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(54) Title: PRODUCTION OF RECOMBINANT BLOOD CLOTTING FACTORS IN HUMAN CELL LINES

(57) Abstract: The present invention relates to an improved method for the production of recombinant human blood clotting factors, in particular of factor VIII and factor IX, utilizing an immortalized human cell line stably expressing viral transcription activator proteins and carrying a vector having a promoter functionally linked to a DNA sequence coding for a blood coagulating factor, provided that said promoter is not a viral promoter which is stimulated by said viral transcription activator proteins; an immortalized human cell line carrying said vector; factor VIII muteins particularly suitable for the above production method; pharmaceutical compositions comprising such factor VIII muteins and the use of such factor VIII muteins for preparing a medicament for treating hemophilia.

Production of Recombinant Blood Clotting Factors in Human Cell Lines

Introduction

The present invention relates to an improved method for the production of recombinant human blood clotting factors, in particular of factor VIII and factor IX, utilizing an immortalized human cell line stably expressing viral transcription activator proteins and carrying a vector having a promoter functionally linked to a DNA sequence coding for a blood coagulating factor, provided that said promoter is not a viral promoter which is stimulated by said viral transcription activator proteins; an immortalized human cell line carrying said vector; factor VIII muteins particularly suitable for the above production method; pharmaceutical compositions comprising such factor VIII muteins and the use of such factor VIII muteins for preparing a medicament for treating hemophilia.

Summary of the Related Art

Hemophiliacs are suffering from hemorrhagic morbidity caused by the disturbed function of protein components of the blood coagulation cascade. Dependent on the affected clotting factor two types of hemophilia can be distinguished. Both have in common the inhibited conversion of soluble brinogen to an insoluble fibrin-clot. They are recessive X-chromosomally-linked genetic diseases affecting mainly the male population.

Hemophilia A affects 1-2 individuals per 10.000 males. It is caused by the deficiency or absence of factor VIII, a very large glycoprotein (Mr approximately 330 kDa (Furie B., Furie B.C., *Cell* (1988) 53, 505-518)), which represents an important element of the blood coagulation cascade. The polypeptide sequence can be subdivided in three regions, an N-

terminal region consisting of the so-called A1 and A2-domains, a central B-domain region and a C-terminal region composed of the A3, C1 and C2 domains. In the blood coagulation factor VIII occurs as an inactive precursor. It is bound tightly and non-covalently to von Willebrand Factor (vWF), which acts as a stabilizing carrier protein. Proteolytical cleavage of factor VIII by thrombin at three specific positions (740, 372, 1689) leads to its dissociation from vWF and releases the procoagulant function within the cascade. In its active form factor VIII functions as a cofactor for factor IXa, thereby accelerating the proteolytic activation of factor X by several orders of magnitude.

Hemophilia B occurs in about 1 of 25,000 males. It is characterized by the deficiency of the serine protease factor IX (Christmas factor). This 415 amino-acid polypeptide is synthesized in the liver as a 56kDa glycoprotein. In order to attain its proper function a posttranslational carboxylation step is required which only occurs in the presence of vitamin K.

Treatment of both types of bleeding disorder traditionally involves infusions of human plasma-derived protein concentrates of factor VIII or factor IX. Although this method represents an efficient therapy for hemophiliacs it carries the risk of transmission of various infectious agents, such as viruses causing hepatitis or AIDS, or thromboembolic factors. Alternatively several recombinant DNA techniques for the production of clotting factors have been described. For this purpose the corresponding cDNAs of wild type factor VIII and factor IX have been isolated and cloned into suitable expression vectors (EP-A-160457; WO-A-86/01961, U.S. Patents 4,770,999, 5,521,070 and 5,521,070).

In the case of factor VIII recombinant expression of subunits for the production of complexes showing coagulant activity is known in the art (e.g., from EP-A-150735, EP-A-232112, EP-A-0500734, WO-91/07490, WO-95/13300 U.S. Patents 5,045,455 and 5,789,203). Moreover, the expression of truncated cDNA-versions partially or entirely lacking the sequence

coding for the highly glycosylated B-domain have been described (e.g. in WO-86/06101, WO-87/04187, WO-87/07144, WO-88/00381, EP-A-251843, EP-A-253455, EP-A-254076, U.S. Patents 4,868,112 and 4,980,456, EP-A-294910, EP-A-265778, EP-A-303540 and WO-91/09122). More recently a variety of selected point mutations have been introduced to inhibit proteolytic inactivation of factor VIII by activated protein C or to reduce the immunogenicity resulting in the formation of inhibitory antibodies by the treated patients (e.g., U.S. Patents 5,859,204, 5,422,260 and 5,451,521, WO-97/49725 and WO-99/29848).

The recombinant clotting factors were usually isolated from the medium of stably transfected eukaryotic and preferably mammalian cell lines. It was, however, general practice to employ non-human cell lines in the production methods disclosed in the references mentioned herein before in order to exclude the risk of copurifying some infectious agents which may be harbored and expressed by human cells.

However, especially for factor VIII, the use of non-human cell lines encountered certain disadvantages. For example unsatisfactory secretion levels of the expressed protein into the medium has been reported. This may be due to slight differences within different types of mammalian cells concerning intracellular pathways for protein translation and modification, which also might have an effect on the biological activity of the expressed polypeptide. Apart from this, there were concerns that the therapeutic proteins purified from non-human expression systems are contaminated with cellular components which can give rise to antigenic reactions in the patients.

Moreover, proteins expressed by non-human expression systems may have non-human glycosylation patterns giving rise to antigenic reactions in the patient. However, biological stability and efficacy of clotting factors is substantially influenced by their N-glycosylation pattern. Especially

peripheral and terminal monosaccharides are important, because they are detected by specific receptors from cells which are responsible for their degradation. Clotting factors carry as terminal monosaccharides sialic acid residues. Modification in the composition of sialic acids in the antennae of glycoproteins as for example the clotting factors can result in heterogenous glycosylation patterns. Thus, biological stability and efficacy is crucially involved when modification occurs. Hence, it is an important consideration in the production of recombinant clotting factors to evaluate the influence of glycosylation from non-human production cell lines versus human cell lines. Generally spoken, it seems plausible that human cell lines are more qualified for the production of recombinant clotting factors than non-human. The reason for this assumption is that probably no extraneous oligosaccharide will be incorporated into the oligosaccharide moiety during synthesis of recombinant factors.

On the other hand, general methods for high level protein expression of a desired gene comprising immortalized, stably transfected mammalian cell lines expressing viral transcription activator proteins have been made available for some time (e.g. U.S. Patent 5,712,119). Further these cell lines are transformed with a vector construct where a suitable viral transcription promoter is operatively associated with a DNA sequence defining a gene of interest, the transcription activator proteins activate the viral transcription promoter and hence initiate the expression of the gene of interest. Again, there were concerns that the transcription activator proteins expressed by these cell lines may give rise to contaminations in the target therapeutic protein.

In view of the above there was still a need for an effective production method for human blood clotting factors.

Surprisingly, it was found that a non-contaminated blood clotting factor can be obtained with the above mentioned imortalized human cell lines. In

particular, the immortalized cell lines - if carrying a vector having a promoter functionally linked to a DNA sequence coding for the blood clotting factor and despite the fact that the promoter is not a viral promoter which is stimulated by said viral transcription activator proteins - are capable of expressing the blood clotting factor. In combination with suitable protein purification and virus-inactivation protocols this method provides an effective system to produce safe and highly active recombinant blood clotting factors for therapeutic applications in humans. Moreover, particular factor VIII muteins were found which are exceptionally stable against proteolytic inactivation and thus allow to be subjected to vigorous virus inactivation protocols.

Summary of the Invention

The present invention provides

(1) a method for the production of recombinant human blood clotting factor which comprises

(a) culturing an immortalized human cell line stably expressing at least one viral transcription activator protein and carrying a vector having a promoter functionally linked to a DNA sequence coding for the human blood clotting factor, provided that said promoter is not a viral promoter which is stimulated by said at least one viral transcription activator protein, and

(b) isolating the blood clotting factor from the culture broth;

(2) a preferred embodiment of the method defined in (1) above, wherein the human blood clotting factor is factor VIII or a mutein thereof;

(3) a preferred embodiment of the method defined in (2) above, wherein the factor VIII is a mutein having at least one of the following mutations:

(a) Val at position 162 has been replaced by another neutral amino acid residue,

(b) Ser at position 2011 has been replaced by another hydrophilic amino acid residue,

(c) Val at position 2223 has been replaced by an acidic amino acid residue, and

(d) the B-domain between positions Arg740 and Glu1649 has been replaced by an Arg-rich linker peptide comprising 10 to 25, preferably 14 to 20 amino acid residues, wherein said factor VIII numbering is relative to the mature wild-type factor VIII sequence shown in SEQ ID NO: 2;

(4) a preferred embodiment of the method defined in (1) above, wherein the human blood clotting factor is factor IX or a mutein thereof;

(5) an immortalized human cell line carrying a vector coding for a human blood clotting factor as defined in (1) to (4) above;

(6) a factor VIII mutein as defined in (3) above;

(7) a DNA sequence coding for the factor VIII mutein as defined in (6) above;

(8) a vector comprising the DNA as defined in (7) above;

(9) a vector as defined in (8) above which is a gene transfer vector;

(10) a host cell being transformed with a vector as defined in (8) above and/or comprising a DNA sequence as defined in (7) above ;

(11) a pharmaceutical composition comprising the factor VIII mutein as defined in (6) above or a gene transfer vector as defined in (9) above;

(12) the use of the factor VIII mutein as defined in (6) above or a gene transfer vector as defined in (9) above for preparing a medicament for treating hemophilia; and

(13) a method for treating hemophilia which comprises administering human hemophiliacs a factor VIII mutein as defined in (6) above or a gene transfer vector as defined in (9) above.

Description of the figures

Fig. 1 shows the fragments utilized for the construction of factor VIII with a deleted B-domain (Example 1).

Fig. 2 shows the vector pTGF8-1, 8720 bps circular DNA, the exact DNA sequence thereof is given in SEQ ID NO: 3 (for the factor VIII protein encoded by said DNA sequence see SEQ ID NO: 4).

Fig. 3 shows vector pTGFG36, 5753 bps circular DNA, the exact DNA sequence thereof is given in SEQ ID NO: 6 (bases 689-2071 within SEQ ID NO: 6 coding for the factor IX protein).

Fig. 4 shows vector pTG36hyg, 8124 bps circular DNA.

Fig. 5A depicts a preferred linker sequence of the present invention (SEQ ID NO: 9).

Fig. 5B shows the coagulation time of recombinant hFVIII as determined in Example 6.

Fig. 6 shows the common molecular structure of pTGF8-2hyg-s and pTGF8-3, 10698 bps circular DNA, the exact DNA sequences thereof are given in SEQ ID NOs: 12 and 14 (for the factor VIII protein encoded by said DNA sequence see SEQ ID NO: 13 and 15).

Fig. 7 A shows the calibration curve of FVIII ELISA as described in Example 5.

Fig. 7 B depicts the results of the determination of recombinant FVIII concentrations in different culture filtrates as described in Example 5.

Fig. 8 shows the results of a factor VIII specific immunofluorescence assay as described in example 9. Upper row: 293T cells stably transfected with pTGF8-3, clone 49/19. Lower row: Negative control: Untransfected 293T cells. A and C: white light, no filter; B and D: Factor VIII detection by fluorescence, filter 550 nm.

Fig. 9 shows the influence of thermal treatment on FIX activity in culture filtrate as described in example 10.

Fig. 10 shows the dependence of expression of active recombinant factor IX on the supplementation of vitamin K into culture medium.

Detailed Description of the Invention

"Functionally linked" refers to configurations of the vector where the promoter is located within the vector in such a manner that it can stimulate transcription of the DNA sequence coding for the human blood clotting factor. "Not functionally linked" refers to a configuration where the promoter is so remotely located from the expressed gene sequence of the blood clotting factor that it cannot stimulate its transcription.

"Gene" refers to a DNA sequence encoding a polypeptide optionally including leader and trailer sequences and introns and exons.

"Vector" refers to any genetic construct, such as plasmid, phage, cosmid, etc., which is capable of replication when associated with the proper control elements. The term includes cloning and expression vehicles. "Carrying a vector" includes both, the stable and transient incorporation of a functional DNA segments into the host cell. The stable incorporation is, however, preferred.

"Gene transfer vector" in accordance with the present invention includes a vector suitable for gene therapy. Such vector comprises functional sequences for the desired purpose as known in the art.

The term "mature" refers to the molecular structure of a given protein directly after its cellular secretion (i.e., lacking its N-terminal export-signal polypeptide).

"Promoter" refers to a region of regulatory DNA sequences for the control of transcription of a gene to which RNA polymerases bind.

"Therapeutically effective dose" of the pharmaceutical composition of the invention refers to a dose effective for treatment or prophylaxis, for example, a dose that yields effective treatment or reduction of the symptoms of hemophilia. The determination of a therapeutically effective dose is within the purview of one skilled in the art.

"Encodes" or "encoding" refers to a property of the nucleic acid sequence being transcribed (in case of DNA) or translated (in case of mRNA) into a polypeptide *in vitro* or *in vivo* when placed under the control of an appropriate regulatory sequence.

For the purpose of this application "express", "expressing" or "expression" refers to the transcription and translation of a gene encoding a protein.

The present invention as described in (1) to (13) above is hereinafter described in more detail. In accordance with embodiment (1) of the invention of the present application the promoter functionally linked to the DNA sequence coding for the human blood clotting factor is not a viral promoter which is stimulated by the at least one viral transcription activator protein expressed by the immortalized human cell line.

The immortalized human cell line preferably is an immortalized kidney, bladder, liver, lung, cardiac muscle, smooth muscle, ovary or gastrointestinal cell. More preferably the immortalized human cell line is derived from an embryonic human kidney cell and most preferably it is cell line 293 T (ECACC: tsa201, ref. 96121229; DSM ACC2494)

The at least one transcription activator protein expressed by the immortalized cell line includes Simian virus T antigen, adenovirus E1A or E1B proteins, a protein encoded by the bovine papilloma virus early region DNA sequence and herpes virus IE proteins. Preferably the immortalized cell expresses at least two viral transcription activator proteins, e.g., a temperature sensitive SV40 T antigen and adenovirus E1A protein (such as the above cell line 293 T).

The promoter functionally linked to the DNA sequence coding for the human blood clotting factor preferably includes
(i) viral promoters being not stimulated by the activator protein expressed by the immortalized cell as defined above (such as SV40 and CMV);
(ii) housekeeping host promoters (albumin); and
(iii) tissue specific promoters (such as α-antitrypsin for liver). The most preferable promoter in accordance with the invention is a CMV promoter (while the transcription activator protein expressed by the immortalized cell is not stimulating said promoter).

In accordance with the invention the vector may carry additional viral promoters which are stimulated by said viral transcription activator proteins, but which are not functionally linked to the blood clotting factor. Such viral promoters are selected from promoters derived from adenovirus, rous sarcoma virus and cytomegalovirus. The vector may further comprise one or more of the following functional sequences: selection markers, regulatory sequences (e.g. PRE), etc.

The human blood clotting factor according to embodiment (1) of the invention includes, but is not limited to, factor IX, factor VIII, factor VII, factor V, von Willebrand factor (vWF) and the like.

In preferred embodiment (2) of the invention, the vector comprises a DNA sequence coding for factor VIII or a mutein thereof. Whereas recombinant

factor IX is in general structurally identical to the wild type protein isolated from blood plasma, several modified factor VIII expression constructs have been designed for recombinant expression. Considering the domain structure of the functional factor VIII polypeptide important interaction sites with vWF are located in the A3-domain (amino acid 1680-1689) and in the C2-domain (Kaufman & Pipe, *Haemophilia* (1998) 4, 370-379). Cleavage after 1689 was proposed to liberate factor VIII from vWF and permit factor VIII to interact with charged phospholipids. Recombinant factor VIII constructs lacking the vWF-binding site were shown to be extremely prone to proteolytic digestion when injected into factor VIII-deficient mice. Recombinant expression of truncated factor VIII constructs in mammalian cell cultures demonstrated that the complete deletion of the B-domain did not alter the biological activity of the corresponding factor VIII–like protein (Eaton et. al., *Biochemistry* (1986) 25, 8343-8347). In addition the observed expression rates of B-domain deleted constructs were significantly higher compared to wild-type factor VIII due to an increased mRNA-level in the cells (Pittman et al., *Blood* (1993) 81, 2925-2935). Four recombinant factor VIII products (Recombinate® Baxter HealthCare; Kogenate® and Kogenate FS® Bayer Corporation and Refacto® Wyeth, Genetics Institute) are currently on the market.

In preferred embodiment (3) of the invention the factor VIII mutein has at least one of the following mutations (a) to (d):

(a) Val at position 162 has been replaced by another neutral amino acid residue;

(b) Ser at position 2011 has been replaced by another hydrophilic amino acid residue;

(c) Val at position 2223 has been replaced by an acidic amino acid residue; and

(d) the B-domain between positions Arg740 and Glu1649 has been replaced by an Arg-rich linker peptide comprising 10 to 25, preferably 14 to 20 amino acid residues,

wherein said factor VIII numbering is relative to the amino acid sequence of wild-type factor VIII shown in SEQ ID NO: 2 (being the amino acid sequence of the mature peptide not including the 19 amino acid signal peptide, but including the entire B-domain (WO 99/29848)).

"Another neutral amino acid residue" in accordance with the present invention includes Gly, Ala, Leu, Ile, Met and Pro and preferably is Ala. The "another hydrophilic amino acid" includes Asn, Thr and Gln and preferably is Asn. The acidic amino acid residue is selected from Glu and Asp and preferably is Glu.

Among the factor VIII muteins of embodiment (3) it is preferred that the factor VIII mutein has at least one of the mutations (a), (b) and (c), more preferably at least one of the mutations (a) and (b), and most preferably all three mutations (a) to (c) as defined above. It is particularly preferred that the mutein comprises all three of the mutations V162A, S2011N and V2223E.

On the same token, the DNA sequence comprised by the vector of embodiment (4) of the invention has the mutations T485C, G6032A and T6668A relative to the DNA sequence of the mature wild-type factor VIII shown in SEQ ID NO: 1. In a preferred embodiment the DNA sequence also contains the quiet (i.e., silent) mutation T6816C (again said numbering being relative to the DNA sequence of the mature wild-type factor VIII).

Among the factor VIII muteins of embodiment (3) it is alternatively preferred that the factor VIII mutein has mutation (d) as defined above.

A preferred expression system of the invention utilizes a unique factor VIII mutein which - besides the point mutation (a) to (c) as defined herein before - partially or entirely lacks its B-domain, preferably a mutein where

the B-domain between position R740 and E1649 is replaced by a characteristic Arg-rich amino acid spacer as defined in (d) above. "Arg-rich" in accordance with the present invention means that said spacer comprises at least 3, preferably at least 4 Arg residues. In a most preferred embodiment said spacer consists of eight amino acids of the wilde type B-domain followed by eight amino acids of a variable domain (see Fig. 5A, SEQ ID NO: 9). In such construct having the B-domain modifications discussed herein before the proposed vWF-binding site remains unchanged to prevent an immediate proteolytic digestion of secreted factor VIII in the cell culture medium or later on in the blood of the treated patients. Only after specific activation by thrombin cleavage factor VIII will be released from vWF. The cDNA for the preferred factor VIII was constructed by assembling four DNA-fragments, e.g., as described in Example 1.

The protein of embodiment (3) of the invention may comprise additional N- or C-terminal sequences including, but not limited to, the natural export signal peptide (corresponding to amino acid residues -19 to -1 of the proteins shown in SEQ ID NOs 4, 13 and 15) or a fragment or analogue thereof, artificial peptides (e.g. oligo-His-tags for high-affinity purification) and the like.

The most preferred vector for the expression of factor VIII is vector pTGF8-1 shown in Fig. 2. The DNA sequence of said vector is shown in SEQ ID NO: 3, and it encompasses all five mutations addressed hereinbefore (the muteins T485C, G6032A, T6668A and T6816C (here: T1217C, G4088A, T4724A and T4872C) and a DNA sequence coding for the B-domain linker of SEQ ID NO: 9) and encodes the factor VIII mutein depicted in SEQ ID NO: 4.

Further most preferred vectors are pTGF8-2hyg-s and pTGF8-3, the common molecular structure of which is depicted in Fig. 6.

pTGF8-2hyg-s shown in SEQ ID NO: 12 contains the silent mutation T6816C only, resulting in a factor VIII mutein having the substitution of the B domain by the linker peptide SEQ ID NO. 9, but no further change in the primary protein structure referring to the wild type sequence SEQ ID NO. 2.

pTGF8-3 shown in SEQ ID NO: 14 contains mutations T485C, T6668A and T6816C, resulting in a factor VIII mutein showing amino acid substitutions V162A and V2223E referring to SEQ ID NO. 2 in addition to the substitution of the B domain as described above.

In the case of the production of factor VIII the culturing is performed in the presence of von Willebrand factor. The von Willebrand factor is preferably used in an amount of 10 to 100, more preferably 50 to 60 mol vWF per mol factor VIII (in the culture broth and/or in the factor VIII solution during the purification procedure (see below).

In preferred embodiment (4) of the present invention the human blood clotting factor is factor IX or a mutein thereof, preferably is wild-type factor IX shown in SEQ ID NO: 5. Suitable muteins of factor IX include point mutated and truncated forms of the factor IX. The most preferred vector for expression of factor IX are vectors pTGFG36 and pTG36hyg shown in Figs. 3 and 4, respectively.

In case of the production of factor IX, the culturing is preferably performed in the presence of vitamin K which may be present in an amount of 0.1 to 100 µg/ml culture broth, more preferably 1 to 20 µg/ml culture broth.

The method according to embodiment (1) of the invention further comprises the steps

(c) purifying the blood clotting factor isolated in step (b) and/or

(d) subjecting the blood clotting factor isolated in step (b) or purified in step (c) to a virus inactivation treatment.

Suitable purification steps include methods which were known in the art to maximize the yield of a pure, stable and highly active product and are selected from immunoaffinity chromatography, anion exchange chromatography, size exclusion chromatography, etc., and combinations thereof. In particular, detailed purification protocols for coagulation factors from human blood plasma are, e.g., disclosed in WO93/15105, EP0813597, WO96/40883 and WO 96/15140/50. They can easly be adapted to the specific requirements needed to isolate recombinant factors VIII and IX. For factor IX an effective protocol has been introduced containing an ammonium sulfate precipitation step followed by DEAE and HIC tentacle chromatography as well as heparin affinity chromatography (US5919909). Quantity and activity of the purified protein during and after the purification procedure may be monitored by ELISA and coagulation assays.

To overcome the problems of possible infectious contaminations in the purified protein samples or in the product directly obtained from the cell culture supernatant containing the secreted recombinant protein of choice, the samples and/or the culture supernatant might be treated with procedures for virus inactivation including heat treatment (dry or in liquid state, with or without the addition of chemical substances including protease inhibitors). After virus inactivation a further purifying step for removing the chemical substances may be necessary. In particular, for factor VIII isolated from blood plasma the recovery of a high purity virus-inactivated protein by anion exchange chromatography was described (WO93/15105). In addition several processes for the production of high-purity, non-infectious coagulation factors from blood plasma or another biological sources have been reported. Lipid coated viruses are effectively inactivated by treating the potentially infectious material with a

hydrophobic phase forming a two-phase system, from which the water-insoluble part is subsequently removed. A further advantage has been proven to complement the hydrophobic phase treatment simultaneously or sequentially with a treatment with non-ionic biocompatible detergents and dialkyl or trialkyl phosphates. (WO 9636369, EP0131740, US 6,007,979). Non-lipid coated viruses require inactivation protocols consisting in treatment with non-ionic detergents follwed by a heating step (60-65 °C) for several hours (WO94/17834).

In view of the above results it is believed that the combination of an effective protein expression system based on a human cell line together with approved methods for inactivation of potentially dangerous infectious agents serve as a safe and easy to use-system for production of recombinant clotting factors.

Moreover, in accordance with embodiment (6) of the invention it is provided a superior factor VIII mutant. Said factor VIII mutant can be part of pharmaceutical compositions, can be used for preparing medicaments for treating hemophilia and can be applied in methods for treating hemophilia (embodiments (11) to (13) of the invention). The above pharmaceutical compositions and the above medicaments may comprise the factor VIII in a therapeutically effective dose, e.g., from 50 to 500 µg (with 200 ng factor VIII corresponding to one International Unit (IU)). Depending on the type of hemophilia, a patient receives an annual dose of factor VIII of up to 200,000 IU, which is usually administered in weekly or twice weekly doses.

The pharmaceutical compositions, medicaments or preparations applied in methods for treating hemophilia of embodiments (11) to (13) contains a therapeutically effective dose of the factor VIII mutein of embodiment (6) or the gene transfer vector of embodiment (9). In case of the former, it may further comprise pharmaceutically acceptable additives including

human serum albumin (HSA; preferably about 1 mg/ml solution); inorganic salts such as $CaCl_2$ (preferably 2 to 5 mM), amino acids such as glycine, lysine, and histidine (preferably 0.1 to 1 M per amino acid); disaccharides such as sucrose and/or trehalose (preferably 0.4 to 1 M); organic salts such as Na-citrate (preferably up to 50 mM); etc. The preparations may be aqueous or non-aqueous. In the latter case the major component is glycerol and/or polyethylene glycol (e.g., PEG-300). The preparation may also be in the dry form (to be dissolved in the desired solvent prior to administration).

As set forth above, the gene transfer vector in accordance with embodiment (9) of the invention can also be part of pharmaceutical compositions, can be used for preparing medicaments for treating hemophilia and can be applied in methods for treating hemophilia (embodiments (11) to (13) of the invention). Said pharmaceutical compositions and medicaments may further comprise suitable matrix formulations, e.g., lipids or hormones as discussed in WO 00/49147 (the disclosure thereof being herewith incorporated by reference). The pharmaceutical composition or medicament comprising the gene transfer vector or the gene transfer vector of the present invention may be administered orally, intravenously, intramuscularly, subcutaneously, tropically, through mucosa (including buccal, nasal spray) or by gene gun. Oral administration (e.g., in a micronized hormone dispersion) is preferred.

The factor VIII mutein of embodiment (6) of the invention is preferably as defined with reference to embodiment (3) above. Said FVIII mutein may further be prepared by standard recombinant techniques, e.g. a method comprising

(a) culturing a host cell transformed with the vector of embodiment (8) and/or comprising the DNA of embodiment (7) (which also includes culturing an immortalized human cell line stably expressing at least one

viral transcription activator protein and carrying a vector having a viral transcription promoter functionally linked to a DNA sequence coding for the human blood clotting factor, wherein said viral promoter is stimulated by said at least one viral transcription activator protein); and
(b) isolating the blood clotting factor from the culture broth. Suitable immortalized human cell lines, transcription activator proteins and viral promoters are those mentioned herein before. The immortalized human cell line utilized in said method preferably expresses two viral trascription activator proteins, most preferably temperature sensitive SV40 T antigen and adenovirus E1A protein. The method may further comprise the purification and virus inactivation steps (c) and (d) described herein before.

The commercially available cell line 293 T (ECACC: tsa201, ref. 96121229) was deposited with the DMSZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, 38124 Braunschweig, Germany) on February 20, 2001 under the depositary no. DSM ACC2494.

The invention is further illustrated by the following examples.

Examples

Example 1 - Cloning of factor VIII: The sequence for the recombinant factor VIII was obtained by reverse transcription from a complete human hepatocellular RNA pool. Afterwards four fragments (1/2, 3/4 , 5/6, 7/8) were amplified by standard PCR using primers designed to contain restriction sites. To fit together the fragments 3/4 and 5/6 the SmaI/SalI fragment from plasmid pBSFVIII3/4 was inserted blunt into the SalI site of pBSFVII5/6 to obtain pBSFVIII3/6. Next, the fragment 3/6 was obtained by digesting pBSFVIII3/6 with XhoI/BspHI and partially with Alw44I. This fragment and the PstI/Alw44I fragment from pBSFVIII1/2 were ligated in one step into the vector backbone of pBSFVIII1/2 digested with PstI and

XhoI by this means obtaining pBSFVIII1/6. The fragment 7/8 was obtained by digesting pBSFVIII7/8 with SmaI and partially with Mva1269I and ligated into pBSFVIII1/6 cut with XhoI and Mva1269I giving rise to pBSFVIII1/8. Finally the SmaI/XhoI fragment from pBSFIII1/8 was inserted blunt into the SalI site of Octagene Vector pTGFG67 (the production of said vector being disclosed in PCT/EP00/01368) resulting in the eukaryotic expression vector for the human factor VIII pTGF8-1 (s. figures 1 and 2). The resulting vector encodes a factor VIII mutein having the mutations V162A, S2011N and V2223E.

Example 2 - Cloning of factor IX: The vector pUC19 (MBI Fermentas) was digested with XbaI, treated with Klenow enzyme and religated. This XbaI deleted vector was then digested with EcoRI, treated with Klenow enzyme and religated in order to delete the EcoRI site. For insertion of an XbaI site into the SacI site of this vector it was digested with SacI, treated with T4-DNA-polymerase, dephosphorylated with alkaline phosphatase and ligated with the XbaI-linker CTCTAGAG (Biolabs #1032). Another XbaI-site was inserted by digesting the newly produced vector with HindIII, treating it with Klenow, dephosphorylating it with alkaline phosphatase and ligating it with the XbaI-linker CTCTAGAG (Biolabs #1032). This vector was named pUC19/X.

In order to destroy the XbaI-site present in the vector phGFP-S65T (Clontech) this vector was digested with XbaI, treated with Klenow enzyme and religated resulting in the vector pGFP/0. A 2.3 kb fragment containing the GFP-Gene was isolated after digesting pGFP/0 with MluI, treating it with Klenow enzyme and digesting it with BamHI. This fragment was inserted into the multiple cloning site of the vector pUC19/X which was digested with SalI, treated with Klenow enzyme and digested with BamHI. The resulting vector was named pTGFG1.

The oligonucleotides (Metabion) PRE-S (5'-GGG GTA CCA GCT TCG TAG CTA GAA CAT CAT GTT CTG GGA TAT CAG CTT CGT AGC TAG AAC ATC ATG TTC TGG TAC CCC-3'; SEQ ID NO: 10) and
PRE-AS (5'-GGG GTA CCA GAA CAT GAT GTT CTA GCT ACG AAG CTG ATA TCC CAG AAC ATG ATG TTC TAG CTA CGA AGC TGG TAC CCC-3'; SEQ ID NO: 11)
were hybridized and phosphorylated by kinase reaction, resulting in the insert PRE(ds).

The vector pTGFG1 was digested with EcoO109I, treated with Klenow enzyme and dephosphorylated with alkaline phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG5.
The vector pUC19 (MBI Fermentas) was digested with SalI, treated with Klenow enzyme and dephosphorylated with alkaline phosphatase. It was ligated to the NotI-linker GCGGCCGC (Biolabs # 1045), resulting in the vector pUC19/N.

Factor IX cDNA was amplified from human liver cDNA (Clontech) using two primers overlapping the start and termination codon of the factor IX open reading frame resulting in a 1387 bp fragment containing the entire open reading frame. Restriction sites for EcoRI (upstream) and BamHI (downstream) were included at the end of each primer to facilitate cloning. Amplification was performed with Pwo DNA-polymerase (Boehringer Mannheim) in 50 µl reaction volume [10 mM Tris HCl pH 8.85, 25 mM KCl, 5 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$] with 30 incubation cycles at 96°C for 1 min, 60°C for 1 min, 72°C for 2 min, followed by a final extension step at 72°C for 10 min.

Reaction products were ligated into the EcoRI- and BamHI-sites of pUC19 and transformed into *E. coli* DH5-α. Positive clones were selected. Sequences were confirmed by cycle sequencing (Amersham) from both

ends with labeled primers (IR-700) and automated analysis on the LiCor sequencing system (MWG, Biotech).

The following primers were used :
GGAATTCCGCAAAGGTTATGCAGCGCGTGAACATGATCATGGC (upstream; SEQ. ID NO: 16)
CGCGGATCCATTAAGTGAGCTTTGTTTTTTCCTTAATCC (downstream; SEQ. ID NO: 17)

A 1.4 kb fragment containing the open reading frame of the human clotting factor IX, isolated from a human cDNA library, was inserted into the PstI site of the above prepared vector pUC19/N which was digested with PstI, treated with T4-polymerase and dephosphorylated with alkaline phosphatase. From the resulting vector pUC19/N-FIX a 1.4 kb fragment containing the open reading frame of the human clotting factor IX was cut out by double-digestion with Hind III and NotI. This fragment was ligated to the 4.3 kb fragment of the HindIII and NotI double-digested vector pTGFG5 resulting in the vector pTGFG36 shown in Fig. 3. This vector is a preferred one for delivery of an expression cassette encoding factor IX into the cell, and its DNA sequence is provided in SEQ ID NO: 6.

Example 3 - Human cell line for protein expression: A preferred cell line is tsA201 (ECACC Ref.: 96121229) which is a transformed embryonal human kidney cell line (293, ECACC Number 85120602) stably expressing an SV40 temperature-sensitive T antigen (J. Membrane Biol. 1996;152:39; Gene 1995;156:235; PNAS USA 1994;91:12785; Pflügers Arch. 1994;427:136; J. Gen. Physiol. 1994;104:507; BioTechniques 1993;15:906). Other names for this cell line include 293tsA1609neo (Mol. Cell. Biol., 1987, 7:379) and 293T. This epithelial-like cell line has been used in a variety of functional expression assays and been reported to produce high levels of recombinant proteins. They can be cultivated in DMEM supplemented with 2mM glutamine and 10% FCS. For efficient pro-

duction of factor IX, the medium can be modified by addition of up to 100 µg/ml vitamin K (US4770999).

To simplify the purification of an expressed polypetide, cells can be cultivated in serum-free or protein-free medium containing suitable supplements. For stability reasons secreted factor VIII requires the presence of vWF in the medium (US5198349). Also the addition of lipoproteins, phospholipids, polyglycols, trace metals, heparin, non-ionic surfactants or cyclodextrin has been reported (EP0254076, US5679549, US5198349, US5250421, US5576194, EP0872487, WO94/11525, US5378612).

Example 4 - Calcium phosphate transfection of 293T cells for the transient production of factors VIII and IX: Confluent 293T cells were plated at low density in 10 cm dishes in 6 ml DMEM/10% FCS (10 µg/ml vitamin K for FIX) the day prior to transfection. Transfection was performed roughly according to Chen and Okayama (Mol. Cell Biol., 7:2745 (1987)). 12 µg of plasmid pTGF8-1 were transfected for the production of factor VIII and pTGFG36 for the production of factor IX. Six hours after transfection the medium was replaced by fresh one and the superantant was harvested three days post transfection and either further purified or analyzed without further purification by ELISA or coagulometry (see Examples 5 and 6).

Example 5 – Determination of FIX and FVIII concentration by ELISA:
Factor IX: Human recombinant factor IX levels in supernatant of transfected 293T cells were determined by ELISA using a goat polyclonal anti-human FIX (Enzyme Research Laboratories) as capture antibody. All incubations were performed for two hours in a humid chamber at 22° C. Plates (Dynex, Immulon-4) were coated with 100 µl of 8.8 µg antibody/ml coating buffer. Blocking is not required under the conditions described. Washing the plate four times (Encore 2000, Merck) with PBS-Tween® (0,1% v/v) is sufficient to block non-specific interactions.

After each further step washing was required to eliminate unbound proteins. 100 µl of supernatant treated with 10 µl 10mM PMSF and 10 µl 0.11 M sodium citrate were added to each well. Dilutions for samples and standard (human factor IX, house standard, Octapharma) were made in dilution buffer (HBS-BSA-EDTA-Tween®) and incubated at 100 µl/well. The detecting antibody was a peroxidase labelled goat polyclonal anti-FIX (Enzyme Research Laboratories) in a concentration of 1 µg antibody /ml dilution buffer and incubated at 100 µl/well. 150 µl ABTS (Roche) was added to each well as substrate, colorimetric reaction was detected at 405 nm after 1-2 hours. Results were calculated by linear regression of standard concentration versus standard absorbance and are summarized in the following table:

Number of cells [/ml]	Factor IX-concentration [ng/ml]	Clotting time [s]
$2,1 \times 10^5$	36	45
$8,7 \times 10^5$	20	79

Normal plasma: 37 – 39 s

Factor IX deficient plasma: 137 – 140 s

Factor VIII: Human recombinant factor VIII levels in culture filtrate of transfected 293T cells were determined by ELISA using an affinity purified polyclonal sheep anti FVIII:C preparation (F8C-EIA-C, Affinity Biologicals) as capture antibody. Coating was performed for two hours in a humid chamber at 22° C. Plates (Dynex, Immulon-4) were coated with 100 µl of a 100-fold antibody dilution in coating buffer (50 mM sodium carbonate pH 9.6). Washing the plate four times (Encore 2000, Merck) with PBS-Tween® (0,1% v/v) was sufficient to block non-specific interactions.

After each further step, washing was required to eliminate unbound proteins. 100 µl each of culture filtrate samples withdrawn from different

293T clones stably transfected with pTGF8-3 after 48h incubation were added to each well. Dilutions of FVIII standard (house standard, Octapharma) were made in dilution buffer (HBS-BSA-EDTA-Tween®) and incubated at 100 µl per well. For detection, a ready-to-use dilution of peroxidase labelled polyclonal anti-FVIII (F8C-EIA-D, Affinity Biologicals) was incubated for 60 min at 100 µl per well. For colorimetric reaction, a 5 mg O-Phenylenediamine (P-6912, Sigma) tablet was dissolved in 12 ml substrate buffer shortly before use and completed with 12 µl 30% H_2O_2. 150 µl of this substrate solution was added to each well and colorimetric recording was done in an MRX Reader (Dynex) at 490 nm after 10 min of incubation at room temperature in the dark and stopping of reaction by addition of 50 µl 2.5 M H_2SO_4 to each well. Results were calculated by linear regression of standard concentrations versus standard absorbances (Fig. 7 A) and are summarized in Fig. 7 B.

Example 6: Detection of Human Clotting Factor VIII and Factor IX Activity

The clotting activity of human recombinant factor VIII in supernatants of cell culture 293T cells (transfected by calcium phosphate precipitation with pTGF8-1 as described in Example 4) was determined as follows:

The clotting activity was assayed based on a partial thromboplastin time assay using Cephalin (phosphatidyl ethanolamine) activation with a manual coagulation instrument (ML-2, Instrumentation Laboratories). For the study, 100 µl undiluted supernatant from transfected 293 T-cells, 100 µl deficiency plasma (Progen) and 100 µl Cephalin (Instrumentation Laboratories) were incubated for 5 minutes at 37°C. Coagulation was started by adding 100 µl $CaCl_2$. Sample coagulation time was compared to normal plasma. The results are summarized in Fig. 5B. As can be seen from Fig. 5B, cell supernatant from cells transfected with pTGF8-1 shows a coagulation activity comparable to normal plasma while non transfected cells give a value equivalent to plasma lacking factor VIII.

A similar assay was performed with regard to factor IX. The results are shown in the Table of Example 5. For dependence of expression on the presence of vitamin K see Fig. 10.

Example 7 - Viral inactivation:

Viral inactivation was performed in accordance with the method of U.S. Patent No. 6,007,979. Namely, to a potentially infectious protein solution the following compounds were added subsequently, with stirring:

1. 0.2 ml of Tween® 80 and 0.06 ml of TNBP were added to 19.74 ml of the solution or

2. 0.2 ml of Triton® X-100 and 0.2 ml of TNBP were added to 19.6 ml of the solution.

1 ml of castor oil was added to preparations 1 and 2 which were then intensely extracted at room temperature for one hour.

Centrifugation was performed in each case for phase separation. For infectiousness control, samples of 1 ml each were repeatedly taken from the aqueous fraction.

Example 8 - Establishment of cell lines stably expressing factor VIII and factor IX: The preferred vectors pTGF8-1 and pTGFG36 comprise constructs for transient expression of factor VIII and factor IX , respectively, in mammalian cells. To enable a selection method for a stably transfected cell clone, a cassette for the hygromycin—B-phosphotransferase (HindIII-Mva 1260I fragment from TK-Hyg, Clontech) was subcloned into the SmaI site being present in both vectors. The resulting constructs (pTGF8-1-hyg and pTG36hyg) then comprise in *cis* the expression cassettes for the human factor VIII or factor IX with a CMV-promotor and a SV40-polyadenylation signal and a hygromycin-B-phosphotransferase expression cassette with the HSV thymidine kinase promoter and HSV thymidine kinase polyadenylation signal (see Fig. 4).

The vectors pTGF8-2hyg-s and pTGF8-3 (Fig. 6, SEQ ID NOs: 12 and 14) are derivatives of pTGF8-1hyg, in that point mutations V162A, S2011N and V2223E (pTGF8-2hyg-s) and S2011N (pTGF8-3) were reverted to wildtype sequence by a PCR- dependent method using the QuikChange® protocol (Stratagene).

The coding sequence for the clotting factors can be replaced by any other gene sequence of choice. These constructs allow for the establishment of stably expressing cell lines by calcium phosphate transfection and subsequent selection for hygromycine resistance. Additionally the plasmids contain a progesterone responsive element (PRE). In transient transfection experiments with pTG36hyg the production of about 40 ng active factor IX per ml culture medium could be shown by ELISA and coagulometric assay (see Examples 5 and 6).

For the production of factor IX 293T cells were cultivated in DMEM supplemented with 10% FCS and 10 µg/ml vitamin K (US Patent No. 4,770,999; see also Fig. 10). First the critical concentration of antibiotics for an effective selection of stably transfected 293T cells had to be established. For this purpose the cells were plated at low dilution and grown in the presence of 10 to 800 µg/ml hygromycin B. After two weeks at 200 µg/ml or higher no cells were growing, so this concentration was chosen for the selection of stably transfected cells.

A typical transfection was performed in 10cm dishes with 293T cells split the day before at a ratio of 1:15. Using the calciumphosphate precipitation method (*Biotechniques* 1988 6:7 632-638) 12 µg plasmid per dish were transfected and two days later the medium was replaced with fresh one containing 200 µg/ml hygromycin B. After 2-3 weeks of selection the medium was tested by ELISA (see Example 5) for the presence of factor VIII or IX. Positive clones were isolated and transferred to a 24-well plate.

After screening by ELISA and activity determination positive clones were subjected to two further rounds of subcloning then expanded and aliquots of them frozen for further use and characterization.

Example 9: Proof of Phenotypic Uniformity of stably transfected cells by *in-situ* Immunofluorescent Detection of Factor VIII Expression:

Each, 5 x 10^7 293T cells stably transfected with pTGF8-3 (clone 49/19) and untransfected 293T cells (negative control) from adhesion cultures in DMEM + 9.1% FBS were detached from the culture dishes by trypsination, washed several times and resuspended in 5 ml PBS buffer.

2 µl of these cell suspensions were transferred to sterile microscopic glass slides and incubated at room temperature until all liquid was evaporated. Cells were fixed in 70% ethanol for 10 min and dried 5 min at room temperature. Slides were blocked against unspecific detection by incubation in a 10% dilution of FBS in PBS buffer. Primary antibody (sh antiFVIII:C F8C-EIA-C, Affinity Biologicals) was diluted 100-fold with PBS buffer containing 10% FBS and 0.1% saponine and incubated for 60 min at room temperature in a humid incubation chamber. After intense washing with PBS, a 100-fold dilution of the secondary antibody (rb anti sh CY3 conjugate 313-165-003, Jackson ImmunoResearch) was prepared and incubated in the way described above. Subsequently, the microscopic preparation was washed intensely and covered by a layer of 50% glycerol and a cover glass. Cells were visualized by white light- and by fluorescence microscopy (emission at 570 nm).
Results are depicted in Fig. 8.

Example 10: Thermal stability test on recombinant factor IX in culture filtrate:

Culture filtrate harvested from 293T cells 48 h after transient transfection with pTGFG36 in the presence of 100 µg/ml vitamin K and stored at -80 °C for 7 days was thawed quickly, distributed into 7 500 µl aliquots

which -then were subsequently submitted to the following thermal incubations:

sample	temp. (°C)	time (min)
1	0	240
2	20	30
3	20	60
4	20	240
5	37	30
6	37	60
7	37	240

Samples were chilled on ice and FIX activity was determined as outlined in Example 6 (double determinations). Results are shown in figure 9. Activity remained almost stable within incubation conditions up to 240 min 37 °C.

Claims

1. A method for the production of recombinant human blood clotting factor which comprises
(a) culturing an immortalized human cell line stably expressing at least one viral transcription activator protein and carrying a vector having a promoter functionally linked to a DNA sequence coding for the human blood clotting factor, provided that said promoter is not a viral promoter which is stimulated by said at least one viral transcription activator protein; and
(b) isolating the blood clotting factor from the culture broth.

2. The method of claim 1, wherein
(i) the immortalized human cell line is an immortalized kidney, bladder, liver, lung, cardiac muscle, smooth muscle, ovary or gastrointestinal cell; and/or
(ii) the at least one viral transcription activator protein is selected from Simian virus T antigen, adenovirus E1A or E1B proteins, a protein encoded by the bovine papilloma virus early region DNA sequence and herpes virus IE proteins; and/or
(iii) the promoter is selected from viral promoters, housekeeping host promoters and tissue specific promoters.

3. The method of claim 2, wherein the immortalized human cell line is derived from an embryonic human kidney cell and preferably is cell line 293T (DSM ACC2494), and the promoter is a CMV promoter.

4. The method according to any one of claims 1 to 3, wherein the vector further comprises a selection marker and/or regulatory sequences.

5. The method according to any one of claims 1 to 4, wherein the vector comprises a DNA sequence coding for human factor VIII or a mutein thereof.

6. The method of claim 5, wherein the vector comprises a DNA sequence coding for the mature wild-type factor VIII shown in SEQ ID NO:2.

7. The method of claim 5, wherein the vector comprises a DNA sequence coding for a factor VIII mutein, said factor VIII mutein being a mutein having point mutations, a mutein being truncated at its C- or N-terminus, and/or a mutein partially or entirely lacking its B-domain, preferably the factor VIII mutein having at least one of the following mutations:
(a) Val at position 162 has been replaced by another neutral amino acid residue;
(b) Ser at position 2011 has been replaced by another hydrophilic amino acid residue;
(c) Val at position 2223 has been replaced by an acidic amino acid residue, and
(d) the B-domain between positions Arg740 and Glu1649 has been replaced by an Arg-rich linker peptide comprising 10 to 25, preferably 14 to 20 amino acid residues,
wherein said factor VIII numbering is relative to the mature wild-type factor VIII sequence shown in SEQ ID NO:2.

8. The method of claim 7, wherein the factor VIII mutein has at least one of the mutations (a), (b) and (c), preferably at least one of the mutations (a) and (b).

9. The method of claim 7 or 8, wherein the factor VIII mutein has all three mutations (a), (b) and (c).

10. The method according to any one of claims 7 to 9, wherein in mutation (a) Val at position 162 has been replaced by Ala, in mutation (b) Ser at position 2011 has been replaced by Asn, and/or in mutation (c) Val has been replaced by Glu.

11. The method of claim 7, wherein the DNA sequence coding for the factor VIII mutein has at least one of the mutations T485C, G6032A and T6668A relative to the DNA sequence of the mature wild-type factor VIII shown in SEQ ID NO: 1, preferably the DNA sequence comprises all three of said mutations.

12. The method of claim 6, 7 or 11, wherein the DNA sequence coding for the factor VIII or mutein thereof contains the quiet mutation T6816C.

13. The method according to any one of claims 8 to 12, wherein the factor VIII mutein further partially or entirely lacks its B-domain.

14. The method of claim 7, 12 or 13, wherein the mutein has mutation (d) as defined in claim 7.

15. The method of claim 7 or 14, wherein the Arg-rich linker peptide comprises at least 3 Arg residues.

16. The method of claims 7, 14 and 15, wherein the linker comprises:
the amino acid sequence SFSQNSRH, and/or
the amino acid sequence QAYRYRRG, and preferably the linker has the sequence SFSQNSRHQAYRYRRG.

17. The method of claim 7, wherein the factor VIII mutein comprises amino acids 1 to 1440 of SEQ ID NO: 4, 13 or 15.

18. The method of claim 5 or 7, wherein the vectors are pTGF8-1 shown in Fig. 2, or pTGF8-2hyg-s or pTGF8-3 depicted in Fig. 6.

19. The method according to any one of claims 5 to 18, wherein the culturing is performed in the presence of von Willebrand Factor (vWF), preferably in an amount of 10 to 100, more preferably 50 to 60 mol vWF per mol factor VIII.

20. The method according to any one of claims 1 to 4, wherein the vector comprises a DNA sequence coding for human factor IX or a mutein thereof.

21. The method of claim 20, wherein the vector comprises a DNA sequence coding for the wild-type factor IX shown in SEQ ID NO: 5 and preferably is vector pTG36hyg shown in Fig. 4.

22. The method of claims 20 or 21, wherein the culturing is performed in the presence of vitamin K, preferably in an amount of 0.1 to 100 µg/ml, more preferably 1 to 20 µg/ml culture broth.

23. The method according to any one of claims 1 to 22 which further comprises
(c) purifying the blood clotting factor isolated in step (b), and/or
(d) subjecting the blood clotting factor isolated in step (b) or purified in step (c) to a virus inactivation treatment.

24. An immortalized human cell line stably expressing at least one viral transcription activator protein and carrying a vector coding for a human blood clotting factor as defined in any one of claims 1 to 18, 20 and 21.

25. A factor VIII mutein as defined in any one of claims 7 to 17 having at least one of the mutations (a) to (d) as defined in claim 7.

26. A DNA sequence coding for the factor VIII mutein as defined in claim 25.

27. A vector comprising the DNA as defined in claim 26.

28. The vector of claim 27 which is a gene transfer vector.

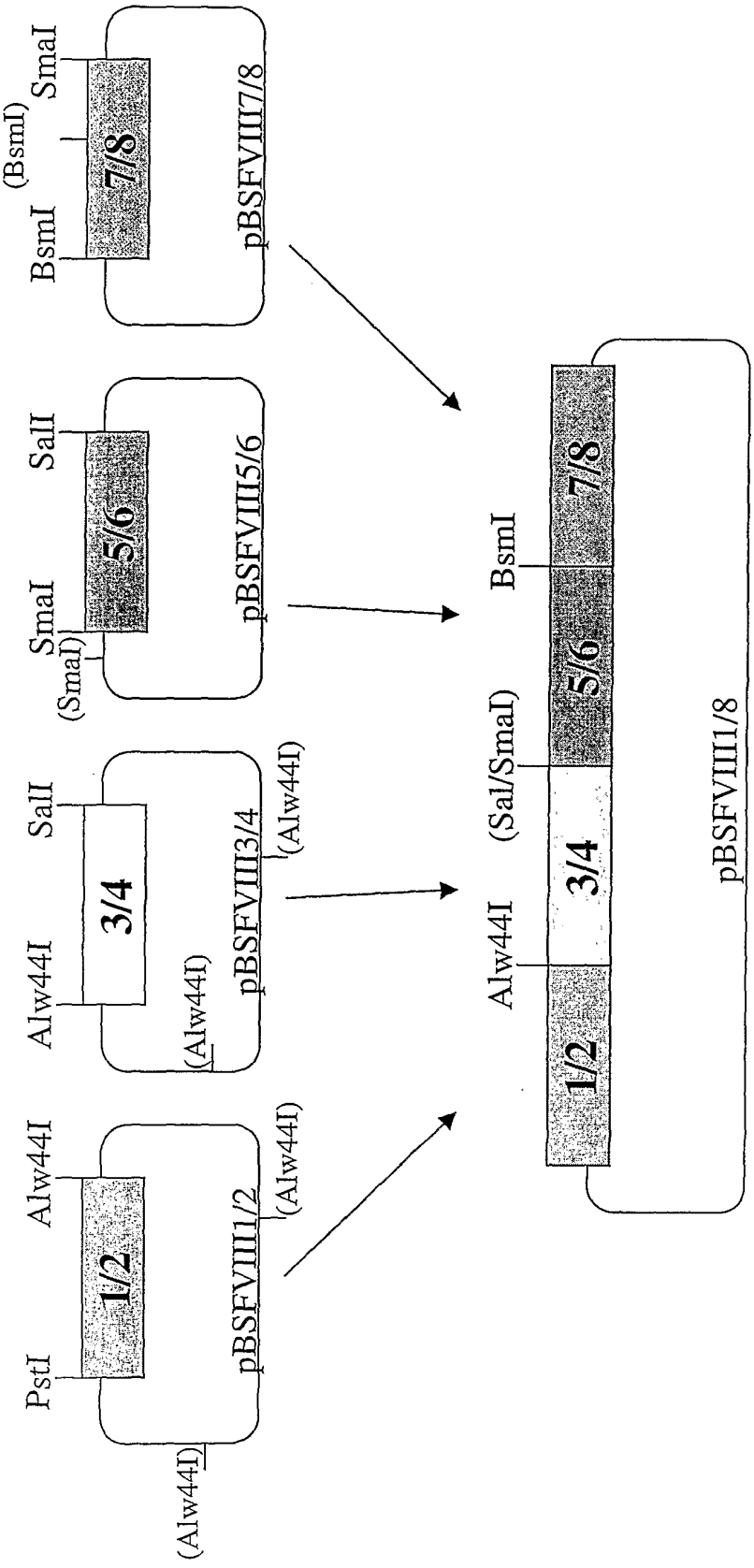
29. A host cell being transformed with a vector as defined in claim 27 and/or comprising a DNA sequence as defined in claim 26.

30. A pharmaceutical composition comprising the factor VIII mutein as defined in claim 25 or a gene transfer vector as defined in claim 28.

31. Use of the factor VIII mutein as defined in claim 25 or a gene transfer vector as defined in claim 28 for preparing a medicament for treating hemophilia, preferably treating hemophilia A.

32. A method for treating hemophilia, preferably for treating hemophilia A, which comprises administering human hemophiliacs a factor VIII mutein as defined in claim 25 or a gene transfer vector as defined in claim 28.

Fig. 1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(Alw44I)
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(SmaI)
SalI
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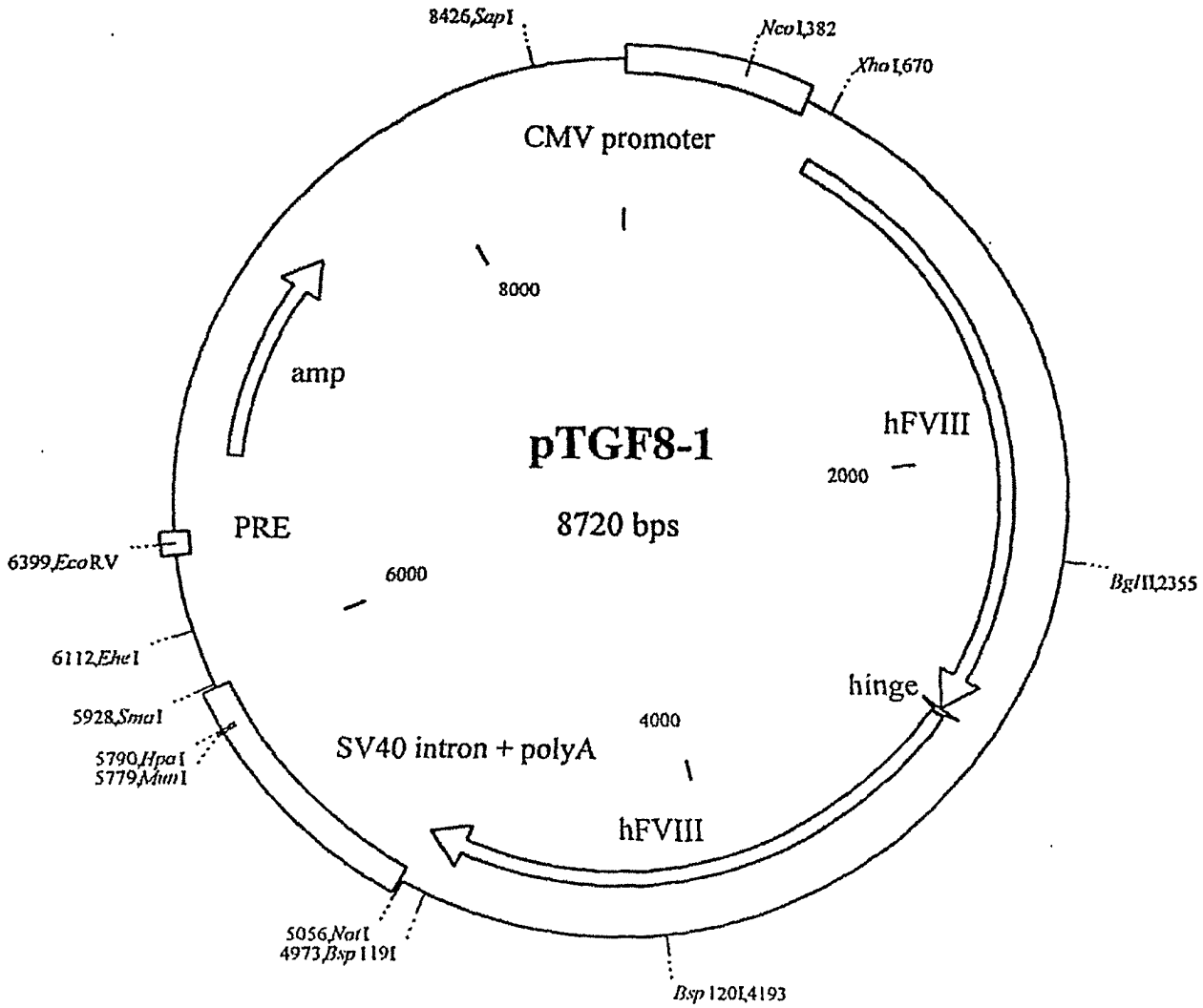
Fig. 2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6000
BglII,2355
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hinge
5928,SmaI
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Fig. 3

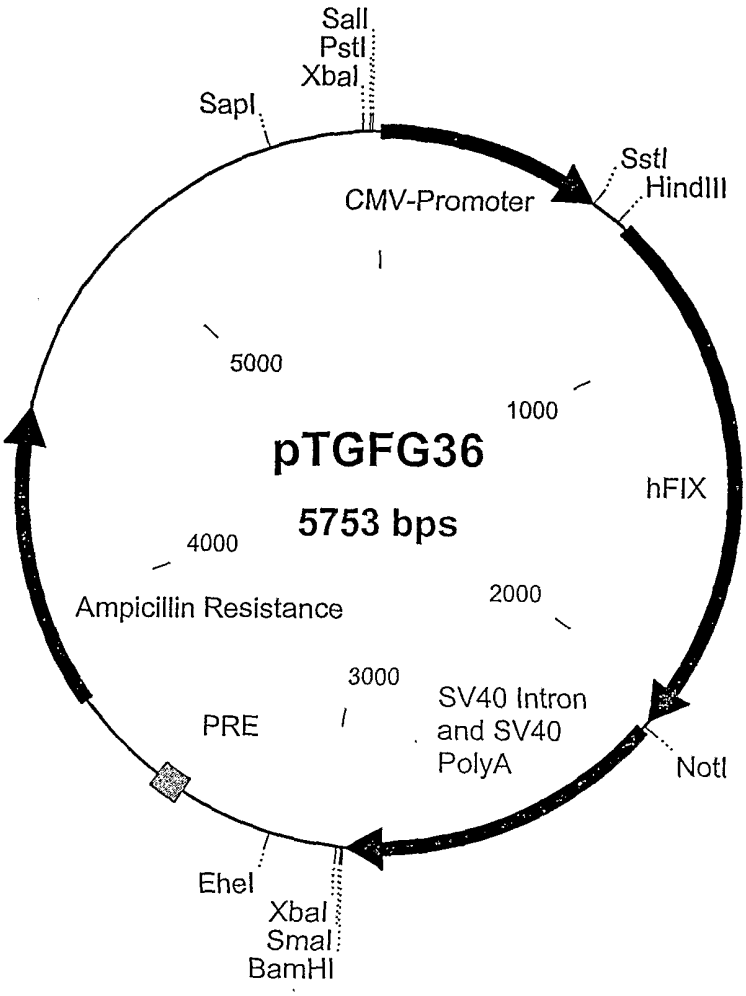
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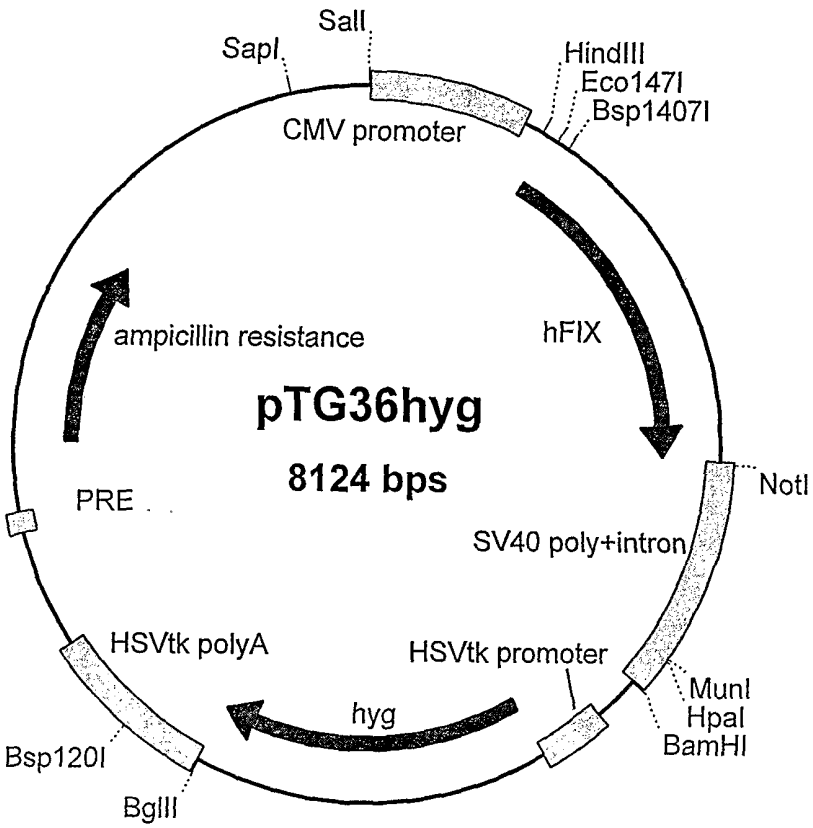
Fig. 4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HSVtk polyA
HSVtk promoter
MunI
HpaI
BamHI
hyg
Bsp120I
BglII

Fig. 5

Fig. 5 A

aga| agc ttc tcc cag aat tca aga cat caa gct tat cga tac cgt cga ggg gaa

R740 S F S Q N S R H Q A Y R Y R R G E1649

wt-B-domain *variable domain*

Fig. 5 B

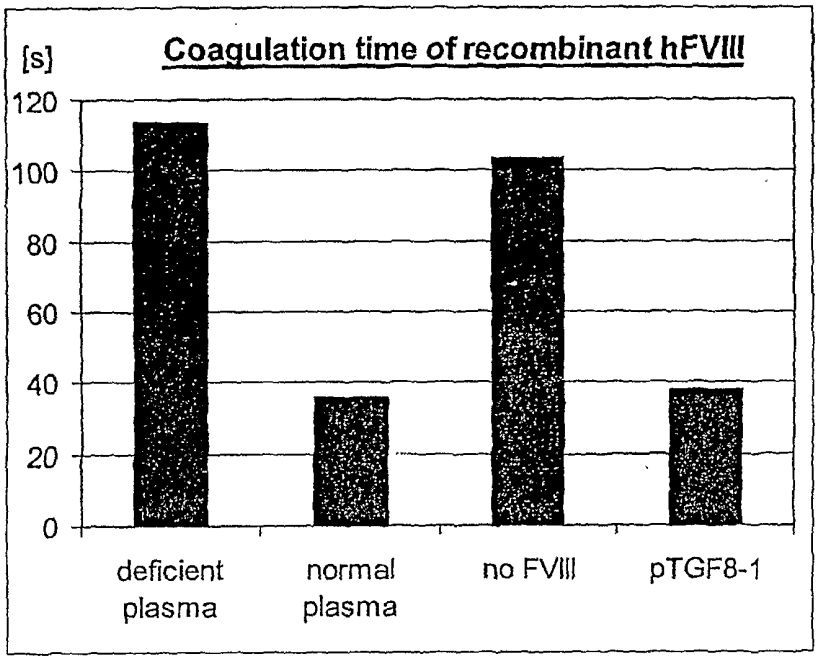
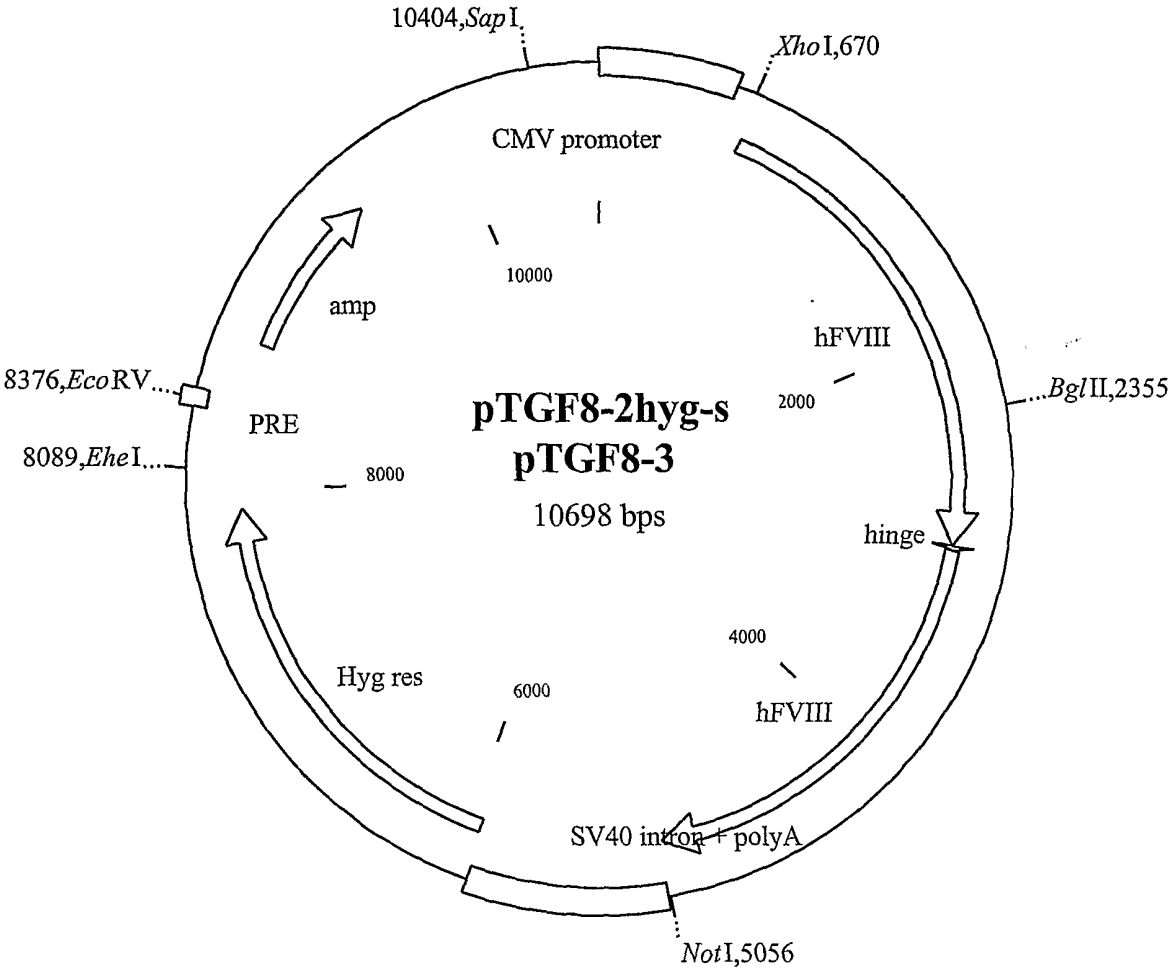


Fig. 6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pTGF8-3
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4000
Hyg res
6000
hFVIII
SV40 intron + polyA
NotI,5056

Fig. 7

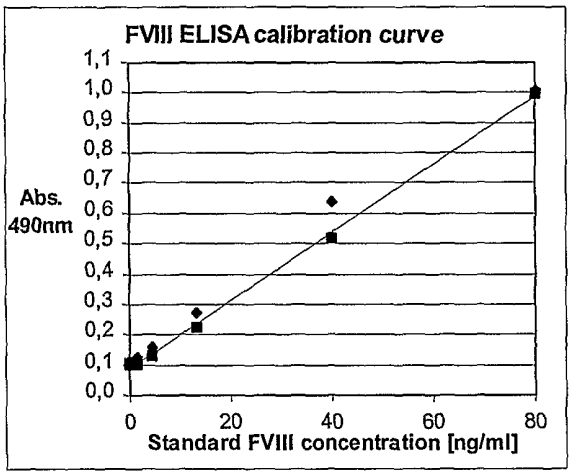


Fig. 7 A

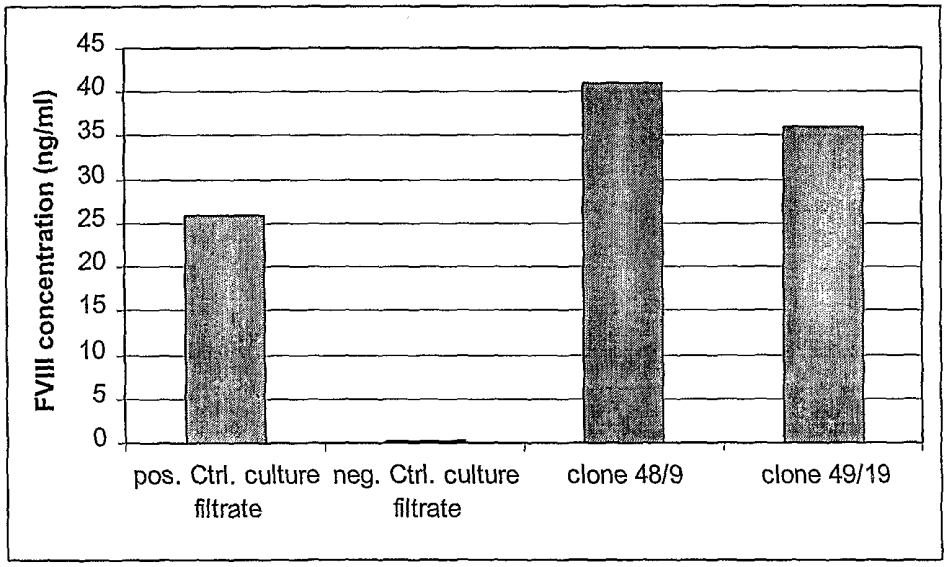


Fig. 7 B

Fig. 8

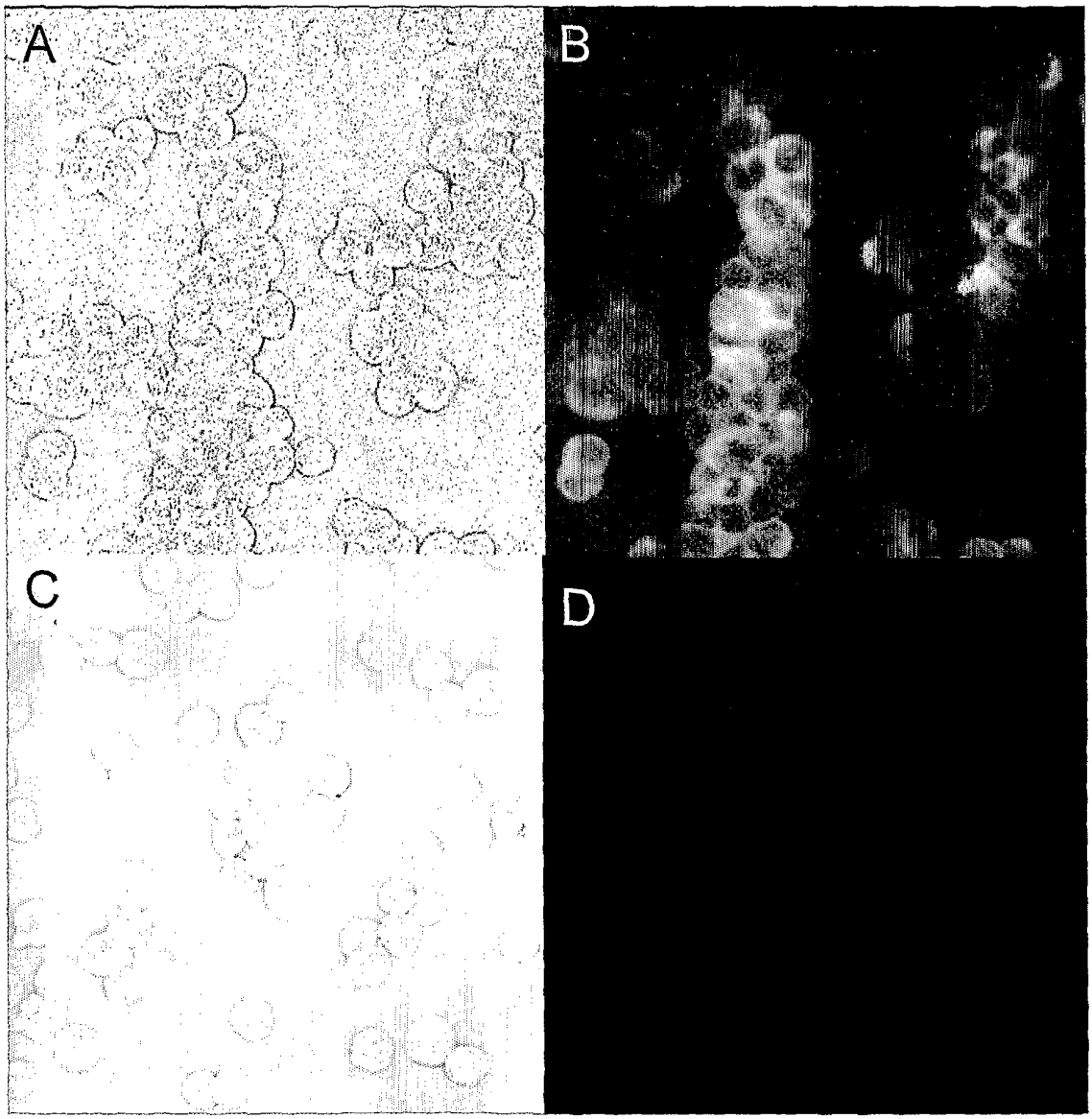


Fig. 9

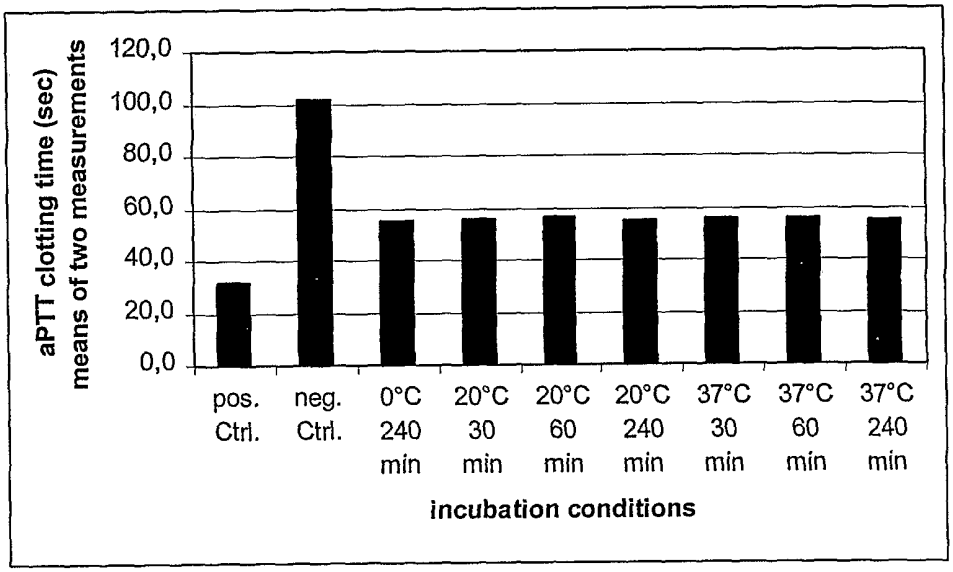
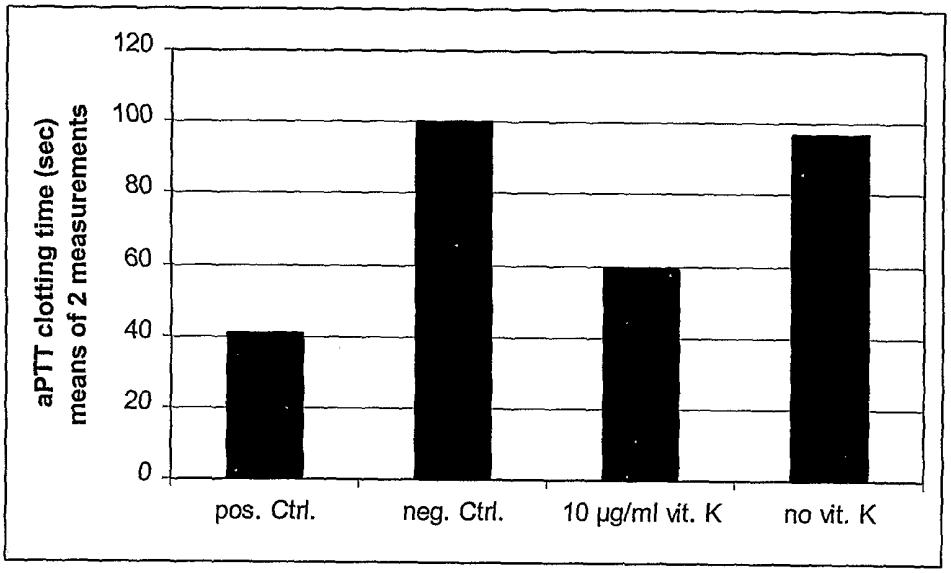

aPTT clotting time (sec)
means of two measurements
120,0
100,0
80,0
60,0
40,0
20,0
0,0
pos. Ctrl.
neg. Ctrl.
0°C 240 min
20°C 30 min
20°C 60 min
20°C 240 min
37°C 30 min
37°C 60 min
37°C 240 min
incubation conditions

Fig. 10


aPTT clotting time (sec)
means of 2 measurements
120
100
80
60
40
20
0
pos. Ctrl.
neg. Ctrl.
10 µg/ml vit. K
no vit. K

SEQUENCE LISTING

<110> Octagene GmbH

<120> Production of Recombinant Blood Clotting Factors in Human Cell Lines

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atg caa agt gat ctc ggt gag ctg cct gtg gac gca aga ttt cct cct   96
Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg Phe Pro Pro
             20                  25                  30

aga gtg cca aaa tct ttt cca ttc aac acc tca gtc gtg tac aaa aag   144
Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val Tyr Lys Lys
         35                  40                  45

act ctg ttt gta gaa ttc acg gtt cac ctt ttc aac atc gct aag cca   192
Thr Leu Phe Val Glu Phe Thr Val His Leu Phe Asn Ile Ala Lys Pro
     50                  55                  60

agg cca ccc tgg atg ggt ctg cta ggt cct acc atc cag gct gag gtt   240
Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln Ala Glu Val
 65                  70                  75                  80

tat gat aca gtg gtc att aca ctt aag aac atg gct tcc cat cct gtc   288
Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser His Pro Val
                 85                  90                  95

agt ctt cat gct gtt ggt gta tcc tac tgg aaa gct tct gag gga gct   336
Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser Glu Gly Ala
            100                 105                 110

gaa tat gat gat cag acc agt caa agg gag aaa gaa gat gat aaa gtc   384
Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp Asp Lys Val
        115                 120                 125

ttc cct ggt gga agc cat aca tat gtc tgg cag gtc ctg aaa gag aat   432
Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu Lys Glu Asn
    130                 135                 140

ggt cca atg gcc tct gac cca ctg tgc ctt acc tac tca tat ctt tct   480
Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser Tyr Leu Ser
145                 150                 155                 160
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cat gtg gac ctg gta aaa gac ttg aat tca ggc ctc att gga gcc cta   528
His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile Gly Ala Leu
                165                 170                 175

cta gta tgt aga gaa ggg agt ctg gcc aag gaa aag aca cag acc ttg   576
Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr Gln Thr Leu
            180                 185                 190

cac aaa ttt ata cta ctt ttt gct gta ttt gat gaa ggg aaa agt tgg   624
His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly Lys Ser Trp
        195                 200                 205

cac tca gaa aca aag aac tcc ttg atg cag gat agg gat gct gca tct   672
His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp Ala Ala Ser
    210                 215                 220

gct cgg gcc tgg cct aaa atg cac aca gtc aat ggt tat gta aac agg   720
Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr Val Asn Arg
225                 230                 235                 240

tct ctg cca ggt ctg att gga tgc cac agg aaa tca gtc tat tgg cat   768
Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val Tyr Trp His
                245                 250                 255

gtg att gga atg ggc acc act cct gaa gtg cac tca ata ttc ctc gaa   816
Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile Phe Leu Glu
            260                 265                 270

ggt cac aca ttt ctt gtg agg aac cat cgc cag gcg tcc ttg gaa atc   864
Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser Leu Glu Ile
        275                 280                 285

tcg cca ata act ttc ctt act gct caa aca ctc ttg atg gac ctt gga   912
Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met Asp Leu Gly
    290                 295                 300

cag ttt cta ctg ttt tgt cat atc tct tcc cac caa cat gat ggc atg   960
Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His Asp Gly Met
305                 310                 315                 320

gaa gct tat gtc aaa gta gac agc tgt cca gag gaa ccc caa cta cga  1008
Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro Gln Leu Arg
                325                 330                 335

atg aaa aat aat gaa gaa gcg gaa gac tat gat gat gat ctt act gat  1056
Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp Leu Thr Asp
            340                 345                 350

tct gaa atg gat gtg gtc agg ttt gat gat gac aac tct cct tcc ttt  1104
Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser Pro Ser Phe
        355                 360                 365

atc caa att cgc tca gtt gcc aag aag cat cct aaa act tgg gta cat  1152
Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr Trp Val His
    370                 375                 380

tac att gct gct gaa gag gag gac tgg gac tat gct ccc tta gtc ctc  1200
Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro Leu Val Leu
385                 390                 395                 400

gcc ccc gat gac aga agt tat aaa agt caa tat ttg aac aat ggc cct  1248
Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn Asn Gly Pro
                405                 410                 415

cag cgg att ggt agg aag tac aaa aaa gtc cga ttt atg gca tac aca  1296
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Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met Ala Tyr Thr
            420                 425                 430

gat gaa acc ttt aag act cgt gaa gct att cag cat gaa tca gga atc   1344
Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu Ser Gly Ile
        435                 440                 445

ttg gga cct tta ctt tat ggg gaa gtt gga gac aca ctg ttg att ata   1392
Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu Leu Ile Ile
    450                 455                 460

ttt aag aat caa gca agc aga cca tat aac atc tac cct cac gga atc   1440
Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro His Gly Ile
465                 470                 475                 480

act gat gtc cgt cct ttg tat tca agg aga tta cca aaa ggt gta aaa   1488
Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys Gly Val Lys
                485                 490                 495

cat ttg aag gat ttt cca att ctg cca gga gaa ata ttc aaa tat aaa   1536
His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe Lys Tyr Lys
            500                 505                 510

tgg aca gtg act gta gaa gat ggg cca act aaa tca gat cct cgg tgc   1584
Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp Pro Arg Cys
        515                 520                 525

ctg acc cgc tat tac tct agt ttc gtt aat atg gag aga gat cta gct   1632
Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg Asp Leu Ala
    530                 535                 540

tca gga ctc att ggc cct ctc ctc atc tgc tac aaa gaa tct gta gat   1680
Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu Ser Val Asp
545                 550                 555                 560

caa aga gga aac cag ata atg tca gac aag agg aat gtc atc ctg ttt   1728
Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val Ile Leu Phe
                565                 570                 575

tct gta ttt gat gag aac cga agc tgg tac ctc aca gag aat ata caa   1776
Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu Asn Ile Gln
            580                 585                 590

cgc ttt ctc ccc aat cca gct gga gtg cag ctt gag gat cca gag ttc   1824
Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp Pro Glu Phe
        595                 600                 605

caa gcc tcc aac atc atg cac agc atc aat ggc tat gtt ttt gat agt   1872
Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val Phe Asp Ser
    610                 615                 620

ttg cag ttg tca gtt tgt ttg cat gag gtg gca tac tgg tac att cta   1920
Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp Tyr Ile Leu
625                 630                 635                 640

agc att gga gca cag act gac ttc ctt tct gtc ttc ttc tct gga tat   1968
Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe Ser Gly Tyr
                645                 650                 655

acc ttc aaa cac aaa atg gtc tat gaa gac aca ctc acc cta ttc cca   2016
Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr Leu Phe Pro
            660                 665                 670

ttc tca gga gaa act gtc ttc atg tcg atg gaa aac cca ggt cta tgg   2064
Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro Gly Leu Trp
        675                 680                 685
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att ctg ggg tgc cac aac tca gac ttt cgg aac aga ggc atg acc gcc   2112
Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly Met Thr Ala
    690                 695                 700

tta ctg aag gtt tct agt tgt gac aag aac act ggt gat tat tac gag   2160
Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp Tyr Tyr Glu
705                 710                 715                 720

gac agt tat gaa gat att tca gca tac ttg ctg agt aaa aac aat gcc   2208
Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys Asn Asn Ala
                725                 730                 735

att gaa cca aga agc ttc tcc cag aat tca aga cac cct agc act agg   2256
Ile Glu Pro Arg Ser Phe Ser Gln Asn Ser Arg His Pro Ser Thr Arg
            740                 745                 750

caa aag caa ttt aat gcc acc aca att cca gaa aat gac ata gag aag   2304
Gln Lys Gln Phe Asn Ala Thr Thr Ile Pro Glu Asn Asp Ile Glu Lys
        755                 760                 765

act gac cct tgg ttt gca cac aga aca cct atg cct aaa ata caa aat   2352
Thr Asp Pro Trp Phe Ala His Arg Thr Pro Met Pro Lys Ile Gln Asn
    770                 775                 780

gtc tcc tct agt gat ttg ttg atg ctc ttg cga cag agt cct act cca   2400
Val Ser Ser Ser Asp Leu Leu Met Leu Leu Arg Gln Ser Pro Thr Pro
785                 790                 795                 800

cat ggg cta tcc tta tct gat ctc caa gaa gcc aaa tat gag act ttt   2448
His Gly Leu Ser Leu Ser Asp Leu Gln Glu Ala Lys Tyr Glu Thr Phe
                805                 810                 815

tct gat gat cca tca cct gga gca ata gac agt aat aac agc ctg tct   2496
Ser Asp Asp Pro Ser Pro Gly Ala Ile Asp Ser Asn Asn Ser Leu Ser
            820                 825                 830

gaa atg aca cac ttc agg cca cag ctc cat cac agt ggg gac atg gta   2544
Glu Met Thr His Phe Arg Pro Gln Leu His His Ser Gly Asp Met Val
        835                 840                 845

ttt acc cct gag tca ggc ctc caa tta aga tta aat gag aaa ctg ggg   2592
Phe Thr Pro Glu Ser Gly Leu Gln Leu Arg Leu Asn Glu Lys Leu Gly
    850                 855                 860

aca act gca gca aca gag ttg aag aaa ctt gat ttc aaa gtt tct agt   2640
Thr Thr Ala Ala Thr Glu Leu Lys Lys Leu Asp Phe Lys Val Ser Ser
865                 870                 875                 880

aca tca aat aat ctg att tca aca att cca tca gac aat ttg gca gca   2688
Thr Ser Asn Asn Leu Ile Ser Thr Ile Pro Ser Asp Asn Leu Ala Ala
                885                 890                 895

ggt act gat aat aca agt tcc tta gga ccc cca agt atg cca gtt cat   2736
Gly Thr Asp Asn Thr Ser Ser Leu Gly Pro Pro Ser Met Pro Val His
            900                 905                 910

tat gat agt caa tta gat acc act cta ttt ggc aaa aag tca tct ccc   2784
Tyr Asp Ser Gln Leu Asp Thr Thr Leu Phe Gly Lys Lys Ser Ser Pro
        915                 920                 925

ctt act gag tct ggt gga cct ctg agc ttg agt gaa gaa aat aat gat   2832
Leu Thr Glu Ser Gly Gly Pro Leu Ser Leu Ser Glu Glu Asn Asn Asp
    930                 935                 940

tca aag ttg tta gaa tca ggt tta atg aat agc caa gaa agt tca tgg   2880
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Ser Lys Leu Leu Glu Ser Gly Leu Met Asn Ser Gln Glu Ser Ser Trp
945                 950                 955                 960

gga aaa aat gta tcg tca aca gag agt ggt agg tta ttt aaa ggg aaa  2928
Gly Lys Asn Val Ser Ser Thr Glu Ser Gly Arg Leu Phe Lys Gly Lys
                965                 970                 975

aga gct cat gga cct gct ttg ttg act aaa gat aat gcc tta ttc aaa  2976
Arg Ala His Gly Pro Ala Leu Leu Thr Lys Asp Asn Ala Leu Phe Lys
            980                 985                 990

gtt agc atc tct ttg tta aag aca aac aaa act tcc aat aat tca gca  3024
Val Ser Ile Ser Leu Leu Lys Thr Asn Lys Thr Ser Asn Asn Ser Ala
        995                1000                1005

act aat aga aag act cac att gat ggc cca tca tta tta att gag aat  3072
Thr Asn Arg Lys Thr His Ile Asp Gly Pro Ser Leu Leu Ile Glu Asn
   1010                1015                1020

agt cca tca gtc tgg caa aat ata tta gaa agt gac act gag ttt aaa  3120
Ser Pro Ser Val Trp Gln Asn Ile Leu Glu Ser Asp Thr Glu Phe Lys
1025                1030                1035                1040

aaa gtg aca cct ttg att cat gac aga atg ctt atg gac aaa aat gct  3168
Lys Val Thr Pro Leu Ile His Asp Arg Met Leu Met Asp Lys Asn Ala
                1045                1050                1055

aca gct ttg agg cta aat cat atg tca aat aaa act act tca tca aaa  3216
Thr Ala Leu Arg Leu Asn His Met Ser Asn Lys Thr Thr Ser Ser Lys
            1060                1065                1070

aac atg gaa atg gtc caa cag aaa aaa gag ggc ccc att cca cca gat  3264
Asn Met Glu Met Val Gln Gln Lys Lys Glu Gly Pro Ile Pro Pro Asp
        1075                1080                1085

gca caa aat cca gat atg tcg ttc ttt aag atg cta ttc ttg cca gaa  3312
Ala Gln Asn Pro Asp Met Ser Phe Phe Lys Met Leu Phe Leu Pro Glu
   1090                1095                1100

tca gca agg tgg ata caa agg act cat gga aag aac tct ctg aac tct  3360
Ser Ala Arg Trp Ile Gln Arg Thr His Gly Lys Asn Ser Leu Asn Ser
1105                1110                1115                1120

ggg caa ggc ccc agt cca aag caa tta gta tcc tta gga cca gaa aaa  3408
Gly Gln Gly Pro Ser Pro Lys Gln Leu Val Ser Leu Gly Pro Glu Lys
                1125                1130                1135

tct gtg gaa ggt cag aat ttc ttg tct gag aaa aac aaa gtg gta gta  3456
Ser Val Glu Gly Gln Asn Phe Leu Ser Glu Lys Asn Lys Val Val Val
            1140                1145                1150

gga aag ggt gaa ttt aca aag gac gta gga ctc aaa gag atg gtt ttt  3504
Gly Lys Gly Glu Phe Thr Lys Asp Val Gly Leu Lys Glu Met Val Phe
        1155                1160                1165

cca agc agc aga aac cta ttt ctt act aac ttg gat aat tta cat gaa  3552
Pro Ser Ser Arg Asn Leu Phe Leu Thr Asn Leu Asp Asn Leu His Glu
   1170                1175                1180

aat aat aca cac aat caa gaa aaa aaa att cag gaa gaa ata gaa aag  3600
Asn Asn Thr His Asn Gln Glu Lys Lys Ile Gln Glu Glu Ile Glu Lys
1185                1190                1195                1200

aag gaa aca tta atc caa gag aat gta gtt ttg cct cag ata cat aca  3648
Lys Glu Thr Leu Ile Gln Glu Asn Val Val Leu Pro Gln Ile His Thr
                1205                1210                1215
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gtg act ggc act aag aat ttc atg aag aac ctt ttc tta ctg agc act   3696
Val Thr Gly Thr Lys Asn Phe Met Lys Asn Leu Phe Leu Leu Ser Thr
          1220                1225                1230

agg caa aat gta gaa ggt tca tat gag ggg gca tat gct cca gta ctt   3744
Arg Gln Asn Val Glu Gly Ser Tyr Glu Gly Ala Tyr Ala Pro Val Leu
       1235                1240                1245

caa gat ttt agg tca tta aat gat tca aca aat aga aca aag aaa cac   3792
Gln Asp Phe Arg Ser Leu Asn Asp Ser Thr Asn Arg Thr Lys Lys His
   1250                1255                1260

aca gct cat ttc tca aaa aaa ggg gag gaa gaa aac ttg gaa ggc ttg   3840
Thr Ala His Phe Ser Lys Lys Gly Glu Glu Glu Asn Leu Glu Gly Leu
1265                1270                1275                1280

gga aat caa acc aag caa att gta gag aaa tat gca tgc acc aca agg   3888
Gly Asn Gln Thr Lys Gln Ile Val Glu Lys Tyr Ala Cys Thr Thr Arg
               1285                1290                1295

ata tct cct aat aca agc cag cag aat ttt gtc acg caa cgt agt aag   3936
Ile Ser Pro Asn Thr Ser Gln Gln Asn Phe Val Thr Gln Arg Ser Lys
          1300                1305                1310

aga gct ttg aaa caa ttc aga ctc cca cta gaa gaa aca gaa ctt gaa   3984
Arg Ala Leu Lys Gln Phe Arg Leu Pro Leu Glu Glu Thr Glu Leu Glu
       1315                1320                1325

aaa agg ata att gtg gat gac acc tca acc cag tgg tcc aaa aac atg   4032
Lys Arg Ile Ile Val Asp Asp Thr Ser Thr Gln Trp Ser Lys Asn Met
   1330                1335                1340

aaa cat ttg acc ccg agc acc ctc aca cag ata gac tac aat gag aag   4080
Lys His Leu Thr Pro Ser Thr Leu Thr Gln Ile Asp Tyr Asn Glu Lys
1345                1350                1355                1360

gag aaa ggg gcc att act cag tct ccc tta tca gat tgc ctt acg agg   4128
Glu Lys Gly Ala Ile Thr Gln Ser Pro Leu Ser Asp Cys Leu Thr Arg
               1365                1370                1375

agt cat agc atc cct caa gca aat aga tct cca tta ccc att gca aag   4176
Ser His Ser Ile Pro Gln Ala Asn Arg Ser Pro Leu Pro Ile Ala Lys
          1380                1385                1390

gta tca tca ttt cca tct att aga cct ata tat ctg acc agg gtc cta   4224
Val Ser Ser Phe Pro Ser Ile Arg Pro Ile Tyr Leu Thr Arg Val Leu
       1395                1400                1405

ttc caa gac aac tct tct cat ctt cca gca gca tct tat aga aag aaa   4272
Phe Gln Asp Asn Ser Ser His Leu Pro Ala Ala Ser Tyr Arg Lys Lys
   1410                1415                1420

gat tct ggg gtc caa gaa agc agt cat ttc tta caa gga gcc aaa aaa   4320
Asp Ser Gly Val Gln Glu Ser Ser His Phe Leu Gln Gly Ala Lys Lys
1425                1430                1435                1440

aat aac ctt tct tta gcc att cta acc ttg gag atg act ggt gat caa   4368
Asn Asn Leu Ser Leu Ala Ile Leu Thr Leu Glu Met Thr Gly Asp Gln
               1445                1450                1455

aga gag gtt ggc tcc ctg ggg aca agt gcc aca aat tca gtc aca tac   4416
Arg Glu Val Gly Ser Leu Gly Thr Ser Ala Thr Asn Ser Val Thr Tyr
          1460                1465                1470

aag aaa gtt gag aac act gtt ctc ccg aaa cca gac ttg ccc aaa aca   4464
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Lys Lys Val Glu Asn Thr Val Leu Pro Lys Pro Asp Leu Pro Lys Thr
      1475                1480                1485

tct ggc aaa gtt gaa ttg ctt cca aaa gtt cac att tat cag aag gac   4512
Ser Gly Lys Val Glu Leu Leu Pro Lys Val His Ile Tyr Gln Lys Asp
   1490                1495                1500

cta ttc cct acg gaa act agc aat ggg tct cct ggc cat ctg gat ctc   4560
Leu Phe Pro Thr Glu Thr Ser Asn Gly Ser Pro Gly His Leu Asp Leu
1505                1510                1515                1520

gtg gaa ggg agc ctt ctt cag gga aca gag gga gcg att aag tgg aat   4608
Val Glu Gly Ser Leu Leu Gln Gly Thr Glu Gly Ala Ile Lys Trp Asn
               1525                1530                1535

gaa gca aac aga cct gga aaa gtt ccc ttt ctg aga gta gca aca gaa   4656
Glu Ala Asn Arg Pro Gly Lys Val Pro Phe Leu Arg Val Ala Thr Glu
           1540                1545                1550

agc tct gca aag act ccc tcc aag cta ttg gat cct ctt gct tgg gat   4704
Ser Ser Ala Lys Thr Pro Ser Lys Leu Leu Asp Pro Leu Ala Trp Asp
      1555                1560                1565

aac cac tat ggt act cag ata cca aaa gaa gag tgg aaa tcc caa gag   4752
Asn His Tyr Gly Thr Gln Ile Pro Lys Glu Glu Trp Lys Ser Gln Glu
   1570                1575                1580

aag tca cca gaa aaa aca gct ttt aag aaa aag gat acc att ttg tcc   4800
Lys Ser Pro Glu Lys Thr Ala Phe Lys Lys Lys Asp Thr Ile Leu Ser
1585                1590                1595                1600

ctg aac gct tgt gaa agc aat cat gca ata gca gca ata aat gag gga   4848
Leu Asn Ala Cys Glu Ser Asn His Ala Ile Ala Ala Ile Asn Glu Gly
               1605                1610                1615

caa aat aag ccc gaa ata gaa gtc acc tgg gca aag caa ggt agg act   4896
Gln Asn Lys Pro Glu Ile Glu Val Thr Trp Ala Lys Gln Gly Arg Thr
           1620                1625                1630

gaa agg ctg tgc tct caa aac cca cca gtc ttg aaa cgc cat caa cgg   4944
Glu Arg Leu Cys Ser Gln Asn Pro Pro Val Leu Lys Arg His Gln Arg
      1635                1640                1645

gaa ata act cgt act act ctt cag tca gat caa gag gaa att gac tat   4992
Glu Ile Thr Arg Thr Thr Leu Gln Ser Asp Gln Glu Glu Ile Asp Tyr
   1650                1655                1660

gat gat acc ata tca gtt gaa atg aag aag gaa gat ttt gac att tat   5040
Asp Asp Thr Ile Ser Val Glu Met Lys Lys Glu Asp Phe Asp Ile Tyr
1665                1670                1675                1680

gat gag gat gaa aat cag agc ccc cgc agc ttt caa aag aaa aca cga   5088
Asp Glu Asp Glu Asn Gln Ser Pro Arg Ser Phe Gln Lys Lys Thr Arg
               1685                1690                1695

cac tat ttt att gct gca gtg gag agg ctc tgg gat tat ggg atg agt   5136
His Tyr Phe Ile Ala Ala Val Glu Arg Leu Trp Asp Tyr Gly Met Ser
           1700                1705                1710

agc tcc cca cat gtt cta aga aac agg gct cag agt ggc agt gtc cct   5184
Ser Ser Pro His Val Leu Arg Asn Arg Ala Gln Ser Gly Ser Val Pro
      1715                1720                1725

cag ttc aag aaa gtt gtt ttc cag gaa ttt act gat ggc tcc ttt act   5232
Gln Phe Lys Lys Val Val Phe Gln Glu Phe Thr Asp Gly Ser Phe Thr
   1730                1735                1740
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cag ccc tta tac cgt gga gaa cta aat gaa cat ttg gga ctc ctg ggg   5280
Gln Pro Leu Tyr Arg Gly Glu Leu Asn Glu His Leu Gly Leu Leu Gly
1745                1750                1755                1760

cca tat ata aga gca gaa gtt gaa gat aat atc atg gta act ttc aga   5328
Pro Tyr Ile Arg Ala Glu Val Glu Asp Asn Ile Met Val Thr Phe Arg
                1765                1770                1775

aat cag gcc tct cgt ccc tat tcc ttc tat tct agc ctt att tct tat   5376
Asn Gln Ala Ser Arg Pro Tyr Ser Phe Tyr Ser Ser Leu Ile Ser Tyr
            1780                1785                1790

gag gaa gat cag agg caa gga gca gaa cct aga aaa aac ttt gtc aag   5424
Glu Glu Asp Gln Arg Gln Gly Ala Glu Pro Arg Lys Asn Phe Val Lys
        1795                1800                1805

cct aat gaa acc aaa act tac ttt tgg aaa gtg caa cat cat atg gca   5472
Pro Asn Glu Thr Lys Thr Tyr Phe Trp Lys Val Gln His His Met Ala
    1810                1815                1820

ccc act aaa gat gag ttt gac tgc aaa gcc tgg gct tat ttc tct gat   5520
Pro Thr Lys Asp Glu Phe Asp Cys Lys Ala Trp Ala Tyr Phe Ser Asp
1825                1830                1835                1840

gtt gac ctg gaa aaa gat gtg cac tca ggc ctg att gga ccc ctt ctg   5568
Val Asp Leu Glu Lys Asp Val His Ser Gly Leu Ile Gly Pro Leu Leu
                1845                1850                1855

gtc tgc cac act aac aca ctg aac cct gct cat ggg aga caa gtg aca   5616
Val Cys His Thr Asn Thr Leu Asn Pro Ala His Gly Arg Gln Val Thr
            1860                1865                1870

gta cag gaa ttt gct ctg ttt ttc acc atc ttt gat gag acc aaa agc   5664
Val Gln Glu Phe Ala Leu Phe Phe Thr Ile Phe Asp Glu Thr Lys Ser
        1875                1880                1885

tgg tac ttc act gaa aat atg gaa aga aac tgc agg gct ccc tgc aat   5712
Trp Tyr Phe Thr Glu Asn Met Glu Arg Asn Cys Arg Ala Pro Cys Asn
    1890                1895                1900

atc cag atg gaa gat ccc act ttt aaa gag aat tat cgc ttc cat gca   5760
Ile Gln Met Glu Asp Pro Thr Phe Lys Glu Asn Tyr Arg Phe His Ala
1905                1910                1915                1920

atc aat ggc tac ata atg gat aca cta cct ggc tta gta atg gct cag   5808
Ile Asn Gly Tyr Ile Met Asp Thr Leu Pro Gly Leu Val Met Ala Gln
                1925                1930                1935

gat caa agg att cga tgg tat ctg ctc agc atg ggc agc aat gaa aac   5856
Asp Gln Arg Ile Arg Trp Tyr Leu Leu Ser Met Gly Ser Asn Glu Asn
            1940                1945                1950

atc cat tct att cat ttc agt gga cat gtg ttc act gta cga aaa aaa   5904
Ile His Ser Ile His Phe Ser Gly His Val Phe Thr Val Arg Lys Lys
        1955                1960                1965

gag gag tat aaa atg gca ctg tac aat ctc tat cca ggt gtt ttt gag   5952
Glu Glu Tyr Lys Met Ala Leu Tyr Asn Leu Tyr Pro Gly Val Phe Glu
    1970                1975                1980

aca gtg gaa atg tta cca tcc aaa gct gga att tgg cgg gtg gaa tgc   6000
Thr Val Glu Met Leu Pro Ser Lys Ala Gly Ile Trp Arg Val Glu Cys
1985                1990                1995                2000

ctt att ggc gag cat cta cat gct ggg atg agc aca ctt ttt ctg gtg   6048
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Leu Ile Gly Glu His Leu His Ala Gly Met Ser Thr Leu Phe Leu Val
                2005                2010                2015

tac agc aat aag tgt cag act ccc ctg gga atg gct tct gga cac att   6096
Tyr Ser Asn Lys Cys Gln Thr Pro Leu Gly Met Ala Ser Gly His Ile
            2020                2025                2030

aga gat ttt cag att aca gct tca gga caa tat gga cag tgg gcc cca   6144
Arg Asp Phe Gln Ile Thr Ala Ser Gly Gln Tyr Gly Gln Trp Ala Pro
        2035                2040                2045

aag ctg gcc aga ctt cat tat tcc gga tca atc aat gcc tgg agc acc   6192
Lys Leu Ala Arg Leu His Tyr Ser Gly Ser Ile Asn Ala Trp Ser Thr
    2050                2055                2060

aag gag ccc ttt tct tgg atc aag gtg gat ctg ttg gca cca atg att   6240
Lys Glu Pro Phe Ser Trp Ile Lys Val Asp Leu Leu Ala Pro Met Ile
2065                2070                2075                2080

att cac ggc atc aag acc cag ggt gcc cgt cag aag ttc tcc agc ctc   6288
Ile His Gly Ile Lys Thr Gln Gly Ala Arg Gln Lys Phe Ser Ser Leu
                2085                2090                2095

tac atc tct cag ttt atc atc atg tat agt ctt gat ggg aag aag tgg   6336
Tyr Ile Ser Gln Phe Ile Ile Met Tyr Ser Leu Asp Gly Lys Lys Trp
            2100                2105                2110

cag act tat cga gga aat tcc act gga acc tta atg gtc ttc ttt ggc   6384
Gln Thr Tyr Arg Gly Asn Ser Thr Gly Thr Leu Met Val Phe Phe Gly
        2115                2120                2125

aat gtg gat tca tct ggg ata aaa cac aat att ttt aac cct cca att   6432
Asn Val Asp Ser Ser Gly Ile Lys His Asn Ile Phe Asn Pro Pro Ile
    2130                2135                2140

att gct cga tac atc cgt ttg cac cca act cat tat agc att cgc agc   6480
Ile Ala Arg Tyr Ile Arg Leu His Pro Thr His Tyr Ser Ile Arg Ser
2145                2150                2155                2160

act ctt cgc atg gag ttg atg ggc tgt gat tta aat agt tgc agc atg   6528
Thr Leu Arg Met Glu Leu Met Gly Cys Asp Leu Asn Ser Cys Ser Met
                2165                2170                2175

cca ttg gga atg gag agt aaa gca ata tca gat gca cag att act gct   6576
Pro Leu Gly Met Glu Ser Lys Ala Ile Ser Asp Ala Gln Ile Thr Ala
            2180                2185                2190

tca tcc tac ttt acc aat atg ttt gcc acc tgg tct cct tca aaa gct   6624
Ser Ser Tyr Phe Thr Asn Met Phe Ala Thr Trp Ser Pro Ser Lys Ala
        2195                2200                2205

cga ctt cac ctc caa ggg agg agt aat gcc tgg aga cct cag gtg aat   6672
Arg Leu His Leu Gln Gly Arg Ser Asn Ala Trp Arg Pro Gln Val Asn
    2210                2215                2220

aat cca aaa gag tgg ctg caa gtg gac ttc cag aag aca atg aaa gtc   6720
Asn Pro Lys Glu Trp Leu Gln Val Asp Phe Gln Lys Thr Met Lys Val
2225                2230                2235                2240

aca gga gta act act cag gga gta aaa tct ctg ctt acc agc atg tat   6768
Thr Gly Val Thr Thr Gln Gly Val Lys Ser Leu Leu Thr Ser Met Tyr
                2245                2250                2255

gtg aag gag ttc ctc atc tcc agc agt caa gat ggc cat cag tgg act   6816
Val Lys Glu Phe Leu Ile Ser Ser Ser Gln Asp Gly His Gln Trp Thr
            2260                2265                2270
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ctc ttt ttt cag aat ggc aaa gta aag gtt ttt cag gga aat caa gac   6864
Leu Phe Phe Gln Asn Gly Lys Val Lys Val Phe Gln Gly Asn Gln Asp
      2275                2280                2285

tcc ttc aca cct gtg gtg aac tct cta gac cca ccg tta ctg act cgc   6912
Ser Phe Thr Pro Val Val Asn Ser Leu Asp Pro Pro Leu Leu Thr Arg
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tac ctt cga att cac ccc cag agt tgg gtg cac cag att gcc ctg agg   6960
Tyr Leu Arg Ile His Pro Gln Ser Trp Val His Gln Ile Ala Leu Arg
2305                2310                2315                2320

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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

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
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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

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
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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

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
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Ser Pro Ser Val Trp Gln Asn Ile Leu Glu Ser Asp Thr Glu Phe Lys
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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
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Gly Gln Gly Pro Ser Pro Lys Gln Leu Val Ser Leu Gly Pro Glu Lys
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Ser Val Glu Gly Gln Asn Phe Leu Ser Glu Lys Asn Lys Val Val Val
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Gly Lys Gly Glu Phe Thr Lys Asp Val Gly Leu Lys Glu Met Val Phe
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Pro Ser Ser Arg Asn Leu Phe Leu Thr Asn Leu Asp Asn Leu His Glu
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Lys Glu Thr Leu Ile Gln Glu Asn Val Val Leu Pro Gln Ile His Thr
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Val Thr Gly Thr Lys Asn Phe Met Lys Asn Leu Phe Leu Leu Ser Thr
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Thr Ala His Phe Ser Lys Lys Gly Glu Glu Glu Asn Leu Glu Gly Leu
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Gly Asn Gln Thr Lys Gln Ile Val Glu Lys Tyr Ala Cys Thr Thr Arg
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Lys Arg Ile Ile Val Asp Asp Thr Ser Thr Gln Trp Ser Lys Asn Met
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Lys His Leu Thr Pro Ser Thr Leu Thr Gln Ile Asp Tyr Asn Glu Lys
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Glu Lys Gly Ala Ile Thr Gln Ser Pro Leu Ser Asp Cys Leu Thr Arg
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Ser His Ser Ile Pro Gln Ala Asn Arg Ser Pro Leu Pro Ile Ala Lys
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Val Ser Ser Phe Pro Ser Ile Arg Pro Ile Tyr Leu Thr Arg Val Leu
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Phe Gln Asp Asn Ser Ser His Leu Pro Ala Ala Ser Tyr Arg Lys Lys
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Asp Ser Gly Val Gln Glu Ser Ser His Phe Leu Gln Gly Ala Lys Lys
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Asn Asn Leu Ser Leu Ala Ile Leu Thr Leu Glu Met Thr Gly Asp Gln
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Lys Lys Val Glu Asn Thr Val Leu Pro Lys Pro Asp Leu Pro Lys Thr
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Ser Gly Lys Val Glu Leu Leu Pro Lys Val His Ile Tyr Gln Lys Asp
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Ser Ser Ala Lys Thr Pro Ser Lys Leu Leu Asp Pro Leu Ala Trp Asp
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Asn His Tyr Gly Thr Gln Ile Pro Lys Glu Glu Trp Lys Ser Gln Glu
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Lys Ser Pro Glu Lys Thr Ala Phe Lys Lys Lys Asp Thr Ile Leu Ser
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Gln Asn Lys Pro Glu Ile Glu Val Thr Trp Ala Lys Gln Gly Arg Thr
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1635 1640 1645

Glu Ile Thr Arg Thr Thr Leu Gln Ser Asp Gln Glu Glu Ile Asp Tyr
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Asp Asp Thr Ile Ser Val Glu Met Lys Lys Glu Asp Phe Asp Ile Tyr
665 1670 1675 1680

Asp Glu Asp Glu Asn Gln Ser Pro Arg Ser Phe Gln Lys Lys Thr Arg
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His Tyr Phe Ile Ala Ala Val Glu Arg Leu Trp Asp Tyr Gly Met Ser
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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
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Gln Pro Leu Tyr Arg Gly Glu Leu Asn Glu His Leu Gly Leu Leu Gly
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1795 1800 1805

Pro Asn Glu Thr Lys Thr Tyr Phe Trp Lys Val Gln His His Met Ala
1810 1815 1820

Pro Thr Lys Asp Glu Phe Asp Cys Lys Ala Trp Ala Tyr Phe Ser Asp
825 1830 1835 1840

Val Asp Leu Glu Lys Asp Val His Ser Gly Leu Ile Gly Pro Leu Leu
1845 1850 1855

Val Cys His Thr Asn Thr Leu Asn Pro Ala His Gly Arg Gln Val Thr
1860 1865 1870

Val Gln Glu Phe Ala Leu Phe Phe Thr Ile Phe Asp Glu Thr Lys Ser
1875 1880 1885

Trp Tyr Phe Thr Glu Asn Met Glu Arg Asn Cys Arg Ala Pro Cys Asn
1890 1895 1900

Ile Gln Met Glu Asp Pro Thr Phe Lys Glu Asn Tyr Arg Phe His Ala
905 1910 1915 1920

Ile Asn Gly Tyr Ile Met Asp Thr Leu Pro Gly Leu Val Met Ala Gln
1925 1930 1935

Asp Gln Arg Ile Arg Trp Tyr Leu Leu Ser Met Gly Ser Asn Glu Asn
1940 1945 1950

Ile His Ser Ile His Phe Ser Gly His Val Phe Thr Val Arg Lys Lys
1955 1960 1965

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1970 1975 1980

Thr Val Glu Met Leu Pro Ser Lys Ala Gly Ile Trp Arg Val Glu Cys
985 1990 1995 2000

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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
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Lys Leu Ala Arg Leu His Tyr Ser Gly Ser Ile Asn Ala Trp Ser Thr
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Lys Glu Pro Phe Ser Trp Ile Lys Val Asp Leu Leu Ala Pro Met Ile
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Ile His Gly Ile Lys Thr Gln Gly Ala Arg Gln Lys Phe Ser Ser Leu
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Tyr Ile Ser Gln Phe Ile Ile Met Tyr Ser Leu Asp Gly Lys Lys Trp
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Gln Thr Tyr Arg Gly Asn Ser Thr Gly Thr Leu Met Val Phe Phe Gly
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Asn Val Asp Ser Ser Gly Ile Lys His Asn Ile Phe Asn Pro Pro Ile
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Ile Ala Arg Tyr Ile Arg Leu His Pro Thr His Tyr Ser Ile Arg Ser
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Thr Leu Arg Met Glu Leu Met Gly Cys Asp Leu Asn Ser Cys Ser Met
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Pro Leu Gly Met Glu Ser Lys Ala Ile Ser Asp Ala Gln Ile Thr Ala
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Arg Leu His Leu Gln Gly Arg Ser Asn Ala Trp Arg Pro Gln Val Asn
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Asn Pro Lys Glu Trp Leu Gln Val Asp Phe Gln Lys Thr Met Lys Val
225 2230 2235 2240

Thr Gly Val Thr Thr Gln Gly Val Lys Ser Leu Leu Thr Ser Met Tyr
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Val Lys Glu Phe Leu Ile Ser Ser Ser Gln Asp Gly His Gln Trp Thr
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Leu Phe Phe Gln Asn Gly Lys Val Lys Val Phe Gln Gly Asn Gln Asp
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Ser Phe Thr Pro Val Val Asn Ser Leu Asp Pro Pro Leu Leu Thr Arg
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ctagagaacc cactgcttac tggcttatcg aaattaatac gactcactat agggagaccc 660

aagcttgacc tcgag atg caa ata gag ctc tcc acc tgc ttc ttt ctg tgc 711
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Val Glu Leu Ser Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro
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gtg gac gca aga ttt cct cct aga gtg cca aaa tct ttt cca ttc aac 855
Val Asp Ala Arg Phe Pro Pro Arg Val Pro Lys Ser Phe Pro Phe Asn
30 35 40

acc tca gtc gtg tac aaa aag act ctg ttt gta gaa ttc acg gat cac 903
Thr Ser Val Val Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr Asp His
45 50 55

ctt ttc aac atc gct aag cca agg cca ccc tgg atg ggt ctg cta ggt 951
Leu Phe Asn Ile Ala Lys Pro Arg Pro Pro Trp Met Gly Leu Leu Gly
60 65 70

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Asn Met Ala Ser His Pro Val Ser Leu His Ala Val Gly Val Ser Tyr
 90                  95                 100                 105

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Trp Lys Ala Ser Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser Gln Arg
                110                 115                 120

gag aaa gaa gat gat aaa gtc ttc cct ggt gga agc cat aca tat gtc  1143
Glu Lys Glu Asp Asp Lys Val Phe Pro Gly Gly Ser His Thr Tyr Val
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tgg cag gtc ctg aaa gag aat ggt cca atg gcc tct gac cca ctg tgc  1191
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 gcg gac ctg gta aaa gac ttg aat  1239
Leu Thr Tyr Ser Tyr Leu Ser His Ala Asp Leu Val Lys Asp Leu Asn
    155                 160                 165

tca ggc ctc att gga gcc cta cta gta tgt aga gaa ggg agt ctg gcc  1287
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  1335
Lys Glu Lys Thr Gln Thr Leu His Lys Phe Ile Leu Leu Phe Ala Val
                190                 195                 200

ttt gat gaa ggg aaa agt tgg cac tca gaa aca aag aac tcc ttg atg  1383
Phe Asp Glu Gly Lys Ser Trp His Ser Glu Thr Lys Asn Ser Leu Met
            205                 210                 215

cag gat agg gat gct gca tct gct cgg gcc tgg cct aaa atg cac aca  1431
Gln Asp Arg Asp Ala Ala Ser Ala Arg Ala Trp Pro Lys Met His Thr
        220                 225                 230

gtc aat ggt tat gta aac agg tct ctg cca ggt ctg att gga tgc cac  1479
Val Asn Gly Tyr Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His
    235                 240                 245

agg aaa tca gtc tat tgg cat gtg att gga atg ggc acc act cct gaa  1527
Arg Lys Ser Val Tyr Trp His Val Ile Gly Met Gly Thr Thr Pro Glu
250                 255                 260                 265

gtg cac tca ata ttc ctc gaa ggt cac aca ttt ctt gtg agg aac cat  1575
Val His Ser Ile Phe Leu Glu Gly His Thr Phe Leu Val Arg Asn His
                270                 275                 280

cgc cag gcg tcc ttg gaa atc tcg cca ata act ttc ctt act gct caa  1623
Arg Gln Ala Ser Leu Glu Ile Ser Pro Ile Thr Phe Leu Thr Ala Gln
            285                 290                 295

aca ctc ttg atg gac ctt gga cag ttt cta ctg ttt tgt cat atc tct  1671
Thr Leu Leu Met Asp Leu Gly Gln Phe Leu Leu Phe Cys His Ile Ser
        300                 305                 310

tcc cac caa cat gat ggc atg gaa gct tat gtc aaa gta gac agc tgt  1719
Ser His Gln His Asp Gly Met Glu Ala Tyr Val Lys Val Asp Ser Cys
    315                 320                 325

cca gag gaa ccc caa cta cga atg aaa aat aat gaa gaa gcg gaa gac  1767
Pro Glu Glu Pro Gln Leu Arg Met Lys Asn Asn Glu Glu Ala Glu Asp
330                 335                 340                 345
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tat gat gat gat ctt act gat tct gaa atg gat gtg gtc agg ttt gat   1815
Tyr Asp Asp Asp Leu Thr Asp Ser Glu Met Asp Val Val Arg Phe Asp
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gat gac aac tct cct tcc ttt atc caa att cgc tca gtt gcc aag aag   1863
Asp Asp Asn Ser Pro Ser Phe Ile Gln Ile Arg Ser Val Ala Lys Lys
            365                 370                 375

cat cct aaa act tgg gta cat tac att gct gct gaa gag gag gac tgg   1911
His Pro Lys Thr Trp Val His Tyr Ile Ala Ala Glu Glu Glu Asp Trp
        380                 385                 390

gac tat gct ccc tta gtc ctc gcc ccc gat gac aga agt tat aaa agt   1959
Asp Tyr Ala Pro Leu Val Leu Ala Pro Asp Asp Arg Ser Tyr Lys Ser
    395                 400                 405

caa tat ttg aac aat ggc cct cag cgg att ggt agg aag tac aaa aaa   2007
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   2055
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   2103
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   2151
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   2199
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   2247
Arg Leu Pro Lys Gly Val Lys His Leu Lys Asp Phe Pro Ile Leu Pro
490                 495                 500                 505

gga gaa ata ttc aaa tat aaa tgg aca gtg act gta gaa gat ggg cca   2295
Gly Glu Ile Phe Lys Tyr Lys Trp Thr Val Thr Val Glu Asp Gly Pro
                510                 515                 520

act aaa tca gat cct cgg tgc ctg acc cgc tat tac tct agt ttc gtt   2343
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   2391
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   2439
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   2487
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   2535
Tyr Leu Thr Glu Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala Gly Val
                590                 595                 600

cag ctt gag gat cca gag ttc caa gcc tcc aac atc atg cac agc atc   2583
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Gln Leu Glu Asp Pro Glu Phe Gln Ala Ser Asn Ile Met His Ser Ile
            605                 610                 615

aat ggc tat gtt ttt gat agt ttg cag ttg tca gtt tgt ttg cat gag   2631
Asn Gly Tyr Val Phe Asp Ser Leu Gln Leu Ser Val Cys Leu His Glu
        620                 625                 630

gtg gca tac tgg tac att cta agc att gga gca cag act gac ttc ctt   2679
Val Ala Tyr Trp Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp Phe Leu
    635                 640                 645

tct gtc ttc ttc tct gga tat acc ttc aaa cac aaa atg gtc tat gaa   2727
Ser Val Phe Phe Ser Gly Tyr Thr Phe Lys His Lys Met Val Tyr Glu
650                 655                 660                 665

gac aca ctc acc cta ttc cca ttc tca gga gaa act gtc ttc atg tcg   2775
Asp Thr Leu Thr Leu Phe Pro Phe Ser Gly Glu Thr Val Phe Met Ser
                670                 675                 680

atg gaa aac cca ggt cta tgg att ctg ggg tgc cac aac tca gac ttt   2823
Met Glu Asn Pro Gly Leu Trp Ile Leu Gly Cys His Asn Ser Asp Phe
            685                 690                 695

cgg aac aga ggc atg acc gcc tta ctg aag gtt tct agt tgt gac aag   2871
Arg Asn Arg Gly Met Thr Ala Leu Leu Lys Val Ser Ser Cys Asp Lys
        700                 705                 710

aac act ggt gat tat tac gag gac agt tat gaa gat att tca gca tac   2919
Asn Thr Gly Asp Tyr Tyr Glu Asp Ser Tyr Glu Asp Ile Ser Ala Tyr
    715                 720                 725

ttg ctg agt aaa aac aat gcc att gaa cca aga agc ttc tcc cag aat   2967
Leu Leu Ser Lys Asn Asn Ala Ile Glu Pro Arg Ser Phe Ser Gln Asn
730                 735                 740                 745

tca aga cat caa gct tat cga tac cgt cga ggg gaa ata act cgt act   3015
Ser Arg His Gln Ala Tyr Arg Tyr Arg Arg Gly Glu Ile Thr Arg Thr
                750                 755                 760

act ctt cag tca gat caa gag gaa att gac tat gat gat acc ata tca   3063
Thr Leu Gln Ser Asp Gln Glu Glu Ile Asp Tyr Asp Asp Thr Ile Ser
            765                 770                 775

gtt gaa atg aag aag gaa gat ttt gac att tat gat gag gat gaa aat   3111
Val Glu Met Lys Lys Glu Asp Phe Asp Ile Tyr Asp Glu Asp Glu Asn
        780                 785                 790

cag agc ccc cgc agc ttt caa aag aaa aca cga cac tat ttt att gct   3159
Gln Ser Pro Arg Ser Phe Gln Lys Lys Thr Arg His Tyr Phe Ile Ala
    795                 800                 805

gca gtg gag agg ctc tgg gat tat ggg atg agt agc tcc cca cat gtt   3207
Ala Val Glu Arg Leu Trp Asp Tyr Gly Met Ser Ser Ser Pro His Val
810                 815                 820                 825

cta aga aac agg gct cag agt ggc agt gtc cct cag ttc aag aaa gtt   3255
Leu Arg Asn Arg Ala Gln Ser Gly Ser Val Pro Gln Phe Lys Lys Val
                830                 835                 840

gtt ttc cag gaa ttt act gat ggc tcc ttt act cag ccc tta tac cgt   3303
Val Phe Gln Glu Phe Thr Asp Gly Ser Phe Thr Gln Pro Leu Tyr Arg
            845                 850                 855

gga gaa cta aat gaa cat ttg gga ctc ctg ggg cca tat ata aga gca   3351
Gly Glu Leu Asn Glu His Leu Gly Leu Leu Gly Pro Tyr Ile Arg Ala
        860                 865                 870
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gaa gtt gaa gat aat atc atg gta act ttc aga aat cag gcc tct cgt   3399
Glu Val Glu Asp Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser Arg
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ccc tat tcc ttc tat tct agc ctt att tct tat gag gaa gat cag agg   3447
Pro Tyr Ser Phe Tyr Ser Ser Leu Ile Ser Tyr Glu Glu Asp Gln Arg
890                 895                 900                 905

caa gga gca gaa cct aga aaa aac ttt gtc aag cct aat gaa acc aaa   3495
Gln Gly Ala Glu Pro Arg Lys Asn Phe Val Lys Pro Asn Glu Thr Lys
                910                 915                 920

act tac ttt tgg aaa gtg caa cat cat atg gca ccc act aaa gat gag   3543
Thr Tyr Phe Trp Lys Val Gln His His Met Ala Pro Thr Lys Asp Glu
            925                 930                 935

ttt gac tgc aaa gcc tgg gct tat ttc tct gat gtt gac ctg gaa aaa   3591
Phe Asp Cys Lys Ala Trp Ala Tyr Phe Ser Asp Val Asp Leu Glu Lys
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gat gtg cac tca ggc ctg att gga ccc ctt ctg gtc tgc cac act aac   3639
Asp Val His Ser Gly Leu Ile Gly Pro Leu Leu Val Cys His Thr Asn
    955                 960                 965

aca ctg aac cct gct cat ggg aga caa gtg aca gta cag gaa ttt gct   3687
Thr Leu Asn Pro Ala His Gly Arg Gln Val Thr Val Gln Glu Phe Ala
970                 975                 980                 985

ctg ttt ttc acc atc ttt gat gag acc aaa agc tgg tac ttc act gaa   3735
Leu Phe Phe Thr Ile Phe Asp Glu Thr Lys Ser Trp Tyr Phe Thr Glu
                990                 995                1000

aat atg gaa aga aac tgc agg gct ccc tgc aat atc cag atg gaa gat   3783
Asn Met Glu Arg Asn Cys Arg Ala Pro Cys Asn Ile Gln Met Glu Asp
            1005                1010                1015

ccc act ttt aaa gag aat tat cgc ttc cat gca atc aat ggc tac ata   3831
Pro Thr Phe Lys Glu Asn Tyr Arg Phe His Ala Ile Asn Gly Tyr Ile
        1020                1025                1030

atg gat aca cta cct ggc tta gta atg gct cag gat caa agg att cga   3879
Met Asp Thr Leu Pro Gly Leu Val Met Ala Gln Asp Gln Arg Ile Arg
    1035                1040                1045

tgg tat ctg ctc agc atg ggc agc aat gaa aac atc cat tct att cat   3927
Trp Tyr Leu Leu Ser Met Gly Ser Asn Glu Asn Ile His Ser Ile His
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ttc agt gga cat gtg ttc act gta cga aaa aaa gag gag tat aaa atg   3975
Phe Ser Gly His Val Phe Thr Val Arg Lys Lys Glu Glu Tyr Lys Met
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gca ctg tac aat ctc tat cca ggt gtt ttt gag aca gtg gaa atg tta   4023
Ala Leu Tyr Asn Leu Tyr Pro Gly Val Phe Glu Thr Val Glu Met Leu
            1085                1090                1095

cca tcc aaa gct gga att tgg cgg gtg gaa tgc ctt att ggc gag cat   4071
Pro Ser Lys Ala Gly Ile Trp Arg Val Glu Cys Leu Ile Gly Glu His
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cta cat gct ggg atg aac aca ctt ttt ctg gtg tac agc aat aag tgt   4119
Leu His Ala Gly Met Asn Thr Leu Phe Leu Val Tyr Ser Asn Lys Cys
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Gln Thr Pro Leu Gly Met Ala Ser Gly His Ile Arg Asp Phe Gln Ile
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Thr Ala Ser Gly Gln Tyr Gly Gln Trp Ala Pro Lys Leu Ala Arg Leu
                1150                1155                1160

cat tat tcc gga tca atc aat gcc tgg agc acc aag gag ccc ttt tct   4263
His Tyr Ser Gly Ser Ile Asn Ala Trp Ser Thr Lys Glu Pro Phe Ser
            1165                1170                1175

tgg atc aag gtg gat ctg ttg gca cca atg att att cac ggc atc aag   4311
Trp Ile Lys Val Asp Leu Leu Ala Pro Met Ile Ile His Gly Ile Lys
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acc cag ggt gcc cgt cag aag ttc tcc agc ctc tac atc tct cag ttt   4359
Thr Gln Gly Ala Arg Gln Lys Phe Ser Ser Leu Tyr Ile Ser Gln Phe
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atc atc atg tat agt ctt gat ggg aag aag tgg cag act tat cga gga   4407
Ile Ile Met Tyr Ser Leu Asp Gly Lys Lys Trp Gln Thr Tyr Arg Gly
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aat tcc act gga acc tta atg gtc ttc ttt ggc aat gtg gat tca tct   4455
Asn Ser Thr Gly Thr Leu Met Val Phe Phe Gly Asn Val Asp Ser Ser
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ggg ata aaa cac aat att ttt aac cct cca att att gct cga tac atc   4503
Gly Ile Lys His Asn Ile Phe Asn Pro Pro Ile Ile Ala Arg Tyr Ile
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cgt ttg cac cca act cat tat agc att cgc agc act ctt cgc atg gag   4551
Arg Leu His Pro Thr His Tyr Ser Ile Arg Ser Thr Leu Arg Met Glu
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ttg atg ggc tgt gat tta aat agt tgc agc atg cca ttg gga atg gag   4599
Leu Met Gly Cys Asp Leu Asn Ser Cys Ser Met Pro Leu Gly Met Glu
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agt aaa gca ata tca gat gca cag att act gct tca tcc tac ttt acc   4647
Ser Lys Ala Ile Ser Asp Ala Gln Ile Thr Ala Ser Ser Tyr Phe Thr
1290                1295                1300                1305

aat atg ttt gcc acc tgg tct cct tca aaa gct cga ctt cac ctc caa   4695
Asn Met Phe Ala Thr Trp Ser Pro Ser Lys Ala Arg Leu His Leu Gln
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ggg agg agt aat gcc tgg aga cct cag gag aat aat cca aaa gag tgg   4743
Gly Arg Ser Asn Ala Trp Arg Pro Gln Glu Asn Asn Pro Lys Glu Trp
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ctg caa gtg gac ttc cag aag aca atg aaa gtc aca gga gta act act   4791
Leu Gln Val Asp Phe Gln Lys Thr Met Lys Val Thr Gly Val Thr Thr
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cag gga gta aaa tct ctg ctt acc agc atg tat gtg aag gag ttc ctc   4839
Gln Gly Val Lys Ser Leu Leu Thr Ser Met Tyr Val Lys Glu Phe Leu
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atc tcc agc agt caa gat ggc cat cag tgg acc ctc ttt ttt cag aat   4887
Ile Ser Ser Ser Gln Asp Gly His Gln Trp Thr Leu Phe Phe Gln Asn
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Val Asn Ser Leu Asp Pro Pro Leu Leu Thr Arg Tyr Leu Arg Ile His
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         -1   1                5                  10

Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg
     15                  20                  25

Phe Pro Pro Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val
 30                  35                  40                  45

Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr Asp His Leu Phe Asn Ile
                 50                  55                  60

Ala Lys Pro Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln
             65                  70                  75

Ala Glu Val Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser
         80                  85                  90

His Pro Val Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser
     95                  100                 105

Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp
110                 115                 120                 125

Asp Lys Val Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu
                130                 135                 140

Lys Glu Asn Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser
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Tyr Leu Ser His Ala Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile
        160                 165                 170

Gly Ala Leu Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr
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Gln Thr Leu His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly
190                 195                 200                 205

Lys Ser Trp His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp
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Ala Ala Ser Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr
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Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val
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Tyr Trp His Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile
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Phe Leu Glu Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser
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Leu Glu Ile Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met
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Asp Gly Met Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro
320 325 330

Gln Leu Arg Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp
335 340 345

Leu Thr Asp Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser
350 355 360 365

Pro Ser Phe Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr
370 375 380

Trp Val His Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro
385 390 395

Leu Val Leu Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn
400 405 410

Asn Gly Pro Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met
415 420 425

Ala Tyr Thr Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu
430 435 440 445

Ser Gly Ile Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu
450 455 460

Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro
465 470 475

His Gly Ile Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys
480 485 490

Gly Val Lys His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe
495 500 505

Lys Tyr Lys Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp
510 515 520 525

Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg
530 535 540

Asp Leu Ala Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu
545 550 555

Ser Val Asp Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val
560 565 570

Ile Leu Phe Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu
575 580 585

Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp
590 595 600 605

Pro Glu Phe Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val
610 615 620

Phe Asp Ser Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp

625 630 635

Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe
640 645 650

Ser Gly Tyr Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr
655 660 665

Leu Phe Pro Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro
670 675 680 685

Gly Leu Trp Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly
690 695 700

Met Thr Ala Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp
705 710 715

Tyr Tyr Glu Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys
720 725 730

Asn Asn Ala Ile Glu Pro Arg Ser Phe Ser Gln Asn Ser Arg His Gln
735 740 745

Ala Tyr Arg Tyr Arg Arg Gly Glu Ile Thr Arg Thr Thr Leu Gln Ser
750 755 760 765

Asp Gln Glu Glu Ile Asp Tyr Asp Asp Thr Ile Ser Val Glu Met Lys
770 775 780

Lys Glu Asp Phe Asp Ile Tyr Asp Glu Asp Glu Asn Gln Ser Pro Arg
785 790 795

Ser Phe Gln Lys Lys Thr Arg His Tyr Phe Ile Ala Ala Val Glu Arg
800 805 810

Leu Trp Asp Tyr Gly Met Ser Ser Ser Pro His Val Leu Arg Asn Arg
815 820 825

Ala Gln Ser Gly Ser Val Pro Gln Phe Lys Lys Val Val Phe Gln Glu
830 835 840 845

Phe Thr Asp Gly Ser Phe Thr Gln Pro Leu Tyr Arg Gly Glu Leu Asn
850 855 860

Glu His Leu Gly Leu Leu Gly Pro Tyr Ile Arg Ala Glu Val Glu Asp
865 870 875

Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser Arg Pro Tyr Ser Phe
880 885 890

Tyr Ser Ser Leu Ile Ser Tyr Glu Glu Asp Gln Arg Gln Gly Ala Glu
895 900 905

Pro Arg Lys Asn Phe Val Lys Pro Asn Glu Thr Lys Thr Tyr Phe Trp
910 915 920 925

Lys Val Gln His His Met Ala Pro Thr Lys Asp Glu Phe Asp Cys Lys
930 935 940

Ala Trp Ala Tyr Phe Ser Asp Val Asp Leu Glu Lys Asp Val His Ser
945 950 955

Gly Leu Ile Gly Pro Leu Leu Val Cys His Thr Asn Thr Leu Asn Pro
960 965 970

Ala His Gly Arg Gln Val Thr Val Gln Glu Phe Ala Leu Phe Phe Thr

975 980 985

Ile Phe Asp Glu Thr Lys Ser Trp Tyr Phe Thr Glu Asn Met Glu Arg
990 995 1000 1005

Asn Cys Arg Ala Pro Cys Asn Ile Gln Met Glu Asp Pro Thr Phe Lys
1010 1015 1020

Glu Asn Tyr Arg Phe His Ala Ile Asn Gly Tyr Ile Met Asp Thr Leu
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Pro Gly Leu Val Met Ala Gln Asp Gln Arg Ile Arg Trp Tyr Leu Leu
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Ser Met Gly Ser Asn Glu Asn Ile His Ser Ile His Phe Ser Gly His
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Val Phe Thr Val Arg Lys Lys Glu Glu Tyr Lys Met Ala Leu Tyr Asn
070 1075 1080 1085

Leu Tyr Pro Gly Val Phe Glu Thr Val Glu Met Leu Pro Ser Lys Ala
1090 1095 1100

Gly Ile Trp Arg Val Glu Cys Leu Ile Gly Glu His Leu His Ala Gly
1105 1110 1115

Met Asn Thr Leu Phe Leu Val Tyr Ser Asn Lys Cys Gln Thr Pro Leu
1120 1125 1130

Gly Met Ala Ser Gly His Ile Arg Asp Phe Gln Ile Thr Ala Ser Gly
1135 1140 1145

Gln Tyr Gly Gln Trp Ala Pro Lys Leu Ala Arg Leu His Tyr Ser Gly
150 1155 1160 1165

Ser Ile Asn Ala Trp Ser Thr Lys Glu Pro Phe Ser Trp Ile Lys Val
1170 1175 1180

Asp Leu Leu Ala Pro Met Ile Ile His Gly Ile Lys Thr Gln Gly Ala
1185 1190 1195

Arg Gln Lys Phe Ser Ser Leu Tyr Ile Ser Gln Phe Ile Ile Met Tyr
1200 1205 1210

Ser Leu Asp Gly Lys Lys Trp Gln Thr Tyr Arg Gly Asn Ser Thr Gly
1215 1220 1225

Thr Leu Met Val Phe Phe Gly Asn Val Asp Ser Ser Gly Ile Lys His
230 1235 1240 1245

Asn Ile Phe Asn Pro Pro Ile Ile Ala Arg Tyr Ile Arg Leu His Pro
1250 1255 1260

Thr His Tyr Ser Ile Arg Ser Thr Leu Arg Met Glu Leu Met Gly Cys
1265 1270 1275

Asp Leu Asn Ser Cys Ser Met Pro Leu Gly Met Glu Ser Lys Ala Ile
1280 1285 1290

Ser Asp Ala Gln Ile Thr Ala Ser Ser Tyr Phe Thr Asn Met Phe Ala
1295 1300 1305

Thr Trp Ser Pro Ser Lys Ala Arg Leu His Leu Gln Gly Arg Ser Asn
310 1315 1320 1325

Ala Trp Arg Pro Gln Glu Asn Asn Pro Lys Glu Trp Leu Gln Val Asp

1330 1335 1340

Phe Gln Lys Thr Met Lys Val Thr Gly Val Thr Thr Gln Gly Val Lys
1345 1350 1355

Ser Leu Leu Thr Ser Met Tyr Val Lys Glu Phe Leu Ile Ser Ser Ser
1360 1365 1370

Gln Asp Gly His Gln Trp Thr Leu Phe Phe Gln Asn Gly Lys Val Lys
1375 1380 1385

Val Phe Gln Gly Asn Gln Asp Ser Phe Thr Pro Val Val Asn Ser Leu
390 1395 1400 1405

Asp Pro Pro Leu Leu Thr Arg Tyr Leu Arg Ile His Pro Gln Ser Trp
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Val His Gln Ile Ala Leu Arg Met Glu Val Leu Gly Cys Glu Ala Gln
1425 1430 1435

Asp Leu Tyr
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Asp His Glu Asn Ala Asn Lys Ile Leu Asn Arg Pro Lys Arg Tyr Asn
35 40 45

Ser Gly Lys Leu Glu Glu Phe Val Gln Gly Asn Leu Glu Arg Glu Cys
50 55 60

Met Glu Glu Lys Cys Ser Phe Glu Glu Ala Arg Glu Val Phe Glu Asn
65 70 75 80

Thr Glu Arg Thr Thr Glu Phe Trp Lys Gln Tyr Val Asp Gly Asp Gln
85 90 95

Cys Glu Ser Asn Pro Cys Leu Asn Gly Gly Ser Cys Lys Asp Asp Ile
100 105 110

Asn Ser Tyr Glu Cys Trp Cys Pro Phe Gly Phe Glu Gly Lys Asn Cys
115 120 125

Glu Leu Asp Val Thr Cys Asn Ile Lys Asn Gly Arg Cys Glu Gln Phe
130 135 140

Cys Lys Asn Ser Ala Asp Asn Lys Val Val Cys Ser Cys Thr Glu Gly
145 150 155 160

Tyr Arg Leu Ala Glu Asn Gln Lys Ser Cys Glu Pro Ala Val Pro Phe
165 170 175

Pro Cys Gly Arg Val Ser Val Ser Gln Thr Ser Lys Leu Thr Arg Ala
180 185 190

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Glu Ala Val Phe Pro Asp Val Asp Tyr Val Asn Ser Thr Glu Ala Glu
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Thr Ile Leu Asp Asn Ile Thr Gln Ser Thr Gln Ser Phe Asn Asp Phe
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Thr Arg Val Val Gly Gly Glu Asp Ala Lys Pro Gly Gln Phe Pro Trp
225                 230                 235                 240

Gln Val Val Leu Asn Gly Lys Val Asp Ala Phe Cys Gly Gly Ser Ile
                245                 250                 255

Val Asn Glu Lys Trp Ile Val Thr Ala Ala His Cys Val Glu Thr Gly
            260                 265                 270

Val Lys Ile Thr Val Val Ala Gly Glu His Asn Ile Glu Glu Thr Glu
        275                 280                 285

His Thr Glu Gln Lys Arg Asn Val Ile Arg Ile Ile Pro His His Asn
    290                 295                 300

Tyr Asn Ala Ala Ile Asn Lys Tyr Asn His Asp Ile Ala Leu Leu Glu
305                 310                 315                 320

Leu Asp Glu Pro Leu Val Leu Asn Ser Tyr Val Thr Pro Ile Cys Ile
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Ala Asp Lys Glu Tyr Thr Asn Ile Phe Leu Lys Phe Gly Ser Gly Tyr
            340                 345                 350

Val Ser Gly Trp Gly Arg Val Phe His Lys Gly Arg Ser Ala Leu Val
        355                 360                 365

Leu Gln Tyr Leu Arg Val Pro Leu Val Asp Arg Ala Thr Cys Leu Arg
    370                 375                 380

Ser Thr Lys Phe Thr Ile Tyr Asn Asn Met Phe Cys Ala Gly Phe His
385                 390                 395                 400

Glu Gly Gly Arg Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro His Val
                405                 410                 415

Thr Glu Val Glu Gly Thr Ser Phe Leu Thr Gly Ile Ile Ser Trp Gly
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Glu Glu Cys Ala Met Lys Gly Lys Tyr Gly Ile Tyr Thr Lys Val Ser
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<211> 5753
<212> DNA
<213> Artificial Sequence

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<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: B-domain linker peptide

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<210> 8
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: B-domain linker peptide

<400> 8
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1 5

<210> 9
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
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<212> DNA
<213> Homo sapiens

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<210> 11
<211> 78
<212> DNA
<213> Homo sapiens

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ctacgaagct ggtacccc 78

<210> 12
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<212> DNA
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<220>
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cgcccaacga cccccgccca ttgacgtcaa taatgacgta tgttcccata gtaacgccaa 180

tagggacttt ccattgacgt caatgggtgg agtatttacg gtaaactgcc cacttggcag 240

tacatcaagt gtatcatatg ccaagtacgc cccctattga cgtcaatgac ggtaaatggc 300

ccgcctggca ttatgcccag tacatgacct tatgggactt tcctacttgg cagtacatct 360

acgtattagt catcgctatt accatggtga tgcggttttg gcagtacatc aatgggcgtg 420

gatagcggtt tgactcacgg ggatttccaa gtctccaccc cattgacgtc aatgggagtt 480

tgttttggca ccaaaatcaa cgggactttc caaaatgtcg taacaactcc gccccattga 540

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ctagagaacc cactgcttac tggcttatcg aaattaatac gactcactat agggagaccc 660

aagcttgacc tcgag atg caa ata gag ctc tcc acc tgc ttc ttt ctg tgc 711
Met Gln Ile Glu Leu Ser Thr Cys Phe Phe Leu Cys
-15 -10

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ctt ttg cga ttc tgc ttt agt gcc acc aga aga tac tac ctg ggt gca   759
Leu Leu Arg Phe Cys Phe Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala
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gtg gaa ctg tca tgg gac tat atg caa agt gat ctc ggt gag ctg cct   807
Val Glu Leu Ser Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro
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gtg gac gca aga ttt cct cct aga gtg cca aaa tct ttt cca ttc aac   855
Val Asp Ala Arg Phe Pro Pro Arg Val Pro Lys Ser Phe Pro Phe Asn
                 30                  35                  40

acc tca gtc gtg tac aaa aag act ctg ttt gta gaa ttc acg gat cac   903
Thr Ser Val Val Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr Asp His
             45                  50                  55

ctt ttc aac atc gct aag cca agg cca ccc tgg atg ggt ctg cta ggt   951
Leu Phe Asn Ile Ala Lys Pro Arg Pro Pro Trp Met Gly Leu Leu Gly
         60                  65                  70

cct acc atc cag gct gag gtt tat gat aca gtg gtc att aca ctt aag   999
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  1047
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  1095
Trp Lys Ala Ser Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser Gln Arg
                110                 115                 120

gag aaa gaa gat gat aaa gtc ttc cct ggt gga agc cat aca tat gtc  1143
Glu Lys Glu Asp Asp Lys Val Phe Pro Gly Gly Ser His Thr Tyr Val
            125                 130                 135

tgg cag gtc ctg aaa gag aat ggt cca atg gcc tct gac cca ctg tgc  1191
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  1239
Leu Thr Tyr Ser Tyr Leu Ser His Val Asp Leu Val Lys Asp Leu Asn
    155                 160                 165

tca ggc ctc att gga gcc cta cta gta tgt aga gaa ggg agt ctg gcc  1287
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  1335
Lys Glu Lys Thr Gln Thr Leu His Lys Phe Ile Leu Leu Phe Ala Val
                190                 195                 200

ttt gat gaa ggg aaa agt tgg cac tca gaa aca aag aac tcc ttg atg  1383
Phe Asp Glu Gly Lys Ser Trp His Ser Glu Thr Lys Asn Ser Leu Met
            205                 210                 215

cag gat agg gat gct gca tct gct cgg gcc tgg cct aaa atg cac aca  1431
Gln Asp Arg Asp Ala Ala Ser Ala Arg Ala Trp Pro Lys Met His Thr
        220                 225                 230

gtc aat ggt tat gta aac agg tct ctg cca ggt ctg att gga tgc cac  1479
Val Asn Gly Tyr Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His
    235                 240                 245

agg aaa tca gtc tat tgg cat gtg att gga atg ggc acc act cct gaa  1527
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Arg Lys Ser Val Tyr Trp His Val Ile Gly Met Gly Thr Thr Pro Glu
250                 255                 260                 265

gtg cac tca ata ttc ctc gaa ggt cac aca ttt ctt gtg agg aac cat   1575
Val His Ser Ile Phe Leu Glu Gly His Thr Phe Leu Val Arg Asn His
                270                 275                 280

cgc cag gcg tcc ttg gaa atc tcg cca ata act ttc ctt act gct caa   1623
Arg Gln Ala Ser Leu Glu Ile Ser Pro Ile Thr Phe Leu Thr Ala Gln
            285                 290                 295

aca ctc ttg atg gac ctt gga cag ttt cta ctg ttt tgt cat atc tct   1671
Thr Leu Leu Met Asp Leu Gly Gln Phe Leu Leu Phe Cys His Ile Ser
        300                 305                 310

tcc cac caa cat gat ggc atg gaa gct tat gtc aaa gta gac agc tgt   1719
Ser His Gln His Asp Gly Met Glu Ala Tyr Val Lys Val Asp Ser Cys
    315                 320                 325

cca gag gaa ccc caa cta cga atg aaa aat aat gaa gaa gcg gaa gac   1767
Pro Glu Glu Pro Gln Leu Arg Met Lys Asn Asn Glu Glu Ala Glu Asp
330                 335                 340                 345

tat gat gat gat ctt act gat tct gaa atg gat gtg gtc agg ttt gat   1815
Tyr Asp Asp Asp Leu Thr Asp Ser Glu Met Asp Val Val Arg Phe Asp
                350                 355                 360

gat gac aac tct cct tcc ttt atc caa att cgc tca gtt gcc aag aag   1863
Asp Asp Asn Ser Pro Ser Phe Ile Gln Ile Arg Ser Val Ala Lys Lys
            365                 370                 375

cat cct aaa act tgg gta cat tac att gct gct gaa gag gag gac tgg   1911
His Pro Lys Thr Trp Val His Tyr Ile Ala Ala Glu Glu Glu Asp Trp
        380                 385                 390

gac tat gct ccc tta gtc ctc gcc ccc gat gac aga agt tat aaa agt   1959
Asp Tyr Ala Pro Leu Val Leu Ala Pro Asp Asp Arg Ser Tyr Lys Ser
    395                 400                 405

caa tat ttg aac aat ggc cct cag cgg att ggt agg aag tac aaa aaa   2007
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   2055
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   2103
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   2151
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   2199
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   2247
Arg Leu Pro Lys Gly Val Lys His Leu Lys Asp Phe Pro Ile Leu Pro
490                 495                 500                 505

gga gaa ata ttc aaa tat aaa tgg aca gtg act gta gaa gat ggg cca   2295
Gly Glu Ile Phe Lys Tyr Lys Trp Thr Val Thr Val Glu Asp Gly Pro
                510                 515                 520
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act aaa tca gat cct cgg tgc ctg acc cgc tat tac tct agt ttc gtt 2343
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 2391
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 2439
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 2487
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 2535
Tyr Leu Thr Glu Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala Gly Val
590 595 600

cag ctt gag gat cca gag ttc caa gcc tcc aac atc atg cac agc atc 2583
Gln Leu Glu Asp Pro Glu Phe Gln Ala Ser Asn Ile Met His Ser Ile
605 610 615

aat ggc tat gtt ttt gat agt ttg cag ttg tca gtt tgt ttg cat gag 2631
Asn Gly Tyr Val Phe Asp Ser Leu Gln Leu Ser Val Cys Leu His Glu
620 625 630

gtg gca tac tgg tac att cta agc att gga gca cag act gac ttc ctt 2679
Val Ala Tyr Trp Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp Phe Leu
635 640 645

tct gtc ttc ttc tct gga tat acc ttc aaa cac aaa atg gtc tat gaa 2727
Ser Val Phe Phe Ser Gly Tyr Thr Phe Lys His Lys Met Val Tyr Glu
650 655 660 665

gac aca ctc acc cta ttc cca ttc tca gga gaa act gtc ttc atg tcg 2775
Asp Thr Leu Thr Leu Phe Pro Phe Ser Gly Glu Thr Val Phe Met Ser
670 675 680

atg gaa aac cca ggt cta tgg att ctg ggg tgc cac aac tca gac ttt 2823
Met Glu Asn Pro Gly Leu Trp Ile Leu Gly Cys His Asn Ser Asp Phe
685 690 695

cgg aac aga ggc atg acc gcc tta ctg aag gtt tct agt tgt gac aag 2871
Arg Asn Arg Gly Met Thr Ala Leu Leu Lys Val Ser Ser Cys Asp Lys
700 705 710

aac act ggt gat tat tac gag gac agt tat gaa gat att tca gca tac 2919
Asn Thr Gly Asp Tyr Tyr Glu Asp Ser Tyr Glu Asp Ile Ser Ala Tyr
715 720 725

ttg ctg agt aaa aac aat gcc att gaa cca aga agc ttc tcc cag aat 2967
Leu Leu Ser Lys Asn Asn Ala Ile Glu Pro Arg Ser Phe Ser Gln Asn
730 735 740 745

tca aga cat caa gct tat cga tac cgt cga ggg gaa ata act cgt act 3015
Ser Arg His Gln Ala Tyr Arg Tyr Arg Arg Gly Glu Ile Thr Arg Thr
750 755 760

act ctt cag tca gat caa gag gaa att gac tat gat gat acc ata tca 3063
Thr Leu Gln Ser Asp Gln Glu Glu Ile Asp Tyr Asp Asp Thr Ile Ser
765 770 775

gtt gaa atg aag aag gaa gat ttt gac att tat gat gag gat gaa aat 3111

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Val Glu Met Lys Lys Glu Asp Phe Asp Ile Tyr Asp Glu Asp Glu Asn
        780                 785                 790

cag agc ccc cgc agc ttt caa aag aaa aca cga cac tat ttt att gct   3159
Gln Ser Pro Arg Ser Phe Gln Lys Lys Thr Arg His Tyr Phe Ile Ala
    795                 800                 805

gca gtg gag agg ctc tgg gat tat ggg atg agt agc tcc cca cat gtt   3207
Ala Val Glu Arg Leu Trp Asp Tyr Gly Met Ser Ser Ser Pro His Val
810                 815                 820                 825

cta aga aac agg gct cag agt ggc agt gtc cct cag ttc aag aaa gtt   3255
Leu Arg Asn Arg Ala Gln Ser Gly Ser Val Pro Gln Phe Lys Lys Val
                830                 835                 840

gtt ttc cag gaa ttt act gat ggc tcc ttt act cag ccc tta tac cgt   3303
Val Phe Gln Glu Phe Thr Asp Gly Ser Phe Thr Gln Pro Leu Tyr Arg
            845                 850                 855

gga gaa cta aat gaa cat ttg gga ctc ctg ggg cca tat ata aga gca   3351
Gly Glu Leu Asn Glu His Leu Gly Leu Leu Gly Pro Tyr Ile Arg Ala
        860                 865                 870

gaa gtt gaa gat aat atc atg gta act ttc aga aat cag gcc tct cgt   3399
Glu Val Glu Asp Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser Arg
    875                 880                 885

ccc tat tcc ttc tat tct agc ctt att tct tat gag gaa gat cag agg   3447
Pro Tyr Ser Phe Tyr Ser Ser Leu Ile Ser Tyr Glu Glu Asp Gln Arg
890                 895                 900                 905

caa gga gca gaa cct aga aaa aac ttt gtc aag cct aat gaa acc aaa   3495
Gln Gly Ala Glu Pro Arg Lys Asn Phe Val Lys Pro Asn Glu Thr Lys
                910                 915                 920

act tac ttt tgg aaa gtg caa cat cat atg gca ccc act aaa gat gag   3543
Thr Tyr Phe Trp Lys Val Gln His His Met Ala Pro Thr Lys Asp Glu
            925                 930                 935

ttt gac tgc aaa gcc tgg gct tat ttc tct gat gtt gac ctg gaa aaa   3591
Phe Asp Cys Lys Ala Trp Ala Tyr Phe Ser Asp Val Asp Leu Glu Lys
        940                 945                 950

gat gtg cac tca ggc ctg att gga ccc ctt ctg gtc tgc cac act aac   3639
Asp Val His Ser Gly Leu Ile Gly Pro Leu Leu Val Cys His Thr Asn
    955                 960                 965

aca ctg aac cct gct cat ggg aga caa gtg aca gta cag gaa ttt gct   3687
Thr Leu Asn Pro Ala His Gly Arg Gln Val Thr Val Gln Glu Phe Ala
970                 975                 980                 985

ctg ttt ttc acc atc ttt gat gag acc aaa agc tgg tac ttc act gaa   3735
Leu Phe Phe Thr Ile Phe Asp Glu Thr Lys Ser Trp Tyr Phe Thr Glu
                990                 995                1000

aat atg gaa aga aac tgc agg gct ccc tgc aat atc cag atg gaa gat   3783
Asn Met Glu Arg Asn Cys Arg Ala Pro Cys Asn Ile Gln Met Glu Asp
            1005                1010                1015

ccc act ttt aaa gag aat tat cgc ttc cat gca atc aat ggc tac ata   3831
Pro Thr Phe Lys Glu Asn Tyr Arg Phe His Ala Ile Asn Gly Tyr Ile
        1020                1025                1030

atg gat aca cta cct ggc tta gta atg gct cag gat caa agg att cga   3879
Met Asp Thr Leu Pro Gly Leu Val Met Ala Gln Asp Gln Arg Ile Arg
    1035                1040                1045
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tgg tat ctg ctc agc atg ggc agc aat gaa aac atc cat tct att cat   3927
Trp Tyr Leu Leu Ser Met Gly Ser Asn Glu Asn Ile His Ser Ile His
1050                1055                1060                1065

ttc agt gga cat gtg ttc act gta cga aaa aaa gag gag tat aaa atg   3975
Phe Ser Gly His Val Phe Thr Val Arg Lys Lys Glu Glu Tyr Lys Met
                1070                1075                1080

gca ctg tac aat ctc tat cca ggt gtt ttt gag aca gtg gaa atg tta   4023
Ala Leu Tyr Asn Leu Tyr Pro Gly Val Phe Glu Thr Val Glu Met Leu
            1085                1090                1095

cca tcc aaa gct gga att tgg cgg gtg gaa tgc ctt att ggc gag cat   4071
Pro Ser Lys Ala Gly Ile Trp Arg Val Glu Cys Leu Ile Gly Glu His
        1100                1105                1110

cta cat gct ggg atg agc aca ctt ttt ctg gtg tac agc aat aag tgt   4119
Leu His Ala Gly Met Ser Thr Leu Phe Leu Val Tyr Ser Asn Lys Cys
    1115                1120                1125

cag act ccc ctg gga atg gct tct gga cac att aga gat ttt cag att   4167
Gln Thr Pro Leu Gly Met Ala Ser Gly His Ile Arg Asp Phe Gln Ile
1130                1135                1140                1145

aca gct tca gga caa tat gga cag tgg gcc cca aag ctg gcc aga ctt   4215
Thr Ala Ser Gly Gln Tyr Gly Gln Trp Ala Pro Lys Leu Ala Arg Leu
                1150                1155                1160

cat tat tcc gga tca atc aat gcc tgg agc acc aag gag ccc ttt tct   4263
His Tyr Ser Gly Ser Ile Asn Ala Trp Ser Thr Lys Glu Pro Phe Ser
            1165                1170                1175

tgg atc aag gtg gat ctg ttg gca cca atg att att cac ggc atc aag   4311
Trp Ile Lys Val Asp Leu Leu Ala Pro Met Ile Ile His Gly Ile Lys
        1180                1185                1190

acc cag ggt gcc cgt cag aag ttc tcc agc ctc tac atc tct cag ttt   4359
Thr Gln Gly Ala Arg Gln Lys Phe Ser Ser Leu Tyr Ile Ser Gln Phe
    1195                1200                1205

atc atc atg tat agt ctt gat ggg aag aag tgg cag act tat cga gga   4407
Ile Ile Met Tyr Ser Leu Asp Gly Lys Lys Trp Gln Thr Tyr Arg Gly
1210                1215                1220                1225

aat tcc act gga acc tta atg gtc ttc ttt ggc aat gtg gat tca tct   4455
Asn Ser Thr Gly Thr Leu Met Val Phe Phe Gly Asn Val Asp Ser Ser
                1230                1235                1240

ggg ata aaa cac aat att ttt aac cct cca att att gct cga tac atc   4503
Gly Ile Lys His Asn Ile Phe Asn Pro Pro Ile Ile Ala Arg Tyr Ile
            1245                1250                1255

cgt ttg cac cca act cat tat agc att cgc agc act ctt cgc atg gag   4551
Arg Leu His Pro Thr His Tyr Ser Ile Arg Ser Thr Leu Arg Met Glu
        1260                1265                1270

ttg atg ggc tgt gat tta aat agt tgc agc atg cca ttg gga atg gag   4599
Leu Met Gly Cys Asp Leu Asn Ser Cys Ser Met Pro Leu Gly Met Glu
    1275                1280                1285

agt aaa gca ata tca gat gca cag att act gct tca tcc tac ttt acc   4647
Ser Lys Ala Ile Ser Asp Ala Gln Ile Thr Ala Ser Ser Tyr Phe Thr
1290                1295                1300                1305

aat atg ttt gcc acc tgg tct cct tca aaa gct cga ctt cac ctc caa   4695
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Asn Met Phe Ala Thr Trp Ser Pro Ser Lys Ala Arg Leu His Leu Gln
                1310                1315                1320

ggg agg agt aat gcc tgg aga cct cag gtg aat aat cca aaa gag tgg   4743
Gly Arg Ser Asn Ala Trp Arg Pro Gln Val Asn Asn Pro Lys Glu Trp
            1325                1330                1335

ctg caa gtg gac ttc cag aag aca atg aaa gtc aca gga gta act act   4791
Leu Gln Val Asp Phe Gln Lys Thr Met Lys Val Thr Gly Val Thr Thr
        1340                1345                1350

cag gga gta aaa tct ctg ctt acc agc atg tat gtg aag gag ttc ctc   4839
Gln Gly Val Lys Ser Leu Leu Thr Ser Met Tyr Val Lys Glu Phe Leu
    1355                1360                1365

atc tcc agc agt caa gat ggc cat cag tgg acc ctc ttt ttt cag aat   4887
Ile Ser Ser Ser Gln Asp Gly His Gln Trp Thr Leu Phe Phe Gln Asn
1370                1375                1380                1385

ggc aaa gta aag gtt ttt cag gga aat caa gac tcc ttc aca cct gtg   4935
Gly Lys Val Lys Val Phe Gln Gly Asn Gln Asp Ser Phe Thr Pro Val
                1390                1395                1400

gtg aac tct cta gac cca ccg tta ctg act cgc tac ctt cga att cac   4983
Val Asn Ser Leu Asp Pro Pro Leu Leu Thr Arg Tyr Leu Arg Ile His
            1405                1410                1415

ccc cag agt tgg gtg cac cag att gcc ctg agg atg gag gtt ctg ggc   5031
Pro Gln Ser Trp Val His Gln Ile Ala Leu Arg Met Glu Val Leu Gly
        1420                1425                1430

tgc gag gca cag gac ctc tac tgagcggccg cgactctact agaggatctt      5082
Cys Glu Ala Gln Asp Leu Tyr
    1435                1440

tgtgaaggaa ccttacttct gtggtgtgac ataattggac aaactaccta cagagattta 5142

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gtggcgataa gtcgtgtctt accgggttgg actcaagacg atagttaccg gataaggcgc 9942
agcggtcggg ctgaacgggg ggttcgtgca cacagcccag cttggagcga acgacctaca 10002

ccgaactgag atacctacag cgtgagctat gagaaagcgc cacgcttccc gaagggagaa 10062

aggcggacag gtatccggta agcggcaggg tcggaacagg agagcgcacg agggagcttc 10122

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cctttttacg gttcctggcc ttttgctggc cttttgctca catgttcttt cctgcgttat 10302

cccctgattc tgtggataac cgtattaccg cctttgagtg agctgatacc gctcgccgca 10362

gccgaacgac cgagcgcagc gagtcagtga gcgaggaagc ggaagagcgc ccaatacgca 10422

aaccgcctct ccccgcgcgt tggccgattc attaatgcag ctggcacgac aggtttcccg 10482

actggaaagc gggcagtgag cgcaacgcaa ttaatgtgag ttagctcact cattaggcac 10542

cccaggcttt acactttatg cttccggctc gtatgttgtg tggaattgtg agcggataac 10602

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tctagagctc tagagagctt gcatgcctgc aggtcg 10698

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Met Gln Ile Glu Leu Ser Thr Cys Phe Phe Leu Cys Leu Leu Arg Phe
-15 -10 -5

Cys Phe Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser
-1 1 5 10

Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg
15 20 25

Phe Pro Pro Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val
30 35 40 45

Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr Asp His Leu Phe Asn Ile
50 55 60

Ala Lys Pro Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln
65 70 75

Ala Glu Val Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser
80 85 90

His Pro Val Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser
95 100 105

Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp
110 115 120 125

Asp Lys Val Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu
130 135 140

Lys Glu Asn Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser
145 150 155

Tyr Leu Ser His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile
160 165 170

Gly Ala Leu Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr
175 180 185

Gln Thr Leu His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly
190 195 200 205

Lys Ser Trp His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp
210 215 220

Ala Ala Ser Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr
225 230 235

Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val
240 245 250

Tyr Trp His Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile
255 260 265

Phe Leu Glu Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser
270 275 280 285

Leu Glu Ile Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met
290 295 300

Asp Leu Gly Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His
305 310 315

Asp Gly Met Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro
320 325 330

Gln Leu Arg Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp
335 340 345

Leu Thr Asp Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser
350 355 360 365

Pro Ser Phe Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr
370 375 380

Trp Val His Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro
385 390 395

Leu Val Leu Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn
400 405 410

Asn Gly Pro Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met
415 420 425

Ala Tyr Thr Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu
430 435 440 445

Ser Gly Ile Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu
450 455 460

Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro
465 470 475

His Gly Ile Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys
480 485 490

Gly Val Lys His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe
495 500 505

Lys Tyr Lys Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp
510 515 520 525

Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg
530 535 540

Asp Leu Ala Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu
545 550 555

Ser Val Asp Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val
560 565 570

Ile Leu Phe Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu
575 580 585

Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp
590 595 600 605

Pro Glu Phe Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val
610 615 620

Phe Asp Ser Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp
625 630 635

Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe
640 645 650

Ser Gly Tyr Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr
655 660 665

Leu Phe Pro Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro
670 675 680 685

Gly Leu Trp Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly
690 695 700

Met Thr Ala Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp
705 710 715

Tyr Tyr Glu Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys
720 725 730

Asn Asn Ala Ile Glu Pro Arg Ser Phe Ser Gln Asn Ser Arg His Gln
735 740 745

Ala Tyr Arg Tyr Arg Arg Gly Glu Ile Thr Arg Thr Thr Leu Gln Ser
750 755 760 765

Asp Gln Glu Glu Ile Asp Tyr Asp Asp Thr Ile Ser Val Glu Met Lys
770 775 780

Lys Glu Asp Phe Asp Ile Tyr Asp Glu Asp Glu Asn Gln Ser Pro Arg
785 790 795

Ser Phe Gln Lys Lys Thr Arg His Tyr Phe Ile Ala Ala Val Glu Arg
800 805 810

Leu Trp Asp Tyr Gly Met Ser Ser Ser Pro His Val Leu Arg Asn Arg
815 820 825

Ala Gln Ser Gly Ser Val Pro Gln Phe Lys Lys Val Val Phe Gln Glu
830 835 840 845

Phe Thr Asp Gly Ser Phe Thr Gln Pro Leu Tyr Arg Gly Glu Leu Asn
850 855 860

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Glu His Leu Gly Leu Leu Gly Pro Tyr Ile Arg Ala Glu Val Glu Asp
            865                 870                 875

Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser Arg Pro Tyr Ser Phe
        880                 885                 890

Tyr Ser Ser Leu Ile Ser Tyr Glu Glu Asp Gln Arg Gln Gly Ala Glu
    895                 900                 905

Pro Arg Lys Asn Phe Val Lys Pro Asn Glu Thr Lys Thr Tyr Phe Trp
910                 915                 920                 925

Lys Val Gln His His Met Ala Pro Thr Lys Asp Glu Phe Asp Cys Lys
                930                 935                 940

Ala Trp Ala Tyr Phe Ser Asp Val Asp Leu Glu Lys Asp Val His Ser
            945                 950                 955

Gly Leu Ile Gly Pro Leu Leu Val Cys His Thr Asn Thr Leu Asn Pro
        960                 965                 970

Ala His Gly Arg Gln Val Thr Val Gln Glu Phe Ala Leu Phe Phe Thr
    975                 980                 985

Ile Phe Asp Glu Thr Lys Ser Trp Tyr Phe Thr Glu Asn Met Glu Arg
990                 995                1000                1005

Asn Cys Arg Ala Pro Cys Asn Ile Gln Met Glu Asp Pro Thr Phe Lys
               1010                1015                1020

Glu Asn Tyr Arg Phe His Ala Ile Asn Gly Tyr Ile Met Asp Thr Leu
           1025                1030                1035

Pro Gly Leu Val Met Ala Gln Asp Gln Arg Ile Arg Trp Tyr Leu Leu
       1040                1045                1050

Ser Met Gly Ser Asn Glu Asn Ile His Ser Ile His Phe Ser Gly His
   1055                1060                1065

Val Phe Thr Val Arg Lys Lys Glu Glu Tyr Lys Met Ala Leu Tyr Asn
070                1075                1080                1085

Leu Tyr Pro Gly Val Phe Glu Thr Val Glu Met Leu Pro Ser Lys Ala
               1090                1095                1100

Gly Ile Trp Arg Val Glu Cys Leu Ile Gly Glu His Leu His Ala Gly
           1105                1110                1115

Met Ser Thr Leu Phe Leu Val Tyr Ser Asn Lys Cys Gln Thr Pro Leu
       1120                1125                1130

Gly Met Ala Ser Gly His Ile Arg Asp Phe Gln Ile Thr Ala Ser Gly
   1135                1140                1145

Gln Tyr Gly Gln Trp Ala Pro Lys Leu Ala Arg Leu His Tyr Ser Gly
150                1155                1160                1165

Ser Ile Asn Ala Trp Ser Thr Lys Glu Pro Phe Ser Trp Ile Lys Val
               1170                1175                1180

Asp Leu Leu Ala Pro Met Ile Ile His Gly Ile Lys Thr Gln Gly Ala
           1185                1190                1195

Arg Gln Lys Phe Ser Ser Leu Tyr Ile Ser Gln Phe Ile Ile Met Tyr
       1200                1205                1210
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Ser Leu Asp Gly Lys Lys Trp Gln Thr Tyr Arg Gly Asn Ser Thr Gly
   1215                1220                1225

Thr Leu Met Val Phe Phe Gly Asn Val Asp Ser Ser Gly Ile Lys His
230                1235                1240                1245

Asn Ile Phe Asn Pro Pro Ile Ile Ala Arg Tyr Ile Arg Leu His Pro
               1250                1255                1260

Thr His Tyr Ser Ile Arg Ser Thr Leu Arg Met Glu Leu Met Gly Cys
           1265                1270                1275

Asp Leu Asn Ser Cys Ser Met Pro Leu Gly Met Glu Ser Lys Ala Ile
       1280                1285                1290

Ser Asp Ala Gln Ile Thr Ala Ser Ser Tyr Phe Thr Asn Met Phe Ala
   1295                1300                1305

Thr Trp Ser Pro Ser Lys Ala Arg Leu His Leu Gln Gly Arg Ser Asn
310                1315                1320                1325

Ala Trp Arg Pro Gln Val Asn Asn Pro Lys Glu Trp Leu Gln Val Asp
               1330                1335                1340

Phe Gln Lys Thr Met Lys Val Thr Gly Val Thr Thr Gln Gly Val Lys
           1345                1350                1355

Ser Leu Leu Thr Ser Met Tyr Val Lys Glu Phe Leu Ile Ser Ser Ser
       1360                1365                1370

Gln Asp Gly His Gln Trp Thr Leu Phe Phe Gln Asn Gly Lys Val Lys
   1375                1380                1385

Val Phe Gln Gly Asn Gln Asp Ser Phe Thr Pro Val Val Asn Ser Leu
390                1395                1400                1405

Asp Pro Pro Leu Leu Thr Arg Tyr Leu Arg Ile His Pro Gln Ser Trp
               1410                1415                1420

Val His Gln Ile Ala Leu Arg Met Glu Val Leu Gly Cys Glu Ala Gln
           1425                1430                1435

Asp Leu Tyr
       1440
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cgcccaacga cccccgccca ttgacgtcaa taatgacgta tgttcccata gtaacgccaa 180

tagggacttt ccattgacgt caatgggtgg agtatttacg gtaaactgcc cacttggcag 240

tacatcaagt gtatcatatg ccaagtacgc cccctattga cgtcaatgac ggtaaatggc 300

ccgcctggca ttatgcccag tacatgacct tatgggactt tcctacttgg cagtacatct 360

acgtattagt catcgctatt accatggtga tgcggttttg gcagtacatc aatgggcgtg 420

gatagcggtt tgactcacgg ggatttccaa gtctccaccc cattgacgtc aatgggagtt 480

tgttttggca ccaaaatcaa cgggactttc caaaatgtcg taacaactcc gccccattga 540

cgcaaatggg cggtaggcgt gtacggtggg aggtctatat aagcagagct ctctggctaa 600

ctagagaacc cactgcttac tggcttatcg aaattaatac gactcactat agggagaccc 660

aagcttgacc tcgag atg caa ata gag ctc tcc acc tgc ttc ttt ctg tgc  711
                Met Gln Ile Glu Leu Ser Thr Cys Phe Phe Leu Cys
                               -15                 -10

ctt ttg cga ttc tgc ttt agt gcc acc aga aga tac tac ctg ggt gca   759
Leu Leu Arg Phe Cys Phe Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala
         -5                -1   1                5

gtg gaa ctg tca tgg gac tat atg caa agt gat ctc ggt gag ctg cct   807
Val Glu Leu Ser Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro
 10                  15                  20                  25

gtg gac gca aga ttt cct cct aga gtg cca aaa tct ttt cca ttc aac   855
Val Asp Ala Arg Phe Pro Pro Arg Val Pro Lys Ser Phe Pro Phe Asn
                30                  35                  40

acc tca gtc gtg tac aaa aag act ctg ttt gta gaa ttc acg gat cac   903
Thr Ser Val Val Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr Asp His
            45                  50                  55

ctt ttc aac atc gct aag cca agg cca ccc tgg atg ggt ctg cta ggt   951
Leu Phe Asn Ile Ala Lys Pro Arg Pro Pro Trp Met Gly Leu Leu Gly
         60                  65                  70

cct acc atc cag gct gag gtt tat gat aca gtg gtc att aca ctt aag   999
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  1047
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  1095
Trp Lys Ala Ser Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser Gln Arg
                110                 115                 120

gag aaa gaa gat gat aaa gtc ttc cct ggt gga agc cat aca tat gtc  1143
Glu Lys Glu Asp Asp Lys Val Phe Pro Gly Gly Ser His Thr Tyr Val
            125                 130                 135

tgg cag gtc ctg aaa gag aat ggt cca atg gcc tct gac cca ctg tgc  1191
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 gcg gac ctg gta aaa gac ttg aat  1239
Leu Thr Tyr Ser Tyr Leu Ser His Ala Asp Leu Val Lys Asp Leu Asn
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    155                 160                 165

tca ggc ctc att gga gcc cta cta gta tgt aga gaa ggg agt ctg gcc   1287
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   1335
Lys Glu Lys Thr Gln Thr Leu His Lys Phe Ile Leu Leu Phe Ala Val
                190                 195                 200

ttt gat gaa ggg aaa agt tgg cac tca gaa aca aag aac tcc ttg atg   1383
Phe Asp Glu Gly Lys Ser Trp His Ser Glu Thr Lys Asn Ser Leu Met
            205                 210                 215

cag gat agg gat gct gca tct gct cgg gcc tgg cct aaa atg cac aca   1431
Gln Asp Arg Asp Ala Ala Ser Ala Arg Ala Trp Pro Lys Met His Thr
        220                 225                 230

gtc aat ggt tat gta aac agg tct ctg cca ggt ctg att gga tgc cac   1479
Val Asn Gly Tyr Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His
    235                 240                 245

agg aaa tca gtc tat tgg cat gtg att gga atg ggc acc act cct gaa   1527
Arg Lys Ser Val Tyr Trp His Val Ile Gly Met Gly Thr Thr Pro Glu
250                 255                 260                 265

gtg cac tca ata ttc ctc gaa ggt cac aca ttt ctt gtg agg aac cat   1575
Val His Ser Ile Phe Leu Glu Gly His Thr Phe Leu Val Arg Asn His
                270                 275                 280

cgc cag gcg tcc ttg gaa atc tcg cca ata act ttc ctt act gct caa   1623
Arg Gln Ala Ser Leu Glu Ile Ser Pro Ile Thr Phe Leu Thr Ala Gln
            285                 290                 295

aca ctc ttg atg gac ctt gga cag ttt cta ctg ttt tgt cat atc tct   1671
Thr Leu Leu Met Asp Leu Gly Gln Phe Leu Leu Phe Cys His Ile Ser
        300                 305                 310

tcc cac caa cat gat ggc atg gaa gct tat gtc aaa gta gac agc tgt   1719
Ser His Gln His Asp Gly Met Glu Ala Tyr Val Lys Val Asp Ser Cys
    315                 320                 325

cca gag gaa ccc caa cta cga atg aaa aat aat gaa gaa gcg gaa gac   1767
Pro Glu Glu Pro Gln Leu Arg Met Lys Asn Asn Glu Glu Ala Glu Asp
330                 335                 340                 345

tat gat gat gat ctt act gat tct gaa atg gat gtg gtc agg ttt gat   1815
Tyr Asp Asp Asp Leu Thr Asp Ser Glu Met Asp Val Val Arg Phe Asp
                350                 355                 360

gat gac aac tct cct tcc ttt atc caa att cgc tca gtt gcc aag aag   1863
Asp Asp Asn Ser Pro Ser Phe Ile Gln Ile Arg Ser Val Ala Lys Lys
            365                 370                 375

cat cct aaa act tgg gta cat tac att gct gct gaa gag gag gac tgg   1911
His Pro Lys Thr Trp Val His Tyr Ile Ala Ala Glu Glu Glu Asp Trp
        380                 385                 390

gac tat gct ccc tta gtc ctc gcc ccc gat gac aga agt tat aaa agt   1959
Asp Tyr Ala Pro Leu Val Leu Ala Pro Asp Asp Arg Ser Tyr Lys Ser
    395                 400                 405

caa tat ttg aac aat ggc cct cag cgg att ggt agg aag tac aaa aaa   2007
Gln Tyr Leu Asn Asn Gly Pro Gln Arg Ile Gly Arg Lys Tyr Lys Lys
410                 415                 420                 425
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gtc cga ttt atg gca tac aca gat gaa acc ttt aag act cgt gaa gct   2055
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   2103
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   2151
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   2199
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   2247
Arg Leu Pro Lys Gly Val Lys His Leu Lys Asp Phe Pro Ile Leu Pro
490                 495                 500                 505

gga gaa ata ttc aaa tat aaa tgg aca gtg act gta gaa gat ggg cca   2295
Gly Glu Ile Phe Lys Tyr Lys Trp Thr Val Thr Val Glu Asp Gly Pro
                510                 515                 520

act aaa tca gat cct cgg tgc ctg acc cgc tat tac tct agt ttc gtt   2343
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   2391
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   2439
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   2487
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   2535
Tyr Leu Thr Glu Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala Gly Val
                590                 595                 600

cag ctt gag gat cca gag ttc caa gcc tcc aac atc atg cac agc atc   2583
Gln Leu Glu Asp Pro Glu Phe Gln Ala Ser Asn Ile Met His Ser Ile
            605                 610                 615

aat ggc tat gtt ttt gat agt ttg cag ttg tca gtt tgt ttg cat gag   2631
Asn Gly Tyr Val Phe Asp Ser Leu Gln Leu Ser Val Cys Leu His Glu
        620                 625                 630

gtg gca tac tgg tac att cta agc att gga gca cag act gac ttc ctt   2679
Val Ala Tyr Trp Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp Phe Leu
    635                 640                 645

tct gtc ttc ttc tct gga tat acc ttc aaa cac aaa atg gtc tat gaa   2727
Ser Val Phe Phe Ser Gly Tyr Thr Phe Lys His Lys Met Val Tyr Glu
650                 655                 660                 665

gac aca ctc acc cta ttc cca ttc tca gga gaa act gtc ttc atg tcg   2775
Asp Thr Leu Thr Leu Phe Pro Phe Ser Gly Glu Thr Val Phe Met Ser
                670                 675                 680

atg gaa aac cca ggt cta tgg att ctg ggg tgc cac aac tca gac ttt   2823
Met Glu Asn Pro Gly Leu Trp Ile Leu Gly Cys His Asn Ser Asp Phe
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            685                 690                 695

cgg aac aga ggc atg acc gcc tta ctg aag gtt tct agt tgt gac aag   2871
Arg Asn Arg Gly Met Thr Ala Leu Leu Lys Val Ser Ser Cys Asp Lys
        700                 705                 710

aac act ggt gat tat tac gag gac agt tat gaa gat att tca gca tac   2919
Asn Thr Gly Asp Tyr Tyr Glu Asp Ser Tyr Glu Asp Ile Ser Ala Tyr
    715                 720                 725

ttg ctg agt aaa aac aat gcc att gaa cca aga agc ttc tcc cag aat   2967
Leu Leu Ser Lys Asn Asn Ala Ile Glu Pro Arg Ser Phe Ser Gln Asn
730                 735                 740                 745

tca aga cat caa gct tat cga tac cgt cga ggg gaa ata act cgt act   3015
Ser Arg His Gln Ala Tyr Arg Tyr Arg Arg Gly Glu Ile Thr Arg Thr
                750                 755                 760

act ctt cag tca gat caa gag gaa att gac tat gat gat acc ata tca   3063
Thr Leu Gln Ser Asp Gln Glu Glu Ile Asp Tyr Asp Asp Thr Ile Ser
            765                 770                 775

gtt gaa atg aag aag gaa gat ttt gac att tat gat gag gat gaa aat   3111
Val Glu Met Lys Lys Glu Asp Phe Asp Ile Tyr Asp Glu Asp Glu Asn
        780                 785                 790

cag agc ccc cgc agc ttt caa aag aaa aca cga cac tat ttt att gct   3159
Gln Ser Pro Arg Ser Phe Gln Lys Lys Thr Arg His Tyr Phe Ile Ala
    795                 800                 805

gca gtg gag agg ctc tgg gat tat ggg atg agt agc tcc cca cat gtt   3207
Ala Val Glu Arg Leu Trp Asp Tyr Gly Met Ser Ser Ser Pro His Val
810                 815                 820                 825

cta aga aac agg gct cag agt ggc agt gtc cct cag ttc aag aaa gtt   3255
Leu Arg Asn Arg Ala Gln Ser Gly Ser Val Pro Gln Phe Lys Lys Val
                830                 835                 840

gtt ttc cag gaa ttt act gat ggc tcc ttt act cag ccc tta tac cgt   3303
Val Phe Gln Glu Phe Thr Asp Gly Ser Phe Thr Gln Pro Leu Tyr Arg
            845                 850                 855

gga gaa cta aat gaa cat ttg gga ctc ctg ggg cca tat ata aga gca   3351
Gly Glu Leu Asn Glu His Leu Gly Leu Leu Gly Pro Tyr Ile Arg Ala
        860                 865                 870

gaa gtt gaa gat aat atc atg gta act ttc aga aat cag gcc tct cgt   3399
Glu Val Glu Asp Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser Arg
    875                 880                 885

ccc tat tcc ttc tat tct agc ctt att tct tat gag gaa gat cag agg   3447
Pro Tyr Ser Phe Tyr Ser Ser Leu Ile Ser Tyr Glu Glu Asp Gln Arg
890                 895                 900                 905

caa gga gca gaa cct aga aaa aac ttt gtc aag cct aat gaa acc aaa   3495
Gln Gly Ala Glu Pro Arg Lys Asn Phe Val Lys Pro Asn Glu Thr Lys
                910                 915                 920

act tac ttt tgg aaa gtg caa cat cat atg gca ccc act aaa gat gag   3543
Thr Tyr Phe Trp Lys Val Gln His His Met Ala Pro Thr Lys Asp Glu
            925                 930                 935

ttt gac tgc aaa gcc tgg gct tat ttc tct gat gtt gac ctg gaa aaa   3591
Phe Asp Cys Lys Ala Trp Ala Tyr Phe Ser Asp Val Asp Leu Glu Lys
        940                 945                 950
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gat gtg cac tca ggc ctg att gga ccc ctt ctg gtc tgc cac act aac   3639
Asp Val His Ser Gly Leu Ile Gly Pro Leu Leu Val Cys His Thr Asn
    955                 960                 965

aca ctg aac cct gct cat ggg aga caa gtg aca gta cag gaa ttt gct   3687
Thr Leu Asn Pro Ala His Gly Arg Gln Val Thr Val Gln Glu Phe Ala
970                 975                 980                 985

ctg ttt ttc acc atc ttt gat gag acc aaa agc tgg tac ttc act gaa   3735
Leu Phe Phe Thr Ile Phe Asp Glu Thr Lys Ser Trp Tyr Phe Thr Glu
                990                 995                1000

aat atg gaa aga aac tgc agg gct ccc tgc aat atc cag atg gaa gat   3783
Asn Met Glu Arg Asn Cys Arg Ala Pro Cys Asn Ile Gln Met Glu Asp
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ccc act ttt aaa gag aat tat cgc ttc cat gca atc aat ggc tac ata   3831
Pro Thr Phe Lys Glu Asn Tyr Arg Phe His Ala Ile Asn Gly Tyr Ile
       1020                1025                1030

atg gat aca cta cct ggc tta gta atg gct cag gat caa agg att cga   3879
Met Asp Thr Leu Pro Gly Leu Val Met Ala Gln Asp Gln Arg Ile Arg
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tgg tat ctg ctc agc atg ggc agc aat gaa aac atc cat tct att cat   3927
Trp Tyr Leu Leu Ser Met Gly Ser Asn Glu Asn Ile His Ser Ile His
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ttc agt gga cat gtg ttc act gta cga aaa aaa gag gag tat aaa atg   3975
Phe Ser Gly His Val Phe Thr Val Arg Lys Lys Glu Glu Tyr Lys Met
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gca ctg tac aat ctc tat cca ggt gtt ttt gag aca gtg gaa atg tta   4023
Ala Leu Tyr Asn Leu Tyr Pro Gly Val Phe Glu Thr Val Glu Met Leu
           1085                1090                1095

cca tcc aaa gct gga att tgg cgg gtg gaa tgc ctt att ggc gag cat   4071
Pro Ser Lys Ala Gly Ile Trp Arg Val Glu Cys Leu Ile Gly Glu His
       1100                1105                1110

cta cat gct ggg atg agc aca ctt ttt ctg gtg tac agc aat aag tgt   4119
Leu His Ala Gly Met Ser Thr Leu Phe Leu Val Tyr Ser Asn Lys Cys
   1115                1120                1125

cag act ccc ctg gga atg gct tct gga cac att aga gat ttt cag att   4167
Gln Thr Pro Leu Gly Met Ala Ser Gly His Ile Arg Asp Phe Gln Ile
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aca gct tca gga caa tat gga cag tgg gcc cca aag ctg gcc aga ctt   4215
Thr Ala Ser Gly Gln Tyr Gly Gln Trp Ala Pro Lys Leu Ala Arg Leu
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cat tat tcc gga tca atc aat gcc tgg agc acc aag gag ccc ttt tct   4263
His Tyr Ser Gly Ser Ile Asn Ala Trp Ser Thr Lys Glu Pro Phe Ser
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tgg atc aag gtg gat ctg ttg gca cca atg att att cac ggc atc aag   4311
Trp Ile Lys Val Asp Leu Leu Ala Pro Met Ile Ile His Gly Ile Lys
       1180                1185                1190

acc cag ggt gcc cgt cag aag ttc tcc agc ctc tac atc tct cag ttt   4359
Thr Gln Gly Ala Arg Gln Lys Phe Ser Ser Leu Tyr Ile Ser Gln Phe
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atc atc atg tat agt ctt gat ggg aag aag tgg cag act tat cga gga   4407
Ile Ile Met Tyr Ser Leu Asp Gly Lys Lys Trp Gln Thr Tyr Arg Gly
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aat tcc act gga acc tta atg gtc ttc ttt ggc aat gtg gat tca tct   4455
Asn Ser Thr Gly Thr Leu Met Val Phe Phe Gly Asn Val Asp Ser Ser
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ggg ata aaa cac aat att ttt aac cct cca att att gct cga tac atc   4503
Gly Ile Lys His Asn Ile Phe Asn Pro Pro Ile Ile Ala Arg Tyr Ile
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cgt ttg cac cca act cat tat agc att cgc agc act ctt cgc atg gag   4551
Arg Leu His Pro Thr His Tyr Ser Ile Arg Ser Thr Leu Arg Met Glu
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ttg atg ggc tgt gat tta aat agt tgc agc atg cca ttg gga atg gag   4599
Leu Met Gly Cys Asp Leu Asn Ser Cys Ser Met Pro Leu Gly Met Glu
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agt aaa gca ata tca gat gca cag att act gct tca tcc tac ttt acc   4647
Ser Lys Ala Ile Ser Asp Ala Gln Ile Thr Ala Ser Ser Tyr Phe Thr
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aat atg ttt gcc acc tgg tct cct tca aaa gct cga ctt cac ctc caa   4695
Asn Met Phe Ala Thr Trp Ser Pro Ser Lys Ala Arg Leu His Leu Gln
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Gly Arg Ser Asn Ala Trp Arg Pro Gln Glu Asn Asn Pro Lys Glu Trp
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ctg caa gtg gac ttc cag aag aca atg aaa gtc aca gga gta act act   4791
Leu Gln Val Asp Phe Gln Lys Thr Met Lys Val Thr Gly Val Thr Thr
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cag gga gta aaa tct ctg ctt acc agc atg tat gtg aag gag ttc ctc   4839
Gln Gly Val Lys Ser Leu Leu Thr Ser Met Tyr Val Lys Glu Phe Leu
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atc tcc agc agt caa gat ggc cat cag tgg acc ctc ttt ttt cag aat   4887
Ile Ser Ser Ser Gln Asp Gly His Gln Trp Thr Leu Phe Phe Gln Asn
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ggc aaa gta aag gtt ttt cag gga aat caa gac tcc ttc aca cct gtg   4935
Gly Lys Val Lys Val Phe Gln Gly Asn Gln Asp Ser Phe Thr Pro Val
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gtg aac tct cta gac cca ccg tta ctg act cgc tac ctt cga att cac   4983
Val Asn Ser Leu Asp Pro Pro Leu Leu Thr Arg Tyr Leu Arg Ile His
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ccc cag agt tgg gtg cac cag att gcc ctg agg atg gag gtt ctg ggc   5031
Pro Gln Ser Trp Val His Gln Ile Ala Leu Arg Met Glu Val Leu Gly
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Cys Glu Ala Gln Asp Leu Tyr
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Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg
15 20 25

Phe Pro Pro Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val

30 35 40 45

Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr Asp His Leu Phe Asn Ile
50 55 60

Ala Lys Pro Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln
65 70 75

Ala Glu Val Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser
80 85 90

His Pro Val Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser
95 100 105

Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp
110 115 120 125

Asp Lys Val Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu
130 135 140

Lys Glu Asn Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser
145 150 155

Tyr Leu Ser His Ala Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile
160 165 170

Gly Ala Leu Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr
175 180 185

Gln Thr Leu His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly
190 195 200 205

Lys Ser Trp His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp
210 215 220

Ala Ala Ser Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr
225 230 235

Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val
240 245 250

Tyr Trp His Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile
255 260 265

Phe Leu Glu Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser
270 275 280 285

Leu Glu Ile Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met
290 295 300

Asp Leu Gly Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His
305 310 315

Asp Gly Met Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro
320 325 330

Gln Leu Arg Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp
335 340 345

Leu Thr Asp Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser
350 355 360 365

Pro Ser Phe Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr
370 375 380

Trp Val His Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro

385 390 395

Leu Val Leu Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn
400 405 410

Asn Gly Pro Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met
415 420 425

Ala Tyr Thr Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu
430 435 440 445

Ser Gly Ile Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu
450 455 460

Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro
465 470 475

His Gly Ile Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys
480 485 490

Gly Val Lys His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe
495 500 505

Lys Tyr Lys Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp
510 515 520 525

Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg
530 535 540

Asp Leu Ala Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu
545 550 555

Ser Val Asp Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val
560 565 570

Ile Leu Phe Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu
575 580 585

Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp
590 595 600 605

Pro Glu Phe Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val
610 615 620

Phe Asp Ser Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp
625 630 635

Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe
640 645 650

Ser Gly Tyr Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr
655 660 665

Leu Phe Pro Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro
670 675 680 685

Gly Leu Trp Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly
690 695 700

Met Thr Ala Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp
705 710 715

Tyr Tyr Glu Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys
720 725 730

Asn Asn Ala Ile Glu Pro Arg Ser Phe Ser Gln Asn Ser Arg His Gln

735 740 745

Ala Tyr Arg Tyr Arg Arg Gly Glu Ile Thr Arg Thr Thr Leu Gln Ser
750 755 760 765

Asp Gln Glu Glu Ile Asp Tyr Asp Asp Thr Ile Ser Val Glu Met Lys
770 775 780

Lys Glu Asp Phe Asp Ile Tyr Asp Glu Asp Glu Asn Gln Ser Pro Arg
785 790 795

Ser Phe Gln Lys Lys Thr Arg His Tyr Phe Ile Ala Ala Val Glu Arg
800 805 810

Leu Trp Asp Tyr Gly Met Ser Ser Ser Pro His Val Leu Arg Asn Arg
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Ala Gln Ser Gly Ser Val Pro Gln Phe Lys Lys Val Val Phe Gln Glu
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850 855 860

Glu His Leu Gly Leu Leu Gly Pro Tyr Ile Arg Ala Glu Val Glu Asp
865 870 875

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880 885 890

Tyr Ser Ser Leu Ile Ser Tyr Glu Glu Asp Gln Arg Gln Gly Ala Glu
895 900 905

Pro Arg Lys Asn Phe Val Lys Pro Asn Glu Thr Lys Thr Tyr Phe Trp
910 915 920 925

Lys Val Gln His His Met Ala Pro Thr Lys Asp Glu Phe Asp Cys Lys
930 935 940

Ala Trp Ala Tyr Phe Ser Asp Val Asp Leu Glu Lys Asp Val His Ser
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Gly Leu Ile Gly Pro Leu Leu Val Cys His Thr Asn Thr Leu Asn Pro
960 965 970

Ala His Gly Arg Gln Val Thr Val Gln Glu Phe Ala Leu Phe Phe Thr
975 980 985

Ile Phe Asp Glu Thr Lys Ser Trp Tyr Phe Thr Glu Asn Met Glu Arg
990 995 1000 1005

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1010 1015 1020

Glu Asn Tyr Arg Phe His Ala Ile Asn Gly Tyr Ile Met Asp Thr Leu
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Pro Gly Leu Val Met Ala Gln Asp Gln Arg Ile Arg Trp Tyr Leu Leu
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Ser Met Gly Ser Asn Glu Asn Ile His Ser Ile His Phe Ser Gly His
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Leu Tyr Pro Gly Val Phe Glu Thr Val Glu Met Leu Pro Ser Lys Ala

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Gln Tyr Gly Gln Trp Ala Pro Lys Leu Ala Arg Leu His Tyr Ser Gly
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Asp Leu Leu Ala Pro Met Ile Ile His Gly Ile Lys Thr Gln Gly Ala
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Arg Gln Lys Phe Ser Ser Leu Tyr Ile Ser Gln Phe Ile Ile Met Tyr
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1295 1300 1305

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