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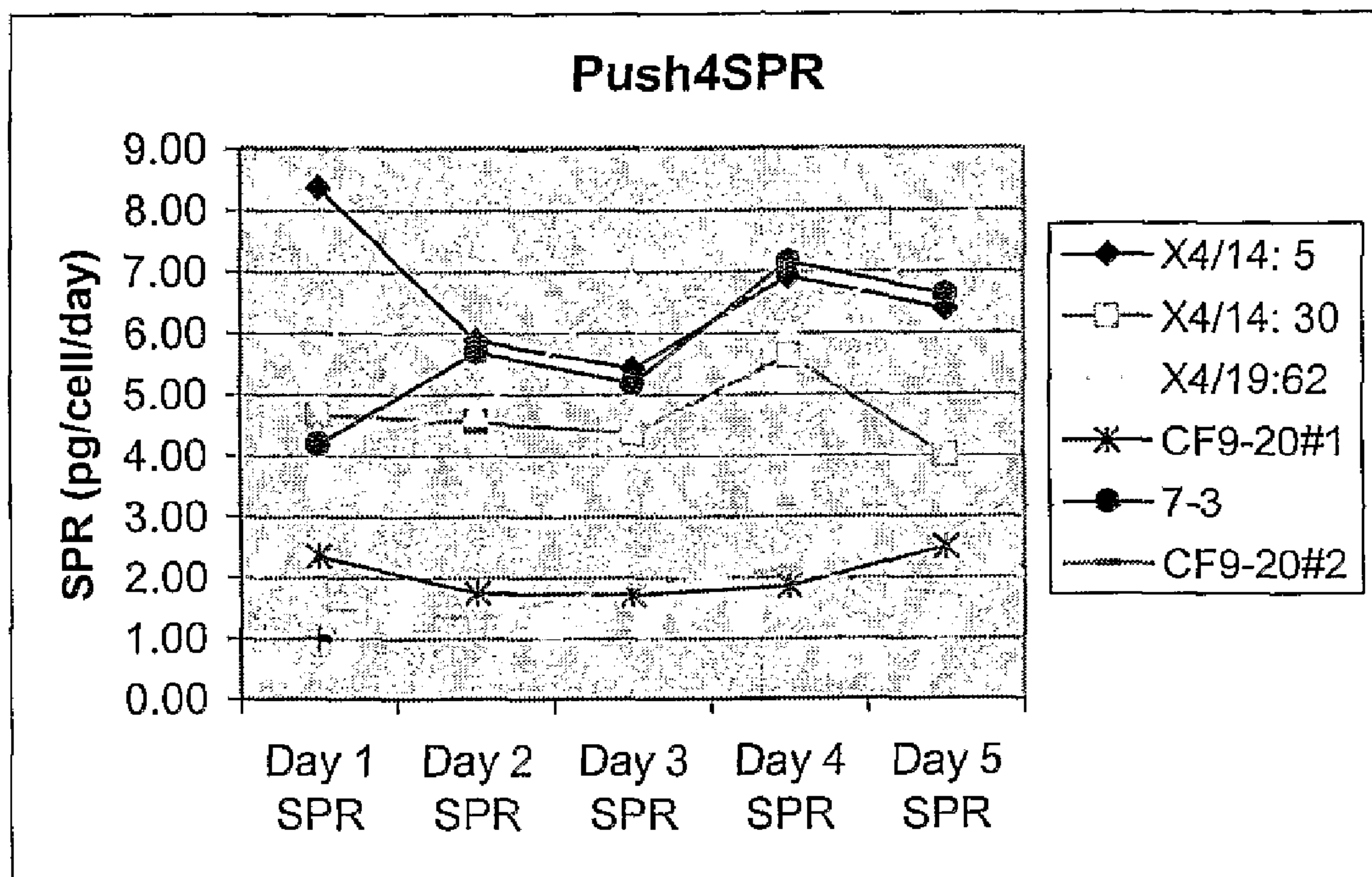
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SECRETEES RECOMBINANTES DANS LES CELLULES MAMMALIENNES

(54) Title: USE OF MOLECULAR CHAPERONES FOR THE ENHANCED PRODUCTION OF SECRETED,
RECOMBINANT PROTEINS IN MAMMALIAN CELLS



(57) Abrégé/Abstract:

The present invention relates to a method for increased production of a secreted, recombinant protein product through the introduction of molecular chaperones in a mammalian host cell. The present invention also relates to a mammalian host cell with enhanced expression of a secreted recombinant protein product by coexpressing at least one chaperone protein.

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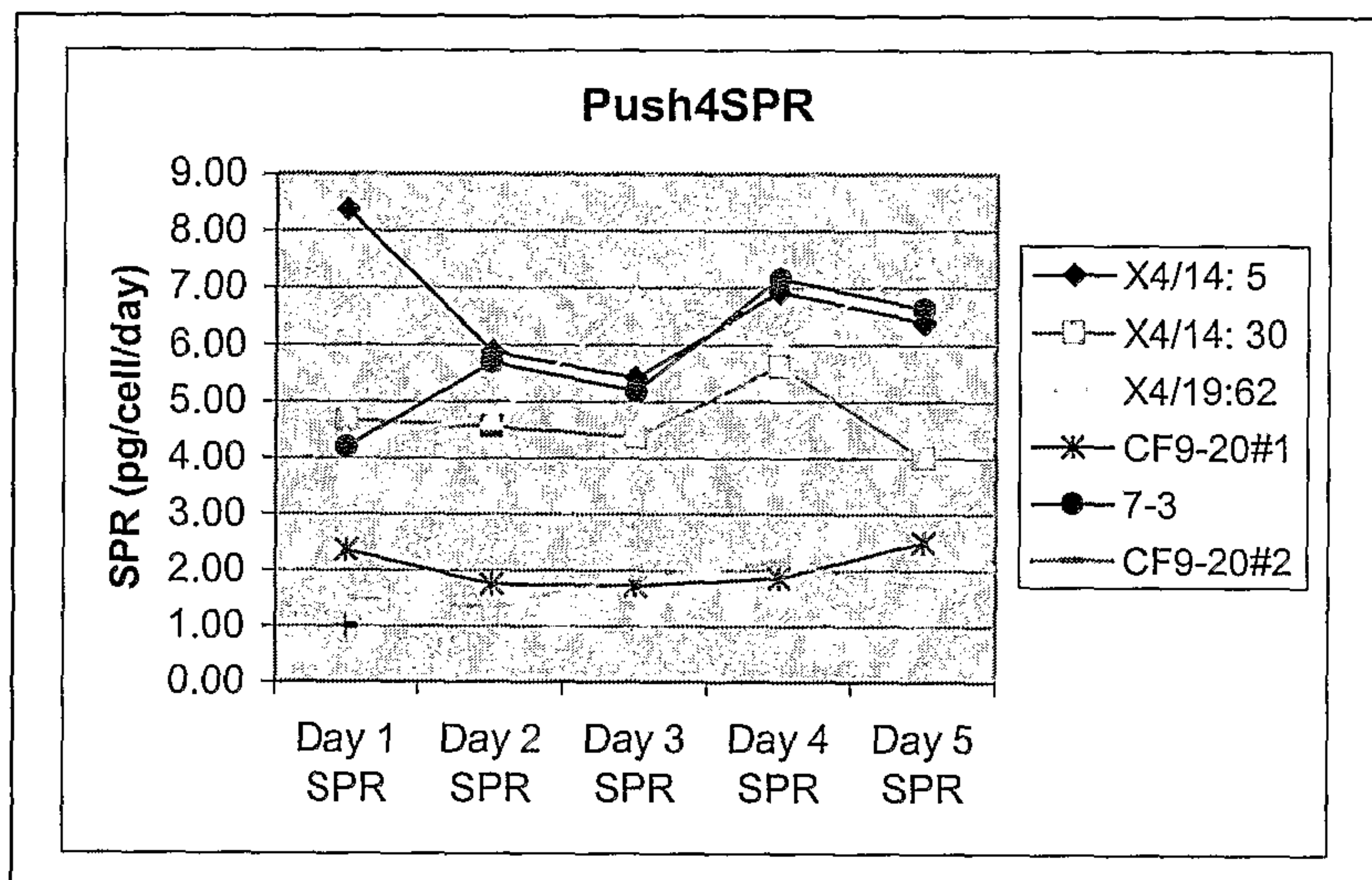
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(54) Title: USE OF MOLECULAR CHAPERONES FOR THE ENHANCED PRODUCTION OF SECRETED, RECOMBINANT PROTEINS IN MAMMALIAN CELLS



(57) Abstract: The present invention relates to a method for increased production of a secreted, recombinant protein product through the introduction of molecular chaperones in a mammalian host cell. The present invention also relates to a mammalian host cell with enhanced expression of a secreted recombinant protein product by coexpressing at least one chaperone protein.

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5 **Use of Molecular Chaperones for the Enhanced Production of Secreted,
 Recombinant Proteins in Mammalian Cells**

10 **Field of the Invention**

 The present invention relates to the general field of recombinant protein
production in a mammalian host cell. Specifically, the present invention relates to
15 enhanced production of a secreted recombinant protein product by coexpressing at least
one chaperone protein in the mammalian host cell.

Background of the Invention

 In both procaryotic and eucaryotic cells, molecular chaperone proteins catalyze
20 disulfide bond exchange and assist in the proper folding of newly synthesized proteins.
This observation has led to a large number of studies and proposed uses for these quality
control proteins. For example, increasing pDI (protein disulfide isomerase) activity in
bacterial, yeast and insect cell expression systems can have beneficial effects on protein
solubility and folding and, in some cases, can lead to an increase in the secretion of
25 heterologous proteins (1-7). In addition, other studies have shown that the molecular
chaperones immunoglobulin heavy chain binding protein (BiP, also referred to as glucose
regulated protein) and human heat shock protein 70 (Hsp 70) have a beneficial effect on
recombinant protein expression in insect cell systems (5, 8-12).

 Molecular chaperones have not had the same level of success on recombinant
30 protein expression and secretion in mammalian cell systems. For example, overexpression
of the pDI chaperone in Chinese hamster ovary (CHO) cells not only had no effect on the
secretion levels of IL-15, but also caused a decrease in secretion, and an increase in
cellular retention of a tumor necrosis factor receptor-Fc fusion protein (TNFR:Fc) (13).
Other studies have shown that overexpression of the BiP chaperone in mammalian cells

can lead to increased cellular retention and decreased secretion of recombinant proteins (14-15 and U.S. Patent No. 4,912,040). The regulatory mechanisms involved in protein processing within the mammalian cell are complex, and probably involve the cooperation of many of these chaperone proteins. Therefore, one cannot predict whether a particular
5 chaperone will lead to an increase in the production of a certain recombinant protein.

Because of the contradictory teaching in the field, the effect of chaperone proteins on the production of a secreted recombinant protein product is not understood and appreciated. U.S. Patent No. 6,451,597 (the '597 patent) describes a method for enhanced production of viral particles, and speculates on the effect of chaperones on improving
10 yield of a recombinant protein in eukaryotic cells. However, no actual expression of a recombinant protein is disclosed. However, other studies had found that over-expression of chaperones in eukaryotic cell lines either had no effect on product yields or had reduced secretion of recombinant proteins (14, 15). See also U.S. Patent No. 4,912,040. In light of the contradictory teaching in the field, the '597 patent does not enable one of
15 skill in the art to use chaperones to improve the production and secretion of a recombinant protein in eukaryotic cells. The state of art does not teach one to predict what effect a particular chaperone will have in the production and secretion of a given recombinant protein in cell culture models such as those described herein. The applicants were therefore surprised to find that when the chaperones described in this study were
20 transfected into mammalian cell lines expressing a secreted, recombinant protein, the resultant effect was an overall increase in the production of the secreted protein.

Summary of the Invention

The present invention relates to mammalian cells, methods and reagents therefor, for
25 enhanced expression of a secreted recombinant protein product in a mammalian host cell.

In accordance with one aspect of the present invention there is provided a mammalian host cell for enhanced expression of a recombinant protein product, said mammalian host cell having genetic material coding for expression of said recombinant protein product and transformed with at least one expression vector comprising DNA
30 encoding chaperone protein Hsp70, wherein the recombinant protein product is Factor VIII or a fragment thereof.

In accordance with another aspect of the present invention there is provided a method for producing a mammalian host cell for enhanced expression of a target recombinant protein or a fragment thereof, wherein the target recombinant protein is Factor VIII or a fragment thereof comprising: providing the mammalian host cell having
5 genetic material coding for expression of the target recombinant protein or a fragment thereof; and transforming the mammalian host cell with at least one expression vector comprising DNA encoding chaperone protein Hsp70.

In accordance with yet another aspect of the present invention there is provided a method for producing a secreted recombinant protein product, wherein the secreted
10 product is Factor VIII or a fragment thereof, comprising the steps of: culturing a mammalian host cell, said mammalian host cell having genetic material coding for expression of said recombinant protein product and transformed with at least one expression vector comprising DNA encoding chaperone protein Hsp70; and recovering from a culture medium the recombinant protein product so produced and secreted.

15 In accordance with still yet another aspect of the present invention there is provided a method for enhancing recombinant Factor VIII yield in a baby hamster kidney (BHK) cell line, wherein genetic material coding for expression of said recombinant Factor VIII has been previously introduced into a first BHK cell line, said method comprising the steps of: inserting at least one chaperone protein expression vector encoding Hsp70 into
20 said first BHK cell line so as to form a modified BHK cell line; and selecting from said modified BHK cell line at least one second cell line exhibiting enhanced yield of the recombinant Factor VIII product.

In accordance with still yet another aspect of the present invention there is provided a method for enhancing recombinant Factor VIII protein production comprising the steps
25 of: culturing a mammalian host cell, said mammalian host cell having genetic material coding for expression of the recombinant Factor VIII protein and transformed with at least one expression vector comprising DNA encoding chaperone protein Hsp70; and recovering from a culture medium the recombinant protein product so produced and secreted.

In one aspect of the invention, a mammalian host cell for enhanced expression of a recombinant protein product is provided, said mammalian cell having genetic material coding for expression of said recombinant protein product and transformed with at least one expression vector comprising DNA encoding a chaperone protein selected from the
5 group consisting of calnexin, calreticulin, Erp57, Hsp40, and Hsp70.

In one embodiment of the first aspect of the invention, the recombinant protein product is secreted.

In another embodiment of the invention, the genetic material coding for expression of said recombinant protein product is integrated into host cell DNA.

In another embodiment of the invention, the mammalian host cell is further transformed with an expression vector comprising DNA encoding a glutamine synthetase protein.

In another embodiment of the invention, the recombinant protein product comprises bikunin, Factor VIII, IL2SA, or fragment thereof.

In another embodiment of the invention, the transformation occurs with an expression vector comprising DNA encoding calnexin, calreticulin, Erp57, Hsp40, and Hsp70.

In another embodiment of the invention, the transformation occurs with a first expression vector comprising DNA encoding calreticulin and a second expression vector.

In a second aspect of the invention, a method for producing a mammalian host cell for enhanced expression of a target recombinant protein or fragment thereof is provided, wherein the method comprises providing a mammalian cell having genetic material coding for expression of a target recombinant protein or fragment thereof; and transforming the mammalian cell with at least one expression vector comprising DNA encoding a chaperone protein selected from the group consisting of calnexin, calreticulin, Erp57, Hsp40, and Hsp70.

In one embodiment of the second aspect of the invention, the recombinant protein product is secreted.

In another embodiment of the invention, the genetic material coding for expression of said recombinant protein product is integrated into host cell DNA.

In another embodiment of the invention, the mammalian host cell is further transformed with an expression vector comprising DNA encoding a glutamine synthetase protein.

In another embodiment of the invention, the recombinant protein product comprises bikunin, Factor VIII, IL2SA, or fragment thereof.

In another embodiment of the invention, the transformation occurs with an expression vector comprising DNA encoding calnexin, calreticulin, Erp57, Hsp40, or Hsp70.

In another embodiment of the invention, the transformation occurs with a first expression vector comprising DNA encoding calreticulin and a second expression vector comprising DNA encoding Erp57.

5 In a third aspect of the invention, a method for producing a secreted recombinant protein product is provided, the method comprising the steps of: culturing a mammalian host cell, said mammalian host cell having genetic material coding for expression of said recombinant protein product and transformed with at least one expression vector comprising DNA encoding a chaperone protein selected from the group consisting of calnexin, calreticulin, Erp57, hsp40, and Hsp70; and recovering from the culture medium
10 the recombinant protein product so produced and secreted.

In one embodiment of the third aspect of the invention, the recombinant protein product is secreted.

In another embodiment of the invention, the genetic material coding for expression of said recombinant protein product is integrated into host cell DNA.

15 In another embodiment of the invention, the mammalian host cell is further transformed with an expression vector comprising DNA encoding a glutamine synthetase protein.

In another embodiment of the invention, the recombinant protein product comprises bikunin, Factor VIII, IL2SA, or fragment thereof.

20 In another embodiment of the invention, the transformation occurs with an expression vector comprising DNA encoding calnexin, calreticulin, Erp57, Hsp40, or Hsp70.

In another embodiment of the invention, the transformation occurs with a first expression vector comprising DNA encoding calreticulin and a second expression vector
25 comprising DNA encoding Erp57.

In a fourth aspect of the invention, a method for enhancing yield of a recombinant protein or fragment thereof in a mammalian cell is provided, the method comprising providing a first cell line having genetic material coding for expression of said recombinant protein product or fragment thereof and introducing at least one chaperone
30 protein expression vector into said first cell line so as to form a modified cell line; and selecting from said modified cell line at least one second cell line exhibiting enhanced yield of the recombinant protein or fragment thereof.

In one embodiment of the forth aspect of the invention, the recombinant protein product is secreted.

In another embodiment of the invention, the genetic material coding for expression of said recombinant protein product is integrated into host cell DNA.

5 In another embodiment of the invention, the mammalian host cell is further transformed with an expression vector comprising DNA encoding a glutamine synthetase protein.

In another embodiment of the invention, the recombinant protein product comprises bikunin, Factor VIII, IL2SA, or fragment thereof.

10 In another embodiment of the invention, the chaperone expression vector comprises DNA encoding calnexin, calreticulin, Erp57, Hsp40, or Hsp70.

In another embodiment of the invention, said introducing occurs with a first chaperone expression vector comprising DNA encoding calreticulin and a second chaperone expression vector comprising DNA encoding Erp57.

15 In another embodiment of the invention, at least one second cell line is produced from said first cell line by selecting a portion of said first cell line exhibiting integration of the chaperone protein expression vector into host DNA.

In a fifth aspect of the invention, a method for enhancing yield of a recombinant protein or fragment thereof in a mammalian cell is provided, the method comprises
20 introducing genetic material coding for a recombinant protein or fragment thereof into a cell line exhibiting enhanced chaperone protein expression.

In one embodiment of this aspect of the invention, the recombinant protein product is secreted.

25 In another embodiment of the invention, the genetic material coding for expression of said recombinant protein product is integrated into host cell DNA.

In another embodiment of the invention, the cell is further transformed with an expression vector comprising DNA encoding a glutamine synthetase protein.

In another embodiment of the invention, the recombinant protein product comprises bikunin, Factor VIII, IL2SA, or fragment thereof.

30 In another embodiment of the invention, the chaperone protein comprises calnexin, calreticulin, Erp57, Hsp40, or Hsp70.

In another embodiment of the invention, the chaperone protein comprises calreticulin and Erp57.

Brief Description of the Drawings

The invention will be better understood from a consideration of the following
5 detailed description and claims, taken in conjunction with the drawings, in which:

Figure 1 depicts the sequences of RT-PCR primers used to amplify cDNA of ER
chaperones from a human cDNA library. Underlined indicates a built in EcoRI (5'
primer) or XbaI (3' primer) restriction site. CNX: calnexin; CRT: calreticulin;

10

Figure 2A depicts the complete nucleotide and amino acid sequences of calnexin cloned
by RT-PCR. The 5' EcoRI and 3' XbaI sites within the primers are underlined. The start
codon and stop codon are shown in bold text;

15 Figure 2B depicts the complete nucleotide and amino acid sequences of calreticulin
cloned by RT-PCR. The 5' EcoRI and 3' XbaI sites are underlined. The start codon and
stop codon are shown in bold text;

Figure 2C depicts the complete nucleotide and amino acid sequences of Erp57 cloned by
20 RT-PCR. The 5' EcoRI and 3' XbaI sites are underlined. The start codon and stop codon
are shown in bold text;

25

Figure 2D depicts the complete nucleotide and amino acid sequences of the coding region
of the human Hsp70 gene;

Figure 2E depicts the complete nucleotide and amino acid sequences of the coding region
of the human Hsp40 gene. The start codon is shown in bold and underlined text;

Figure 2F depicts the complete nucleotide and amino acid sequences of the coding region
30 of the glutamine synthetase gene. The start codon is shown in bold and underlined text;

Figure 3 is an illustration of overexpression of bikunin in clones super transfected with
calnexin (X4.14:5, X4/14:30), Hsp70 (7-3) or Erp57(X4/19:62). The specific Bikunin

production rate for all cell lines is expressed as pg Bikunin/cell/day (SPR). Each day cells were harvested and transferred into fresh media and incubated for 24 hours at 37°C in shaking flasks. The following day, cells were harvested again, counted and re-suspended into fresh media of the same volume and incubated similarly for another 24 hours.

5 Bikunin activity measurements (pg/cell/day) were conducted on samples of the spent media. The same procedure was repeated every day until the cell number and viability started to decrease. The control cell line (CF 9-20) expresses bikunin but does not express any of chaperone proteins;

10 Figure 4 is an illustration of overexpression of bikunin in clones super transfected with Hsp70. All clones except CF9-20 (control cells) are super transfected with Hsp70. The experiment procedure is the same as that described in Figure 3; and

Figure 5 depicts the amino acid sequence of bikunin.

15

Detailed Description of the Invention

The present invention relates to a method and reagents therefor, for enhanced expression of a secreted recombinant protein product in a mammalian host cell.

20 In one embodiment of the invention, a mammalian host cell for enhanced expression of a recombinant protein product is provided, wherein said mammalian cell comprises genetic material coding for expression of said recombinant protein product and is further transformed with at least one expression vector comprising DNA encoding a chaperone protein selected from the group consisting of calnexin, calreticulin, Erp57, Hsp40, and Hsp70.

25 In another embodiment of the invention, the mammalian host cell is stably transformed with the genetic material coding for expression of said recombinant protein product.

30 The term "mammalian host cell" is used to refer to a mammalian cell which has been transfected, or is capable of being transfected with a nucleic acid sequence and then of expressing a selected gene of interest. The term includes the progeny of the parent cell, whether or not the progeny is identical in morphology or in genetic make-up to the original parent, so long as the selected gene is present.

Suitable mammalian cells for use in the present invention include, but are not limited to Chinese hamster ovary (CHO) cells, baby hamster kidney (BHK) cells, human HeLa cells, monkey COS-1 cell, human embryonic kidney 293 cells, mouse myeloma NSO and human HKB cells (US Patent No. 6,136,599). The other cell lines are readily
5 available from the ATCC.

The term "transfection" is used to refer to the uptake of foreign or exogenous DNA by a cell, and a cell has been "transfected" when the exogenous DNA has been introduced inside the cell membrane. A number of transfection techniques are well known in the art and are disclosed herein. See, e.g., Graham *et al.*, 1973, *Virology*
10 52:456; Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual* (Cold Spring Harbor Laboratories, 1989); Davis *et al.*, *Basic Methods in Molecular Biology* (Elsevier, 1986); and Chu *et al.*, 1981, *Gene* 13:197. Such techniques can be used to introduce one or more exogenous DNA moieties into suitable host cells.

Suitable techniques of transfection for use in the present invention include, but are not limited to calcium phosphate-mediated transfection, DEAE-dextran mediated
15 transfection, and electroporation. Cationic lipid transfection using commercially available reagents including the Boehringer Mannheim Transfection Reagent (N->1-(2,3-Dioleoyloxy)propyl-N,N,N-trimethyl ammoniummethanesulfate, Boehringer Mannheim, Indianapolis, Ind.) or LIPOFECTIN or LIPOFECTAMIN or DMRIE reagent (GIBCO-
20 BRL, Gaithersburg, MD) may also be used.

As used herein the term "super transfection" refers to transfecting more than one expression vectors to a host cell already expressing a recombinant gene.

The term "transformation" as used herein refers to a change in a cell's genetic characteristics, and a cell has been transformed when it has been modified to contain a
25 new DNA. For example, a cell is transformed where it is genetically modified from its native state. Following transfection, the transforming DNA may recombine with that of the cell by physically integrating into a chromosome of the cell, may be maintained transiently as an episomal element without being replicated, or may replicate independently as a plasmid. A cell is considered to have been stably transformed when
30 the DNA is replicated with the division of the cell.

As used herein the term "modified cell line" refers to a cell line either transiently or stably transformed with one or more DNA constructs.

Polynucleotides, genetic material, recombinant DNA molecules, expression vectors, and such, used in the practice of the present invention may be isolated using standard cloning methods such as those described by Sambrook et al. (Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., 1989).

5 Alternatively, the polynucleotides coding for a recombinant protein product of the present invention may be synthesized using standard techniques that are well known in the art, such as by synthesis on an automated DNA synthesizer. For example, in one embodiment of the invention, DNA sequences encoding the calnexin protein are synthesized by RT-PCR using primers depicted in Figure 1.

10 As used herein an "expression vector" refers to a DNA molecule, or a clone of such a molecule, which has been modified through human intervention to contain segments of DNA combined and juxtaposed in a manner that would not otherwise exist in nature. DNA constructs may be engineered to include a first DNA segment encoding a polypeptide of the present invention operably linked to additional DNA segments
15 required for the expression of the first DNA segment. Within the context of the present invention additional DNA segments will generally include promoters and transcription terminators and may further include enhancers and other elements. One or more selectable markers may also be included. DNA constructs useful for expressing cloned DNA segments in a variety of prokaryotic and eukaryotic host cells can be prepared from
20 readily available components or purchased from commercial suppliers.

DNA constructs may also contain DNA segments necessary to direct the secretion of a polypeptide or protein of interest. Such DNA segments may include at least one secretory signal sequence. Secretory signal sequences, also called leader sequences, prepro sequences and/or pre sequences, are amino acid sequences that act to direct the
25 secretion of mature polypeptides or proteins from a cell. Such sequences are characterized by a core of hydrophobic amino acids and are typically (but not exclusively) found at the amino termini of newly synthesized proteins. Very often the secretory peptide is cleaved from the mature protein during secretion. Such secretory peptides contain processing sites that allow cleavage of the secretory peptide from the mature protein as it passes through
30 the secretory pathway. A recombinant protein may contain a secretory signal sequence in its original amino acid sequence, or may be engineered to become a secreted protein by

inserting an engineered secretory signal sequence into its original amino acid sequence. The choice of suitable promoters, terminators and secretory signals is well within the level of ordinary skill in the art. Expression of cloned genes in cultured mammalian cells and in *E. coli*, for example, is discussed in detail in Sambrook et al. (Molecular Cloning:
5 A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., 1989).

As used herein, the term “recombinant protein product” refers to a recombinant protein or fragment thereof expressed from the genetic material introduced into the host mammalian cell.

After transfection, the cell may be maintained either transiently transformed or
10 stably transformed with said DNA construct. Introduction of multiple DNA constructs, and selection of cells containing the multiple DNA constructs can be done either simultaneously or, more preferably, sequentially. The technique of establishing a cell line stably transformed with a genetic material or expression vector is well known in the art (Current Protocols in Molecular Biology). In general, after transfection, the growth
15 medium will select for cells containing the DNA construct by, for example, drug selection or deficiency in an essential nutrient, which is complemented by a selectable marker on the DNA construct or co-transfected with the DNA construct. Cultured mammalian cells are generally cultured in commercially available serum-containing or serum-free medium. Selection of a medium appropriate for the particular host cell used is within the level of
20 ordinary skill in the art.

Suitable selectable markers for drug selection used in this invention include, but are not limited to, neomycin (G418), hygromycin, puromycin, zeocin, colchicine, methotrexate, and methionine sulfoximine.

Once a drug resistant cell population is established, individual clones may be
25 selected and screened for high expressing clones. Methods of establishing cloned cell line are well known in the art, including, but not limited to, using a cloning cylinder, or by limiting dilution. Expression of the recombinant product of interest from each clone can be measured by methods such as, but not limited to, immunoassay, enzymatic assay, or chromogenic assay.

Cell line stably transformed with a first DNA construct may be then used as host cell for transfection with a second or more DNA constructs, and subjected to different drug selections.

5 In one particular embodiment there is provided a mammalian host cell for enhanced expression of a recombinant protein product, said mammalian cell having genetic material coding for expression of said recombinant protein product and transformed with at least one expression vector comprising DNA encoding chaperone protein Hsp70.

10 In another particular embodiment there is provided a mammalian host cell for enhanced expression of bikunin or a fragment thereof, said mammalian cell having genetic material coding for expression of bikunin or a fragment thereof and transformed with at least one expression vector comprising DNA encoding chaperone protein Hsp70.

15 In yet another particular embodiment there is provided a mammalian host cell for enhanced expression of Factor VIII or a fragment thereof, said mammalian cell having genetic material coding for expression of Factor VIII or a fragment thereof and transformed with at least one expression vector comprising DNA encoding chaperone protein Hsp70.

20 In still yet another particular embodiment there is provided a mammalian host cell for enhanced expression of IL2SA or a fragment thereof, said mammalian cell having genetic material coding for expression of IL2SA or a fragment thereof and transformed with at least one expression vector comprising DNA encoding chaperone protein Hsp70.

25 In still yet another particular embodiment there is provided a method for producing a mammalian host cell for enhanced expression of a target recombinant protein or a fragment thereof comprising: providing a mammalian cell having genetic material coding for expression of a target recombinant protein or a fragment thereof; and transforming the mammalian cell with at least one expression vector comprising DNA encoding chaperone protein Hsp70.

30 In still yet another particular embodiment there is provided a method for producing a secreted recombinant protein product comprising the steps of: culturing a mammalian host cell, said mammalian host cell having genetic material coding for

expression of said recombinant protein product and transformed with at least one expression vector comprising DNA encoding chaperone protein Hsp70; and recovering from the culture medium the recombinant protein product so produced and secreted.

5 In still yet another particular embodiment there is provided a method for enhancing recombinant bikunin protein yield in a Chinese hamster ovary (CHO) cell line, wherein genetic material coding for expression of said recombinant bikunin has been previously introduced into a first CHO cell line, said method comprising the steps of: inserting at least one chaperone protein expression vector encoding Hsp70 into said first CHO cell line so as to form a modified CHO cell line; and selecting from said modified
10 CHO cell line at least one second cell line exhibiting enhanced yield of the recombinant bikunin protein.

In still yet another particular embodiment there is provided a method for enhancing recombinant Factor VIII yield in a baby hamster kidney (BHK) cell line, wherein genetic material coding for expression of said recombinant Factor VIII has been
15 previously introduced into a first BHK cell line, said method comprising the steps of: inserting at least one chaperone protein expression vector encoding Hsp70 into said first BHK cell line so as to form a modified BHK cell line; and selecting from said modified BHK cell line at least one second cell line exhibiting enhanced yield of the recombinant Factor VIII product.

20 In still yet another particular embodiment there is provided a method for enhancing recombinant IL2SA protein yield in a CHO cell line, wherein genetic material coding for expression of said recombinant IL2SA has been previously introduced into a first CHO cell line, said method comprising the steps of: inserting at least one chaperone protein expression vector encoding Hsp70 into said first CHO cell line so as to form a
25 modified CHO cell line; and selecting from said modified CHO cell line at least one second cell line exhibiting enhanced yield of the recombinant IL2SA protein.

In still yet another particular embodiment there is provided a method for enhancing yield of a recombinant Factor VIII or a fragment thereof in a BHK cell line comprising introducing genetic material coding for such Factor VIII or a fragment thereof
30 into a BHK cell line exhibiting enhanced chaperone protein expression, wherein the chaperone protein is Hsp70.

In still yet another particular embodiment there is provided a method for enhancing yield of a recombinant IL2SA or a fragment thereof in a CHO cell line comprising introducing genetic material coding for such IL2SA into a CHO cell line exhibiting enhanced chaperone protein Hsp70 expression.

5 In still yet another particular embodiment there is provided a method for enhancing recombinant Factor VIII protein production comprising the steps of: culturing a mammalian host cell, said mammalian host cell having genetic material coding for expression of the recombinant Factor VIII protein and transformed with at least one expression vector comprising DNA encoding chaperone protein Hsp70; and recovering
10 from the culture medium the recombinant protein product so produced and secreted.

In still yet another particular embodiment there is provided a method for enhancing recombinant IL2SA protein production comprising the steps of: culturing a mammalian host cell, said mammalian host cell having genetic material coding for expression of the recombinant IL2SA protein and transformed with at least one
15 expression vector comprising DNA encoding chaperone protein Hsp70; and recovering from the culture medium the recombinant protein product so produced and secreted.

In one embodiment of the invention, a mammalian host cell with enhanced expression and secretion of bikunin protein or fragment thereof is provided, wherein the

mammalian host cell is further transformed with at least one expression vector comprising DNA encoding a chaperone protein selected from the group consisting of calnexin, calreticulin, Erp57, Hsp40, and Hsp70.

5 In a preferred embodiment of the invention, the mammalian host cell with enhanced expression and secretion of bikunin is a CHO cell.

As used herein the term "bikunin" refers to any protein, which has at least one Kunitz domain. Kunitz-type domains have been described in references such as Laskowski et al., 1980, Ann Rev Biochem. 49:593-626; and U.S. Patent No. 5,914,315 (June 22, 1999). In one preferred embodiment, the term bikunin used herein refers to the
10 amino acid sequence shown in Figure 5. Other bikunin proteins and fragments thereof are described in U.S. Patent No. 6,583,208, U.S. Patent Publication No. 2003-0194398 A1, and PCT Application serial numbers US97/03894, published as WO 97/33996, and US99/04381, published as WO 00/37099).

In another embodiment of the invention, the invention provides a mammalian host
15 cell with enhanced expression and secretion of Factor VIII protein or fragment thereof, and the mammalian host cell is further transformed with at least one expression vector comprising DNA encoding a chaperone protein selected from the group consisting of calnexin, calreticulin, Erp57, Hsp40, and Hsp70.

In one preferred embodiment, the Factor VIII protein has the sequence depicted in
20 U.S. Patent No. 4,965,199.

In yet another preferred embodiment, the mammalian host cell with enhanced expression and secretion of Factor VIII is a BHK cell.

In another embodiment of the invention, the invention provides a mammalian host cell with enhanced expression and secretion of IL2SA protein or fragment thereof, and
25 the mammalian host cell is further transformed with at least one expression vector comprising DNA encoding a chaperone protein selected from the group consisting of calnexin, calreticulin, Erp57, Hsp40, and Hsp70.

In one preferred embodiment, the IL2SA protein has the sequence depicted in US patent No. 6,348,192.

30 In yet another preferred embodiment, the mammalian host cell with enhanced expression and secretion of IL2SA is a CHO cell.

In still another embodiment of the invention, the mammalian host cell is further transformed with an expression vector encoding a glutamine synthetase protein.

The present invention also provides a method for producing a mammalian host cell for enhanced expression of a target recombinant protein or fragment thereof comprising: providing a mammalian cell having genetic material coding for expression of a target recombinant protein or fragment thereof; and transforming the mammalian cell with at least one expression vector comprising DNA encoding a chaperone protein selected from the group consisting of calnexin, calreticulin, Erp57, Hsp40, and Hsp70.

In one embodiment of the invention, the genetic material coding for expression of said recombinant protein product is integrated into host cell DNA.

In another embodiment of the invention, the mammalian host cell is further transformed with an expression vector comprising DNA encoding a glutamine synthetase protein.

In one preferred embodiment of the invention, the recombinant protein product is bikunin or fragment thereof and the transformation occurs with an expression vector comprising DNA encoding calnexin, Erp57, calreticulin, or Hsp70.

In another preferred embodiment of the invention, the recombinant protein product is Factor VIII or fragment thereof and the transformation occurs with a first expression vector comprising DNA encoding calreticulin and a second expression vector comprising DNA encoding Erp57.

In another preferred embodiment of the invention, the recombinant protein product is Factor VIII or fragment thereof and the transformation occurs with an expression vector comprising DNA encoding calnexin or Hsp70.

In another preferred embodiment of the invention, the recombinant protein product is IL2SA or fragment thereof and the transformation occurs with an expression vector comprising DNA encoding Hsp70.

The present invention also provides a method for producing a secreted recombinant protein product comprising culturing a mammalian host cell, said mammalian host cell having a genetic material coding for expression of said recombinant product and further transformed with at least one expression vector comprising DNA encoding a chaperone protein elected from the group consisting of calnexin, calreticulin,

Erp57, Hsp40, and Hsp70; and recovering from the culture medium the bikunin protein or fragment thereof so produced and secreted.

In one embodiment of the invention, the method for producing a secreted recombinant protein product comprising culturing a mammalian host cell, wherein the
5 mammalian host cell is stably transformed with a genetic material coding for the expression of said recombinant product.

In another embodiment of the invention, the method for producing a secreted recombinant protein product further comprises transfecting the mammalian host cell with an expression vector encoding a glutamine synthetase protein.

10 One embodiment of the invention provides a method of producing a bikunin protein or fragment thereof, comprising culturing a mammalian host cell expressing bikunin or fragment thereof, and at least one of the chaperone proteins selected from the group consisting of calnexin, calreticulin, Erp57, Hsp40, and Hsp70; and recovering from the culture medium the bikunin protein or fragment thereof so produced and secreted.

15 In one embodiment of the invention, a method for enhanced production of a recombinant bikunin protein in a CHO cell is provided, wherein a genetic material coding for expression of said recombinant bikunin has been previously introduced into a first CHO cell line (as described in U.S. Patent Publication No. 2004-0235715 to Chan published November 25, 2004), comprising the steps of: inserting at least one chaperone
20 protein expression vector into said first CHO cell line so as to form a modified CHO cell line; and selecting from said modified CHO cell line at least one second cell exhibiting enhanced yield of the recombinant bikunin protein.

In another embodiment of the invention, the method for enhancing recombinant bikunin yield in a CHO cell line comprises introducing a genetic material for such
25 bikunin into a CHO cell line, wherein the CHO cell line exhibits enhanced chaperone protein expression.

In yet another embodiment of the invention, a method for enhanced production of a recombinant Factor VIII protein in a BHK cells is provided, wherein a genetic material coding for expression of said recombinant Factor VIII has been previously introduced
30 into a first BHK cell line, comprising the steps of: inserting at least one chaperone protein expression vector into said first BHK cell line so as to form a modified BHK cell line; and selecting from said modified BHK cell line at least one second cell exhibiting enhanced yield of the recombinant Factor VIII protein.

In still another embodiment of the invention, the method for enhancing recombinant Factor VIII yield in a BHK cell line comprises introducing a genetic material for such Factor VIII into a BHK cell line, wherein the BHK cell line exhibits enhanced chaperone protein expression.

5 The present invention also provides a method for enhanced production of a recombinant IL2SA protein into a CHO cell, wherein a genetic material coding for expression of said recombinant IL2SA has been previously introduced into a first CHO cell line, comprising the steps of: inserting at least one chaperone protein expression vector into said first CHO cell line so as to form a modified CHO cell line; and selecting
10 from said modified CHO cell line at least one second cell exhibiting enhanced yield of the recombinant IL2SA protein.

In another embodiment of the invention, the method for enhancing recombinant IL2SA yield in a CHO cell line comprises introducing a genetic material for such IL2SA into a CHO cell line, wherein the CHO cell line exhibits enhanced chaperone protein
15 expression.

The following examples are intended for illustration purposes only, and should not be construed as limiting the scope of the invention in any way.

20 **Examples**

Example 1. Cloning of chaperone cDNA.

All chaperone sequences were cloned from human cDNA libraries followed by verification of the nucleotide sequences. DNA sequences representing the three ER
25 chaperones were cloned by RT-PCR from a human cDNA library. The RT-PCR primers used in these reactions were designed to amplify the entire coding region using the appropriate sequences obtained from Genbank. Each pair of 5' and 3' primers include either an EcoRI (5' primer) or XbaI (3' primer) restriction site (Figure 1) to facilitate cloning of the PCR product into the expression vector, pCI-neo (Promega).

30 The PCR reactions were performed using high fidelity PFU enzyme (Stratagene). Bands of the expected size were purified, digested with EcoR I and Xba I and cloned into the similarly digested pCI-neo vector. Recombinant vectors from this step were propagated in E. Coli followed by isolation and purification of the vector sequences. The

sequence inserts representing the chaperones were sequenced using primers binding just outside the multiple cloning sites of the vector as well as within the chaperone sequence. Sequencing was done using the Big Dye™ terminator method on MJ Research's thermal cyclers and analyzed using an ABI 310 Genetic Analyzer. The cDNA sequences of human calnexin, clareticulin and Erp57 are shown in Figures 2A-2C.

The full-length human Hsp70 cDNA fragment was obtained by RT-PCR using human brain polyA⁺ RNA (CLONTECH Cat: 6516-1) and two primers designated F-Hsp70 = 5'AGG GAA CCG CAT GGC CAA AG and R-Hsp70 = 5' GAA AGG CCCCTA ATC TAC CTC CTC A. The primer sequences of Hsp 70 were derived from the previously published sequence for the human heat shock protein (Hsp70) gene [9]. The F-Hsp70 and R-Hsp70 primers included either an EcoRI or XbaI sequence respectively. The desired PCR fragment was purified by agarose gel electrophoresis and confirmed by nucleotide sequencing. The full-length human Hsp70 cDNA fragment was then inserted into the EcoRI and XbaI cloning sites of the pCI-neo vector to form the pCI-neo-Hsp70 vector. The pCI-neo-Hsp70 vector was propagated in E. Coli followed by isolation and purification of the vector sequences. pCI-neo-Hsp70 plasmid DNA was sequenced by ABI PRISM™ 310 Genetic Analyzer. The sequence of human Hsp70 is shown in Figure 2D.

Example 2. Bikunin production is increased in CHO cells after transfection of an ER chaperone such as calnexin, calreticulin, Erp57 or Hsp70.

A CHO cell line secreting the Bikunin recombinant protein (U.S. Patent Publication No. 2004-0235715) was super transfected with various combinations of the ER chaperones, calnexin (CNX), calreticulin (CRT), ERp57 or Hsp70 followed by selection with G418. Populations were obtained and screened by kallikrein assay (U.S. Publication No. 2004-0235715). Briefly, bikunin standards or culture fluid was serially diluted and incubated with an equal volume of kallikrein at 37°C for 30 minutes, after which a chromogenic substrate, N-benzoyl-Pro-Phe-Arg-pNA, was added. The reaction was incubated for 15 minutes before the addition of 50% acetic acid. The amount of p-nitroanilide released was measured at 405 nM. Populations showing the highest Bikunin

titers were then single cell cloned and growth expanded over a period of several weeks. Clones showing consistently higher Bikunin titers (2-4x) relative to the control CF9-20 cells were retained and expanded into shake flasks for further analysis. These clones were further narrowed based on Bikunin titers and growth characteristics demonstrated while growing in the shake flask environment. Final candidate clones were selected after several rounds and extensive analyses at the shake flask stage.

The specific Bikunin production rate for all cell lines is expressed as pg Bikunin/cell/day (SPR). Each day cells were harvested and transferred into fresh media and incubated for 24 hours at 37°C in shaking flasks. The following day, cells were harvested again, counted and re-suspended into fresh media of the same volume and incubated similarly for another 24 hours. Bikunin activity measurements (pg/cell/day) were conducted on samples of the spent media. The same procedure was repeated every day until the cell number and viability started to decrease.

The effect of chaperone proteins on bikunin expression is shown in Figures 3 and 4. The control cell line (CF9-20) expresses Bikunin but does not express any of chaperone proteins. The effect of calnexin, calreticulin, and Erp57 on bikunin expression is further summarized in Table 1.

Table 1. Overall Bikunin production levels are 2-4 fold higher in clones that have been super transfected with a chaperone

	<u>Clone</u>	<u>Bikunin Increase Relative to Control</u>	<u>Chaperone</u>
25	X4/14:5	2-4	CNX
	X4/14:30	2-4	CNX
	X4/19:62	2-4	ERp57
	T4/13:22	1.5-2	CRT

Fold activity measurements are relative to a control cell line that expresses Bikunin but does not express any of the chaperone proteins. Cells were grown in serum free media in shake flask cultures.

Example 3. Recombinant Factor VIII production is increased in BHK cells after transfection with ER chaperones.

Stable Factor VIII producing cells (MWCB1) (U.S. Patent No. 4,965,199; ATCC No. CRL 8544) were transfected with chaperone expression vectors in addition to pPUR, a vector containing puromycin-resistant gene, in a 10 : 1 ratio. Approximately 4×10^6 MWCB1 cells were transfected with a total of 5 μ g of DNA using the DMRIE-C reagent and OPTI-MEM medium (Life Technology, MD) in 6-well plates. Three days post transfection, 100,000 cells were seeded in 6-well plates and then selected in the presence of 1 – 2 μ g/ ml puromycin with OPTI-MEM medium containing 2% FBS for 2 weeks. Puromycin resistant colonies were manually picked and seeded into 96 well plates and expanded without the presence of drug. Individual clonal populations were screened for Factor VIII production using a COATEST kit (Chromogenix, Italy) according to manufacturer's instructions. The high producing clones were sequentially expanded from the 6 well dish, to T75 flask, followed by shake flask stage for stability and productivity tests. The Calnexin (CNX), Calreticulin (CRT), Erp57, Hsp40 and Hsp70 chaperones were then transfected into cells individually or in combinations of two. A significant 2 to 3 fold increase of productivity of Factor VIII was observed in clones transfected with CNX, CRT and Erp57, Hsp70, and Hsp40 while the empty vector control (PCI-Neo) showed no difference compared to the parent MWCB1 cells (Table 2).

20

Table 2. Recombinant Factor VIII productivity in clones

	Factor VIII (U/ml)	Fold of Inc (SPR)
MWCB1(27000JC)	0.11	1.00
PCI-Neo + pPUR	0.09	1.00
CNX + pPUR	0.31	2.88
CRT + pPUR	0.13	1.25
Erp57 + pPUR	0.05	0.91
CRT, Erp57 + pPUR	0.29	2.50
Hsp70 + pPUR	0.37	2.50
Hsp40 + pPUR	0.11	1.00
Hsp70, 40 + pPUR	0.28	1.66

Cells were seeded at 1×10^6 per ml, total 15 ml in shake flask 2-day

25

Example 4. Co-expression of BiP and PDI does not enhance the expression of Factor VIII and anti-TNF antibody in BHK and CHO cells.

Recombinant CHO cells (as described in Example 2) expressing high levels of bikunin, and recombinant BHK cells (as described in Example 3) expressing high levels of recombinant Factor VIII (rFVIII) were super-transfected with pHyg (plasmid conferring hygromycin resistance) and pBiP. The transfection conditions and selection conditions were same as in Example 2. After selection in hygromycin and limiting dilution cloning, clones were evaluated for productivity for bikunin and rFVIII activity. No significant difference in the specific productivity of clones derived from cells transfected only with the control vector (pHyg) and clones derived from cells transfected with pBiP.

Example 5. Transfection of IL2SA-producing clone with Glutamine Synthetase (GS) and Hsp70.

IL2SA (IL2 selective agonist; U.S. Patent No. 6,348,192) producing CHO cell line, 49-19-H42 (a clonal variant of ATCC deposit PTA-8), was co-transfected with PCI-GS and PCI-neo-Hsp70. 4×10^6 cells were transfected with 2.5 μ g of plasmid DNA using DMRIE-C reagents and OPTI-MEM medium (Life Technology, MD) in 6-well plates according to manufacturer's instructions. Three days after transfection, cells were seeded in 150-mm and 96 well plates and then selected in the presence of 10 μ M MSX (methionine sulfoximine) and 250 μ g/ml G418 with DME:F12 (1:1) medium deficient in glutamine containing 2% dialyzed FBS for 2 weeks. Single cell colonies were picked and re-seeded in 96 wells. The clones were selected for another week with increased concentrations of MSX (20 μ M) and G418 (400 μ g/ml). A pool is generated from a 150-mm plate after 3 weeks' selection. The pool and clones were gradually expanded to shake flasks and screened for IL2 productivity using ELISA. The expression of GS and Hsp70 proteins were confirmed by FACSTM analysis using a flow cytometer. The "GS positive" cells were cultured in a glutamine-free medium supplement with 5.6 mM glutamate and 4 g/L glucose. The doubling time of these clones varied from 24 to 48 hr. A comparison of the productivity of the parent and clones is shown in Table 3. A 2- 4 fold increase in overall titer and a 2 -3 fold increase in specific productivity was observed in all the single cell clones when compared against either the pool or the parental line.

Table 3. Productivity of IL2SA producing cells

	Titer ($\mu\text{g/ml}$)	Cell density ($10^6/\text{ml}$)	SPR (pg/c/d)	GS	Hsp70
49-19H42 parent line	18.78	3.51	2.67	(-)	(-)

5

49-19H42 GShsp70-SC#12	33.87	2.63	6.44	+++	+++
49-19H42 GShsp70-SC#14	22.08	1.83	6.03	+++	+++
49-19H42 GShsp70-SC#17	64.00	3.05	10.50	+++	+++
49-19H42 GShsp70-pool	10.59	1.74	3.04	+++	+

Cells were seeded at 1 million per ml at day 0 in 15 ml of complete (for the parental line) or glutamine-free medium. Samples were taken at 2 day after seeding and analyzed using ELISA. For GS and Hsp70 expression, cells were fixed with 70% EtOH, labeled with proper antibodies, and analyzed by FACS.

10

+++ = all cells expressed GS or Hsp70; + = 30% of cells expressed GS or Hsp70; (-) = no expression.

:

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SEQUENCE LISTING

<110> BAYER PHARMACEUTICALS CORPORATION

<120> USE OF MOLECULAR CHAPERONES FOR THE ENHANCED PRODUCTION OF
SECRETED, RECOMBINANT PROTEINS IN MAMMALIAN CELLS

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Asp Val Lys Glu Glu Glu Glu Glu Lys Glu Glu Glu Lys Asp Lys Gly
525 530 535

gat gag gag gag gaa gga gaa gag aaa ctt gaa gag aaa cag aaa agt 1684
Asp Glu Glu Glu Glu Gly Glu Glu Lys Leu Glu Glu Lys Gln Lys Ser
540 545 550

gat gct gaa gaa gat ggt ggc act gtc agt caa gag gag gaa gac aga 1732
Asp Ala Glu Glu Asp Gly Gly Thr Val Ser Gln Glu Glu Glu Asp Arg
555 560 565 570

aaa cct aaa gca gag gag gat gaa att ttg aac aga tca cca aga aac 1780
Lys Pro Lys Ala Glu Glu Asp Glu Ile Leu Asn Arg Ser Pro Arg Asn
575 580 585

aga aag cca cga aga gag tga aacaatctta agagcttgat ctgtgatttc 1831
Arg Lys Pro Arg Arg Glu
590

ttctccctcc tcccctgcat ctaga 1856

<210> 8
<211> 592
<212> PRT
<213> Homo sapiens

<400> 8
Met Glu Gly Lys Trp Leu Leu Cys Met Leu Leu Val Leu Gly Thr Ala
1 5 10 15

Ile Val Glu Ala His Asp Gly His Asp Asp Asp Val Ile Asp Ile Glu
20 25 30

Asp Asp Leu Asp Asp Val Ile Glu Glu Val Glu Asp Ser Lys Pro Asp
35 40 45

Thr	Thr	Ala	Pro	Pro	Ser	Ser	Pro	Lys	Val	Thr	Tyr	Lys	Ala	Pro	Val
50						55					60				
Pro	Thr	Gly	Glu	Val	Tyr	Phe	Ala	Asp	Ser	Phe	Asp	Arg	Gly	Thr	Leu
65					70					75					80
Ser	Gly	Trp	Ile	Leu	Ser	Lys	Ala	Lys	Lys	Asp	Asp	Thr	Asp	Asp	Glu
				85					90					95	
Ile	Ala	Lys	Tyr	Asp	Gly	Lys	Trp	Glu	Val	Glu	Glu	Met	Lys	Glu	Ser
				100				105						110	
Lys	Leu	Pro	Gly	Asp	Lys	Gly	Leu	Val	Leu	Met	Ser	Arg	Ala	Lys	His
		115					120					125			
His	Ala	Ile	Ser	Ala	Lys	Leu	Asn	Lys	Pro	Phe	Leu	Phe	Asp	Thr	Lys
	130						135					140			
Pro	Leu	Ile	Val	Gln	Tyr	Glu	Val	Asn	Phe	Gln	Asn	Gly	Ile	Glu	Cys
145					150					155					160
Gly	Gly	Ala	Tyr	Val	Lys	Leu	Leu	Ser	Lys	Thr	Pro	Glu	Leu	Asn	Leu
				165					170					175	
Asp	Gln	Phe	His	Asp	Lys	Thr	Pro	Tyr	Thr	Ile	Met	Phe	Gly	Pro	Asp
			180					185					190		
Lys	Cys	Gly	Glu	Asp	Tyr	Lys	Leu	His	Phe	Ile	Phe	Arg	His	Lys	Asn
		195					200					205			
Pro	Lys	Thr	Gly	Ile	Tyr	Glu	Glu	Lys	His	Ala	Lys	Arg	Pro	Asp	Ala
	210						215				220				
Asp	Leu	Lys	Thr	Tyr	Phe	Thr	Asp	Lys	Lys	Thr	His	Leu	Tyr	Thr	Leu
225					230					235					240
Ile	Leu	Asn	Pro	Asp	Asn	Ser	Phe	Glu	Ile	Leu	Val	Asp	Gln	Ser	Val
				245					250					255	

Val Asn Ser Gly Asn Leu Leu Asn Asp Met Thr Pro Pro Val Asn Pro
 260 265 270

Ser Arg Glu Ile Glu Asp Pro Glu Asp Arg Lys Pro Glu Asp Trp Asp
 275 280 285

Glu Arg Pro Lys Ile Pro Asp Pro Glu Ala Val Lys Pro Asp Asp Trp
 290 295 300

Asp Glu Asp Ala Pro Ala Lys Ile Pro Asp Glu Glu Ala Thr Lys Pro
 305 310 315 320

Glu Gly Trp Leu Asp Asp Glu Pro Glu Tyr Val Pro Asp Pro Asp Ala
 325 330 335

Glu Lys Pro Glu Asp Trp Asp Glu Asp Met Asp Gly Glu Trp Glu Ala
 340 345 350

Pro Gln Ile Ala Asn Pro Arg Cys Glu Ser Ala Pro Gly Cys Gly Val
 355 360 365

Trp Gln Arg Pro Val Ile Asp Asn Pro Asn Tyr Lys Gly Lys Trp Lys
 370 375 380

Pro Pro Met Ile Asp Asn Pro Ser Tyr Gln Gly Ile Trp Lys Pro Arg
 385 390 395 400

Lys Ile Pro Asn Pro Asp Phe Phe Glu Asp Leu Glu Pro Phe Arg Met
 405 410 415

Thr Pro Phe Ser Ala Ile Gly Leu Glu Leu Trp Ser Met Thr Ser Asp
 420 425 430

Ile Phe Phe Asp Asn Phe Ile Ile Cys Ala Asp Arg Arg Ile Val Asp
 435 440 445

Asp Trp Ala Asn Asp Gly Trp Gly Leu Lys Lys Ala Ala Asp Gly Ala
 450 455 460

Ala Glu Pro Gly Val Val Gly Gln Met Ile Glu Ala Ala Glu Glu Arg
465 470 475 480

Pro Trp Leu Trp Val Val Tyr Ile Leu Thr Val Ala Leu Pro Val Phe
485 490 495

Leu Val Ile Leu Phe Cys Cys Ser Gly Lys Lys Gln Thr Ser Gly Met
500 505 510

Glu Tyr Lys Lys Thr Asp Ala Pro Gln Pro Asp Val Lys Glu Glu Glu
515 520 525

Glu Glu Lys Glu Glu Glu Lys Asp Lys Gly Asp Glu Glu Glu Glu Gly
530 535 540

Glu Glu Lys Leu Glu Glu Lys Gln Lys Ser Asp Ala Glu Glu Asp Gly
545 550 555 560

Gly Thr Val Ser Gln Glu Glu Glu Asp Arg Lys Pro Lys Ala Glu Glu
565 570 575

Asp Glu Ile Leu Asn Arg Ser Pro Arg Asn Arg Lys Pro Arg Arg Glu
580 585 590

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<211> 1287
<212> DNA
<213> Homo sapiens

<220>
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<222> (12)..(1265)

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1 5 10

ctg gcc gtc gcc gag cct gcc gtc tac ttc aag gag cag ttt ctg gac	98
Leu Ala Val Ala Glu Pro Ala Val Tyr Phe Lys Glu Gln Phe Leu Asp	
15 20 25	
gga gac ggg tgg act tcc cgc tgg atc gaa tcc aaa cac aag tca gat	146
Gly Asp Gly Trp Thr Ser Arg Trp Ile Glu Ser Lys His Lys Ser Asp	
30 35 40 45	
ttt ggc aaa ttc gtt ctc agt tcc ggc aag ttc tac ggt gac gag gag	194
Phe Gly Lys Phe Val Leu Ser Ser Gly Lys Phe Tyr Gly Asp Glu Glu	
50 55 60	
aaa gat aaa ggt ttg cag aca agc cag gat gca cgc ttt tat gct ctg	242
Lys Asp Lys Gly Leu Gln Thr Ser Gln Asp Ala Arg Phe Tyr Ala Leu	
65 70 75	
tcg gcc agt ttc gag cct ttc agc aac aaa ggc cag acg ctg gtg gtg	290
Ser Ala Ser Phe Glu Pro Phe Ser Asn Lys Gly Gln Thr Leu Val Val	
80 85 90	
cag ttc acg gtg aaa cat gag cag aac atc gac tgt ggg ggc ggc tat	338
Gln Phe Thr Val Lys His Glu Gln Asn Ile Asp Cys Gly Gly Gly Tyr	
95 100 105	
gtg aag ctg ttt cct aat agt ttg gac cag aca gac atg cac gga gac	386
Val Lys Leu Phe Pro Asn Ser Leu Asp Gln Thr Asp Met His Gly Asp	
110 115 120 125	
tca gaa tac aac atc atg ttt ggt ccc gac atc tgt ggc cct ggc acc	434
Ser Glu Tyr Asn Ile Met Phe Gly Pro Asp Ile Cys Gly Pro Gly Thr	
130 135 140	
aag aag gtt cat gtc atc ttc aac tac aag ggc aag aac gtg ctg atc	482
Lys Lys Val His Val Ile Phe Asn Tyr Lys Gly Lys Asn Val Leu Ile	
145 150 155	
aac aag gac atc cgt tgc aag gat gat gag ttt aca cac ctg tac aca	530
Asn Lys Asp Ile Arg Cys Lys Asp Asp Glu Phe Thr His Leu Tyr Thr	
160 165 170	

ctg att gtg cgg cca gac aac acc tat gag gtg aag att gac aac agc	578
Leu Ile Val Arg Pro Asp Asn Thr Tyr Glu Val Lys Ile Asp Asn Ser	
175 180 185	
cag gtg gag tcc ggc tcc ttg gaa gac gat tgg gac ttc ctg cca ccc	626
Gln Val Glu Ser Gly Ser Leu Glu Asp Asp Trp Asp Phe Leu Pro Pro	
190 195 200 205	
aag aag ata aag gat cct gat gct tca aaa ccg gaa gac tgg gat gag	674
Lys Lys Ile Lys Asp Pro Asp Ala Ser Lys Pro Glu Asp Trp Asp Glu	
210 215 220	
cgg gcc aag atc gat gat ccc aca gac tcc aag cct gag gac tgg gac	722
Arg Ala Lys Ile Asp Asp Pro Thr Asp Ser Lys Pro Glu Asp Trp Asp	
225 230 235	
aag ccc gag cat atc cct gac cct gat gct aag aag ccc gag gac tgg	770
Lys Pro Glu His Ile Pro Asp Pro Asp Ala Lys Lys Pro Glu Asp Trp	
240 245 250	
gat gaa gag atg gac gga gag tgg gaa ccc cca gtg att cag aac cct	818
Asp Glu Glu Met Asp Gly Glu Trp Glu Pro Pro Val Ile Gln Asn Pro	
255 260 265	
gag tac aag ggt gag tgg aag ccc cgg cag atc gac aac cca gat tac	866
Glu Tyr Lys Gly Glu Trp Lys Pro Arg Gln Ile Asp Asn Pro Asp Tyr	
270 275 280 285	
aag ggc act tgg atc cac cca gaa att gac aac ccc gag tat tct ccc	914
Lys Gly Thr Trp Ile His Pro Glu Ile Asp Asn Pro Glu Tyr Ser Pro	
290 295 300	
gat ccc agt atc tat gcc tat gat aac ttt ggc gtg ctg ggc ctg gac	962
Asp Pro Ser Ile Tyr Ala Tyr Asp Asn Phe Gly Val Leu Gly Leu Asp	
305 310 315	
ctc tgg cag gtc aag tct ggc acc atc ttt gac aac ttc ctc atc acc	1010
Leu Trp Gln Val Lys Ser Gly Thr Ile Phe Asp Asn Phe Leu Ile Thr	
320 325 330	

aac gat gag gca tac gct gag gag ttt ggc aac gag acg tgg ggc gta 1058
 Asn Asp Glu Ala Tyr Ala Glu Glu Phe Gly Asn Glu Thr Trp Gly Val
 335 340 345

aca aag gca gca gag aaa caa atg aag gac aaa cag gac gag gag cag 1106
 Thr Lys Ala Ala Glu Lys Gln Met Lys Asp Lys Gln Asp Glu Glu Gln
 350 355 360 365

agg ctt aag gag gag gaa gaa gac aag aaa cgc aaa gag gag gag gag 1154
 Arg Leu Lys Glu Glu Glu Glu Asp Lys Lys Arg Lys Glu Glu Glu Glu
 370 375 380

gca gag gac aag gag gat gat gag gac aaa gat gag gat gag gag gat 1202
 Ala Glu Asp Lys Glu Asp Asp Glu Asp Lys Asp Glu Asp Glu Glu Asp
 385 390 395

gag gag gac aag gag gaa gat gag gag gaa gat gtc ccc ggc cag gcc 1250
 Glu Glu Asp Lys Glu Glu Asp Glu Glu Glu Asp Val Pro Gly Gln Ala
 400 405 410

aag gac gag ctg tag agaggcctgc ctccagtcta ga 1287
 Lys Asp Glu Leu
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<210> 10

<211> 417

<212> PRT

<213> Homo sapiens

<400> 10

Met Leu Leu Ser Val Pro Leu Leu Leu Gly Leu Leu Gly Leu Ala Val
 1 5 10 15

Ala Glu Pro Ala Val Tyr Phe Lys Glu Gln Phe Leu Asp Gly Asp Gly
 20 25 30

Trp Thr Ser Arg Trp Ile Glu Ser Lys His Lys Ser Asp Phe Gly Lys
 35 40 45

Phe Val Leu Ser Ser Gly Lys Phe Tyr Gly Asp Glu Glu Lys Asp Lys
 50 55 60

Gly Leu Gln Thr Ser Gln Asp Ala Arg Phe Tyr Ala Leu Ser Ala Ser
 65 70 75 80

Phe Glu Pro Phe Ser Asn Lys Gly Gln Thr Leu Val Val Gln Phe Thr
 85 90 95

Val Lys His Glu Gln Asn Ile Asp Cys Gly Gly Gly Tyr Val Lys Leu
 100 105 110

Phe Pro Asn Ser Leu Asp Gln Thr Asp Met His Gly Asp Ser Glu Tyr
 115 120 125

Asn Ile Met Phe Gly Pro Asp Ile Cys Gly Pro Gly Thr Lys Lys Val
 130 135 140

His Val Ile Phe Asn Tyr Lys Gly Lys Asn Val Leu Ile Asn Lys Asp
 145 150 155 160

Ile Arg Cys Lys Asp Asp Glu Phe Thr His Leu Tyr Thr Leu Ile Val
 165 170 175

Arg Pro Asp Asn Thr Tyr Glu Val Lys Ile Asp Asn Ser Gln Val Glu
 180 185 190

Ser Gly Ser Leu Glu Asp Asp Trp Asp Phe Leu Pro Pro Lys Lys Ile
 195 200 205

Lys Asp Pro Asp Ala Ser Lys Pro Glu Asp Trp Asp Glu Arg Ala Lys
 210 215 220

Ile Asp Asp Pro Thr Asp Ser Lys Pro Glu Asp Trp Asp Lys Pro Glu
 225 230 235 240

His Ile Pro Asp Pro Asp Ala Lys Lys Pro Glu Asp Trp Asp Glu Glu
 245 250 255

Met Asp Gly Glu Trp Glu Pro Pro Val Ile Gln Asn Pro Glu Tyr Lys
 260 265 270

Gly Glu Trp Lys Pro Arg Gln Ile Asp Asn Pro Asp Tyr Lys Gly Thr
 275 280 285

Trp Ile His Pro Glu Ile Asp Asn Pro Glu Tyr Ser Pro Asp Pro Ser
 290 295 300

Ile Tyr Ala Tyr Asp Asn Phe Gly Val Leu Gly Leu Asp Leu Trp Gln
 305 310 315 320

Val Lys Ser Gly Thr Ile Phe Asp Asn Phe Leu Ile Thr Asn Asp Glu
 325 330 335

Ala Tyr Ala Glu Glu Phe Gly Asn Glu Thr Trp Gly Val Thr Lys Ala
 340 345 350

Ala Glu Lys Gln Met Lys Asp Lys Gln Asp Glu Glu Gln Arg Leu Lys
 355 360 365

Glu Glu Glu Glu Asp Lys Lys Arg Lys Glu Glu Glu Glu Ala Glu Asp
 370 375 380

Lys Glu Asp Asp Glu Asp Lys Asp Glu Asp Glu Glu Asp Glu Glu Asp
 385 390 395 400

Lys Glu Glu Asp Glu Glu Glu Asp Val Pro Gly Gln Ala Lys Asp Glu
 405 410 415

Leu

<210> 11

<211> 1696

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (65)..(1582)

<400> 11

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cgcc atg cgc ctc cgc cgc cta gcg ctg ttc ccg ggt gtg gcg ctg ctt 109

Met Arg Leu Arg Arg Leu Ala Leu Phe Pro Gly Val Ala Leu Leu

1

5

10

15

ctt gcc gcg gcc cgc ctc gcc gct gcc tcc gac gtg cta gaa ctc acg 157

Leu Ala Ala Ala Arg Leu Ala Ala Ala Ser Asp Val Leu Glu Leu Thr

20

25

30

gac gac aac ttc gag agt cgc atc tcc gac acg ggc tct gcg ggc ctc 205

Asp Asp Asn Phe Glu Ser Arg Ile Ser Asp Thr Gly Ser Ala Gly Leu

35

40

45

atg ctc gtc gag ttc ttc gct ccc tgg tgt gga cac tgc aag aga ctt 253

Met Leu Val Glu Phe Phe Ala Pro Trp Cys Gly His Cys Lys Arg Leu

50

55

60

gca cct gag tat gaa gct gca gct acc aga tta aaa gga ata gtc cca 301

Ala Pro Glu Tyr Glu Ala Ala Ala Thr Arg Leu Lys Gly Ile Val Pro

65

70

75

tta gca aag gtt gat tgc act gcc aac act aac acc tgt aat aaa tat 349

Leu Ala Lys Val Asp Cys Thr Ala Asn Thr Asn Thr Cys Asn Lys Tyr

80

85

90

95

gga gtc agt gga tat cca acc ctg aag ata ttt aga gat ggt gaa gaa 397

Gly Val Ser Gly Tyr Pro Thr Leu Lys Ile Phe Arg Asp Gly Glu Glu

100

105

110

gca ggt gct tat gat gga cct agg act gct gat gga att gtc agc cac 445

Ala Gly Ala Tyr Asp Gly Pro Arg Thr Ala Asp Gly Ile Val Ser His

115

120

125

ttg aag aag cag gca gga cca gct tca gtg cct ctc agg act gag gaa	493
Leu Lys Lys Gln Ala Gly Pro Ala Ser Val Pro Leu Arg Thr Glu Glu	
130 135 140	
 gaa ttt aag aaa ttc att agt gat aaa gat gcc tct ata gta ggt ttt	541
Glu Phe Lys Lys Phe Ile Ser Asp Lys Asp Ala Ser Ile Val Gly Phe	
145 150 155	
 ttc gat gat tca ttc agt gag gct cac tcc gag ttc cta aaa gca gcc	589
Phe Asp Asp Ser Phe Ser Glu Ala His Ser Glu Phe Leu Lys Ala Ala	
160 165 170 175	
 agc aac ttg agg gat aac tac cga ttt gca cat acg aat gtt gag tct	637
Ser Asn Leu Arg Asp Asn Tyr Arg Phe Ala His Thr Asn Val Glu Ser	
180 185 190	
 ctg gtg aac gag tat gat gat aat gga gag ggt atc atc tta ttt cgt	685
Leu Val Asn Glu Tyr Asp Asp Asn Gly Glu Gly Ile Ile Leu Phe Arg	
195 200 205	
 cct tca cat ctc act aac aag ttt gag gac aag act gtg gca tat aca	733
Pro Ser His Leu Thr Asn Lys Phe Glu Asp Lys Thr Val Ala Tyr Thr	
210 215 220	
 gag caa aaa atg acc agt ggc aaa att aaa aag ttt atc cag gaa aac	781
Glu Gln Lys Met Thr Ser Gly Lys Ile Lys Lys Phe Ile Gln Glu Asn	
225 230 235	
 att ttt ggt atc tgc cct cac atg aca gaa gac aat aaa gat ttg ata	829
Ile Phe Gly Ile Cys Pro His Met Thr Glu Asp Asn Lys Asp Leu Ile	
240 245 250 255	
 cag ggc aag gac tta ctt att gct tac tat gat gtg gac tat gaa aag	877
Gln Gly Lys Asp Leu Leu Ile Ala Tyr Tyr Asp Val Asp Tyr Glu Lys	
260 265 270	
 aac gct aaa ggt tcc aac tac tgg aga aac agg gta atg atg gtg gca	925
Asn Ala Lys Gly Ser Asn Tyr Trp Arg Asn Arg Val Met Met Val Ala	
275 280 285	

aag aaa ttc ctg gat gct ggg cac aaa ctc aac ttt gct gta gct agc	973
Lys Lys Phe Leu Asp Ala Gly His Lys Leu Asn Phe Ala Val Ala Ser	
290 295 300	
cgc aaa acc ttt agc cat gaa ctt tct gat ttt ggc ttg gag agc act	1021
Arg Lys Thr Phe Ser His Glu Leu Ser Asp Phe Gly Leu Glu Ser Thr	
305 310 315	
gct gga gag att cct gtt gtt gct atc aga act gct aaa gga gag aag	1069
Ala Gly Glu Ile Pro Val Val Ala Ile Arg Thr Ala Lys Gly Glu Lys	
320 325 330 335	
ttt gtc atg cag gag gag ttc tcg cgt gat ggg aag gct ctg gag agg	1117
Phe Val Met Gln Glu Glu Phe Ser Arg Asp Gly Lys Ala Leu Glu Arg	
340 345 350	
ttc ctg cag gat tac ttt gat ggc aat ctg aag aga tac ctg aag tct	1165
Phe Leu Gln Asp Tyr Phe Asp Gly Asn Leu Lys Arg Tyr Leu Lys Ser	
355 360 365	
gaa cct atc cca gag agc aat gat ggg cct gtg aag gta gtg gta gca	1213
Glu Pro Ile Pro Glu Ser Asn Asp Gly Pro Val Lys Val Val Val Ala	
370 375 380	
gag aat ttt gat gaa ata gtg aat aat gaa aat aaa gat gtg ctg att	1261
Glu Asn Phe Asp Glu Ile Val Asn Asn Glu Asn Lys Asp Val Leu Ile	
385 390 395	
gaa ttt tat gcc cct tgg tgt ggt cat tgt aag aac ctg gag ccc aag	1309
Glu Phe Tyr Ala Pro Trp Cys Gly His Cys Lys Asn Leu Glu Pro Lys	
400 405 410 415	
tat aaa gaa ctt ggc gag aag ctc agc aaa gac cca aat atc gtc ata	1357
Tyr Lys Glu Leu Gly Glu Lys Leu Ser Lys Asp Pro Asn Ile Val Ile	
420 425 430	
gcc aag atg gat gcc aca gcc aat gat gtg cct tct cca tat gaa gtc	1405
Ala Lys Met Asp Ala Thr Ala Asn Asp Val Pro Ser Pro Tyr Glu Val	
435 440 445	

aga ggt ttt cct acc ata tac ttc tct cca gcc aac aag aag cta aat 1453
Arg Gly Phe Pro Thr Ile Tyr Phe Ser Pro Ala Asn Lys Lys Leu Asn
450 455 460

cca aag aaa tat gaa ggt ggc cgt gaa tta agt gat ttt att agc tat 1501
Pro Lys Lys Tyr Glu Gly Gly Arg Glu Leu Ser Asp Phe Ile Ser Tyr
465 470 475

cta caa aga gaa gct aca aac ccc cct gta att caa gaa gaa aaa ccc 1549
Leu Gln Arg Glu Ala Thr Asn Pro Pro Val Ile Gln Glu Glu Lys Pro
480 485 490 495

aag aag aag aag aag gca cag gag gat ctc taa agcagtagcc aaacaccact 1602
Lys Lys Lys Lys Lys Ala Gln Glu Asp Leu
500 505

ttgtaaaagg actcttccat cagagatggg aaaaccattg gggaggacta ggacccatat 1662
gggaattatt acctctcagg gccgagagtc taga 1696

<210> 12
<211> 505
<212> PRT
<213> Homo sapiens

<400> 12
Met Arg Leu Arg Arg Leu Ala Leu Phe Pro Gly Val Ala Leu Leu Leu
1 5 10 15

Ala Ala Ala Arg Leu Ala Ala Ala Ser Asp Val Leu Glu Leu Thr Asp
20 25 30

Asp Asn Phe Glu Ser Arg Ile Ser Asp Thr Gly Ser Ala Gly Leu Met
35 40 45

Leu Val Glu Phe Phe Ala Pro Trp Cys Gly His Cys Lys Arg Leu Ala
50 55 60

Pro Glu Tyr Glu Ala Ala Ala Thr Arg Leu Lys Gly Ile Val Pro Leu
65 70 75 80

Ala Lys Val Asp Cys Thr Ala Asn Thr Asn Thr Cys Asn Lys Tyr Gly
85 90 95

Val Ser Gly Tyr Pro Thr Leu Lys Ile Phe Arg Asp Gly Glu Glu Ala
100 105 110

Gly Ala Tyr Asp Gly Pro Arg Thr Ala Asp Gly Ile Val Ser His Leu
115 120 125

Lys Lys Gln Ala Gly Pro Ala Ser Val Pro Leu Arg Thr Glu Glu Glu
130 135 140

Phe Lys Lys Phe Ile Ser Asp Lys Asp Ala Ser Ile Val Gly Phe Phe
145 150 155 160

Asp Asp Ser Phe Ser Glu Ala His Ser Glu Phe Leu Lys Ala Ala Ser
165 170 175

Asn Leu Arg Asp Asn Tyr Arg Phe Ala His Thr Asn Val Glu Ser Leu
180 185 190

Val Asn Glu Tyr Asp Asp Asn Gly Glu Gly Ile Ile Leu Phe Arg Pro
195 200 205

Ser His Leu Thr Asn Lys Phe Glu Asp Lys Thr Val Ala Tyr Thr Glu
210 215 220

Gln Lys Met Thr Ser Gly Lys Ile Lys Lys Phe Ile Gln Glu Asn Ile
225 230 235 240

Phe Gly Ile Cys Pro His Met Thr Glu Asp Asn Lys Asp Leu Ile Gln
245 250 255

Gly Lys Asp Leu Leu Ile Ala Tyr Tyr Asp Val Asp Tyr Glu Lys Asn
260 265 270

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

Gln Arg Glu Ala Thr Asn Pro Pro Val Ile Gln Glu Glu Lys Pro Lys
485 490 495

Lys Lys Lys Lys Ala Gln Glu Asp Leu
500 505

<210> 13
<211> 1926
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1926)

<400> 13
atg gcc aaa gcc gcg gcg atc ggc atc gac ctg ggc acc acc tac tcc 48
Met Ala Lys Ala Ala Ala Ile Gly Ile Asp Leu Gly Thr Thr Tyr Ser
1 5 10 15

tgc gtg ggg gtg ttc caa cac ggc aag gtg gag atc atc gcc aac gac 96
Cys Val Gly Val Phe Gln His Gly Lys Val Glu Ile Ile Ala Asn Asp
20 25 30

cag ggc aac cgc acc acc ccc agc tac gtg gcc ttc acg gac acc gag 144
Gln Gly Asn Arg Thr Thr Pro Ser Tyr Val Ala Phe Thr Asp Thr Glu
35 40 45

cgg ctc atc ggg gat gcg gcc aag aac cag gtg gcg ctg aac ccg cag 192
Arg Leu Ile Gly Asp Ala Ala Lys Asn Gln Val Ala Leu Asn Pro Gln
50 55 60

aac acc gtg ttt gac gcg aag cgg ctg atc ggc cgc aag ttc ggc gac 240
Asn Thr Val Phe Asp Ala Lys Arg Leu Ile Gly Arg Lys Phe Gly Asp
65 70 75 80

ccg gtg gtg cag tcg gac atg aag cac tgg cct ttc cag gtg atc aac	288
Pro Val Val Gln Ser Asp Met Lys His Trp Pro Phe Gln Val Ile Asn	
85 90 95	
gac gga gac aag ccc aag gtg cag gtg agc tac aag ggg gag acc aag	336
Asp Gly Asp Lys Pro Lys Val Gln Val Ser Tyr Lys Gly Glu Thr Lys	
100 105 110	
gca ttc tac ccc gag gag atc tcg tcc atg gtg ctg acc aag atg aag	384
Ala Phe Tyr Pro Glu Glu Ile Ser Ser Met Val Leu Thr Lys Met Lys	
115 120 125	
gag atc gcc gag gcg tac ctg ggc tac ccg gtg acc aac gcg gtg atc	432
Glu Ile Ala Glu Ala Tyr Leu Gly Tyr Pro Val Thr Asn Ala Val Ile	
130 135 140	
acc gtg ccg gcc tac ttc aac gac tcg cag cgc cag gcc acc aag gat	480
Thr Val Pro Ala Tyr Phe Asn Asp Ser Gln Arg Gln Ala Thr Lys Asp	
145 150 155 160	
gcg ggt gtg atc gcg ggg ctc aac gtg ctg cgg atc atc aac gag ccc	528
Ala Gly Val Ile Ala Gly Leu Asn Val Leu Arg Ile Ile Asn Glu Pro	
165 170 175	
acg gcc gcc gcc atc gcc tac ggc ctg gac aga acg ggc aag ggg gag	576
Thr Ala Ala Ala Ile Ala Tyr Gly Leu Asp Arg Thr Gly Lys Gly Glu	
180 185 190	
cgc aac gtg ctc atc ttt gac ctg ggc ggg ggc acc ttc gac gtg tcc	624
Arg Asn Val Leu Ile Phe Asp Leu Gly Gly Gly Thr Phe Asp Val Ser	
195 200 205	
atc ctg acg atc gac gac ggc atc ttc gag gtg aag gcc acg gcc ggg	672
Ile Leu Thr Ile Asp Asp Gly Ile Phe Glu Val Lys Ala Thr Ala Gly	
210 215 220	
gac acc cac ctg ggt ggg gag gac ttt gac aac agg ctg gtg aac cac	720
Asp Thr His Leu Gly Gly Glu Asp Phe Asp Asn Arg Leu Val Asn His	
225 230 235 240	

ttc gtg gag gag ttc aag aga aaa cac aag aag gac atc agc cag aac	768
Phe Val Glu Glu Phe Lys Arg Lys His Lys Lys Asp Ile Ser Gln Asn	
245 250 255	
aag cga gcc gtg agg cgg ctg cgc acc gcc tgc gag agg gcc aag agg	816
Lys Arg Ala Val Arg Arg Leu Arg Thr Ala Cys Glu Arg Ala Lys Arg	
260 265 270	
acc ctg tcg tcc agc acc cag gcc agc ctg gag atc gac tcc ctg ttt	864
Thr Leu Ser Ser Ser Thr Gln Ala Ser Leu Glu Ile Asp Ser Leu Phe	
275 280 285	
gag ggc atc gac ttc tac acg tcc atc acc agg gcg agg ttc gag gag	912
Glu Gly Ile Asp Phe Tyr Thr Ser Ile Thr Arg Ala Arg Phe Glu Glu	
290 295 300	
ctg tgc tcc gac ctg ttc cga agc acc ctg gag ccc gtg gag aag gct	960
Leu Cys Ser Asp Leu Phe Arg Ser Thr Leu Glu Pro Val Glu Lys Ala	
305 310 315 320	
ctg cgc gac gcc aag ctg gac aag gcc cag att cac gac ctg gtc ctg	1008
Leu Arg Asp Ala Lys Leu Asp Lys Ala Gln Ile His Asp Leu Val Leu	
325 330 335	
gtc ggg ggc tcc acc cgc atc ccc aag gtg cag aag ctg ctg cag gac	1056
Val Gly Gly Ser Thr Arg Ile Pro Lys Val Gln Lys Leu Leu Gln Asp	
340 345 350	
ttc ttc aac ggg cgc gac ctg aac aag agc atc aac ccc gac gag gct	1104
Phe Phe Asn Gly Arg Asp Leu Asn Lys Ser Ile Asn Pro Asp Glu Ala	
355 360 365	
gtg gcc tac ggg gcg gcg gtg cag gcg gcc atc ctg atg ggg gac aag	1152
Val Ala Tyr Gly Ala Ala Val Gln Ala Ala Ile Leu Met Gly Asp Lys	
370 375 380	
tcc gag aac gtg cag gac ctg ctg ctg ctg gac gtg gct ccc ctg tcg	1200
Ser Glu Asn Val Gln Asp Leu Leu Leu Leu Asp Val Ala Pro Leu Ser	
385 390 395 400	

ctg ggg ctg gag acg gcc gga ggc gtg atg act gcc ctg atc aag cgc	1248
Leu Gly Leu Glu Thr Ala Gly Gly Val Met Thr Ala Leu Ile Lys Arg	
405 410 415	
aac tcc acc atc ccc acc aag cag acg cag atc ttc acc acc tac tcc	1296
Asn Ser Thr Ile Pro Thr Lys Gln Thr Gln Ile Phe Thr Thr Tyr Ser	
420 425 430	
gac aac caa ccc ggg gtg ctg atc cag gtg tac gag ggc gag agg gcc	1344
Asp Asn Gln Pro Gly Val Leu Ile Gln Val Tyr Glu Gly Glu Arg Ala	
435 440 445	
atg acg aaa gac aac aat ctg ttg ggg cgc ttc gag ctg agc ggc atc	1392
Met Thr Lys Asp Asn Asn Leu Leu Gly Arg Phe Glu Leu Ser Gly Ile	
450 455 460	
cct ccg gcc ccc agg ggc gtg ccc cag atc gag gtg acc ttc gac atc	1440
Pro Pro Ala Pro Arg Gly Val Pro Gln Ile Glu Val Thr Phe Asp Ile	
465 470 475 480	
gat gcc aac ggc atc ctg aac gtc acg gcc acg gac aag agc acc ggc	1488
Asp Ala Asn Gly Ile Leu Asn Val Thr Ala Thr Asp Lys Ser Thr Gly	
485 490 495	
aag gcc aac aag atc acc atc acc aac gac aag ggc cgc ctg agc aag	1536
Lys Ala Asn Lys Ile Thr Ile Thr Asn Asp Lys Gly Arg Leu Ser Lys	
500 505 510	
gag gag atc gag cgc atg gtg cag gag gcg gag aag tac aaa gcg gag	1584
Glu Glu Ile Glu Arg Met Val Gln Glu Ala Glu Lys Tyr Lys Ala Glu	
515 520 525	
gac gag gtg cag cgc gag agg gtg tca gcc aag aac gcc ctg gag tcc	1632
Asp Glu Val Gln Arg Glu Arg Val Ser Ala Lys Asn Ala Leu Glu Ser	
530 535 540	
tac gcc ttc aac atg aag agc gcc gtg gag gat gag ggg ctc aag ggc	1680
Tyr Ala Phe Asn Met Lys Ser Ala Val Glu Asp Glu Gly Leu Lys Gly	
545 550 555 560	

aag atc agc gag gcc gac aag aag aag gtg ctg gac aag tgt caa gag 1728
Lys Ile Ser Glu Ala Asp Lys Lys Lys Val Leu Asp Lys Cys Gln Glu
565 570 575

gtc atc tcg tgg ctg gac gcc aac acc ttg gcc gag aag gac gag ttt 1776
Val Ile Ser Trp Leu Asp Ala Asn Thr Leu Ala Glu Lys Asp Glu Phe
580 585 590

gag cac aag agg aag gag ctg gag cag gtg tgt aac ccc atc atc agc 1824
Glu His Lys Arg Lys Glu Leu Glu Gln Val Cys Asn Pro Ile Ile Ser
595 600 605

gga ctg tac cag ggt gcc ggt ggt ccc ggg cct ggg ggc ttc ggg gct 1872
Gly Leu Tyr Gln Gly Ala Gly Gly Pro Gly Pro Gly Gly Phe Gly Ala
610 615 620

cag ggt ccc aag gga ggg tct ggg tca ggc ccc acc att gag gag gta 1920
Gln Gly Pro Lys Gly Gly Ser Gly Ser Gly Pro Thr Ile Glu Glu Val
625 630 635 640

gat tag 1926
Asp

<210> 14
<211> 641
<212> PRT
<213> Homo sapiens

<400> 14
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20 25 30

Gln Gly Asn Arg Thr Thr Pro Ser Tyr Val Ala Phe Thr Asp Thr Glu
35 40 45

Arg	Leu	Ile	Gly	Asp	Ala	Ala	Lys	Asn	Gln	Val	Ala	Leu	Asn	Pro	Gln
50							55					60			
Asn	Thr	Val	Phe	Asp	Ala	Lys	Arg	Leu	Ile	Gly	Arg	Lys	Phe	Gly	Asp
65					70					75				80	
Pro	Val	Val	Gln	Ser	Asp	Met	Lys	His	Trp	Pro	Phe	Gln	Val	Ile	Asn
				85					90					95	
Asp	Gly	Asp	Lys	Pro	Lys	Val	Gln	Val	Ser	Tyr	Lys	Gly	Glu	Thr	Lys
			100						105				110		
Ala	Phe	Tyr	Pro	Glu	Glu	Ile	Ser	Ser	Met	Val	Leu	Thr	Lys	Met	Lys
		115						120					125		
Glu	Ile	Ala	Glu	Ala	Tyr	Leu	Gly	Tyr	Pro	Val	Thr	Asn	Ala	Val	Ile
	130						135					140			
Thr	Val	Pro	Ala	Tyr	Phe	Asn	Asp	Ser	Gln	Arg	Gln	Ala	Thr	Lys	Asp
145						150				155					160
Ala	Gly	Val	Ile	Ala	Gly	Leu	Asn	Val	Leu	Arg	Ile	Ile	Asn	Glu	Pro
					165					170				175	
Thr	Ala	Ala	Ala	Ile	Ala	Tyr	Gly	Leu	Asp	Arg	Thr	Gly	Lys	Gly	Glu
				180					185					190	
Arg	Asn	Val	Leu	Ile	Phe	Asp	Leu	Gly	Gly	Gly	Thr	Phe	Asp	Val	Ser
		195						200					205		
Ile	Leu	Thr	Ile	Asp	Asp	Gly	Ile	Phe	Glu	Val	Lys	Ala	Thr	Ala	Gly
	210						215					220			
Asp	Thr	His	Leu	Gly	Gly	Glu	Asp	Phe	Asp	Asn	Arg	Leu	Val	Asn	His
225					230					235					240
Phe	Val	Glu	Glu	Phe	Lys	Arg	Lys	His	Lys	Lys	Asp	Ile	Ser	Gln	Asn
					245					250				255	

Lys Arg Ala Val Arg Arg Leu Arg Thr Ala Cys Glu Arg Ala Lys Arg
 260 265 270

Thr Leu Ser Ser Ser Thr Gln Ala Ser Leu Glu Ile Asp Ser Leu Phe
 275 280 285

Glu Gly Ile Asp Phe Tyr Thr Ser Ile Thr Arg Ala Arg Phe Glu Glu
 290 295 300

Leu Cys Ser Asp Leu Phe Arg Ser Thr Leu Glu Pro Val Glu Lys Ala
 305 310 315 320

Leu Arg Asp Ala Lys Leu Asp Lys Ala Gln Ile His Asp Leu Val Leu
 325 330 335

Val Gly Gly Ser Thr Arg Ile Pro Lys Val Gln Lys Leu Leu Gln Asp
 340 345 350

Phe Phe Asn Gly Arg Asp Leu Asn Lys Ser Ile Asn Pro Asp Glu Ala
 355 360 365

Val Ala Tyr Gly Ala Ala Val Gln Ala Ala Ile Leu Met Gly Asp Lys
 370 375 380

Ser Glu Asn Val Gln Asp Leu Leu Leu Leu Asp Val Ala Pro Leu Ser
 385 390 395 400

Leu Gly Leu Glu Thr Ala Gly Gly Val Met Thr Ala Leu Ile Lys Arg
 405 410 415

Asn Ser Thr Ile Pro Thr Lys Gln Thr Gln Ile Phe Thr Thr Tyr Ser
 420 425 430

Asp Asn Gln Pro Gly Val Leu Ile Gln Val Tyr Glu Gly Glu Arg Ala
 435 440 445

Met Thr Lys Asp Asn Asn Leu Leu Gly Arg Phe Glu Leu Ser Gly Ile
 450 455 460

Pro Pro Ala Pro Arg Gly Val Pro Gln Ile Glu Val Thr Phe Asp Ile
 465 470 475 480

Asp Ala Asn Gly Ile Leu Asn Val Thr Ala Thr Asp Lys Ser Thr Gly
 485 490 495

Lys Ala Asn Lys Ile Thr Ile Thr Asn Asp Lys Gly Arg Leu Ser Lys
 500 505 510

Glu Glu Ile Glu Arg Met Val Gln Glu Ala Glu Lys Tyr Lys Ala Glu
 515 520 525

Asp Glu Val Gln Arg Glu Arg Val Ser Ala Lys Asn Ala Leu Glu Ser
 530 535 540

Tyr Ala Phe Asn Met Lys Ser Ala Val Glu Asp Glu Gly Leu Lys Gly
 545 550 555 560

Lys Ile Ser Glu Ala Asp Lys Lys Lys Val Leu Asp Lys Cys Gln Glu
 565 570 575

Val Ile Ser Trp Leu Asp Ala Asn Thr Leu Ala Glu Lys Asp Glu Phe
 580 585 590

Glu His Lys Arg Lys Glu Leu Glu Gln Val Cys Asn Pro Ile Ile Ser
 595 600 605

Gly Leu Tyr Gln Gly Ala Gly Gly Pro Gly Pro Gly Gly Phe Gly Ala
 610 615 620

Gln Gly Pro Lys Gly Gly Ser Gly Ser Gly Pro Thr Ile Glu Glu Val
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Asp

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<212> DNA
<213> Homo sapiens

<220>
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<222> (1)..(1023)

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Met Gly Lys Asp Tyr Tyr Gln Thr Leu Gly Leu Ala Arg Gly Ala Ser
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gac gag gag atc aag cgg gcc tac cgc cgc cag gcg ctg cgc tac cac 96
Asp Glu Glu Ile Lys Arg Ala Tyr Arg Arg Gln Ala Leu Arg Tyr His
20 25 30

ccg gac aag aac aag gag ccc ggc gcc gag gag aag ttc aag gag atc 144
Pro Asp Lys Asn Lys Glu Pro Gly Ala Glu Glu Lys Phe Lys Glu Ile
35 40 45

gct gag gcc tac gac gtg ctc agc gac ccg cgc aag cgc gag atc ttc 192
Ala Glu Ala Tyr Asp Val Leu Ser Asp Pro Arg Lys Arg Glu Ile Phe
50 55 60

gac cgc tac ggg gag gaa ggc cta aag ggg agt ggc ccc agt ggc ggt 240
Asp Arg Tyr Gly Glu Glu Gly Leu Lys Gly Ser Gly Pro Ser Gly Gly
65 70 75 80

agc ggc ggt ggt gcc aat ggt acc tct ttc agc tac aca ttc cat gga 288
Ser Gly Gly Gly Ala Asn Gly Thr Ser Phe Ser Tyr Thr Phe His Gly
85 90 95

gac cct cat gcc atg ttt gct gag ttc ttc ggt ggc aga aat ccc ttt 336
Asp Pro His Ala Met Phe Ala Glu Phe Phe Gly Gly Arg Asn Pro Phe
100 105 110

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gac acc ttt ttt ggg cag cgg aac ggg gag gaa ggc atg gac att gat	384
Asp Thr Phe Phe Gly Gln Arg Asn Gly Glu Glu Gly Met Asp Ile Asp	
115 120 125	
gac cca ttc tct ggc ttc cct atg ggc atg ggt ggc ttc acc aac gtg	432
Asp Pro Phe Ser Gly Phe Pro Met Gly Met Gly Gly Phe Thr Asn Val	
130 135 140	
aac ttt ggc cgc tcc cgc tct gcc caa gag ccc gcc cga aag aag caa	480
Asn Phe Gly Arg Ser Arg Ser Ala Gln Glu Pro Ala Arg Lys Lys Gln	
145 150 155 160	
gat ccc cca gtc acc cac gac ctt cga gtc tcc ctt gaa gag atc tac	528
Asp Pro Pro Val Thr His Asp Leu Arg Val Ser Leu Glu Glu Ile Tyr	
165 170 175	
agc ggc tgt acc aag aag atg aaa atc tcc cac aag cgg cta aac ccc	576
Ser Gly Cys Thr Lys Lys Met Lys Ile Ser His Lys Arg Leu Asn Pro	
180 185 190	
gac gga aag agc att cga aac gaa gac aaa ata ttg acc atc gaa gtg	624
Asp Gly Lys Ser Ile Arg Asn Glu Asp Lys Ile Leu Thr Ile Glu Val	
195 200 205	
aag aag ggg tgg aaa gaa gga acc aaa atc act ttc ccc aag gaa gga	672
Lys Lys Gly Trp Lys Glu Gly Thr Lys Ile Thr Phe Pro Lys Glu Gly	
210 215 220	
gac cag acc tcc aac aac att cca gct gat atc gtc ttt gtt tta aag	720
Asp Gln Thr Ser Asn Asn Ile Pro Ala Asp Ile Val Phe Val Leu Lys	
225 230 235 240	
gac aag ccc cac aat atc ttt aag aga gat ggc tct gat gtc att tat	768
Asp Lys Pro His Asn Ile Phe Lys Arg Asp Gly Ser Asp Val Ile Tyr	
245 250 255	
cct gcc agg atc agc ctc cgg gag gct ctg tgt ggc tgc aca gtg aac	816
Pro Ala Arg Ile Ser Leu Arg Glu Ala Leu Cys Gly Cys Thr Val Asn	
260 265 270	

gtc ccc act ctg gac ggc agg acg ata ccc gtc gta ttc aaa gat gtt 864
 Val Pro Thr Leu Asp Gly Arg Thr Ile Pro Val Val Phe Lys Asp Val
 275 280 285

atc agg cct ggc atg cgg cga aaa gtt cct gga gaa ggc ctc ccc ctc 912
 Ile Arg Pro Gly Met Arg Arg Lys Val Pro Gly Glu Gly Leu Pro Leu
 290 295 300

ccc aaa aca ccc gag aaa cgt ggg gac ctc att att gag ttt gaa gtg 960
 Pro Lys Thr Pro Glu Lys Arg Gly Asp Leu Ile Ile Glu Phe Glu Val
 305 310 315 320

atc ttc ccc gaa agg att ccc cag aca tca aga acc gta ctt gag cag 1008
 Ile Phe Pro Glu Arg Ile Pro Gln Thr Ser Arg Thr Val Leu Glu Gln
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gtt ctt cca ata tag 1023
 Val Leu Pro Ile
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<210> 16
 <211> 340
 <212> PRT
 <213> Homo sapiens

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

Ala Glu Ala Tyr Asp Val Leu Ser Asp Pro Arg Lys Arg Glu Ile Phe
 50 55 60

Asp Arg Tyr Gly Glu Glu Gly Leu Lys Gly Ser Gly Pro Ser Gly Gly
 65 70 75 80

Ser Gly Gly Gly Ala Asn Gly Thr Ser Phe Ser Tyr Thr Phe His Gly
 85 90 95

Asp Pro His Ala Met Phe Ala Glu Phe Phe Gly Gly Arg Asn Pro Phe
 100 105 110

Asp Thr Phe Phe Gly Gln Arg Asn Gly Glu Glu Gly Met Asp Ile Asp
 115 120 125

Asp Pro Phe Ser Gly Phe Pro Met Gly Met Gly Gly Phe Thr Asn Val
 130 135 140

Asn Phe Gly Arg Ser Arg Ser Ala Gln Glu Pro Ala Arg Lys Lys Gln
 145 150 155 160

Asp Pro Pro Val Thr His Asp Leu Arg Val Ser Leu Glu Glu Ile Tyr
 165 170 175

Ser Gly Cys Thr Lys Lys Met Lys Ile Ser His Lys Arg Leu Asn Pro
 180 185 190

Asp Gly Lys Ser Ile Arg Asn Glu Asp Lys Ile Leu Thr Ile Glu Val
 195 200 205

Lys Lys Gly Trp Lys Glu Gly Thr Lys Ile Thr Phe Pro Lys Glu Gly
 210 215 220

Asp Gln Thr Ser Asn Asn Ile Pro Ala Asp Ile Val Phe Val Leu Lys
 225 230 235 240

Asp Lys Pro His Asn Ile Phe Lys Arg Asp Gly Ser Asp Val Ile Tyr
 245 250 255

Pro Ala Arg Ile Ser Leu Arg Glu Ala Leu Cys Gly Cys Thr Val Asn
 260 265 270

Val Pro Thr Leu Asp Gly Arg Thr Ile Pro Val Val Phe Lys Asp Val
275 280 285

Ile Arg Pro Gly Met Arg Arg Lys Val Pro Gly Glu Gly Leu Pro Leu
290 295 300

Pro Lys Thr Pro Glu Lys Arg Gly Asp Leu Ile Ile Glu Phe Glu Val
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325 330 335

Val Leu Pro Ile
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<210> 17
<211> 1122
<212> DNA
<213> Homo sapiens

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<221> CDS
<222> (1)..(1122)

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tac atg tcc ctg cct cag ggt gag aaa gtc cag gcc atg tat atc tgg 96
Tyr Met Ser Leu Pro Gln Gly Glu Lys Val Gln Ala Met Tyr Ile Trp
20 25 30

atc gat ggt act gga gaa gga ctg cgc tgc aag acc cgg acc ctg gac 144
Ile Asp Gly Thr Gly Glu Gly Leu Arg Cys Lys Thr Arg Thr Leu Asp
35 40 45

agt gag ccc aag tgt gtg gaa gag ttg cct gag tgg aat ttc gat ggc	192
Ser Glu Pro Lys Cys Val Glu Glu Leu Pro Glu Trp Asn Phe Asp Gly	
50 55 60	
tcc agt act tta cag tct gag ggt tcc aac agt gac atg tat ctc gtg	240
Ser Ser Thr Leu Gln Ser Glu Gly Ser Asn Ser Asp Met Tyr Leu Val	
65 70 75 80	
cct gct gcc atg ttt cgg gac ccc ttc cgt aag gac cct aac aag ctg	288
Pro Ala Ala Met Phe Arg Asp Pro Phe Arg Lys Asp Pro Asn Lys Leu	
85 90 95	
gtg tta tgt gaa gtt ttc aag tac aat cga agg cct gca gag acc aat	336
Val Leu Cys Glu Val Phe Lys Tyr Asn Arg Arg Pro Ala Glu Thr Asn	
100 105 110	
ttg agg cac acc tgt aaa cgg ata atg gac atg gtg agc aac cag cac	384
Leu Arg His Thr Cys Lys Arg Ile Met Asp Met Val Ser Asn Gln His	
115 120 125	
ccc tgg ttt ggc atg gag cag gag tat acc ctc atg ggg aca gat ggg	432
Pro Trp Phe Gly Met Glu Gln Glu Tyr Thr Leu Met Gly Thr Asp Gly	
130 135 140	
cac ccc ttt ggt tgg cct tcc aac ggc ttc cca ggg ccc cag ggt cca	480
His Pro Phe Gly Trp Pro Ser Asn Gly Phe Pro Gly Pro Gln Gly Pro	
145 150 155 160	
tat tac tgt ggt gtg gga gca gac aga gcc tat ggc agg gac atc gtg	528
Tyr Tyr Cys Gly Val Gly Ala Asp Arg Ala Tyr Gly Arg Asp Ile Val	
165 170 175	
gag gcc cat tac cgg gcc tgc ttg tat gct gga gtc aag att gcg ggg	576
Glu Ala His Tyr Arg Ala Cys Leu Tyr Ala Gly Val Lys Ile Ala Gly	
180 185 190	
act aat gcc gag gtc atg cct gcc cag tgg gaa ttt cag att gga cct	624
Thr Asn Ala Glu Val Met Pro Ala Gln Trp Glu Phe Gln Ile Gly Pro	
195 200 205	

tgt gaa gga atc agc atg gga gat cat ctc tgg gtg gcc cgt ttc atc	672
Cys Glu Gly Ile Ser Met Gly Asp His Leu Trp Val Ala Arg Phe Ile	
210 215 220	
ttg cat cgt gtg tgt gaa gac ttt gga gtg ata gca acc ttt gat cct	720
Leu His Arg Val Cys Glu Asp Phe Gly Val Ile Ala Thr Phe Asp Pro	
225 230 235 240	
aag ccc att cct ggg aac tgg aat ggt gca ggc tgc cat acc aac ttc	768
Lys Pro Ile Pro Gly Asn Trp Asn Gly Ala Gly Cys His Thr Asn Phe	
245 250 255	
agc acc aag gcc atg cgg gag gag aat ggt ctg aag tac atc gag gag	816
Ser Thr Lys Ala Met Arg Glu Glu Asn Gly Leu Lys Tyr Ile Glu Glu	
260 265 270	
gcc att gag aaa cta agc aag cgg cac cag tac cac atc cgt gcc tat	864
Ala Ile Glu Lys Leu Ser Lys Arg His Gln Tyr His Ile Arg Ala Tyr	
275 280 285	
gat ccc aag gga ggc ctg gac aat gcc cga cgt cta act gga ttc cat	912
Asp Pro Lys Gly Gly Leu Asp Asn Ala Arg Arg Leu Thr Gly Phe His	
290 295 300	
gaa acc tcc aac atc aac gac ttt tct gct ggt gta gcc aat cgt agc	960
Glu Thr Ser Asn Ile Asn Asp Phe Ser Ala Gly Val Ala Asn Arg Ser	
305 310 315 320	
gcc agc ata cgc att ccc cgg act gtt ggc cag gag aag aag ggt tac	1008
Ala Ser Ile Arg Ile Pro Arg Thr Val Gly Gln Glu Lys Lys Gly Tyr	
325 330 335	
ttt gaa gat cgt cgc ccc tct gcc aac tgc gac ccc ttt tcg gtg aca	1056
Phe Glu Asp Arg Arg Pro Ser Ala Asn Cys Asp Pro Phe Ser Val Thr	
340 345 350	
gaa gcc ctc atc cgc acg tgt ctt ctc aat gaa acc ggc gat gag ccc	1104
Glu Ala Leu Ile Arg Thr Cys Leu Leu Asn Glu Thr Gly Asp Glu Pro	
355 360 365	

ttc cag tac aaa aat taa

1122

Phe Gln Tyr Lys Asn

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<211> 373

<212> PRT

<213> Homo sapiens

<400> 18

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20 25 30

Ile Asp Gly Thr Gly Glu Gly Leu Arg Cys Lys Thr Arg Thr Leu Asp
35 40 45

Ser Glu Pro Lys Cys Val Glu Glu Leu Pro Glu Trp Asn Phe Asp Gly
50 55 60

Ser Ser Thr Leu Gln Ser Glu Gly Ser Asn Ser Asp Met Tyr Leu Val
65 70 75 80

Pro Ala Ala Met Phe Arg Asp Pro Phe Arg Lys Asp Pro Asn Lys Leu
85 90 95

Val Leu Cys Glu Val Phe Lys Tyr Asn Arg Arg Pro Ala Glu Thr Asn
100 105 110

Leu Arg His Thr Cys Lys Arg Ile Met Asp Met Val Ser Asn Gln His
115 120 125

Pro Trp Phe Gly Met Glu Gln Glu Tyr Thr Leu Met Gly Thr Asp Gly
130 135 140

His	Pro	Phe	Gly	Trp	Pro	Ser	Asn	Gly	Phe	Pro	Gly	Pro	Gln	Gly	Pro
145					150					155					160
Tyr	Tyr	Cys	Gly	Val	Gly	Ala	Asp	Arg	Ala	Tyr	Gly	Arg	Asp	Ile	Val
				165					170					175	
Glu	Ala	His	Tyr	Arg	Ala	Cys	Leu	Tyr	Ala	Gly	Val	Lys	Ile	Ala	Gly
				180					185					190	
Thr	Asn	Ala	Glu	Val	Met	Pro	Ala	Gln	Trp	Glu	Phe	Gln	Ile	Gly	Pro
				195				200					205		
Cys	Glu	Gly	Ile	Ser	Met	Gly	Asp	His	Leu	Trp	Val	Ala	Arg	Phe	Ile
								215					220		
Leu	His	Arg	Val	Cys	Glu	Asp	Phe	Gly	Val	Ile	Ala	Thr	Phe	Asp	Pro
225						230					235				240
Lys	Pro	Ile	Pro	Gly	Asn	Trp	Asn	Gly	Ala	Gly	Cys	His	Thr	Asn	Phe
					245				250						255
Ser	Thr	Lys	Ala	Met	Arg	Glu	Glu	Asn	Gly	Leu	Lys	Tyr	Ile	Glu	Glu
				260					265					270	
Ala	Ile	Glu	Lys	Leu	Ser	Lys	Arg	His	Gln	Tyr	His	Ile	Arg	Ala	Tyr
				275					280					285	
Asp	Pro	Lys	Gly	Gly	Leu	Asp	Asn	Ala	Arg	Arg	Leu	Thr	Gly	Phe	His
								295					300		
Glu	Thr	Ser	Asn	Ile	Asn	Asp	Phe	Ser	Ala	Gly	Val	Ala	Asn	Arg	Ser
305											315				320
Ala	Ser	Ile	Arg	Ile	Pro	Arg	Thr	Val	Gly	Gln	Glu	Lys	Lys	Gly	Tyr
										330				335	
Phe	Glu	Asp	Arg	Arg	Pro	Ser	Ala	Asn	Cys	Asp	Pro	Phe	Ser	Val	Thr
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Glu Ala Leu Ile Arg Thr Cys Leu Leu Asn Glu Thr Gly Asp Glu Pro
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Phe Gln Tyr Lys Asn
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<210> 19

<211> 170

<212> PRT

<213> Homo sapiens

<400> 19

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Asp Gly Ser Cys Gln Leu Phe Val Tyr Gly Gly Cys Asp Gly Asn Ser
 35 40 45

Asn Asn Tyr Leu Thr Lys Glu Glu Cys Leu Lys Lys Cys Ala Thr Val
 50 55 60

Thr Glu Asn Ala Thr Gly Asp Leu Ala Thr Ser Arg Asn Ala Ala Asp
 65 70 75 80

Ser Ser Val Pro Ser Ala Pro Arg Arg Gln Asp Ser Glu Asp His Ser
 85 90 95

Ser Asp Met Phe Asn Tyr Glu Glu Tyr Cys Thr Ala Asn Ala Val Thr
 100 105 110

Gly Pro Cys Arg Ala Ser Phe Pro Arg Trp Tyr Phe Asp Val Glu Arg
 115 120 125

Asn Ser Cys Asn Asn Phe Ile Tyr Gly Gly Cys Arg Gly Asn Lys Asn
 130 135 140

Ser Tyr Arg Ser Glu Glu Ala Cys Met Leu Arg Cys Phe Arg Gln Gln
 145 150 155 160

Glu Asn Pro Pro Leu Pro Leu Gly Ser Lys
 165 170

<210> 20

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<212> DNA

<213> Artificial sequence

<220>

<223> synthetic oligonucleotide

<400> 20

agggaaccgc atggccaaag

20

<210> 21

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic oligonucleotide

<400> 21

gaaaggcccc taatctacct cctca

25

<210> 22

<211> 3

<212> PRT

<213> Artificial sequence

<220>

<223> synthetic peptide

<220>

<221> MISC_FEATURE

<222> (1)..(1)

<223> N-benzoyl

<220>

<221> MISC_FEATURE

<222> (3)..(3)

<223> Xaa is Arg-pNA

<400> 22

Pro Phe Xaa

1

WHAT IS CLAIMED:

1. A mammalian host cell for enhanced expression of a recombinant protein product, said mammalian host cell having genetic material coding for expression of said recombinant protein product and transformed with at least one expression vector comprising DNA encoding chaperone protein Hsp70, wherein the recombinant protein product is Factor VIII or a biologically active fragment thereof.
5
2. The mammalian host cell according to claim 1, wherein the recombinant protein product is secreted.
10
3. The mammalian host cell according to claim 2, wherein the genetic material coding for expression of said recombinant protein product is integrated into host cell DNA.
15
4. The mammalian host cell according to claim 3, further transformed with an expression vector comprising DNA encoding a glutamine synthetase protein.
5. A method for producing a mammalian host cell for enhanced expression of a target recombinant protein or a biologically active fragment thereof, wherein the target recombinant protein is Factor VIII, the method comprising:
20
 providing a mammalian host cell having genetic material coding for expression of the target recombinant protein or the biologically active fragment thereof; and
 transforming the mammalian host cell with at least one expression vector comprising DNA encoding chaperone protein Hsp70.
25
6. The method according to claim 5, wherein the target recombinant protein or a fragment thereof is secreted.

7. The method according to claim 5, wherein the genetic material coding for expression of said target recombinant protein or a fragment thereof is integrated into host cell DNA.

5 8. The method according to claim 7, wherein said host cell is further transformed with an expression vector comprising DNA encoding a glutamine synthetase protein.

9. A method for enhancing recombinant Factor VIII or a biologically active
10 fragment thereof yield in a baby hamster kidney (BHK) cell line, wherein genetic material coding for expression of said recombinant Factor VIII or said biologically active fragment thereof has been previously introduced into a first BHK cell line, said method comprising the steps of:

inserting at least one chaperone protein expression vector encoding Hsp70 into
15 said first BHK cell line so as to form a modified BHK cell line; and

selecting from said modified BHK cell line at least one second cell line exhibiting enhanced yield of said recombinant Factor VIII or said biologically active fragment thereof.

20 10. The method according to claim 9, wherein the genetic material coding for expression of said recombinant Factor VIII or said active fragment thereof is integrated into the first BHK cell DNA.

11. The method according to claim 9, wherein the second cell line is further
25 transformed with an expression vector comprising DNA encoding a glutamine synthetase protein.

12. The method according to claim 9, wherein said at least one second cell line
is produced from said first cell line by selecting a portion of said first cell line exhibiting
30 integration of the chaperone protein expression vector into host DNA.

13. The method according to claim 9, wherein said BHK cell line is further transfected with a vector including a puromycin-resistant gene.

14. The method according to claim 13, wherein the selection occurs in the presence of puromycin.

15. A method for enhancing yield of a recombinant Factor VIII or a biologically active fragment thereof in a BHK cell line comprising introducing genetic material coding for said Factor VIII or said biologically active fragment thereof into a BHK cell line exhibiting enhanced chaperone protein expression, wherein the chaperone protein is Hsp70.

16. The method according to claim 15, wherein the BHK cell line is further transformed with an expression vector comprising DNA encoding a glutamine synthetase protein.

17. A method for producing recombinant Factor VIII or a biologically active fragment thereof comprising the steps of:

culturing a mammalian host cell, said mammalian host cell having genetic material coding for expression of said recombinant Factor VIII or said biologically active fragment thereof and transformed with at least one expression vector comprising DNA encoding chaperone protein Hsp70; and

recovering from a culture medium said recombinant Factor VIII or said biologically active fragment thereof so produced and secreted.

18. The method according to claim 17, wherein said mammalian host cell is a BHK cell.

19. The method according to claim 17, wherein said genetic material coding for expression of said recombinant Factor VIII protein or said active fragment thereof is integrated into the host cell DNA.

20. The method according to claim 17, wherein said mammalian host cell is further transformed with an expression vector comprising DNA encoding a glutamine synthetase protein.

5 21. A BHK cell line with enhanced expression of Factor VIII or a biologically active fragment thereof generated by introducing genetic material coding for said Factor VIII or biologically active fragment into a BHK cell line exhibiting enhanced chaperone protein expression, wherein the chaperone protein is Hsp70.

10 22. The BHK cell line according to claim 21, wherein the BHK cell line is further transformed with an expression vector comprising DNA encoding a glutamine synthetase protein.

CNX: 5' primer: ATGAATTCCGGGAGGCTAGAGATCATGG
3' primer: ATTCTAGATGCAGGGGAGGAGGGAGAAG
CRT: 5' primer: ATGAATTCCTCCGCATGCTGCTATCCGTG
3' primer: ATTCTAGACTGGAGGCAGGCCTCTCTAC
Erp57: 5' primer: ATGAATTCCTCCGCAGTCCCAGCCGAGC
3' primer: ATTCTAGACTCTCGGCCCTGAGAGGTAA

FIG. 1

M E G K W L

1 GAATTCCGGG AGGCTAGAGA TC**ATG**GGAAGG GAAGTGGTTG
 L C M L L V L G T A I V E A .
 41 CTGTGTATGT TACTGGTGCT TGGAAGTGCT ATTGTTGAGG
 . H D G H D D D V I D I E D .
 81 CTCATGATGG ACATGATGAT GATGTGATTG ATATTGAGGA
 . D L D D V I E E V E D S K
 121 TGACCTTGAC GATGTCATTG AAGAGGTAGA AGACTCAAAA
 P D T T A P P S S P K V T Y .
 161 CCAGATACCA CTGCTCCTCC TTCATCTCCC AAGGTTACTT
 . K A P V P T G E V Y F A D .
 201 ACAAGCTCC AGTTCCAACA GGGGAAGTAT ATTTTGCTGA
 . S F D R G T L S G W I L S
 241 TTCTTTTGAC AGAGGAACTC TGTCAGGGTG GATTTTATCC
 K A K K D D T D D E I A K Y .
 281 AAAGCCAAGA AAGACGATAC CGATGATGAA ATTGCCAAAT
 . D G K W E V E E M K E S K .
 321 ATGATGGAAA GTGGGAGGTA GAGGAAATGA AGGAGTCAAA
 . L P G D K G L V L M S R A
 361 GCTTCCAGGT GATAAAGGAC TTGTGTTGAT GTCTCGGGCC
 K H H A I S A K L N K P F L .
 401 AAGCATCATG CCATCTCTGC TAAACTGAAC AAGCCCTTCC
 . F D T K P L I V Q Y E V N .
 441 TGTTTGACAC CAAGCCTCTC ATTGTTTCAGT ATGAGGTTAA
 . F Q N G I E C G G A Y V K
 481 TTTCCAAAAT GGAATAGAAT GTGGTGGTGC CTATGTGAAA
 L L S K T P E L N L D Q F H .
 521 CTGCTTTCTA AAACACCAGA ACTCAACCTG GATCAGTTCC
 . D K T P Y T I M F G P D K .
 561 ATGACAAGAC CCCTTATACG ATTATGTTTG GTCCAGATAA
 . C G E D Y K L H F I F R H
 601 ATGTGGAGAG GACTATAAAC TGCACTTCAT CTTCCGACAC
 K N P K T G I Y E E K H A K .
 641 AAAAACCCCA AAACGGGTAT CTATGAAGAA AAACATGCTA
 . R P D A D L K T Y F T D K .
 681 AGAGGCCAGA TGCAGATCTG AAGACCTATT TTACTGATAA
 . K T H L Y T L I L N P D N

FIG. 2A

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721 GAAAACACAT CTTTACACAC TAATCTTGAA TCCAGATAAT
    S F E I L V D Q S V V N S G .
761 AGTTTTGAAA TACTGGTTGA CCAATCTGTG GTGAATAGTG
    . N L L N D M T P P V N P S .
801 GAAATCTGCT CAATGACATG ACTCCTCCTG TAAATCCTTC
    . R E I E D P E D R K P E D
841 ACGTGAAATT GAGGACCCAG AAGACCGGAA GCCCGAGGAT
    W D E R P K I P D P E A V K .
881 TGGGATGAAA GACCAAAAAT CCCAGATCCA GAAGCTGTCA
    . P D D W D E D A P A K I P .
921 AGCCAGATGA CTGGGATGAA GATGCCCCTG CTAAGATTCC
    . D E E A T K P E G W L D D
961 AGATGAAGAG GCCACAAAAC CCGAAGGCTG GTTAGATGAT
    E P E Y V P D P D A E K P E .
1001 GAGCCTGAGT ACGTACCTGA TCCAGACGCA GAGAAACCTG
    . D W D E D M D G E W E A P .
1041 AGGATTGGGA TGAAGACATG GATGGAGAAT GGGAGGCTCC
    . Q I A N P R C E S A P G C
1081 TCAGATTGCC AACCTAGAT GTGAGTCAGC TCCTGGATGT
    G V W Q R P V I D N P N Y K .
1121 GGTGTCTGGC AGCGACCTGT GATTGACAAC CCCAATTATA
    . G K W K P P M I D N P S Y .
1161 AAGGCAAATG GAAGCCTCCT ATGATTGACA ATCCCAGTTA
    . Q G I W K P R K I P N P D
1201 CCAGGGAATC TGGAAACCCA GGAAAATACC AAATCCAGAT
    F F E D L E P F R M T P F S .
1241 TTCTTTGAAG ATCTGGAACC TTTCAGAATG ACTCCTTTTA
    . A I G L E L W S M T S D I .
1281 GTGCTATTGG TTTGGAGCTG TGGTCCATGA CCTCTGACAT
    . F F D N F I I C A D R R I
1321 TTTTTTTGAC AACTTTATCA TTTGTGCTGA TCGAAGAATA
    V D D W A N D G W G L K K A .
1361 GTTGATGATT GGGCCAATGA TGGATGGGGC CTGAAGAAAG
    . A D G A A E P G V V G Q M .
1401 CTGCTGATGG GGCTGCTGAG CCAGGCGTTG TGGGGCAGAT
    . I E A A E E R P W L W V V
1441 GATCGAGGCA GCTGAAGAGC GCCCGTGGCT GTGGGTAGTC
    Y I L T V A L P V F L V I L .
1481 TATATTCTAA CTGTAGCCCT TCCTGTGTTC CTGGTTATCC
    . F C C S G K K Q T S G M E .

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FIG. 2A (Continued)

1521 TCTTCTGCTG TTCTGGAAAG AAACAGACCA GTGGTATGGA
· Y K K T D A P Q P D V K E
1561 GTATAAGAAA ACTGATGCAC CTCAACCGGA TGTGAAGGAA
E E E E K E E E K D K G D E ·
1601 GAGGAAGAAG AGAAGGAAGA GGAAAAGGAC AAGGGAGATG
· E E E G E E K L E E K Q K ·
1641 AGGAGGAGGA AGGAGAAGAG AAAGTTGAAG AGAAACAGAA
· S D A E E D G G T V S Q E
1681 AAGTGATGCT GAAGAAGATG GTGGCACTGT CAGTCAAGAG
E E D R K P K A E E D E I L ·
1721 GAGGAAGACA GAAAACCTAA AGCAGAGGAG GATGAAATTT
· N R S P R N R K P R R E * ·
1761 TGAACAGATC ACCAAGAAAC AGAAAGCCAC GAAGAGAGTG
· *
1801 ~~AA~~ACAATCTT AAGAGCTTGA TCTGTGATTT CTTCTCCCTC
1841 CTCCCCTGCA TCTAGA

FIG. 2A(Continued)

M L L S V P L L L G .
 1 GAATTCCCGC **CATG**CTGCTA TCCGTGCCGC TGCTGCTCGG
 . L L G L A V A E P A V Y F
 41 CCTCCTCGGC CTGGCCGTCG CCGAGCCTGC CGTCTACTTC
 K E Q F L D G D G W T S R W .
 81 AAGGAGCAGT TTCTGGACGG AGACGGGTGG ACTTCCCGCT
 . I E S K H K S D F G K F V .
 121 GGATCGAATC CAAACACAAG TCAGATTTTG GCAAATTCGT
 . L S S G K F Y G D E E K D
 161 TCTCAGTTCC GGCAAGTTCT ACGGTGACGA GGAGAAAGAT
 K G L Q T S Q D A R F Y A L .
 201 AAAGGTTTGC AGACAAGCCA GGATGCACGC TTTTATGCTC
 . S A S F E P F S N K G Q T .
 241 TGTCGGCCAG TTTCGAGCCT TTCAGCAACA AAGGCCAGAC
 . L V V Q F T V K H E Q N I
 281 GCTGGTGGTG CAGTTCACGG TGAAACATGA GCAGAACATC
 D C G G G Y V K L F P N S L .
 321 GACTGTGGGG GCGGCTATGT GAAGCTGTTT CCTAATAGTT
 . D Q T D M H G D S E Y N I .
 361 TGGACCAGAC AGACATGCAC GGAGACTCAG AATACAACAT
 . M F G P D I C G P G T K K
 401 CATGTTTGGT CCCGACATCT GTGGCCCTGG CACCAAGAAG
 V H V I F N Y K G K N V L I .
 441 GTTCATGTCA TCTTCAACTA CAAGGGCAAG AACGTGCTGA
 . N K D I R C K D D E F T H .
 481 TCAACAAGGA CATCCGTTGC AAGGATGATG AGTTTACACA
 . L Y T L I V R P D N T Y E
 521 CCTGTACACA CTGATTGTGC GGCCAGACAA CACCTATGAG
 V K I D N S Q V E S G S L E .
 561 GTGAAGATTG ACAACAGCCA GGTGGAGTCC GGCTCCTTGG
 . D D W D F L P P K K I K D .
 601 AAGACGATTG GGA~~CT~~TCCTG CCACCCAAGA AGATAAAGGA
 . P D A S K P E D W D E R A
 641 TCCTGATGCT TCAAAACCGG AAGACTGGGA TGAGCGGGCC
 K I D D P T D S K P E D W D .
 681 AAGATCGATG ATCCCACAGA CTCCAAGCCT GAGGACTGGG
 . K P E H I P D P D A K K P .
 721 ACAAGCCCGA GCATATCCCT GACCCTGATG CTAAGAAGCC
 . E D W D E E M D G E W E P

FIG. 2B

761 CGAGGACTGG GATGAAGAGA TGGACGGAGA GTGGGAACCC
P V I Q N P E Y K G E W K P .
801 CCAGTGATTC AGAACCCCTGA GTACAAGGGT GAGTGGAAGC
. R Q I D N P D Y K G T W I .
841 CCCGGCAGAT CGACAACCCA GATTACAAGG GCACTTGGAT
. H P E I D N P E Y S P D P
881 CCACCCAGAA ATTGACAACC CCGAGTATTC TCCCGATCCC
S I Y A Y D N F G V L G L D .
921 AGTATCTATG CCTATGATAA CTTTGGCGTG CTGGGCCTGG
. L W Q V K S G T I F D N F .
961 ACCTCTGGCA GGTCAAGTCT GGCACCATCT TTGACAACCTT
. L I T N D E A Y A E E F G
1001 CCTCATCACC AACGATGAGG CATACGCTGA GGAGTTTGGC
N E T W G V T K A A E K Q M .
1041 AACGAGACGT GGGGCGTAAC AAAGGCAGCA GAGAAACAAA
. K D K Q D E E Q R L K E E .
1081 TGAAGGACAA ACAGGACGAG GAGCAGAGGC TTAAGGAGGA
. E E D K K R K E E E E A E
1121 GGAAGAAGAC AAGAAACGCA AAGAGGAGGA GGAGGCAGAG
D K E D D E D K D E D E E D .
1161 GACAAGGAGG ATGATGAGGA CAAAGATGAG GATGAGGAGG
. E E D K E E D E E E D V P .
1201 ATGAGGAGGA CAAGGAGGAA GATGAGGAGG AAGATGTCCC
. G Q A K D E L *
1241 CGGCCAGGCC AAGGACGAGC TG**T**AGAGAGG CCTGCCTCCA
1281 GTCTAGA

FIG. 2B(Continued)

FIG. 2C

801 ACATGACAGA AGACAATAAA GATTTGATAC AGGGCAAGGA
· L L I A Y Y D V D Y E K N
841 CTTACTTATT GCTTACTATG ATGTGGACTA TGAAAAGAAC
A K G S N Y W R N R V M M V ·
881 GCTAAAGGTT CCAACTACTG GAGAAACAGG GTAATGATGG
· A K K F L D A G H K L N F ·
921 TGGCAAAGAA ATTCCTGGAT GCTGGGCACA AACTCAACTT
· A V A S R K T F S H E L S
961 TGCTGTAGCT AGCCGCAAAA CCTTTAGCCA TGAAC TTTCT
D F G L E S T A G E I P V V ·
1001 GATTTTGGCT TGGAGAGCAC TGCTGGAGAG ATTCCTGTTG
· A I R T A K G E K F V M Q ·
1041 TTGCTATCAG AACTGCTAAA GGAGAGAAGT TTGTCATGCA
· E E F S R D G K A L E R F
1081 GGAGGAGTTC TCGCGTGATG GGAAGGCTCT GGAGAGGTTC
L Q D Y F D G N L K R Y L K ·
1121 CTGCAGGATT ACTTTGATGG CAATCTGAAG AGATACCTGA
· S E P I P E S N D G P V K ·
1161 AGTCTGAACC TATCCCAGAG AGCAATGATG GGCCTGTGAA
· V V V A E N F D E I V N N
1201 GGTAGTGGTA GCAGAGAATT TTGATGAAAT AGTGAATAAT
E N K D V L I E F Y A P W C ·
1241 GAAAATAAAG ATGTGCTGAT TGAATTTTAT GCCCCTTGGT
· G H C K N L E P K Y K E L ·
1281 GTGGTCATTG TAAGAACCTG GAGCCCAAGT ATAAAGAACT
· G E K L S K D P N I V I A
1321 TGGCGAGAAG CTCAGCAAAG ACCCAAATAT CGTCATAGCC
K M D A T A N D V P S P Y E ·
1361 AAGATGGATG CCACAGCCAA TGATGTGCCT TCTCCATATG
· V R G F P T I Y F S P A N ·
1401 AAGTCAGAGG TTTTCCTACC ATATACTTCT CTCCAGCCAA
· K K L N P K K Y E G G R E
1441 CAAGAAGCTA AATCCAAAGA AATATGAAGG TGGCCGTGAA
L S D F I S Y L Q R E A T N ·
1481 TTAAGTGATT TTATTAGCTA TCTACAAAGA GAAGCTACAA
· P P V I Q E E K P K K K K ·

FIG. 2C (Continued)

1521 ACCCCCCTGT AATTCAAGAA GAAAAACCCA AGAAGAAGAA
· K A Q E D L *
1561 GAAGGCACAG GAGGATCTCT **AA**AGCAGTAG CCAAACACCA
1601 CTTTGTAATA GGACTCTTCC ATCAGAGATG GGAAAACCAT
1641 TGGGGAGGAC TAGGACCCAT ATGGGAATTA TTACCTCTCA
1681 GGGCCGAGAG TCTAGA

FIG. 2C (Continued)

M A K A A A I G I D L G T T Y S C .
 1 ATGGCCAAAG CCGCGGCGAT CGGCATCGAC CTGGGCACCA CCTACTCCTG
 . V G V F Q H G K V E I I A N D Q G .
 51 CGTGGGGGTG TTCCAACACG GCAAGGTGGA GATCATCGCC AACGACCAGG
 . N R T T P S Y V A F T D T E R L
 101 GCAACCGCAC CACCCCCAGC TACGTGGCCT TCACGGACAC CGAGCGGCTC
 I G D A A K N Q V A L N P Q N T V .
 151 ATCGGGGATG CGGCCAAGAA CCAGGTGGCG CTGAACCCGC AGAACACCGT
 . F D A K R L I G R K F G D P V V Q .
 201 GTTTGACGCG AAGCGGCTGA TCGGCCGCAA GTTCGGCGAC CCGGTGGTGC
 . S D M K H W P F Q V I N D G D K
 251 AGTCGGACAT GAAGCACTGG CCTTTCCAGG TGATCAACGA CGGAGACAAG
 P K V Q V S Y K G E T K A F Y P E .
 301 CCCAAGGTGC AGGTGAGCTA CAAGGGGGAG ACCAAGGCAT TCTACCCCGA
 . E I S S M V L T K M K E I A E A Y .
 351 GGAGATCTCG TCCATGGTGC TGACCAAGAT GAAGGAGATC GCCGAGGCGT
 . L G Y P V T N A V I T V P A Y F
 401 ACCTGGGCTA CCCGGTGACC AACGCGGTGA TCACCGTGCC GGCCTACTTC
 N D S Q R Q A T K D A G V I A G L .
 451 AACGACTCGC AGCGCCAGGC CACCAAGGAT GCGGGTGTGA TCGCGGGGCT
 . N V L R I I N E P T A A A I A Y G .
 501 CAACGTGCTG CGGATCATCA ACGAGCCCAC GGCCGCCGCC ATCGCCTACG
 . L D R T G K G E R N V L I F D L
 551 GCCTGGACAG AACGGGCAAG GGGGAGCGCA ACGTGCTCAT CTTTGACCTG
 G G G T F D V S I L T I D D G I F .
 601 GGCGGGGGCA CCTTCGACGT GTCCATCCTG ACGATCGACG ACGGCATCTT
 . E V K A T A G D T H L G G E D F D .
 651 CGAGGTGAAG GCCACGGCCG GGGACACCCA CCTGGGTGGG GAGGACTTTG
 . N R L V N H F V E E F K R K H K
 701 ACAACAGGCT GGTGAACCAC TTCGTGGAGG AGTTCAAGAG AAAACACAAG
 K D I S Q N K R A V R R L R T A C .
 751 AAGGACATCA GCCAGAACAA GCGAGCCGTG AGGCGGCTGC GCACCGCCTG
 . E R A K R T L S S S T Q A S L E I .
 801 CGAGAGGGCC AAGAGGACCC TGTCGTCCAG CACCCAGGCC AGCCTGGAGA
 . D S L F E G I D F Y T S I T R A
 851 TCGACTCCCT GTTTGAGGGC ATCGACTTCT ACACGTCCAT CACCAGGGCG
 R F E E L C S D L F R S T L E P V .
 901 AGGTTCGAGG AGCTGTGCTC CGACCTGTTC CGAAGCACCC TGGAGCCCGT
 . E K A L R D A K L D K A Q I H D L .
 951 GGAGAAGGCT CTGCGCGACG CCAAGCTGGA CAAGGCCAG ATTCACGACC
 . V L V G G S T R I P K V Q K L L
 1001 TGGTCCTGGT CGGGGGCTCC ACCCGCATCC CCAAGGTGCA GAAGCTGCTG
 Q D F F N G R D L N K S I N P D E .
 1051 CAGGACTTCT TCAACGGGCG CGACCTGAAC AAGAGCATCA ACCCCGACGA
 . A V A Y G A A V Q A A I L M G D K .
 1101 GGCTGTGGCC TACGGGGCGG CGGTGCAGGC GGCCATCCTG ATGGGGGACA
 . S E N V Q D L L L L D V A P L S
 1151 AGTCCGAGAA CGTGCAGGAC CTGCTGCTGC TGGACGTGGC TCCCCTGTCTG

FIG. 2D

```

      L G L E T A G G V M T A L I K R N .
1201 CTGGGGCTGG AGACGGCCGG AGGCGTGATG ACTGCCCTGA TCAAGCGCAA
      . S T I P T K Q T Q I F T T Y S D N .
1251 CTCCACCATC CCCACCAAGC AGACGCAGAT CTTCACCACC TACTCCGACA
      . Q P G V L I Q V Y E G E R A M T
1301 ACCAACCCGG GGTGCTGATC CAGGTGTACG AGGGCGAGAG GGCCATGACG
      K D N N L L G R F E L S G I P P A .
1351 AAAGACAACA ATCTGTTGGG GCGCTTCGAG CTGAGCGGCA TCCCTCCGGC
      . P R G V P Q I E V T F D I D A N G .
1401 CCCCAGGGGC GTGCCCCAGA TCGAGGTGAC CTTCGACATC GATGCCAACG
      . I L N V T A T D K S T G K A N K
1451 GCATCCTGAA CGTCACGGCC ACGGACAAGA GCACCGGCAA GGCCAACAAG
      I T I T N D K G R L S K E E I E R .
1501 ATCACCATCA CCAACGACAA GGGCCGCCTG AGCAAGGAGG AGATCGAGCG
      . M V Q E A E K Y K A E D E V Q R E .
1551 CATGGTGCAG GAGGCGGAGA AGTACAAAGC GGAGGACGAG GTGCAGCGCG
      . R V S A K N A L E S Y A F N M K
1601 AGAGGGTGTC AGCCAAGAAC GCCCTGGAGT CCTACGCCTT CAACATGAAG
      S A V E D E G L K G K I S E A D K .
1651 AGCGCCGTGG AGGATGAGGG GCTCAAGGGC AAGATCAGCG AGGCCGACAA
      . K K V L D K C Q E V I S W L D A N .
1701 GAAGAAGGTG CTGGACAAGT GTCAAGAGGT CATCTCGTGG CTGGACGCCA
      . T L A E K D E F E H K R K E L E
1751 ACACCTTGGC CGAGAAGGAC GAGTTTGAGC ACAAGAGGAA GGAGCTGGAG
      Q V C N P I I S G L Y Q G A G G P .
1801 CAGGTGTGTA ACCCCATCAT CAGCGGACTG TACCAGGGTG CCGGTGGTCC
      . G P G G F G A Q G P K G G S G S G .
1851 CGGGCCTGGG GGCTTCGGGG CTCAGGGTCC CAAGGGAGGG TCTGGGTCAG
      . P T I E E V D *
1901 GCCCCACCAT TGAGGAGGTA GATTAG

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FIG. 2D (Continued)

M G K D Y Y Q T L G L A R G A S D .
 1 ATGGGTAAAG ACTACTACCA GACGTTGGGC CTGGCCCGCG GCGCGTCGGA
 . E E I K R A Y R R Q A L R Y H P D .
 51 CGAGGAGATC AAGCGGGCCT ACCGCCGCCA GGCGCTGCGC TACCACCCGG
 . K N K E P G A E E K F K E I A E
 101 ACAAGAACAA GGAGCCCGGC GCCGAGGAGA AGTTCAAGGA GATCGCTGAG
 A Y D V L S D P R K R E I F D R Y .
 151 GCCTACGACG TGCTCAGCGA CCCGCGCAAG CGCGAGATCT TCGACCGCTA
 . G E E G L K G S G P S G G S G G G .
 201 CGGGGAGGAA GGCCTAAAGG GGAGTGGCCC CAGTGGCGGT AGCGGCGGTG
 . A N G T S F S Y T F H G D P H A
 251 GTGCCAATGG TACCTCTTTC AGCTACACAT TCCATGGAGA CCCTCATGCC
 M F A E F F G G R N P F D T F F G .
 301 ATGTTTGCTG AGTTCTTCGG TGGCAGAAAT CCCTTTGACA CCTTTTTTGG
 . Q R N G E E G M D I D D P F S G F .
 351 GCAGCGGAAC GGGGAGGAAG GCATGGACAT TGATGACCCA TTCTCTGGCT
 . P M G M G G F T N V N F G R S R
 401 TCCCTATGGG CATGGGTGGC TTCACCAACG TGAAC TTG CCGCTCCCGC
 S A Q E P A R K K Q D P P V T H D .
 451 TCTGCCCAAG AGCCCGCCCG AAAGAAGCAA GATCCCCCAG TCACCCACGA
 . L R V S L E E I Y S G C T K K M K .
 501 CCTTCGAGTC TCCCTTGAAG AGATCTACAG CGGCTGTACC AAGAAGATGA
 . I S H K R L N P D G K S I R N E
 551 AAATCTCCCA CAAGCGGCTA AACCCCGACG GAAAGAGCAT TCGAAACGAA
 D K I L T I E V K K G W K E G T K .
 601 GACAAAATAT TGACCATCGA AGTGAAGAAG GGGTGGAAAG AAGGAACCAA
 . I T F P K E G D Q T S N N I P A D .
 651 AATCACTTTC CCCAAGGAAG GAGACCAGAC CTCCAACAAC ATTCCAGCTG
 . I V F V L K D K P H N I F K R D
 701 ATATCGTCTT TGTTTTAAAG GACAAGCCCC ACAATATCTT TAAGAGAGAT

FIG. 2E

G S D V I Y P A R I S L R E A L C .
751 GGCTCTGATG TCATTTATCC TGCCAGGATC AGCCTCCGGG AGGCTCTGTG
· G C T V N V P T L D G R T I P V V .
801 TGGCTGCACA GTGAACGTCC CCACTCTGGA CGGCAGGACG ATACCCGTCG
· F K D V I R P G M R R K V P G E
851 TATTCAAAGA TGTTATCAGG CCTGGCATGC GGCGAAAAGT TCCTGGAGAA
G L P L P K T P E K R G D L I I E .
901 GGCCTCCCCC TCCCCAAAAC ACCCGAGAAA CGTGGGGACC TCATTATTGA
· F E V I F P E R I P Q T S R T V L .
951 GTTTGAAGTG ATCTTCCCCG AAAGGATTCC CCAGACATCA AGAACCGTAC
· E Q V L P I *
1001 TTGAGCAGGT TCTTCCAATA TAG

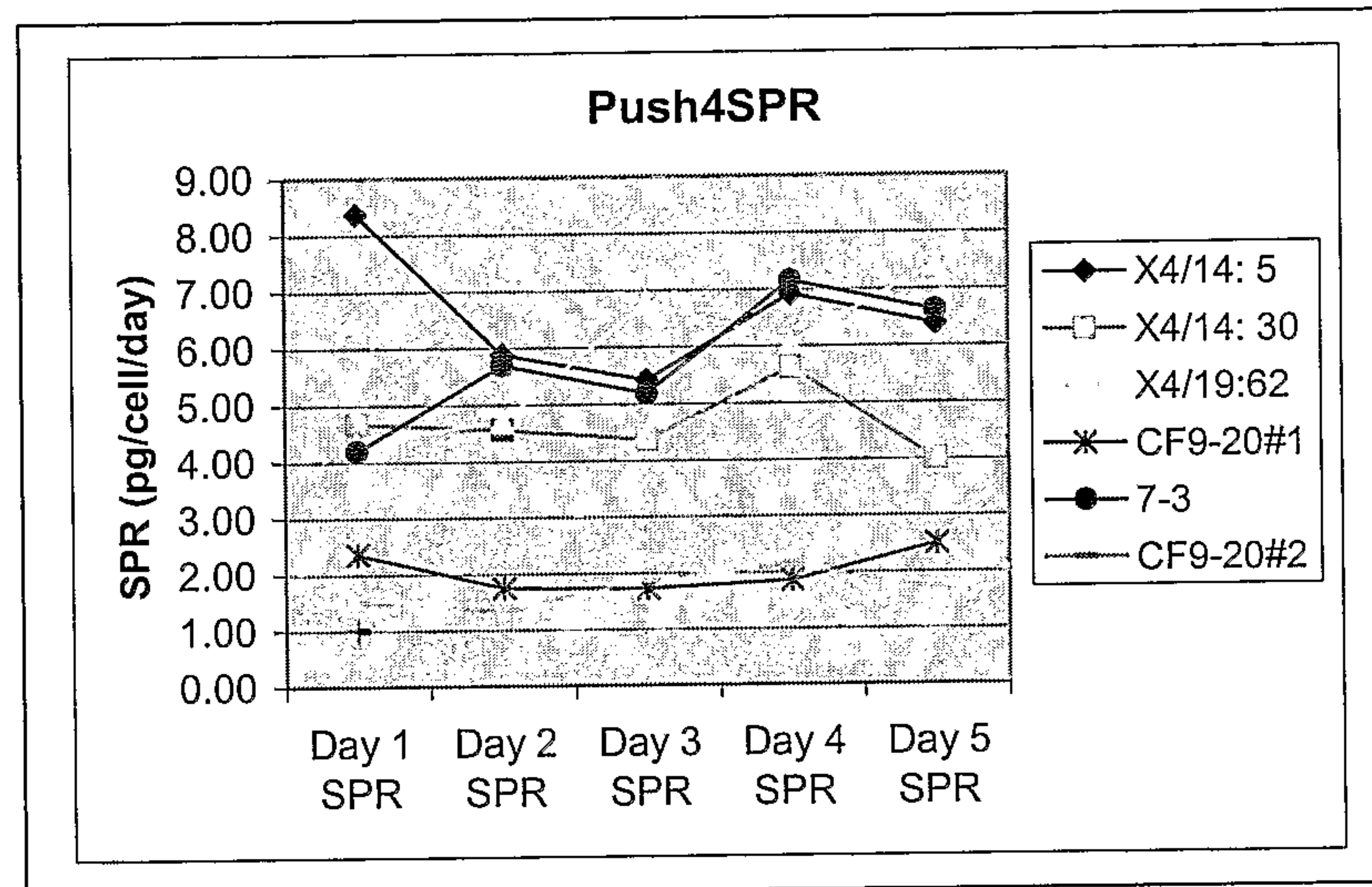
FIG. 2E (Continued)

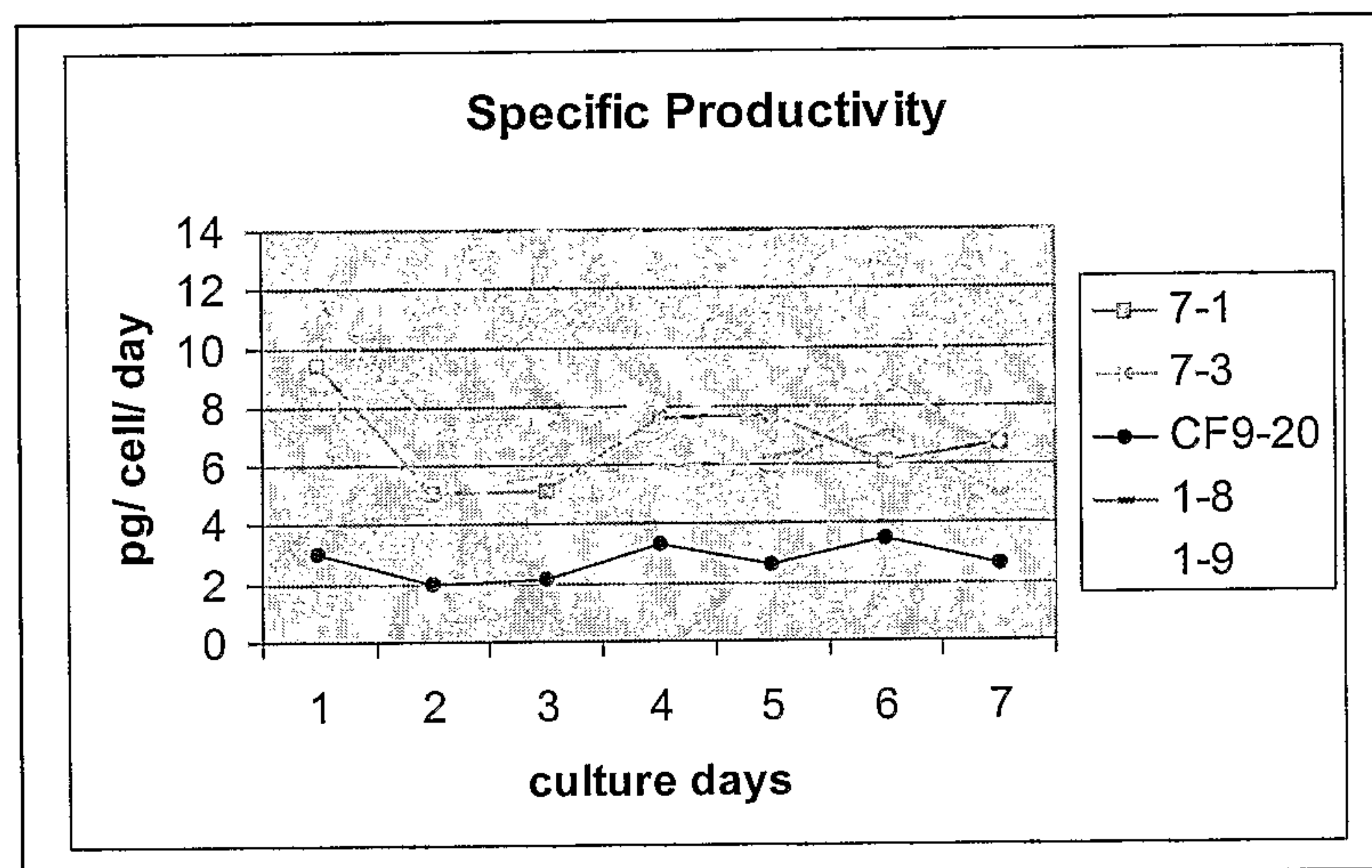
M T T S A S S H L N K G I K Q V Y .
 1 ATGACCACCT CAGCAAGTTC CCACTTAAAT AAAGGCATCA AGCAGGTGTA
 · M S L P Q G E K V Q A M Y I W I D ·
 51 CATGTCCCTG CCTCAGGGTG AGAAAGTCCA GGCCATGTAT ATCTGGATCG
 · G T G E G L R C K T R T L D S E
 101 ATGGTACTGG AGAAGGACTG CGCTGCAAGA CCCGGACCCT GGACAGTGAG
 P K C V E E L P E W N F D G S S T ·
 151 CCCAAGTGTG TGGAAGAGTT GCCTGAGTGG AATTTCGATG GCTCCAGTAC
 · L Q S E G S N S D M Y L V P A A M ·
 201 TTTACAGTCT GAGGGTTCCA ACAGTGACAT GTATCTCGTG CCTGCTGCCA
 · F R D P F R K D P N K L V L C E
 251 TGTTTCGGGA CCCCTTCCGT AAGGACCCTA ACAAGCTGGT GTTATGTGAA
 V F K Y N R R P A E T N L R H T C ·
 301 GTTTTCAAGT ACAATCGAAG GCCTGCAGAG ACCAATTTGA GGCACACCTG.
 · K R I M D M V S N Q H P W F G M E ·
 351 TAAACGGATA ATGGACATGG TGAGCAACCA GCACCCCTGG TTTGGCATGG
 · Q E Y T L M G T D G H P F G W P
 401 AGCAGGAGTA TACCCTCATG GGGACAGATG GGCACCCCTT TGGTTGGCCT
 S N G F P G P Q G P Y Y C G V G A ·
 451 TCCAACGGCT TCCCAGGGCC CCAGGGTCCA TATTACTGTG GTGTGGGAGC
 · D R A Y G R D I V E A H Y R A C L ·
 501 AGACAGAGCC TATGGCAGGG ACATCGTGGA GGCCCATTAC CGGGCCTGCT
 · Y A G V K I A G T N A E V M P A
 551 TGTATGCTGG AGTCAAGATT GCGGGGACTA ATGCCGAGGT CATGCCTGCC
 Q W E F Q I G P C E G I S M G D H ·
 601 CAGTGGGAAT TTCAGATTGG ACCTTGTGAA GGAATCAGCA TGGGAGATCA
 · L W V A R F I L H R V C E D F G V ·
 651 TCTCTGGGTG GCCCGTTTCA TCTTGCATCG TGTGTGTGAA GACTTTGGAG
 · I A T F D P K P I P G N W N G A
 701 TGATAGCAAC CTTTGATCCT AAGCCCATTC CTGGGAACTG GAATGGTGCA

FIG. 2F

G C H T N F S T K A M R E E N G L .
751 GGCTGCCATA CCAACTTCAG CACCAAGGCC ATGCGGGAGG AGAATGGTCT
· K Y I E E A I E K L S K R H Q Y H .
801 GAAGTACATC GAGGAGGCCA TTGAGAACT AAGCAAGCGG CACCAGTACC
· I R A Y D P K G G L D N A R R L
851 ACATCCGTGC CTATGATCCC AAGGGAGGCC TGGACAATGC CCGACGTCTA
T G F H E T S N I N D F S A G V A .
901 ACTGGATTCC ATGAAACCTC CAACATCAAC GACTTTTCTG CTGGTGTAGC
· N R S A S I R I P R T V G Q E K K .
951 CAATCGTAGC GCCAGCATAC GCATTCCCCG GACTGTTGGC CAGGAGAAGA
· G Y F E D R R P S A N C D P F S
1001 AGGGTTACTT TGAAGATCGT CGCCCCTCTG CCAACTGCGA CCCCTTTTCG
V T E A L I R T C L L N E T G D E .
1051 GTGACAGAAG CCCTCATCCG CACGTGTCTT CTCAATGAAA CCGGCGATGA
· P F Q Y K N *
1101 GCCCTTCCAG TACAAAAATT AA

FIG. 2F (Continued)

**FIG. 3**

**FIG. 4**

ADRERSIHDF	CLVSKVVGRC	RASMPRWWYN	30
VTDGSCQLFV	YGGCDGNSNN	YLTKEECLKK	60
CATVTENATG	DLATSRNAAD	SSVPSAPRRQ	90
DSEDHSSDMF	NYEEYCTANA	VTGPCRASFP	120
RWYFDVERNS	CNNFIYGGCR	GNKNSYRSEE	150
ACMLRCFRQQ	ENPPLPLGSK		170

FIG. 5

Push4SPR

