



Office de la Propriété
Intellectuelle
du Canada

Un organisme
d'Industrie Canada

Canadian
Intellectual Property
Office

An agency of
Industry Canada

CA 2400295 A1 2001/09/20

(21) **2 400 295**

(12) **DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION**

(13) **A1**

(86) Date de dépôt PCT/PCT Filing Date: 2001/02/16

(87) Date publication PCT/PCT Publication Date: 2001/09/20

(85) Entrée phase nationale/National Entry: 2002/08/23

(86) N° demande PCT/PCT Application No.: US 2001/005076

(87) N° publication PCT/PCT Publication No.: 2001/068109

(30) Priorité/Priority: 2000/03/10 (09/523,656) US

(51) Cl.Int.⁷/Int.Cl.⁷ A61K 35/14, A61K 38/00, C12P 21/00,
C07K 1/00

(71) Demandeur/Applicant:
EMORY UNIVERSITY, US

(72) Inventeur/Inventor:
LOLLAR, JOHN S., US

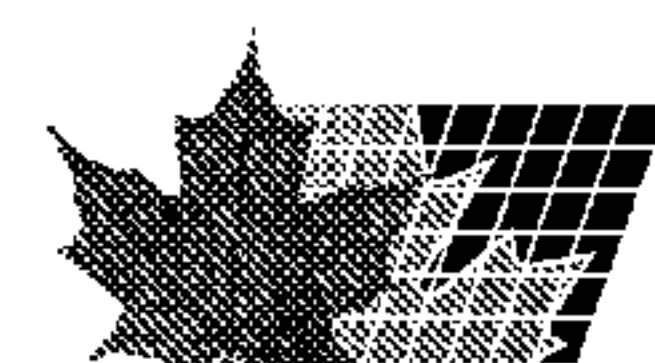
(74) Agent: MCKAY-CAREY & COMPANY

(54) Titre : FACTEUR VIII MODIFIE

(54) Title: MODIFIED FACTOR VIII

(57) **Abrégé/Abstract:**

The invention relates to a modified B-domainless form of porcine factor VIII, to a DNA encoding the same, and to the use thereof for treatment of hemophilia.



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

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
20 September 2001 (20.09.2001)

PCT
(10) International Publication Number
WO 01/68109 A1

- (51) International Patent Classification⁷: **A61K 35/14**, 38/00, C07K 1/00, C12P 21/00

(21) International Application Number: PCT/US01/05076

(22) International Filing Date: 16 February 2001 (16.02.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/523,656 10 March 2000 (10.03.2000) US

(71) Applicant: **EMORY UNIVERSITY** [US/US]; 2009 Ridgewood Drive, Atlanta, GA 30322 (US).

(72) Inventor: **LOLLAR, John, S.**; 2568 Oak Crossing Drive, Decatur, GA 30033 (US).

(74) Agents: **GREENLEE, Lorance, L.** et al.; Greenlee, Winner and Sullivan, P.C., Suite 201, 5370 Manhattan Circle, Boulder, CO 80303 (US).
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 01/68109 A1

- (54) Title: MODIFIED FACTOR VIII
- (57) Abstract: The invention relates to a modified B-domainless form of porcine factor VIII, to a DNA encoding the same, and to the use thereof for treatment of hemophilia.

MODIFIED FACTOR VIII

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority from United States Patent Application No. 09/037,601 filed March 10, 1998; which is a continuation-in-part of United States Patent Application No. 08/670,707 filed June 26, 1996, which issued as U.S. Patent No. 5,859,204, and of International Patent Application No. PCT/US97/11155 filed June 26, 1997.

ACKNOWLEDGEMENT OF FEDERAL RESEARCH SUPPORT

The government has rights in this invention arising from National Institutes of Health Grant No. HL46215 that partially funded the research leading to this invention.

BACKGROUND OF THE INVENTION

Blood clotting begins when platelets adhere to the cut wall of an injured blood vessel at a lesion site. Subsequently, in a cascade of enzymatically regulated reactions, soluble fibrinogen molecules are converted by the enzyme thrombin to insoluble strands of fibrin that hold the platelets together in a thrombus. At each step in the cascade, a protein precursor is converted to a protease that cleaves the next protein precursor in the series. Cofactors are required at most of the steps.

Factor VIII circulates as an inactive precursor in blood, bound tightly and non-covalently to von Willebrand factor. Factor VIII is proteolytically activated by thrombin or factor Xa, which dissociates it from von Willebrand factor and activates its procoagulant function in the cascade. In its active form, the protein factor VIIIa is a cofactor that increases the catalytic efficiency of factor IXa toward factor X activation by several orders of magnitude.

People with deficiencies in factor VIII or antibodies against factor VIII who are not treated with factor VIII suffer uncontrolled internal bleeding that may cause a range of serious symptoms, from inflammatory reactions in joints to early death. Severe hemophiliacs, who number about 10,000 in the United States, can be treated with infusion of human factor VIII, which will restore the blood's normal clotting ability if administered with sufficient frequency and concentration. The classic definition of factor VIII, in fact, is that substance present in normal blood plasma that corrects the clotting defect in plasma derived from individuals with hemophilia A.

The development of antibodies ("inhibitors" or "inhibitory antibodies") that inhibit the activity of factor VIII is a serious complication in the management of patients with hemophilia. Autoantibodies develop in approximately 20% of patients with hemophilia A in response to therapeutic infusions of factor VIII. In previously untreated patients with hemophilia A who develop inhibitors, the inhibitor usually develops within one year of treatment. Additionally, autoantibodies that inactivate factor VIII occasionally develop in individuals with previously normal factor VIII levels. If the inhibitor titer is low enough, patients can be managed by increasing the dose of factor VIII. However, often the inhibitor titer is so high that it cannot be overwhelmed by factor VIII. An alternative strategy is to bypass the need for factor VIII during normal hemostasis using factor IX complex preparations (for example, KONYNE[®], Proplex[®]) or recombinant human factor VIIa. Additionally, since porcine factor VIII usually has substantially less reactivity with inhibitors than human factor VIII, a partially purified porcine factor VIII preparation (HYATE:C[®]) has been used. Many patients who have developed inhibitory antibodies to human factor VIII have been successfully treated with porcine factor VIII and have tolerated such treatment for long periods of time. However, administration of porcine factor VIII is not a complete solution because inhibitors may develop to porcine factor VIII after one or more infusions in some patients.

Several preparations of human plasma-derived factor VIII of varying degrees of purity are available commercially for the treatment of hemophilia A. These include a partially-purified factor VIII derived from the pooled blood of many donors that is heat- and detergent-

treated for viruses but contain a significant level of antigenic proteins; a monoclonal antibody-purified factor VIII that has lower levels of antigenic impurities and viral contamination; and recombinant human factor VIII, clinical trials for which are underway. Unfortunately, human factor VIII is unstable at physiologic concentrations and pH, is present in blood at an extremely low concentration (0.2 $\mu\text{g/ml}$ plasma), and has low specific clotting activity. Public health concerns regarding the risk of viruses or other blood-borne contaminants have limited the usefulness of porcine factor VIII purified from porcine blood.

Hemophiliacs require daily replacement of factor VIII to prevent bleeding and the resulting deforming hemophilic arthropathy. However, supplies have been inadequate and problems in therapeutic use occur due to difficulty in isolation and purification, immunogenicity, and the necessity of removing the AIDS and hepatitis infectivity risk. The use of recombinant human factor VIII or partially-purified porcine factor VIII will not resolve all the problems.

The problems associated with the commonly used, commercially available, plasma-derived factor VIII have stimulated significant interest in the development of a better factor VIII product. There is a need for a more potent factor VIII molecule so that more units of clotting activity can be delivered per molecule; a factor VIII molecule that is stable at a selected pH and physiologic concentration; a factor VIII molecule that is less apt to cause production of inhibitory antibodies; and a factor VIII molecule that evades immune detection in patients who have already acquired antibodies to human factor VIII.

It is therefore an object of the present invention to provide a factor VIII that corrects hemophilia in a patient deficient in factor VIII or having inhibitors to human factor VIII.

It is a further object of the present invention to provide methods for treatment of hemophiliacs.

It is still another object of the present invention to provide a factor VIII that is stable at a selected pH and physiologic concentration.

It is yet another object of the present invention to provide a factor VIII that has greater coagulant activity than human factor VIII.

It is an additional object of the present invention to provide a factor VIII against which less antibody is produced.

It is a further object of the invention to provide a method for making recombinant porcine factor VIII and specifically modified porcine factor VIII.

SUMMARY OF THE INVENTION

The determination of the entire DNA sequence encoding porcine factor VIII set forth herein has enabled, for the first time, the synthesis of full-length porcine factor VIII by expressing the DNA encoding porcine factor VIII in a suitable host cell. Purified recombinant porcine factor VIII is therefore an aspect of the present invention. The DNA encoding each domain of porcine factor VIII as well as any specified fragment thereof, can be similarly expressed. Furthermore, porcine fVIII having all or part of the B domain deleted (B-domainless porcine fVIII) is made available as part of the present invention, by expression DNA encoding porcine fVIII having a deletion of one or more codons of the B-domain.

Also provided are pharmaceutical compositions and methods for treating patients having factor VIII deficiency comprising administering recombinant porcine factor VIII or a modified recombinant porcine factor VIII, in particular a B-domainless porcine factor VIII.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A-1H taken together provide an aligned sequence comparison of the human, pig and mouse factor VIII acid sequences.

DETAILED DESCRIPTION OF THE INVENTION

Unless otherwise specified or indicated, as used herein, "factor VIII" denotes any functional factor VIII protein molecule from any mammal.

As used herein, "mammalian factor VIII" includes factor VIII with amino acid sequence derived from any non-human mammal, unless otherwise specified. "Animal", as used herein, refers to pig and other non-human mammals.

A "fusion protein" or "fusion factor VIII or fragment thereof", as used herein, is the product of a hybrid gene in which the coding sequence for one protein is altered, for example, by joining part of it to the coding sequence for a second protein from a different gene in proper reading frame register such that uninterrupted transcription and translation of the joined segments can occur to produce a hybrid gene that encodes the fusion protein.

A "corresponding" nucleic acid or amino acid or sequence of either, as used herein, is one present at a site in a factor VIII molecule or fragment thereof that has the same structure and/or function as a site in the factor VIII molecule of another species, although the nucleic acid or amino acid number may not be identical. A DNA sequence "corresponding to" another factor VIII sequence substantially corresponds to such sequence, and hybridizes to the sequence of the designated SEQ ID NO. under stringent conditions. A DNA sequence "corresponding to" another factor VIII sequence also includes a sequence that results in the expression of a factor VIII or fragment thereof and would hybridize to the designated SEQ ID NO. but for the redundancy of the genetic code.

A "unique" amino acid residue or sequence, as used herein, refers to an amino acid sequence or residue in the factor VIII molecule of one species that is different from the homologous residue or sequence in the factor VIII molecule of another species.

"Specific activity," as used herein, refers to the activity that will correct the coagulation defect of human factor VIII deficient plasma. Specific activity is measured in units of clotting

activity per milligram total factor VIII protein in a standard assay in which the clotting time of human factor VIII deficient plasma is compared to that of normal human plasma. One unit of factor VIII activity is the activity present in one milliliter of normal human plasma. In the assay, the shorter the time for clot formation, the greater the activity of the factor VIII being assayed. Porcine factor VIII has coagulation activity in a human factor VIII assay.

"Expression" refers to the set of processes that occur whereby genetic information is utilized to yield a product. A DNA encoding the amino acid sequence of porcine factor VIII can be "expressed" within a mammalian host cell to yield porcine factor VIII protein. The materials, genetic structures, host cells and conditions which permit expression of a given DNA sequence to occur are well-known in the art and can be manipulated to affect the time and amount of expression, as well as the intra- or extra-cellular location of the expressed protein. For example, by including DNA encoding a signal peptide at the 5' end of the DNA encoding porcine factor VIII (the 5' end being, by convention, that end encoding the NH₂ terminus of the protein) the expressed protein becomes exported from the interior of the host cell into the culture medium. Providing a signal peptide coding DNA in combination with the porcine factor VIII coding DNA is advantageous because the expressed factor VIII is exported into the culture medium which simplifies the process of purification. A preferred signal peptide is a mammalian factor VIII signal peptide.

The human factor VIII cDNA nucleotide and predicted amino acid sequences are shown in SEQ ID NOs:1 and 2, respectively. Factor VIII is synthesized as an approximately 300 kDa single chain protein with internal sequence homology that defines the "domain" sequence NH₂-A1-A2-B-A3-C1-C2-COOH. In a factor VIII molecule, a "domain", as used herein, is a continuous sequence of amino acids that is defined by internal amino acid sequence identity and sites of proteolytic cleavage by thrombin. Unless otherwise specified, factor VIII domains include the following amino acid residues, when the sequences are aligned with the human amino acid sequence (SEQ ID NO:2): A1, residues Ala1-Arg372; A2, residues Ser373-Arg740; B, residues Ser741-Arg1648; A3, residues Ser1690-Ile2032; C1, residues Arg2033-Asn2172; C2, residues Ser2173-Tyr2332. The A3-C1-C2 sequence includes residues Ser1690-

Tyr2332. The remaining segment, residues Glu1649-Arg1689, is usually referred to as the factor VIII light chain activation peptide. Factor VIII is proteolytically activated by thrombin or factor Xa, which dissociates it from von Willebrand factor, forming factor VIIIa, which has procoagulant function. The biological function of factor VIIIa is to increase the catalytic efficiency of factor IXa toward factor X activation by several orders of magnitude. Thrombin-activated factor VIIIa is a 160 kDa A1/A2/A3-C1-C2 heterotrimer that forms a complex with factor IXa and factor X on the surface of platelets or monocytes. A "partial domain" as used herein is a continuous sequence of amino acids forming part of a domain.

"Subunits" of human or animal factor VIII, as used herein, are the heavy and light chains of the protein. The heavy chain of factor VIII contains three domains, A1, A2, and B. The light chain of factor VIII also contains three domains, A3, C1, and C2.

The terms "epitope," "antigenic site," and "antigenic determinant," as used herein, are used synonymously and are defined as a portion of the human, or animal factor VIII or fragment thereof that is specifically recognized by an antibody. It can consist of any number of amino acid residues, and it can be dependent upon the primary, secondary, or tertiary structure of the protein.

The term "immunogenic site," as used herein, is defined as a region of the human or animal factor VIII, or fragment thereof, that specifically elicits the production of antibody to the factor VIII, or fragment, in a human or animal, as measured by routine protocols, such as immunoassay, e.g. ELISA, or the Bethesda assay, described herein. It can consist of any number of amino acid residues, and it can be dependent upon the primary, secondary, or tertiary structure of the protein. In some embodiments, the hybrid or hybrid equivalent factor VIII or fragment thereof is nonimmunogenic or less immunogenic in an animal or human than human or porcine factor VIII.

"Factor VIII deficiency," as used herein, includes deficiency in clotting activity caused by production of defective factor VIII, by inadequate or no production of factor VIII, or by

partial or total inhibition of factor VIII by inhibitors. Hemophilia A is a type of factor VIII deficiency resulting from a defect in an X-linked gene and the absence or deficiency of the factor VIII protein it encodes.

As used herein, "diagnostic assays" include assays that in some manner utilize the antigen-antibody interaction to detect and/or quantify the amount of a particular antibody that is present in a test sample to assist in the selection of medical therapies. There are many such assays known to those of skill in the art. As used herein, human, porcine or modified porcine factor VIII DNA or fragment thereof and protein expressed therefrom, in whole or in part, can be substituted for the corresponding reagents in the otherwise known assays, whereby the modified assays may be used to detect and/or quantify antibodies to factor VIII. It is the use of these reagents, the factor VIII DNA or fragment thereof or protein expressed therefrom, that permits modification of known assays for detection of antibodies to human or animal factor VIII. Such assays include, but are not limited to ELISAs, immunodiffusion assays, and immunoblots. Suitable methods for practicing any of these assays are known to those of skill in the art. As used herein, the factor VIII or fragment thereof that includes at least one epitope of the protein can be used as the diagnostic reagent. Examples of other assays in which human, porcine or modified porcine factor VIII or fragment thereof can be used include the Bethesda assay and anticoagulation assays.

The term "DNA encoding a protein, such as porcine factor VIII" means a polydeoxynucleic acid whose nucleotide sequence embodies coding information to a host cell for the amino acid sequence of the protein, e.g. porcine factor VIII, according to the known relationships of the genetic code.

The "expression product" of a DNA encoding a human or animal factor VIII or a modified factor VIII is the product obtained from expression of the referenced DNA in a suitable host cell, including such features of pre- or post-translational modification of protein encoded by the referenced DNA, including but not limited to glycosylation, proteolytic cleavage and the like. It is known in the art that such modifications can occur and can differ

somewhat depending upon host cell type and other factors, and can result in molecular isoforms of the product, with retention of procoagulant activity. See, e.g. Lind, P. et al., *Eur. J. Biochem.* **232**:1927 (1995), incorporated herein by reference.

An "expression vector" is a DNA element, often of circular structure, having the ability to replicate autonomously in a desired host cell, or to integrate into a host cell genome and also possessing certain well-known features which permit expression of a coding DNA inserted into the vector sequence at the proper site and in proper orientation. Such features can include, but are not limited to, one or more promoter sequences to direct transcription initiation of the coding DNA and other DNA elements such as enhancers, polyadenylation sites and the like, all as well known in the art. The term "expression vector" is used to denote both a vector having a DNA coding sequence to be expressed inserted within its sequence, and a vector having the requisite expression control elements so arranged with respect to an insertion site that it can serve to express any coding DNA inserted into the site, all as well-known in the art. Thus, for example, a vector lacking a promoter can become an expression vector by the insertion of a promoter combined with a coding DNA.

GENERAL DESCRIPTION OF METHODS

U.S. Patent 5,364,771 described the discovery of hybrid human/porcine factor VIII molecules having coagulant activity, in which elements of the factor VIII molecule of human or pig are substituted for corresponding elements of the factor VIII molecule of the other species. U.S. Patent 5,663,060 describes procoagulant hybrid human/animal and hybrid equivalent factor VIII molecules, in which elements of the factor VIII molecule of one species are substituted for corresponding elements of the factor VIII molecule of the other species.

Since current information indicates that the B domain has no inhibitory epitope and has no known effect on factor VIII function, in some embodiments the B domain is wholly or partially deleted in the active hybrid or hybrid equivalent factor VIII molecules or fragments thereof ("B(-) factor VIII") prepared by any of the methods described herein.

The human factor VIII gene was isolated and expressed in mammalian cells, as reported by Toole, J.J. et al. (1984) *Nature* 312:342-347 (Genetics Institute); Gitschier, J. et al. (1984) *Nature* 312:326-330 (Genentech); Wood, W.I. et al. (1984) *Nature* 312:330-337 (Genentech); Vehar, G.A. et al. (1984) *Nature* 312:337-342 (Genentech); WO 87/04187; WO 88/08035; WO 88/03558; U.S. Patent No. 4,757,006, and the amino acid sequence was deduced from cDNA. U.S. Patent No. 4,965,199 to Capon et al. discloses a recombinant DNA method for producing factor VIII in mammalian host cells and purification of human factor VIII. Human factor VIII expression on CHO (Chinese hamster ovary) cells and BHKC (baby hamster kidney cells) has been reported. Human factor VIII has been modified to delete part or all of the B domain (U.S. Patent No. 4,868,112), and replacement of the human factor VIII B domain with the human factor V B domain has been attempted (U.S. Patent No. 5,004,803). The cDNA sequence encoding human factor VIII and predicted amino acid sequence are shown in SEQ ID NOs:1 and 2, respectively. In SEQ ID NO:1, the coding region begins at nucleotide position 208, the triplet GCC being the codon for amino acid number 1 (Ala) of the mature protein as given in SEQ ID NO:2.

Porcine factor VIII has been isolated from plasma [Fass, D.N. et al. (1982) *Blood* 59:594]. Partial amino acid sequence of porcine factor VIII corresponding to portions of the N-terminal light chain sequence having homology to ceruloplasmin and coagulation factor V were described by Church et al. (1984) *Proc. Natl. Acad. Sci. USA* 81:6934. Toole, J.J. et al. (1984) *Nature* 312:342-347 described the partial sequencing of the N-terminal end of four amino acid fragments of porcine factor VIII but did not characterize the fragments as to their positions in the factor VIII molecule. The amino acid sequence of the B and part of the A2 domains of porcine factor VIII were reported by Toole, J.J. et al. (1986) *Proc. Natl. Acad. Sci, USA* 83:5939-5942. The cDNA sequence encoding the complete A2 domain of porcine factor VIII and predicted amino acid sequence and hybrid human/porcine factor VIII having substitutions of all domains, all subunits, and specific amino acid sequences were disclosed in U.S. Patent 5,364,771 entitled "Hybrid Human/Porcine factor VIII" issued on November 15, 1994, and in WO 93/20093 published October 14, 1993. The cDNA sequence encoding the A2 domain of porcine factor VIII corresponding to residues 373-740 in mature human factor

VIII, as shown in SEQ ID NO:1, and the predicted amino acid sequence are shown in SEQ ID NOs:3 and 4, respectively. More recently, the nucleotide and corresponding amino acid sequences of part of the A1 domain lacking the first 198 amino acid and of the A2 domain of porcine factor VIII were reported in WO 94/11503, published May 26, 1994. The entire nucleotide sequence encoding porcine factor VIII, including the complete A1 domain, activation peptide, A3, C1 and C2 domains, as well as the encoded amino acid sequence, was finally obtained by Lollar, as disclosed in U.S. Patent 5,859,204, issued January 12, 1999, and in WO 97/49725, published December 31, 1997, both incorporated herein by reference..

Both porcine and human factor VIII are isolated from plasma as a two subunit protein. The subunits, known as the heavy chain and light chain, are held together by a non-covalent bond that requires calcium or other divalent metal ions. The heavy chain of factor VIII contains three domains, A1, A2, and B, which are linked covalently. The light chain of factor VIII also contains three domains, designated A3, C1, and C2. The B domain has no known biological function and can be removed, or partially removed from the molecule proteolytically or by recombinant DNA technology methods without significant alteration in any measurable parameter of factor VIII. Human recombinant factor VIII has a similar structure and function to plasma-derived factor VIII, though it is not glycosylated unless expressed in mammalian cells.

Both human and porcine activated factor VIII ("factor VIIIa") have three subunits due to cleavage of the heavy chain between the A1 and A2 domains. This structure is designated A1/A2/A3-C1-C2. Human factor VIIIa is not stable under the conditions that stabilize porcine factor VIIIa, presumably because of the weaker association of the A2 subunit of human factor VIIIa. Dissociation of the A2 subunit of human and porcine factor VIIIa is associated with loss of activity in the factor VIIIa molecule. Yakhyev, A. et al. (1997) *Blood* 90:Suppl. 1, Abstract

#126, reported binding of A2 domain by low density lipoprotein receptor-related protein, suggesting that cellular uptake of A2 mediated by such binding acts to down-regulate factor VIII activity.

Expression of "B-domainless factor VIII" is enhanced by including portions of the B-domain. The inclusion of those parts of the B domain designated "SQ" [Lind, P. et al. (1995) *supra*] was reported to result in favorable expression. "SQ" constructs lack all of the human B domain except for 5 amino acids of the B domain N-terminus and 9 amino acids of the B domain C-terminus.

The purified hybrid factor VIII or fragment thereof can be assayed for immunoreactivity and coagulation activity by standard assays including, for example, the plasma-free factor VIII assay, the one-stage clotting assay, and the enzyme-linked immunosorbent assay using purified recombinant human factor VIII as a standard.

Other vectors, including both plasmid and eukaryotic viral vectors, may be used to express a recombinant gene construct in eukaryotic cells depending on the preference and judgment of the skilled practitioner (see, for example, Sambrook et al., Chapter 16). Other vectors and expression systems, including bacterial, yeast, and insect cell systems, can be used but are not preferred due to differences in, or lack of, glycosylation.

Recombinant factor VIII protein can be expressed in a variety of cells commonly used for culture and recombinant mammalian protein expression. In particular, a number of rodent cell lines have been found to be especially useful hosts for expression of large proteins. Preferred cell lines, available from the American Type Culture Collection, Rockville, MD, include baby hamster kidney cells, and chinese hamster ovary (CHO) cells which are cultured using routine procedures and media.

The basis for the greater coagulant activity of porcine factor VIII appears to be the more rapid spontaneous dissociation of the human A2 subunit from human factor VIIIa than the porcine A2 subunit from porcine factor VIIIa. Dissociation of the A2 subunit leads to loss of activity, [Lollar, P. et al. (1990) *J. Biol. Chem.* 265:1688-1692; Lollar, P. et al. (1992) *J. Biol. Chem.* 267:23652-23657; Fay, P.J. et al. (1992) *J. Biol. Chem.* 267:13246-13250].

Factor VIII molecules with reduced immunoreactivity:

Epitopes that are immunoreactive with antibodies that inhibit the coagulant activity of factor VIII ("inhibitors" or "inhibitory antibodies") have been characterized based on known structure-function relationships in factor VIII. Presumably, inhibitors could act by disrupting any of the macromolecular interactions associated with the domain structure of factor VIII or its associations with von Willebrand factor, thrombin, factor Xa, factor IXa, or factor X. However, most inhibitory antibodies to human factor VIII act by binding to epitopes located in the 40 kDa A2 domain or 20 kDa C2 domain of factor VIII, disrupting specific functions associated with these domains, as described by Fulcher et al. (1985) *Proc. Natl. Acad. Sci USA* 82:7728-7732; and Scandella et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:6152-6156. In addition to the A2 and C2 epitopes, there may be a third epitope in the A3 or C1 domain of the light chain of factor VIII, according to Scandella et al. (1993) *Blood* 82:1767-1775. The significance of this putative third epitope is unknown, but it appears to account for a minor fraction of the epitope reactivity in factor VIII.

Anti-A2 antibodies block factor X activation, as shown by Lollar et al. (1994) *J. Clin. Invest.* 93:2497-2504. Previous mapping studies by deletion mutagenesis described by Ware et al. (1992) *Blood Coagul. Fibrinolysis* 3:703-716, located the A2 epitope to within a 20 kDa region of the NH₂-terminal end of the 40 kDa A2 domain. Competition immunoradiometric assays have indicated that A2 inhibitors recognize either a common epitope or narrowly clustered epitopes, as described by Scandella et al. (1992) *Throm. Haemostas* 67:665-671, and as demonstrated in U.S. Patent 5,859,204.

Animal or modified animal factor VIII molecules can be tested in humans for their reduced antigenicity and/or immunogenicity in clinical trials. In one type of trial, designed to determine whether the factor VIII is immunoreactive with inhibitory antibodies, factor VIII is administered, preferably by intravenous infusion, to approximately 25 patients having factor VIII deficiency who have antibodies that inhibit the coagulant activity of therapeutic human factor VIII. The dosage of the animal or modified animal factor VIII is in a range between 5 and 50 Units/kg body weight, preferably 10-50 Units/kg, and most preferably 40 Units/kg

body weight. Approximately 1 hour after each administration, the recovery of factor VIII from blood samples is measured in a one-stage coagulation assay. Samples are taken again approximately 5 hours after infusion, and recovery is measured. Total recovery and the rate of disappearance of factor VIII from the samples is predictive of the antibody titer and inhibitory activity. If the antibody titer is high, factor VIII recovery usually cannot be measured. The recovery results are compared to the recovery results in patients treated with plasma-derived human factor VIII, recombinant human factor VIII, plasma-derived porcine factor VIII, and other commonly used therapeutic forms of factor VIII or factor VIII substitutes.

After identification of clinically significant epitopes, recombinant factor VIII molecules can be expressed that have less than or equal cross-reactivity compared with plasma-derived porcine factor VIII when tested *in vitro* against a broad survey of inhibitor plasmas. Additional mutagenesis in epitopic regions can be done to reduce cross-reactivity. Reduced cross-reactivity, although desirable, is not necessary to produce a product that may have advantages over the existing plasma-derived porcine factor VIII concentrate, which can produce side effects due to contaminant porcine proteins or contaminant infectious agents such as viruses or prions. A recombinant porcine or modified porcine factor VIII molecule will not contain foreign porcine proteins.

Diagnostic Assays.

The factor VIII cDNA and/or protein expressed therefrom, in whole or in part, can be used in assays as diagnostic reagents for the detection of inhibitory antibodies to human or animal factor VIII or modified animal VIII in substrates, including, for example, samples of serum and body fluids of human patients with factor VIII deficiency. These antibody assays include assays such as ELISA assays, immunoblots, radioimmunoassays, immunodiffusion assays, and assay of factor VIII biological activity (e.g., by coagulation assay). Techniques for preparing these reagents and methods for use thereof are known to those skilled in the art. For example, an immunoassay for detection of inhibitory antibodies in a patient serum sample can include reacting the test sample with a sufficient amount of the factor VIII to be tested that

a detectable complex can be formed with the inhibitory antibodies in the sample of the test factor VIII is indeed antigenic..

Nucleic acid and amino acid probes can be prepared based on the sequence of the hybrid factor VIII cDNA or protein molecule or fragments thereof. In some embodiments, these can be labeled using dyes or enzymatic, fluorescent, chemiluminescent, or radioactive labels that are commercially available. The amino acid probes can be used, for example, to screen sera or other body fluids where the presence of inhibitors to human, animal, or hybrid human/animal factor VIII is suspected. Levels of inhibitors can be quantitated in patients and compared to healthy controls, and can be used, for example, to determine whether a patient with a factor VIII deficiency can be treated with an animal or modified animal factor VIII. The cDNA probes can be used, for example, for research purposes in screening DNA libraries.

Pharmaceutical Compositions.

Pharmaceutical compositions containing recombinant porcine or modified porcine factor VIII, alone or in combination with appropriate pharmaceutical stabilization compounds, delivery vehicles, and/or carrier vehicles, are prepared according to known methods, as described in Remington's *Pharmaceutical Sciences* by E.W. Martin.

In one preferred embodiment, the preferred carriers or delivery vehicles for intravenous infusion are physiological saline or phosphate buffered saline.

In another preferred embodiment, suitable stabilization compounds, delivery vehicles, and carrier vehicles include but are not limited to other human or animal proteins such as albumin.

Phospholipid vesicles or liposomal suspensions are also preferred as pharmaceutically acceptable carriers or delivery vehicles. These can be prepared according to methods known to those skilled in the art and can contain, for example, phosphatidylserine/-phosphatidylcholine or other compositions of phospholipids or detergents that together impart

a negative charge to the surface, since factor VIII binds to negatively charged phospholipid membranes. Liposomes may be prepared by dissolving appropriate lipid(s) (such as stearyl phosphatidyl ethanolamine, stearyl phosphatidyl choline, arachadoyl phosphatidyl choline, and cholesterol) in an inorganic solvent that is then evaporated, leaving behind a thin film of dried lipid on the surface of the container. An aqueous solution of the hybrid factor VIII is then introduced into the container. The container is then swirled by hand to free lipid material from the sides of the container and to disperse lipid aggregates, thereby forming the liposomal suspension.

The recombinant porcine or modified porcine factor VIII can be combined with other suitable stabilization compounds, delivery vehicles, and/or carrier vehicles, including vitamin K dependent clotting factors, tissue factor, and von Willebrand factor (vWf) or a fragment of vWf that contains the factor VIII binding site, and polysaccharides such as sucrose.

Recombinant porcine or modified porcine factor VIII can also be delivered by gene therapy in the same way that human factor VIII can be delivered, using delivery means such as retroviral vectors. This method consists of incorporation of the desired factor VIII construct cDNA into human cells that are transplanted directly into a factor VIII deficient patient or that are placed in an implantable device, permeable to the factor VIII molecules but impermeable to cells, that is then transplanted. The preferred method will be retroviral-mediated gene transfer. In this method, an exogenous gene (e.g., a factor VIII cDNA) is cloned into the genome of a modified retrovirus. The gene is inserted into the genome of the host cell by viral machinery where it will be expressed by the cell. The retroviral vector is modified so that it will not produce virus, preventing viral infection of the host. The general principles for this type of therapy are known to those skilled in the art and have been reviewed in the literature [e.g., Kohn, D.B. et al. (1989) *Transufusion* 29:812-820].

Porcine or modified porcine factor VIII can be stored bound to vWf to increase the half-life and shelf-life of the hybrid molecule. Additionally, lyophilization of factor VIII can improve the yields of active molecules in the presence of vWf. Current methods for storage

of human and animal factor VIII used by commercial suppliers can be employed for storage of recombinant factor VIII. These methods include: (1) lyophilization of factor VIII in a partially-purified state (as a factor VIII "concentrate" that is infused without further purification); (2) immunoaffinity-purification of factor VIII by the Zimmerman method and lyophilization in the presence of albumin, which stabilizes the factor VIII; (3) lyophilization of recombinant factor VIII in the presence of albumin.

Additionally, porcine or modified porcine factor VIII has been found to be indefinitely stable at 4° C in 0.6 M NaCl, 20 mM MES, and 5 mM CaCl₂ at pH 6.0 and also can be stored frozen in these buffers and thawed with minimal loss of activity.

Methods of Treatment.

Recombinant porcine or modified porcine factor VIII is used to treat uncontrolled bleeding due to factor VIII deficiency (e.g., intraarticular, intracranial, or gastrointestinal hemorrhage) in hemophiliacs with and without inhibitory antibodies and in patients with acquired factor VIII deficiency due to the development of inhibitory antibodies. The active materials are preferably administered intravenously.

Additionally, recombinant porcine or modified porcine factor VIII can be administered by transplant of cells genetically engineered to produce the protein by implantation of a device containing such cells, as described above.

In a preferred embodiment, pharmaceutical compositions of recombinant porcine or modified porcine factor VIII alone or in combination with stabilizers, delivery vehicles, and/or carriers are infused into patients intravenously according to the same procedure that is used for infusion of human or animal factor VIII.

The treatment dosages of recombinant porcine or modified porcine factor VIII composition that must be administered to a patient in need of such treatment will vary depending on the severity of the factor VIII deficiency. Generally, dosage level is adjusted in

frequency, duration, and units in keeping with the severity and duration of each patient's bleeding episode. Accordingly, the factor VIII is included in a pharmaceutically acceptable carrier, delivery vehicle, or stabilizer in an amount sufficient to deliver to a patient a therapeutically effective amount of the protein to stop bleeding, as measured by standard clotting assays.

Factor VIII is classically defined as that substance present in normal blood plasma that corrects the clotting defect in plasma derived from individuals with hemophilia A. The coagulant activity *in vitro* of purified and partially-purified forms of factor VIII is used to calculate the dose of factor VIII for infusions in human patients and is a reliable indicator of activity recovered from patient plasma and of correction of the *in vivo* bleeding defect. There are no reported discrepancies between standard assay of novel factor VIII molecules *in vitro* and their behavior in the dog infusion model or in human patients, according to Lusher, J.M. et al. 328 *New Engl. J. Med.* 328:453-459; Pittman, D.D. et al. (1992) *Blood* 79:389-397; and Brinkhous et al. (1985) *Proc. Natl. Acad. Sci.* 82:8752-8755.

Usually, the desired plasma factor VIII activity level to be achieved in the patient through administration of the recombinant porcine or modified porcine factor VIII is in the range of 30-100% of normal. In a preferred mode of administration of the therapeutic factor VIII, the composition is given intravenously at a preferred dosage in the range from about 5 to 50 units/kg body weight, more preferably in a range of 10-50 units/kg body weight, and most preferably at a dosage of 20-40 units/kg body weight; the interval frequency is in the range from about 8 to 24 hours (in severely affected hemophiliacs); and the duration of treatment in days is in the range from 1 to 10 days or until the bleeding episode is resolved. See, e.g., Roberts, H.R., and M.R. Jones, "Hemophilia and Related Conditions - Congenital Deficiencies of Prothrombin (Factor II, Factor V, and Factors VII to XII)," Ch. 153, 1453-1474, 1460, in Hematology, Williams, W. J., et al., ed. (1990). Patients with inhibitors may require a different amount of recombinant porcine or modified porcine factor VIII than their previous form of factor VIII. For example, patients may require less recombinant porcine or modified porcine factor VIII because of its higher specific activity than human factor VIII and

its decreased antibody reactivity. As in treatment with human or plasma-derived porcine factor VIII, the amount of therapeutic factor VIII infused is defined by the one-stage factor VIII coagulation assay and, in selected instances, *in vivo* recovery is determined by measuring the factor VIII in the patient's plasma after infusion. It is to be understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

Treatment can take the form of a single intravenous administration of the composition or periodic or continuous administration over an extended period of time, as required. Alternatively, therapeutic factor VIII can be administered subcutaneously or orally with liposomes in one or several doses at varying intervals of time.

Recombinant porcine or modified porcine factor VIII can also be used to treat uncontrolled bleeding due to factor VIII deficiency in hemophiliacs who have developed antibodies to human factor VIII. In this case, coagulant activity that is superior to that of human or animal factor VIII alone is not necessary. Coagulant activity that is inferior to that of human factor VIII (i.e., less than 3,000 units/mg) will be useful if that activity is not neutralized by antibodies in the patient's plasma.

It has been demonstrated herein that recombinant porcine and modified porcine factor VIII's can differ in specific activity from human factor VIII. Factor VIII proteins having greater procoagulant activity from human factor VIII are useful in treatment of hemophilia because lower dosages will be required to correct a patient's factor VIII deficiency. Factor VIII's having lower procoagulant activity than human factor VIII are also suitable for therapeutic use provided they have at least 1% of specific activity compared to normal human factor VIII. A factor VIII of the present invention having procoagulant activity is therefore defined as having at least 1% of the specific activity of human factor VIII.

The recombinant porcine or modified porcine factor VIII molecule and the methods for isolation, characterization, making, and using it generally described above will be further understood with reference to the following non-limiting examples.

Example 1: Assay of porcine factor VIII and hybrid human/porcine factor VIII.

Porcine factor VIII has more coagulant activity than human factor VIII, based on specific activity of the molecule. This conclusion is based on the use of appropriate standard curves that allow human porcine factor VIII to be fairly compared. Coagulation assays are based on the ability of factor VIII to shorten the clotting time of plasma derived from a patient with hemophilia A. Two types of assays were employed: the one-stage and the two stage assay.

In the one-stage assay, 0.1 ml hemophilia A plasma (George King Biomedical, Inc.) was incubated with 0.1 ml activated partial thromboplastin reagent (APTT) (Organon Teknika) and 0.01 ml sample or standard, consisting of diluted, citrated normal human plasma, for 5 min at 37°C in a water bath. Incubation was followed by addition of 0.1 ml 20 mM CaCl₂, and the time for development of a fibrin clot was determined by visual inspection.

A unit of factor VIII is defined as the amount present in 1 ml of citrated normal human plasma. With human plasma as the standard, porcine and human factor VIII activity were compared directly. Dilutions of the plasma standard or purified proteins were made into 0.15 M NaCl, 0.02 M HEPES, pH 7.4. The standard curve was constructed based on 3 or 4 dilutions of plasma, the highest dilution being 1/50, and on log₁₀ clotting time plotted against log₁₀ plasma concentration, which results in a linear plot. The units of factor VIII in an unknown sample were determined by interpolation from the standard curve.

The one-stage assay relies on endogenous activation of factor VIII by activators formed in the hemophilia A plasma, whereas the two-stage assay measures the procoagulant activity of preactivated factor VIII. In the two-stage assay, samples containing factor VIII that had been reacted with thrombin were added to a mixture of activated partial thromboplastin and

human hemophilia A plasma that had been preincubated for 5 min at 37°C. The resulting clotting times were then converted to units/ml, based on the same human standard curve described above. The relative activity in the two-stage assay was higher than in the one-stage assay because the factor VIII had been preactivated.

Example 2: Characterization of the functional difference between human and porcine factor VIII.

The isolation of porcine and human plasma-derived factor VIII and human recombinant factor VIII have been described in the literature in Fulcher, C.A. et al. (1982) *Proc. Natl. Acad. Sci. USA* 79:1648-1652; Toole et al. (1984) *Nature* 312:342-347 (Genetics Institute); Gitschier et al. (1984) *Nature* 312:326-330 (Genentech); Wood et al. (1984) *Nature* 312:330-337 (Genentech); Vehar et al. 312 *Nature* 312:337-342 (Genentech); Fass et al. (1982) *Blood* 59:594; Toole et al. (1986) *Proc. Natl. Acad. Sci. USA* 83:5939-5942. This can be accomplished in several ways. All these preparations are similar in subunit composition, although there is a functional difference in stability between human and porcine factor VIII.

For comparison of human recombinant and porcine factor VIII, preparations of highly-purified human recombinant factor VIII (Cutter Laboratories, Berkeley, CA) and porcine factor VIII [immunopurified as described in Fass et al. (1982) *Blood* 59:594] were subjected to high-pressure liquid chromatography (HPLC) over a Mono QTM (Pharmacia-LKB, Piscataway, NJ) anion-exchange column (Pharmacia, Inc.). The purposes of the Mono QTM HPLC step were elimination of minor impurities of exchange of human and porcine factor VIII into a common buffer for comparative purposes. Vials containing 1000-2000 units of factor VIII were reconstituted with 5 ml H₂O. Hepes (2 M at pH 7.4) was then added to a final concentration of 0.02 M. Factor VIII was applied to a Mono QTM HR 5/5 column equilibrated in 0.15 M NaCl, 0.02 M Hepes, 5mM CaCl₂, at pH 7.4 (Buffer A plus 0.15 M NaCl); washed with 10 ml Buffer A + 0.15 M NaCl; and eluted with a 20 ml linear gradient, 0.15 M to 0.90 M NaCl in Buffer A at a flow rate of 1 ml/min.

For comparison of human plasma-derived factor VIII (purified by Mono QTM HPLC) and porcine factor VIII, immunoaffinity-purified, plasma-derived porcine factor VIII was

diluted 1:4 with 0.04 M Hepes, 5 mM CaCl₂, 0.01 % Tween-80, at pH 7.4, and subjected to Mono QTM HPLC under the same conditions described in the previous paragraph for human factor VIII. These procedures for the isolation of human and porcine factor VIII are standard for those skilled in the art.

Column fractions were assayed for factor VIII activity by a one-stage coagulation assay. The average results of the assays, expressed in units of activity per A₂₈₀ of material, are given in Table II, and indicate that porcine factor VIII has at least six times greater activity than human factor VIII when the one-stage assay is used.

TABLE II
COMPARISON OF HUMAN AND PORCINE FACTOR VIII
COAGULANT ACTIVITY

	Activity (U/A ₂₈₀)
Porcine	21,300
Human plasma-derived	3,600
Human recombinant	2,400

Example 3: Comparison of the stability of human and porcine factor VIII.

The results of the one-stage assay for factor VIII reflect activation of factor VIII to factor VIIIa in the sample and possibly loss of formed factor VIIIa activity. A direct comparison of the stability of human and porcine factor VIII was made. Samples from Mono QTM HPLC (Pharmacia, Inc., Piscataway, N.J.) were diluted to the same concentration and buffer composition and reacted with thrombin. At various times, samples were removed for two-stage coagulation assay. Typically, peak activity (at 2 min) was 10-fold greater for porcine than human factor VIIIa, and the activities of both porcine and human factor VIIIa subsequently decreased, with human factor VIIIa activity decreasing more rapidly.

Generally, attempts to isolate stable human factor VIIIa are not successful even when conditions that produce stable porcine factor VIIIa are used. To demonstrate this, Mono QTM HPLC-purified human factor VIII was activated with thrombin and subjected to Mono STM

cation-exchange (Pharmacia, Inc.) HPLC under conditions that produce stable porcine factor VIIIa, as described by Lollar et al. (1989) *Biochemistry* 28:666.

Human factor VIII, 43 $\mu\text{g/ml}$ (0.2 μM) in 0.2 M NaCl, 0.01 M Hepes, 2.5 mM CaCl_2 , at pH 7.4, in 10 ml total volume, was reacted with thrombin (0.036 μM) for 10 min, at which time FPR- CH_2Cl D-phenyl-prolyl-arginyl-chloromethyl ketone was added to a concentration of 0.2 μM for irreversible inactivation of thrombin. The mixture then was diluted 1:1 with 40 mM 2-(N-morpholino) ethane sulfonic acid (MES), 5 mM CaCl_2 , at pH 6.0, and loaded at 2 ml/min onto a Mono STM HR 5/5 HPLC column (Pharmacia, Inc.) equilibrated in 5 mM MES, 5 mM CaCl_2 , at pH 6.0 (Buffer B) plus 0.1 M NaCl. Factor VIIIa was eluted without column washing with a 20 ml gradient from 0.1 M NaCl to 0.9 M NaCl in Buffer B at 1 ml/min.

The fraction with coagulant activity in the two-stage assay eluted as a single peak under these conditions. The specific activity of the peak fraction was approximately 7,500 U/A₂₈₀. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the Mono STM factor VIIIa peak, followed by silver staining of the protein, revealed two bands corresponding to a heterodimeric (A3-C1-C2/A1) derivative of factor VIII. Although the A2 fragment was not identified by silver staining under these conditions because of its low concentration, it was identified as a trace constituent by ¹²⁵I-labeling.

In contrast to the results with human factor VIII, porcine factor VIIIa isolated by Mono STM HPLC under the same conditions had a specific activity 1.6×10^6 U/A₂₈₀. Analysis of porcine factor VIIIa by SDS-PAGE revealed 3 fragments corresponding to A1, A2, and A3-C1-C2 subunits, demonstrating that porcine factor VIIIa possesses three subunits.

The results of Mono STM HPLC of human thrombin-activated factor VIII preparations at pH 6.0 indicate that human factor VIIIa is labile under conditions that yield stable porcine factor VIIIa. However, although trace amounts of A2 fragment were identified in the peak fraction, determination of whether the coagulant activity resulted from small amounts of

heterotrimeric factor VIIIa or from heterodimeric factor VIIIa that has a low specific activity was not possible from this method alone.

A way to isolate human factor VIIIa before it loses its A2 subunit is desirable to resolve this question. To this end, isolation was accomplished in a procedure that involves reduction of the pH of the Mono STM buffers to pH 5. Mono QTM-purified human factor VIII (0.5 mg) was diluted with H₂O to give a final composition of 0.25 mg/ml (1 μ M) factor VIII in 0.25 M NaCl, 0.01 M Hepes, 2.5 mM CaCl₂, 0.005% Tween-80, at pH 7.4 (total volume 7.0 ml). Thrombin was added to a final concentration of 0.072 μ M and allowed to react for 3 min. Thrombin was then inactivated with FPR-CH₂Cl (0.2 μ M). The mixture then was diluted 1:1 with 40 mM sodium acetate, 5 mM CaCl₂, 0.01% Tween-80, at pH 5.0, and loaded at 2 ml/min onto a Mono STM HR 5/5 HPLC column equilibrated in 0.01 M sodium acetate, 5 mM CaCl₂, 0.01% Tween-80, at pH 5.0, plus 0.1 M NaCl. Factor VIIIa was eluted without column washing with a 20 ml gradient from 0.1 M NaCl to 1.0 M NaCl in the same buffer at 1 ml/min. This resulted in recovery of coagulant activity in a peak that contained detectable amounts of the A2 fragment as shown by SDS-PAGE and silver staining. The specific activity of the peak fraction was tenfold greater than that recovered at pH 6.0 (75,000 U/A₂₈₀ v. 7,500 U/A₂₈₀). However, in contrast to porcine factor VIIIa isolated at pH 6.0, which is indefinitely stable at 4°C, human factor VIIIa activity decreased steadily over a period of several hours after elution from Mono STM. Additionally, the specific activity of factor VIIIa purified at pH 5.0 and assayed immediately is only 5% that of porcine factor VIIIa, indicating that substantial dissociation occurred prior to assay.

These results demonstrate that both human and porcine factor VIIIa are composed of three subunits (A1, A2, and A3-C1-C2). Dissociation of the A2 subunit is responsible for the loss of activity of both human and porcine factor VIIIa under certain conditions, such as physiological ionic strength, pH, and concentration. The relative stability of porcine factor VIIIa under certain conditions is because of stronger association of the A2 subunit.

Example 4: Isolation and sequencing of DNA encoding the A2 domain of porcine factor VIII.

Only the nucleotide sequence encoding the B domain and part of the A2 domain of porcine factor VIII has been sequenced previously [Toole et al. (1986) *Proc. Natl. Acad. Sci. USA* 83:5939-5942]. The cDNA and predicted amino acid sequences (SEQ ID NOs: 3 and 4, respectively) for the entire porcine factor VIII A2 domain are disclosed herein.

The porcine factor VIII A2 domain was cloned by reverse transcription of porcine spleen total RNA and PCR amplification; degenerate primers based on the known human factor VIII cDNA sequence and an exact porcine primer based on a part of the porcine factor VIII sequence were used. A 1 kb PCR product was isolated and amplified by insertion into a Bluescript™ (Stratagene) phagemid vector.

The porcine A2 domain was completely sequenced by dideoxy sequencing. The cDNA and predicted amino acid sequences are as described in SEQ ID NOs: 3 and 4, respectively.

Example 5: Complete sequence of DNA encoding porcine factor VIII.

Klenow fragment, phosphorylated ClaI linkers, NotI linkers, T4 ligase, and *Taq* DNA polymerase were purchased from Promega (Madison, Wisconsin). Polynucleotide kinase was purchased from Life Technologies, Inc., Gaithersburg, Maryland. $\gamma^{32}\text{P}$ -ATP (Redivue, >5000Ci/mmol) was purchased from Amersham. pBluescript II KS- and *E. coli* Epicurean XL1-Blue cells were purchased from Stratagene (La Jolla, California). Synthetic oligonucleotides were purchased from Life Technologies, Inc. or Cruachem, Inc. 5'-phosphorylated primers were used when PCR products were produced for cloning purposes. Nucleotide (nt) numbering of oligonucleotides used as primers for polymerase chain reaction (PCR) amplification of porcine fVIII cDNA or genomic DNA uses the human fVIII cDNA as reference (Wood et al. (1984) *supra*).

Porcine spleen total RNA was isolated by acid guanidinium thiocyanate-phenol-chloroform extraction [Chomczynski et al. (1987) *Anal. Biochem.* 162:156-159]. Porcine cDNA was prepared from total spleen RNA using Moloney murine leukemia virus reverse

transcriptase (RT) and random hexamers to prime the reaction (First-Strand cDNA Synthesis Kit, Pharmacia Biotech) unless otherwise indicated. RT reactions contained 45 mM Tris-Cl, pH 8.3, 68 mM KCl, 15 mM DTT, 9 mM MgCl₂, 0.08 mg/ml bovine serum albumin and 1.8 mM deoxynucleotide triphosphate (dNTP). Porcine genomic DNA was isolated from spleen using a standard procedure (Strauss, W.M. (1995) In Current Protocols in Molecular Biology, F. M. Ausubel et al., editors, John Wiley & Sons, pp. 2.2.1-2.2.3). Isolation of DNA from agarose gels was done using Geneclean II (Bio 101) or Quiex II Gel Extraction Kit (Qiagen).

PCR reactions were done using a Hybaid OmniGene thermocycler. For PCR reactions employing *Taq* DNA polymerase, reactions included 0.6 mM MgCl₂, 0.2 mM dNTPs, 0.5 μM oligonucleotide primers, 50 U/ml polymerase and 0.1 volume of first strand cDNA reaction mix. Except where indicated otherwise, PCR products were gel purified, blunt-ended with Klenow fragment, precipitated with ethanol, and either ligated to the EcoRV site of dephosphorylated pBluescript II KS- or ligated with phosphorylated ClaI linkers using T4 ligase, digested with ClaI, purified by Sephacryl S400 chromatography, and ligated to ClaI-cut, dephosphorylated pBluescript II KS-. Ligations were done using T4 DNA ligase (Rapid DNA ligation kit, Boehringer Mannheim) except where indicated otherwise. Insert-containing pBluescript II KS- plasmids were used to transform *E. coli* Epicurean XL1-Blue cells.

Sequencing of plasmid DNA was done using an Applied Biosystems 373a automated DNA sequencer and the PRISM dye terminator kit or manually using Sequenase v. 2.0 sequencing kit (Amersham Corporation). Direct sequencing of PCR products, including ³²P-end labelling of oligonucleotides was done using a cycle sequencing protocol (dsDNA Cycle Sequencing System, Life Technologies).

Isolation of porcine fVIII cDNA clones containing 5' UTR sequence, signal peptide and A1 domain codons.

The porcine fVIII cDNA 5' to the A2 domain was amplified by nested RT-PCR of female pig spleen total RNA using a 5' rapid amplification of cDNA ends (5'-RACE) protocol (Marathon cDNA Amplification, Clontech, Version PR55453). This included first strand

cDNA synthesis using a lock-docking oligo(dT) primer [Borson, N.D. et al. (1992) *PCR Methods Appl.* 2:144-148], second strand cDNA synthesis using *E. coli* DNA polymerase I, and ligation with a 5' extended double stranded adaptor, SEQ ID NO:5

5'-CTA ATA CGA CTC ACT ATA GGG CTC GAG CGG CCG CCC GGG CAG GT-3
3'-H₂N-CCCGTCCA-PO₄-5'

whose short strand was blocked at the 3' end with an amino group to reduce non-specific PCR priming and which was complementary to the 8 nucleotides at the 3' end (Siebert, P.D., et al. (1995) *Nucleic. Acids. Res.* 23:1087-1088). The first round of PCR was done using an adaptor-specific oligonucleotide, SEQ ID NO:6 5'-CCA TCC TAA TAC GAC TCA CTA TAG GGC-3' (designated AP1) as sense primer, and a porcine fVIII A2 domain specific oligonucleotide SEQ ID NO:7 5'-CCA TTG ACA TGA AGA CCG TTT CTC-3' (nt 2081-2104) as antisense primer. The second round of PCR was done using a nested, adaptor-specific oligonucleotide, SEQ ID NO:8 5'-ACT CAC TAT AGG GCT CGA GCG GC-3' (designated AP2) as sense primer, and a nested, porcine A2 domain-specific oligonucleotide SEQ ID NO:9 5'-GGG TGC AAA GCG CTG ACA TCA GTG-3' (nt 1497-1520) as antisense primer. PCR was carried out using a commercial kit (Advantage cDNA PCR core kit) which employs an antibody-mediated hot start protocol [Kellogg, D.E. et al. (1994) *BioTechniques* 16:1134-1137]. PCR conditions included denaturation at 94°C for 60 sec, followed by 30 cycles (first PCR) or 25 cycles (second PCR) of denaturation for 30 sec at 94°C, annealing for 30 sec at 60°C and elongation for 4 min at 68°C using tube temperature control. This procedure yielded a prominent ≈1.6 kb product which was consistent with amplification of a fragment extending approximately 150 bp into the 5' UTR. The PCR product was cloned into pBluescript using ClaI linkers. The inserts of four clones were sequenced in both directions.

The sequence of these clones included regions corresponding to 137 bp of the 5' UTR, the signal peptide, the A1 domain and part of the A2 domain. A consensus was reached in at least 3 of 4 sites. However, the clones contained an average of 4 apparent PCR-generated mutations, presumably due to the multiple rounds of PCR required to generate a clonable product. Therefore, we used sequence obtained from the signal peptide region to design a sense strand phosphorylated PCR primer, SEQ ID NO:10 5'-CCT CTC GAG CCA CCA TGT CGA GCC ACC ATG CAG CTA GAG CTC TCC ACC TG-3', designated RENEOPIGSP, for

synthesis of another PCR product to confirm the sequence and for cloning into an expression vector. The sequence in bold represents the start codon. The sequence 5' to this represents sequence identical to that 5' of the insertion site into the mammalian expression vector ReNeo used for expression of fVIII (Lubin et al. (1994) *supra*). This site includes an Xho1 cleavage site (underlined). RENEOPIGSP and the nt 1497-1520 oligonucleotide were used to prime a Taq DNA polymerase-mediated PCR reaction using porcine female spleen cDNA as a template. DNA polymerases from several other manufacturers failed to yield a detectable product. PCR conditions included denaturation at 94°C for four min, followed by 35 cycles of denaturation for 1 min at 94°C, annealing for 2 min at 55°C and elongation for 2 min at 72°C, followed by a final elongation step for 5 min at 72°C. The PCR product was cloned into pBluescript using ClaI linkers. The inserts of two of these clones were sequenced in both directions and matched the consensus sequence.

Isolation of porcine fVIII cDNA clones containing A3, C1 and 5' half of the C2 domain codons.

Initially, two porcine spleen RT-PCR products, corresponding to a B-A3 domain fragment (nt 4519-5571) and a C1-C2 domain fragment (nt 6405-6990) were cloned. The 3' end of the C2 domain that was obtained extended into the exon 26 region, which is the terminal exon in fVIII. The B-A3 product was made using the porcine-specific B domain primer, SEQ ID NO:11 5' CGC GCG GCC GCG CAT CTG GCA AAG CTG AGT T 3', where the underlined region corresponds to a region in porcine fVIII that aligns with nt 4519-4530 in human fVIII. The 5' region of the oligonucleotide includes a NotI site that was originally intended for cloning purposes. The antisense primer used in generating the B-A3 product, SEQ ID NO:12 5'-GAA ATA AGC CCA GGC TTT GCA GTC RAA-3' was based on the reverse complement of the human fVIII cDNA sequence at nt 5545-5571. The PCR reaction contained 50 mM KCl, 10 mM Tris-Cl, pH 9.0, 0.1 % Triton X-100, 1.5 mM MgCl₂, 2.5 mM dNTPs, 20 µM primers, 25 units/ml Taq DNA polymerase and 1/20 volume of RT reaction mix. PCR conditions were denaturation at 94°C for 3 min, followed by 30 cycles of denaturation for 1 min at 94° C, annealing for 2 min at 50°C and elongation for 2 min at 72°C. The PCR products were phosphorylated using T4 DNA kinase and NotI linkers were added. After

cutting with NotI, the PCR fragments were cloned into the NotI site of BlueScript II KS- and transformed into XL1-Blue cells.

The C1-C2 product was made using the known human cDNA sequence to synthesize sense and antisense primers, SEQ ID NO:13 5'-AGG AAA TTC CAC TGG AAC CTT N-3' (nt 6405-6426) and SEQ ID NO:14 5'-CTG GGG GTG AAT TCG AAG GTA GCG N-3' (reverse complement of nt 6966-6990), respectively. PCR conditions were identical to those used to generate the B-A2 product. The resulting fragment was ligated to the pNOT cloning vector using the Prime PCR Cloner Cloning System (5 Prime-3 Prime, Inc., Boulder, Colorado) and grown in JM109 cells.

The B-A3 and C1-C2 plasmids were partially sequenced to make the porcine-specific sense and antisense oligonucleotides, SEQ ID NO:15 5'-GAG TTC ATC GGG AAG ACC TGT TG-3' (nt 4551-4573) and SEQ ID NO:16 5'-ACA GCC CAT CAA CTC CAT GCG AAG-3' (nt 6541-6564), respectively. These oligonucleotides were used as primers to generate a 2013 bp RT-PCR product using a Clontech Advantage cDNA PCR kit. This product, which corresponds to human nt 4551-6564, includes the region corresponding to the light chain activation peptide (nt 5002-5124), A3 domain (nt 5125-6114) and most of the C1 domain (nt 6115-6573). The sequence of the C1-C2 clone had established that human and porcine cDNAs from nt 6565 to the 3' end of the C1 domain were identical. The PCR product cloned into the EcoRV site of pBluescript II KS-. Four clones were completely sequenced in both directions. A consensus was reached in at least 3 of 4 sites.

Isolation of porcine fVIII cDNA clones containing the 3' half of the C2 domain codons.

The C2 domain of human fVIII (nucleotides 6574-7053) is contained within exons 24-26 [Gitschier J. et al. (1984) *Nature* 312:326-330]. Human exon 26 contains 1958 bp, corresponding nucleotides 6901-8858. It includes 1478 bp of 3' untranslated sequence. Attempts to clone the exon 26 cDNA corresponding to the 3' end of the C2 domain and the 3'UTR by 3' RACE [Siebert et al. (1995) *supra*], inverse PCR [Ochman, H. et al. (1990) *Biotechnology (N.Y.)* 8:759-760], restriction site PCR [Sarkar, G. et al. (1993) *PCR Meth.*

Appl. 2:318-322], "unpredictably primed" PCR [Dominguez, O. et al. (1994) *Nucleic. Acids Res.* 22:3247-3248] and by screening a porcine liver cDNA library failed. 3' RACE was attempted using the same adaptor-ligated double stranded cDNA library that was used to successfully used to clone the 5' end of the porcine fVIII cDNA. Thus, the failure of this method was not due to the absence of cDNA corresponding to exon 26.

A targeted gene walking PCR procedure [Parker, J.D. et al. (1991) *Nucleic. Acids. Res.* 19:3055-3060] was used to clone the 3' half of the C2 domain. A porcine-specific sense primer, SEQ ID NO:17 5'-TCAGGGCAATCAGGACTCC-3' (nt 6904-6924) was synthesized based on the initial C2 domain sequence and was used in a PCR reaction with nonspecific "walking" primers selected from oligonucleotides available in the laboratory. The PCR products were then targeted by primer extension analysis [Parker et al. (1991) *BioTechniques* 10:94-101] using a ³²P-end labelled porcine-specific internal primer, SEQ ID NO:18 5'-CCGTGGTGAACGCTCTGGACC-3' (nt 6932-6952). Interestingly, of the 40 nonspecific primers tested, only two yielded positive products on primer extension analysis and these two corresponded to an exact and a degenerate human sequence at the 3' end of the C2 domain: SEQ ID NO:19 5'-GTAGAGGTCCTGTGCCTCGCAGCC-3' (nt 7030-7053) and SEQ ID NO:20 5'-GTAGAGSTSCTGKGCCTCRCAKCCYAG-3', (nt 7027-7053). These primers had initially been designed to yield a product by conventional RT-PCR but failed to yield sufficient product that could be visualized by ethidium bromide dye binding. However, a PCR product could be identified by the more sensitive primer extension method. This product was gel-purified and directly sequenced. This extended the sequence of porcine fVIII 3' to nt 7026.

Additional sequence was obtained by primer extension analysis of a nested PCR product generated using the adaptor-ligated double-stranded cDNA library used in the 5'-RACE protocol described previously. The first round reaction used the porcine exact primer SEQ ID NO:21 5'-CTTCGCATGGAGTTGATGGGCTGT-3' (nt 6541-6564) and the AP1 primer. The second round reaction used SEQ ID NO:22 5'-AATCAGGACTCCTCCACCCCCG-3' (nt 6913-6934) and the AP2 primer. Direct PCR sequencing extended the sequence 3' to the end of the C2 domain (nt 7053). The C2 domain sequence was unique except at nt 7045 near the

3' end of the C2 domain. Analysis of repeated PCR reactions yielded either A, G or a double read of A/G at this site.

Sequencing was extended into the 3'UTR using two additional primers, SEQ ID NO:23 5'-GGA TCC ACC CCA CGA GCT GG-3' (nt 6977-6996) and SEQ ID NO:24 5'-CGC CCT GAG GCT CGA GGT TCT AGG-3' (nt 7008-7031). Approximately 15 bp of 3' UTR sequence were obtained, although the sequence was unclear at several sites. Several antisense primers then were synthesized based on the best estimates of the 3' untranslated sequence. These primers included the reverse complement of the TGA stop codon at their 3' termini. PCR products were obtained from both porcine spleen genomic DNA and porcine spleen cDNA that were visualized by agarose gel electrophoresis and ethidium bromide staining using a specific sense primer SEQ ID NO:25 5'-AAT CAG GAC TCC TCC ACC CCC G-3' (nt 6913-6934) and the 3' UTR antisense primer, SEQ ID NO:26 5'-CCTTGCAGGAATTCGATTCA-3'. To obtain sufficient quantities of material for cloning purposes, a second round of PCR was done using a nested sense primer, SEQ ID NO:27 5'-CCGTGGTGAACGCTCTGGACC-3' (nt 6932-6952) and the same antisense primer. The 141 bp PCR product was cloned into EcoRV-cut pBluescript II KS-. Sequence of three clones derived from genomic DNA and three clones derived from cDNA was obtained in both directions. The sequence was unambiguous except at nt 7045, where genomic DNA was always A and cDNA was always G.

Multiple DNA sequence alignments of human, porcine, and mouse fVIII (Fig. 1A-1H).

Alignments of the signal peptide, A1, A2, A3, C1, and C2 regions were done using the CLUSTALW program [Thompson, J.D. et al. (1994) *Nucleic. Acids. Res.* 22:4673-4680]. Gap open and gap extension penalties were 10 and 0.05 respectively. The alignments of the human, mouse, and pig B domains have been described previously [Elder et al. (1993) *supra*]. The human A2 sequence corresponds to amino acids 373-740 in SEQ ID NO:2. The porcine A2 amino acid sequence is given in SEQ ID NO:4, and the mouse A2 domain amino acid sequence is given in SEQ ID NO:28, amino acids 392-759.

Example 6: Expression of active, recombinant B-domainless porcine factor VIII (PB⁻).

Materials

Citrated hemophilia A and normal pooled human plasmas were purchased from George King Biomedical, Inc. Fetal bovine serum, geneticin, penicillin, streptomycin, DMEM/F12 medium and AIM-V medium were purchased from Life Technologies, Inc. *Taq* DNA polymerase was purchased from Promega. *Vent* DNA polymerase was purchased from New England Biolabs. *Pfu* DNA polymerase and the phagemid pBlueScript II KS⁻ were purchased from Stratagene. Synthetic oligonucleotides were purchased from Life Technologies or Cruachem, Inc. Restriction enzymes were purchased from New England Biolabs or Promega. 5'-phosphorylated primers were used when PCR products were produced for cloning purposes. Nucleotide (nt) numbering of oligonucleotides used as primers for polymerase chain reaction (PCR) amplification of porcine fVIII cDNA or genomic DNA uses the human fVIII cDNA as reference [Wood et al. (1984) *Nature* 312:330-337]. A fVIII expression vector, designated HB⁻/ReNeo, was obtained from Biogen, Inc. HB⁻/ReNeo contains ampicillin and geneticin resistance genes and a human fVIII cDNA that lacks the entire B domain, defined as the Ser741-Arg1648 cleavage fragment produced by thrombin. To simplify mutagenesis of fVIII C2 domain cDNA, which is at the 3' end of the fVIII insert in ReNeo, a *NotI* site was introduced two bases 3' to the stop codon of HB⁻/ReNeo by splicing-by-overlap extension (SOE) mutagenesis [Horton, R.M. et al. (1993) *Methods Enzymol.* 217:270-279]. This construct is designated HB⁻ReNeo/*NotI*.

Total RNA was isolated by acid guanidinium thiocyanate-phenol-chloroform extraction [Chomczynski, P. et al. (1987) *Anal. Biochem.* 162:156-159]. cDNA was synthesized from mRNA using Moloney murine leukemia virus reverse transcriptase (RT) and random hexamers according to instructions supplied by the manufacturer (First-Strand cDNA Synthesis Kit, Pharmacia Biotech). Plasmid DNA was purified using a Qiagen Plasmid Maxi Kit (Qiagen, Inc.). PCR reactions were done using a Hybaid OmniGene thermocycler using *Taq*, *Vent*, or *Pfu* DNA polymerases. PCR products were gel purified, precipitated with ethanol, and ligated into plasmid DNA using T4 DNA ligase (Rapid DNA ligation kit, Boehringer Mannheim). Insert-containing plasmids were used to transform *E. coli* Epicurean XL1-Blue cells. All novel

fVIII DNA sequences generated by PCR were confirmed by dideoxy sequencing using an Applied Biosystems 373a automated DNA sequencer and the PRISM dye terminator kit.

Construction of a hybrid fVIII expression vector, HP20, containing the porcine C2 domain.

A porcine fVIII cDNA corresponding to the 3' end of the C1 domain and all of the C2 domain was cloned into pBluescript by RT-PCR from spleen total RNA using primers based on known porcine fVIII cDNA sequence [Healey, J.F. et al. (1996) *Blood* 88:4209-4214]. This construct and HB⁻/ReNeo were used as templates to construct a human C1-porcine C2 fusion product in pBlueScript by SOE mutagenesis. The C1-C2 fragment in this plasmid was removed with *ApaI* and *NotI* and ligated into *ApaI/NotI*-cut HB⁻/ReNeo/*NotI* to produce HP20/ReNeo/*NotI*.

Construction of B-domain deleted hybrid human/porcine fVIII containing the porcine light chain (HP18)-

The human fVIII light chain consists of amino acid residues Asp1649-Tyr2332. The corresponding residues in the porcine fVIII cDNA were substituted for this region of HB⁻ to produce a hybrid human/porcine fVIII molecule designated HP18. This was done by substituting a PCR product corresponding to porcine A2 region, the A3 domain, the C1 domain, and part of the C2 domain for the corresponding region in HP20. To facilitate constructions, a synonymous *AvrII* site was introduced into nt 2273 at the junction of the A2 and A3 domains of HP20 by SOE mutagenesis.

Construction of B-domain deleted hybrid human/porcine fVIII containing the porcine signal peptide, A1 domain and A2 domain (HP22)-

The human fVIII signal peptide, A1 domain and A2 domains consist of amino acid residues Met(-19)-Arg740. The corresponding residues in the porcine fVIII cDNA were substituted for this region of HB⁻ to produce a molecule designated HP22. Additionally, a synonymous *AvrII* site was introduced into nt 2273 at the junction of the A2 and A3 domains of HP22 by SOE mutagenesis. HP22 was constructed by fusion of a porcine signal peptide-A1-partial A2 fragment in pBlueScript [Healy et al. (1996) *supra*] with a B-domainless hybrid

human/porcine fVIII containing the porcine A2 domain, designated HP1 [Lubin et al. (1994) *supra*].

Construction of porcine B domainless fVIII-(PB⁻)

A SpeI/NotI fragment of HP18/BS (+ *AvrII*) was digested with *AvrII*/NotI and ligated into *AvrII*/NotI-digested HP22/BS (+ *AvrII*) to produce a construct PB⁻/BS (+ *AvrII*), which consists of the porcine fVIII lacking the entire B domain. PB⁻ was cloned into ReNeo by ligating an *Xba*/NotI fragment of PB⁻/BS (+ *AvrII*) into HP22/ReNeo/NotI (+ *AvrII*).

Expression of recombinant fVIII molecules

PB⁻/ReNeo/NotI (+ *AvrII*) and HP22/ReNeo/NotI (+ *AvrII*) were transiently transfected into COS cells and expressed as described previously [Lubin, I.M. et al. (1994) *J. Biol. Chem.* 269:8639-8641]. HB⁻/ReNeo/NotI and no DNA (mock) were transfected as a control.

The fVIII activity of PB⁻, HP22, and HB⁻ were measured by a chromogenic assay as follows. Samples of fVIII in COS cell culture supernatants were activated by 40 nM thrombin in a 0.15 M NaCl, 20 mM HEPES, 5mM cAC12, 0.01% Tween-80, pH 7.4 in the presence of 10 nM factor IXa, 425 nM factor X, and 50 μ M unilamellar phosphatidylserine-[phosphatidylcholine (25/75 w/w) vesicles. After 5 min, the reaction was stopped with 0.05 M EDTA and 100 nM recombinant desulfatohirudin and the resultant factor Xa was measured by chromogenic substrate assay. In the chromogenic substrate assay, 0.4 mM Spectrozyme Xa was added and the rate of para-nitroanilide release was measured by measuring the absorbance of the solution at 405 nm.

Results of independently transfected duplicate cell culture supernatants (absorbance at 405 nm per minute)

HB⁻: 13.9

PB⁻: 139

HP22: 100

mock: <0.2

These results indicate that porcine B-domainless fVIII and a B-domainless fVIII consisting of the porcine A1 and A2 subunits are active and suggest that they have superior activity to human B-domainless fVIII.

PB⁻ was partially purified and concentrated from the growth medium by heparin-Sepharose chromatography. Heparin-Sepharose (10 ml) was equilibrated with 0.075 M NaCl, 10 mM HEPES, 2.5 mM CaCl₂, 0.005 % Tween-80, 0.02 % sodium azide, pH 7.40. Medium (100-200 ml) from expressing cells was applied to the heparin-Sepharose, which then was washed with 30 ml of equilibration buffer without sodium azide. PB⁻ was eluted with 0.65 M NaCl, 20 mM HEPES, 5 mM CaCl₂, 0.01 % Tween-80, pH 7.40 and was stored at -80 °C. The yield of fVIII coagulant activity was typically 50-75 %.

Stable expression of porcine B-domainless fVIII (PB⁻)

Transfected cell lines were maintained in Dulbecco's modified Eagle's medium-F12 containing 10 % fetal bovine serum, 50 U/ml penicillin, 50 µg/ml streptomycin. Fetal bovine serum was heat inactivated at 50 °C for one hour before use. HB⁻/ReNeo and PB⁻ReNeo/*NotI* (+ *AvrII*) were stably transfected into BHK cells and selected for geneticin resistance using a general protocol that has been described previously [Lubin et al. (1994) *Biol. Chem.* **269**:8639-8641] except that expressing cells were maintained in growth medium containing 600 µg/ml geneticin. Cells from Corning T-75 flasks grown to confluence were transferred to Nunc triple flasks in medium containing 600 µg/ml geneticin and grown to confluence. The medium was removed and replaced with serum-free, AIM-V medium (Life Technologies, Inc.) without geneticin. Factor VIII expression was monitored by one-stage factor VIII coagulant activity (*vide supra*) and 100-150 ml of medium was collected once daily for four to five days. Maximum expression levels in medium for HB⁻ and PB⁻ were 102 units per ml and 10-12 units per ml of factor VIII coagulant activity, respectively.

Purification of PB⁻

PB⁻ was precipitated from culture supernatant using 60 % saturated ammonium sulfate and then purified by W3-3 immunoaffinity chromatography and mono Q high pressure liquid

chromatography as described previously for the purification of plasma-derived porcine factor VIII [Lollar et al. (1993) Factor VIII/factor VIIIa. *Methods Enzymol.* 222:128-143]. The specific coagulant activity of PB⁻ was measured by a one-stage coagulation assay [Lollar et al. (1993) *supra*] and was similar to plasma-derived porcine factor VIII.

When analyzed by SDS-polyacrylamide gel electrophoresis, the PB⁻ preparation contained three bands of apparent molecular masses 160 kDa, 82 kDa, and 76 kDa. The 82 kDa and 76 kDa bands have been previously described as heterodimer containing the A1-A2 and ap-A3-C1-C2 domains (where ap refers to an activation peptide) [Toole et al. (1984) *Nature* 312:342-347]. The 160 kDa band was transferred to a polyvinylidene fluoride membrane and subjected to NH₂-terminal sequencing, which yielded Arg-Ile-Xx-Xx-Tyr (where Xx represents undermined) which is the NH₂-terminal sequence of single chain factor VIII [Toole et al. (1984) *supra*]. Thus, PB⁻ is partially processed by cleavage between the A2 and A3 domains, such that it consists of two forms, a single chain A1-A2-ap-A3-C1-C2 protein and a A1-A2/ap-A3-C1-C2 heterodimer. Similar processing of recombinant HB⁻ has been reported [Lind et al. (1995) *Eur. J. Biochem.* 232:19-27].

Characterization of Porcine factor VIII

We have determined the cDNA sequence of porcine fVIII corresponding to 137 bp of the 5' UTR, the signal peptide coding region (57 bp), and the A1 (1119 bp), A3 (990 bp), C1 (456 bp), and C2 (483 bp) domains. Along with previously published sequence of the B domain and light chain activation peptide regions [Toole et al. (1986) *supra*] and the A2 domain [Lubin et al. (1994) *supra*], the sequence reported here completes the determination of the porcine fVIII cDNA corresponding to the translated product. A fragment that included the 5' UTR region, signal peptide, and A1 domain cDNA was cloned using a 5'-RACE RT-PCR protocol. A primer based on human C2 sequence was successful in producing an RT-PCR product that led to cloning of the A3, C1, and 5' half of the C2 domain. The cDNA corresponding to the 3' half of the C2 domain and 3' UTR cDNA proved difficult to clone. The remainder of the C2 domain ultimately was cloned by a targeted gene walking PCR procedure [Parker et al. (1991) *supra*].

The sequence reported herein SEQ ID NO:29 was unambiguous except at nt 7045 near the 3' end of the C2 domain, which is either A or G as described hereinabove. The corresponding codon is GAC (Asp) or AAC (Asn). The human and mouse codons are GAC and CAG (Gln), respectively. Whether this represents a polymorphism or a reproducible PCR artifact is unknown. Recombinant hybrid human/porcine B-domainless fVIII cDNAs containing porcine C2 domain substitutions corresponding to both the GAC and AAC codons have been stably expressed with no detectable difference in procoagulant activity. This indicates that there is not a functional difference between these two C2 domain variants.

The alignment of the predicted amino acid sequence of full-length porcine fVIII SEQ ID NO:30 with the published human [Wood et al. (1984) *supra*] and murine [Elder et al. (1993) *supra*] sequences is shown in Fig. 1A-1H along with sites for post-translational modification, proteolytic cleavage, and recognition by other macromolecules. The degree of identity of the aligned sequences is shown in Table VII. As noted previously, the B domains of these species are more divergent than the A or C domains. This is consistent with the observation that the B domain has no known function, despite its large size [Elder et al. (1993) *supra*; Toole et al. (1986) *supra*]. The results of the present invention confirm that the B domain of porcine fVIII is not necessary for activity. Based on the sequence data presented herein, porcine fVIII having all or part of the B-domain deleted can be synthesized by expressing the porcine fVIII coding DNA having deleted therefrom all or part of codons of the porcine B domain. There is also more divergence of sequences corresponding to the A1 domain APC/factor IXa cleavage peptide (residues 337-372) and the light chain activation peptide (Table VII). The thrombin cleavage site at position 336 to generate the 337-372 peptide is apparently lost in the mouse since this residue is glutamine instead of arginine [Elder et al. (1993) *supra*]. The relatively rapid divergence of thrombin cleavage peptides (or in mouse fVIII a possibly vestigial 337-372 activation peptide) has been previously noted for the fibrinopeptides [Creighton, T. E. (1993) In Proteins: Structures and Molecular Properties, W.H. Freeman, New York, pp. 105-138]. Lack of biological function of these peptides once cleaved has been cited as a possible reason for the rapid divergence. Arg562 in human fVIII has been proposed to be the more important cleavage site for activated protein C during the

inactivation of fVIII and fVIIIa [Fay, P.J. et al. (1991) *J. Biol. Chem.* 266:20139-20145]. This site is conserved in human, porcine and mouse fVIII.

Potential N-linked glycosylation sites (NXS/T where X is not proline) can be seen in Fig. 1A-1H. There are eight conserved N-linked glycosylation sites: one in the A1 domain, one in the A2 domain, four in the B domain, one in the A3 domain, and one in the C1 domain. The 19 A and C domain cysteines are conserved, whereas there is divergence of B domain cysteines. Six of the seven disulfide linkages in fVIII are found at homologous sites in factor V and ceruloplasmin, and both C domain disulfide linkages are found in factor V [McMullen, B.A. et al. (1995) *Protein Sci.* 4:740-746]. Human fVIII contains sulfated tyrosines at positions 346, 718, 719, 723, 1664, and 1680 [Pittman, D.D. et al. (1992) *Biochemistry* 31:3315-3325; Michnick, D.A. et al. (1994) *J. Biol. Chem.* 269:20095-20102]. These residues are conserved in mouse fVIII and porcine fVIII (Fig. 1), although the CLUSTALW program failed to align the mouse tyrosine corresponding to Tyr346 in human fVIII.

Mouse and pig plasma can correct the clotting defect in human hemophilia A plasma, which is consistent with the level of conservation of residues in the A and C domains of these species. The procoagulant activity of porcine fVIII is superior to that of human fVIII [Lollar, P. et al. (1992) *J. Biol. Chem.* 267:23652-23657]. The recombinant porcine factor VIII (B domain-deleted) expressed and purified as herein described also displays greater specific coagulant activity than human fVIII, being comparable to plasma-derived porcine fVIII. This may be due to a decreased spontaneous dissociation rate of the A2 subunit from the active A1/A2/A3-C1-C2 fVIIIa heterotrimer. Whether this difference in procoagulant activity reflects an evolutionary change in function as an example of species adaptation [Perutz, M.F. (1996) *Adv. Protein Chem.* 36:213-244] is unknown. Now that the porcine fVIII cDNA sequence corresponding to the translated product is complete, homolog scanning mutagenesis [Cunningham, B.C., et al. (1989) *Science* 243:1330-1336] may provide a way to identify structural differences between human and porcine fVIII that are responsible for the superior activity of the latter.

Porcine fVIII is typically less reactive with inhibitory antibodies that arise in hemophiliacs who have been transfused with fVIII or which arise as autoantibodies in the general population. This is the basis for using porcine fVIII concentrate in the management of patients with inhibitory antibodies [Hay and Lozier (1995) *supra*]. Most inhibitors are directed against epitopes located in the A2 domain or C2 domain [Fulcher, C.A. et al. (1985) *Proc. Natl. Acad. Sci. USA* 82:7728-7732; Scandella, D. et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:6152-6156; Scandella, D. et al. (1989) *Blood* 74:1618-1626]. Additionally, an epitope of unknown significance has been identified that is in either the A3 or C1 domain [Scandella et al. (1989) *supra*; Scandella, D. et al. (1993) *Blood* 82:1767-1775; Nakai, H. et al. (1994) *Blood* 84:224a]. The A2 epitope has been mapped to residues 484-508 by homolog scanning mutagenesis [Healey et al. (1995) *supra*]. In this 25 residue segment, there is relatively low proportion of identical sequence (16/25 or 64%). It is interesting that this region, which appears to be functionally important based on the fact that antibodies to it are inhibitory, apparently has been subjected to relatively more rapid genetic drift. Alignment of the porcine A2 domain and A3 domains indicate that the A2 epitope shares no detectable homology with the corresponding region in the A3 domain.

The C2 inhibitor epitope of human fVIII has been proposed to be located to within residues 2248-2312 by deletion mapping [Scandella, D. et al. (1995) *Blood* 86:1811-1819]. Human and porcine fVIII are 83 % identical in this 65 residue segment. However, homolog scanning mutagenesis of this region to characterize the C2 epitope has revealed that a major determinant of the C2 epitope was unexpectedly located in the region corresponding to human amino acids 2181-2243 (SEQ ID NO:2) and Fig. 1H.

Human-porcine hybrid factor VIII proteins were made in which various portions of the C2 domain of human factor VIII were replaced by the corresponding portions of porcine factor VIII, using the strategy herein described. (Example 5) The synthesis of the various C2-hybrid factor VIIIs was accomplished by constructing hybrid coding DNA, using the nucleotide sequence encoding the porcine C2 region given in SEQ ID NO:30. Each hybrid DNA was expressed in transfected cells, such that the hybrid factor VIIIs could be partially purified from

the growth medium. Activity, in the absence of any inhibitor, was measured by the one-stage clotting assay.

A battery of five human inhibitors was used to test each hybrid factor VIII. The inhibitor plasmas containing anti factor VIII antibody had been previously shown to be directed against human C2 domain, based on the ability of recombinant human C2 domain to neutralize the inhibition. In all the test plasmas, the inhibitor titer was neutralized greater than 79% by C2 domain or light chain but less than 10% by recombinant human A2 domain. In addition the C2-hybrid factor VIIIs were tested against a murine monoclonal antibody, which binds the C2 domain, and like human C2 inhibitor antibodies, it inhibited the binding of factor VIII to phospholipid and to von Willebrand factor.

By comparing the antibody inhibitor titers against the C2-hybrid factor VIIIs, the major determinant of the human C2 inhibitor epitope was shown to be the region of residues 2181-2243 (SEQ ID NO:2, see also Fig. 1H). Anti-C2 antibodies directed to a region COOH-terminal to residue 2253 were not identified in four of the five patient sera. In comparing hybrids having porcine sequence corresponding to human amino acid residues numbers 2181-2199 and 2207-2243, it was apparent that both regions contribute to antibody binding. The porcine amino acid sequence corresponding to human residues 2181-2243 is numbered 1982-2044 in SEQ ID NO:30. The sequence of porcine DNA encoding porcine amino acids numbered 1982-2044 is nucleotides numbered 5944-6132 in SEQ ID NO:29.

Referring to Fig. 1H, it can be seen that in the region 2181-2243, there are 16 amino acid differences between the human and porcine sequences. The differences are found at residues 2181, 2182, 2188, 2195-2197, 2199, 2207, 2216, 2222, 2224-2227, 2234, 2238 and 2243. Amino acid replacement at one or more of these numbered residues can be carried out to make a modified human factor VIII non-reactive to human anti-C2 inhibitor antibodies. Alanine scanning mutagenesis provides a convenient method for generating alanine substitutions for naturally-occurring residues, as previously described. Amino acids other than alanine can be substituted as well, as described herein. Alanine substitutions for individual

amino acids, especially those which are non-identical between human/porcine or human/mouse or which are most likely to contribute to antibody binding, can yield a modified factor VIII with reduced reactivity to inhibitory antibodies.

Figs. 1A-1H taken together provide an aligned sequence comparison of the human, pig and mouse factor VIII amino acid sequences. Fig. 1A compares signal peptide regions (human, SEQ ID NO:31; porcine, SEQ ID NO:30, amino acids 1-19; murine, SEQ ID NO:28, amino acids 1-19). Note that the amino acids in Fig. 1A-1H are numbered at the first Alanine of the mature protein as number 1, with amino acids of the signal peptide assigned negative numbers. The Human fVIII sequence in SEQ ID NO:2 also begins with the first Alanine of the mature protein as amino acid number 1. In the amino acid sequences of mouse fVIII (SEQ ID NO:28) and porcine fVIII (SEQ ID NO:30), the first amino acid (alanine) of the mature sequence is amino acid number 20. Fig. 1A-1H shows an alignment of the corresponding sequences of human, mouse and pig fVIII, such that the regions of greatest amino acid identity are juxtaposed. The amino acid numbers in Fig. 1A-1H apply to human fVIII only. Fig. 1B gives the amino acid sequences for the A1 domain of human (SEQ ID NO:2, amino acids 1-372), porcine (SEQ ID NO:30, amino acids 20-391), and murine (SEQ ID NO:28, amino acids 20-391). Fig. 1C provides amino acid sequences for the Factor VIII A2 domains from human (SEQ ID NO:2, amino acids 373-740), pig (SEQ ID NO:30, amino acids 392-759) and mouse (SEQ ID NO:28, amino acids 392-759). Fig. 1D provides the amino acid sequences of B domains of human factor VIII (SEQ ID NO:2, amino acids 741-1648), pig (SEQ ID NO:30, amino acids 760-1449) and mouse (SEQ ID NO:28, amino acids 760-1640). Fig. 1E compares the amino acid sequences of Factor VIII light chain activation peptides of human, pig and mouse (SEQ ID NO:2, amino acids 1649-1689; SEQ ID NO:30, amino acids 1450-1490; and SEQ ID NO:28, amino acids 1641-1678, respectively). Fig. 1F provides the sequence comparison for human, pig and mouse Factor VIII A3 domains (SEQ ID NO:2, amino acids 1690-2019; SEQ ID NO:30, amino acids 1491-1820; and SEQ ID NO:28, amino acids 1679-2006, respectively). Fig. 1G provides the amino acid sequences of the Factor VIII C1 domains of human, pig and mouse (SEQ ID NO:2, amino acids 2020-2172; SEQ ID NO:30, amino acids 1821-1973; and SEQ ID NO:28, amino acids 2007-2159, respectively). Fig. 1H

provides sequence data for the C2 domains of the Factor VIII C2 domains of human, pig and mouse (SEQ ID NO:2, amino acids 2173-2332; SEQ ID NO:30, amino acids 1974-2133; and SEQ ID NO:28, amino acids 2160-2319, respectively).

The diamonds represent tyrosine sulfation sites, proposed binding sites for Factor IXa, phospholipid and Protein C are double-underlined, and regions involved in binding anti-A2 and anti-C2 inhibitory antibodies are italicized. Asterisks highlight amino acid sequences which are conserved. See also SEQ ID NO:29 (porcine factor VIII cDNA) and SEQ ID NO:30 (deduced amino acid sequence of porcine factor VIII). The human numbering system is used as the reference [Wood et al. (1984) *supra*]. The A1, A2, and B domains are defined by thrombin cleavage sites at positions 372 and 740 and an unknown protease cleavage site at 1648 as residues 1-372, 373-740, and 741-1648, respectively [Eaton, D.L. et al. (1986) *Biochemistry* 25:8343-8347]. The A3, C1, and C2 domains are defined as residues 1690-2019, 2020-2172, and 2173-2332, respectively [Vehar et al. (1984) *supra*]. Cleavage sites for thrombin (factor IIa), factor IXa, factor Xa and APC [Fay et al. (1991) *supra*; Eaton, D. et al. (1986) *Biochemistry* 25:505-512; Lamphear, B.J. et al. (1992) *Blood* 80:3120-3128] are shown by placing the enzyme name over the reactive arginine. An acidic peptide is cleaved from the fVIII light chain by thrombin or factor Xa at position 1689. Proposed binding sites for factor IXa [Fay, P.J. et al. (1994) *J. Biol. Chem.* 269:20522-20527; Lenting, P.J. et al. (1994) *J. Biol. Chem.* 269:7150-7155), phospholipid (Foster, P.A. et al. (1990) *Blood* 75:1999-2004) and protein C (Walker, F.J. et al. (1990) *J. Biol. Chem.* 265:1484-1489] are doubly underlined. Regions involved in binding anti-A2 [Lubin et al. (1994) *supra*; Healey et al. (1995) *supra*]; and previously proposed for anti-C2 inhibitory antibodies are italicized. The C2 inhibitor epitope identified as herein described (human amino acids 2181-2243) is shown by a single underline in Fig. 1H. Tyrosine sulfation sites [Pittman et al. (1992) *supra*; Michnick et al. (1994) *supra*] are shown by ♦.

Example 7: Construction of POL1212 and Expression in Baby Hamster Kidney Cells.

POL1212 is a partially B-domainless porcine factor VIII, having the B-domain deleted except that 12 amino acids of the NH₂ terminus of the B-domain and 12 amino acids of the -COOH terminus are retained.

The cDNAs encoding for the sequences for the porcine fVIII domains A1, A2, *ap*-A3-C1, and C2 were obtained as described in Example 5. The DNA nucleotide sequence and derived amino acid sequence of porcine factor VIII are presented as SEQ ID NO:29 and SEQ ID NO:30, respectively. The amplified fragments were separately cloned into the plasmid pBluescript II KS⁻ (pBS).

POL1212 refers to the cDNA encoding porcine fVIII lacking most of the B domain but containing DNA sequence encoding a 24 amino acid linker between the A2 and *ap* domains. POL1212 was constructed in a mammalian expression vector, ReNeo, which was obtained from Biogen. ReNeo can replicate in bacteria, replicate as an episome in COS cells for transient expression of factor VIII, or be stably integrated into a variety of mammalian cells. It consists of 1) sequences derived from plasmid pBR322 that include an origin of replication and ampicillin resistance gene, 2) a neomycin resistance gene whose expression is under control of the SV40 promoter/enhancer, SV40 small t intron, and the SV40 polyadenylation signal regulatory elements, 3) a site for insertion of fVIII and its signal peptide, the expression of which is under control of the SV40 enhancer, adenovirus type 2 major late promoter, and adenovirus type 2 tripartite leader sequence. Any vector having similar functional components can be used in place of the ReNeo vector.

POL1212/ReNeo was prepared in several steps. First, the cDNAs encoding for porcine fVIII heavy chain (A1-A2) and the cDNAs encoding for porcine fVIII light chain (*ap*-A3-C1-C2) were separately assembled in pBS. From these constructs, the DNA encoding for porcine B-domainless fVIII was assembled in pBS (PB-/pBS). This form of porcine fVIII lacks the entire B domain, defined as amino acids corresponding to residues 741 – 1648 in human fVIII (human nucleotides 2278 – 5001). Next, the DNA encoding for porcine A2 was substituted for

the human A2 domain in the human B-domainless fVIII expression vector ReNeo (HB-/ReNeo). The DNA encoding the remainder of the porcine heavy chain and the DNA encoding the porcine light chain was substituted for the human domains in two additional steps using the porcine heavy chain/pBS and PB-/pBS constructs made previously. A fragment of the human B domain encoding the 5 C-terminal and 9 N-terminal amino acids was inserted between the A2 and A3 domains producing a construct called PSQ/ReNeo [Healey et al. (1998) 92:3701-3709]. Residues Glu2181-Val2243 contain a major determinant of the inhibitory epitope in the C2 domain of human factor VIII). This construct was used as a template to make a fragment of the porcine B domain encoding for the 12 C-terminal and 12 N-terminal amino acids. This fragment was inserted between the A2 and A3 domains resulting in the final construct, POL1212/ReNeo.

The POL1212 24 amino acid linker consists of the first 12 and last 12 residues of the porcine fVIII B domain. The POL1212 linker has the following sequence:

SFAQNSRPPSASAPKPPVLR RHQR. (SEQ ID NO:32)

The nucleotide sequence corresponding to the 1212 linker and surrounding amino acids is:

GTC ATT GAA CCT AGG AGC TTT GCC CAG AAT TCA AGA CCC CCT AGT GCG
(SEQ ID NO: 33)

V I E P R S F A Q N S R P P S A

AGC GCT CCA AAG CCT CCG GTC CTG CGA CGG CAT CAG AGG GAC ATA

S A P K P P V L R R H Q R D I

AGC CTT CCT ACT

S L P T

The POL1212 linker was synthesized by splicing-by-overlap extension (SOE) mutagenesis, as follows:

PCR reactions used to make SOE products were as follows:

REACTION #1

Outside primer: Rev 4, which is a porcine A2 primer, nucleotides 1742-1761. (SEQ ID NO:29) The sequence is: 5'-GAGGAAAACCAGATGATGTCA-3' (SEQ ID NO:34)

Inside primer: OL12, which is a porcine reverse primer covering the first (5') 15 amino acids of OL1212 and the last (3') 5 amino acids of porcine A2. The sequence is: 5'-CTTTGGAGCGCTCGCACTAGGGGGTCTTGAATTCTGGGCAAAGCTCCTAGGTTC AATGAC-3' (SEQ ID NO:35)

Template: PSQ/ReNeo

Product: porcine DNA from nucleotide 1742 in the A2 domain to 2322 in OL1212, 580 bp

REACTION #2

Outside primer: P2949 is a porcine reverse A3 primer, nucleotides 2998-3021 of SEQ ID NO:29. The sequence is: 5'-GGTCACTTGTCTACCGTGAGCAGC -3' (see SEQ ID NO:29)

Inside primer: OL12+, a porcine primer covering the last (3') 16 amino acids of OL1212 and the first (5') 6 amino acids of the activation peptide, nucleotide 2302-2367 of SEQ ID NO:29. The sequence is: 5'-CCTAGTGCGAGCGCTCCAAAGCCTCCGGTCCTGCGACGGCATCAGAGGGACATA AGCCTTCCTACT-3' (SEQ ID NO:36)

Template: PSQ/ReNeo

Product: porcine from nucleotide 2302 in OL1212 to nucleotide 3021 in the A3 domain, 719 bp

SOE REACTION

Primers: Rev 4, P2949-

Templates: Fragment from rxn #1 (bp) and low melt fragment from rxn #2 (bp)

Product: porcine DNA from nucleotide 1742 in the A2 domain to nucleotide 3021 in the A3 domain (SEQ ID NO:29) including OL1212, 1279 bp. The reaction product was ethanol precipitated.

The 1212 linker was inserted into PSQ/ReNeo by cutting the SOE product (insert) and PSQ/ReNeo (vector) with *BsaB I*. The vector and insert were ligated using T4 ligase and the product was used to transform *E. coli* XL1-Blue cells. Plasmid DNA was prepared from several colonies and the sequence of the 1212 linker and other PCR-generated sequence was verified by DNA sequence analysis.

CULTURE OF BABY HAMSTER KIDNEY (BHK) CRL-1632 CELLS

A BHK cell line was obtained from the ATCC, accession identification CRL-1632 and was stored frozen at -20°C until further use. The cells were thawed at 37°C and put into 10 ml of complete medium, defined as DMEM/F12, 50 U/ml penicillin, 50 $\mu\text{g/ml}$ streptomycin plus 10 % fetal bovine serum (FBS). FBS was purchased from Hyclone, Logan Utah. The cells were centrifuged for 2 minutes at 300 RPM. The medium was aspirated and the cells were resuspended in two ml complete medium in a T-75 flask containing 20 ml of complete medium.

POL1212 has been expressed in both baby hamster kidney (BHK) and Chinese hamster ovary (CHO) cells. Two BHK lines were used, the CRL-1632 line from ATCC and another BHK line obtained from R. McGillivray, University of British Columbia, [Funk, et al. (1990) *Biochemistry* 29:1654-1660]. The latter were subcultured without selection in the inventors' lab and designated BHK1632 (Emory). The CHO cell line was CHO-K1, ATCC accession CCL-61. The expression of the average clone from the Emory cell line and from CHO-K1 cells was somewhat higher than from CRL-1632 cells as judged by chromogenic assay activity.

The cells grown in the T-75 flask formed a confluent monolayer. A 60 ml culture of *E. coli* XL1-Blue cells in LB/ampicillin (50 mg/ml) carrying the POL1212/ReNeo plasmid was prepared.

TRANSFECTION OF CRL-1632 BHK CELLS WITH POL1212/ReNeo

DNA from the overnight culture of the POL1212/ReNeo XL1-Blue cells was prepared using a Qiagen, Valencia, CA Spin Miniprep kit. One flask of CRL-1632 cells was split into a stock flask with 0.2 ml and a flask for transfection with 0.3 ml from 2 ml total. The other flask was fed fresh medium. Medium was DMEM/F12 + 10% Hyclone FBS + 50 U/ml penicillin, 50 µg/ml streptomycin. CRL-1632 cells were split into 6 well plates aiming for 50-90% confluence for transfection (0.3 ml of cells from the T-75 flask in 2 ml 1:5000 Versene [Life Technologies, Gaithersburg, MD] in each well) using fresh DMEM/F12 + 10% Hyclone FBS + 50 U/ml penicillin, 50 µg/ml streptomycin.

The following solutions were prepared in sterile 1-2 ml test tubes;

A) 48 µl (10µg) Miniprep POL1212/ReNeo DNA plus µl medium without serum (DMEM/F12) plus 10 µl Lipofectin™ (Life Technologies, Gaithersburg, MD).

B) 10 µl Lipofectin plus 190 µl medium (mock transfection) was gently mixed and the DNA and Lipofectin allowed to react for 15 minutes at room temperature. During this time, the cells were washed twice with 2 ml of DMEM/F12. 1.8 ml of DMEM/F12 was then added to the cells. The DNA/Lipofectin complex was added dropwise to the cells, and swirled gently to mix. The cells remained in the incubator overnight. Removed the DNA/Lipofectin and added 3 ml of medium with serum to the cells. Incubated the cells 30 - 48 hours. Geneticin was purchased from Life Technologies, Gaithersburg, MD. The cell cultures were divided 1:20, 1:50 and 1:100, 1:250, 1:500 onto 10 cm dishes in 10 ml of medium with serum containing 535 µg/ml geneticin. Over the next several days, cells that did not take up the POL1212/ReNeo plasmid were killed due to the presence of geneticin. The remaining cells continued to replicate in geneticin, forming visible monolayer colonies on the dishes.

EXPRESSION AND ASSAY OF POL1212 from BHK CRL-1632 CELLS

Small plastic cylindrical rings were placed around the colonies. The colonies were aspirated separately using complete medium and transferred to test tubes. These colonies are referred to as ring cloned colonies. Ring cloned colonies were plated separately onto 24 well plates and grown in complete medium.

CHROMOGENIC SUBSTRATE ASSAY FOR FACTOR VIII EXPRESSION BY TRANSFECTED CRL-1632 CELLS

Samples of POL1212 from cell culture supernatants were mixed with 50 nM purified porcine factor IXa and 0,05 mM phosphatidylcholine/phosphatidylserine (PCPS) vesicles in 0.15M NaCl, 20 mM HEPES, 5mM CaCl₂, 0.01 % Tween 80, pH 7.4. As a control, cell culture medium from mock-transfected cells was used. Thrombin and factor X were added simultaneously to final concentrations of 40 and 425 nM, respectively. thrombin activates factor VIII, which then, along with PCPS, serves as a cofactor for factor IXa during the activation of factor X.

After 5 min, the activation of factor X by factor IXa/factor VIIIa/PCPS was stopped by the addition of EDTA to a final concentration of 50 mM. At the same time the activation of factor VIII by thrombin was stopped by the addition of the thrombin inhibitor, recombinant desulfatohirudin, to a final concentration of 100 nM. A 25- μ l sample of the reaction mix was transferred to a microtiter well, to which was added 74 μ l of Spectrozyme Xa (America Diagnostica, Greenwich, CT), which is a chromogenic substrate for factor Xa. The final concentration of Spectrozyme Xa was 0.6 mM. The absorbance at 405 nm due to the cleavage of Spectrozyme Xa by factor Xa was monitored continuously for 5 minutes with a Vmax Kinetic Plate Reader (Molecular Devices, Inc., Menlo park, CA). The results are expressed in terms of A₄₀₅/min.

Factor VIII chromogenic assay of ten ring-cloned colonies:

Colony number	A_{405}/min ($\times 10^3$)
Buffer	0.2
1	2.1
2	8.4
3	6.4
4	10.7
5	12.5
6	7.6
7	51.3
8	139.5
9	3.8
10	8.4

These results show that all ten colonies that were selected express factor VIII activity that is at least ten-fold greater than background.

The activity from medium of colony 8, which was the highest expressing colony, was further examined by one-stage factor VIII clotting assay. In this assay, 50 μ l of factor VIII deficient plasma (George King Biomedical Overland Park, KA), 5 μ l sample or standard, and 50 μ l of activated particulate thromboplastin time reagent (Organon Teknika, Durham, NC) were incubated 3 min at 37° C. Samples include colony 8 medium diluted in 0.15 M NaCl, mM hepes, pH 7.4 (HBS) or, as a control, complete medium. Clotting was initiated by addition of 50 μ l of 20 mM CaCl_2 . The clotting time was measured using an ST4 BIO Coagulation Instrument (Diagnostics Stago, Parsippany, NJ). A standard curve was obtained by making dilutions of pooled, citrated normal human plasma, lot 0641 (George King Biomedical, Overland Park, KA). The factor VIII concentration of the standard was 0.9 units per ml.

Standard curve:

	<u>Dilution</u>	<u>U/ml</u>	<u>Clot Time</u>
1)	Undiluted	0.96	45.2
2)	1/3 (HBS)	0.32	53.7
3)	1/11 (HBS)	0.087	62.5
4)	1/21 (HBS)	0.046	68.9

Linear regression of the clotting times versus the logarithm of the concentration of standard yielded a correlation coefficient of 0.997.

Test substances gave the following clotting times, which were converted to units per ml using the standard curve:

	<u>Sample</u>	<u>Clot Time (sec)</u>	<u>Units/ml</u>
1)	Colony 8 (24h), 1/10 in HBS	40.6	$1.74 \times 10 \doteq 17.4$
2)	Colony 8 (24h), 1/10 in HBS	41.1	$1.63 \times 10 = 16.3$
3)	Colony 8 (24h), 1/20 in HBS	47.7	$0.69 \times 20 = 13.8$
4)	Colony 8 (24h), 1/20 in HBS	47.2	$0.73 \times 20 = 14.6$
5)	Complete medium	82.9	0.007
6)	Complete medium	83.3	0.006

These results show that colony 8 clotting activity that is approximately 2000-fold higher than the control sample.

The DNA sequence encoding POL1212 is set forth as SEQ ID NO:37. The encoded amino acid sequence of POL1212 is set forth as SEQ ID NO:38. Further purification of POL1212 can be carried out using a variety of known methods such as immunoaffinity chromatography and HPLC chromatography - see Examples 2 and 3.

GENERAL CONCLUDING REMARKS

It will be understood that minor variations of amino acid sequence or the DNA encoding such sequence relating to POL1212 can be introduced without affecting the essential

attributes of function. For example, the length of B-domain sequence retained as a linker /between the A2 domain and the activation peptide can be increased or decreased within limits known in the art. Sequence variants can be introduced in the linker region while retaining the equivalent functional attributes of POL1212 as taught herein and of porcine B-domainless factor VIII as taught herein and as known in the art. Based on comparisons of known factor VIII amino acid sequences having coagulant activity in human blood, sequence variants such as individual amino acid substitutions or substitution of peptide segments with known functional variants can be made in the basic POL1212 amino acid sequence, while retaining the equivalent functional attributes thereof. The foregoing types of variation are not intended as exhaustive, but are merely exemplary of the sequence modifications that could be made by those of ordinary skill in the art, without substantially modifying the functional attributes of the protein. All such variants and modifications are deemed to fall within the scope of the invention as claimed or as equivalents thereof.

Sequence ID listing:

<u>SEQ ID NO:</u>	<u>Identification</u>
1	Human factor VIII cDNA. Coding for amino acid number 1 of the mature protein begins at nucleotide number 208.
2	Human factor amino acid sequence.
3	Porcine factor VIII A2 domain cDNA
4	Porcine factor VIII A2 domain amino acid sequence
5 thru 27	Oligonucleotide primer seq. (Example 5)
28	Murine factor VIII amino acid sequence
29	Porcine factor VIII cDNA
30	Porcine factor VIII amino acid sequence
31	Human factor VIII signal peptide amino acid sequence
32 thru 36	Oligonucleotide primer (Example 7)
37	POL1212 coding DNA
38	POL1212 amino acid sequence

WO 01/68109

PCT/US01/05076

SEQUENCE LISTING

<110> Emory University

<120> MODIFIED FACTOR VIII

<130> 75-95I WO

<140> NOT ASSIGNED YET

<141> 2001-02-16

<150> US 09/523,656

<151> 2000-03-10

<150> US 09/037,601

<151> 1998-03-10

<150> US 08/670,707

<151> 1996-06-26

<160> 38

<170> PatentIn Ver. 2.0

<210> 1

<211> 9009

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (208)..(7203)

<400> 1

```

cagtgggtaa gttccttaaa tgctctgcaa agaaattggg acttttcatt aaatcagaaa 60
ttttactttt ttcccctcct gggagctaaa gatatttttag agaagaatta accttttgct 120
tctccagttg aacatttgta gcaataagtc atgcaaatag agctctccac ctgcttcttt 180
ctgtgccttt tgcgattctg ctttagt gcc acc aga aga tac tac ctg ggt gca 234
                Ala Thr Arg Arg Tyr Tyr Leu Gly Ala
                  1                      5

gtg gaa ctg tca tgg gac tat atg caa agt gat ctc ggt gag ctg cct 282
Val Glu Leu Ser Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro
  10                      15                      20                      25

gtg gac gca aga ttt cct cct aga gtg cca aaa tct ttt cca ttc aac 330
Val Asp Ala Arg Phe Pro Pro Arg Val Pro Lys Ser Phe Pro Phe Asn
                30                      35                      40

acc tca gtc gtg tac aaa aag act ctg ttt gta gaa ttc acg gtt cac 378
Thr Ser Val Val Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr Val His
                45                      50                      55

```

WO 01/68109

PCT/US01/05076

ctt ttc aac atc gct aag cca agg cca ccc tgg atg ggt ctg cta ggt	426
Leu Phe Asn Ile Ala Lys Pro Arg Pro Pro Trp Met Gly Leu Leu Gly	
60 65 70	
cct acc atc cag gct gag gtt tat gat aca gtg gtc att aca ctt aag	474
Pro Thr Ile Gln Ala Glu Val Tyr Asp Thr Val Val Ile Thr Leu Lys	
75 80 85	
aac atg gct tcc cat cct gtc agt ctt cat gct gtt ggt gta tcc tac	522
Asn Met Ala Ser His Pro Val Ser Leu His Ala Val Gly Val Ser Tyr	
90 95 100 105	
tgg aaa gct tct gag gga gct gaa tat gat gat cag acc agt caa agg	570
Trp Lys Ala Ser Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser Gln Arg	
110 115 120	
gag aaa gaa gat gat aaa gtc ttc cct ggt gga agc cat aca tat gtc	618
Glu Lys Glu Asp Asp Lys Val Phe Pro Gly Gly Ser His Thr Tyr Val	
125 130 135	
tgg cag gtc ctg aaa gag aat ggt cca atg gcc tct gac cca ctg tgc	666
Trp Gln Val Leu Lys Glu Asn Gly Pro Met Ala Ser Asp Pro Leu Cys	
140 145 150	
ctt acc tac tca tat ctt tct cat gtg gac ctg gta aaa gac ttg aat	714
Leu Thr Tyr Ser Tyr Leu Ser His Val Asp Leu Val Lys Asp Leu Asn	
155 160 165	
tca ggc ctc att gga gcc cta cta gta tgt aga gaa ggg agt ctg gcc	762
Ser Gly Leu Ile Gly Ala Leu Leu Val Cys Arg Glu Gly Ser Leu Ala	
170 175 180 185	
aag gaa aag aca cag acc ttg cac aaa ttt ata cta ctt ttt gct gta	810
Lys Glu Lys Thr Gln Thr Leu His Lys Phe Ile Leu Leu Phe Ala Val	
190 195 200	
ttt gat gaa ggg aaa agt tgg cac tca gaa aca aag aac tcc ttg atg	858
Phe Asp Glu Gly Lys Ser Trp His Ser Glu Thr Lys Asn Ser Leu Met	
205 210 215	
cag gat agg gat gct gca tct gct cgg gcc tgg cct aaa atg cac aca	906
Gln Asp Arg Asp Ala Ala Ser Ala Arg Ala Trp Pro Lys Met His Thr	
220 225 230	
gtc aat ggt tat gta aac agg tct ctg cca ggt ctg att gga tgc cac	954
Val Asn Gly Tyr Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His	
235 240 245	
agg aaa tca gtc tat tgg cat gtg att gga atg ggc acc act cct gaa	1002
Arg Lys Ser Val Tyr Trp His Val Ile Gly Met Gly Thr Thr Pro Glu	
250 255 260 265	
gtg cac tca ata ttc ctc gaa ggt cac aca ttt ctt gtg agg aac cat	1050
Val His Ser Ile Phe Leu Glu Gly His Thr Phe Leu Val Arg Asn His	
270 275 280	

WO 01/68109

PCT/US01/05076

cgc	cag	gcg	tcc	ttg	gaa	atc	tcg	cca	ata	act	ttc	ctt	act	gct	caa	1098
Arg	Gln	Ala	Ser	Leu	Glu	Ile	Ser	Pro	Ile	Thr	Phe	Leu	Thr	Ala	Gln	
			285					290					295			
aca	ctc	ttg	atg	gac	ctt	gga	cag	ttt	cta	ctg	ttt	tgt	cat	atc	tct	1146
Thr	Leu	Leu	Met	Asp	Leu	Gly	Gln	Phe	Leu	Leu	Phe	Cys	His	Ile	Ser	
		300					305					310				
tcc	cac	caa	cat	gat	ggc	atg	gaa	gct	tat	gtc	aaa	gta	gac	agc	tgt	1194
Ser	His	Gln	His	Asp	Gly	Met	Glu	Ala	Tyr	Val	Lys	Val	Asp	Ser	Cys	
		315				320					325					
cca	gag	gaa	ccc	caa	cta	cga	atg	aaa	aat	aat	gaa	gaa	gcg	gaa	gac	1242
Pro	Glu	Glu	Pro	Gln	Leu	Arg	Met	Lys	Asn	Asn	Glu	Glu	Ala	Glu	Asp	
330					335					340					345	
tat	gat	gat	gat	ctt	act	gat	tct	gaa	atg	gat	gtg	gtc	agg	ttt	gat	1290
Tyr	Asp	Asp	Asp	Leu	Thr	Asp	Ser	Glu	Met	Asp	Val	Val	Arg	Phe	Asp	
				350				355						360		
gat	gac	aac	tct	cct	tcc	ttt	atc	caa	att	cgc	tca	gtt	gcc	aag	aag	1338
Asp	Asp	Asn	Ser	Pro	Ser	Phe	Ile	Gln	Ile	Arg	Ser	Val	Ala	Lys	Lys	
			365					370					375			
cat	cct	aaa	act	tgg	gta	cat	tac	att	gct	gct	gaa	gag	gag	gac	tgg	1386
His	Pro	Lys	Thr	Trp	Val	His	Tyr	Ile	Ala	Ala	Glu	Glu	Glu	Asp	Trp	
		380					385					390				
gac	tat	gct	ccc	tta	gtc	ctc	gcc	ccc	gat	gac	aga	agt	tat	aaa	agt	1434
Asp	Tyr	Ala	Pro	Leu	Val	Leu	Ala	Pro	Asp	Asp	Arg	Ser	Tyr	Lys	Ser	
		395				400					405					
caa	tat	ttg	aac	aat	ggc	cct	cag	cgg	att	ggt	agg	aag	tac	aaa	aaa	1482
Gln	Tyr	Leu	Asn	Asn	Gly	Pro	Gln	Arg	Ile	Gly	Arg	Lys	Tyr	Lys	Lys	
410					415					420					425	
gtc	cga	ttt	atg	gca	tac	aca	gat	gaa	acc	ttt	aag	act	cgt	gaa	gct	1530
Val	Arg	Phe	Met	Ala	Tyr	Thr	Asp	Glu	Thr	Phe	Lys	Thr	Arg	Glu	Ala	
				430				435						440		
att	cag	cat	gaa	tca	gga	atc	ttg	gga	cct	tta	ctt	tat	ggg	gaa	gtt	1578
Ile	Gln	His	Glu	Ser	Gly	Ile	Leu	Gly	Pro	Leu	Leu	Tyr	Gly	Glu	Val	
			445				450					455				
gga	gac	aca	ctg	ttg	att	ata	ttt	aag	aat	caa	gca	agc	aga	cca	tat	1626
Gly	Asp	Thr	Leu	Leu	Ile	Ile	Phe	Lys	Asn	Gln	Ala	Ser	Arg	Pro	Tyr	
		460					465					470				
aac	atc	tac	cct	cac	gga	atc	act	gat	gtc	cgt	cct	ttg	tat	tca	agg	1674
Asn	Ile	Tyr	Pro	His	Gly	Ile	Thr	Asp	Val	Arg	Pro	Leu	Tyr	Ser	Arg	
		475				480					485					
aga	tta	cca	aaa	ggt	gta	aaa	cat	ttg	aag	gat	ttt	cca	att	ctg	cca	1722
Arg	Leu	Pro	Lys	Gly	Val	Lys	His	Leu	Lys	Asp	Phe	Pro	Ile	Leu	Pro	
490					495					500					505	

WO 01/68109

PCT/US01/05076

gga gaa ata ttc aaa tat aaa tgg aca gtg act gta gaa gat ggg cca	1770
Gly Glu Ile Phe Lys Tyr Lys Trp Thr Val Thr Val Glu Asp Gly Pro	
510 515 520	
act aaa tca gat cct cgg tgc ctg acc cgc tat tac tct agt ttc gtt	1818
Thr Lys Ser Asp Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser Phe Val	
525 530 535	
aat atg gag aga gat cta gct tca gga ctc att ggc cct ctc ctc atc	1866
Asn Met Glu Arg Asp Leu Ala Ser Gly Leu Ile Gly Pro Leu Leu Ile	
540 545 550	
tgc tac aaa gaa tct gta gat caa aga gga aac cag ata atg tca gac	1914
Cys Tyr Lys Glu Ser Val Asp Gln Arg Gly Asn Gln Ile Met Ser Asp	
555 560 565	
aag agg aat gtc atc ctg ttt tct gta ttt gat gag aac cga agc tgg	1962
Lys Arg Asn Val Ile Leu Phe Ser Val Phe Asp Glu Asn Arg Ser Trp	
570 575 580 585	
tac ctc aca gag aat ata caa cgc ttt ctc ccc aat cca gct gga gtg	2010
Tyr Leu Thr Glu Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala Gly Val	
590 595 600	
cag ctt gag gat cca gag ttc caa gcc tcc aac atc atg cac agc atc	2058
Gln Leu Glu Asp Pro Glu Phe Gln Ala Ser Asn Ile Met His Ser Ile	
605 610 615	
aat ggc tat gtt ttt gat agt ttg cag ttg tca gtt tgt ttg cat gag	2106
Asn Gly Tyr Val Phe Asp Ser Leu Gln Leu Ser Val Cys Leu His Glu	
620 625 630	
gtg gca tac tgg tac att cta agc att gga gca cag act gac ttc ctt	2154
Val Ala Tyr Trp Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp Phe Leu	
635 640 645	
tct gtc ttc ttc tct gga tat acc ttc aaa cac aaa atg gtc tat gaa	2202
Ser Val Phe Phe Ser Gly Tyr Thr Phe Lys His Lys Met Val Tyr Glu	
650 655 660 665	
gac aca ctc acc cta ttc cca ttc tca gga gaa act gtc ttc atg tcg	2250
Asp Thr Leu Thr Leu Phe Pro Phe Ser Gly Glu Thr Val Phe Met Ser	
670 675 680	
atg gaa aac cca ggt cta tgg att ctg ggg tgc cac aac tca gac ttt	2298
Met Glu Asn Pro Gly Leu Trp Ile Leu Gly Cys His Asn Ser Asp Phe	
685 690 695	
cgg aac aga ggc atg acc gcc tta ctg aag gtt tct agt tgt gac aag	2346
Arg Asn Arg Gly Met Thr Ala Leu Leu Lys Val Ser Ser Cys Asp Lys	
700 705 710	
aac act ggt gat tat tac gag gac agt tat gaa gat att tca gca tac	2394
Asn Thr Gly Asp Tyr Tyr Glu Asp Ser Tyr Glu Asp Ile Ser Ala Tyr	
715 720 725	

WO 01/68109

PCT/US01/05076

ttg ctg agt aaa aac aat gcc att gaa cca aga agc ttc tcc cag aat	2442
Leu Leu Ser Lys Asn Asn Ala Ile Glu Pro Arg Ser Phe Ser Gln Asn	
730 735 740 745	
tca aga cac cct agc act agg caa aag caa ttt aat gcc acc aca att	2490
Ser Arg His Pro Ser Thr Arg Gln Lys Gln Phe Asn Ala Thr Thr Ile	
750 755 760	
cca gaa aat gac ata gag aag act gac cct tgg ttt gca cac aga aca	2538
Pro Glu Asn Asp Ile Glu Lys Thr Asp Pro Trp Phe Ala His Arg Thr	
765 770 775	
cct atg cct aaa ata caa aat gtc tcc tct agt gat ttg ttg atg ctc	2586
Pro Met Pro Lys Ile Gln Asn Val Ser Ser Ser Asp Leu Leu Met Leu	
780 785 790	
ttg cga cag agt cct act cca cat ggg cta tcc tta tct gat ctc caa	2634
Leu Arg Gln Ser Pro Thr Pro His Gly Leu Ser Leu Ser Asp Leu Gln	
795 800 805	
gaa gcc aaa tat gag act ttt tct gat gat cca tca cct gga gca ata	2682
Glu Ala Lys Tyr Glu Thr Phe Ser Asp Asp Pro Ser Pro Gly Ala Ile	
810 815 820 825	
gac agt aat aac agc ctg tct gaa atg aca cac ttc agg cca cag ctc	2730
Asp Ser Asn Asn Ser Leu Ser Glu Met Thr His Phe Arg Pro Gln Leu	
830 835 840	
cat cac agt ggg gac atg gta ttt acc cct gag tca ggc ctc caa tta	2778
His His Ser Gly Asp Met Val Phe Thr Pro Glu Ser Gly Leu Gln Leu	
845 850 855	
aga tta aat gag aaa ctg ggg aca act gca gca aca gag ttg aag aaa	2826
Arg Leu Asn Glu Lys Leu Gly Thr Thr Ala Ala Thr Glu Leu Lys Lys	
860 865 870	
ctt gat ttc aaa gtt tct agt aca tca aat aat ctg att tca aca att	2874
Leu Asp Phe Lys Val Ser Ser Thr Ser Asn Asn Leu Ile Ser Thr Ile	
875 880 885	
cca tca gac aat ttg gca gca ggt act gat aat aca agt tcc tta gga	2922
Pro Ser Asp Asn Leu Ala Ala Gly Thr Asp Asn Thr Ser Ser Leu Gly	
890 895 900 905	
ccc cca agt atg cca gtt cat tat gat agt caa tta gat acc act cta	2970
Pro Pro Ser Met Pro Val His Tyr Asp Ser Gln Leu Asp Thr Thr Leu	
910 915 920	
ttt ggc aaa aag tca tct ccc ctt act gag tct ggt gga cct ctg agc	3018
Phe Gly Lys Lys Ser Ser Pro Leu Thr Glu Ser Gly Gly Pro Leu Ser	
925 930 935	
ttg agt gaa gaa aat aat gat tca aag ttg tta gaa tca ggt tta atg	3066
Leu Ser Glu Glu Asn Asn Asp Ser Lys Leu Leu Glu Ser Gly Leu Met	
940 945 950	

WO 01/68109

PCT/US01/05076

aat agc caa gaa agt tca tgg gga aaa aat gta tcg tca aca gag agt	3114
Asn Ser Gln Glu Ser Ser Trp Gly Lys Asn Val Ser Ser Thr Glu Ser	
955 960 965	
ggt agg tta ttt aaa ggg aaa aga gct cat gga cct gct ttg ttg act	3162
Gly Arg Leu Phe Lys Gly Lys Arg Ala His Gly Pro Ala Leu Leu Thr	
970 975 980 985	
aaa gat aat gcc tta ttc aaa gtt agc atc tct ttg tta aag aca aac	3210
Lys Asp Asn Ala Leu Phe Lys Val Ser Ile Ser Leu Leu Lys Thr Asn	
990 995 1000	
aaa act tcc aat aat tca gca act aat aga aag act cac att gat ggc	3258
Lys Thr Ser Asn Asn Ser Ala Thr Asn Arg Lys Thr His Ile Asp Gly	
1005 1010 1015	
cca tca tta tta att gag aat agt cca tca gtc tgg caa aat ata tta	3306
Pro Ser Leu Leu Ile Glu Asn Ser Pro Ser Val Trp Gln Asn Ile Leu	
1020 1025 1030	
gaa agt gac act gag ttt aaa aaa gtg aca cct ttg att cat gac aga	3354
Glu Ser Asp Thr Glu Phe Lys Lys Val Thr Pro Leu Ile His Asp Arg	
1035 1040 1045	
atg ctt atg gac aaa aat gct aca gct ttg agg cta aat cat atg tca	3402
Met Leu Met Asp Lys Asn Ala Thr Ala Leu Arg Leu Asn His Met Ser	
1050 1055 1060 1065	
aat aaa act act tca tca aaa aac atg gaa atg gtc caa cag aaa aaa	3450
Asn Lys Thr Thr Ser Ser Lys Asn Met Glu Met Val Gln Gln Lys Lys	
1070 1075 1080	
gag ggc ccc att cca cca gat gca caa aat cca gat atg tcg ttc ttt	3498
Glu Gly Pro Ile Pro Pro Asp Ala Gln Asn Pro Asp Met Ser Phe Phe	
1085 1090 1095	
aag atg cta ttc ttg cca gaa tca gca agg tgg ata caa agg act cat	3546
Lys Met Leu Phe Leu Pro Glu Ser Ala Arg Trp Ile Gln Arg Thr His	
1100 1105 1110	
gga aag aac tct ctg aac tct ggg caa ggc ccc agt cca aag caa tta	3594
Gly Lys Asn Ser Leu Asn Ser Gly Gln Gly Pro Ser Pro Lys Gln Leu	
1115 1120 1125	
gta tcc tta gga cca gaa aaa tct gtg gaa ggt cag aat ttc ttg tct	3642
Val Ser Leu Gly Pro Glu Lys Ser Val Glu Gly Gln Asn Phe Leu Ser	
1130 1135 1140 1145	
gag aaa aac aaa gtg gta gta gga aag ggt gaa ttt aca aag gac gta	3690
Glu Lys Asn Lys Val Val Val Gly Lys Gly Glu Phe Thr Lys Asp Val	
1150 1155 1160	
gga ctc aaa gag atg gtt ttt cca agc agc aga aac cta ttt ctt act	3738
Gly Leu Lys Glu Met Val Phe Pro Ser Ser Arg Asn Leu Phe Leu Thr	
1165 1170 1175	

WO 01/68109

PCT/US01/05076

aac ttg gat aat tta cat gaa aat aat aca cac aat caa gaa aaa aaa	3786
Asn Leu Asp Asn Leu His Glu Asn Asn Thr His Asn Gln Glu Lys Lys	
1180 1185 1190	
att cag gaa gaa ata gaa aag aag gaa aca tta atc caa gag aat gta	3834
Ile Gln Glu Glu Ile Glu Lys Lys Glu Thr Leu Ile Gln Glu Asn Val	
1195 1200 1205	
gtt ttg cct cag ata cat aca gtg act ggc act aag aat ttc atg aag	3882
Val Leu Pro Gln Ile His Thr Val Thr Gly Thr Lys Asn Phe Met Lys	
1210 1215 1220 1225	
aac ctt ttc tta ctg agc act agg caa aat gta gaa ggt tca tat gag	3930
Asn Leu Phe Leu Leu Ser Thr Arg Gln Asn Val Glu Gly Ser Tyr Glu	
1230 1235 1240	
ggg gca tat gct cca gta ctt caa gat ttt agg tca tta aat gat tca	3978
Gly Ala Tyr Ala Pro Val Leu Gln Asp Phe Arg Ser Leu Asn Asp Ser	
1245 1250 1255	
aca aat aga aca aag aaa cac aca gct cat ttc tca aaa aaa ggg gag	4026
Thr Asn Arg Thr Lys Lys His Thr Ala His Phe Ser Lys Lys Gly Glu	
1260 1265 1270	
gaa gaa aac ttg gaa ggc ttg gga aat caa acc aag caa att gta gag	4074
Glu Glu Asn Leu Glu Gly Leu Gly Asn Gln Thr Lys Gln Ile Val Glu	
1275 1280 1285	
aaa tat gca tgc acc aca agg ata tct cct aat aca agc cag cag aat	4122
Lys Tyr Ala Cys Thr Thr Arg Ile Ser Pro Asn Thr Ser Gln Gln Asn	
1290 1295 1300 1305	
ttt gtc acg caa cgt agt aag aga gct ttg aaa caa ttc aga ctc cca	4170
Phe Val Thr Gln Arg Ser Lys Arg Ala Leu Lys Gln Phe Arg Leu Pro	
1310 1315 1320	
cta gaa gaa aca gaa ctt gaa aaa agg ata att gtg gat gac acc tca	4218
Leu Glu Glu Thr Glu Leu Glu Lys Arg Ile Ile Val Asp Asp Thr Ser	
1325 1330 1335	
acc cag tgg tcc aaa aac atg aaa cat ttg acc ccg agc acc ctc aca	4266
Thr Gln Trp Ser Lys Asn Met Lys His Leu Thr Pro Ser Thr Leu Thr	
1340 1345 1350	
cag ata gac tac aat gag aag gag aaa ggg gcc att act cag tct ccc	4314
Gln Ile Asp Tyr Asn Glu Lys Glu Lys Gly Ala Ile Thr Gln Ser Pro	
1355 1360 1365	
tta tca gat tgc ctt acg agg agt cat agc atc cct caa gca aat aga	4362
Leu Ser Asp Cys Leu Thr Arg Ser His Ser Ile Pro Gln Ala Asn Arg	
1370 1375 1380 1385	
tct cca tta ccc att gca aag gta tca tca ttt cca tct att aga cct	4410
Ser Pro Leu Pro Ile Ala Lys Val Ser Ser Phe Pro Ser Ile Arg Pro	
1390 1395 1400	

ata tat ctg acc agg gtc cta ttc caa gac aac tct tct cat ctt cca	4458
Ile Tyr Leu Thr Arg Val Leu Phe Gln Asp Asn Ser Ser His Leu Pro	
1405 1410 1415	
gca gca tct tat aga aag aaa gat tct ggg gtc caa gaa agc agt cat	4506
Ala Ala Ser Tyr Arg Lys Lys Asp Ser Gly Val Gln Glu Ser Ser His	
1420 1425 1430	
ttc tta caa gga gcc aaa aaa aat aac ctt tct tta gcc att cta acc	4554
Phe Leu Gln Gly Ala Lys Lys Asn Asn Leu Ser Leu Ala Ile Leu Thr	
1435 1440 1445	
ttg gag atg act ggt gat caa aga gag gtt ggc tcc ctg ggg aca agt	4602
Leu Glu Met Thr Gly Asp Gln Arg Glu Val Gly Ser Leu Gly Thr Ser	
1450 1455 1460 1465	
gcc aca aat tca gtc aca tac aag aaa gtt gag aac act gtt ctc ccg	4650
Ala Thr Asn Ser Val Thr Tyr Lys Lys Val Glu Asn Thr Val Leu Pro	
1470 1475 1480	
aaa cca gac ttg ccc aaa aca tct ggc aaa gtt gaa ttg ctt cca aaa	4698
Lys Pro Asp Leu Pro Lys Thr Ser Gly Lys Val Glu Leu Leu Pro Lys	
1485 1490 1495	
gtt cac att tat cag aag gac cta ttc cct acg gaa act agc aat ggg	4746
Val His Ile Tyr Gln Lys Asp Leu Phe Pro Thr Glu Thr Ser Asn Gly	
1500 1505 1510	
tct cct ggc cat ctg gat ctc gtg gaa ggg agc ctt ctt cag gga aca	4794
Ser Pro Gly His Leu Asp Leu Val Glu Gly Ser Leu Leu Gln Gly Thr	
1515 1520 1525	
gag gga gcg att aag tgg aat gaa gca aac aga cct gga aaa gtt ccc	4842
Glu Gly Ala Ile Lys Trp Asn Glu Ala Asn Arg Pro Gly Lys Val Pro	
1530 1535 1540 1545	
ttt ctg aga gta gca aca gaa agc tct gca aag act ccc tcc aag cta	4890
Phe Leu Arg Val Ala Thr Glu Ser Ser Ala Lys Thr Pro Ser Lys Leu	
1550 1555 1560	
ttg gat cct ctt gct tgg gat aac cac tat ggt act cag ata cca aaa	4938
Leu Asp Pro Leu Ala Trp Asp Asn His Tyr Gly Thr Gln Ile Pro Lys	
1565 1570 1575	
gaa gag tgg aaa tcc caa gag aag tca cca gaa aaa aca gct ttt aag	4986
Glu Glu Trp Lys Ser Gln Glu Lys Ser Pro Glu Lys Thr Ala Phe Lys	
1580 1585 1590	
aaa aag gat acc att ttg tcc ctg aac gct tgt gaa agc aat cat gca	5034
Lys Lys Asp Thr Ile Leu Ser Leu Asn Ala Cys Glu Ser Asn His Ala	
1595 1600 1605	
ata gca gca ata aat gag gga caa aat aag ccc gaa ata gaa gtc acc	5082
Ile Ala Ala Ile Asn Glu Gly Gln Asn Lys Pro Glu Ile Glu Val Thr	
1610 1615 1620 1625	

WO 01/68109

PCT/US01/05076

tgg gca aag caa ggt agg act gaa agg ctg tgc tct caa aac cca cca	5130
Trp Ala Lys Gln Gly Arg Thr Glu Arg Leu Cys Ser Gln Asn Pro Pro	
1630 1635 1640	
gtc ttg aaa cgc cat caa cgg gaa ata act cgt act act ctt cag tca	5178
Val Leu Lys Arg His Gln Arg Glu Ile Thr Arg Thr Thr Leu Gln Ser	
1645 1650 1655	
gat caa gag gaa att gac tat gat gat acc ata tca gtt gaa atg aag	5226
Asp Gln Glu Glu Ile Asp Tyr Asp Asp Thr Ile Ser Val Glu Met Lys	
1660 1665 1670	
aag gaa gat ttt gac att tat gat gag gat gaa aat cag agc ccc cgc	5274
Lys Glu Asp Phe Asp Ile Tyr Asp Glu Asp Glu Asn Gln Ser Pro Arg	
1675 1680 1685	
agc ttt caa aag aaa aca cga cac tat ttt att gct gca gtg gag agg	5322
Ser Phe Gln Lys Lys Thr Arg His Tyr Phe Ile Ala Ala Val Glu Arg	
1690 1695 1700 1705	
ctc tgg gat tat ggg atg agt agc tcc cca cat gtt cta aga aac agg	5370
Leu Trp Asp Tyr Gly Met Ser Ser Ser Pro His Val Leu Arg Asn Arg	
1710 1715 1720	
gct cag agt ggc agt gtc cct cag ttc aag aaa gtt gtt ttc cag gaa	5418
Ala Gln Ser Gly Ser Val Pro Gln Phe Lys Lys Val Val Phe Gln Glu	
1725 1730 1735	
ttt act gat ggc tcc ttt act cag ccc tta tac cgt gga gaa cta aat	5466
Phe Thr Asp Gly Ser Phe Thr Gln Pro Leu Tyr Arg Gly Glu Leu Asn	
1740 1745 1750	
gaa cat ttg gga ctc ctg ggg cca tat ata aga gca gaa gtt gaa gat	5514
Glu His Leu Gly Leu Leu Gly Pro Tyr Ile Arg Ala Glu Val Glu Asp	
1755 1760 1765	
aat atc atg gta act ttc aga aat cag gcc tct cgt ccc tat tcc ttc	5562
Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser Arg Pro Tyr Ser Phe	
1770 1775 1780 1785	
tat tct agc ctt att tct tat gag gaa gat cag agg caa gga gca gaa	5610
Tyr Ser Ser Leu Ile Ser Tyr Glu Glu Asp Gln Arg Gln Gly Ala Glu	
1790 1795 1800	
cct aga aaa aac ttt gtc aag cct aat gaa acc aaa act tac ttt tgg	5658
Pro Arg Lys Asn Phe Val Lys Pro Asn Glu Thr Lys Thr Tyr Phe Trp	
1805 1810 1815	
aaa gtg caa cat cat atg gca ccc act aaa gat gag ttt gac tgc aaa	5706
Lys Val Gln His His Met Ala Pro Thr Lys Asp Glu Phe Asp Cys Lys	
1820 1825 1830	
gcc tgg gct tat ttc tct gat gtt gac ctg gaa aaa gat gtg cac tca	5754
Ala Trp Ala Tyr Phe Ser Asp Val Asp Leu Glu Lys Asp Val His Ser	
1835 1840 1845	

WO 01/68109

PCT/US01/05076

ggc ctg att gga ccc ctt ctg gtc tgc cac act aac aca ctg aac cct	5802
Gly Leu Ile Gly Pro Leu Leu Val Cys His Thr Asn Thr Leu Asn Pro	
1850 1855 1860 1865	
gct cat ggg aga caa gtg aca gta cag gaa ttt gct ctg ttt ttc acc	5850
Ala His Gly Arg Gln Val Thr Val Gln Glu Phe Ala Leu Phe Phe Thr	
1870 1875 1880	
atc ttt gat gag acc aaa agc tgg tac ttc act gaa aat atg gaa aga	5898
Ile Phe Asp Glu Thr Lys Ser Trp Tyr Phe Thr Glu Asn Met Glu Arg	
1885 1890 1895	
aac tgc agg gct ccc tgc aat atc cag atg gaa gat ccc act ttt aaa	5946
Asn Cys Arg Ala Pro Cys Asn Ile Gln Met Glu Asp Pro Thr Phe Lys	
1900 1905 1910	
gag aat tat cgc ttc cat gca atc aat ggc tac ata atg gat aca cta	5994
Glu Asn Tyr Arg Phe His Ala Ile Asn Gly Tyr Ile Met Asp Thr Leu	
1915 1920 1925	
cct ggc tta gta atg gct cag gat caa agg att cga tgg tat ctg ctc	6042
Pro Gly Leu Val Met Ala Gln Asp Gln Arg Ile Arg Trp Tyr Leu Leu	
1930 1935 1940 1945	
agc atg ggc agc aat gaa aac atc cat tct att cat ttc agt gga cat	6090
Ser Met Gly Ser Asn Glu Asn Ile His Ser Ile His Phe Ser Gly His	
1950 1955 1960	
gtg ttc act gta cga aaa aaa gag gag tat aaa atg gca ctg tac aat	6138
Val Phe Thr Val Arg Lys Lys Glu Glu Tyr Lys Met Ala Leu Tyr Asn	
1965 1970 1975	
ctc tat cca ggt gtt ttt gag aca gtg gaa atg tta cca tcc aaa gct	6186
Leu Tyr Pro Gly Val Phe Glu Thr Val Glu Met Leu Pro Ser Lys Ala	
1980 1985 1990	
gga att tgg cgg gtg gaa tgc ctt att ggc gag cat cta cat gct ggg	6234
Gly Ile Trp Arg Val Glu Cys Leu Ile Gly Glu His Leu His Ala Gly	
1995 2000 2005	
atg agc aca ctt ttt ctg gtg tac agc aat aag tgt cag act ccc ctg	6282
Met Ser Thr Leu Phe Leu Val Tyr Ser Asn Lys Cys Gln Thr Pro Leu	
2010 2015 2020 2025	
gga atg gct tct gga cac att aga gat ttt cag att aca gct tca gga	6330
Gly Met Ala Ser Gly His Ile Arg Asp Phe Gln Ile Thr Ala Ser Gly	
2030 2035 2040	
caa tat gga cag tgg gcc cca aag ctg gcc aga ctt cat tat tcc gga	6378
Gln Tyr Gly Gln Trp Ala Pro Lys Leu Ala Arg Leu His Tyr Ser Gly	
2045 2050 2055	
tca atc aat gcc tgg agc acc aag gag ccc ttt tct tgg atc aag gtg	6426
Ser Ile Asn Ala Trp Ser Thr Lys Glu Pro Phe Ser Trp Ile Lys Val	
2060 2065 2070	

WO 01/68109

PCT/US01/05076

gat ctg ttg gca cca atg att att cac ggc atc aag acc cag ggt gcc	6474
Asp Leu Leu Ala Pro Met Ile Ile His Gly Ile Lys Thr Gln Gly Ala	
2075 2080 2085	
cgt cag aag ttc tcc agc ctc tac atc tct cag ttt atc atc atg tat	6522
Arg Gln Lys Phe Ser Ser Leu Tyr Ile Ser Gln Phe Ile Ile Met Tyr	
2090 2095 2100 2105	
agt ctt gat ggg aag aag tgg cag act tat cga gga aat tcc act gga	6570
Ser Leu Asp Gly Lys Lys Trp Gln Thr Tyr Arg Gly Asn Ser Thr Gly	
2110 2115 2120	
acc tta atg gtc ttc ttt ggc aat gtg gat tca tct ggg ata aaa cac	6618
Thr Leu Met Val Phe Phe Gly Asn Val Asp Ser Ser Gly Ile Lys His	
2125 2130 2135	
aat att ttt aac cct cca att att gct cga tac atc cgt ttg cac cca	6666
Asn Ile Phe Asn Pro Pro Ile Ile Ala Arg Tyr Ile Arg Leu His Pro	
2140 2145 2150	
act cat tat agc att cgc agc act ctt cgc atg gag ttg atg ggc tgt	6714
Thr His Tyr Ser Ile Arg Ser Thr Leu Arg Met Glu Leu Met Gly Cys	
2155 2160 2165	
gat tta aat agt tgc agc atg cca ttg gga atg gag agt aaa gca ata	6762
Asp Leu Asn Ser Cys Ser Met Pro Leu Gly Met Glu Ser Lys Ala Ile	
2170 2175 2180 2185	
tca gat gca cag att act gct tca tcc tac ttt acc aat atg ttt gcc	6810
Ser Asp Ala Gln Ile Thr Ala Ser Ser Tyr Phe Thr Asn Met Phe Ala	
2190 2195 2200	
acc tgg tct cct tca aaa gct cga ctt cac ctc caa ggg agg agt aat	6858
Thr Trp Ser Pro Ser Lys Ala Arg Leu His Leu Gln Gly Arg Ser Asn	
2205 2210 2215	
gcc tgg aga cct cag gtg aat aat cca aaa gag tgg ctg caa gtg gac	6906
Ala Trp Arg Pro Gln Val Asn Asn Pro Lys Glu Trp Leu Gln Val Asp	
2220 2225 2230	
ttc cag aag aca atg aaa gtc aca gga gta act act cag gga gta aaa	6954
Phe Gln Lys Thr Met Lys Val Thr Gly Val Thr Thr Gln Gly Val Lys	
2235 2240 2245	
tct ctg ctt acc agc atg tat gtg aag gag ttc ctc atc tcc agc agt	7002
Ser Leu Leu Thr Ser Met Tyr Val Lys Glu Phe Leu Ile Ser Ser Ser	
2250 2255 2260 2265	
caa gat ggc cat cag tgg act ctc ttt ttt cag aat ggc aaa gta aag	7050
Gln Asp Gly His Gln Trp Thr Leu Phe Phe Gln Asn Gly Lys Val Lys	
2270 2275 2280	
gtt ttt cag gga aat caa gac tcc ttc aca cct gtg gtg aac tct cta	7098
Val Phe Gln Gly Asn Gln Asp Ser Phe Thr Pro Val Val Asn Ser Leu	
2285 2290 2295	

gac cca ccg tta ctg act cgc tac ctt cga att cac ccc cag agt tgg 7146
 Asp Pro Pro Leu Leu Thr Arg Tyr Leu Arg Ile His Pro Gln Ser Trp
 2300 2305 2310

gtg cac cag att gcc ctg agg atg gag gtt ctg ggc tgc gag gca cag 7194
 Val His Gln Ile Ala Leu Arg Met Glu Val Leu Gly Cys Glu Ala Gln
 2315 2320 2325

gac ctc tac tgaggggtggc cactgcagca cctgccactg ccgtcacctc 7243
 Asp Leu Tyr
 2330

tccctcctca gctccagggc agtgtccctc cctggcttgc cttctacctt tgtgctaaat 7303

cctagcagac actgccttga agcctcctga attaactatc atcagtcctg catttctttg 7363

gtgggggggcc aggaggggtgc atccaattta acttaactct tacctatttt ctgcagctgc 7423

tcccagatta ctccctcctt ccaatataac taggcaaaaa gaagtgagga gaaacctgca 7483

tgaaagcatt cttccctgaa aagttaggcc tctcagagtc accacttcct ctgttgtaga 7543

aaaactatgt gatgaaactt tgaaaaagat atttatgatg ttaacatttc aggttaagcc 7603

tcatacgttt aaaataaaaac tctcagttgt ttattatcct gatcaagcat ggaacaaagc 7663

atgtttcagg atcagatcaa tacaatcttg gagtcaaaag gcaaatcatt tggacaatct 7723

gcaaaatgga gagaatacaa taactactac agtaaagtct gtttctgctt ccttacacat 7783

agatataatt atgttattta gtcattatga ggggcacatt cttatctcca aaactagcat 7843

tcttaaactg agaattatag atgggggttca agaatcccta agtcccctga aattatataa 7903

ggcattctgt ataaatgcaa atgtgcattt ttctgacgag tgtccataga tataaagcca 7963

ttgggtcttaa ttctgaccaa taaaaaaata agtcaggagg atgcaattgt tgaaagcttt 8023

gaaataaaat aacatgtctt cttgaaattt gtgatggcca agaaagaaaa tgatgatgac 8083

attaggcttc taaaggacat acatttaata tttctgtgga aatatgagga aaatccatgg 8143

ttatctgaga taggagatac aaactttgta attctaataa tgcactcagt ttactctctc 8203

cctctactaa tttcctgctg aaaataaacac aacaaaaatg taacagggga aattatatac 8263

cgtgactgaa aactagagtc ctacttacat agttgaaata tcaaggaggt cagaagaaaa 8323

ttggactggg gaaaacagaa aaaacactcc agtctgccat atcaccacac aataggatcc 8383

cccttcttgc cctccacccc cataagattg tgaagggttt actgctcctt ccatctgcct 8443

gcacccttc actatgacta cacagaactc tcctgatagt aaagggggct ggaggcaagg 8503

ataagttata gagcagttgg aggaagcatc caaagactgc aaccagggc aaatggaaaa 8563

<400> 2															
Ala	Thr	Arg	Arg	Tyr	Tyr	Leu	Gly	Ala	Val	Glu	Leu	Ser	Trp	Asp	Tyr
1				5					10					15	
Met	Gln	Ser	Asp	Leu	Gly	Glu	Leu	Pro	Val	Asp	Ala	Arg	Phe	Pro	Pro
			20					25					30		
Arg	Val	Pro	Lys	Ser	Phe	Pro	Phe	Asn	Thr	Ser	Val	Val	Tyr	Lys	Lys
		35					40					45			
Thr	Leu	Phe	Val	Glu	Phe	Thr	Val	His	Leu	Phe	Asn	Ile	Ala	Lys	Pro
	50						55				60				
Arg	Pro	Pro	Trp	Met	Gly	Leu	Leu	Gly	Pro	Thr	Ile	Gln	Ala	Glu	Val
65					70					75					80
Tyr	Asp	Thr	Val	Val	Ile	Thr	Leu	Lys	Asn	Met	Ala	Ser	His	Pro	Val
				85					90					95	
Ser	Leu	His	Ala	Val	Gly	Val	Ser	Tyr	Trp	Lys	Ala	Ser	Glu	Gly	Ala
			100					105					110		
Glu	Tyr	Asp	Asp	Gln	Thr	Ser	Gln	Arg	Glu	Lys	Glu	Asp	Asp	Lys	Val
		115					120					125			
Phe	Pro	Gly	Gly	Ser	His	Thr	Tyr	Val	Trp	Gln	Val	Leu	Lys	Glu	Asn
	130					135					140				
Gly	Pro	Met	Ala	Ser	Asp	Pro	Leu	Cys	Leu	Thr	Tyr	Ser	Tyr	Leu	Ser
145					150					155					160
His	Val	Asp	Leu	Val	Lys	Asp	Leu	Asn	Ser	Gly	Leu	Ile	Gly	Ala	Leu
				165					170					175	

WO 01/68109

PCT/US01/05076

Leu	Val	Cys	Arg	Glu	Gly	Ser	Leu	Ala	Lys	Glu	Lys	Thr	Gln	Thr	Leu	180	185	190	
His	Lys	Phe	Ile	Leu	Leu	Phe	Ala	Val	Phe	Asp	Glu	Gly	Lys	Ser	Trp	195	200	205	
His	Ser	Glu	Thr	Lys	Asn	Ser	Leu	Met	Gln	Asp	Arg	Asp	Ala	Ala	Ser	210	215	220	
Ala	Arg	Ala	Trp	Pro	Lys	Met	His	Thr	Val	Asn	Gly	Tyr	Val	Asn	Arg	225	230	235	240
Ser	Leu	Pro	Gly	Leu	Ile	Gly	Cys	His	Arg	Lys	Ser	Val	Tyr	Trp	His	245	250	255	
Val	Ile	Gly	Met	Gly	Thr	Thr	Pro	Glu	Val	His	Ser	Ile	Phe	Leu	Glu	260	265	270	
Gly	His	Thr	Phe	Leu	Val	Arg	Asn	His	Arg	Gln	Ala	Ser	Leu	Glu	Ile	275	280	285	
Ser	Pro	Ile	Thr	Phe	Leu	Thr	Ala	Gln	Thr	Leu	Leu	Met	Asp	Leu	Gly	290	295	300	
Gln	Phe	Leu	Leu	Phe	Cys	His	Ile	Ser	Ser	His	Gln	His	Asp	Gly	Met	305	310	315	320
Glu	Ala	Tyr	Val	Lys	Val	Asp	Ser	Cys	Pro	Glu	Glu	Pro	Gln	Leu	Arg	325	330	335	
Met	Lys	Asn	Asn	Glu	Glu	Ala	Glu	Asp	Tyr	Asp	Asp	Asp	Leu	Thr	Asp	340	345	350	
Ser	Glu	Met	Asp	Val	Val	Arg	Phe	Asp	Asp	Asp	Asn	Ser	Pro	Ser	Phe	355	360	365	
Ile	Gln	Ile	Arg	Ser	Val	Ala	Lys	Lys	His	Pro	Lys	Thr	Trp	Val	His	370	375	380	
Tyr	Ile	Ala	Ala	Glu	Glu	Glu	Asp	Trp	Asp	Tyr	Ala	Pro	Leu	Val	Leu	385	390	395	400
Ala	Pro	Asp	Asp	Arg	Ser	Tyr	Lys	Ser	Gln	Tyr	Leu	Asn	Asn	Gly	Pro	405	410	415	
Gln	Arg	Ile	Gly	Arg	Lys	Tyr	Lys	Lys	Val	Arg	Phe	Met	Ala	Tyr	Thr	420	425	430	
Asp	Glu	Thr	Phe	Lys	Thr	Arg	Glu	Ala	Ile	Gln	His	Glu	Ser	Gly	Ile	435	440	445	
Leu	Gly	Pro	Leu	Leu	Tyr	Gly	Glu	Val	Gly	Asp	Thr	Leu	Leu	Ile	Ile	450	455	460	

WO 01/68109

PCT/US01/05076

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

WO 01/68109

PCT/US01/05076

Gln	Lys	Gln	Phe	Asn	Ala	Thr	Thr	Ile	Pro	Glu	Asn	Asp	Ile	Glu	Lys	755	760	765
Thr	Asp	Pro	Trp	Phe	Ala	His	Arg	Thr	Pro	Met	Pro	Lys	Ile	Gln	Asn	770	775	780
Val	Ser	Ser	Ser	Asp	Leu	Leu	Met	Leu	Leu	Arg	Gln	Ser	Pro	Thr	Pro	785	790	795
His	Gly	Leu	Ser	Leu	Ser	Asp	Leu	Gln	Glu	Ala	Lys	Tyr	Glu	Thr	Phe	805	810	815
Ser	Asp	Asp	Pro	Ser	Pro	Gly	Ala	Ile	Asp	Ser	Asn	Asn	Ser	Leu	Ser	820	825	830
Glu	Met	Thr	His	Phe	Arg	Pro	Gln	Leu	His	His	Ser	Gly	Asp	Met	Val	835	840	845
Phe	Thr	Pro	Glu	Ser	Gly	Leu	Gln	Leu	Arg	Leu	Asn	Glu	Lys	Leu	Gly	850	855	860
Thr	Thr	Ala	Ala	Thr	Glu	Leu	Lys	Lys	Leu	Asp	Phe	Lys	Val	Ser	Ser	865	870	875
Thr	Ser	Asn	Asn	Leu	Ile	Ser	Thr	Ile	Pro	Ser	Asp	Asn	Leu	Ala	Ala	885	890	895
Gly	Thr	Asp	Asn	Thr	Ser	Ser	Leu	Gly	Pro	Pro	Ser	Met	Pro	Val	His	900	905	910
Tyr	Asp	Ser	Gln	Leu	Asp	Thr	Thr	Leu	Phe	Gly	Lys	Lys	Ser	Ser	Pro	915	920	925
Leu	Thr	Glu	Ser	Gly	Gly	Pro	Leu	Ser	Leu	Ser	Glu	Glu	Asn	Asn	Asp	930	935	940
Ser	Lys	Leu	Leu	Glu	Ser	Gly	Leu	Met	Asn	Ser	Gln	Glu	Ser	Ser	Trp	945	950	955
Gly	Lys	Asn	Val	Ser	Ser	Thr	Glu	Ser	Gly	Arg	Leu	Phe	Lys	Gly	Lys	965	970	975
Arg	Ala	His	Gly	Pro	Ala	Leu	Leu	Thr	Lys	Asp	Asn	Ala	Leu	Phe	Lys	980	985	990
Val	Ser	Ile	Ser	Leu	Leu	Lys	Thr	Asn	Lys	Thr	Ser	Asn	Asn	Ser	Ala	995	1000	1005
Thr	Asn	Arg	Lys	Thr	His	Ile	Asp	Gly	Pro	Ser	Leu	Leu	Ile	Glu	Asn	1010	1015	1020
Ser	Pro	Ser	Val	Trp	Gln	Asn	Ile	Leu	Glu	Ser	Asp	Thr	Glu	Phe	Lys	1025	1030	1035
																		1040

Lys Val Thr Pro Leu Ile His Asp Arg Met Leu Met Asp Lys Asn Ala
 1045 1050 1055
 Thr Ala Leu Arg Leu Asn His Met Ser Asn Lys Thr Thr Ser Ser Lys
 1060 1065 1070
 Asn Met Glu Met Val Gln Gln Lys Lys Glu Gly Pro Ile Pro Pro Asp
 1075 1080 1085
 Ala Gln Asn Pro Asp Met Ser Phe Phe Lys Met Leu Phe Leu Pro Glu
 1090 1095 1100
 Ser Ala Arg Trp Ile Gln Arg Thr His Gly Lys Asn Ser Leu Asn Ser
 105 1110 1115 1120
 Gly Gln Gly Pro Ser Pro Lys Gln Leu Val Ser Leu Gly Pro Glu Lys
 1125 1130 1135
 Ser Val Glu Gly Gln Asn Phe Leu Ser Glu Lys Asn Lys Val Val Val
 1140 1145 1150
 Gly Lys Gly Glu Phe Thr Lys Asp Val Gly Leu Lys Glu Met Val Phe
 1155 1160 1165
 Pro Ser Ser Arg Asn Leu Phe Leu Thr Asn Leu Asp Asn Leu His Glu
 1170 1175 1180
 Asn Asn Thr His Asn Gln Glu Lys Lys Ile Gln Glu Glu Ile Glu Lys
 185 1190 1195 1200
 Lys Glu Thr Leu Ile Gln Glu Asn Val Val Leu Pro Gln Ile His Thr
 1205 1210 1215
 Val Thr Gly Thr Lys Asn Phe Met Lys Asn Leu Phe Leu Leu Ser Thr
 1220 1225 1230
 Arg Gln Asn Val Glu Gly Ser Tyr Glu Gly Ala Tyr Ala Pro Val Leu
 1235 1240 1245
 Gln Asp Phe Arg Ser Leu Asn Asp Ser Thr Asn Arg Thr Lys Lys His
 1250 1255 1260
 Thr Ala His Phe Ser Lys Lys Gly Glu Glu Glu Asn Leu Glu Gly Leu
 265 1270 1275 1280
 Gly Asn Gln Thr Lys Gln Ile Val Glu Lys Tyr Ala Cys Thr Thr Arg
 1285 1290 1295
 Ile Ser Pro Asn Thr Ser Gln Gln Asn Phe Val Thr Gln Arg Ser Lys
 1300 1305 1310
 Arg Ala Leu Lys Gln Phe Arg Leu Pro Leu Glu Glu Thr Glu Leu Glu
 1315 1320 1325

Lys Arg Ile Ile Val Asp Asp Thr Ser Thr Gln Trp Ser Lys Asn Met
 1330 1335 1340
 Lys His Leu Thr Pro Ser Thr Leu Thr Gln Ile Asp Tyr Asn Glu Lys
 345 1350 1355 1360
 Glu Lys Gly Ala Ile Thr Gln Ser Pro Leu Ser Asp Cys Leu Thr Arg
 1365 1370 1375
 Ser His Ser Ile Pro Gln Ala Asn Arg Ser Pro Leu Pro Ile Ala Lys
 1380 1385 1390
 Val Ser Ser Phe Pro Ser Ile Arg Pro Ile Tyr Leu Thr Arg Val Leu
 1395 1400 1405
 Phe Gln Asp Asn Ser Ser His Leu Pro Ala Ala Ser Tyr Arg Lys Lys
 1410 1415 1420
 Asp Ser Gly Val Gln Glu Ser Ser His Phe Leu Gln Gly Ala Lys Lys
 425 1430 1435 1440
 Asn Asn Leu Ser Leu Ala Ile Leu Thr Leu Glu Met Thr Gly Asp Gln
 1445 1450 1455
 Arg Glu Val Gly Ser Leu Gly Thr Ser Ala Thr Asn Ser Val Thr Tyr
 1460 1465 1470
 Lys Lys Val Glu Asn Thr Val Leu Pro Lys Pro Asp Leu Pro Lys Thr
 1475 1480 1485
 Ser Gly Lys Val Glu Leu Leu Pro Lys Val His Ile Tyr Gln Lys Asp
 1490 1495 1500
 Leu Phe Pro Thr Glu Thr Ser Asn Gly Ser Pro Gly His Leu Asp Leu
 505 1510 1515 1520
 Val Glu Gly Ser Leu Leu Gln Gly Thr Glu Gly Ala Ile Lys Trp Asn
 1525 1530 1535
 Glu Ala Asn Arg Pro Gly Lys Val Pro Phe Leu Arg Val Ala Thr Glu
 1540 1545 1550
 Ser Ser Ala Lys Thr Pro Ser Lys Leu Leu Asp Pro Leu Ala Trp Asp
 1555 1560 1565
 Asn His Tyr Gly Thr Gln Ile Pro Lys Glu Glu Trp Lys Ser Gln Glu
 1570 1575 1580
 Lys Ser Pro Glu Lys Thr Ala Phe Lys Lys Lys Asp Thr Ile Leu Ser
 585 1590 1595 1600
 Leu Asn Ala Cys Glu Ser Asn His Ala Ile Ala Ala Ile Asn Glu Gly
 1605 1610 1615

Gln Asn Lys Pro Glu Ile Glu Val Thr Trp Ala Lys Gln Gly Arg Thr
 1620 1625 1630
 Glu Arg Leu Cys Ser Gln Asn Pro Pro Val Leu Lys Arg His Gln Arg
 1635 1640 1645
 Glu Ile Thr Arg Thr Thr Leu Gln Ser Asp Gln Glu Glu Ile Asp Tyr
 1650 1655 1660
 Asp Asp Thr Ile Ser Val Glu Met Lys Lys Glu Asp Phe Asp Ile Tyr
 665 1670 1675 1680
 Asp Glu Asp Glu Asn Gln Ser Pro Arg Ser Phe Gln Lys Lys Thr Arg
 1685 1690 1695
 His Tyr Phe Ile Ala Ala Val Glu Arg Leu Trp Asp Tyr Gly Met Ser
 1700 1705 1710
 Ser Ser Pro His Val Leu Arg Asn Arg Ala Gln Ser Gly Ser Val Pro
 1715 1720 1725
 Gln Phe Lys Lys Val Val Phe Gln Glu Phe Thr Asp Gly Ser Phe Thr
 1730 1735 1740
 Gln Pro Leu Tyr Arg Gly Glu Leu Asn Glu His Leu Gly Leu Leu Gly
 745 1750 1755 1760
 Pro Tyr Ile Arg Ala Glu Val Glu Asp Asn Ile Met Val Thr Phe Arg
 1765 1770 1775
 Asn Gln Ala Ser Arg Pro Tyr Ser Phe Tyr Ser Ser Leu Ile Ser Tyr
 1780 1785 1790
 Glu Glu Asp Gln Arg Gln Gly Ala Glu Pro Arg Lys Asn Phe Val Lys
 1795 1800 1805
 Pro Asn Glu Thr Lys Thr Tyr Phe Trp Lys Val Gln His His Met Ala
 1810 1815 1820
 Pro Thr Lys Asp Glu Phe Asp Cys Lys Ala Trp Ala Tyr Phe Ser Asp
 825 1830 1835 1840
 Val Asp Leu Glu Lys Asp Val His Ser Gly Leu Ile Gly Pro Leu Leu
 1845 1850 1855
 Val Cys His Thr Asn Thr Leu Asn Pro Ala His Gly Arg Gln Val Thr
 1860 1865 1870
 Val Gln Glu Phe Ala Leu Phe Phe Thr Ile Phe Asp Glu Thr Lys Ser
 1875 1880 1885
 Trp Tyr Phe Thr Glu Asn Met Glu Arg Asn Cys Arg Ala Pro Cys Asn
 1890 1895 1900

WO 01/68109

PCT/US01/05076

Ile Gln Met Glu Asp Pro Thr Phe Lys Glu Asn Tyr Arg Phe His Ala		
905	1910	1915 1920
Ile Asn Gly Tyr Ile Met Asp Thr Leu Pro Gly Leu Val Met Ala Gln		
	1925	1930 1935
Asp Gln Arg Ile Arg Trp Tyr Leu Leu Ser Met Gly Ser Asn Glu Asn		
	1940	1945 1950
Ile His Ser Ile His Phe Ser Gly His Val Phe Thr Val Arg Lys Lys		
	1955	1960 1965
Glu Glu Tyr Lys Met Ala Leu Tyr Asn Leu Tyr Pro Gly Val Phe Glu		
	1970	1975 1980
Thr Val Glu Met Leu Pro Ser Lys Ala Gly Ile Trp Arg Val Glu Cys		
985	1990	1995 2000
Leu Ile Gly Glu His Leu His Ala Gly Met Ser Thr Leu Phe Leu Val		
	2005	2010 2015
Tyr Ser Asn Lys Cys Gln Thr Pro Leu Gly Met Ala Ser Gly His Ile		
	2020	2025 2030
Arg Asp Phe Gln Ile Thr Ala Ser Gly Gln Tyr Gly Gln Trp Ala Pro		
	2035	2040 2045
Lys Leu Ala Arg Leu His Tyr Ser Gly Ser Ile Asn Ala Trp Ser Thr		
	2050	2055 2060
Lys Glu Pro Phe Ser Trp Ile Lys Val Asp Leu Leu Ala Pro Met Ile		
065	2070	2075 2080
Ile His Gly Ile Lys Thr Gln Gly Ala Arg Gln Lys Phe Ser Ser Leu		
	2085	2090 2095
Tyr Ile Ser Gln Phe Ile Ile Met Tyr Ser Leu Asp Gly Lys Lys Trp		
	2100	2105 2110
Gln Thr Tyr Arg Gly Asn Ser Thr Gly Thr Leu Met Val Phe Phe Gly		
	2115	2120 2125
Asn Val Asp Ser Ser Gly Ile Lys His Asn Ile Phe Asn Pro Pro Ile		
	2130	2135 2140
Ile Ala Arg Tyr Ile Arg Leu His Pro Thr His Tyr Ser Ile Arg Ser		
145	2150	2155 2160
Thr Leu Arg Met Glu Leu Met Gly Cys Asp Leu Asn Ser Cys Ser Met		
	2165	2170 2175
Pro Leu Gly Met Glu Ser Lys Ala Ile Ser Asp Ala Gln Ile Thr Ala		
	2180	2185 2190

WO 01/68109

PCT/US01/05076

Ser Ser Tyr Phe Thr Asn Met Phe Ala Thr Trp Ser Pro Ser Lys Ala
 2195 2200 2205

Arg Leu His Leu Gln Gly Arg Ser Asn Ala Trp Arg Pro Gln Val Asn
 2210 2215 2220

Asn Pro Lys Glu Trp Leu Gln Val Asp Phe Gln Lys Thr Met Lys Val
 225 2230 2235 2240

Thr Gly Val Thr Thr Gln Gly Val Lys Ser Leu Leu Thr Ser Met Tyr
 2245 2250 2255

Val Lys Glu Phe Leu Ile Ser Ser Ser Gln Asp Gly His Gln Trp Thr
 2260 2265 2270

Leu Phe Phe Gln Asn Gly Lys Val Lys Val Phe Gln Gly Asn Gln Asp
 2275 2280 2285

Ser Phe Thr Pro Val Val Asn Ser Leu Asp Pro Pro Leu Leu Thr Arg
 2290 2295 2300

Tyr Leu Arg Ile His Pro Gln Ser Trp Val His Gln Ile Ala Leu Arg
 305 2310 2315 2320

Met Glu Val Leu Gly Cys Glu Ala Gln Asp Leu Tyr
 2325 2330

<210> 3

<211> 1130

<212> DNA

<213> Porcine

<400> 3

taagcaccct aagacgtggg tgcactacat ctctgcagag gaggaggact gggactacgc 60
 ccccgcggtc cccagcccca gtgacagaag ttataaaagt ctctacttga acagtgggtcc 120
 tcagcgaatt ggtaggaaat acaaaaaagc tcgattcgtc gcttacacgg atgtaacatt 180
 taagactcgt aaagctattc cgtatgaatc aggaatcctg ggacctttac tttatggaga 240
 agttggagac acacttttga ttatatTTaa gaataaagcg agccgaccat ataacatcta 300
 ccctcatgga atcactgatg tcagcgcttt gcacccaggg agacttctaa aaggttggaa 360
 acatttgaaa gacatgccaa ttctgccagg agagactttc aagtataaat ggacagtgac 420
 tgtggaagat gggccaacca agtccgatcc tcggtgcctg acccgctact actcgagctc 480
 cattaatcta gagaaagatc tggcttcggg actcattggc cctctcctca tctgctacaa 540
 agaatctgta gaccaaagag gaaaccagat gatgtcagac aagagaaacg tcatcctgtt 600
 ttctgtattc gatgagaatc aaagctggta cctcgcagag aatattcagc gcttcctccc 660

caatccggat ggattacagc cccaggatcc agagttccaa gcttctaaca tcatgcacag 720
 catcaatggc tatgtttttg atagcttgca gctgtcgggt tgtttgcacg aggtggcata 780
 ctggtacatt ctaagtgttg gagcacagac ggacttcctc tccgtcttct tctctggcta 840
 caccttcaaa cacaaaatgg tctatgaaga cacactcacc ctgttcccct tctcaggaga 900
 aacggtcttc atgtcaatgg aaaaccagg tctctgggtc ctaggggtgcc acaactcaga 960
 cttgcggaac agagggatga cagccttact gaaggtgtat agttgtgaca gggacattgg 1020
 tgattattat gacaacactt atgaagatat tccaggcttc ttgctgagtg gaaagaatgt 1080
 cattgaaccc agaagctttg cccagaattc aagaccccct agtgcgagca 1130

<210> 4
 <211> 368
 <212> PRT
 <213> Porcine

<400> 4
 Ser Val Ala Lys Lys His Pro Lys Thr Trp Val His Tyr Ile Ser Ala
 1 5 10 15
 Glu Glu Glu Asp Trp Asp Tyr Ala Pro Ala Val Pro Ser Pro Ser Asp
 20 25 30
 Arg Ser Tyr Lys Ser Leu Tyr Leu Asn Ser Gly Pro Gln Arg Ile Gly
 35 40 45
 Arg Lys Tyr Lys Lys Ala Arg Phe Val Ala Tyr Thr Asp Val Thr Phe
 50 55 60
 Lys Thr Arg Lys Ala Ile Pro Tyr Glu Ser Gly Ile Leu Gly Pro Leu
 65 70 75 80
 Leu Tyr Gly Glu Val Gly Asp Thr Leu Leu Ile Ile Phe Lys Asn Lys
 85 90 95
 Ala Ser Arg Pro Tyr Asn Ile Tyr Pro His Gly Ile Thr Asp Val Ser
 100 105 110
 Ala Leu His Pro Gly Arg Leu Leu Lys Gly Trp Lys His Leu Lys Asp
 115 120 125
 Met Pro Ile Leu Pro Gly Glu Thr Phe Lys Tyr Lys Trp Thr Val Thr
 130 135 140
 Val Glu Asp Gly Pro Thr Lys Ser Asp Pro Arg Cys Leu Thr Arg Tyr
 145 150 155 160
 Tyr Ser Ser Ser Ile Asn Leu Glu Lys Asp Leu Ala Ser Gly Leu Ile
 165 170 175

WO 01/68109

PCT/US01/05076

Gly Pro Leu Leu Ile Cys Tyr Lys Glu Ser Val Asp Gln Arg Gly Asn
 180 185 190
 Gln Met Met Ser Asp Lys Arg Asn Val Ile Leu Phe Ser Val Phe Asp
 195 200 205
 Glu Asn Gln Ser Trp Tyr Leu Ala Glu Asn Ile Gln Arg Phe Leu Pro
 210 215 220
 Asn Pro Asp Gly Leu Gln Pro Gln Asp Pro Glu Phe Gln Ala Ser Asn
 225 230 235 240
 Ile Met His Ser Ile Asn Gly Tyr Val Phe Asp Ser Leu Gln Leu Ser
 245 250 255
 Val Cys Leu His Glu Val Ala Tyr Trp Tyr Ile Leu Ser Val Gly Ala
 260 265 270
 Gln Thr Asp Phe Leu Ser Val Phe Phe Ser Gly Tyr Thr Phe Lys His
 275 280 285
 Lys Met Val Tyr Glu Asp Thr Leu Thr Leu Phe Pro Phe Ser Gly Glu
 290 295 300
 Thr Val Phe Met Ser Met Glu Asn Pro Gly Leu Trp Val Leu Gly Cys
 305 310 315 320
 His Asn Ser Asp Leu Arg Asn Arg Gly Met Thr Ala Leu Leu Lys Val
 325 330 335
 Tyr Ser Cys Asp Arg Asp Ile Gly Asp Tyr Tyr Asp Asn Thr Tyr Glu
 340 345 350
 Asp Ile Pro Gly Phe Leu Leu Ser Gly Lys Asn Val Ile Glu Pro Arg
 355 360 365

<210> 5
 <211> 44
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:oligonucleotide
 primer

<400> 5
 ctaatacgac tcactatagg gctcgagcgg ccgcccgggc aggt

44

<210> 6
 <211> 27
 <212> DNA
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:oligonucleotide
primer

<400> 6

ccatcctaatac gactcact atagggc

27

<210> 7

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:oligonucleotide
primer

<400> 7

ccattgacat gaagaccgtt tctc

24

<210> 8

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:oligonucleotide
primer

<400> 8

actcactata gggctcgagc ggc

23

<210> 9

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:oligonucleotide
primer

<400> 9

gggtgcaaag cgctgacatc agtg

24

<210> 10

<211> 50

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:oligonucleotide
primer

<400> 10
cctctcgagc caccatgtcg agccaccatg cagctagagc tctccacctg 50

<210> 11
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide
primer

<400> 11.
cgcgcgggccg cgcattctggc aaagctgagt t 31

<210> 12
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide
primer

<400> 12
gaaataagcc caggctttgc agtcraa 27

<210> 13
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide
primer

<400> 13
aggaaattcc actggaacct tn 22

<210> 14
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide
primer

<400> 14
ctgggggtga attcgaaggt agcgn 25

<210> 15
 <211> 23
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:oligonucleotide
 primer

<400> 15
 gagttcatcg ggaagacctg ttg 23

<210> 16
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:
 Oligonucleotide primer

<400> 16
 acagcccatc aactccatgc gaag 24

<210> 17
 <211> 19
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:oligonucleotide
 primer

<400> 17
 tcagggcaat caggactcc 19

<210> 18
 <211> 21
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:oligonucleotide
 primer

<400> 18
 ccgtggtgaa cgctctggac c 21

<210> 19
 <211> 24
 <212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:oligonucleotide primer

<400> 19

gtagaggtcc tgtgcctcgc agcc

24

<210> 20

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:oligonucleotide primer

<400> 20

gtagagstsc tgkgcctcgc akccyag

27

<210> 21

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:oligonucleotide primer

<400> 21

cttcgcatgg agttgatggg ctgt

24

<210> 22

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:oligonucleotide primer

<400> 22

aatcaggact cctccacccc g

21

<210> 23

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:oligonucleotide primer

<400> 23

ggatccacccc cacgagctgg

20

<210> 24

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:oligonucleotide primer

<400> 24

cgccctgagg ctcgaggttc tagg

24

<210> 25

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:oligonucleotide primer

<400> 25

aatcaggact cctccacccc cg

22

<210> 26

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:oligonucleotide primer

<400> 26

ccttgcagga attcgattca

20

<210> 27

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:oligonucleotide primer

WO 01/68109

PCT/US01/05076

<400> 27

ccgtggtgaa cgctctggac c

21

<210> 28

<211> 2319

<212> PRT

<213> Mus musculus

<400> 28

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

Cys	Ser	Ser	Ala	Ile	Arg	Arg	Tyr	Tyr	Leu	Gly	Ala	Val	Glu	Leu	Ser
			20				25						30		

Trp	Asn	Tyr	Ile	Gln	Ser	Asp	Leu	Leu	Ser	Val	Leu	His	Thr	Asp	Ser
		35					40					45			

Arg	Phe	Leu	Pro	Arg	Met	Ser	Thr	Ser	Phe	Pro	Phe	Asn	Thr	Ser	Ile
	50					55					60				

Met	Tyr	Lys	Lys	Thr	Val	Phe	Val	Glu	Tyr	Lys	Asp	Gln	Leu	Phe	Asn
65					70					75					80

Ile	Ala	Lys	Pro	Arg	Pro	Pro	Trp	Met	Gly	Leu	Leu	Gly	Pro	Thr	Ile
				85					90					95	

Trp	Thr	Glu	Val	His	Asp	Thr	Val	Val	Ile	Thr	Leu	Lys	Asn	Met	Ala
			100					105					110		

Ser	His	Pro	Val	Ser	Leu	His	Ala	Val	Gly	Val	Ser	Tyr	Trp	Lys	Ala
		115					120					125			

Ser	Glu	Gly	Asp	Glu	Tyr	Glu	Asp	Gln	Thr	Ser	Gln	Met	Glu	Lys	Glu
	130					135					140				

Asp	Asp	Lys	Val	Phe	Pro	Gly	Glu	Ser	His	Thr	Tyr	Val	Trp	Gln	Val
145					150					155					160

Leu	Lys	Glu	Asn	Gly	Pro	Met	Ala	Ser	Asp	Pro	Pro	Cys	Leu	Thr	Tyr
				165					170					175	

Ser	Tyr	Met	Ser	His	Val	Asp	Leu	Val	Lys	Asp	Leu	Asn	Ser	Gly	Leu
			180					185					190		

Ile	Gly	Ala	Leu	Leu	Val	Cys	Lys	Glu	Gly	Ser	Leu	Ser	Lys	Glu	Arg
		195				200						205			

Thr	Gln	Met	Leu	Tyr	Gln	Phe	Val	Leu	Leu	Phe	Ala	Val	Phe	Asp	Glu
	210					215					220				

Gly	Lys	Ser	Trp	His	Ser	Glu	Thr	Asn	Asp	Ser	Tyr	Thr	Gln	Ser	Met
225					230					235					240

WO 01/68109

PCT/US01/05076

Asp	Ser	Ala	Ser	Ala	Arg	Asp	Trp	Pro	Lys	Met	His	Thr	Val	Asn	Gly	245	250	255	
Tyr	Val	Asn	Arg	Ser	Leu	Pro	Gly	Leu	Ile	Gly	Cys	His	Arg	Lys	Ser	260	265	270	
Val	Tyr	Trp	His	Val	Ile	Gly	Met	Gly	Thr	Thr	Pro	Glu	Ile	His	Ser	275	280	285	
Ile	Phe	Leu	Glu	Gly	His	Thr	Phe	Phe	Val	Arg	Asn	His	Arg	Gln	Ala	290	295	300	
Ser	Leu	Glu	Ile	Ser	Pro	Ile	Thr	Phe	Leu	Thr	Ala	Gln	Thr	Leu	Leu	305	310	315	320
Ile	Asp	Leu	Gly	Gln	Phe	Leu	Leu	Phe	Cys	His	Ile	Ser	Ser	His	Lys	325	330	335	
His	Asp	Gly	Met	Glu	Ala	Tyr	Val	Lys	Val	Asp	Ser	Cys	Pro	Glu	Glu	340	345	350	
Ser	Gln	Trp	Gln	Lys	Lys	Asn	Asn	Asn	Glu	Glu	Met	Glu	Asp	Tyr	Asp	355	360	365	
Asp	Asp	Leu	Tyr	Ser	Glu	Met	Asp	Met	Phe	Thr	Leu	Asp	Tyr	Asp	Ser	370	375	380	
Ser	Pro	Phe	Ile	Gln	Ile	Arg	Ser	Val	Ala	Lys	Lys	Tyr	Pro	Lys	Thr	385	390	395	400
Trp	Ile	His	Tyr	Ile	Ser	Ala	Glu	Glu	Glu	Asp	Trp	Asp	Tyr	Ala	Pro	405	410	415	
Ser	Val	Pro	Thr	Ser	Asp	Asn	Gly	Ser	Tyr	Lys	Ser	Gln	Tyr	Leu	Ser	420	425	430	
Asn	Gly	Pro	His	Arg	Ile	Gly	Arg	Lys	Tyr	Lys	Lys	Val	Arg	Phe	Ile	435	440	445	
Ala	Tyr	Thr	Asp	Glu	Thr	Phe	Lys	Thr	Arg	Glu	Thr	Ile	Gln	His	Glu	450	455	460	
Ser	Gly	Leu	Leu	Gly	Pro	Leu	Leu	Tyr	Gly	Glu	Val	Gly	Asp	Thr	Leu	465	470	475	480
Leu	Ile	Ile	Phe	Lys	Asn	Gln	Ala	Ser	Arg	Pro	Tyr	Asn	Ile	Tyr	Pro	485	490	495	
His	Gly	Ile	Thr	Asp	Val	Ser	Pro	Leu	His	Ala	Arg	Arg	Leu	Pro	Arg	500	505	510	
Gly	Ile	Lys	His	Val	Lys	Asp	Leu	Pro	Ile	His	Pro	Gly	Glu	Ile	Phe	515	520	525	

31

WO 01/68109

PCT/US01/05076

His	Pro	Thr	Pro	His	Gly	Leu	Phe	Leu	Ser	Asp	Gly	Gln	Glu	Ala	Ile	
			820					825					830			
Tyr	Glu	Ala	Ile	His	Asp	Asp	His	Ser	Pro	Asn	Ala	Ile	Asp	Ser	Asn	
		835					840					845				
Glu	Gly	Pro	Ser	Lys	Val	Thr	Gln	Leu	Arg	Pro	Glu	Ser	His	His	Ser	
	850					855					860					
Glu	Lys	Ile	Val	Phe	Thr	Pro	Gln	Pro	Gly	Leu	Gln	Leu	Arg	Ser	Asn	
865					870				875						880	
Lys	Ser	Leu	Glu	Thr	Thr	Ile	Glu	Val	Lys	Trp	Lys	Lys	Leu	Gly	Leu	
				885					890					895		
Gln	Val	Ser	Ser	Leu	Pro	Ser	Asn	Leu	Met	Thr	Thr	Thr	Ile	Leu	Ser	
			900					905					910			
Asp	Asn	Leu	Lys	Ala	Thr	Phe	Glu	Lys	Thr	Asp	Ser	Ser	Gly	Phe	Pro	
		915					920					925				
Asp	Met	Pro	Val	His	Ser	Ser	Ser	Lys	Leu	Ser	Thr	Thr	Ala	Phe	Gly	
	930					935					940					
Lys	Lys	Ala	Tyr	Ser	Leu	Val	Gly	Ser	His	Val	Pro	Leu	Asn	Ala	Ser	
945					950					955					960	
Glu	Glu	Asn	Ser	Asp	Ser	Asn	Ile	Leu	Asp	Ser	Thr	Leu	Met	Tyr	Ser	
				965					970					975		
Gln	Glu	Ser	Leu	Pro	Arg	Asp	Asn	Ile	Leu	Ser	Ile	Glu	Asn	Asp	Arg	
			980					985					990			
Leu	Leu	Arg	Glu	Lys	Arg	Phe	His	Gly	Ile	Ala	Leu	Leu	Thr	Lys	Asp	
		995					1000					1005				
Asn	Thr	Leu	Phe	Lys	Asp	Asn	Val	Ser	Leu	Met	Lys	Thr	Asn	Lys	Thr	
	1010					1015					1020					
Tyr	Asn	His	Ser	Thr	Thr	Asn	Glu	Lys	Leu	His	Thr	Glu	Ser	Pro	Thr	
1025					1030					1035					1040	
Ser	Ile	Glu	Asn	Ser	Thr	Thr	Asp	Leu	Gln	Asp	Ala	Ile	Leu	Lys	Val	
				1045					1050					1055		
Asn	Ser	Glu	Ile	Gln	Glu	Val	Thr	Ala	Leu	Ile	His	Asp	Gly	Thr	Leu	
		1060					1065					1070				
Leu	Gly	Lys	Asn	Ser	Thr	Tyr	Leu	Arg	Leu	Asn	His	Met	Leu	Asn	Arg	
	1075						1080				1085					
Thr	Thr	Ser	Thr	Lys	Asn	Lys	Asp	Ile	Phe	His	Arg	Lys	Asp	Glu	Asp	
	1090					1095					1100					

WO 01/68109

PCT/US01/05076

Pro Ile Pro Gln Asp Glu Glu Asn Thr Ile Met Pro Phe Ser Lys Met			
1105	1110	1115	1120
Leu Phe Leu Ser Glu Ser Ser Asn Trp Phe Lys Lys Thr Asn Gly Asn			
	1125	1130	1135
Asn Ser Leu Asn Ser Glu Gln Glu His Ser Pro Lys Gln Leu Val Tyr			
	1140	1145	1150
Leu Met Phe Lys Lys Tyr Val Lys Asn Gln Ser Phe Leu Ser Glu Lys			
	1155	1160	1165
Asn Lys Val Thr Val Glu Gln Asp Gly Phe Thr Lys Asn Ile Gly Leu			
	1170	1175	1180
Lys Asp Met Ala Phe Pro His Asn Met Ser Ile Phe Leu Thr Thr Leu			
1185	1190	1195	1200
Ser Asn Val His Glu Asn Gly Arg His Asn Gln Glu Lys Asn Ile Gln			
	1205	1210	1215
Glu Glu Ile Glu Lys Glu Ala Leu Ile Glu Glu Lys Val Val Leu Pro			
	1220	1225	1230
Gln Val His Glu Ala Thr Gly Ser Lys Asn Phe Leu Lys Asp Ile Leu			
	1235	1240	1245
Ile Leu Gly Thr Arg Gln Asn Ile Ser Leu Tyr Glu Val His Val Pro			
	1250	1255	1260
Val Leu Gln Asn Ile Thr Ser Ile Asn Asn Ser Thr Asn Thr Val Gln			
1265	1270	1275	1280
Ile His Met Glu His Phe Phe Lys Arg Arg Lys Asp Lys Glu Thr Asn			
	1285	1290	1295
Ser Glu Gly Leu Val Asn Lys Thr Arg Glu Met Val Lys Asn Tyr Pro			
	1300	1305	1310
Ser Gln Lys Asn Ile Thr Thr Gln Arg Ser Lys Arg Ala Leu Gly Gln			
	1315	1320	1325
Phe Arg Leu Ser Thr Gln Trp Leu Lys Thr Ile Asn Cys Ser Thr Gln			
	1330	1335	1340
Cys Ile Ile Lys Gln Ile Asp His Ser Lys Glu Met Lys Lys Phe Ile			
1345	1350	1355	1360
Thr Lys Ser Ser Leu Ser Asp Ser Ser Val Ile Lys Ser Thr Thr Gln			
	1365	1370	1375
Thr Asn Ser Ser Asp Ser His Ile Val Lys Thr Ser Ala Phe Pro Pro			
	1380	1385	1390

WO 01/68109

PCT/US01/05076

Ile	Asp	Leu	Lys	Arg	Ser	Pro	Phe	Gln	Asn	Lys	Phe	Ser	His	Val	Gln	1395	1400	1405	
Ala	Ser	Ser	Tyr	Ile	Tyr	Asp	Phe	Lys	Thr	Lys	Ser	Ser	Arg	Ile	Gln	1410	1415	1420	
Glu	Ser	Asn	Asn	Phe	Leu	Lys	Glu	Thr	Lys	Ile	Asn	Asn	Pro	Ser	Leu	1425	1430	1435	1440
Ala	Ile	Leu	Pro	Trp	Asn	Met	Phe	Ile	Asp	Gln	Gly	Lys	Phe	Thr	Ser	1445	1450	1455	
Pro	Gly	Lys	Ser	Asn	Thr	Asn	Ser	Val	Thr	Tyr	Lys	Lys	Arg	Glu	Asn	1460	1465	1470	
Ile	Ile	Phe	Leu	Lys	Pro	Thr	Leu	Pro	Glu	Glu	Ser	Gly	Lys	Ile	Glu	1475	1480	1485	
Leu	Leu	Pro	Gln	Val	Ser	Ile	Gln	Glu	Glu	Glu	Ile	Leu	Pro	Thr	Glu	1490	1495	1500	
Thr	Ser	His	Gly	Ser	Pro	Gly	His	Leu	Asn	Leu	Met	Lys	Glu	Val	Phe	1505	1510	1515	1520
Leu	Gln	Lys	Ile	Gln	Gly	Pro	Thr	Lys	Trp	Asn	Lys	Ala	Lys	Arg	His	1525	1530	1535	
Gly	Glu	Ser	Ile	Lys	Gly	Lys	Thr	Glu	Ser	Ser	Lys	Asn	Thr	Arg	Ser	1540	1545	1550	
Lys	Leu	Leu	Asn	His	His	Ala	Trp	Asp	Tyr	His	Tyr	Ala	Ala	Gln	Ile	1555	1560	1565	
Pro	Lys	Asp	Met	Trp	Lys	Ser	Lys	Glu	Lys	Ser	Pro	Glu	Ile	Ile	Ser	1570	1575	1580	
Ile	Lys	Gln	Glu	Asp	Thr	Ile	Leu	Ser	Leu	Arg	Pro	His	Gly	Asn	Ser	1585	1590	1595	1600
His	Ser	Ile	Gly	Ala	Asn	Glu	Lys	Gln	Asn	Trp	Pro	Gln	Arg	Glu	Thr	1605	1610	1615	
Thr	Trp	Val	Lys	Gln	Gly	Gln	Thr	Gln	Arg	Thr	Cys	Ser	Gln	Ile	Pro	1620	1625	1630	
Pro	Val	Leu	Lys	Arg	His	Gln	Arg	Glu	Leu	Ser	Ala	Phe	Gln	Ser	Glu	1635	1640	1645	
Gln	Glu	Ala	Thr	Asp	Tyr	Asp	Asp	Ala	Ile	Thr	Ile	Glu	Thr	Ile	Glu	1650	1655	1660	
Asp	Phe	Asp	Ile	Tyr	Ser	Glu	Asp	Ile	Lys	Gln	Gly	Pro	Arg	Ser	Phe	1665	1670	1675	1680

WO 01/68109

PCT/US01/05076

Gln Gln Lys Thr Arg His Tyr Phe Ile Ala Ala Val Glu Arg Leu Trp
 1685 1690 1695
 Asp Tyr Gly Met Ser Thr Ser His Val Leu Arg Asn Arg Tyr Gln Ser
 1700 1705 1710
 Asp Asn Val Pro Gln Phe Lys Lys Val Val Phe Gln Glu Phe Thr Asp
 1715 1720 1725
 Gly Ser Phe Ser Gln Pro Leu Tyr Arg Gly Glu Leu Asn Glu His Leu
 1730 1735 1740
 Gly Leu Leu Gly Pro Tyr Ile Arg Ala Glu Val Glu Asp Asn Ile Met
 1745 1750 1755 1760
 Val Thr Phe Lys Asn Gln Ala Ser Arg Pro Tyr Ser Phe Tyr Ser Ser
 1765 1770 1775
 Leu Ile Ser Tyr Lys Glu Asp Gln Arg Gly Glu Glu Pro Arg Arg Asn
 1780 1785 1790
 Phe Val Lys Pro Asn Glu Thr Lys Ile Tyr Phe Trp Lys Val Gln His
 1795 1800 1805
 His Met Ala Pro Thr Glu Asp Glu Phe Asp Cys Lys Ala Trp Ala Tyr
 1810 1815 1820
 Phe Ser Asp Val Asp Leu Glu Arg Asp Met His Ser Gly Leu Ile Gly
 1825 1830 1835 1840
 Pro Leu Leu Ile Cys His Ala Asn Thr Leu Asn Pro Ala His Gly Arg
 1845 1850 1855
 Gln Val Ser Val Gln Glu Phe Ala Leu Leu Phe Thr Ile Phe Asp Glu
 1860 1865 1870
 Thr Lys Ser Trp Tyr Phe Thr Glu Asn Val Lys Arg Asn Cys Lys Thr
 1875 1880 1885
 Pro Cys Asn Phe Gln Met Glu Asp Pro Thr Leu Lys Glu Asn Tyr Arg
 1890 1895 1900
 Phe His Ala Ile Asn Gly Tyr Val Met Asp Thr Leu Pro Gly Leu Val
 1905 1910 1915 1920
 Met Ala Gln Asp Gln Arg Ile Arg Trp Tyr Leu Leu Ser Met Gly Asn
 1925 1930 1935
 Asn Glu Asn Ile Gln Ser Ile His Phe Ser Gly His Val Phe Thr Val
 1940 1945 1950
 Arg Lys Lys Glu Glu Tyr Lys Met Ala Val Tyr Asn Leu Tyr Pro Gly
 1955 1960 1965

WO 01/68109

PCT/US01/05076

Val Phe Glu Thr Leu Glu Met Ile Pro Ser Arg Ala Gly Ile Trp Arg		
1970	1975	1980
Val Glu Cys Leu Ile Gly Glu His Leu Gln Ala Gly Met Ser Thr Leu		
1985	1990	2000
Phe Leu Val Tyr Ser Lys Gln Cys Gln Ile Pro Leu Gly Met Ala Ser		
	2005	2015
Gly Ser Ile Arg Asp Phe Gln Ile Thr Ala Ser Gly His Tyr Gly Gln		
	2020	2030
Trp Ala Pro Asn Leu Ala Arg Leu His Tyr Ser Gly Ser Ile Asn Ala		
	2035	2045
Trp Ser Thr Lys Glu Pro Phe Ser Trp Ile Lys Val Asp Leu Leu Ala		
	2050	2060
Pro Met Ile Val His Gly Ile Lys Thr Gln Gly Ala Arg Gln Lys Phe		
	2065	2080
Ser Ser Leu Tyr Ile Ser Gln Phe Ile Ile Met Tyr Ser Leu Asp Gly		
	2085	2095
Lys Lys Trp Leu Ser Tyr Gln Gly Asn Ser Thr Gly Thr Leu Met Val		
	2100	2110
Phe Phe Gly Asn Val Asp Ser Ser Gly Ile Lys His Asn Ser Phe Asn		
	2115	2125
Pro Pro Ile Ile Ala Arg Tyr Ile Arg Leu His Pro Thr His Ser Ser		
	2130	2140
Ile Arg Ser Thr Leu Arg Met Glu Leu Met Gly Cys Asp Leu Asn Ser		
	2145	2160
Cys Ser Ile Pro Leu Gly Met Glu Ser Lys Val Ile Ser Asp Thr Gln		
	2165	2175
Ile Thr Ala Ser Ser Tyr Phe Thr Asn Met Phe Ala Thr Trp Ser Pro		
	2180	2190
Ser Gln Ala Arg Leu His Leu Gln Gly Arg Thr Asn Ala Trp Arg Pro		
	2195	2205
Gln Val Asn Asp Pro Lys Gln Trp Leu Gln Val Asp Leu Gln Lys Thr		
	2210	2220
Met Lys Val Thr Gly Ile Ile Thr Gln Gly Val Lys Ser Leu Phe Thr		
	2225	2240
Ser Met Phe Val Lys Glu Phe Leu Ile Ser Ser Ser Gln Asp Gly His		
	2245	2255

WO 01/68109

PCT/US01/05076

His Trp Thr Gln Ile Leu Tyr Asn Gly Lys Val Lys Val Phe Gln Gly
 2260 2265 2270

Asn Gln Asp Ser Ser Thr Pro Met Met Asn Ser Leu Asp Pro Pro Leu
 2275 2280 2285

Leu Thr Arg Tyr Leu Arg Ile His Pro Gln Ile Trp Glu His Gln Ile
 2290 2295 2300

Ala Leu Arg Leu Glu Ile Leu Gly Cys Glu Ala Gln Gln Gln Tyr
 2305 2310 2315

<210> 29

<211> 6402

<212> DNA

<213> Porcine

<220>

<221> CDS

<222> (1)..(6399)

<400> 29

atg cag cta gag ctc tcc acc tgt gtc ttt ctg tgt ctc ttg cca ctc 48
 Met Gln Leu Glu Leu Ser Thr Cys Val Phe Leu Cys Leu Leu Pro Leu
 1 5 10 15

ggc ttt agt gcc atc agg aga tac tac ctg ggc gca gtg gaa ctg tcc 96
 Gly Phe Ser Ala Ile Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser
 20 25 30

tgg gac tac cgg caa agt gaa ctc ctc cgt gag ctg cac gtg gac acc 144
 Trp Asp Tyr Arg Gln Ser Glu Leu Leu Arg Glu Leu His Val Asp Thr
 35 40 45

aga ttt cct gct aca gcg cca gga gct ctt ccg ttg ggc ccg tca gtc 192
 Arg Phe Pro Ala Thr Ala Pro Gly Ala Leu Pro Leu Gly Pro Ser Val
 50 55 60

ctg tac aaa aag act gtg ttc gta gag ttc acg gat caa ctt ttc agc 240
 Leu Tyr Lys Lys Thr Val Phe Val Glu Phe Thr Asp Gln Leu Phe Ser
 65 70 75 80

gtt gcc agg ccc agg cca cca tgg atg ggt ctg ctg ggt cct acc atc 288
 Val Ala Arg Pro Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile
 85 90 95

cag gct gag gtt tac gac acg gtg gtc gtt acc ctg aag aac atg gct 336
 Gln Ala Glu Val Tyr Asp Thr Val Val Val Thr Leu Lys Asn Met Ala
 100 105 110

tct cat ccc gtt agt ctt cac gct gtc ggc gtc tcc ttc tgg aaa tct 384
 Ser His Pro Val Ser Leu His Ala Val Gly Val Ser Phe Trp Lys Ser
 115 120 125

WO 01/68109

PCT/US01/05076

tcc gaa ggc gct gaa tat gag gat cac acc agc caa agg gag aag gaa	432
Ser Glu Gly Ala Glu Tyr Glu Asp His Thr Ser Gln Arg Glu Lys Glu	
130 135 140	
gac gat aaa gtc ctt ccc ggt aaa agc caa acc tac gtc tgg cag gtc	480
Asp Asp Lys Val Leu Pro Gly Lys Ser Gln Thr Tyr Val Trp Gln Val	
145 150 155 160	
ctg aaa gaa aat ggt cca aca gcc tct gac cca cca tgt ctc acc tac	528
Leu Lys Glu Asn Gly Pro Thr Ala Ser Asp Pro Pro Cys Leu Thr Tyr	
165 170 175	
tca tac ctg tct cac gtg gac ctg gtg aaa gac ctg aat tcg ggc ctc	576
Ser Tyr Leu Ser His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu	
180 185 190	
att gga gcc ctg ctg gtt tgt aga gaa ggg agt ctg acc aga gaa agg	624
Ile Gly Ala Leu Leu Val Cys Arg Glu Gly Ser Leu Thr Arg Glu Arg	
195 200 205	
acc cag aac ctg cac gaa ttt gta cta ctt ttt gct gtc ttt gat gaa	672
Thr Gln Asn Leu His Glu Phe Val Leu Leu Phe Ala Val Phe Asp Glu	
210 215 220	
ggg aaa agt tgg cac tca gca aga aat gac tcc tgg aca cgg gcc atg	720
Gly Lys Ser Trp His Ser Ala Arg Asn Asp Ser Trp Thr Arg Ala Met	
225 230 235 240	
gat ccc gca cct gcc agg gcc cag cct gca atg cac aca gtc aat ggc	768
Asp Pro Ala Pro Ala Arg Ala Gln Pro Ala Met His Thr Val Asn Gly	
245 250 255	
tat gtc aac agg tct ctg cca ggt ctg atc gga tgt cat aag aaa tca	816
Tyr Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His Lys Lys Ser	
260 265 270	
gtc tac tgg cac gtg att gga atg ggc acc agc ccg gaa gtg cac tcc	864
Val Tyr Trp His Val Ile Gly Met Gly Thr Ser Pro Glu Val His Ser	
275 280 285	
att ttt ctt gaa ggc cac acg ttt ctc gtg agg cac cat cgc cag gct	912
Ile Phe Leu Glu Gly His Thr Phe Leu Val Arg His His Arg Gln Ala	
290 295 300	
tcc ttg gag atc tcg cca cta act ttc ctc act gct cag aca ttc ctg	960
Ser Leu Glu Ile Ser Pro Leu Thr Phe Leu Thr Ala Gln Thr Phe Leu	
305 310 315 320	
atg gac ctt ggc cag ttc cta ctg ttt tgt cat atc tct tcc cac cac	1008
Met Asp Leu Gly Gln Phe Leu Leu Phe Cys His Ile Ser Ser His His	
325 330 335	
cat ggt ggc atg gag gct cac gtc aga gta gaa agc tgc gcc gag gag	1056
His Gly Gly Met Glu Ala His Val Arg Val Glu Ser Cys Ala Glu Glu	
340 345 350	

WO 01/68109

PCT/US01/05076

ccc	cag	ctg	cgg	agg	aaa	gct	gat	gaa	gag	gaa	gat	tat	gat	gac	aat	1104
Pro	Gln	Leu	Arg	Arg	Lys	Ala	Asp	Glu	Glu	Glu	Asp	Tyr	Asp	Asp	Asn	
		355					360					365				
ttg	tac	gac	tcg	gac	atg	gac	gtg	gtc	cgg	ctc	gat	ggg	gac	gac	gtg	1152
Leu	Tyr	Asp	Ser	Asp	Met	Asp	Val	Val	Arg	Leu	Asp	Gly	Asp	Asp	Val	
	370					375					380					
tct	ccc	ttt	atc	caa	atc	cgc	tcg	gtt	gcc	aag	aag	cat	ccc	aaa	acc	1200
Ser	Pro	Phe	Ile	Gln	Ile	Arg	Ser	Val	Ala	Lys	Lys	His	Pro	Lys	Thr	
385					390					395					400	
tgg	gtg	cac	tac	atc	tct	gca	gag	gag	gag	gac	tgg	gac	tac	gcc	ccc	1248
Trp	Val	His	Tyr	Ile	Ser	Ala	Glu	Glu	Glu	Asp	Trp	Asp	Tyr	Ala	Pro	
				405					410					415		
gcg	gtc	ccc	agc	ccc	agt	gac	aga	agt	tat	aaa	agt	ctc	tac	ttg	aac	1296
Ala	Val	Pro	Ser	Pro	Ser	Asp	Arg	Ser	Tyr	Lys	Ser	Leu	Tyr	Leu	Asn	
			420					425					430			
agt	ggg	cct	cag	cga	att	ggg	agg	aaa	tac	aaa	aaa	gct	cga	ttc	gtc	1344
Ser	Gly	Pro	Gln	Arg	Ile	Gly	Arg	Lys	Tyr	Lys	Lys	Ala	Arg	Phe	Val	
		435					440					445				
gct	tac	acg	gat	gta	aca	ttt	aag	act	cgt	aaa	gct	att	ccg	tat	gaa	1392
Ala	Tyr	Thr	Asp	Val	Thr	Phe	Lys	Thr	Arg	Lys	Ala	Ile	Pro	Tyr	Glu	
	450					455					460					
tca	gga	atc	ctg	gga	cct	tta	ctt	tat	gga	gaa	gtt	gga	gac	aca	ctt	1440
Ser	Gly	Ile	Leu	Gly	Pro	Leu	Leu	Tyr	Gly	Glu	Val	Gly	Asp	Thr	Leu	
465					470					475					480	
ttg	att	ata	ttt	aag	aat	aaa	gcg	agc	cga	cca	tat	aac	atc	tac	cct	1488
Leu	Ile	Ile	Phe	Lys	Asn	Lys	Ala	Ser	Arg	Pro	Tyr	Asn	Ile	Tyr	Pro	
				485					490					495		
cat	gga	atc	act	gat	gtc	agc	gct	ttg	cac	cca	ggg	aga	ctt	cta	aaa	1536
His	Gly	Ile	Thr	Asp	Val	Ser	Ala	Leu	His	Pro	Gly	Arg	Leu	Leu	Lys	
			500					505					510			
ggg	tgg	aaa	cat	ttg	aaa	gac	atg	cca	att	ctg	cca	gga	gag	act	ttc	1584
Gly	Trp	Lys	His	Leu	Lys	Asp	Met	Pro	Ile	Leu	Pro	Gly	Glu	Thr	Phe	
		515					520					525				
aag	tat	aaa	tgg	aca	gtg	act	gtg	gaa	gat	ggg	cca	acc	aag	tcc	gat	1632
Lys	Tyr	Lys	Trp	Thr	Val	Thr	Val	Glu	Asp	Gly	Pro	Thr	Lys	Ser	Asp	
	530					535					540					
cct	cgg	tgc	ctg	acc	cgc	tac	tac	tcg	agc	tcc	att	aat	cta	gag	aaa	1680
Pro	Arg	Cys	Leu	Thr	Arg	Tyr	Tyr	Ser	Ser	Ser	Ile	Asn	Leu	Glu	Lys	
545					550					555					560	
gat	ctg	gct	tcg	gga	ctc	att	ggc	cct	ctc	ctc	atc	tgc	tac	aaa	gaa	1728
Asp	Leu	Ala	Ser	Gly	Leu	Ile	Gly	Pro	Leu	Leu	Ile	Cys	Tyr	Lys	Glu	
				565					570					575		

WO 01/68109

PCT/US01/05076

tct gta gac caa aga gga aac cag atg atg tca gac aag aga aac gtc	1776
Ser Val Asp Gln Arg Gly Asn Gln Met Met Ser Asp Lys Arg Asn Val	
580 585 590	
atc ctg ttt tct gta ttc gat gag aat caa agc tgg tac ctc gca gag	1824
Ile Leu Phe Ser Val Phe Asp Glu Asn Gln Ser Trp Tyr Leu Ala Glu	
595 600 605	
aat att cag cgc ttc ctc ccc aat ccg gat gga tta cag ccc cag gat	1872
Asn Ile Gln Arg Phe Leu Pro Asn Pro Asp Gly Leu Gln Pro Gln Asp	
610 615 620	
cca gag ttc caa gct tct aac atc atg cac agc atc aat ggc tat gtt	1920
Pro Glu Phe Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val	
625 630 635 640	
ttt gat agc ttg cag ctg tcg gtt tgt ttg cac gag gtg gca tac tgg	1968
Phe Asp Ser Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp	
645 650 655	
tac att cta agt gtt gga gca cag acg gac ttc ctc tcc gtc ttc ttc	2016
Tyr Ile Leu Ser Val Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe	
660 665 670	
tct ggc tac acc ttc aaa cac aaa atg gtc tat gaa gac aca ctc acc	2064
Ser Gly Tyr Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr	
675 680 685	
ctg ttc ccc ttc tca gga gaa acg gtc ttc atg tca atg gaa aac cca	2112
Leu Phe Pro Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro	
690 695 700	
ggc ctc tgg gtc cta ggg tgc cac aac tca gac ttg cgg aac aga ggg	2160
Gly Leu Trp Val Leu Gly Cys His Asn Ser Asp Leu Arg Asn Arg Gly	
705 710 715 720	
atg aca gcc tta ctg aag gtg tat agt tgt gac agg gac att ggt gat	2208
Met Thr Ala Leu Leu Lys Val Tyr Ser Cys Asp Arg Asp Ile Gly Asp	
725 730 735	
tat tat gac aac act tat gaa gat att cca ggc ttc ttg ctg agt gga	2256
Tyr Tyr Asp Asn Thr Tyr Glu Asp Ile Pro Gly Phe Leu Leu Ser Gly	
740 745 750	
aag aat gtc att gaa ccc aga agc ttt gcc cag aat tca aga ccc cct	2304
Lys Asn Val Ile Glu Pro Arg Ser Phe Ala Gln Asn Ser Arg Pro Pro	
755 760 765	
agt gcg agc caa aag caa ttc caa acc atc aca agt cca gaa gat gac	2352
Ser Ala Ser Gln Lys Gln Phe Gln Thr Ile Thr Ser Pro Glu Asp Asp	
770 775 780	
gtg gag ctt gac ccg cag tct gga gag aga acc caa gca ctg gaa gaa	2400
Val Glu Leu Asp Pro Gln Ser Gly Glu Arg Thr Gln Ala Leu Glu Glu	
785 790 795 800	

WO 01/68109

PCT/US01/05076

cta agt gtc ccc tct ggt gat ggg tcg atg ctc ttg gga cag aat cct	2448
Leu Ser Val Pro Ser Gly Asp Gly Ser Met Leu Leu Gly Gln Asn Pro	
805 810 815	
gct cca cat ggc tca tcc tca tct gat ctt caa gaa gcc agg aat gag	2496
Ala Pro His Gly Ser Ser Ser Ser Asp Leu Gln Glu Ala Arg Asn Glu	
820 825 830	
gct gat gat tat tta cct gga gca aga gaa aga ggc acg gcc cca tcc	2544
Ala Asp Asp Tyr Leu Pro Gly Ala Arg Glu Arg Gly Thr Ala Pro Ser	
835 840 845	
gca gcg gca cgt ctc aga cca gag ctg cat cac agt gcc gaa aga gta	2592
Ala Ala Ala Arg Leu Arg Pro Glu Leu His His Ser Ala Glu Arg Val	
850 855 860	
ctt act cct gag cca gag aaa gag ttg aag aaa ctt gat tca aaa atg	2640
Leu Thr Pro Glu Pro Glu Lys Glu Leu Lys Lys Leu Asp Ser Lys Met	
865 870 875 880	
tct agt tca tca gac ctt cta aag act tcg cca aca att cca tca gac	2688
Ser Ser Ser Ser Asp Leu Leu Lys Thr Ser Pro Thr Ile Pro Ser Asp	
885 890 895	
acg ttg tca gcg gag act gaa agg aca cat tcc tta ggc ccc cca cac	2736
Thr Leu Ser Ala Glu Thr Glu Arg Thr His Ser Leu Gly Pro Pro His	
900 905 910	
ccg cag gtt aat ttc agg agt caa tta ggt gcc att gta ctt ggc aaa	2784
Pro Gln Val Asn Phe Arg Ser Gln Leu Gly Ala Ile Val Leu Gly Lys	
915 920 925	
aat tca tct cac ttt att ggg gct ggt gtc cct ttg ggc tcg act gag	2832
Asn Ser Ser His Phe Ile Gly Ala Gly Val Pro Leu Gly Ser Thr Glu	
930 935 940	
gag gat cat gaa agc tcc ctg gga gaa aat gta tca cca gtg gag agt	2880
Glu Asp His Glu Ser Ser Leu Gly Glu Asn Val Ser Pro Val Glu Ser	
945 950 955 960	
gac ggg ata ttt gaa aag gaa aga gct cat gga cct gct tca ctg acc	2928
Asp Gly Ile Phe Glu Lys Glu Arg Ala His Gly Pro Ala Ser Leu Thr	
965 970 975	
aaa gac gat gtt tta ttt aaa gtt aat atc tct ttg gta aag aca aac	2976
Lys Asp Asp Val Leu Phe Lys Val Asn Ile Ser Leu Val Lys Thr Asn	
980 985 990	
aag gca cga gtt tac tta aaa act aat aga aag att cac att gat gac	3024
Lys Ala Arg Val Tyr Leu Lys Thr Asn Arg Lys Ile His Ile Asp Asp	
995 1000 1005	
gca gct tta tta act gag aat agg gca tct gca acg ttt atg gac aaa	3072
Ala Ala Leu Leu Thr Glu Asn Arg Ala Ser Ala Thr Phe Met Asp Lys	
1010 1015 1020	

WO 01/68109

PCT/US01/05076

aat act aca gct tcg gga tta aat cat gtg tca aat tgg ata aaa ggg	3120
Asn Thr Thr Ala Ser Gly Leu Asn His Val Ser Asn Trp Ile Lys Gly	
1025 1030 1035 1040	
ccc ctt ggc aag aac ccc cta agc tcg gag cga ggc ccc agt cca gag	3168
Pro Leu Gly Lys Asn Pro Leu Ser Ser Glu Arg Gly Pro Ser Pro Glu	
1045 1050 1055	
ctt ctg aca tct tca gga tca gga aaa tct gtg aaa ggt cag agt tct	3216
Leu Leu Thr Ser Ser Gly Ser Gly Lys Ser Val Lys Gly Gln Ser Ser	
1060 1065 1070	
ggg cag ggc aga ata cgg gtg gca gtg gaa gag gaa gaa ctg agc aaa	3264
Gly Gln Gly Arg Ile Arg Val Ala Val Glu Glu Glu Glu Leu Ser Lys	
1075 1080 1085	
ggc aaa gag atg atg ctt ccc aac agc gag ctc acc ttt ctc act aac	3312
Gly Lys Glu Met Met Leu Pro Asn Ser Glu Leu Thr Phe Leu Thr Asn	
1090 1095 1100	
tcg gct gat gtc caa gga aac gat aca cac agt caa gga aaa aag tct	3360
Ser Ala Asp Val Gln Gly Asn Asp Thr His Ser Gln Gly Lys Lys Ser	
1105 1110 1115 1120	
cgg gaa gag atg gaa agg aga gaa aaa tta gtc caa gaa aaa gtc gac	3408
Arg Glu Glu Met Glu Arg Arg Glu Lys Leu Val Gln Glu Lys Val Asp	
1125 1130 1135	
ttg cct cag gtg tat aca gcg act gga act aag aat ttc ctg aga aac	3456
Leu Pro Gln Val Tyr Thr Ala Thr Gly Thr Lys Asn Phe Leu Arg Asn	
1140 1145 1150	
att ttt cac caa agc act gag ccc agt gta gaa ggg ttt gat ggg ggg	3504
Ile Phe His Gln Ser Thr Glu Pro Ser Val Glu Gly Phe Asp Gly Gly	
1155 1160 1165	
tca cat gcg ccg gtg cct caa gac agc agg tca tta aat gat tcg gca	3552
Ser His Ala Pro Val Pro Gln Asp Ser Arg Ser Leu Asn Asp Ser Ala	
1170 1175 1180	
gag aga gca gag act cac ata gcc cat ttc tca gca att agg gaa gag	3600
Glu Arg Ala Glu Thr His Ile Ala His Phe Ser Ala Ile Arg Glu Glu	
1185 1190 1195 1200	
gca ccc ttg gaa gcc ccg gga aat cga aca ggt cca ggt ccg agg agt	3648
Ala Pro Leu Glu Ala Pro Gly Asn Arg Thr Gly Pro Gly Pro Arg Ser	
1205 1210 1215	
gcg gtt ccc cgc cgc gtt aag cag agc ttg aaa cag atc aga ctc ccg	3696
Ala Val Pro Arg Arg Val Lys Gln Ser Leu Lys Gln Ile Arg Leu Pro	
1220 1225 1230	
cta gaa gaa ata aag cct gaa agg ggg gtg gtt ctg aat gcc acc tca	3744
Leu Glu Glu Ile Lys Pro Glu Arg Gly Val Val Leu Asn Ala Thr Ser	
1235 1240 1245	

acc cgg tgg tct gaa agc agt cct atc tta caa gga gcc aaa aga aat	3792
Thr Arg Trp Ser Glu Ser Ser Pro Ile Leu Gln Gly Ala Lys Arg Asn	
1250 1255 1260	
aac ctt tct tta cct ttc ctg acc ttg gaa atg gcc gga ggt caa gga	3840
Asn Leu Ser Leu Pro Phe Leu Thr Leu Glu Met Ala Gly Gly Gln Gly	
1265 1270 1275 1280	
aag atc agc gcc ctg ggg aaa agt gcc gca ggc ccg ctg gcg tcc ggg	3888
Lys Ile Ser Ala Leu Gly Lys Ser Ala Ala Gly Pro Leu Ala Ser Gly	
1285 1290 1295	
aag ctg gag aag gct gtt ctc tct tca gca ggc ttg tct gaa gca tct	3936
Lys Leu Glu Lys Ala Val Leu Ser Ser Ala Gly Leu Ser Glu Ala Ser	
1300 1305 1310	
ggc aaa gct gag ttt ctt cct aaa gtt cga gtt cat cgg gaa gac ctg	3984
Gly Lys Ala Glu Phe Leu Pro Lys Val Arg Val His Arg Glu Asp Leu	
1315 1320 1325	
ttg cct caa aaa acc agc aat gtt tct tgc gca cac ggg gat ctc ggc	4032
Leu Pro Gln Lys Thr Ser Asn Val Ser Cys Ala His Gly Asp Leu Gly	
1330 1335 1340	
cag gag atc ttc ctg cag aaa aca cgg gga cct gtt aac ctg aac aaa	4080
Gln Glu Ile Phe Leu Gln Lys Thr Arg Gly Pro Val Asn Leu Asn Lys	
1345 1350 1355 1360	
gta aat aga cct gga agg act ccc tcc aag ctt ctg ggt ccc ccg atg	4128
Val Asn Arg Pro Gly Arg Thr Pro Ser Lys Leu Leu Gly Pro Pro Met	
1365 1370 1375	
ccc aaa gag tgg gaa tcc cta gag aag tca cca aaa agc aca gct ctc	4176
Pro Lys Glu Trp Glu Ser Leu Glu Lys Ser Pro Lys Ser Thr Ala Leu	
1380 1385 1390	
agg acg aaa gac atc atc agt tta ccc ctg gac cgt cac gaa agc aat	4224
Arg Thr Lys Asp Ile Ile Ser Leu Pro Leu Asp Arg His Glu Ser Asn	
1395 1400 1405	
cat tca ata gca gca aaa aat gaa gga caa gcc gag acc caa aga gaa	4272
His Ser Ile Ala Ala Lys Asn Glu Gly Gln Ala Glu Thr Gln Arg Glu	
1410 1415 1420	
gcc gcc tgg acg aag cag gga ggg cct gga agg ctg tgc gct cca aag	4320
Ala Ala Trp Thr Lys Gln Gly Gly Pro Gly Arg Leu Cys Ala Pro Lys	
1425 1430 1435 1440	
cct ccg gtc ctg cga cgg cat cag agg gac ata agc ctt cct act ttt	4368
Pro Pro Val Leu Arg Arg His Gln Arg Asp Ile Ser Leu Pro Thr Phe	
1445 1450 1455	
cag ccg gag gaa gac aaa atg gac tat gat gat atc ttc tca act gaa	4416
Gln Pro Glu Glu Asp Lys Met Asp Tyr Asp Asp Ile Phe Ser Thr Glu	
1460 1465 1470	

WO 01/68109

PCT/US01/05076

acg aag gga gaa gat ttt gac att tac ggt gag gat gaa aat cag gac	4464
Thr Lys Gly Glu Asp Phe Asp Ile Tyr Gly Glu Asp Glu Asn Gln Asp	
1475 1480 1485	
cct cgc agc ttt cag aag aga acc cga cac tat ttc att gct gcg gtg	4512
Pro Arg Ser Phe Gln Lys Arg Thr Arg His Tyr Phe Ile Ala Ala Val	
1490 1495 1500	
gag cag ctc tgg gat tac ggg atg agc gaa tcc ccc cgg gcg cta aga	4560
Glu Gln Leu Trp Asp Tyr Gly Met Ser Glu Ser Pro Arg Ala Leu Arg	
1505 1510 1515 1520	
aac agg gct cag aac gga gag gtg cct cgg ttc aag aag gtg gtc ttc	4608
Asn Arg Ala Gln Asn Gly Glu Val Pro Arg Phe Lys Lys Val Val Phe	
1525 1530 1535	
cgg gaa ttt gct gac ggc tcc ttc acg cag ccg tcg tac cgc ggg gaa	4656
Arg Glu Phe Ala Asp Gly Ser Phe Thr Gln Pro Ser Tyr Arg Gly Glu	
1540 1545 1550	
ctc aac aaa cac ttg ggg ctc ttg gga ccc tac atc aga gcg gaa gtt	4704
Leu Asn Lys His Leu Gly Leu Leu Gly Pro Tyr Ile Arg Ala Glu Val	
1555 1560 1565	
gaa gac aac atc atg gta act ttc aaa aac cag gcg tct cgt ccc tat	4752
Glu Asp Asn Ile Met Val Thr Phe Lys Asn Gln Ala Ser Arg Pro Tyr	
1570 1575 1580	
tcc ttc tac tcg agc ctt att tct tat ccg gat gat cag gag caa ggg	4800
Ser Phe Tyr Ser Ser Leu Ile Ser Tyr Pro Asp Asp Gln Glu Gln Gly	
1585 1590 1595 1600	
gca gaa cct cga cac aac ttc gtc cag cca aat gaa acc aga act tac	4848
Ala Glu Pro Arg His Asn Phe Val Gln Pro Asn Glu Thr Arg Thr Tyr	
1605 1610 1615	
ttt tgg aaa gtg cag cat cac atg gca ccc aca gaa gac gag ttt gac	4896
Phe Trp Lys Val Gln His His Met Ala Pro Thr Glu Asp Glu Phe Asp	
1620 1625 1630	
tgc aaa gcc tgg gcc tac ttt tct gat gtt gac ctg gaa aaa gat gtg	4944
Cys Lys Ala Trp Ala Tyr Phe Ser Asp Val Asp Leu Glu Lys Asp Val	
1635 1640 1645	
cac tca ggc ttg atc ggc ccc ctt ctg atc tgc cgc gcc aac acc ctg	4992
His Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Arg Ala Asn Thr Leu	
1650 1655 1660	
aac gct gct cac ggt aga caa gtg acc gtg caa gaa ttt gct ctg ttt	5040
Asn Ala Ala His Gly Arg Gln Val Thr Val Gln Glu Phe Ala Leu Phe	
1665 1670 1675 1680	
ttc act att ttt gat gag aca aag agc tgg tac ttc act gaa aat gtg	5088
Phe Thr Ile Phe Asp Glu Thr Lys Ser Trp Tyr Phe Thr Glu Asn Val	
1685 1690 1695	

gaa agg aac tgc cgg gcc ccc tgc cac ctg cag atg gag gac ccc act	5136
Glu Arg Asn Cys Arg Ala Pro Cys His Leu Gln Met Glu Asp Pro Thr	
1700 1705 1710	
ctg aaa gaa aac tat cgc ttc cat gca atc aat ggc tat gtg atg gat	5184
Leu Lys Glu Asn Tyr Arg Phe His Ala Ile Asn Gly Tyr Val Met Asp	
1715 1720 1725	
aca ctc cct ggc tta gta atg gct cag aat caa agg atc cga tgg tat	5232
Thr Leu Pro Gly Leu Val Met Ala Gln Asn Gln Arg Ile Arg Trp Tyr	
1730 1735 1740	
ctg ctc agc atg ggc agc aat gaa aat atc cat tcg att cat ttt agc	5280
Leu Leu Ser Met Gly Ser Asn Glu Asn Ile His Ser Ile His Phe Ser	
1745 1750 1755 1760	
gga cac gtg ttc agt gta cgg aaa aag gag gag tat aaa atg gcc gtg	5328
Gly His Val Phe Ser Val Arg Lys Lys Glu Glu Tyr Lys Met Ala Val	
1765 1770 1775	
tac aat ctc tat ccg ggt gtc ttt gag aca gtg gaa atg cta ccg tcc	5376
Tyr Asn Leu Tyr Pro Gly Val Phe Glu Thr Val Glu Met Leu Pro Ser	
1780 1785 1790	
aaa gtt gga att tgg cga ata gaa tgc ctg att ggc gag cac ctg caa	5424
Lys Val Gly Ile Trp Arg Ile Glu Cys Leu Ile Gly Glu His Leu Gln	
1795 1800 1805	
gct ggg atg agc acg act ttc ctg gtg tac agc aag gag tgt cag gct	5472
Ala Gly Met Ser Thr Thr Phe Leu Val Tyr Ser Lys Glu Cys Gln Ala	
1810 1815 1820	
cca ctg gga atg gct tct gga cgc att aga gat ttt cag atc aca gct	5520
Pro Leu Gly Met Ala Ser Gly Arg Ile Arg Asp Phe Gln Ile Thr Ala	
1825 1830 1835 1840	
tca gga cag tat gga cag tgg gcc cca aag ctg gcc aga ctt cat tat	5568
Ser Gly Gln Tyr Gly Gln Trp Ala Pro Lys Leu Ala Arg Leu His Tyr	
1845 1850 1855	
tcc gga tca atc aat gcc tgg agc acc aag gat ccc cac tcc tgg atc	5616
Ser Gly Ser Ile Asn Ala Trp Ser Thr Lys Asp Pro His Ser Trp Ile	
1860 1865 1870	
aag gtg gat ctg ttg gca cca atg atc att cac ggc atc atg acc cag	5664
Lys Val Asp Leu Leu Ala Pro Met Ile Ile His Gly Ile Met Thr Gln	
1875 1880 1885	
ggt gcc cgt cag aag ttt tcc agc ctc tac atc tcc cag ttt atc atc	5712
Gly Ala Arg Gln Lys Phe Ser Ser Leu Tyr Ile Ser Gln Phe Ile Ile	
1890 1895 1900	
atg tac agt ctt gac ggg agg aac tgg cag agt tac cga ggg aat tcc	5760
Met Tyr Ser Leu Asp Gly Arg Asn Trp Gln Ser Tyr Arg Gly Asn Ser	
1905 1910 1915 1920	

WO 01/68109

PCT/US01/05076

acg ggc acc tta atg gtc ttc ttt ggc aat gtg gac gca tct ggg att	5808
Thr Gly Thr Leu Met Val Phe Phe Gly Asn Val Asp Ala Ser Gly Ile	
1925 1930 1935	
aaa cac aat att ttt aac cct ccg att gtg gct cgg tac atc cgt ttg	5856
Lys His Asn Ile Phe Asn Pro Pro Ile Val Ala Arg Tyr Ile Arg Leu	
1940 1945 1950	
cac cca aca cat tac agc atc cgc agc act ctt cgc atg gag ttg atg	5904
His Pro Thr His Tyr Ser Ile Arg Ser Thr Leu Arg Met Glu Leu Met	
1955 1960 1965	
ggc tgt gat tta aac agt tgc agc atg ccc ctg gga atg cag aat aaa	5952
Gly Cys Asp Leu Asn Ser Cys Ser Met Pro Leu Gly Met Gln Asn Lys	
1970 1975 1980	
gcg ata tca gac tca cag atc acg gcc tcc tcc cac cta agc aat ata	6000
Ala Ile Ser Asp Ser Gln Ile Thr Ala Ser Ser His Leu Ser Asn Ile	
1985 1990 1995 2000	
ttt gcc acc tgg tct cct tca caa gcc cga ctt cac ctc cag ggg cgg	6048
Phe Ala Thr Trp Ser Pro Ser Gln Ala Arg Leu His Leu Gln Gly Arg	
2005 2010 2015	
acg aat gcc tgg cga ccc cgg gtg agc agc gca gag gag tgg ctg cag	6096
Thr Asn Ala Trp Arg Pro Arg Val Ser Ser Ala Glu Glu Trp Leu Gln	
2020 2025 2030	
gtg gac ctg cag aag acg gtg aag gtc aca ggc atc acc acc cag ggc	6144
Val Asp Leu Gln Lys Thr Val Lys Val Thr Gly Ile Thr Thr Gln Gly	
2035 2040 2045	
gtg aag tcc ctg ctc agc agc atg tat gtg aag gag ttc ctc gtg tcc	6192
Val Lys Ser Leu Leu Ser Ser Met Tyr Val Lys Glu Phe Leu Val Ser	
2050 2055 2060	
agt agt cag gac ggc cgc cgc tgg acc ctg ttt ctt cag gac ggc cac	6240
Ser Ser Gln Asp Gly Arg Arg Trp Thr Leu Phe Leu Gln Asp Gly His	
2065 2070 2075 2080	
acg aag gtt ttt cag ggc aat cag gac tcc tcc acc ccc gtg gtg aac	6288
Thr Lys Val Phe Gln Gly Asn Gln Asp Ser Ser Thr Pro Val Val Asn	
2085 2090 2095	
gct ctg gac ccc ccg ctg ttc acg cgc tac ctg agg atc cac ccc acg	6336
Ala Leu Asp Pro Pro Leu Phe Thr Arg Tyr Leu Arg Ile His Pro Thr	
2100 2105 2110	
agc tgg gcg cag cac atc gcc ctg agg ctc gag gtt cta gga tgt gag	6384
Ser Trp Ala Gln His Ile Ala Leu Arg Leu Glu Val Leu Gly Cys Glu	
2115 2120 2125	
gca cag gat ctc tac tga	6402
Ala Gln Asp Leu Tyr	
2130	

WO 01/68109

PCT/US01/05076

<210> 30

<211> 2133

<212> PRT

<213> Porcine

<400> 30

```

Met Gln Leu Glu Leu Ser Thr Cys Val Phe Leu Cys Leu Leu Pro Leu
 1              5              10              15

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

Trp Asp Tyr Arg Gln Ser Glu Leu Leu Arg Glu Leu His Val Asp Thr
      35              40              45

Arg Phe Pro Ala Thr Ala Pro Gly Ala Leu Pro Leu Gly Pro Ser Val
      50              55              60

Leu Tyr Lys Lys Thr Val Phe Val Glu Phe Thr Asp Gln Leu Phe Ser
 65              70              75              80

Val Ala Arg Pro Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile
      85              90              95

Gln Ala Glu Val Tyr Asp Thr Val Val Val Thr Leu Lys Asn Met Ala
      100              105              110

Ser His Pro Val Ser Leu His Ala Val Gly Val Ser Phe Trp Lys Ser
      115              120              125

Ser Glu Gly Ala Glu Tyr Glu Asp His Thr Ser Gln Arg Glu Lys Glu
      130              135              140

Asp Asp Lys Val Leu Pro Gly Lys Ser Gln Thr Tyr Val Trp Gln Val
      145              150              155              160

Leu Lys Glu Asn Gly Pro Thr Ala Ser Asp Pro Pro Cys Leu Thr Tyr
      165              170              175

Ser Tyr Leu Ser His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu
      180              185              190

Ile Gly Ala Leu Leu Val Cys Arg Glu Gly Ser Leu Thr Arg Glu Arg
      195              200              205

Thr Gln Asn Leu His Glu Phe Val Leu Leu Phe Ala Val Phe Asp Glu
      210              215              220

Gly Lys Ser Trp His Ser Ala Arg Asn Asp Ser Trp Thr Arg Ala Met
      225              230              235              240

Asp Pro Ala Pro Ala Arg Ala Gln Pro Ala Met His Thr Val Asn Gly
      245              250              255

```

Tyr Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His Lys Lys Ser
 260 265 270
 Val Tyr Trp His Val Ile Gly Met Gly Thr Ser Pro Glu Val His Ser
 275 280 285
 Ile Phe Leu Glu Gly His Thr Phe Leu Val Arg His His Arg Gln Ala
 290 295 300
 Ser Leu Glu Ile Ser Pro Leu Thr Phe Leu Thr Ala Gln Thr Phe Leu
 305 310 315 320
 Met Asp Leu Gly Gln Phe Leu Leu Phe Cys His Ile Ser Ser His His
 325 330 335
 His Gly Gly Met Glu Ala His Val Arg Val Glu Ser Cys Ala Glu Glu
 340 345 350
 Pro Gln Leu Arg Arg Lys Ala Asp Glu Glu Glu Asp Tyr Asp Asp Asn
 355 360 365
 Leu Tyr Asp Ser Asp Met Asp Val Val Arg Leu Asp Gly Asp Asp Val
 370 375 380
 Ser Pro Phe Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr
 385 390 395 400
 Trp Val His Tyr Ile Ser Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro
 405 410 415
 Ala Val Pro Ser Pro Ser Asp Arg Ser Tyr Lys Ser Leu Tyr Leu Asn
 420 425 430
 Ser Gly Pro Gln Arg Ile Gly Arg Lys Tyr Lys Lys Ala Arg Phe Val
 435 440 445
 Ala Tyr Thr Asp Val Thr Phe Lys Thr Arg Lys Ala Ile Pro Tyr Glu
 450 455 460
 Ser Gly Ile Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu
 465 470 475 480
 Leu Ile Ile Phe Lys Asn Lys Ala Ser Arg Pro Tyr Asn Ile Tyr Pro
 485 490 495
 His Gly Ile Thr Asp Val Ser Ala Leu His Pro Gly Arg Leu Leu Lys
 500 505 510
 Gly Trp Lys His Leu Lys Asp Met Pro Ile Leu Pro Gly Glu Thr Phe
 515 520 525
 Lys Tyr Lys Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp
 530 535 540

WO 01/68109

PCT/US01/05076

Pro	Arg	Cys	Leu	Thr	Arg	Tyr	Tyr	Ser	Ser	Ser	Ile	Asn	Leu	Glu	Lys	545	550	555	560
Asp	Leu	Ala	Ser	Gly	Leu	Ile	Gly	Pro	Leu	Leu	Ile	Cys	Tyr	Lys	Glu	565	570	575	
Ser	Val	Asp	Gln	Arg	Gly	Asn	Gln	Met	Met	Ser	Asp	Lys	Arg	Asn	Val	580	585	590	
Ile	Leu	Phe	Ser	Val	Phe	Asp	Glu	Asn	Gln	Ser	Trp	Tyr	Leu	Ala	Glu	595	600	605	
Asn	Ile	Gln	Arg	Phe	Leu	Pro	Asn	Pro	Asp	Gly	Leu	Gln	Pro	Gln	Asp	610	615	620	
Pro	Glu	Phe	Gln	Ala	Ser	Asn	Ile	Met	His	Ser	Ile	Asn	Gly	Tyr	Val	625	630	635	640
Phe	Asp	Ser	Leu	Gln	Leu	Ser	Val	Cys	Leu	His	Glu	Val	Ala	Tyr	Trp	645	650	655	
Tyr	Ile	Leu	Ser	Val	Gly	Ala	Gln	Thr	Asp	Phe	Leu	Ser	Val	Phe	Phe	660	665	670	
Ser	Gly	Tyr	Thr	Phe	Lys	His	Lys	Met	Val	Tyr	Glu	Asp	Thr	Leu	Thr	675	680	685	
Leu	Phe	Pro	Phe	Ser	Gly	Glu	Thr	Val	Phe	Met	Ser	Met	Glu	Asn	Pro	690	695	700	
Gly	Leu	Trp	Val	Leu	Gly	Cys	His	Asn	Ser	Asp	Leu	Arg	Asn	Arg	Gly	705	710	715	720
Met	Thr	Ala	Leu	Leu	Lys	Val	Tyr	Ser	Cys	Asp	Arg	Asp	Ile	Gly	Asp	725	730	735	
Tyr	Tyr	Asp	Asn	Thr	Tyr	Glu	Asp	Ile	Pro	Gly	Phe	Leu	Leu	Ser	Gly	740	745	750	
Lys	Asn	Val	Ile	Glu	Pro	Arg	Ser	Phe	Ala	Gln	Asn	Ser	Arg	Pro	Pro	755	760	765	
Ser	Ala	Ser	Gln	Lys	Gln	Phe	Gln	Thr	Ile	Thr	Ser	Pro	Glu	Asp	Asp	770	775	780	
Val	Glu	Leu	Asp	Pro	Gln	Ser	Gly	Glu	Arg	Thr	Gln	Ala	Leu	Glu	Glu	785	790	795	800
Leu	Ser	Val	Pro	Ser	Gly	Asp	Gly	Ser	Met	Leu	Leu	Gly	Gln	Asn	Pro	805	810	815	
Ala	Pro	His	Gly	Ser	Ser	Ser	Ser	Asp	Leu	Gln	Glu	Ala	Arg	Asn	Glu	820	825	830	

WO 01/68109

PCT/US01/05076

Ala	Asp	Asp	Tyr	Leu	Pro	Gly	Ala	Arg	Glu	Arg	Gly	Thr	Ala	Pro	Ser	835	840	845	
Ala	Ala	Ala	Arg	Leu	Arg	Pro	Glu	Leu	His	His	Ser	Ala	Glu	Arg	Val	850	855	860	
Leu	Thr	Pro	Glu	Pro	Glu	Lys	Glu	Leu	Lys	Lys	Leu	Asp	Ser	Lys	Met	865	870	875	880
Ser	Ser	Ser	Ser	Asp	Leu	Leu	Lys	Thr	Ser	Pro	Thr	Ile	Pro	Ser	Asp	885	890	895	
Thr	Leu	Ser	Ala	Glu	Thr	Glu	Arg	Thr	His	Ser	Leu	Gly	Pro	Pro	His	900	905	910	
Pro	Gln	Val	Asn	Phe	Arg	Ser	Gln	Leu	Gly	Ala	Ile	Val	Leu	Gly	Lys	915	920	925	
Asn	Ser	Ser	His	Phe	Ile	Gly	Ala	Gly	Val	Pro	Leu	Gly	Ser	Thr	Glu	930	935	940	
Glu	Asp	His	Glu	Ser	Ser	Leu	Gly	Glu	Asn	Val	Ser	Pro	Val	Glu	Ser	945	950	955	960
Asp	Gly	Ile	Phe	Glu	Lys	Glu	Arg	Ala	His	Gly	Pro	Ala	Ser	Leu	Thr	965	970	975	
Lys	Asp	Asp	Val	Leu	Phe	Lys	Val	Asn	Ile	Ser	Leu	Val	Lys	Thr	Asn	980	985	990	
Lys	Ala	Arg	Val	Tyr	Leu	Lys	Thr	Asn	Arg	Lys	Ile	His	Ile	Asp	Asp	995	1000	1005	
Ala	Ala	Leu	Leu	Thr	Glu	Asn	Arg	Ala	Ser	Ala	Thr	Phe	Met	Asp	Lys	1010	1015	1020	
Asn	Thr	Thr	Ala	Ser	Gly	Leu	Asn	His	Val	Ser	Asn	Trp	Ile	Lys	Gly	1025	1030	1035	1040
Pro	Leu	Gly	Lys	Asn	Pro	Leu	Ser	Ser	Glu	Arg	Gly	Pro	Ser	Pro	Glu	1045	1050	1055	
Leu	Leu	Thr	Ser	Ser	Gly	Ser	Gly	Lys	Ser	Val	Lys	Gly	Gln	Ser	Ser	1060	1065	1070	
Gly	Gln	Gly	Arg	Ile	Arg	Val	Ala	Val	Glu	Glu	Glu	Glu	Leu	Ser	Lys	1075	1080	1085	
Gly	Lys	Glu	Met	Met	Leu	Pro	Asn	Ser	Glu	Leu	Thr	Phe	Leu	Thr	Asn	1090	1095	1100	
Ser	Ala	Asp	Val	Gln	Gly	Asn	Asp	Thr	His	Ser	Gln	Gly	Lys	Lys	Ser	1105	1110	1115	1120

Arg Glu Glu Met Glu Arg Arg Glu Lys Leu Val Gln Glu Lys Val Asp
 1125 1130 1135
 Leu Pro Gln Val Tyr Thr Ala Thr Gly Thr Lys Asn Phe Leu Arg Asn
 1140 1145 1150
 Ile Phe His Gln Ser Thr Glu Pro Ser Val Glu Gly Phe Asp Gly Gly
 1155 1160 1165
 Ser His Ala Pro Val Pro Gln Asp Ser Arg Ser Leu Asn Asp Ser Ala
 1170 1175 1180
 Glu Arg Ala Glu Thr His Ile Ala His Phe Ser Ala Ile Arg Glu Glu
 1185 1190 1195 1200
 Ala Pro Leu Glu Ala Pro Gly Asn Arg Thr Gly Pro Gly Pro Arg Ser
 1205 1210 1215
 Ala Val Pro Arg Arg Val Lys Gln Ser Leu Lys Gln Ile Arg Leu Pro
 1220 1225 1230
 Leu Glu Glu Ile Lys Pro Glu Arg Gly Val Val Leu Asn Ala Thr Ser
 1235 1240 1245
 Thr Arg Trp Ser Glu Ser Ser Pro Ile Leu Gln Gly Ala Lys Arg Asn
 1250 1255 1260
 Asn Leu Ser Leu Pro Phe Leu Thr Leu Glu Met Ala Gly Gly Gln Gly
 1265 1270 1275 1280
 Lys Ile Ser Ala Leu Gly Lys Ser Ala Ala Gly Pro Leu Ala Ser Gly
 1285 1290 1295
 Lys Leu Glu Lys Ala Val Leu Ser Ser Ala Gly Leu Ser Glu Ala Ser
 1300 1305 1310
 Gly Lys Ala Glu Phe Leu Pro Lys Val Arg Val His Arg Glu Asp Leu
 1315 1320 1325
 Leu Pro Gln Lys Thr Ser Asn Val Ser Cys Ala His Gly Asp Leu Gly
 1330 1335 1340
 Gln Glu Ile Phe Leu Gln Lys Thr Arg Gly Pro Val Asn Leu Asn Lys
 1345 1350 1355 1360
 Val Asn Arg Pro Gly Arg Thr Pro Ser Lys Leu Leu Gly Pro Pro Met
 1365 1370 1375
 Pro Lys Glu Trp Glu Ser Leu Glu Lys Ser Pro Lys Ser Thr Ala Leu
 1380 1385 1390
 Arg Thr Lys Asp Ile Ile Ser Leu Pro Leu Asp Arg His Glu Ser Asn
 1395 1400 1405

His Ser Ile Ala Ala Lys Asn Glu Gly Gln Ala Glu Thr Gln Arg Glu
 1410 1415 1420
 Ala Ala Trp Thr Lys Gln Gly Gly Pro Gly Arg Leu Cys Ala Pro Lys
 1425 1430 1435 1440
 Pro Pro Val Leu Arg Arg His Gln Arg Asp Ile Ser Leu Pro Thr Phe
 1445 1450 1455
 Gln Pro Glu Glu Asp Lys Met Asp Tyr Asp Asp Ile Phe Ser Thr Glu
 1460 1465 1470
 Thr Lys Gly Glu Asp Phe Asp Ile Tyr Gly Glu Asp Glu Asn Gln Asp
 1475 1480 1485
 Pro Arg Ser Phe Gln Lys Arg Thr Arg His Tyr Phe Ile Ala Ala Val
 1490 1495 1500
 Glu Gln Leu Trp Asp Tyr Gly Met Ser Glu Ser Pro Arg Ala Leu Arg
 1505 1510 1515 1520
 Asn Arg Ala Gln Asn Gly Glu Val Pro Arg Phe Lys Lys Val Val Phe
 1525 1530 1535
 Arg Glu Phe Ala Asp Gly Ser Phe Thr Gln Pro Ser Tyr Arg Gly Glu
 1540 1545 1550
 Leu Asn Lys His Leu Gly Leu Leu Gly Pro Tyr Ile Arg Ala Glu Val
 1555 1560 1565
 Glu Asp Asn Ile Met Val Thr Phe Lys Asn Gln Ala Ser Arg Pro Tyr
 1570 1575 1580
 Ser Phe Tyr Ser Ser Leu Ile Ser Tyr Pro Asp Asp Gln Glu Gln Gly
 1585 1590 1595 1600
 Ala Glu Pro Arg His Asn Phe Val Gln Pro Asn Glu Thr Arg Thr Tyr
 1605 1610 1615
 Phe Trp Lys Val Gln His His Met Ala Pro Thr Glu Asp Glu Phe Asp
 1620 1625 1630
 Cys Lys Ala Trp Ala Tyr Phe Ser Asp Val Asp Leu Glu Lys Asp Val
 1635 1640 1645
 His Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Arg Ala Asn Thr Leu
 1650 1655 1660
 Asn Ala Ala His Gly Arg Gln Val Thr Val Gln Glu Phe Ala Leu Phe
 1665 1670 1675 1680
 Phe Thr Ile Phe Asp Glu Thr Lys Ser Trp Tyr Phe Thr Glu Asn Val
 1685 1690 1695

WO 01/68109

PCT/US01/05076

Glu Arg Asn Cys Arg Ala Pro Cys His Leu Gln Met Glu Asp Pro Thr
 1700 1705 1710

Leu Lys Glu Asn Tyr Arg Phe His Ala Ile Asn Gly Tyr Val Met Asp
 1715 1720 1725

Thr Leu Pro Gly Leu Val Met Ala Gln Asn Gln Arg Ile Arg Trp Tyr
 1730 1735 1740

Leu Leu Ser Met Gly Ser Asn Glu Asn Ile His Ser Ile His Phe Ser
 1745 1750 1755 1760

Gly His Val Phe Ser Val Arg Lys Lys Glu Glu Tyr Lys Met Ala Val
 1765 1770 1775

Tyr Asn Leu Tyr Pro Gly Val Phe Glu Thr Val Glu Met Leu Pro Ser
 1780 1785 1790

Lys Val Gly Ile Trp Arg Ile Glu Cys Leu Ile Gly Glu His Leu Gln
 1795 1800 1805

Ala Gly Met Ser Thr Thr Phe Leu Val Tyr Ser Lys Glu Cys Gln Ala
 1810 1815 1820

Pro Leu Gly Met Ala Ser Gly Arg Ile Arg Asp Phe Gln Ile Thr Ala
 1825 1830 1835 1840

Ser Gly Gln Tyr Gly Gln Trp Ala Pro Lys Leu Ala Arg Leu His Tyr
 1845 1850 1855

Ser Gly Ser Ile Asn Ala Trp Ser Thr Lys Asp Pro His Ser Trp Ile
 1860 1865 1870

Lys Val Asp Leu Leu Ala Pro Met Ile Ile His Gly Ile Met Thr Gln
 1875 1880 1885

Gly Ala Arg Gln Lys Phe Ser Ser Leu Tyr Ile Ser Gln Phe Ile Ile
 1890 1895 1900

Met Tyr Ser Leu Asp Gly Arg Asn Trp Gln Ser Tyr Arg Gly Asn Ser
 1905 1910 1915 1920

Thr Gly Thr Leu Met Val Phe Phe Gly Asn Val Asp Ala Ser Gly Ile
 1925 1930 1935

Lys His Asn Ile Phe Asn Pro Pro Ile Val Ala Arg Tyr Ile Arg Leu
 1940 1945 1950

His Pro Thr His Tyr Ser Ile Arg Ser Thr Leu Arg Met Glu Leu Met
 1955 1960 1965

Gly Cys Asp Leu Asn Ser Cys Ser Met Pro Leu Gly Met Gln Asn Lys
 1970 1975 1980

WO 01/68109

PCT/US01/05076

Ala Ile Ser Asp Ser Gln Ile Thr Ala Ser Ser His Leu Ser Asn Ile
 1985 1990 1995 2000

Phe Ala Thr Trp Ser Pro Ser Gln Ala Arg Leu His Leu Gln Gly Arg
 2005 2010 2015

Thr Asn Ala Trp Arg Pro Arg Val Ser Ser Ala Glu Glu Trp Leu Gln
 2020 2025 2030

Val Asp Leu Gln Lys Thr Val Lys Val Thr Gly Ile Thr Thr Gln Gly
 2035 2040 2045

Val Lys Ser Leu Leu Ser Ser Met Tyr Val Lys Glu Phe Leu Val Ser
 2050 2055 2060

Ser Ser Gln Asp Gly Arg Arg Trp Thr Leu Phe Leu Gln Asp Gly His
 2065 2070 2075 2080

Thr Lys Val Phe Gln Gly Asn Gln Asp Ser Ser Thr Pro Val Val Asn
 2085 2090 2095

Ala Leu Asp Pro Pro Leu Phe Thr Arg Tyr Leu Arg Ile His Pro Thr
 2100 2105 2110

Ser Trp Ala Gln His Ile Ala Leu Arg Leu Glu Val Leu Gly Cys Glu
 2115 2120 2125

Ala Gln Asp Leu Tyr
 2130

<210> 31
 <211> 19
 <212> PRT
 <213> Homo sapiens

<400> 31
 Met Gln Ile Glu Leu Ser Thr Cys Phe Phe Leu Cys Leu Leu Arg Phe
 1 5 10 15

Cys Phe Ser

<210> 32
 <211> 24
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:linker

<400> 32
 Ser Phe Ala Gln Asn Ser Arg Pro Pro Ser Ala Ser Ala Pro Lys Pro
 1 5 10 15

WO 01/68109

PCT/US01/05076

Pro Val Leu Arg Arg His Gln Arg
20

<210> 33
<211> 105
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:linker

<400> 33
gtcattgaac ctaggagctt tgcccagaat tcaagacccc ctagtgcgag cgctccaaag 60

cctccggtcc tgcgacggca tcagagggac ataagccttc ctact 105

<210> 34
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide
primer

<400> 34
gaggaaaacc agatgatgtc a 21

<210> 35
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide
primer

<400> 35
ctttggagcg ctcgcactag ggggtcttga attctgggca aagctcctag gttcaatgac 60

<210> 36
<211> 66
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide
primer

<400> 36
cctagtgcga gcgctccaaa gcctccggtc ctgcgacggc atcagaggga cataagcctt 60

WO 01/68109

PCT/US01/05076

cctact

66

<210> 37

<211> 4404

<212> DNA

<213> Porcine

<220>

<221> CDS

<222> (1) .. (4401)

<400> 37

atg cag cta gag ctc tcc acc tgt gtc ttt ctg tgt ctc ttg cca ctc	48
Met Gln Leu Glu Leu Ser Thr Cys Val Phe Leu Cys Leu Leu Pro Leu	
1 5 10 15	
ggc ttt agt gcc atc agg aga tac tac ctg ggc gca gtg gaa ctg tcc	96
Gly Phe Ser Ala Ile Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser	
20 25 30	
tgg gac tac cgg caa agt gaa ctc ctc cgt gag ctg cac gtg gac acc	144
Trp Asp Tyr Arg Gln Ser Glu Leu Leu Arg Glu Leu His Val Asp Thr	
35 40 45	
aga ttt cct gct aca gcg cca gga gct ctt ccg ttg ggc ccg tca gtc	192
Arg Phe Pro Ala Thr Ala Pro Gly Ala Leu Pro Leu Gly Pro Ser Val	
50 55 60	
ctg tac aaa aag act gtg ttc gta gag ttc acg gat caa ctt ttc agc	240
Leu Tyr Lys Lys Thr Val Phe Val Glu Phe Thr Asp Gln Leu Phe Ser	
65 70 75 80	
gtt gcc agg ccc agg cca cca tgg atg ggt ctg ctg ggt cct acc atc	288
Val Ala Arg Pro Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile	
85 90 95	
cag gct gag gtt tac gac acg gtg gtc gtt acc ctg aag aac atg gct	336
Gln Ala Glu Val Tyr Asp Thr Val Val Val Thr Leu Lys Asn Met Ala	
100 105 110	
tct cat ccc gtt agt ctt cac gct gtc ggc gtc tcc ttc tgg aaa tct	384
Ser His Pro Val Ser Leu His Ala Val Gly Val Ser Phe Trp Lys Ser	
115 120 125	
tcc gaa ggc gct gaa tat gag gat cac acc agc caa agg gag aag gaa	432
Ser Glu Gly Ala Glu Tyr Glu Asp His Thr Ser Gln Arg Glu Lys Glu	
130 135 140	
gac gat aaa gtc ctt ccc ggt aaa agc caa acc tac gtc tgg cag gtc	480
Asp Asp Lys Val Leu Pro Gly Lys Ser Gln Thr Tyr Val Trp Gln Val	
145 150 155 160	
ctg aaa gaa aat ggt cca aca gcc tct gac cca cca tgt ctt acc tac	528
Leu Lys Glu Asn Gly Pro Thr Ala Ser Asp Pro Pro Cys Leu Thr Tyr	
165 170 175	

WO 01/68109

PCT/US01/05076

tca tac ctg tct cac gtg gac ctg gtg aaa gac ctg aat tcg ggc ctc	576
Ser Tyr Leu Ser His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu	
180 185 190	
att gga gcc ctg ctg gtt tgt aga gaa ggg agt ctg acc aga gaa agg	624
Ile Gly Ala Leu Leu Val Cys Arg Glu Gly Ser Leu Thr Arg Glu Arg	
195 200 205	
acc cag aac ctg cac gaa ttt gta cta ctt ttt gct gtc ttt gat gaa	672
Thr Gln Asn Leu His Glu Phe Val Leu Leu Phe Ala Val Phe Asp Glu	
210 215 220	
ggg aaa agt tgg cac tca gca aga aat gac tcc tgg aca cgg gcc atg	720
Gly Lys Ser Trp His Ser Ala Arg Asn Asp Ser Trp Thr Arg Ala Met	
225 230 235 240	
gat ccc gca cct gcc agg gcc cag cct gca atg cac aca gtc aat ggc	768
Asp Pro Ala Pro Ala Arg Ala Gln Pro Ala Met His Thr Val Asn Gly	
245 250 255	
tat gtc aac agg tct ctg cca ggt ctg atc gga tgt cat aag aaa tca	816
Tyr Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His Lys Lys Ser	
260 265 270	
gtc tac tgg cac gtg att gga atg ggc acc agc ccg gaa gtg cac tcc	864
Val Tyr Trp His Val Ile Gly Met Gly Thr Ser Pro Glu Val His Ser	
275 280 285	
att ttt ctt gaa ggc cac acg ttt ctc gtg agg cac cat cgc cag gct	912
Ile Phe Leu Glu Gly His Thr Phe Leu Val Arg His His Arg Gln Ala	
290 295 300	
tcc ttg gag atc tcg cca cta act ttc ctc act gct cag aca ttc ctg	960
Ser Leu Glu Ile Ser Pro Leu Thr Phe Leu Thr Ala Gln Thr Phe Leu	
305 310 315 320	
atg gac ctt ggc cag ttc cta ctg ttt tgt cat atc tct tcc cac cac	1008
Met Asp Leu Gly Gln Phe Leu Leu Phe Cys His Ile Ser Ser His His	
325 330 335	
cat ggt ggc atg gag gct cac gtc aga gta gaa agc tgc gcc gag gag	1056
His Gly Gly Met Glu Ala His Val Arg Val Glu Ser Cys Ala Glu Glu	
340 345 350	
ccc cag ctg cgg agg aaa gct gat gaa gag gaa gat tat gat gac aat	1104
Pro Gln Leu Arg Arg Lys Ala Asp Glu Glu Glu Asp Tyr Asp Asp Asn	
355 360 365	
ttg tac gac tcg gac atg gac gtg gtc cgg ctc gat ggt gac gac gtg	1152
Leu Tyr Asp Ser Asp Met Asp Val Val Arg Leu Asp Gly Asp Asp Val	
370 375 380	
tct ccc ttt atc caa atc cgc tcg gtt gcc aag aag cat ccc aaa acc	1200
Ser Pro Phe Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr	
385 390 395 400	

WO 01/68109

PCT/US01/05076

tgg	gtg	cac	tac	atc	tct	gca	gag	gag	gag	gac	tgg	gac	tac	gcc	ccc	1248
Trp	Val	His	Tyr	Ile	Ser	Ala	Glu	Glu	Glu	Asp	Trp	Asp	Tyr	Ala	Pro	
				405					410					415		
gcg	gtc	ccc	agc	ccc	agt	gac	aga	agt	tat	aaa	agt	ctc	tac	ttg	aac	1296
Ala	Val	Pro	Ser	Pro	Ser	Asp	Arg	Ser	Tyr	Lys	Ser	Leu	Tyr	Leu	Asn	
			420					425					430			
agt	ggt	cct	cag	cga	att	ggt	agg	aaa	tac	aaa	aaa	gct	cga	ttc	gtc	1344
Ser	Gly	Pro	Gln	Arg	Ile	Gly	Arg	Lys	Tyr	Lys	Lys	Ala	Arg	Phe	Val	
			435				440					445				
gct	tac	acg	gat	gta	aca	ttt	aag	act	cgt	aaa	gct	att	ccg	tat	gaa	1392
Ala	Tyr	Thr	Asp	Val	Thr	Phe	Lys	Thr	Arg	Lys	Ala	Ile	Pro	Tyr	Glu	
	450					455					460					
tca	gga	atc	ctg	gga	cct	tta	ctt	tat	gga	gaa	gtt	gga	gac	aca	ctt	1440
Ser	Gly	Ile	Leu	Gly	Pro	Leu	Leu	Tyr	Gly	Glu	Val	Gly	Asp	Thr	Leu	
465					470					475					480	
ttg	att	ata	ttt	aag	aat	aaa	gcg	agc	cga	cca	tat	aac	atc	tac	cct	1488
Leu	Ile	Ile	Phe	Lys	Asn	Lys	Ala	Ser	Arg	Pro	Tyr	Asn	Ile	Tyr	Pro	
				485					490					495		
cat	gga	atc	act	gat	gtc	agc	gct	ttg	cac	cca	ggg	aga	ctt	cta	aaa	1536
His	Gly	Ile	Thr	Asp	Val	Ser	Ala	Leu	His	Pro	Gly	Arg	Leu	Leu	Lys	
			500					505					510			
ggt	tgg	aaa	cat	ttg	aaa	gac	atg	cca	att	ctg	cca	gga	gag	act	ttc	1584
Gly	Trp	Lys	His	Leu	Lys	Asp	Met	Pro	Ile	Leu	Pro	Gly	Glu	Thr	Phe	
		515					520					525				
aag	tat	aaa	tgg	aca	gtg	act	gtg	gaa	gat	ggg	cca	acc	aag	tcc	gat	1632
Lys	Tyr	Lys	Trp	Thr	Val	Thr	Val	Glu	Asp	Gly	Pro	Thr	Lys	Ser	Asp	
	530					535					540					
cct	cgg	tgc	ctg	acc	cgc	tac	tac	tcg	agc	tcc	att	aat	cta	gag	aaa	1680
Pro	Arg	Cys	Leu	Thr	Arg	Tyr	Tyr	Ser	Ser	Ser	Ile	Asn	Leu	Glu	Lys	
545					550					555					560	
gat	ctg	gct	tcg	gga	ctc	att	ggc	cct	ctc	ctc	atc	tgc	tac	aaa	gaa	1728
Asp	Leu	Ala	Ser	Gly	Leu	Ile	Gly	Pro	Leu	Leu	Ile	Cys	Tyr	Lys	Glu	
				565				570						575		
tct	gta	gac	caa	aga	gga	aac	cag	atg	atg	tca	gac	aag	aga	aac	gtc	1776
Ser	Val	Asp	Gln	Arg	Gly	Asn	Gln	Met	Met	Ser	Asp	Lys	Arg	Asn	Val	
			580				585						590			
atc	ctg	ttt	tct	gta	ttc	gat	gag	aat	caa	agc	tgg	tac	ctc	gca	gag	1824
Ile	Leu	Phe	Ser	Val	Phe	Asp	Glu	Asn	Gln	Ser	Trp	Tyr	Leu	Ala	Glu	
		595					600					605				
aat	att	cag	cgc	ttc	ctc	ccc	aat	ccg	gat	gga	tta	cag	ccc	cag	gat	1872
Asn	Ile	Gln	Arg	Phe	Leu	Pro	Asn	Pro	Asp	Gly	Leu	Gln	Pro	Gln	Asp	
	610					615					620					

WO 01/68109

PCT/US01/05076

cca gag ttc caa gct tct aac atc atg cac agc atc aat ggc tat gtt	1920
Pro Glu Phe Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val	
625 630 635 640	
ttt gat agc ttg cag ctg tcg gtt tgt ttg cac gag gtg gca tac tgg	1968
Phe Asp Ser Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp	
645 650 655	
tac att cta agt gtt gga gca cag acg gac ttc ctc tcc gtc ttc ttc	2016
Tyr Ile Leu Ser Val Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe	
660 665 670	
tct ggc tac acc ttc aaa cac aaa atg gtc tat gaa gac aca ctc acc	2064
Ser Gly Tyr Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr	
675 680 685	
ctg ttc ccc ttc tca gga gaa acg gtc ttc atg tca atg gaa aac cca	2112
Leu Phe Pro Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro	
690 695 700	
ggc ctc tgg gtc ctt ggg tgc cac aac tca gac ttg cgg aac aga ggg	2160
Gly Leu Trp Val Leu Gly Cys His Asn Ser Asp Leu Arg Asn Arg Gly	
705 710 715 720	
atg aca gcc tta ctg aag gtg tat agt tgt gac agg gac att ggt gat	2208
Met Thr Ala Leu Leu Lys Val Tyr Ser Cys Asp Arg Asp Ile Gly Asp	
725 730 735	
tat tat gac aac act tat gaa gat att cca ggc ttc ttg ctg agt gga	2256
Tyr Tyr Asp Asn Thr Tyr Glu Asp Ile Pro Gly Phe Leu Leu Ser Gly	
740 745 750	
aag aat gtc att gaa cct agg agc ttt gcc cag aat tca aga ccc cct	2304
Lys Asn Val Ile Glu Pro Arg Ser Phe Ala Gln Asn Ser Arg Pro Pro	
755 760 765	
agt gcg agc gct cca aag cct ccg gtc ctg cga cgg cat cag agg gac	2352
Ser Ala Ser Ala Pro Lys Pro Pro Val Leu Arg Arg His Gln Arg Asp	
770 775 780	
ata agc ctt cct act ttt cag ccg gag gaa gac aaa atg gac tat gat	2400
Ile Ser Leu Pro Thr Phe Gln Pro Glu Glu Asp Lys Met Asp Tyr Asp	
785 790 795 800	
gat atc ttc tca act gaa acg aag gga gaa gat ttt gac att tac ggt	2448
Asp Ile Phe Ser Thr Glu Thr Lys Gly Glu Asp Phe Asp Ile Tyr Gly	
805 810 815	
gag gat gaa aat cag gac cct cgc agc ttt cag aag aga acc cga cac	2496
Glu Asp Glu Asn Gln Asp Pro Arg Ser Phe Gln Lys Arg Thr Arg His	
820 825 830	
tat ttc att gct gcg gtg gag cag ctc tgg gat tac ggg atg agc gaa	2544
Tyr Phe Ile Ala Ala Val Glu Gln Leu Trp Asp Tyr Gly Met Ser Glu	
835 840 845	

WO 01/68109

PCT/US01/05076

tcc ccc cgg gcg cta aga aac agg gct cag aac gga gag gtg cct cgg	2592
Ser Pro Arg Ala Leu Arg Asn Arg Ala Gln Asn Gly Glu Val Pro Arg	
850 855 860	
ttc aag aag gtg gtc ttc cgg gaa ttt gct gac ggc tcc ttc acg cag	2640
Phe Lys Lys Val Val Phe Arg Glu Phe Ala Asp Gly Ser Phe Thr Gln	
865 870 875 880	
ccg tcg tac cgc ggg gaa ctc aac aaa cac ttg ggg ctc ttg gga ccc	2688
Pro Ser Tyr Arg Gly Glu Leu Asn Lys His Leu Gly Leu Leu Gly Pro	
885 890 895	
tac atc aga gcg gaa gtt gaa gac aac atc atg gta act ttc aaa aac	2736
Tyr Ile Arg Ala Glu Val Glu Asp Asn Ile Met Val Thr Phe Lys Asn	
900 905 910	
cag gcg tct cgt ccc tat tcc ttc tac tcg agc ctt att tct tat ccg	2784
Gln Ala Ser Arg Pro Tyr Ser Phe Tyr Ser Ser Leu Ile Ser Tyr Pro	
915 920 925	
gat gat cag gag caa ggg gca gaa cct cga cac aac ttc gtc cag cca	2832
Asp Asp Gln Glu Gln Gly Ala Glu Pro Arg His Asn Phe Val Gln Pro	
930 935 940	
aat gaa acc aga act tac ttt tgg aaa gtg cag cat cac atg gca ccc	2880
Asn Glu Thr Arg Thr Tyr Phe Trp Lys Val Gln His His Met Ala Pro	
945 950 955 960	
aca gaa gac gag ttt gac tgc aaa gcc tgg gcc tac ttt tct gat gtt	2928
Thr Glu Asp Glu Phe Asp Cys Lys Ala Trp Ala Tyr Phe Ser Asp Val	
965 970 975	
gac ctg gaa aaa gat gtg cac tca ggc ttg atc ggc ccc ctt ctg atc	2976
Asp Leu Glu Lys Asp Val His Ser Gly Leu Ile Gly Pro Leu Leu Ile	
980 985 990	
tgc cgc gcc aac acc ctg aac gct gct cac ggt aga caa gtg acc gtg	3024
Cys Arg Ala Asn Thr Leu Asn Ala Ala His Gly Arg Gln Val Thr Val	
995 1000 1005	
caa gaa ttt gct ctg ttt ttc act att ttt gat gag aca aag agc tgg	3072
Gln Glu Phe Ala Leu Phe Phe Thr Ile Phe Asp Glu Thr Lys Ser Trp	
1010 1015 1020	
tac ttc act gaa aat gtg gaa agg aac tgc cgg gcc ccc tgc cat ctg	3120
Tyr Phe Thr Glu Asn Val Glu Arg Asn Cys Arg Ala Pro Cys His Leu	
1025 1030 1035 1040	
cag atg gag gac ccc act ctg aaa gaa aac tat cgc ttc cat gca atc	3168
Gln Met Glu Asp Pro Thr Leu Lys Glu Asn Tyr Arg Phe His Ala Ile	
1045 1050 1055	
aat ggc tat gtg atg gat aca ctc cct ggc tta gta atg gct cag aat	3216
Asn Gly Tyr Val Met Asp Thr Leu Pro Gly Leu Val Met Ala Gln Asn	
1060 1065 1070	

WO 01/68109

PCT/US01/05076

caa agg atc cga tgg tat ctg ctc agc atg ggc agc aat gaa aat atc	3264
Gln Arg Ile Arg Trp Tyr Leu Leu Ser Met Gly Ser Asn Glu Asn Ile	
1075 1080 1085	
cat tcg att cat ttt agc gga cac gtg ttc agt gta cgg aaa aag gag	3312
His Ser Ile His Phe Ser Gly His Val Phe Ser Val Arg Lys Lys Glu	
1090 1095 1100	
gag tat aaa atg gcc gtg tac aat ctc tat ccg ggt gtc ttt gag aca	3360
Glu Tyr Lys Met Ala Val Tyr Asn Leu Tyr Pro Gly Val Phe Glu Thr	
1105 1110 1115 1120	
gtg gaa atg cta ccg tcc aaa gtt gga att tgg cga ata gaa tgc ctg	3408
Val Glu Met Leu Pro Ser Lys Val Gly Ile Trp Arg Ile Glu Cys Leu	
1125 1130 1135	
att ggc gag cac ctg caa gct ggg atg agc acg act ttc ctg gtg tac	3456
Ile Gly Glu His Leu Gln Ala Gly Met Ser Thr Thr Phe Leu Val Tyr	
1140 1145 1150	
agc aag gag tgt cag gct cca ctg gga atg gct tct gga cgc att aga	3504
Ser Lys Glu Cys Gln Ala Pro Leu Gly Met Ala Ser Gly Arg Ile Arg	
1155 1160 1165	
gat ttt cag atc aca gct tca gga cag tat gga cag tgg gcc cca aag	3552
Asp Phe Gln Ile Thr Ala Ser Gly Gln Tyr Gly Gln Trp Ala Pro Lys	
1170 1175 1180	
ctg gcc aga ctt cat tat tcc gga tca atc aat gcc tgg agc acc aag	3600
Leu Ala Arg Leu His Tyr Ser Gly Ser Ile Asn Ala Trp Ser Thr Lys	
1185 1190 1195 1200	
gat ccc cac tcc tgg atc aag gtg gat ctg ttg gca cca atg atc att	3648
Asp Pro His Ser Trp Ile Lys Val Asp Leu Leu Ala Pro Met Ile Ile	
1205 1210 1215	
cac ggc atc atg acc cag ggt gcc cgt cag aag ttt tcc agc ctc tac	3696
His Gly Ile Met Thr Gln Gly Ala Arg Gln Lys Phe Ser Ser Leu Tyr	
1220 1225 1230	
atc tcc cag ttt atc atc atg tac agt ctt gac ggg agg aac tgg cag	3744
Ile Ser Gln Phe Ile Ile Met Tyr Ser Leu Asp Gly Arg Asn Trp Gln	
1235 1240 1245	
agt tac cga ggg aat tcc acg ggc acc tta atg gtc ttc ttt ggc aat	3792
Ser Tyr Arg Gly Asn Ser Thr Gly Thr Leu Met Val Phe Phe Gly Asn	
1250 1255 1260	
gtg gac gca tct ggg att aaa cac aat att ttt aac cct ccg att gtg	3840
Val Asp Ala Ser Gly Ile Lys His Asn Ile Phe Asn Pro Pro Ile Val	
1265 1270 1275 1280	
gct cgg tac atc cgt ttg cac cca aca cat tac agc atc cgc agc act	3888
Ala Arg Tyr Ile Arg Leu His Pro Thr His Tyr Ser Ile Arg Ser Thr	
1285 1290 1295	

WO 01/68109

PCT/US01/05076

ctt cgc atg gag ttg atg ggc tgt gat tta aac agt tgc agc atg ccc 3936
 Leu Arg Met Glu Leu Met Gly Cys Asp Leu Asn Ser Cys Ser Met Pro
 1300 1305 1310

ctg gga atg cag aat aaa gcg ata tca gac tca cag atc acg gcc tcc 3984
 Leu Gly Met Gln Asn Lys Ala Ile Ser Asp Ser Gln Ile Thr Ala Ser
 1315 1320 1325

tcc cac cta agc aat ata ttt gcc acc tgg tct cct tca caa gcc cga 4032
 Ser His Leu Ser Asn Ile Phe Ala Thr Trp Ser Pro Ser Gln Ala Arg
 1330 1335 1340

ctt cac ctc cag ggg cgg acg aat gcc tgg cga ccc cgg gtg agc agc 4080
 Leu His Leu Gln Gly Arg Thr Asn Ala Trp Arg Pro Arg Val Ser Ser
 1345 1350 1355 1360

gca gag gag tgg ctg cag gtg gac ctg cag aag acg gtg aag gtc aca 4128
 Ala Glu Glu Trp Leu Gln Val Asp Leu Gln Lys Thr Val Lys Val Thr
 1365 1370 1375

ggc atc acc acc cag ggc gtg aag tcc ctg ctc agc agc atg tat gtg 4176
 Gly Ile Thr Thr Gln Gly Val Lys Ser Leu Leu Ser Ser Met Tyr Val
 1380 1385 1390

aag gag ttc ctc gtg tcc agt agt cag gac ggc cgc cgc tgg acc ctg 4224
 Lys Glu Phe Leu Val Ser Ser Ser Gln Asp Gly Arg Arg Trp Thr Leu
 1395 1400 1405

ttt ctt cag gac ggc cac acg aag gtt ttt cag ggc aat cag gac tcc 4272
 Phe Leu Gln Asp Gly His Thr Lys Val Phe Gln Gly Asn Gln Asp Ser
 1410 1415 1420

tcc acc ccc gtg gtg aac gct ctg gac ccc ccg ctg ttc acg cgc tac 4320
 Ser Thr Pro Val Val Asn Ala Leu Asp Pro Pro Leu Phe Thr Arg Tyr
 1425 1430 1435 1440

ctg agg atc cac ccc acg agc tgg gcg cag cac atc gcc ctg agg ctc 4368
 Leu Arg Ile His Pro Thr Ser Trp Ala Gln His Ile Ala Leu Arg Leu
 1445 1450 1455

gag gtt cta gga tgt gag gca cag gat ctc tac tga 4404
 Glu Val Leu Gly Cys Glu Ala Gln Asp Leu Tyr
 1460 1465

<210> 38
 <211> 1467
 <212> PRT
 <213> Porcine

<400> 38
 Met Gln Leu Glu Leu Ser Thr Cys Val Phe Leu Cys Leu Leu Pro Leu
 1 5 10 15
 Gly Phe Ser Ala Ile Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser
 20 25 30

Trp	Asp	Tyr	Arg	Gln	Ser	Glu	Leu	Leu	Arg	Glu	Leu	His	Val	Asp	Thr			
		35					40					45						
Arg	Phe	Pro	Ala	Thr	Ala	Pro	Gly	Ala	Leu	Pro	Leu	Gly	Pro	Ser	Val			
	50					55					60							
Leu	Tyr	Lys	Lys	Thr	Val	Phe	Val	Glu	Phe	Thr	Asp	Gln	Leu	Phe	Ser			
65					70					75					80			
Val	Ala	Arg	Pro	Arg	Pro	Pro	Trp	Met	Gly	Leu	Leu	Gly	Pro	Thr	Ile			
				85					90					95				
Gln	Ala	Glu	Val	Tyr	Asp	Thr	Val	Val	Val	Thr	Leu	Lys	Asn	Met	Ala			
			100					105					110					
Ser	His	Pro	Val	Ser	Leu	His	Ala	Val	Gly	Val	Ser	Phe	Trp	Lys	Ser			
		115					120					125						
Ser	Glu	Gly	Ala	Glu	Tyr	Glu	Asp	His	Thr	Ser	Gln	Arg	Glu	Lys	Glu			
	130					135					140							
Asp	Asp	Lys	Val	Leu	Pro	Gly	Lys	Ser	Gln	Thr	Tyr	Val	Trp	Gln	Val			
145					150					155					160			
Leu	Lys	Glu	Asn	Gly	Pro	Thr	Ala	Ser	Asp	Pro	Pro	Cys	Leu	Thr	Tyr			
				165					170					175				
Ser	Tyr	Leu	Ser	His	Val	Asp	Leu	Val	Lys	Asp	Leu	Asn	Ser	Gly	Leu			
			180					185					190					
Ile	Gly	Ala	Leu	Leu	Val	Cys	Arg	Glu	Gly	Ser	Leu	Thr	Arg	Glu	Arg			
		195					200					205						
Thr	Gln	Asn	Leu	His	Glu	Phe	Val	Leu	Leu	Phe	Ala	Val	Phe	Asp	Glu			
	210					215					220							
Gly	Lys	Ser	Trp	His	Ser	Ala	Arg	Asn	Asp	Ser	Trp	Thr	Arg	Ala	Met			
225					230					235					240			
Asp	Pro	Ala	Pro	Ala	Arg	Ala	Gln	Pro	Ala	Met	His	Thr	Val	Asn	Gly			
				245					250					255				
Tyr	Val	Asn	Arg	Ser	Leu	Pro	Gly	Leu	Ile	Gly	Cys	His	Lys	Lys	Ser			
			260					265					270					
Val	Tyr	Trp	His	Val	Ile	Gly	Met	Gly	Thr	Ser	Pro	Glu	Val	His	Ser			
		275					280					285						
Ile	Phe	Leu	Glu	Gly	His	Thr	Phe	Leu	Val	Arg	His	His	Arg	Gln	Ala			
	290					295					300							
Ser	Leu	Glu	Ile	Ser	Pro	Leu	Thr	Phe	Leu	Thr	Ala	Gln	Thr	Phe	Leu			
305					310					315					320			

WO 01/68109

PCT/US01/05076

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

WO 01/68109

PCT/US01/05076

Asn	Ile	Gln	Arg	Phe	Leu	Pro	Asn	Pro	Asp	Gly	Leu	Gln	Pro	Gln	Asp	610	615	620
Pro	Glu	Phe	Gln	Ala	Ser	Asn	Ile	Met	His	Ser	Ile	Asn	Gly	Tyr	Val	625	630	635
Phe	Asp	Ser	Leu	Gln	Leu	Ser	Val	Cys	Leu	His	Glu	Val	Ala	Tyr	Trp	645	650	655
Tyr	Ile	Leu	Ser	Val	Gly	Ala	Gln	Thr	Asp	Phe	Leu	Ser	Val	Phe	Phe	660	665	670
Ser	Gly	Tyr	Thr	Phe	Lys	His	Lys	Met	Val	Tyr	Glu	Asp	Thr	Leu	Thr	675	680	685
Leu	Phe	Pro	Phe	Ser	Gly	Glu	Thr	Val	Phe	Met	Ser	Met	Glu	Asn	Pro	690	695	700
Gly	Leu	Trp	Val	Leu	Gly	Cys	His	Asn	Ser	Asp	Leu	Arg	Asn	Arg	Gly	705	710	715
Met	Thr	Ala	Leu	Leu	Lys	Val	Tyr	Ser	Cys	Asp	Arg	Asp	Ile	Gly	Asp	725	730	735
Tyr	Tyr	Asp	Asn	Thr	Tyr	Glu	Asp	Ile	Pro	Gly	Phe	Leu	Leu	Ser	Gly	740	745	750
Lys	Asn	Val	Ile	Glu	Pro	Arg	Ser	Phe	Ala	Gln	Asn	Ser	Arg	Pro	Pro	755	760	765
Ser	Ala	Ser	Ala	Pro	Lys	Pro	Pro	Val	Leu	Arg	Arg	His	Gln	Arg	Asp	770	775	780
Ile	Ser	Leu	Pro	Thr	Phe	Gln	Pro	Glu	Glu	Asp	Lys	Met	Asp	Tyr	Asp	785	790	795
Asp	Ile	Phe	Ser	Thr	Glu	Thr	Lys	Gly	Glu	Asp	Phe	Asp	Ile	Tyr	Gly	805	810	815
Glu	Asp	Glu	Asn	Gln	Asp	Pro	Arg	Ser	Phe	Gln	Lys	Arg	Thr	Arg	His	820	825	830
Tyr	Phe	Ile	Ala	Ala	Val	Glu	Gln	Leu	Trp	Asp	Tyr	Gly	Met	Ser	Glu	835	840	845
Ser	Pro	Arg	Ala	Leu	Arg	Asn	Arg	Ala	Gln	Asn	Gly	Glu	Val	Pro	Arg	850	855	860
Phe	Lys	Lys	Val	Val	Phe	Arg	Glu	Phe	Ala	Asp	Gly	Ser	Phe	Thr	Gln	865	870	875
Pro	Ser	Tyr	Arg	Gly	Glu	Leu	Asn	Lys	His	Leu	Gly	Leu	Leu	Gly	Pro	885	890	895

Tyr Ile Arg Ala Glu Val Glu Asp Asn Ile Met Val Thr Phe Lys Asn
 900 905 910
 Gln Ala Ser Arg Pro Tyr Ser Phe Tyr Ser Ser Leu Ile Ser Tyr Pro
 915 920 925
 Asp Asp Gln Glu Gln Gly Ala Glu Pro Arg His Asn Phe Val Gln Pro
 930 935 940
 Asn Glu Thr Arg Thr Tyr Phe Trp Lys Val Gln His His Met Ala Pro
 945 950 955 960
 Thr Glu Asp Glu Phe Asp Cys Lys Ala Trp Ala Tyr Phe Ser Asp Val
 965 970 975
 Asp Leu Glu Lys Asp Val His Ser Gly Leu Ile Gly Pro Leu Leu Ile
 980 985 990
 Cys Arg Ala Asn Thr Leu Asn Ala Ala His Gly Arg Gln Val Thr Val
 995 1000 1005
 Gln Glu Phe Ala Leu Phe Phe Thr Ile Phe Asp Glu Thr Lys Ser Trp
 1010 1015 1020
 Tyr Phe Thr Glu Asn Val Glu Arg Asn Cys Arg Ala Pro Cys His Leu
 1025 1030 1035 1040
 Gln Met Glu Asp Pro Thr Leu Lys Glu Asn Tyr Arg Phe His Ala Ile
 1045 1050 1055
 Asn Gly Tyr Val Met Asp Thr Leu Pro Gly Leu Val Met Ala Gln Asn
 1060 1065 1070
 Gln Arg Ile Arg Trp Tyr Leu Leu Ser Met Gly Ser Asn Glu Asn Ile
 1075 1080 1085
 His Ser Ile His Phe Ser Gly His Val Phe Ser Val Arg Lys Lys Glu
 1090 1095 1100
 Glu Tyr Lys Met Ala Val Tyr Asn Leu Tyr Pro Gly Val Phe Glu Thr
 1105 1110 1115 1120
 Val Glu Met Leu Pro Ser Lys Val Gly Ile Trp Arg Ile Glu Cys Leu
 1125 1130 1135
 Ile Gly Glu His Leu Gln Ala Gly Met Ser Thr Thr Phe Leu Val Tyr
 1140 1145 1150
 Ser Lys Glu Cys Gln Ala Pro Leu Gly Met Ala Ser Gly Arg Ile Arg
 1155 1160 1165
 Asp Phe Gln Ile Thr Ala Ser Gly Gln Tyr Gly Gln Trp Ala Pro Lys
 1170 1175 1180

WO 01/68109

PCT/US01/05076

Leu Ala Arg Leu His Tyr Ser Gly Ser Ile Asn Ala Trp Ser Thr Lys
 1185 1190 1195 1200
 Asp Pro His Ser Trp Ile Lys Val Asp Leu Leu Ala Pro Met Ile Ile
 1205 1210 1215
 His Gly Ile Met Thr Gln Gly Ala Arg Gln Lys Phe Ser Ser Leu Tyr
 1220 1225 1230
 Ile Ser Gln Phe Ile Ile Met Tyr Ser Leu Asp Gly Arg Asn Trp Gln
 1235 1240 1245
 Ser Tyr Arg Gly Asn Ser Thr Gly Thr Leu Met Val Phe Phe Gly Asn
 1250 1255 1260
 Val Asp Ala Ser Gly Ile Lys His Asn Ile Phe Asn Pro Pro Ile Val
 1265 1270 1275 1280
 Ala Arg Tyr Ile Arg Leu His Pro Thr His Tyr Ser Ile Arg Ser Thr
 1285 1290 1295
 Leu Arg Met Glu Leu Met Gly Cys Asp Leu Asn Ser Cys Ser Met Pro
 1300 1305 1310
 Leu Gly Met Gln Asn Lys Ala Ile Ser Asp Ser Gln Ile Thr Ala Ser
 1315 1320 1325
 Ser His Leu Ser Asn Ile Phe Ala Thr Trp Ser Pro Ser Gln Ala Arg
 1330 1335 1340
 Leu His Leu Gln Gly Arg Thr Asn Ala Trp Arg Pro Arg Val Ser Ser
 1345 1350 1355 1360
 Ala Glu Glu Trp Leu Gln Val Asp Leu Gln Lys Thr Val Lys Val Thr
 1365 1370 1375
 Gly Ile Thr Thr Gln Gly Val Lys Ser Leu Leu Ser Ser Met Tyr Val
 1380 1385 1390
 Lys Glu Phe Leu Val Ser Ser Ser Gln Asp Gly Arg Arg Trp Thr Leu
 1395 1400 1405
 Phe Leu Gln Asp Gly His Thr Lys Val Phe Gln Gly Asn Gln Asp Ser
 1410 1415 1420
 Ser Thr Pro Val Val Asn Ala Leu Asp Pro Pro Leu Phe Thr Arg Tyr
 1425 1430 1435 1440
 Leu Arg Ile His Pro Thr Ser Trp Ala Gln His Ile Ala Leu Arg Leu
 1445 1450 1455
 Glu Val Leu Gly Cys Glu Ala Gln Asp Leu Tyr
 1460 1465

WHAT IS CLAIMED IS:

1. DNA encoding the amino acid sequence of POL1212 as set forth in SEQ ID NO:39
2. An expression vector comprising a DNA according to claim 1.
3. DNA according to claim 1 having the nucleotide sequence of SEQ ID NO:38.
4. An expression vector comprising a DNA according to claim 3.
5. A modified porcine factor VIII having the amino acid sequence of SEQ ID NO:39.
6. A therapeutic composition comprising a modified porcine factor VIII according to claim 5 and a physiologically acceptable carrier.
7. A method for producing a modified porcine factor VIII protein having the amino acid sequence of SEQ ID NO:39 comprising
expressing in a mammalian host cell a DNA encoding the amino acid sequence of SEQ ID NO:39.
8. The method of claim 7 wherein the DNA encoding the amino acid sequence of SEQ ID NO:39 also encodes a signal peptide, whereby the modified porcine factor VIII protein is exported from the mammalian host cell.
9. The method of claim 8 wherein the signal peptide has the sequence of amino acids 1-19 of SEQ ID NO:30.
10. A mammalian cell containing and replicating an expression vector comprising DNA encoding the amino acid sequence of POL1212 as set forth in SEQ ID NO:39.
11. A mammalian cell according to claim 10 wherein the vector comprising DNA has the nucleotide sequence of SEQ ID NO:38.
12. A cell according to claim 11 wherein the host cell is BHK CRL-1632.

1/6

Signal peptide

Human	-19	MQIELSTCFF	LCLLRFCFS
Pig		MQLELSTCVF	LCLLPLGFS
Mouse		MQIALFACFF	LSLFNFCSS
		** * * *	* * *

FIG. 1A

A1 domain

Human	1	ATTRYYLGAV	ELSWDYMQSD	LG-ELPVDAR	FPPRVPKSFP	FNTSVVYKKT
Pig		AIRRYYLGA	ELSWDYRQSE	LLRELHVDTR	FPATAPGALP	LGPSVLYKKT
Mouse		AIRRYYLGA	ELSWNYIQSD	LLSVLHTDSR	FLPRMSTSFP	FNTSIMYKKT
		*****	*****	* * * *	* * *	* * *

FIG. 1B

50	LFVEFTDHLF	NIAKPRPPWM	GLLGPTIQAE	VYDTVVITLK	NMASHPVSLH
	VFVEFTDQLF	SVARPRPPWM	GLLGPTIQAE	VYDTVVVTLK	NMASHPVSLH
	VFVEYKDQLF	NIAKPRPPWM	GLLGPTIWTE	VHDTVVITLK	NMASHPVSLH
	***	* **	* *****	* *****	* *****

100	AVGVSYWKAS	EGA EYDDQTS	QREKEDDKVF	PGGSHTYVWQ	VLKENGPMAS
	AVGVSFWKSS	EGA EYEDHTS	QREKEDDKVL	PGKSQTYVWQ	VLKENGPTAS
	AVGVSYWKAS	EGDEYEDQTS	QMEKEDDKVF	PGESHTYVWQ	VLKENGPMAS
	*****	** *	* *****	** * *****	***** **

150	DPLCLTYSYL	SHVDLVKDLN	SGLIGALLVC	REGSLAKEKT	QTLHKFILLF
	DPPCLTYSYL	SHVDLVKDLN	SGLIGALLVC	REGSLTRERT	QNLHEFVLLF
	DPPCLTYSYM	SHVDLVKDLN	SGLIGALLVC	KEGSLSKERT	QMLYQFVLLF
	*****	*****	*****	*****	* * * *

200	AVFDEGKSWH	SETKNSLMQD	RDAASARAWP	KMHTVNGYVN	RSLPGLIGCH
	AVFDEGKSWH	SARNDSWTRA	MDPAPARAQP	AMHTVNGYVN	RSLPGLIGCH
	AVFDEGKSWH	SETNDSYTQS	MDSASARDWP	KMHTVNGYVN	RSLPGLIGCH
	*****	*	* * *	*****	*****

250	RKSVYWHVIG	MGTTPEVHSI	FLEGHTFLVR	NHRQASLEIS	PITFLTAQTL
	KKSVYWHVIG	MGTSPEVHSI	FLEGHTFLVR	HHRQASLEIS	PLTFLTAQTF
	RKSVYWHVIG	MGTTPEIHSI	FLEGHTFFVR	NHRQASLEIS	PITFLTAQTL
	*****	*** **	*****	*****	*****

APC/IXa

300	LMDLGQFLLF	CHISSHQHDG	MEAYVKVDSC	PEEPQLRMKN	NEEAEDYDDD
	LMDLGQFLLF	CHISSHHHGG	MEAHVRVESC	AEEPQLRRKA	DE-EEDYDDN
	LIDLQFLLF	CHISSHKHDG	MEAYVKVDSC	PEESQWQKN	NN-EEMEDYD
	* *****	*****	* * *	** *	* *

IIa/Xa

350	LTDSEMDVVR	FDDDNPSFI	QIR
	LYDSDMDVVR	LDGDDVSPFI	QIR
	DDLSEMDMF	TLDYDSSPFI	QIR
		**	***

2/6

A2 domain

Human 373 SVAKKHPKTW VHYIAAEEED WDYAPLV LAP DDRSYKSQYL NNGPQRIGRK
 Pig SVAKKHPKTW VHYISAEED WDYAPAVPSP SDRSYKSLYL NSGPQRIGRK
 Mouse SVAKKYPKTW IHYISAEED WDYAPSVPTS DNGSYKSQYL SNGPHRIGRK
 ***** **

FIG. 1C

423 YKKVRFMAYT DETFKTREAI QHESGILGPL LYGEVGD TLL IIFKNQASRP
 YKKARFVAYT DVTFKTRKAI PYESGILGPL LYGEVGD TLL IIFKNKASRP
 YKKVRFIAYT DETFKTRET I QHESGILLGPL LYGEVGD TLL IIFKNQASRP
 *** **

A2 Inhibitor epitope

473 YNIYPHGITD VRPLYSRRLP KGVKHLKDFP ILPGEIFKYK WTVTVEDGPT
 YNIYPHGITD VSALHPGRLL KGWKHLKDMP ILPGETFKYK WTVTVEDGPT
 YNIYPHGITD VSPLHARRLP RGIKHKVDLP IHPGEIFKYK WTVTVEDGPT
 ***** *

F.IXa binding

APC

523 KSDPRCLTRY YSSFVNMERD LASGLIGPLL ICYKESVDQR GNQIMSDKRN
 KSDPRCLTRY YSSSINLEKD LASGLIGPLL ICYKESVDQR GNQMMSDKRN
 KSDPRCLTRY YSSFINPERD LASGLIGPLL ICYKESVDQR GNQMMSDKRN
 ***** **

573 VILFSVFDEN RSWYLTENIQ RFLPNPAGVQ LEDPEFQASN IMHSINGYVF
 VILFSVFDEN QSWYLAENIQ RFLPNPDGLQ PQDPEFQASN IMHSINGYVF
 VILFSIFDEN QSWYITENMQ RFLPNAKTQ PQDPGFQASN IMHSINGYVF
 ***** **

623 DSLQLSVCLH EVAYWYILSI GAQTDFLSVF FSGYTFFHKM VYEDTLTLFP
 DSLQLSVCLH EVAYWYILSV GAQTDFLSVF FSGYTFFHKM VYEDTLTLFP
 DSLELTVCLH EVAYWHILSV GAQTDFLSIF FSGYTFFHKM VYEDTLTLFP
 *** * *****

673 FSGETVFMSM ENPGLWILGC HNSDFRNRGM TALLKVSSCD KNTGDYYEDS
 FSGETVFMSM ENPGLWVLGC HNSDLRNRGM TALLKVYSCD RDIGDYDNT
 FSGETVFMSM ENPGLWVLGC HNSDFRKRGM TALLKVSSCD KSTSDYYEEI
 ***** *

IIa/Xa/APC

723 YEDISAYLLS KNAIEPR
 YEDIPGFLS GKNVIEPR
 YEDIPTQLVN ENNVIDPR
 ***** *

3/6

B domain

Human 741 SFSQNSRHPS TRQKQFNATT IPENDIEKTD PWFHRTMPMP KIQNVSSSDL
 Pig SFAQNSRPPS ASQKQFQTIT SPEDDVE-LD PQSGERTQAL EELSVPSGDG
 Mouse SFFQNTNHPN TRKKKFKDST IPKNDMEKIE PQFEEIAEML KVQSVSVSDM
 ** ** * * * * * *

FIG. 1D

791 LMLLRQS-PT PHGLSLSDLQ EAKYETFSDD PSPGAIDSNN SLSEMTHFRP
 SMLLGQN-PA PHGSSSSDLQ EARNEA--DD YLPGARERNT APSAAARLRP
 LMLLGQSHPT PHGLFLSDGQ EAIYEAIHDD HSPNAIDSNE GPSKVTQLRP
 *** * *** * * * * *

840 QLHHSGDMVF TPESGLQLRL NEKLGTTAAT ELKKLDFKVS ST-SNNLIS-
 ELHHSARVL TPEP----- -----EK ELKKLDSKMS SSSDLLKTSP
 ESHHSEKIVF TPQGLQLRS NKSLETTIEV KWKKLGLQVS SLPSNLMTT-
 *** * ** *** * *

888 TIPSDNLAAGT DNTSSLGPPS MPVHYDSQLD TTLFGKKSSP LTESGGPLSL
 TIPSDTLAET ERTHSLGPPH PQVNFRSQLG AIVLGKNSSH FIGAGVPLGS
 TILSDNLKATF EKTDSSGFDP MPVHSSSKLS TTAFGKKAYS LVGSHVPLNA
 ** ** * * * * * *

939 SEENNSKLL ESGLMNSQES SWGKNVSSTE SGRLFKGKRA HGPALLTKDN
 TEED----- -----HES SLGENVSPVE SDGIFEKERA HGPASLTKDD
 SEENSOSNIL DSTLMYSQES LPRDNILSIE NDRLLREKRF HGIALLTKN
 ** ** * * *

989 ALFKVISILL KTNKTSNNSA TNRKTHIDGP SLLIENSPSV WQNILESDE
 VLFKVNISLV KTNKARVYLK TNRKIHIDDA ALLTENRAS- -----
 TLFKDNVSLM KTNKTYNHST TNEKLHTESE TSIENSTTDL QDAILKVNSE
 *** ** **** ** *

1039 FKKVTPLIHD RMLMDKNATA LRLNHMSNKT TSSKNMEMVQ QKKEGPIPPD
 ----- ATFMKNNTA SGLNHVSN-- -----
 IQEVTALIHD GTLLGKNSTY LRLNHMLNRT TSTKNKDIFH RKDEDPIPD
 * *** ** *** *

1089 AQNPDMSEFFK MLFLPESARW IQRTHGKNSL NSGQGPSKQ LVSLGPEKSV
 -----W IKGPLGKNPL SSERGPSPEL LTSSGSGKSV
 EENTIMPFSK MLFLSESSNW FKKTNGNNSL NSEQEHSPKQ LVYLMFKKYV
 * * * * *

1139 EGQNFLSEKN KVVVGKGEFT KDVGLKEMVF PSSRNFLFTN LDNLHENNTH
 KGQSSGQGRI RVAVEEEELS KG---KEMML PNELTFTN SADVQGNTH
 KNQSFLSEKN KVTVEQDGF KNIGLKDMAF PHNMSIFLTT LSNVHENGHR
 * * * * *

1189 NQEKKIQEEI EKKETLIQEN VVLPQIHTVT GTKNFMKNLF LLSTRQNVG
 SQGKKSREEM ERREKLVEK VDLPPQVYAT GTKNFLRNIF HQSTEPSVEG
 NQEKNIQEEI EK-EALIEEK VVLPQVHEAT GSKNFKDIL ILGTRQNI--
 * * : * * * * * *

1239 SYDGAYAPVL QDFRSLNDST NRTKKHTAHF SK--KGEEEN LEGLGNQTKQ
 FDGGSHAPVP QDSRSLNDSA ERAETHIAHF SAIR--EEAP LEAPGNRT..
 SLYEVHVPVL QNITSINNST NTVQIHMEHF FKRRKDKETN SEGLVNKTRE
 ** * * * * *

4/6

1287 IVEKYACTTR ISPNTSQNF VTQRSKRALK QFRLPLEETE LEKRIIVDDT
 ----- ---GPGRSA VPRRVKQSLK QIRLPLEEIK PERGVVLNAT
 MVKNYP---- -----SQKNI TTQRSKRALG QFRL-----

1337 STQWSKNMKH LTPSTLTQID YNEKEKGAIT QSPLSDCLTR SHSIPQANRS
 STRWS-----
 STQWLKTINC STQCIKQID HSKEMKKFIT KSSLSDS-SV IKSTTQTNSS
 ** *

1387 PLPIAKVSSF PSIRPIYLTR VLFQDNSSHL PAASY----R KKDSGVQESS

 DSHIVKTSAF P---PIDLKR SPFQNKFSHV QASSYIYDFK TKSSRIQESN
 **

1433 HFLQGAKKNN LSLAILTLEM TGDQREVGSL GTSATNSVTY KKVENTVLPK
 PILQGAKRNN LSLPFLTLEM AGGQGKISAL GKSAAGPLAS GKLEKAVLSS
 NFLKETKINN PSLAILPWNM FIDQGKFTSP GKSNTNSVTY KKRENIIFLK
 * * ** * * *

1483 PDLPKTSGKV ELLPKVHIYQ KDLFPTETSN GSPGHLDLVE GSLLQGTEGA
 AGLSEASGKA EFLPKVRVHR EDLLPQKTSN VSCAHGDLGQ EIFLQKTRGP
 PTLPEESGKI ELLPQVSIQE EEILPTTETSH GSPGHLNLMK EVFLQKIQGP
 *** * * * * *

1533 IKWNEANRPG KVPFLRVATE SSAKTPSKLL DPLAWDNHYG TQIPKEEWKS
 VNLNKVNRPG ----- ---RTPSKLL -----G PPMPKE-WES
 TKWNKAKRHG ESIKGKTES- -SKNTRSKLL NHHAWDYHYA AQIPKDMWKS
 * * * * * *

1583 QEKSPEKTAF KKKDTI-LSLN ACESNHAIAA INEGQNKPEI EVTWAKQGRT
 LEKSPKSTAL RTKDIISLPLD RHESNHSIAA KNEGQAETQR EAAWTKQGGP
 KEKSPEIISI KQEDTI-LSLR PHGNSHSIGA -NEKQNWQQR ETTWVKQGQT
 ***** * * ** * *

1633 ERLGSONPPY LKRHRQ
 GRLCAPKPPV LRRHRQ
 QRTCSQIPPV LKRHRQ
 * * * * *

Light chain activation peptide

Human 1649 EITRTTLQSDQEEIDYDDTISVEMKKEDFDIYDEDENQSPR
 Pig DISLPTFQPEEDKMDYDDIFSTETKGEDFDIYGEDENQDPR
 Mouse EL--SAFQSEQEATDYDDAITIET-IEDFDIYSEDIKQGPR
 * * * * * *

I Ia/Xa

FIG. 1E

5/6

A3 domain

IXa Xa

Human 1690	SFQKKTRHYF	IAAVERLWDY	GMSSSPHVLR	NRAQSGSVPQ	FKKVVFQFT
Pig	SFQKRTRHYF	IAAVEQLWDY	GMSESPRALR	NRAQNGEVPR	FKKVVFREFA
Mouse	SVQKKTRHYF	IAAVERLWDY	GMSTS-HVLR	NRYQSDNVPQ	FKKVVFQFT
	* *	*****	*****	*****	*****

FIG. 1F

1740	DGSFTQPLYR	GELNEHLGLL	GPYIRAEVED	NIMVTFRNQA	SRPYSFYSSL
	DGSFTQPSYR	GELNKHLGLL	GPYIRAEVED	NIMVTFRNQA	SRPYSFYSSL
	DGSFSQPLYR	GELNEHLGLL	GPYIRAEVED	NIMVTFRNQA	SRPYSFYSSL
	****	** *	*****	*****	*****

Factor IXa binding

1790	ISYEEDQRQG	AEPRKNFVKP	NETKTYFWKV	QHHMAPTKDE	FDCKAWAYFS
	ISYPDDQEQG	AEPRHNFVQP	NETRTYFWKV	QHHMAPTEDE	FDCKAWAYFS
	ISYKEDQR-G	EEPRRNFVKP	NETKIYFWKV	QHHMAPTEDE	FDCKAWAYFS
	***	** *	***	*****	*****

1840	DVDLEKDVHS	GLIGPLLVCH	TNTLNPAHGR	QVTVQEFALF	FTIFDETKSW
	DVDLEKDVHS	GLIGPLLICR	ANTLNAAHGR	QVTVQEFALF	FTIFDETKSW
	DVDLERDMHS	GLIGPLlich	ANTLNPAHGR	QVSVQEFALL	FTIFDETKSW
	*****	* **	*****	** *****	*****

1890	YFTENMERNc	RAPcNIQMED	PTFKENYRFH	AINGYIMDTL	PGLVMAQDQR
	YFTENVERNc	RAPCHLQMED	PTLKENYRFH	AINGYVMDTL	PGLVMAQNQR
	YFTENVKRNC	KTPCNFQMED	PTLKENYRFH	AINGYVMDTL	PGLVMAQDQR
	*****	***	** *****	*****	*****

1940	IRWYLLSMGS	NENIHSIHFS	GHVFTVRKKE	EYKMALYNLY	PGVFETVEML
	IRWYLLSMGS	NENIHSIHFS	GHVFSVRKKE	EYKMAVYNLY	PGVFETVEML
	IRWYLLSMGN	NENIQSIHFS	GHVFTVRKKE	EYKMAVYNLY	PGVFETLEMI
	*****	****	*****	*****	*****

Protein C binding

1990	PSKAGIWRVE	CLIGEHLHAG	MSTLFLVYSN
	PSKVGIWRIE	CLIGEHLQAG	MSTTFLVYSK
	PSRAGIWRVE	CLIGEHLQAG	MSTLFLVYSK
	**	*****	*****

6/6

C1 domain

Human 2020 KCQTPLGMAS GHIRDFQITA SGQYGQWAPK LARLHYSGLI NAWSTKEPFS
 Pig ECQAPLGMAS GRIRDFQITA SGQYGQWAPK LARLHYSGLI NAWSTKDPHS
 Mouse QCQIPLGMAS GSIRDFQITA SGHYGQWAPN LARLHYSGLI NAWSTKEPFS
 ** * * * * *

FIG. 1G

2070 WIKVDLLAPM IIHGKKTQGA RQKFSSLYIS QFIIMYSLDG KKWQTYRGNS
 WIKVDLLAPM IIHGIMTQGA RQKFSSLYIS QFIIMYSLDG RNWQSYRGNS
 WIKVDLLAPM IVHGKKTQGA RQKFSSLYIS QFIIMYSLDG KKWLSYQGNS
 * * * * *

2120 TGTLMVFFGN VDSSGIKHNI FNPPIIARYI RLHPHTHSIR STLRMELMGCDLN
 TGTLMVFFGN VDASGIKHNI FNPPIVARYI RLHPHTHSIR STLRMELMGCDLN
 TGTLMVFFGN VDSSGIKHNS FNPPIIARYI RLHPHTSSIR STLRMELMGCDLN
 * * * * *

C2 domain

inhibitor epitope

Human 2173 SCSMPLGMES KAISDAQITA SSYFTNMFAT WSPSKARLHL QGRSNAWRPQ
 Pig SCSMPLGMQN KAISDSQITA SSHLSNIFAT WSPSQARLHL QGRTNAWRPR
 Mouse SCSIPLGMES KVISDTQITA SSYFTNMFAT WSPSQARLHL QGRTNAWRPQ
 * * * * *

FIG. 1H

C2

2223 VNNPKEWLQV DFQKTMKVTG VTTQGVKSLL TSMYVKEFLI SSSQDGHQWT
 VSSAEEWLQV DLQKTVKVTG ITTQGVKSLL SSMYVKEFLV SSSQDGRRW
 VNDPKQWLQV DLQKTMKVTG IITQGVKSLL TSMFYVKEFLI SSSQDGHHT
 * * * * *

Phospholipid

2273 LFFQNGKVKV FQGNQDSFTP VVNSLDPPLL TRYLRHPQS WVHQIALRME
 LFLQDGHTKV FQGNQDSSTP VVNALDPPLF TRYLRHPQS WAHQIALRLE
 QILYNGKVKV FQGNQDSSTP MMNSLDPPLL TRYLRHPQI WEHQIALRLE
 * * * * *

binding

2323 VLGCEAQQDY
 VLGCEAQQDY
 ILGCEAQQDY
 * * * * *