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 μg/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 NH_2 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 NH_2-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. Yakhyæv, 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 NH_2-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 stearoyl phosphatidyl ethanolamine, stearoyl 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 $CaCl_2$ 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 $CaCl_2$, 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 $\log_{10}$ clotting time plotted against $\log_{10}$ 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 Q™ (Pharmacia-LKB, Piscataway, NJ) anion-exchange column (Pharmacia, Inc.). The purposes of the Mono Q™ 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 H_20. 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 Q™ HR 5/5 column equilibrated in 0.15 M NaCl, 0.02 M Hepes, 5mM $CaCl_2$, 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 Q™ HPLC) and porcine factor VIII, immunoaffinity-purified, plasma-derived porcine factor VIII was

diluted 1:4 with 0.04 M Hepes, 5 mM $CaCl_2$, 0.01% Tween-80, at pH 7.4, and subjected to Mono Q™ 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 A_{280} 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 Q™ 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 Q™ HPLC-purified human factor VIII was activated with thrombin and subjected to Mono S™

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 μg/ml (0.2 μM) in O.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 S™ 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/A_{280}. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the Mono S™ 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 ^{125}I-labeling.

In contrast to the results with human factor VIII, porcine factor VIIIa isolated by Mono S™ HPLC under the same conditions had a specific activity 1.6×10^6 U/A_{280}. 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 S™ 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 S™ buffers to pH 5. Mono Q™-purified human factor VIII (0.5 mg) was diluted with H_2O to give a final composition of 0.25 mg/ml (1 μm) factor VIII in 0.25 M NaCl, 0.01 M Hepes, 2.5 mM $CaCl_2$, 0.005% Tween-80, at pH 7.4 (total volume 7.0 ml). Thrombin was added to a final concentration of 0.072 μm and allowed to react for 3 min. Thrombin was then inactivated with FPR-CH_2Cl (0.2 μm). The mixture then was diluted 1:1 with 40 mM sodium acetate, 5 mM $CaCl_2$, 0.01% Tween-80, at pH 5.0, and loaded at 2 ml/min onto a Mono S™ HR 5/5 HPLC column equilibrated in 0.01 M sodium acetate, 5 mM $CaCl_2$, 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/A_{280} v. 7,500 U/A_{280}). 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 S™. 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. γ^{32}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 $MgCl_2$, 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 $MgCl_2$, 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 ^{32}P-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'-H_2N-CCCGTCCA-PO_4-5'

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 $MgCl_2$, 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 ^{32}P-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 μM unilamellar phosphatidylserine-[phosphatidycholine (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 $CaCl_2$, 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 $CaCl_2$, 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:

SFAQNSRPPSASAPKPPVLRRHQR. (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 µg/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 phosphtidylcholine/phosphatidylserine (PCPS) vesicles in 0.15M NaCl, 20 m 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-μl sample of the reaction mix was transferred to a microtiter well, to which was added 74 μl 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 ($x 10^3$)
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-state factor VIII clotting assay. In this assay, 50 ml of factor VIII deficient plasma (George King Biomedical Overland Park, KA), 5 ml sample or standard, and 50 ml 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 ml of 20 mM CaCl2. The clotting time was measured using an ST4 BIO Coagulation Instrument (Diagnostica 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:

	Dilution	U/ml	Clot Time
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:

	Sample	Clot Time (sec)	Units/ml
1)	Colony 8 (24h), 1/10 in HBS	40.6	1.74 x 10 = 17.4
2)	Colony 8 (24h), 1/10 in HBS	41.1	1.63 x 10 = 16.3
3)	Colony 8 (24h), 1/20 in HBS	47.7	0.69 x 20 = 13.8
4)	Colony 8 (24h), 1/20 in HBS	47.2	0.73 x 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:

SEQ ID NO:	Identification
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	Oligonucleotiode primer (Example 7)
37	POL1212 coding DNA
38	POL1212 amino acid sequence

WHAT IS CLAIMED IS:

1. DNA encoding the amino acid sequence of POL1212 as set forth in SEQ ID NO:38.

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:37.

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:38.

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:38 comprising

expressing in a mammalian host cell a DNA encoding the amino acid sequence of SEQ ID NO:38.

8. The method of claim 7 wherein the DNA encoding the amino acid sequence of SEQ ID NO:38 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:38.

11. A mammalian cell according to claim 10 wherein the vector comprising DNA has the nucleotide sequence of SEQ ID NO:37.

12. A cell according to claim 11 wherein the host cell is BHK CRL-1632.

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
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<151> 1996-06-26

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1 5

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30 35 40

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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
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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
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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
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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
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Val Asn Gly Tyr Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His
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agg aaa tca gtc tat tgg cat gtg att gga atg ggc acc act cct gaa   1002
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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
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cgc cag gcg tcc ttg gaa atc tcg cca ata act ttc ctt act gct caa   1098
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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
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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
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cat cct aaa act tgg gta cat tac att gct gct gaa gag gag gac tgg   1386
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        380                 385                 390

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Asp Tyr Ala Pro Leu Val Leu Ala Pro Asp Asp Arg Ser Tyr Lys Ser
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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
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gga gaa ata ttc aaa tat aaa tgg aca gtg act gta gaa gat ggg cca   1770
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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
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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
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Val Ala Tyr Trp Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp Phe Leu
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Ser Val Phe Phe Ser Gly Tyr Thr Phe Lys His Lys Met Val Tyr Glu
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Asp Thr Leu Thr Leu Phe Pro Phe Ser Gly Glu Thr Val Phe Met Ser
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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
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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
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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
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Pro Ser Asp Asn Leu Ala Ala Gly Thr Asp Asn Thr Ser Ser Leu Gly
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Gly Arg Leu Phe Lys Gly Lys Arg Ala His Gly Pro Ala Leu Leu Thr
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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
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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
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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
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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
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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
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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 tgagggtggc cactgcagca cctgccactg ccgtcacctc 7243
Asp Leu Tyr
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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

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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
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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
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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
785 790 795 800

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
865 870 875 880

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
945 950 955 960

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
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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
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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
1105 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
1185 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
1265 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
1345 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
1425 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
1505 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
1585 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
1665 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
1745 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
1825 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

Ile Gln Met Glu Asp Pro Thr Phe Lys Glu Asn Tyr Arg Phe His Ala
1905 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
1985 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
2065 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
2145 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

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
2225 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
2305 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
taagcacect aagacgtggg tgcactacat ctctgcagag gaggaggact gggactacgc 60

ccccgcggtc cccagcccca gtgacagaag ttataaaagt ctctacttga acagtggtcc 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 agactttctaa 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 gctgtcggtt tgtttgcacg aggtggcata 780

ctggtacatt ctaagtgttg gagcacagac ggacttcctc tccgtcttct tctctggcta 840

caccttcaaa cacaaaatgg tctatgaaga cacactcacc ctgttccgct tctcaggaga 900

aacggtcttc atgtcaatgg aaaacccagg tctctgggtc ctagggtgcc acaactcaga 960

cttgcggaac agagggatga cagccttact gaaggtgtat agttgtgaca gggacattgg 1020

tgattattat gacaacactt atgaagatat tccaggcttc ttgctgagtg gaaagaatgt 1080

cattgaaccc agaagctttg cccagaattc aagacccgct 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

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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
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<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
ccatcctaat acgactcact 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
cgcgcggccg cgcatctggc 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 tgkgcctcrc 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
ggatccaccc 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

<400> 27
ccgtggtgaa cgctctggac c 21

<210> 28
<211> 2319
<212> PRT
<213> Mus musculus

<400> 28

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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
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```
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
```

```
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 Phe Ile Asn Pro Glu Arg
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 Ile Phe Asp Glu Asn Gln Ser Trp Tyr Ile Thr Glu
        595                 600                 605

Asn Met Gln Arg Phe Leu Pro Asn Ala Ala Lys Thr Gln Pro Gln Asp
    610                 615                 620

Pro Gly Phe Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val
625                 630                 635                 640

Phe Asp Ser Leu Glu Leu Thr Val Cys Leu His Glu Val Ala Tyr Trp
                645                 650                 655

His Ile Leu Ser Val Gly Ala Gln Thr Asp Phe Leu Ser Ile 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 Phe Arg Lys Arg Gly
705                 710                 715                 720

Met Thr Ala Leu Leu Lys Val Ser Ser Cys Asp Lys Ser Thr Ser Asp
                725                 730                 735

Tyr Tyr Glu Glu Ile Tyr Glu Asp Ile Pro Thr Gln Leu Val Asn Glu
            740                 745                 750

Asn Asn Val Ile Asp Pro Arg Ser Phe Phe Gln Asn Thr Asn His Pro
        755                 760                 765

Asn Thr Arg Lys Lys Lys Phe Lys Asp Ser Thr Ile Pro Lys Asn Asp
    770                 775                 780

Met Glu Lys Ile Glu Pro Gln Phe Glu Glu Ile Ala Glu Met Leu Lys
785                 790                 795                 800

Val Gln Ser Val Ser Val Ser Asp Met Leu Met Leu Leu Gly Gln Ser
                805                 810                 815
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```
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
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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
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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
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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
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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 1995 2000

Phe Leu Val Tyr Ser Lys Gln Cys Gln Ile Pro Leu Gly Met Ala Ser
2005 2010 2015

Gly Ser Ile Arg Asp Phe Gln Ile Thr Ala Ser Gly His Tyr Gly Gln
2020 2025 2030

Trp Ala Pro Asn Leu Ala Arg Leu His Tyr Ser Gly Ser Ile Asn Ala
2035 2040 2045

Trp Ser Thr Lys Glu Pro Phe Ser Trp Ile Lys Val Asp Leu Leu Ala
2050 2055 2060

Pro Met Ile Val His Gly Ile Lys Thr Gln Gly Ala Arg Gln Lys Phe
2065 2070 2075 2080

Ser Ser Leu Tyr Ile Ser Gln Phe Ile Ile Met Tyr Ser Leu Asp Gly
2085 2090 2095

Lys Lys Trp Leu Ser Tyr Gln Gly Asn Ser Thr Gly Thr Leu Met Val
2100 2105 2110

Phe Phe Gly Asn Val Asp Ser Ser Gly Ile Lys His Asn Ser Phe Asn
2115 2120 2125

Pro Pro Ile Ile Ala Arg Tyr Ile Arg Leu His Pro Thr His Ser Ser
2130 2135 2140

Ile Arg Ser Thr Leu Arg Met Glu Leu Met Gly Cys Asp Leu Asn Ser
2145 2150 2155 2160

Cys Ser Ile Pro Leu Gly Met Glu Ser Lys Val Ile Ser Asp Thr Gln
2165 2170 2175

Ile Thr Ala Ser Ser Tyr Phe Thr Asn Met Phe Ala Thr Trp Ser Pro
2180 2185 2190

Ser Gln Ala Arg Leu His Leu Gln Gly Arg Thr Asn Ala Trp Arg Pro
2195 2200 2205

Gln Val Asn Asp Pro Lys Gln Trp Leu Gln Val Asp Leu Gln Lys Thr
2210 2215 2220

Met Lys Val Thr Gly Ile Ile Thr Gln Gly Val Lys Ser Leu Phe Thr
2225 2230 2235 2240

Ser Met Phe Val Lys Glu Phe Leu Ile Ser Ser Ser Gln Asp Gly His
2245 2250 2255

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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


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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
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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
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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

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
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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

ggt 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
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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
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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 ggg 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
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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
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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
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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
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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
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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

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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
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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
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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
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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

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

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
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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

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Cys Phe Ser

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Pro Val Leu Arg Arg His Gln Arg
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cctccggtcc tgcgacggca tcagagggac ataagccttc ctact 105

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  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
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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
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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
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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

ggt 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
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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
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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
```

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

<400> 38

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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
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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
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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
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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 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                 800

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                 880

Pro Ser Tyr Arg Gly Glu Leu Asn Lys His Leu Gly Leu Leu Gly Pro
                885                 890                 895
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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
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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
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