalian cells

35 This example illustrates preparation of a potentially glycosylated form of PRO by recombinant expression in mammalian cells.

The vector, pRK5 (see EP 307,247, published March 15, 1989), is employed as the expression 40 vector. Optionally, the PRO DNA is ligated into pRK5 with selected restriction enzymes to allow insertion of the PRO DNA using ligation methods such as described in Sambrook et al., supra. The resulting vector is called pRK5-PRO.

In one embodiment, the selected host cells may be 293 cells. Human 293 cells (ATCC CCL 1573) are grown to confluence in tissue culture plates in medium such as DMEM supplemented with fetal calf serum and optionally, nutrient components and/or antibiotics. About 10 μ g pRK5-PRO DNA is mixed with about 1 μ g DNA encoding the VA RNA gene [Thimmapaya et al., *Cell*, 31:543 (1982)] and dissolved in 5 500 μ l of 1 mM Tris-HCl, 0.1 mM EDTA, 0.227 M CaCl_2 . To this mixture is added, dropwise, 500 μ l of 50 mM HEPES (pH 7.35), 280 mM NaCl, 1.5 mM NaPO_4 , and a precipitate is allowed to form for 10 minutes at 25°C. The precipitate is suspended and added to the 293 cells and allowed to settle for about four hours at 37°C. The culture medium is aspirated off and 2 ml of 20% glycerol in PBS is added for 30 seconds. The 293 cells are then washed with serum free medium, fresh medium is added and the cells are incubated for 10 about 5 days.

Approximately 24 hours after the transfections, the culture medium is removed and replaced with culture medium (alone) or culture medium containing 200 $\mu\text{Ci}/\text{ml}$ ^{35}S -cysteine and 200 $\mu\text{Ci}/\text{ml}$ ^{35}S -methionine. After a 12 hour incubation, the conditioned medium is collected, concentrated on a spin filter, and loaded onto a 15% SDS gel. The processed gel may be dried and exposed to film for a selected period 15 of time to reveal the presence of PRO polypeptide. The cultures containing transfected cells may undergo further incubation (in serum free medium) and the medium is tested in selected bioassays.

In an alternative technique, PRO may be introduced into 293 cells transiently using the dextran sulfate method described by Sompanyrac et al., *Proc. Natl. Acad. Sci.*, 78:7575 (1981). 293 cells are grown to maximal density in a spinner flask and 700 μ g pRK5-PRO DNA is added. The cells are first concentrated 20 from the spinner flask by centrifugation and washed with PBS. The DNA-dextran precipitate is incubated on the cell pellet for four hours. The cells are treated with 20% glycerol for 90 seconds, washed with tissue culture medium, and re-introduced into the spinner flask containing tissue culture medium, 5 $\mu\text{g}/\text{ml}$ bovine insulin and 0.1 $\mu\text{g}/\text{ml}$ bovine transferrin. After about four days, the conditioned media is centrifuged and filtered to remove cells and debris. The sample containing expressed PRO can then be concentrated and 25 purified by any selected method, such as dialysis and/or column chromatography.

In another embodiment, PRO can be expressed in CHO cells. The pRK5-PRO can be transfected into CHO cells using known reagents such as CaPO_4 or DEAE-dextran. As described above, the cell cultures can be incubated, and the medium replaced with culture medium (alone) or medium containing a radiolabel such as ^{35}S -methionine. After determining the presence of PRO polypeptide, the culture medium 30 may be replaced with serum free medium. Preferably, the cultures are incubated for about 6 days, and then the conditioned medium is harvested. The medium containing the expressed PRO can then be concentrated and purified by any selected method.

Epitope-tagged PRO may also be expressed in host CHO cells. The PRO may be subcloned out of the pRK5 vector. The subclone insert can undergo PCR to fuse in frame with a selected epitope tag such as 35 a poly-his tag into a Baculovirus expression vector. The poly-his tagged PRO insert can then be subcloned into a SV40 promoter/enhancer containing vector containing a selection marker such as DHFR for selection of stable clones. Finally, the CHO cells can be transfected (as described above) with the SV40 promoter/enhancer containing vector. Labeling may be performed, as described above, to verify expression. The culture medium containing the expressed poly-His tagged PRO can then be concentrated and purified 40 by any selected method, such as by Ni^{2+} -chelate affinity chromatography.

PRO may also be expressed in CHO and/or COS cells by a transient expression procedure or in CHO cells by another stable expression procedure.

Stable expression in CHO cells is performed using the following procedure. The proteins are expressed as an IgG construct (immunoadhesin), in which the coding sequences for the soluble forms (e.g. 5 extracellular domains) of the respective proteins are fused to an IgG1 constant region sequence containing the hinge, CH2 and CH2 domains and/or is a poly-His tagged form.

Following PCR amplification, the respective DNAs are subcloned in a CHO expression vector using standard techniques as described in Ausubel et al., *Current Protocols of Molecular Biology*, Unit 3.16, John Wiley and Sons (1997). CHO expression vectors are constructed to have compatible restriction sites 5' 10 and 3' of the DNA of interest to allow the convenient shuttling of cDNA's. The vector used expression in CHO cells is as described in Lucas et al., *Nucl. Acids Res.* 24:9 (1774-1779 (1996), and uses the SV40 early promoter/enhancer to drive expression of the cDNA of interest and dihydrofolate reductase (DHFR). DHFR expression permits selection for stable maintenance of the plasmid following transfection.

Twelve micrograms of the desired plasmid DNA is introduced into approximately 10 million CHO 15 cells using commercially available transfection reagents Superfect® (Qiagen), Dospel® or Fugene® (Boehringer Mannheim). The cells are grown as described in Lucas et al., *supra*. Approximately 3×10^7 cells are frozen in an ampule for further growth and production as described below.

The ampules containing the plasmid DNA are thawed by placement into water bath and mixed by 20 vortexing. The contents are pipetted into a centrifuge tube containing 10 mL of media and centrifuged at 1000 rpm for 5 minutes. The supernatant is aspirated and the cells are resuspended in 10 mL of selective media (0.2 μ m filtered PS20 with 5% 0.2 μ m diafiltered fetal bovine serum). The cells are then aliquoted into a 100 mL spinner containing 90 mL of selective media. After 1-2 days, the cells are transferred into a 250 mL spinner filled with 150 mL selective growth medium and incubated at 37°C. After another 2-3 days, 250 mL, 500 mL and 2000 mL spinners are seeded with 3×10^5 cells/mL. The cell media is exchanged with 25 fresh media by centrifugation and resuspension in production medium. Although any suitable CHO media may be employed, a production medium described in U.S. Patent No. 5,122,469, issued June 16, 1992 may actually be used. A 3L production spinner is seeded at 1.2×10^6 cells/mL. On day 0, pH is determined. On day 1, the spinner is sampled and sparging with filtered air is commenced. On day 2, the spinner is sampled, the temperature shifted to 33°C, and 30 mL of 500 g/L glucose and 0.6 mL of 10% antifoam (e.g., 35% 30 polydimethylsiloxane emulsion, Dow Corning 365 Medical Grade Emulsion) taken. Throughout the production, the pH is adjusted as necessary to keep it at around 7.2. After 10 days, or until the viability dropped below 70%, the cell culture is harvested by centrifugation and filtering through a 0.22 μ m filter. The filtrate was either stored at 4°C or immediately loaded onto columns for purification.

For the poly-His tagged constructs, the proteins are purified using a Ni-NTA column (Qiagen). 35 Before purification, imidazole is added to the conditioned media to a concentration of 5 mM. The conditioned media is pumped onto a 6 ml Ni-NTA column equilibrated in 20 mM Hepes, pH 7.4, buffer containing 0.3 M NaCl and 5 mM imidazole at a flow rate of 4-5 mL/min. at 4°C. After loading, the column is washed with additional equilibration buffer and the protein eluted with equilibration buffer containing 0.25 M imidazole. The highly purified protein is subsequently desalted into a storage buffer containing 10

mM Hepes, 0.14 M NaCl and 4% mannitol, pH 6.8, with a 25 ml G25 Superfine (Pharmacia) column and stored at -80°C.

5 Immunoadhesin (Fc-containing) constructs are purified from the conditioned media as follows. The conditioned medium is pumped onto a 5 ml Protein A column (Pharmacia) which had been equilibrated in 20 mM Na phosphate buffer, pH 6.8. After loading, the column is washed extensively with equilibration buffer before elution with 100 mM citric acid, pH 3.5. The eluted protein is immediately neutralized by collecting 1 ml fractions into tubes containing 275 µl of 1 M Tris buffer, pH 9. The highly purified protein is subsequently desalted into storage buffer as described above for the poly-His tagged proteins. The homogeneity is assessed by SDS polyacrylamide gels and by N-terminal amino acid sequencing by Edman 10 degradation.

Many of the PRO polypeptides disclosed herein were successfully expressed as described above.

EXAMPLE 5: Expression of PRO in Yeast

The following method describes recombinant expression of PRO in yeast.

15 First, yeast expression vectors are constructed for intracellular production or secretion of PRO from the ADH2/GAPDH promoter. DNA encoding PRO and the promoter is inserted into suitable restriction enzyme sites in the selected plasmid to direct intracellular expression of PRO. For secretion, DNA encoding PRO can be cloned into the selected plasmid, together with DNA encoding the ADH2/GAPDH promoter, a native PRO signal peptide or other mammalian signal peptide, or, for example, a yeast alpha-factor or 20 invertase secretory signal/leader sequence, and linker sequences (if needed) for expression of PRO.

Yeast cells, such as yeast strain AB110, can then be transformed with the expression plasmids described above and cultured in selected fermentation media. The transformed yeast supernatants can be analyzed by precipitation with 10% trichloroacetic acid and separation by SDS-PAGE, followed by staining of the gels with Coomassie Blue stain.

25 Recombinant PRO can subsequently be isolated and purified by removing the yeast cells from the fermentation medium by centrifugation and then concentrating the medium using selected cartridge filters. The concentrate containing PRO may further be purified using selected column chromatography resins.

Many of the PRO polypeptides disclosed herein were successfully expressed as described above.

30 **EXAMPLE 6: Expression of PRO in Baculovirus-Infected Insect Cells**

The following method describes recombinant expression of PRO in Baculovirus-infected insect cells.

The sequence coding for PRO is fused upstream of an epitope tag contained within a baculovirus expression vector. Such epitope tags include poly-his tags and immunoglobulin tags (like Fc regions of 35 IgG). A variety of plasmids may be employed, including plasmids derived from commercially available plasmids such as pVL1393 (Novagen). Briefly, the sequence encoding PRO or the desired portion of the coding sequence of PRO such as the sequence encoding the extracellular domain of a transmembrane protein or the sequence encoding the mature protein if the protein is extracellular is amplified by PCR with primers complementary to the 5' and 3' regions. The 5' primer may incorporate flanking (selected) restriction

enzyme sites. The product is then digested with those selected restriction enzymes and subcloned into the expression vector.

Recombinant baculovirus is generated by co-transfected the above plasmid and BaculoGoldTM virus DNA (Pharmingen) into *Spodoptera frugiperda* ("Sf9") cells (ATCC CRL 1711) using lipofectin (commercially available from GIBCO-BRL). After 4 - 5 days of incubation at 28°C, the released viruses are harvested and used for further amplifications. Viral infection and protein expression are performed as described by O'Reilley et al., *Baculovirus expression vectors: A Laboratory Manual*, Oxford: Oxford University Press (1994).

Expressed poly-his tagged PRO can then be purified, for example, by Ni²⁺-chelate affinity chromatography as follows. Extracts are prepared from recombinant virus-infected Sf9 cells as described by Rupert et al., *Nature*, 362:175-179 (1993). Briefly, Sf9 cells are washed, resuspended in sonication buffer (25 mL Hepes, pH 7.9; 12.5 mM MgCl₂; 0.1 mM EDTA; 10% glycerol; 0.1% NP-40; 0.4 M KCl), and sonicated twice for 20 seconds on ice. The sonicates are cleared by centrifugation, and the supernatant is diluted 50-fold in loading buffer (50 mM phosphate, 300 mM NaCl, 10% glycerol, pH 7.8) and filtered through a 0.45 μm filter. A Ni²⁺-NTA agarose column (commercially available from Qiagen) is prepared with a bed volume of 5 mL, washed with 25 mL of water and equilibrated with 25 mL of loading buffer. The filtered cell extract is loaded onto the column at 0.5 mL per minute. The column is washed to baseline A₂₈₀ with loading buffer, at which point fraction collection is started. Next, the column is washed with a secondary wash buffer (50 mM phosphate; 300 mM NaCl, 10% glycerol, pH 6.0), which elutes nonspecifically bound protein. After reaching A₂₈₀ baseline again, the column is developed with a 0 to 500 mM Imidazole gradient in the secondary wash buffer. One mL fractions are collected and analyzed by SDS-PAGE and silver staining or Western blot with Ni²⁺-NTA-conjugated to alkaline phosphatase (Qiagen). Fractions containing the eluted His₁₀-tagged PRO are pooled and dialyzed against loading buffer.

Alternatively, purification of the IgG tagged (or Fc tagged) PRO can be performed using known chromatography techniques, including for instance, Protein A or protein G column chromatography.

Many of the PRO polypeptides disclosed herein were successfully expressed as described above.

EXAMPLE 7: Preparation of Antibodies that Bind PRO

This example illustrates preparation of monoclonal antibodies which can specifically bind PRO. Techniques for producing the monoclonal antibodies are known in the art and are described, for instance, in Goding, *supra*. Immunogens that may be employed include purified PRO, fusion proteins containing PRO, and cells expressing recombinant PRO on the cell surface. Selection of the immunogen can be made by the skilled artisan without undue experimentation.

Mice, such as Balb/c, are immunized with the PRO immunogen emulsified in complete Freund's adjuvant and injected subcutaneously or intraperitoneally in an amount from 1-100 micrograms. Alternatively, the immunogen is emulsified in MPL-TDM adjuvant (Ribi Immunochemical Research, Hamilton, MT) and injected into the animal's hind foot pads. The immunized mice are then boosted 10 to 12 days later with additional immunogen emulsified in the selected adjuvant. Thereafter, for several weeks, the mice may also be boosted with additional immunization injections. Serum samples may be periodically obtained from the mice by retro-orbital bleeding for testing in ELISA assays to detect anti-PRO antibodies.

After a suitable antibody titer has been detected, the animals "positive" for antibodies can be injected with a final intravenous injection of PRO. Three to four days later, the mice are sacrificed and the spleen cells are harvested. The spleen cells are then fused (using 35% polyethylene glycol) to a selected murine myeloma cell line such as P3X63AgU.1, available from ATCC, No. CRL 1597. The fusions 5 generate hybridoma cells which can then be plated in 96 well tissue culture plates containing HAT (hypoxanthine, aminopterin, and thymidine) medium to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

The hybridoma cells will be screened in an ELISA for reactivity against PRO. Determination of "positive" hybridoma cells secreting the desired monoclonal antibodies against PRO is within the skill in the 10 art.

The positive hybridoma cells can be injected intraperitoneally into syngeneic Balb/c mice to produce ascites containing the anti-PRO monoclonal antibodies. Alternatively, the hybridoma cells can be grown in tissue culture flasks or roller bottles. Purification of the monoclonal antibodies produced in the ascites can be accomplished using ammonium sulfate precipitation, followed by gel exclusion 15 chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can be employed.

EXAMPLE 8: Purification of PRO Polypeptides Using Specific Antibodies

Native or recombinant PRO polypeptides may be purified by a variety of standard techniques in the 20 art of protein purification. For example, pro-PRO polypeptide, mature PRO polypeptide, or pre-PRO polypeptide is purified by immunoaffinity chromatography using antibodies specific for the PRO polypeptide of interest. In general, an immunoaffinity column is constructed by covalently coupling the anti-PRO polypeptide antibody to an activated chromatographic resin.

25 Polyclonal immunoglobulins are prepared from immune sera either by precipitation with ammonium sulfate or by purification on immobilized Protein A (Pharmacia LKB Biotechnology, Piscataway, N.J.). Likewise, monoclonal antibodies are prepared from mouse ascites fluid by ammonium sulfate precipitation or chromatography on immobilized Protein A. Partially purified immunoglobulin is covalently attached to a chromatographic resin such as CnBr-activated SEPHAROSETM (Pharmacia LKB Biotechnology). The antibody is coupled to the resin, the resin is blocked, and the derivative resin is washed 30 according to the manufacturer's instructions.

Such an immunoaffinity column is utilized in the purification of PRO polypeptide by preparing a fraction from cells containing PRO polypeptide in a soluble form. This preparation is derived by solubilization of the whole cell or of a subcellular fraction obtained via differential centrifugation by the addition of detergent or by other methods well known in the art. Alternatively, soluble PRO polypeptide 35 containing a signal sequence may be secreted in useful quantity into the medium in which the cells are grown.

A soluble PRO polypeptide-containing preparation is passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of PRO polypeptide (e.g., high ionic strength buffers in the presence of detergent). Then, the column is eluted under conditions that disrupt

antibody/PRO polypeptide binding (*e.g.*, a low pH buffer such as approximately pH 2-3, or a high concentration of a chaotrope such as urea or thiocyanate ion), and PRO polypeptide is collected.

EXAMPLE 9: Drug Screening

5 This invention is particularly useful for screening compounds by using PRO polypeptides or binding fragment thereof in any of a variety of drug screening techniques. The PRO polypeptide or fragment employed in such a test may either be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the PRO polypeptide or fragment.

10 Drugs are screened against such transformed cells in competitive binding assays. Such cells, either in viable or fixed form, can be used for standard binding assays. One may measure, for example, the formation of complexes between PRO polypeptide or a fragment and the agent being tested. Alternatively, one can examine the diminution in complex formation between the PRO polypeptide and its target cell or target receptors caused by the agent being tested.

15 Thus, the present invention provides methods of screening for drugs or any other agents which can affect a PRO polypeptide-associated disease or disorder. These methods comprise contacting such an agent with a PRO polypeptide or fragment thereof and assaying (i) for the presence of a complex between the agent and the PRO polypeptide or fragment, or (ii) for the presence of a complex between the PRO polypeptide or fragment and the cell, by methods well known in the art. In such competitive binding assays, 20 the PRO polypeptide or fragment is typically labeled. After suitable incubation, free PRO polypeptide or fragment is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of the particular agent to bind to PRO polypeptide or to interfere with the PRO polypeptide/cell complex.

25 Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to a polypeptide and is described in detail in WO 84/03564, published on September 13, 1984. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. As applied to a PRO polypeptide, the peptide test compounds are reacted with PRO polypeptide and washed. Bound PRO polypeptide is detected by methods well known in the art. Purified PRO polypeptide can also be coated directly onto plates for use in the 30 aforementioned drug screening techniques. In addition, non-neutralizing antibodies can be used to capture the peptide and immobilize it on the solid support.

35 This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding PRO polypeptide specifically compete with a test compound for binding to PRO polypeptide or fragments thereof. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with PRO polypeptide.

EXAMPLE 10: Rational Drug Design

The goal of rational drug design is to produce structural analogs of biologically active polypeptide of interest (*i.e.*, a PRO polypeptide) or of small molecules with which they interact, *e.g.*, agonists, 40 antagonists, or inhibitors. Any of these examples can be used to fashion drugs which are more active or

stable forms of the PRO polypeptide or which enhance or interfere with the function of the PRO polypeptide *in vivo* (c.f., Hodgson, *Bio/Technology*, 9: 19-21 (1991)).

In one approach, the three-dimensional structure of the PRO polypeptide, or of a PRO polypeptide-inhibitor complex, is determined by x-ray crystallography, by computer modeling or, most typically, by a combination of the two approaches. Both the shape and charges of the PRO polypeptide must be ascertained to elucidate the structure and to determine active site(s) of the molecule. Less often, useful information regarding the structure of the PRO polypeptide may be gained by modeling based on the structure of homologous proteins. In both cases, relevant structural information is used to design analogous PRO polypeptide-like molecules or to identify efficient inhibitors. Useful examples of rational drug design may include molecules which have improved activity or stability as shown by Braxton and Wells, *Biochemistry*, 31:7796-7801 (1992) or which act as inhibitors, agonists, or antagonists of native peptides as shown by Athauda *et al.*, *J. Biochem.*, 113:742-746 (1993).

It is also possible to isolate a target-specific antibody, selected by functional assay, as described above, and then to solve its crystal structure. This approach, in principle, yields a pharmacore upon which subsequent drug design can be based. It is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies (anti-ids) to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-ids would be expected to be an analog of the original receptor. The anti-id could then be used to identify and isolate peptides from banks of chemically or biologically produced peptides. The isolated peptides would then act as the pharmacore.

By virtue of the present invention, sufficient amounts of the PRO polypeptide may be made available to perform such analytical studies as X-ray crystallography. In addition, knowledge of the PRO polypeptide amino acid sequence provided herein will provide guidance to those employing computer modeling techniques in place of or in addition to x-ray crystallography.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by the construct deposited, since the deposited embodiment is intended as a single illustration of certain aspects of the invention and any constructs that are functionally equivalent are within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustrations that it represents. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

The claims defining the invention are as follows:

1. A method of diagnosing an immune related disease in a mammal, said method comprising detecting the level of expression of a gene encoding a PRO polypeptide of the invention as described in Figure 1400 (SEQ ID NO: 1400), (a) in a test sample of tissue cells obtained from the mammal, and (b) in a control sample of known normal tissue cells of the same cell type, wherein a higher or lower level of expression of said gene in the test sample as compared to the control sample is indicative of the presence of an immune related disease in the mammal from which the test tissue cells were obtained, wherein the immune related disease is systemic lupus erythematosis, an idiopathic inflammatory myopathy, Sjögren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, thyroiditis, diabetes mellitus, immune-mediated renal disease, a demyelinating disease of the central or peripheral nervous system, idiopathic demyelinating polyneuropathy, Guillain-Barré syndrome, a chronic inflammatory demyelinating polyneuropathy, a hepatobiliary disease, infectious or autoimmune chronic active hepatitis, primary biliary cirrhosis, granulomatous hepatitis, sclerosing cholangitis, inflammatory bowel disease, gluten-sensitive enteropathy, Whipple's disease, an autoimmune or immune-mediated skin disease, a bullous skin disease, erythema multiforme, contact dermatitis, psoriasis, an allergic disease, asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity, urticaria, an immunologic disease of the lung, eosinophilic pneumonias, idiopathic pulmonary fibrosis, hypersensitivity pneumonitis, a transplantation associated disease, graft rejection or graft-versus-host-disease.
2. A method of diagnosing an immune related disease in a mammal, said method comprising detecting the level of expression of a gene as described in Figure 1399 (SEQ ID NO: 1399), (a) in a test sample of tissue cells obtained from the mammal, and (b) in a control sample of known normal tissue cells of the same cell

type, wherein a higher or lower level of expression of said gene in the test sample as compared to the control sample is indicative of the presence of an immune related disease in the mammal from which the test tissue cells were obtained,
5 wherein the immune related disease is systemic lupus erythematosus, an idiopathic inflammatory myopathy, Sjögren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, thyroiditis, diabetes mellitus, immune-mediated renal disease, a demyelinating disease of the central or peripheral nervous system, idiopathic demyelinating polyneuropathy, Guillain-Barré syndrome, a chronic inflammatory demyelinating polyneuropathy, a hepatobiliary disease, infectious or autoimmune chronic active hepatitis, primary biliary cirrhosis, granulomatous hepatitis, sclerosing cholangitis, inflammatory bowel disease, gluten-sensitive enteropathy, Whipple's disease, an autoimmune or immune-mediated skin disease, a bullous skin disease, erythema multiforme, contact dermatitis, psoriasis, an allergic disease, asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity,
10 15 20 25
urticaria, an immunologic disease of the lung, eosinophilic pneumonias, idiopathic pulmonary fibrosis, hypersensitivity pneumonitis, a transplantation associated disease, graft rejection or graft-versus-host-disease.

3. A method of diagnosing an immune related disease in a mammal, said method comprising:
(a) contacting an anti-PRO antibody with a test sample of tissue cells obtained from said mammal and
(b) detecting the formation of a complex between the antibody and a PRO polypeptide as described in Figure 1400 (SEQ ID NO:1400) in the test sample, wherein formation of said complex is indicative of the presence of an immune related disease in the mammal from which the test tissue cells were obtained,
30 wherein the immune related disease is systemic lupus erythematosus, an idiopathic inflammatory myopathy, Sjögren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic
35

anemia, autoimmune thrombocytopenia, thyroiditis, diabetes
mellitus, immune-mediated renal disease, a demyelinating disease
of the central or peripheral nervous system, idiopathic
demyelinating polyneuropathy, Guillain-Barré syndrome, a chronic
5 inflammatory demyelinating polyneuropathy, a hepatobiliary
disease, infectious or autoimmune chronic active hepatitis,
primary biliary cirrhosis, granulomatous hepatitis, sclerosing
cholangitis, inflammatory bowel disease, gluten-sensitive
enteropathy, Whipple's disease, an autoimmune or immune-mediated
10 skin disease, a bullous skin disease, erythema multiforme,
contact dermatitis, psoriasis, an allergic disease, asthma,
allergic rhinitis, atopic dermatitis, food hypersensitivity,
urticaria, an immunologic disease of the lung, eosinophilic
pneumonias, idiopathic pulmonary fibrosis, hypersensitivity
15 pneumonitis, a transplantation associated disease, graft
rejection or graft-versus-host-disease.

4. A method of diagnosing an inflammatory immune response in a
mammal, said method comprising detecting the level of expression
20 of a gene encoding a PRO polypeptide of the invention as
described in Figure 1400 (SEQ ID NO: 1400), (a) in a test sample
of tissue cells obtained from the mammal, and (b) in a control
sample of known normal tissue cells of the same cell type,
wherein a higher or lower level of expression of said gene in
25 the test sample as compared to the control sample is indicative
of the presence of an inflammatory immune response in the mammal
from which the test tissue cells were obtained,
wherein the inflammatory immune response is systemic lupus
erythematosus, an idiopathic inflammatory myopathy, Sjögren's
30 syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic
anemia, autoimmune thrombocytopenia, thyroiditis, diabetes
mellitus, immune-mediated renal disease, a demyelinating disease
of the central or peripheral nervous system, idiopathic
demyelinating polyneuropathy, Guillain-Barré syndrome, a chronic
35 inflammatory demyelinating polyneuropathy, a hepatobiliary
disease, infectious or autoimmune chronic active hepatitis,
primary biliary cirrhosis, granulomatous hepatitis, sclerosing

cholangitis, inflammatory bowel disease, gluten-sensitive
enteropathy, Whipple's disease, an autoimmune or immune-mediated
skin disease, a bullous skin disease, erythema multiforme,
contact dermatitis, psoriasis, an allergic disease, asthma,
5 allergic rhinitis, atopic dermatitis, food hypersensitivity,
urticaria, an immunologic disease of the lung, eosinophilic
pneumonias, idiopathic pulmonary fibrosis, hypersensitivity
pneumonitis, a transplantation associated disease, graft
rejection or graft-versus-host-disease.

10 5. A method of diagnosing an inflammatory immune response in a
mammal, said method comprising detecting the level of expression
of a gene as described in Figure 1399 (SEQ ID NO: 1399), (a) in
a test sample of tissue cells obtained from the mammal, and (b)
15 in a control sample of known normal tissue cells of the same
cell type, wherein a higher or lower level of expression of said
gene in the test sample as compared to the control sample is
indicative of the presence of an inflammatory immune response in
the mammal from which the test tissue cells were obtained,
20 wherein the inflammatory immune response is systemic lupus
erythematosus, an idiopathic inflammatory myopathy, Sjögren's
syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic
anemia, autoimmune thrombocytopenia, thyroiditis, diabetes
mellitus, immune-mediated renal disease, a demyelinating disease
25 of the central or peripheral nervous system, idiopathic
demyelinating polyneuropathy, Guillain-Barré syndrome, a chronic
inflammatory demyelinating polyneuropathy, a hepatobiliary
disease, infectious or autoimmune chronic active hepatitis,
primary biliary cirrhosis, granulomatous hepatitis, sclerosing
30 cholangitis, inflammatory bowel disease, gluten-sensitive
enteropathy, Whipple's disease, an autoimmune or immune-mediated
skin disease, a bullous skin disease, erythema multiforme,
contact dermatitis, psoriasis, an allergic disease, asthma,
allergic rhinitis, atopic dermatitis, food hypersensitivity,
35 urticaria, an immunologic disease of the lung, eosinophilic
pneumonias, idiopathic pulmonary fibrosis, hypersensitivity
pneumonitis, a transplantation associated disease, graft

2003295401 22 Mar 2010

- 102 -

rejection or graft-versus-host-disease.

6. A method of diagnosing an immune related disorder, substantially as hereinbefore described with reference to any one of the examples.
- 5
7. A method of diagnosing an immune related disease, substantially as hereinbefore described with reference to any one of the examples.
- 10
8. A method of diagnosing an inflammatory immune response, substantially as hereinbefore described with reference to any one of the examples.

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Editorial Note
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