PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/81, 15/79 // 15/17

A1

(11) International Publication Number: **WO 95/34666**

(43) International Publication Date: 21 December 1995 (21.12.95)

(21) International Application Number: PCT/DK95/00249

(22) International Filing Date: 16 June 1995 (16.06.95)

(30) Priority Data:

0705/94	16 June 1994 (16.06.94)	DK
08/282,852	29 July 1994 (29.07.94)	US

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(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

Published
With international search report.

(54) Title: SYNTHETIC LEADER PEPTIDE SEQUENCES

(57) Abstract

The present invention relates to synthetic leader peptide sequences for secreting polypeptides in yeast.

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SYNTHETIC LEADER PEPTIDE SEQUENCES

FIELD OF INVENTION

The present invention relates to synthetic leader peptide sequences for secreting polypeptides in yeast.

BACKGROUND OF THE INVENTION

Yeast organisms produce a number of proteins which are synthesized intracellularly, but which have a function outside the cell. Such extracellular proteins are referred to as secreted proteins. These secreted proteins are expressed initially inside the cell in a precursor or a pre-protein form containing a presequence ensuring effective direction of the expressed product across the membrane of the endoplasmic reticulum (ER). The presequence, normally named a signal peptide, is generally cleaved off from the desired product during translocation. Once entered in the secretory pathway, the protein is transported to the Golgi apparatus. From the Golgi the protein can follow different routes that lead to compartments such as the cell vacuole or the cell membrane, or it can be routed out of the cell to be secreted to the external medium (Pfeffer, S.R. and Rothman, J.E. Ann.Rev.Biochem. 56 (1987) 829-852).

Several approaches have been suggested for the expression and secretion in yeast of proteins heterologous to yeast. European published patent application No. 88 632 describes a process by which proteins heterologous to yeast are expressed, processed and secreted by transforming a yeast organism with an expression vehicle harbouring DNA encoding the desired protein and a signal peptide, preparing a culture of the transformed organism, growing the culture and recovering the protein from the culture medium. The signal peptide may be the signal peptide of the desired protein itself, a heterologous signal

peptide or a hybrid of native and heterologous signal peptide.

A problem encountered with the use of signal peptides heterologous to yeast might be that the heterologous signal peptide does not ensure efficient translocation and/or cleavage after the signal peptide.

The Saccharomyces cerevisiae MFα1 (α-factor) is synthesized as a prepro form of 165 amino acids comprising signal- or prepeptide of 19 amino acids followed by a "leader" or propeptide of 64 amino acids, encompassing three N-linked glycosylation sites followed by $(LysArg((Asp/Glu)Ala)_{2-3}\alpha\text{-factor})_4$ (Kurjan, J. and Herskowitz, I. Cell 30 (1982) 933-943). The signal-leader part of the preproMFα1 has been widely employed to obtain synthesis and secretion of heterologous proteins in S. cerevisiae.

Use of signal/leader peptides homologous to yeast is known from i.a. US patent specification No. 4,546,082, European published patent applications Nos. 116 201, 123 294, 123 544, 163 529 and 123 289 and DK patent application No. 3614/83.

In EP 123 289 utilization of the S. cerevisiae a-factor precursor is described whereas WO 84/01153 indicates utilization of the S. cerevisiae invertase signal peptide and DK 3614/83 utilization of the S. cerevisiae PH05 signal peptide for secretion of foreign proteins.

US patent specification No. 4,546,082, EP 16 201, 123 294, 123 544 and 163 529 describe processes by which the α-factor signal-leader from S. cerevisiae (MFα1 or MFα2) is utilized in the secretion process of expressed heterologous proteins in yeast. By fusing a DNA sequence encoding the S. cerevisiae MFα1 signal/leader sequence at the 5' end of the gene for the desired protein secretion and processing of the desired protein was demonstrated.

EP 206 783 discloses a system for the secretion of polypeptides from S. cerevisiae using an α-factor leader sequence which has been truncated to eliminate the four α-factor units present on the native leader sequence so as to leave the leader peptide itself fused to a heterologous polypeptide via the α-factor processing site LysArgGluAlaGluAla. This construction is indicated to lead to an efficient processing of smaller peptides (less than 50 amino acids). For the secretion and processing of larger polypeptides, the native α-factor leader sequence has been truncated to leave one or two of the α-factor units between the leader peptide and the polypeptide.

A number of secreted proteins are routed so as to be exposed to a proteolytic processing system which can cleave the peptide bond at the carboxy end of two consecutive basic amino acids. This enzymatic activity is in S. cerevisiae encoded by the KEX 2 gene (Julius, D.A. et al., Cell 37 (1984b) 1075). Processing of the product by the KEX 2 protease is needed for the secretion of active S. cerevisiae mating factor α1 (MFα1 or α-factor) whereas KEX 2 is not involved in the secretion of active S. cerevisiae mating factor a.

Secretion and correct processing of a polypeptide intended to be secreted is obtained in some cases when culturing a yeast organism which is transformed with a vector constructed as indicated in the references given above. In many cases, however, the level of secretion is very low or there is no secretion, or the proteolytic processing may be incorrect or incomplete. It is therefore the object of the present invention to provide leader peptides which ensure a more efficient expression and/or processing of polypeptides.

SUMMARY OF THE INVENTION

Surprisingly, a new type of leader peptide has been found which allows secretion in high yield of a polypeptide in yeast.

Accordingly, the present invention relates to a DNA expression cassette comprising the following sequence:

$$5'\text{-P-SP-LS-PS-*gene*-(T)}_i\text{-}3'$$

wherein

P is a promoter sequence,

SP is a DNA sequence encoding a signal peptide,

LS is a DNA sequence encoding a leader peptide with the general formula I:

$$\text{GlnProIle(Asp/Glu)(Asp/Glu)}X^1\text{(Glu/Asp)}X^2\text{AsnZ(Thr/Ser)}X^3 \quad \text{(I)}$$

wherein

X^1 is a peptide bond or a codable amino acid;

X^2 is a peptide bond, a codable amino acid or a sequence of up to 4 codable amino acids which may be the same or different;

Z is a codable amino acid except Pro; and

X^3 is a sequence of from 4 to 30 codable amino acids which may be the same or different;

PS is a DNA sequence encoding a processing site;

gene is a DNA sequence encoding a polypeptide;

T is a terminator sequence; and

i is 0 or 1.

In the present context, the expression "leader peptide" is understood to indicate a peptide whose function is to allow the expressed polypeptide to be directed from the endoplasmic reticulum to the Golgi apparatus and further to a secretory vesicle for secretion into the medium, (i.e. exportation of the expressed polypeptide across the cell wall or at least through the cellular membrane into the periplasmic space of the cell). The term "synthetic" used in connection with leader peptides is intended to indicate that the leader peptide is one not found in nature.

The term "signal peptide" is understood to mean a presequence which is predominantly hydrophobic in nature and present as an N-terminal sequence of the precursor form of an extracellular protein expressed in yeast. The function of the signal peptide is to allow the expressed protein to be secreted to enter the endoplasmic reticulum. The signal peptide is normally cleaved off in the course of this process. The signal peptide may be heterologous or homologous to the yeast organism producing the protein.

The expression "polypeptide" is intended to indicate a heterologous polypeptide, i.e. a polypeptide which is not produced by the host yeast organism in nature as well as a homologous polypeptide, i.e. a polypeptide which is produced by the host yeast organism in nature and any preform thereof. In a preferred embodiment, the expression cassette of the present invention encodes a heterologous polypeptide.

The expression "a codable amino acid" is intended to indicate an amino acid which can be coded for by a triplet ("codon") of nucleotides.

When, in the amino acid sequences given in the present specification, the three letter codes of two amino acids, separated by a slash, are given in brackets, e.g. (Asp/Glu), this is intended to indicate that the sequence has either the one or the other of these amino acids in the pertinent position.

In a further aspect, the present invention relates to a process for producing a polypeptide in yeast, the process comprising culturing a yeast cell, which is capable of expressing a polypeptide and which is transformed with a yeast expression vector as described above including a leader peptide sequence of the invention, in a suitable medium to obtain expression and secretion of the polypeptide, after which the polypeptide is recovered from the medium.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention is further illustrated with reference to the appended drawings wherein

Fig. 1 schematically shows the plasmid pAK492;

Fig. 2 shows part of the DNA sequence encoding the signal peptide/leader/MI3 insulin precursor;

Fig. 3 shows the construction of the plasmid pAK546;

Fig. 4 shows the amino acid sequence of the leader SEQ ID No. 4 and the DNA sequence encoding it;

Fig. 5 shows the DNA sequence of S. cerevisiae expression plasmid pAK546 encoding the YAP3 signal peptide, leader SEQ ID No. 4 and the MI3 insulin precursor and the encoded amino acid sequence;

Fig. 6 shows the amino acid sequence of the leader SEQ ID No. 6 and the DNA sequence encoding it;

Fig. 7 shows the amino acid sequence of the leader SEQ ID No. 8 and the DNA sequence encoding it;

Fig. 8 shows the amino acid sequence of the leader SEQ ID No. 17 and the DNA sequence encoding it;

Fig. 9 shows the amino acid sequence of the leader SEQ ID No. 16 and the DNA sequence encoding it;

Fig. 10 shows the amino acid sequence of the leader SEQ ID No. 19 and the DNA sequence encoding it;

Fig. 11 shows the amino acid sequence of the leader SEQ ID No. 20 and the DNA sequence encoding it;

Fig. 12 shows the amino acid sequence of the leader SEQ ID No. 21 and the DNA sequence encoding it;

Fig. 13 shows the DNA fragment of pAK527 used as the direct template in the construction of SEQ ID Nos. 4 and 6;

Fig. 14 shows the DNA fragment of pAK531 used as the direct template in the construction of SEQ ID No. 8;

Fig. 15 shows the DNA fragment of pAK555 used as the direct template in the construction of SEQ ID Nos. 16 and 17;

Fig. 16 shows the DNA fragment of pAK559 used as the direct template in the construction of SEQ ID Nos. 19 and 20; and

Fig. 17 shows the DNA fragment of pAK562 used as the direct template in the construction of SEQ ID No. 21;

Fig. 18 shows the amino acid sequence of the leader SEQ ID No. 27 and the DNA sequence SEQ ID No. 66 encoding it;

Fig. 19 shows the amino acid sequence SEQ ID No. 70 of an N-terminally extended MI3 insulin precursor and the DNA sequence SEQ ID No. 71 encoding it;

Fig. 20 shows the amino acid sequence of the leader SEQ ID No. 67 and the DNA sequence SEQ ID No. 69 encoding it;

Fig. 21 shows the DNA fragment SEQ ID No. 72 of pAK614 used as the direct template in the construction of SEQ ID No. 27; and

Fig. 22 shows the DNA fragment SEQ ID No. 73 of pAK625 used as the direct template in the construction of SEQ ID No. 67.

DETAILED DISCLOSURE OF THE INVENTION

When X^1 in general formula I designates an amino acid, it is preferably Ser, Thr or Ala. When X^2 in general formula I designates one amino acid, it is preferably Ser, Thr or Ala. When X^2 in general formula I designates a sequence of two amino acids, it is preferably SerIle. When X^2 in general formula I designates a sequence of three amino acids, it is preferably SerAlaIle. When X^2 in general formula I designates a sequence of four amino acids it is preferably SerPheAlaThr. In a preferred embodiment, X^3 is an amino acid sequence of the general formula II

$$X^4\text{-}X^5\text{-}X^6 \qquad \text{(II)}$$

wherein X^4 is a sequence of from 1 to 21 codable amino acids which may be the same or different, X^5 is Pro or one of the amino acid sequences ValAsnLeu or LeuAlaAsnValAlaMetAla, and X^6 is a sequence of from 1 to 8 codable amino acids which may be the same or different.

In general formula II, X4 is preferably an amino acid sequence which includes one or more of the motifs LeuValAsnLeu, SerValAsnLeu, MetAlaAsp, ThrGluSer, ArgPheAlaThr or ValAlaMetAla; or X^4 is an amino acid sequence which includes the sequence AsnSerThr or AsnThrThr; or X^4 is an amino acid sequence which includes the sequence

(Ser/Leu)ValAsnLeu,
(Ser/Leu)ValAsnLeuMetAlaAsp,
(Ser/Leu)ValAsnLeuMetAlaAspAsp,
(Ser/Leu)ValAsnLeuMetAlaAspAspThrGluSer,
(Ser/Leu)ValAsnLeuMetAlaAspAspThrGluSerIle or
(Ser/Leu)ValAsnLeuMetAlaAspAspThrGluSerArgPheAlaThr;

or X^4 is an amino acid sequence which includes the sequence

Asn(Thr/Ser)ThrLeu,
Asn(Thr/Ser)ThrLeuAsnLeu or
Asn(Thr/Ser)ThrLeuValAsnLeu; or any combination

thereof.

In general formula II, X^5 is preferably Pro or an amino acid sequence which includes the sequence ValAsnLeu, LeuAlaAsnValAlaMetAla, LeuAspValValAsnLeuProGly or LeuAspValValAsnLeuIleSerMet.

When X^6, in general formula II, designates one amino acid, it is preferably Ala, Gly, Leu, Thr, Val or Ser. When X^6, in general formula II, designates a sequence of two amino acids, it is preferably GlyAla or SerAla. When X^6, in general formula II, designates a sequence of three amino acids, it is preferably AlaValAla. When X^6, in general formula II, designates a sequence of eight amino acids, it is preferably GlyAlaAspSerLysThrValGlu.

Examples of preferred leader peptides coded for by the DNA sequence LS are:

SEQ ID No. 1 GlnProIleAspGluAspAsnAspThrSerValAsnLeuProAla;

SEQ ID No. 2 GlnProIleAspAspGluAsnThrThrSerValAsnLeuProAla;

SEQ ID No. 3 GlnProIleAspAspGluSerAsnThrThrSerValAsnLeuProAla;

SEQ ID No. 4 GlnProIleAspAspGluAsnThrThrSerValAsnLeuProVal;

SEQ ID No. 5 GlnProIleAspAspThrGluAsnThrThrSerValAsnLeuProAla;

SEQ ID No. 6 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuPro-Ala;

SEQ ID No. 7 GlnProIleAspAspGluAsnThrThrSerValAsnLeuMetAla;

SEQ ID No. 8 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuPro-GlyAla;

SEQ ID No. 9 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMet-Ala;

SEQ ID No. 10 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnValPro-Thr;

SEQ ID No. 11 GlnProIleAspAspThrGluSerAsnThrThrLeuValAsnValPro-Thr;

SEQ ID No. 12 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuPro-Thr;

SEQ ID No. 13 GlnProIleAspAspThrGluSerAsnThrThrLeuValAsnValPro-GlyAla;

SEQ ID No. 14 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMet-AlaProAlaValAla;

SEQ ID No. 15 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMet-AspLeuAlaValGlyLeuProGlyAla;

SEQ ID No. 16 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMet-AlaAspAspThrGluSerIleAsnThrThrLeuValAsnLeuProGly-Ala;

SEQ ID No. 17 GlnProIleAspAspThrGluSerIleAsnThrThrLeuValAsnLeu-ProGlyAla;

SEQ ID No. 18 GlnProIleAspAspThrGluSerAsnThrThrLeuValAsnLeuPro-GlyAla;

SEQ ID No. 19 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMet-AlaAspAspThrGluSerArgPheAlaThrAsnThrThrLeuValAsn-LeuProLeu;

SEQ ID No. 20 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMet-AlaAspAspThrGluSerIleAsnThrThrLeuValAsnLeuAlaAsn-

ValAlaMetAla;

SEQ ID No. 21 GlnProIleAspAspThrGluSerAlaIleAsnThrThrLeuValAsn-LeuProGlyAla;

SEQ ID No. 22 GlnProIleAspAspThrGluSerPheAlaThrAsnThrThrLeuVal-AsnLeuProGlyAla;

SEQ ID No. 23 GlnProIleAspAspThrGluSerIleAsnThrThrLeuValAsnLeu-MetAlaAspAspThrGluSerArgPheAlaThrAsnThrThrLeuVal-AsnLeuProLeu;

SEQ ID No. 24 GlnProIleAspAspThrGluSerIleAsnThrThrLeuValAsnLeu-MetAlaAspAspThrGluSerArgPheAlaThrAsnThrThrLeuAsp-ValValAsnLeuProGlyAla;

SEQ ID No. 25 GlnProIleAspAspThrGluSerAlaAlaIleAsnThrThrLeuVal-AsnLeuProGlyAla;

SEQ ID No. 26 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMet-AlaAspAspThrGluSerArgPheAlaThrAsnThrThrLeuValAsn-LeuAlaAsnValAlaMetAla;

SEQ ID No. 27 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMet-AlaAspAspThrGluSerArgPheAlaThrAsnThrThrLeuAspVal-ValAsnLeuIleSerMetAla;

SEQ ID No. 28 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMet-AlaAsnThrThrGluSerArgPheAlaThrAsnThrThrLeuAspVal-ValAsnLeuIleSerMetAla; and

SEQ ID No. 67 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMet-AlaAspAspThrGluSerArgPheAlaThrAsnThrThrLeuAlaLeu-AspValValAsnLeuIleSerMetAlaLysArg.

Particularly preferred leader peptides coded for by the DNA sequence LS are:

SEQ ID No. 15 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMet-AspLeuAlaValGlyLeuProGlyAla;

SEQ ID No. 16 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMet-AlaAspAspThrGluSerIleAsnThrThrLeuValAsnLeuProGly-Ala;

SEQ ID No. 17 GlnProIleAspAspThrGluSerIleAsnThrThrLeuValAsnLeu-ProGlyAla;

SEQ ID No. 18 GlnProIleAspAspThrGluSerAsnThrThrLeuValAsnLeuPro-GlyAla;

SEQ ID No. 19 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMet-AlaAspAspThrGluSerArgPheAlaThrAsnThrThrLeuValAsn-LeuProLeu;

SEQ ID No. 20 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMet-AlaAspAspThrGluSerIleAsnThrThrLeuValAsnLeuAlaAsn-ValAlaMetAla;

SEQ ID No. 21 GlnProIleAspAspThrGluSerAlaIleAsnThrThrLeuValAsn-LeuProGlyAla;

SEQ ID No. 22 GlnProIleAspAspThrGluSerPheAlaThrAsnThrThrLeuVal-AsnLeuProGlyAla;

SEQ ID No. 23 GlnProIleAspAspThrGluSerIleAsnThrThrLeuValAsnLeu-MetAlaAspAspThrGluSerArgPheAlaThrAsnThrThrLeuVal-AsnLeuProLeu;

SEQ ID No. 24 GlnProIleAspAspThrGluSerIleAsnThrThrLeuValAsnLeu-MetAlaAspAspThrGluSerArgPheAlaThrAsnThrThrLeuAsp-ValValAsnLeuProGlyAla;

SEQ ID No. 25 GlnProIleAspAspThrGluSerAlaAlaIleAsnThrThrLeuVal-AsnLeuProGlyAla;

SEQ ID No. 26 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMet-AlaAspAspThrGluSerArgPheAlaThrAsnThrThrLeuValAsn-LeuAlaAsnValAlaMetAla; and

SEQ ID No. 28 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMet-AlaAsnThrThrGluSerArgPheAlaThrAsnThrThrLeuAspVal-ValAsnLeuIleSerMetAla.

SEQ ID No. 67 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMet-AlaAspAspThrGluSerArgPheAlaThrAsnThrThrLeuAlaLeu-AspValValAsnLeuIleSerMetAlaLysArg.

The signal sequence (SP) may encode any signal peptide which ensures an effective direction of the expressed polypeptide into the secretory pathway of the cell. The signal peptide may be a naturally occurring signal peptide or functional parts thereof or it may be a synthetic peptide. Suitable signal peptides have been found to be the α-factor signal peptide, the signal peptide of mouse salivary amylase, a modified carboxypeptidase signal peptide, the yeast BAR1 signal peptide or the Humicola lanuginosa lipase signal peptide or a derivative thereof. The mouse salivary amylase signal sequence is described by Hagenbüchle, O. et al., Nature 289 (1981) 643-646. The carboxypeptidase signal sequence is described by Valls, L.A. et al., Cell 48 (1987) 887-897. The BAR1 signal peptide is disclosed in WO 87/02670. The yeast aspartic protease 3 signal peptide is described in Danish patent application No. 0828/93.

The yeast processing site encoded by the DNA sequence PS may suitably be any paired combination of Lys and Arg, such as LysArg, ArgLys, ArgArg or LysLys which permits processing of the polypeptide by the KEX2 protease of Saccharomyces cerevisiae or the equivalent protease in other yeast species (Julius, D.A. et al., Cell 37 (1984) 1075). If KEX2 processing is not convenient, e.g. if it would lead to cleavage of the polypeptide product, e.g. due to the presence of two

consecutive basic amino acid internally in the desired product, a processing site for another protease may be selected comprising an amino acid combination which is not found in the polypeptide product, e.g. the processing site for FX_a, IleGluGlyArg (cf. Sambrook, J., Fritsch, E.F. and Maniatis, T., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York, 1989).

The protein produced by the method of the invention may be any protein which may advantageously be produced in yeast. Examples of such proteins are heterologous proteins such as aprotinin, tissue factor pathway inhibitor or other protease inhibitors, insulin or insulin precursors, human or bovine growth hormone, interleukin, glucagon, GLP-1, IGF-I, IGF-II, tissue plasminogen activator, transforming growth factor α or β, platelet-derived growth factor, enzymes or a functional analogue thereof. In the present context, the term "functional analogue" is meant to indicate a protein with a similar function as the native protein (this is intended to be understood as relating to the nature rather than the level of biological activity of the native protein). The protein may be structurally similar to the native protein and may be derived from the native protein by addition of one or more amino acids to either or both the C- and N-terminal end of the native protein, substitution of one or more amino acids at one or a number of different sites in the native amino acid sequence, deletion of one or more amino acids at either or both ends of the native protein or at one or several sites in the amino acid sequence, or insertion of one or more amino acids at one or more sites in the native amino acid sequence. Such modifications are well known for several of the proteins mentioned above. Also, precursors or intermediates for other proteins may be produced by the method of the invention. An example of such a precursor is the MI3 insulin precursor which comprises the amino acid sequence B(1-29)AlaAlaLysA(1-21) wherein A(1-21) is the A chain of human insulin and B(1-29) is the B chain of human insulin in which Thr(B30) is missing.

Preferred DNA constructs encoding leader sequences are as shown in Figs. 4 - 12 or suitable modifications thereof. Examples of suitable modifications of the DNA sequence are nucleotide substitutions which do not give rise to another amino acid sequence of the protein, but which may correspond to the codon usage of the yeast organism into which the DNA construct is inserted or nucleotide substitutions which do give rise to a different amino acid sequence and therefore, possibly, a different protein structure. Other examples of possible modifications are insertion of one or more codons into the sequence, addition of one or more codons at either end of the sequence and deletion of one or more codons at either end of or within the sequence.

The recombinant expression vector carrying the expression casette

$$5'\text{-P-SP-LS-PS-*gene*-}(T)_i\text{-}3'$$

wherein P, SP, LS, *gene*, T and i are as defined above may be any vector which is capable of replicating in yeast organisms. The promoter may be any DNA sequence which shows transcriptional activity in yeast and may be derived from genes encoding proteins either homologous or heterologous to yeast. The promoter is preferably derived from a gene encoding a protein homologous to yeast. Examples of suitable promoters are the Saccharomyces cerevisiae MFα1, TPI, ADH or PGK promoters.

The sequences shown above should preferably also be operably connected to a suitable terminator, e.g. the TPI terminator (cf. Alber, T. and Kawasaki, G., J. Mol. Appl. Genet. 1 (1982) 419-434).

The recombinant expression vector of the invention further comprises a DNA sequence enabling the vector to replicate in yeast. Examples of such sequences are the yeast plasmid 2μ replication genes REP 1-3 and origin of replication. The vector may also comprise a selectable marker, e.g. the Schizo-

saccharomyces pombe TPI gene as described by Russell, P.R., Gene 40 (1985) 125-130.

The methods used to ligate the sequence 5'-P-SP-LS-PS-*gene*-$(T)_i$-3' and to insert it into suitable yeast vectors containing the information necessary for yeast replication, are well known to persons skilled in the art (cf., for instance, Sambrook, J., Fritsch, E.F. and Maniatis, T., op.cit.). It will be understood that the vector may be constructed either by first preparing a DNA construct containing the entire sequence 5'-P-SP-LS-PS-*gene*-$(T)_i$-3' and subsequently inserting this fragment into a suitable expression vector, or by sequentially inserting DNA fragments into a suitable vector containing genetic information for the individual elements (such as the promoter sequence, the signal peptide, the leader sequence GlnProIle(Asp/Glu)(Asp/Glu)X^1(Glu/Asp)X^2AsnZ(Thr/Ser)X^3, the processing site, the polypeptide, and, if present, the terminator sequence) followed by ligation.

The yeast organism used in the method of the invention may be any suitable yeast organism which, on cultivation, produces large amounts of the desired polypeptide. Examples of suitable yeast organisms may be strains of the yeast species Saccharomyces cerevisiae, Saccharomyces kluyveri, Schizosaccharomyces pombe or Saccharomyces uvarum. The transformation of the yeast cells may for instance be effected by protoplast formation followed by transformation in a manner known per se. The medium used to cultivate the cells may be any conventional medium suitable for growing yeast organisms. The secreted polypeptide, a significant proportion of which will be present in the medium in correctly processed form, may be recovered from the medium by conventional procedures including separating the yeast cells from the medium by centrifugation or filtration, precipitating the proteinaceous components of the supernatant or filtrate by means of a salt, e.g. ammonium sulphate, followed by purification by a variety of chromatographic procedures, e.g. ion exchange chromatography,

affinity chromatography or the like.

The invention is further described in the following examples which are not to be construed as limiting the scope of the invention as claimed.

EXAMPLES

Plasmids and DNA material

All expression plasmids are of the C-POT type. Such plasmids are described in EP patent application No. 171 142 and are characterized in containing the Schizosaccharomyces pombe triose phosphate isomerase gene (POT) for the purpose of plasmid selection and stabilization. A plasmid containing the POT-gene is available from a deposited E. coli strain (ATCC 39685). The plasmids furthermore contain the S. cerevisiae triose phosphate isomerase promoter and terminator (P_{TPI} and T_{TPI}). They are identical to pMT742 (Egel-Mitani, M. et al., Gene 73 (1988) 113-120) (see Fig. 1) except for the region defined by the EcoR I-Xba I restriction sites encompassing the coding region for signal/leader/product.

The plasmids pAK527, pAK531, pAK555, pAK559, pAK562, pAK614 and pAK625 were used as DNA templates in the PCR reactions applied in the construction of the leaders described in the examples. The synthetic DNA fragments serving as the direct template are shown in Figs. 13 - 17. With the exception of the shown DNA regions the plasmids are identical to pAK492 shown in Fig. 1.

Synthetic DNA fragments were synthesized on an automatic DNA synthesizer (Applied Biosystems model 380A) using phosphoramidite chemistry and commercially available reagents (Beaucage, S.L. and Caruthers, M.H., Tetrahedron Letters 22 (1981) 1859-1869).

All other methods and materials used are common state of the art knowledge (see, e.g. Sambrook, J., Fritsch, E.F. and Maniatis, T., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York, 1989).

EXAMPLE 1

Synthesis of the leader SEQ ID No. 4 for expression of the MI3 insulin precursor in S. cerevisiae (strain yAK546).

The leader SEQ ID No. 4 has the following amino acid sequence:
GlnProIleAspAspGluAsnThrThrSerValAsnLeuProVal

The following oligonucleotides were synthesised:

# 94	5'-TAAATCTATAACTACAAAAAACACATA-3'	**SEQ ID No. 29**
# 333	5'-GACTCTCTTAACTGGCAAGTTGACA-3'	**SEQ ID No. 30**
# 312	5'-AAGTACAAAGCTTCAACCAAGTGAGAACCACACAAGTGTT GGTTAACGAATCTCTT-3'	**SEQ ID No. 31**
# 1845	5'-CATACACAATATAAACGACGG-3'	**SEQ ID No. 32**

The following polymerase chain reactions (PCR) were performed using the Gene Amp PCR reagent kit (Perkin Elmer, 761 Main Avewalk, CT 06859, USA) according to the manufacturers instructions. During the reaction, the PCR mixtures were overlayed with 100 µl of mineral oil (Sigma Chemical CO, St. Louis MO, USA):

Polymerase chain reaction No. 1

5 µl of oligonucleotide # 94 (50 pmol)
5 µl of oligonucleotide # 333 (50 pmol)
10 µl of 10X PCR buffer
16 µl of dNTP mix
0.5 µl of Taq enzyme
0.5 µl of pAK527 plasmid (Fig. 13) as template (0.2 µg of DNA)

63 µl of water

A total of 12 cycles were performed, one cycle was 94°C for 1 min; 37°C for 2 min; 72°C for 3 min. The PCR mixture was then loaded onto a 2% agarose gel and electrophoresis was performed using standard techniques (Sambrook, J., Fritsch, E.F. and Maniatis, T., op.cit.). The resulting DNA fragment was cut out of the agarose gel and isolated using the Gene Clean kit (Bio 101 inc., PO BOX 2284, La Jolla, CA 92038, USA) according to the manufacturers instructions.

Polymerase chain reaction No. 2

5 µl of oligonucleotide # 312 (50 pmol)
5 µl of oligonucleotide # 94 (50 pmol)
10 µl of 10X PCR buffer
16 µl of dNTP mix
0.5 µl of Taq enzyme
10 µl of purified DNA fragment from PCR No. 1
53.5 µl of water

A total of 12 cycles were performed, one cycle was 94°C for 1 min; 37°C for 2 min; 72°C for 3 min.
The DNA fragment from polymerase chain reaction No. 2 was isolated and purified using the Gene Clean kit (Bio 101 inc., PO BOX 2284, La Jolla, CA 92038, USA) according to the manufacturers instructions.

The purified PCR DNA fragment was dissolved in 10 µl of water and restriction endonuclease buffer and cut with the restriction endonucleases Asp 718 and Hind III in a total volume of 15 µl according to standard techniques (Sambrook, J., Fritsch, E.F. and Maniatis, T., op.cit.). The 167 bp Asp 718/Hind III DNA fragment was subjected to electrophoresis on agarose gel and purified using The Gene Clean Kit as described. The S. cerevisiae expression plasmid pAK492 (shown in Fig. 1) is a derivative of the previously described plasmid pMT742 in

which the fragment encoding the signal/leader/insulin precursor has been replaced by the EcoR I-Xba I fragment shown in Fig. 2. This fragment has been synthesized on an Applied Biosystems DNA synthesizer in accordance with the manufacturer's instructions. The plasmid pAK492 was cut with the restriction endonucleases Asp 718 and Xba I and the vector fragment of 10986 bp was isolated. The plasmid pAK492 was cut with the restriction endonucleases Hind III and Xba I and the DNA fragment of 140 bp encoding part of the MI3 insulin precursor was isolated. The three DNA fragments were ligated together using T4 DNA ligase under standard conditions (Sambrook, J., Fritsch, E.F. and Maniatis, T., op.cit.). The ligation mixture was then transformed into a competent E. coli strain (R-, M+) and transformants were identified by ampicillin resistance. Plasmids were isolated from the resulting E. coli colonies using standard DNA miniprep technique (Sambrook, J., Fritsch, E.F. and Maniatis, T., op.cit.), checked with appropriate restrictions endonucleases i.e. EcoR I, Xba I, Nco I and Hind III. The selected plasmid, pAK546, was shown by DNA sequencing analysis (Sequenase, U.S. Biochemical Corp.) using the primer # 94 to contain a DNA sequence encoding the leader SEQ ID No. 4. For the DNA sequence encoding the leader SEQ ID No. 4, see Fig. 4). The plasmid pAK546 was transformed into S. cerevisiae strain MT663 as described in European published patent application No. 214 826 and the resulting strain was named yAK546. The DNA sequence of the protein coding region of the expression plasmid is given in Fig. 5.

EXAMPLE 2

Synthesis of the leader SEQ ID No. 6 for expression of the MI3 insulin precursor in S. cerevisiae (strain yAK531).

The leader SEQ ID No. 6 has the following amino acid sequence:
GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuProAla

The following oligonucleotide was synthesised:

331 5'-GAATCTCTTAGCTGGCAAGTTGACAGAAGTAGTGTTAG
TTTCAGAGTCGTCAATT-3' **SEQ ID No. 33**

The polymerase chain reaction was performed as described in Example 1 with the expection that oligonucleotide # 331 was used insted of oligonucleotide # 333.

The Asp 718/Hind III DNA fragment of 168 bp was subjected to electrophoresis on agarose gel and purified as described in Example 1. The Asp 718/Hind III DNA fragment was subcloned into the S. cerevisiae expression plasmid as described in Example 1. The selected plasmid, pAK531, was shown by DNA sequencing analysis, as described in Example 1, to contain a DNA sequence encoding the leader SEQ ID No. 6. For the DNA sequence encoding the leader SEQ ID No. 6, see Fig. 6. The plasmid pAK531 was transformed into S. cerevisiae strain MT663 as described in European patent application 86306721.1 and the resulting strain was named yAK531. The DNA sequences encoding the signal peptide and the insulin precursor MI3 were the same as those shown in Fig. 5.

EXAMPLE 3

Synthesis of the leader SEQ ID No. 8 for expression of the MI3 insulin precursor in S. cerevisiae (strain yAK547).

The leader SEQ ID No. 8 has the following amino acid sequence:
GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuProGlyAla

The following oligonucleotide was synthesised:

345 5'-AACGAATCTCTTAGCACCTGGCAAGTTGACAGAAGT-3' **SEQ ID No. 34**

The polymerase chain reaction was performed as described in Example 1 with the expection that oligonucleotide # 345 was used insted of oligonucleotide # 333 and plasmid pAK531 (Fig. 14) was used as template.

The Asp 718/Hind III DNA fragment of 171 bp was subjected to electrophoresis on agarose gel and purified as described in Example 1. The Asp 718/Hind III DNA fragment was subcloned into the S. cerevisiae expression plasmid as described in Example 1. The selected plasmid, pAK547, was shown by DNA sequencing analysis, as described in Example 1, to contain a DNA sequence encoding the leader SEQ ID No. 8. For the DNA sequence encoding the leader SEQ ID No. 8, see Fig. 7. The plasmid pAK547 was transformed into S. cerevisiae strain MT663 as described in European patent application No. 86306721.1 and the resulting strain was named yAK547. The DNA sequences encoding the signal peptide and the insulin precursor MI3 were the same as those shown in Fig. 5.

EXAMPLE 4

Synthesis of the leader SEQ ID No. 17 for expression of the MI3 insulin precursor in S. cerevisiae (strain yAK561).

The leader SEQ ID No. 17 has the following amino acid sequence:
GlnProIleAspAspThrGluSerIleAsnThrThrLeuValAsnLeuProGlyAla

The following oligonucleotide was synthesised:

376 5'-AACGAATCTCTTAGCACCTGGCAAGTTGACCAAAGTAG
TGTTGATAGATTCAGTGTCGTC-3' **SEQ ID No. 35**

The polymerase chain reaction was performed as described in Example 1 with the expection that oligonucleotide # 376 was used insted of oligonucleotide # 333 and plasmid pAK555 (Fig. 15) was used as template.

The Asp 718/Hind III DNA fragment of 180 bp was subjected to electrophoresis on agarose gel and purified as described in Example 1. The Asp 718/Hind III DNA fragment was subcloned into the S. cerevisiae expression plasmid as described in Example 1. The selected plasmid, pAK561, was shown by DNA sequencing analysis, as described in Example 1, to contain a DNA sequence encoding the leader SEQ ID No. 17. For the DNA sequence encoding the leader SEQ ID No. 17, see Fig. 8. The plasmid pAK561 was transformed into S. cerevisiae strain MT663 as described in European patent application No. 86306721.1 and the resulting strain was named yAK561. The DNA sequences encoding the signal peptide and the insulin precursor MI3 were the same as those shown in Fig. 5.

EXAMPLE 5

Synthesis of the leader SEQ ID No. 16 for expression of the MI3 insulin precursor in S. cerevisiae (strain yAK559).

The leader SEQ ID No. 16 has the following amino acid sequence:
GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMetAlaAspAspThr-GluSerIleAsnThrThrLeuValAsnLeuProGlyAla

The following oligonucleotide was synthesised:

375 5'-AACGAATCTCTTAGCACCTGGCAAGTTAACCAAAGTAGT
GTTGATAGATTCAGTGTCGTCAGCCATCAAGTTGAC-3' **SEQ ID No. 36**

The polymerase chain reaction was performed as described in Example 1 with the expection that oligonucleotide # 375 was used insted of oligonucleotide # 333 and plasmid pAK555 (Fig. 15) was used as template.

The Asp 718/Hind III DNA fragment of 222 bp was subjected to electrophoresis on agarose gel and purified as described in Example 1. The Asp 718/Hind III DNA fragment was subcloned into

the S. cerevisiae expression plasmid as described in Example 1. The selected plasmid, pAK559, was shown by DNA sequencing analysis, as described in Example 1, to contain a DNA sequence encoding the leader SEQ ID No. 16. For the DNA sequence encoding the leader SEQ ID No. 16, see Fig. 9. The plasmid pAK559 was transformed into S. cerevisiae strain MT663 as described in European patent application No. 86306721.1 and the resulting strain was named yAK559. The DNA sequences encoding the signal peptide and the insulin precursor MI3 were the same as those shown in Fig. 5.

EXAMPLE 6

Synthesis of the leader SEQ ID No. 19 for expression of the MI3 insulin precursor in S. cerevisiae (strain yAK580).

The leader SEQ ID No. 19 has the following amino acid sequence:
GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMetAlaAspAspThr-GluSerArgPheAlaThrAsnThrThrLeuValAsnLeuProLeu

The following oligonucleotide was synthesised:

384 5'-AACGAATCTCTTCAATGGCAAGTTAACCAAAGTAGTGT
TAGTAGCGAATCTAGATTCAGTGTCGTCAGCCAT-3' **SEQ ID No. 37**

The polymerase chain reaction was performed as described in Example 1 with the expection that oligonucleotide # 384 was used insted of oligonucleotide # 333 and plasmid pAK559 (Fig. 16) was used as template.

The Asp 718/Hind III DNA fragment of 228 bp was subjected to electrophoresis on agarose gel and purified as described in Example 1. The Asp 718/Hind III DNA fragment was subcloned into the S. cerevisiae expression plasmid as described in Example 1. The selected plasmid, pAK580, was shown by DNA sequencing analysis, as described in Example 1, to contain a DNA sequence

encoding the leader SEQ ID No. 19. For the DNA sequence encoding the leader SEQ ID No. 19, see Fig. 10. The plasmid pAK580 was transformed into S. cerevisiae strain MT663 as described in European patent application No. 86306721.1 and the resulting strain was named yAK580. The DNA sequences encoding the signal peptide and the insulin precursor MI3 were the same as those shown in Fig. 5.

EXAMPLE 7

Synthesis of the leader SEQ ID No. 20 for expression of the MI3 insulin precursor in S. cerevisiae (strain yAK583).

The leader SEQ ID No. 20 has the following amino acid sequence:
GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMetAlaAspAspThr-GluSerIleAsnThrThrLeuValAsnLeuAlaAsnValAlaMetAla

The following oligonucleotide was synthesised:

390 5'-AACGAATCTCTTAGCCATGGCAACGTTAGCCAAGTTAA
CCAAAGT-3' **SEQ ID No. 38**

The polymerase chain reaction was performed as described in Example 1 with the expection that oligonucleotide # 390 was used insted of oligonucleotide # 333 and plasmid pAK559 (Fig. 16) was used as template.

The Asp 718/Hind III DNA fragment of 231 bp was subjected to electrophoresis on agarose gel and purified as described in Example 1. The Asp 718/Hind III DNA fragment was subcloned into the S. cerevisiae expression plasmid as described in Example 1. The selected plasmid, pAK583, was shown by DNA sequencing analysis, as described in Example 1, to contain a DNA sequence encoding the leader SEQ ID No. 20. For the DNA sequence encoding the leader SEQ ID No. 20, see Fig. 11. The plasmid pAK583 was transformed into S. cerevisiae strain MT663 as

described in European patent application No. 86306721.1 and the resulting strain was named yAK583. The DNA sequences encoding the signal peptide and the insulin precursor MI3 were the same as those shown in Fig. 5.

EXAMPLE 8

Synthesis of the leader SEQ ID No. 21 for expression of the MI3 insulin precursor in S. cerevisiae (strain yAK586).

The leader SEQ ID No. 21 has the following amino acid sequence:
GlnProIleAspAspThrGluSerAlaIleAsnThrThrLeuValAsnLeuProGlyAla

The following oligonucleotide was synthesised:

401 5'-AACGAATCTCTTAGCACCTGGCAAGTTGACCAAAGTAG
TGTTGATAGCAGATTCAGTGTCG-3' **SEQ ID No. 39**

The polymerase chain reaction was performed as described in Example 1 with the exception that oligonucleotide # 401 was used instead of oligonucleotide # 333 and plasmid pAK562 (Fig. 17) was used as template.

The Asp 718/Hind III DNA fragment of 183 bp was subjected to electrophoresis on agarose gel and purified as described in Example 1. The Asp 718/Hind III DNA fragment was subcloned into the S. cerevisiae expression plasmid as described in Example 1. The selected plasmid, pAK586, was shown by DNA sequencing analysis, as described in Example 1, to contain a DNA sequence encoding the leader SEQ ID No. 21, see Fig. 12. The plasmid pAK586 was transformed into S. cerevisiae strain MT663 as described in European patent application No. 86306721.1 and the resulting strain was named yAK586. The DNA sequences encoding the signal peptide and the insulin precursor MI3 were the same as those shown in Fig. 5.

EXAMPLE 9

Expression of the MI3 insulin precursor using selected leader sequences according to the present invention.

Yeast strains harbouring plasmids as described above, were grown in YPD medium (Sherman, F. et al., Methods in Yeast Genetics, Cold Spring Harbor Laboratory Press, 1981). For each strain 6 individual 5 ml cultures were shaken at 30°C for 72 hours, with a final OD_{600} of approx. 15. After centrifugation the supernatant was removed for HPLC analysis by which method the concentration of secreted insulin precursor was measured by a method described by Snel, L. et al. Chromatographia 24 (1987) 329-332.

In Table 1 the expression levels of the insulin precursor, MI3, obtained by use of selected leader sequences according to the present invention, are given as a percentage of the level obtained with transformants of pMT742, utilizing the MFα(1) leader of S. cerevisiae.

Table 1

Leader	Expression level, %
MT748 α-leader	100
SEQ ID No. 15	87
SEQ ID No. 16	215
SEQ ID No. 17	157
SEQ ID No. 19	166
SEQ ID No. 20	86
SEQ ID No. 21	145
SEQ ID No. 22	137
SEQ ID No. 23	121

EXAMPLE 10

Synthesis of the leader SEQ ID No. 27 for expression of the extended MI3 insulin precursor in S. cerevisiae (strain yAK677).

The leader SEQ ID No. 27 has the following amino acid sequence: GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMetAlaAspAspThr-GluSerArgPheAlaThrAsnThrThrLeuAspValValAsnLeuIleSerMetAla

The following oligonucleotides were synthesised:

440 5'-GGTTAACGAACTTTGGAGCTTCAGCTTCAGCTTCTTCTCTCTTAGCCAT GGAGATCAAGTTAACAACATCCAAAGTAGTGTT-3' **SEQ ID No. 64**

and

441 5'-CAAGTACAAAGCTTCAACCAAGTGGGAACCGCACAAGTGTTGGTTAACG AACTT-3' **SEQ ID No. 65**

Polymerase chain reactions were performed as described in Example in 1 with the exception that oligonucleotide # 440 was used instead of oligonucleotide # 333 and plasmid pAK614 was used as template. For the second polymerase chain reaction, oligonucleotide # 441 was used instead of oligonucleotide # 312.

The purified PCR DNA fragment was isolated and digested with the restriction endonucleases Asp 718 and Hind III as described in Example 1. The Asp 718/Hind III DNA fragment of 268 bp was subjected to electrophoresis on agarose gel and purified as described in Example 1. The Asp 718/Hind III DNA fragment was subcloned into the S. cerevisiae expression plasmid as described in Example 1, with the exception that the 140 bp Hind III/Xba I DNA fragment was derived from pAK602 and encodes Asp^{B28} human insulin. The selected plasmid, pAK616, was shown by DNA sequencing analysis, as

described in Example 1, to contain the DNA sequence encoding the leader SEQ ID No. 27. For the DNA sequence, SEQ ID No. 66, encoding the leader SEQ ID No. 27, see Fig. 18. The Asp 718/Hind III DNA fragment of 268 bp from pAK616 was isolated and ligated with the 10986 bp Asp 718/Xba I DNA fragment from pAK601 and the 140 bp DNA fragment Hind III/Xba I from pAK464 (encoding an extended version of Asp^{B28} human insulin) and named pAK 625. The 180 bp Asp 718/Nco I DNA fragment from pAK625 was isolated and ligated with the 221 bp Nco I/Xba I DNA fragment from pJB146 (encoding and extended version of the insulin precursor) and the 10824 bp Asp 718/Xba I DNA fragment from pAK601 and the resulting plasmid was named pAK677. The plasmid pAK677 was transformed into S. cerevisiae strain MT663 as described in European patent application 86306721.1 and the resulting strain was named yAK677. With the exception of the DNA sequence encoding the leader, the DNA sequence encoding the signal peptide is as described in Fig. 5. The DNA sequence coding for the extended MI3 insulin precursor is as described in Fig. 19.

EXAMPLE 11

Synthesis of the leader SEQ ID No. 67 for expression of the extended MI3 insulin precursor in S. cerevisiae (yAK680)

The leader SEQ ID No. 67 has the following amino acid sequence:

GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMetAlaAspAspThr GluSerArgPheAlaThrAsnThrThrLeuAlaLeuAspValValAsnLeuIleSerMet Ala

The following oligonucleotide was synthesised:

577 5'-TCTCTTAGCCATGGAGATCAAGTTAACAACATCCAAAG
CCAAAGTAGTGTT-3' **SEQ ID No. 68**

The PCR was performed as described in Example in 1 with the exception that oligonucleotide # 577 was used instead of oligonucleotide # 333 and plasmid pAK625 was used as template and the second PCR was not performed. The PCR fragment was digested with the restriction endonucleases Asp 718 and Nco I as described in Example 1.

The Asp 718/Nco I DNA fragment of 190 bp was subjected to electrophoresis on agarose gel and purified as described in Example 1 expect that the 10824 bp Asp 718/Xba I vector DNA fragment was isolated from and from pAK601. The 190 bp Asp 718/Nco I DNA fragment was subcloned into the S. cerevisiae expression plasmid as described in Example 1, expect that the 221 bp DNA fragment Nco I/Xba I (encoding an extended version of the MI3 insulin precursor) was isolated from pAK677 and used instead of the Hind III/Xba I DNA fragment. The selected plasmid was shown by DNA sequencing analysis as described in Example 1 to contain the DNA sequence encoding the leader SEQ ID No. 67 and named pAK680. For the DNA sequence, SEQ ID No. 69, encoding the leader SEQ ID No. 67, see Fig. 20. The plasmid pAK680 was transformed into S. cerevisiae strain MT663 as described in European patent application 86306721.1 and the resulting strain was named yAK680. With the exception of the DNA sequence encoding the leader, the DNA sequence encoding the signal peptide is as described in Fig. 5 and the extended insulin precursor MI3 DNA sequence is as described in Fig. 19.

EXAMPLE 12

Expression of N-terminally extended MI3 insulin precursors using the leader sequences SEQ ID No. 27 and SEQ ID No. 67 according to the present invention.

Yeast strains harbouring plasmids as described above, were grown in YPD medium (Sherman, F. et al., Methods in Yeast

Genetics, Cold Spring Harbor Laboratory Press, 1981). For each strain 6 individual 5 ml cultures were shaken at 30°C for 72 hours, with a final OD_{600} of approximately 15. After centrifugation the supernatant was removed for HPLC analysis by which method the concentration of secreted insulin precursor was measured by a method described by Snel, L. et al. Chromatographia 24 (1987) 329-332.

In Table 2 the expression levels of some N-terminally extended MI3 insulin precursors, obtained by use of the leader sequences SEQ ID No. 27 and SEQ ID No. 67 according to the present invention, are given as a percentage of the level obtained with transformants of pMT742, utilizing the MFα(1) leader of S. cerevisiae.

Table 2

Strain	Signal peptide	Leader	Extension	Relative to MT748
MT748	α	α		
yAK675	YAP3	SEQ ID No. 27	EEAEAEAPK	251%
yAK677	YAP3	SEQ ID No. 27	EEAEAEAEPK	224%
yAK681	YAP3	SEQ ID No. 67	EEAEAEAPK	248%
yAK680	YAP3	SEQ ID No. 67	EEAEAEAEPK	362%

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: SYNTHETIC LEADERS PEPTIDE SEQUENCES

(iii) NUMBER OF SEQUENCES: 73

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: DK 0705/94 and US 08/282,852
(B) FILING DATE: 16-JUN-1994 and 29-JUL-1994

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Gln Pro Ile Asp Glu Asp Asn Asp Thr Ser Val Asn Leu Pro Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Gln Pro Ile Asp Asp Glu Asn Thr Thr Ser Val Asn Leu Pro Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Gln Pro Ile Asp Asp Glu Ser Asn Thr Thr Ser Val Asn Leu Pro Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Gln Pro Ile Asp Asp Glu Asn Thr Thr Ser Val Asn Leu Pro Val
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Gln Pro Ile Asp Asp Thr Glu Asn Thr Thr Ser Val Asn Leu Pro Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr Ser Val Asn Leu Pro
1 5 10 15

Ala

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Gln Pro Ile Asp Asp Glu Asn Thr Thr Ser Val Asn Leu Met Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr Ser Val Asn Leu Pro
1 5 10 15

Gly Ala

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr Ser Val Asn Leu Met
1 5 10 15

Ala

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr Ser Val Asn Val Pro
1 5 10 15

Thr

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr Leu Val Asn Val Pro
1 5 10 15

Thr

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr Ser Val Asn Leu Pro
1 5 10 15

Thr

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr Leu Val Asn Val Pro
1 5 10 15

Gly Ala

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr Ser Val Asn Leu Met
1 5 10 15

Ala Pro Ala Val Ala
20

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr Ser Val Asn Leu Met
1 5 10 15

Asp Leu Ala Val Gly Leu Pro Gly Ala
20 25

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr Ser Val Asn Leu Met
1 5 10 15

Ala Asp Asp Thr Glu Ser Ile Asn Thr Thr Leu Val Asn Leu Pro Gly
20 25 30

Ala

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Gln Pro Ile Asp Asp Thr Glu Ser Ile Asn Thr Thr Leu Val Asn Leu
1 5 10 15

Pro Gly Ala

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr Leu Val Asn Leu Pro
1 5 10 15

Gly Ala

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr Ser Val Asn Leu Met
1 5 10 15

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

Leu Pro Leu
35

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr Ser Val Asn Leu Met
1 5 10 15

Ala Asp Asp Thr Glu Ser Ile Asn Thr Thr Leu Val Asn Leu Ala Asn
20 25 30

Val Ala Met Ala
35

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Gln Pro Ile Asp Asp Thr Glu Ser Ala Ile Asn Thr Thr Leu Val Asn
1 5 10 15

Leu Pro Gly Ala
20

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Gln Pro Ile Asp Asp Thr Glu Ser Phe Ala Thr Asn Thr Thr Leu Val
1 5 10 15

Asn Leu Pro Gly Ala
20

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Gln Pro Ile Asp Asp Thr Glu Ser Ile Asn Thr Thr Leu Val Asn Leu
1 5 10 15

Met Ala Asp Asp Thr Glu Ser Arg Phe Ala Thr Asn Thr Thr Leu Val
20 25 30

Asn Leu Pro Leu
35

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Gln Pro Ile Asp Asp Thr Glu Ser Ile Asn Thr Thr Leu Val Asn Leu
1 5 10 15

Met Ala Asp Asp Thr Glu Ser Arg Phe Ala Thr Asn Thr Thr Leu Asp
20 25 30

Val Val Asn Leu Pro Gly Ala
35

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Gln Pro Ile Asp Asp Thr Glu Ser Ala Ala Ile Asn Thr Thr Leu Val
1 5 10 15

Asn Leu Pro Gly Ala
20

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr Ser Val Asn Leu Met
1 5 10 15

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

Leu Ala Asn Val Ala Met Ala
35

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr Ser Val Asn Leu Met
1 5 10 15

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

Val Asn Leu Ile Ser Met Ala
35

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr Ser Val Asn Leu Met
1 5 10 15

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

Val Asn Leu Ile Ser Met Ala
35

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

TAAATCTATA ACTACAAAAA ACACATA 27

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GACTCTCTTA ACTGGCAAGT TGACA 25

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 56 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

AAGTACAAAG CTTCAACCAA GTGAGAACCA CACAAGTGTT GGTTAACGAA TCTCTT 56

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CATACACAAT ATAAACGACG G 21

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 55 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GAATCTCTTA GCTGGCAAGT TGACAGAAGT AGTGTTAGTT TCAGAGTCGT CAATT 55

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

AACGAATCTC TTAGCACCTG GCAAGTTGAC AGAAGT 36

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

AACGAATCTC TTAGCACCTG GCAAGTTGAC CAAAGTAGTG TTGATAGATT CAGTGTCGTC 60

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 75 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

AACGAATCTC TTAGCACCTG GCAAGTTAAC CAAAGTAGTG TTGATAGATT CAGTGTCGTC 60

AGCCATCAAG TTGAC 75

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

AACGAATCTC TTCAATGGCA AGTTAACCAA AGTAGTGTTA GTAGCGAATC TAGATTCAGT 60

GTCGTCAGCC AT 72

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

AACGAATCTC TTAGCCATGG CAACGTTAGC CAAGTTAACC AAAGT 45

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 61 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

```
AACGAATCTC TTAGCACCTG GCAAGTTGAC CAAAGTAGTG TTGATAGCAG ATTCAGTGTC      60

G                                                                      61
```

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 372 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 82..351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

```
GAATTCATTC AAGAATAGTT CAAACAAGAA GATTACAAAC TATCAATTTC ATACACAATA      60

TAAACGACGG GTACCAAAAT A ATG AAA CTG AAA ACT GTA AGA TCT GCG GTC        111
                        Met Lys Leu Lys Thr Val Arg Ser Ala Val
                          1               5                  10

CTT TCG TCA CTC TTT GCA TCT CAG GTC CTT GGC CAA CCA ATA GAC GAA        159
Leu Ser Ser Leu Phe Ala Ser Gln Val Leu Gly Gln Pro Ile Asp Glu
          15                  20                  25

GAC AAC GAC ACT TCT TCC ATG GCT AAG AGA TTC GTT AAC CAA CAC TTG        207
Asp Asn Asp Thr Ser Ser Met Ala Lys Arg Phe Val Asn Gln His Leu
             30                  35                  40

TGC GGT TCC CAC TTG GTT GAA GCT TTG TAC TTG GTT TGC GGT GAA AGA        255
Cys Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg
         45                  50                  55

GGT TTC TTC TAC ACT CCT AAG GCT GCT AAG GGT ATT GTC GAG CAA TGC        303
Gly Phe Phe Tyr Thr Pro Lys Ala Ala Lys Gly Ile Val Glu Gln Cys
     60                  65                  70

TGT ACC TCC ATC TGC TCC TTG TAC CAA TTG GAA AAC TAC TGC AAC TAGACGCAGC
358
Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
 75                  80                  85                  90

CCGCAGGCTC TAGA                                                        372
```

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 89 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

```
Met Lys Leu Lys Thr Val Arg Ser Ala Val Leu Ser Ser Leu Phe Ala
 1               5                  10                  15

Ser Gln Val Leu Gly Gln Pro Ile Asp Glu Asp Asn Asp Thr Ser Ser
            20                  25                  30

Met Ala Lys Arg Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val
        35                  40                  45

Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro
    50                  55                  60

Lys Ala Ala Lys Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser
65                  70                  75                  80

Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
                85
```

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

```
CAA CCA ATT GAC GAC GAA AAC ACT ACT TCT GTC AAC TTG CCA GTT          45
Gln Pro Ile Asp Asp Glu Asn Thr Thr Ser Val Asn Leu Pro Val
 1               5                  10                  15
```

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

```
Gln Pro Ile Asp Asp Glu Asn Thr Thr Ser Val Asn Leu Pro Val
 1               5                  10                  15
```

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 297 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..276

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

```
ATG AAA CTG AAA ACT GTA AGA TCT GCG GTC CTT TCG TCA CTC TTT GCA        48
Met Lys Leu Lys Thr Val Arg Ser Ala Val Leu Ser Ser Leu Phe Ala
 1               5                  10                  15

TCT CAG GTC CTT GGC CAA CCA ATT GAC GAC GAA AAC ACT ACT TCT GTC        96
Ser Gln Val Leu Gly Gln Pro Ile Asp Asp Glu Asn Thr Thr Ser Val
            20                  25                  30

AAC TTG CCA GTT AAG AGA TTC GTT AAC CAA CAC TTG TGT GGT TCT CAC       144
Asn Leu Pro Val Lys Arg Phe Val Asn Gln His Leu Cys Gly Ser His
        35                  40                  45

TTG GTT GAA GCT TTG TAC TTG GTT TGC GGT GAA AGA GGT TTC TTC TAC       192
Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr
    50                  55                  60

ACT CCT AAG GCT GCT AAG GGT ATT GTC GAA CAA TGC TGT ACC TCC ATC       240
Thr Pro Lys Ala Ala Lys Gly Ile Val Glu Gln Cys Cys Thr Ser Ile
65                  70                  75                  80

TGC TCC TTG TAC CAA TTG GAA AAC TAC TGC AAC TAGACGCAGC CCGCAGGCTC     293
Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
                85                  90

TAGA                                                                 297
```

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 91 amino acids
(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

```
Met Lys Leu Lys Thr Val Arg Ser Ala Val Leu Ser Ser Leu Phe Ala
 1               5                  10                  15

Ser Gln Val Leu Gly Gln Pro Ile Asp Asp Glu Asn Thr Thr Ser Val
            20                  25                  30

Asn Leu Pro Val Lys Arg Phe Val Asn Gln His Leu Cys Gly Ser His
        35                  40                  45

Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr
    50                  55                  60

Thr Pro Lys Ala Ala Lys Gly Ile Val Glu Gln Cys Cys Thr Ser Ile
65                  70                  75                  80

Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
                85                  90
```

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..51

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

```
CAA CCA ATT GAC GAC ACT GAA TCT AAC ACT ACT TCT GTC AAC TTG CCA      48
Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr Ser Val Asn Leu Pro
 1               5                  10                  15

GCT                                                                 51
Ala
```

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

```
Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr Ser Val Asn Leu Pro
 1               5                  10                  15

Ala
```

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..54

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

```
CAA CCA ATT GAC GAC ACT GAA TCT AAC ACT ACT TCT GTC AAC TTG CCA      48
Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr Ser Val Asn Leu Pro
 1               5                  10                  15

GGT GCT                                                            54
Gly Ala
```

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..57

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

```
CAA CCA ATT GAC GAC ACT GAA TCT ATC AAC ACT ACT TTG GTC AAC TTG      48
Gln Pro Ile Asp Asp Thr Glu Ser Ile Asn Thr Thr Leu Val Asn Leu
 1               5                  10                  15
```

```
CCA GGT GCT                                                              57
Pro Gly Ala
```

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 99 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..99

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

```
CAA CCA ATT GAC GAC ACT GAA TCT AAC ACT ACT TCT GTC AAC TTG ATG          48
Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr Ser Val Asn Leu Met
  1               5                  10                  15

GCT GAC GAC ACT GAA TCT ATC AAC ACT ACT TTG GTT AAC TTG CCA GGT          96
Ala Asp Asp Thr Glu Ser Ile Asn Thr Thr Leu Val Asn Leu Pro Gly
            20                  25                  30

GCT                                                                      99
Ala
```

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 105 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..105

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

```
CAA CCA ATT GAC GAC ACT GAA TCT AAC ACT ACT TCT GTC AAC TTG ATG          48
Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr Ser Val Asn Leu Met
  1               5                  10                  15
```

```
GCT GAC GAC ACT GAA TCT AGA TTC GCT ACT AAC ACT ACT TTG GTT AAC        96
Ala Asp Asp Thr Glu Ser Arg Phe Ala Thr Asn Thr Thr Leu Val Asn
            20                  25                  30

TTG CCA TTG                                                          105
Leu Pro Leu
        35
```

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 108 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..108

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

```
CAA CCA ATT GAC GAC ACT GAA TCT AAC ACT ACT TCT GTC AAC TTG ATG        48
Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr Ser Val Asn Leu Met
  1               5                  10                  15

GCT GAC GAC ACT GAA TCT ATC AAC ACT ACT TTG GTT AAC TTG GCT AAC        96
Ala Asp Asp Thr Glu Ser Ile Asn Thr Thr Leu Val Asn Leu Ala Asn
            20                  25                  30

GTT GCC ATG GCT                                                      108
Val Ala Met Ala
        35
```

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

```
CAA CCA ATT GAC GAC ACT GAA TCT GCT ATC AAC ACT ACT TTG GTC AAC        48
Gln Pro Ile Asp Asp Thr Glu Ser Ala Ile Asn Thr Thr Leu Val Asn
  1               5                  10                  15

TTG CCA GGT GCT                                                      60
Leu Pro Gly Ala
            20
```

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 276 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 113..274

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

```
TTAAATCTAT AACTACAAAA AACACATACA GGAATTCATT CAAGAATAGT TCAAACAAGA      60

AGATTACAAA CTATCAATTT CATACACAAT ATAAACGACG GGTACCAAAA TA ATG         115
                                                           Met
                                                            1

AAA CTG AAA ACT GTA AGA TCT GCG GTC CTT TCG TCA CTC TTT GCA TCT       163
Lys Leu Lys Thr Val Arg Ser Ala Val Leu Ser Ser Leu Phe Ala Ser
            5                  10                  15

CAG GTC CTT GGC CAA CCA ATT GAC GAC GAA AAC ACT ACT TCT GTT AAC       211
Gln Val Leu Gly Gln Pro Ile Asp Asp Glu Asn Thr Thr Ser Val Asn
        20                  25                  30

TTG CCA GCT AAG AGA TTC GTT AAC CAA CAC TTG TGC GGT TCC CAC TTG       259
Leu Pro Ala Lys Arg Phe Val Asn Gln His Leu Cys Gly Ser His Leu
    35                  40                  45

GTT GAA GCT TTG TAC TT                                               276
Val Glu Ala Leu Tyr
 50
```

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

```
Met Lys Leu Lys Thr Val Arg Ser Ala Val Leu Ser Ser Leu Phe Ala
 1               5                  10                  15

Ser Gln Val Leu Gly Gln Pro Ile Asp Asp Glu Asn Thr Thr Ser Val
            20                  25                  30

Asn Leu Pro Ala Lys Arg Phe Val Asn Gln His Leu Cys Gly Ser His
        35                  40                  45

Leu Val Glu Ala Leu Tyr
    50
```

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 282 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 113..280

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

```
TTAAATCTAT AACTACAAAA AACACATACA GGAATTCATT CAAGAATAGT TCAAACAAGA      60

AGATTACAAA CTATCAATTT CATACACAAT ATAAACGACG GGTACCAAAA TA ATG         115
                                                          Met
                                                           1

AAA CTG AAA ACT GTA AGA TCT GCG GTC CTT TCG TCA CTC TTT GCA TCT       163
Lys Leu Lys Thr Val Arg Ser Ala Val Leu Ser Ser Leu Phe Ala Ser
             5                  10                  15

CAG GTC CTT GGC CAA CCA ATT GAC GAC ACT GAA TCT AAC ACT ACT TCT       211
Gln Val Leu Gly Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr Ser
        20                  25                  30

GTC AAC TTG CCA GCT AAG AGA TTC GTT AAC CAA CAC TTG TGC GGT TCC       259
Val Asn Leu Pro Ala Lys Arg Phe Val Asn Gln His Leu Cys Gly Ser
    35                  40                  45

CAC TTG GTT GAA GCT TTG TAC TT                                       282
His Leu Val Glu Ala Leu Tyr
 50                  55
```

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 56 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

```
Met Lys Leu Lys Thr Val Arg Ser Ala Val Leu Ser Ser Leu Phe Ala
 1               5                  10                  15

Ser Gln Val Leu Gly Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr
            20                  25                  30

Ser Val Asn Leu Pro Ala Lys Arg Phe Val Asn Gln His Leu Cys Gly
        35                  40                  45

Ser His Leu Val Glu Ala Leu Tyr
    50                  55
```

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 282 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 113..280

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

```
TTAAATCTAT AACTACAAAA AACACATACA GGAATTCATT CAAGAATAGT TCAAACAAGA      60

AGATTACAAA CTATCAATTT CATACACAAT ATAAACGACG GGTACCAAAA TA ATG          115
                                                          Met
                                                           1

AAA CTG AAA ACT GTA AGA TCT GCG GTC CTT TCG TCA CTC TTT GCA TCT       163
Lys Leu Lys Thr Val Arg Ser Ala Val Leu Ser Ser Leu Phe Ala Ser
             5                  10                  15

CAG GTC CTT GGC CAA CCA ATT GAC GAC ACT GAA TCT AAC ACT ACT TCT       211
Gln Val Leu Gly Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr Ser
        20                  25                  30

GTC AAC TTG ATG GCT AAG AGA TTC GTT AAC CAA CAC TTG TGC GGT TCC       259
Val Asn Leu Met Ala Lys Arg Phe Val Asn Gln His Leu Cys Gly Ser
    35                  40                  45
```

```
CAC TTG GTT GAA GCT TTG TAC TT                                          282
His Leu Val Glu Ala Leu Tyr
 50                  55
```

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 56 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

```
Met Lys Leu Lys Thr Val Arg Ser Ala Val Leu Ser Ser Leu Phe Ala
  1               5                  10                  15

Ser Gln Val Leu Gly Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr
            20                  25                  30

Ser Val Asn Leu Met Ala Lys Arg Phe Val Asn Gln His Leu Cys Gly
         35                  40                  45

Ser His Leu Val Glu Ala Leu Tyr
     50                  55
```

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 330 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 113..328

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

```
TTAAATCTAT AACTACAAAA AACACATACA GGAATTCATT CAAGAATAGT TCAAACAAGA       60

AGATTACAAA CTATCAATTT CATACACAAT ATAAACGACG GGTACCAAAA TA ATG           115
                                                          Met
                                                           1

AAA CTG AAA ACT GTA AGA TCT GCG GTC CTT TCG TCA CTC TTT GCA TCT         163
Lys Leu Lys Thr Val Arg Ser Ala Val Leu Ser Ser Leu Phe Ala Ser
             5                  10                  15
```

```
CAG GTC CTT GGC CAA CCA ATT GAC GAC ACT GAA TCT AAC ACT ACT TCT      211
Gln Val Leu Gly Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr Ser
        20                  25                  30

GTC AAC TTG ATG GCT GAC GAC ACT GAA TCT ATC AAC ACT ACT TTG GTT      259
Val Asn Leu Met Ala Asp Asp Thr Glu Ser Ile Asn Thr Thr Leu Val
    35                  40                  45

AAC TTG CCA GGT GCT AAG AGA TTC GTT AAC CAA CAC TTG TGC GGT TCC      307
Asn Leu Pro Gly Ala Lys Arg Phe Val Asn Gln His Leu Cys Gly Ser
50                  55                  60                  65

CAC TTG GTT GAA GCT TTG TAC TT                                      330
His Leu Val Glu Ala Leu Tyr
                70
```

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

```
Met Lys Leu Lys Thr Val Arg Ser Ala Val Leu Ser Ser Leu Phe Ala
 1               5                  10                  15

Ser Gln Val Leu Gly Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr
            20                  25                  30

Ser Val Asn Leu Met Ala Asp Asp Thr Glu Ser Ile Asn Thr Thr Leu
        35                  40                  45

Val Asn Leu Pro Gly Ala Lys Arg Phe Val Asn Gln His Leu Cys Gly
    50                  55                  60

Ser His Leu Val Glu Ala Leu Tyr
65                  70
```

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 288 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS

(B) LOCATION: 113..286

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

```
TTAAATCTAT AACTACAAAA AACACATACA GGAATTCATT CAAGAATAGT TCAAACAAGA      60

AGATTACAAA CTATCAATTT CATACACAAT ATAAACGACG GGTACCAAAA TA ATG          115
                                                            Met
                                                             1

AAA CTG AAA ACT GTA AGA TCT GCG GTC CTT TCG TCA CTC TTT GCA TCT        163
Lys Leu Lys Thr Val Arg Ser Ala Val Leu Ser Ser Leu Phe Ala Ser
             5                  10                  15


CAG GTC CTT GGC CAA CCA ATT GAC GAC ACT GAA TCT ATC AAC ACT ACT        211
Gln Val Leu Gly Gln Pro Ile Asp Asp Thr Glu Ser Ile Asn Thr Thr
        20                  25                  30

TTG GTC AAC TTG CCA GGT GCT AAG AGA TTC GTT AAC CAA CAC TTG TGC        259
Leu Val Asn Leu Pro Gly Ala Lys Arg Phe Val Asn Gln His Leu Cys
    35                  40                  45

GGT TCC CAC TTG GTT GAA GCT TTG TAC TT                                 288
Gly Ser His Leu Val Glu Ala Leu Tyr
 50                  55
```

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 58 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

```
Met Lys Leu Lys Thr Val Arg Ser Ala Val Leu Ser Ser Leu Phe Ala
 1               5                  10                  15

Ser Gln Val Leu Gly Gln Pro Ile Asp Asp Thr Glu Ser Ile Asn Thr
            20                  25                  30

Thr Leu Val Asn Leu Pro Gly Ala Lys Arg Phe Val Asn Gln His Leu
        35                  40                  45

Cys Gly Ser His Leu Val Glu Ala Leu Tyr
    50                  55
```

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 82 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

GGTTAACGAA CTTTGGAGCT TCAGCTTCAG CTTCTTCTCT CTTAGCCATG GAGATCAAGT 60

TAACAACATC CAAAGTAGTG TT 82

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

CAAGTACAAA GCTTCAACCA AGTGGGAACC GCACAAGTGT TGGTTAACGA ACTT 54

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

CAACCAATTG ACGACACTGA ATCTAACACT ACTTCTGTCA ACTTGATGGC TGACGACACT 60

GAATCTAGAT TCGCTACTAA CACTACTTTG GATGTTGTTA ACTTGATCTC CATGGCT 117

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

Gln Pro Ile Asp Asp Thr Glu Ser Asn Thr Thr Ser Val Asn Leu Met
1 5 10 15

Ala Asp Asp Thr Glu Ser Arg Phe Ala Thr Asn Thr Thr Leu Ala Leu
20 25 30

Asp Val Val Asn Leu Ile Ser Met Ala
35 40

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

TCTCTTAGCC ATGGAGATCA AGTTAACAAC ATCCAAAGCC AAAGTAGTGT T 51

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 123 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

CAACCAATTG ACGACACTGA ATCTAACACT ACTTCTGTCA ACTTGATGGC TGACGACACT 60

GAATCTAGAT TCGCTACTAA CACTACTTTG GCTTTGGATG TTGTTAACTT GATCTCCATG 120

GCT 123

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 65 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

Lys Arg Glu Glu Ala Glu Ala Glu Ala Glu Pro Lys Phe Val Asn Gln
1 5 10 15

His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly
20 25 30

Glu Arg Gly Phe Phe Tyr Thr Pro Lys Ala Ala Lys Gly Ile Val Glu
35 40 45

Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys
50 55 60

Asn
65

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 219 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

AAGAGAGAAG AAGCTGAAGC TGAAGCTGAA CCAAAGTTCG TTAACCAACA CTTGTGTGGT 60

TCTCACTTGG TTGAAGCTTT GTACTTGGTT TGCGGTGAAA GAGGTTTCTT CTACACTCCT 120

AAGGCTGCTA AGGGTATTGT CGAACAATGC TGTACCTCCA TCTGCTCCTT GTACCAATTG 180

GAAAACTACT GCAACTAGAC GCAGCCCGCA GGCTCTAGA 219

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 348 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

TTAAATCTAT AACTACAAAA AACACATACA GGAATTCCAT TCAAGAATAG TTCAAACAAG 60

AAGATTACAA ACTATCAATT TCATACACAA TATAAACGAC GGTACCAAAA TAATGAAACT 120

GAAAACTGTA AGATCTGCGG TCCTTTCGTC ACTCTTTGCA TCTCAGGTCC TTGGCCAACC 180

AATTGACGAC ACTGAATCTA ACACTACTTC TGTCAACTTG ATGGCTGACG ACACTGAATC 240

TAGATTCGCT ACTAACACTA CTTTGGTTAA CTTGGCTAAC GTTGCCAACC AACACTTGTG 300

TGGTTCTCAC TTGGTTGAAG CTTTGTACTT ATGGCTAAGA GATTCGTT 348

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 379 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

TTAAATCTAT AACTACAAAA AACACATACA GGAATTCCAT TCAAGAATAG TTCAAACAAG 60

AAGATTACAA ACTATCAATT TCATACACAA TATAAACGAC GGTACCAAAA TAATGAAACT 120

GAAAACTGTA AGATCTGCGG TCCTTTCGTC ACTCTTTGCA TCTCAGGTCC TTGGCCAACC 180

AATTGACGAC ACTGAATCTA ACACTACTTC TGTCAACTTG ATGGCTGACG ACACTGAATC 240

TAGATTCGCT ACTAACACTA CTTTGGATGT TGTTAACTTG ATCTCCATGG CTAAGAGAGA 300

AGAAGCTGAA GCTGAAGCTG AACCAAAGTT CGTTAACCAA CACTTGTGTG GTTCTCACTT 360

GGTTGAAGCT TTGTACTTG 379

CLAIMS

1. A DNA expression cassette comprising the following sequence:

5'-P-SP-LS-PS-*gene*-$(T)_i$-3'

wherein
P is a promoter sequence,
SP is a DNA sequence encoding a signal peptide,
LS is a DNA sequence encoding a leader peptide with the general formula I:

GlnProIle(Asp/Glu)(Asp/Glu)X^1(Glu/Asp)X^2AsnZ(Thr/Ser)X^3 (I)

wherein
X^1 is a peptide bond or a codable amino acid;
X^2 is a peptide bond, a codable amino acid or a sequence of up to 4 codable amino acids which may be the same or different;
Z is a codable amino acid except Pro; and
X^3 is a sequence of from 4 to 30 codable amino acids which may be the same or different;

PS is a DNA sequence encoding a processing site;
gene is a DNA sequence encoding a polypeptide;
T is a terminator sequence; and
i is 0 or 1.

2. An expression cassette according to claim 1, wherein X^1, in general formula I, is Ser, Thr or Ala.

3. An expression cassette according to claim 1, wherein X^2, in general formula I, is Ser, Thr or Ala.

4. An expression cassette according to claim 1, wherein X^2, in general formula I, is SerIle.

5. An expression cassette according to claim 1, wherein X^2, in general formula I, is SerAlaIle.

6. An expression cassette according to claim 1, wherein X^2, in general formula I, is SerPheAlaThr.

7. An expression cassette according to claim 1, wherein X^3, in general formula I, is an amino acid sequence of the general formula II

$$X^4\text{-}X^5\text{-}X^6 \qquad \text{(II)}$$

wherein
X^4 is a sequence of from 1 to 21 codable amino acids;
X^5 is Pro or an amino acid sequence including the amino acid sequence ValAsnLeu, LeuAlaAsnValAlaMetAla, LeuAspValValAsnLeuProGly, or LeuAspValValAsnLeuIleSerMet;
and X^6 is a sequence of from 1 to 8 codable amino acids.

8. An expression cassette according to claim 7, wherein X^4, in general formula II, is an amino acid sequence including one or more of the motifs LeuValAsnLeu, SerValAsnLeu, MetAlaAsp, ThrGluSer, ArgPheAlaThr and ValAlaMetAla.

9. An expression cassette according to claim 7, wherein X^4, in general formula II, is an amino acid sequence including the sequence AsnSerThr or AsnThrThr.

10. An expression cassette according to claim 7, wherein X^4, in general formula II, is an amino acid sequence including the sequence
(Ser/Leu)ValAsnLeu,
(Ser/Leu)ValAsnLeuMetAlaAsp,
(Ser/Leu)ValAsnLeuMetAlaAspAsp,
(Ser/Leu)ValAsnLeuMetAlaAspAspThrGluSer,
(Ser/Leu)ValAsnLeuMetAlaAspAspThrGluSerIle, or
(Ser/Leu)ValAsnLeuMetAlaAspAspThrGluSerArgPheAlaThr.

11. An expression cassette according to claim 7, wherein X4, in general formula II, is an amino acid sequence including the sequence
Asn(Thr/Ser)ThrLeu,
Asn(Thr/Ser)ThrLeuAsnLeu, or
Asn(Thr/Ser)ThrLeuValAsnLeu.

12. An expression cassette according to claim 7, wherein X^5, in general formula II, is Pro.

13. An expression cassette according to claim 7, wherein X^5, in general formula II, is the amino acid sequence ValAsnLeu.

14. An expression cassette according to claim 7, wherein X^5, in general formula II, is the amino acid sequence LeuAlaAsnValAlaMetAla.

15. An expression cassette according to claim 7, wherein X^5, in general formula II, is the amino acid sequence LeuAspValValAsnLeuProGly.

16. An expression cassette according to claim 7, wherein X^5, in general formula II, is the amino acid sequence LeuAspValValAsnLeuIleSerMet.

17. An expression cassette according to claim 7, wherein X^6, in general formula II, is Ala, Gly, Leu, Thr, Val or Ser.

18. An expression cassette according to claim 7, wherein X^6, in general formula II, is GlyAla or SerAla.

19. An expression cassette according to claim 7, wherein X^6, in general formula II, is AlaValAla.

20. An expression cassette according to claim 7, wherein X^6, in general formula II, is GlyAlaAspSerLysThrValGlu.

21. An expression cassette according to claim 1, wherein the leader peptide coded for by the DNA sequence LS is selected from the group comprising:

SEQ ID No. 1 GlnProIleAspGluAspAsnAspThrSerValAsnLeuProAla;

SEQ ID No. 2 GlnProIleAspAspGluAsnThrThrSerValAsnLeuProAla;

SEQ ID No. 3 GlnProIleAspAspGluSerAsnThrThrSerValAsnLeuPro-Ala;

SEQ ID No. 4 GlnProIleAspAspGluAsnThrThrSerValAsnLeuProVal;

SEQ ID No. 5 GlnProIleAspAspThrGluAsnThrThrSerValAsnLeuPro-Ala;

SEQ ID No. 6 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeu-ProAla;

SEQ ID No. 7 GlnProIleAspAspGluAsnThrThrSerValAsnLeuMetAla;

SEQ ID No. 8 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeu ProGlyAla;

SEQ ID No. 9 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeu-MetAla;

SEQ ID No. 10 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnVal-ProThr;

SEQ ID No. 11 GlnProIleAspAspThrGluSerAsnThrThrLeuValAsnVal-ProThr;

SEQ ID No. 12 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeu-ProThr;

SEQ ID No. 13 GlnProIleAspAspThrGluSerAsnThrThrLeuValAsnVal-ProGlyAla;

SEQ ID No. 14 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeu-MetAlaProAlaValAla;

SEQ ID No. 15 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeu-MetAspLeuAlaValGlyLeuProGlyAla;

SEQ ID No. 16 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeu-MetAlaAspAspThrGluSerIleAsnThrThrLeuValAsnLeu-ProGlyAla;

SEQ ID No. 17 GlnProIleAspAspThrGluSerIleAsnThrThrLeuValAsn-LeuProGlyAla;

SEQ ID No. 18 GlnProIleAspAspThrGluSerAsnThrThrLeuValAsnLeu-ProGlyAla;

SEQ ID No. 19 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeu-MetAlaAspAspThrGluSerArgPheAlaThrAsnThrThrLeu ValAsnLeuProLeu;

SEQ ID No. 20 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeu-MetAlaAspAspThrGluSerIleAsnThrThrLeuValAsnLeu-AlaAsnValAlaMetAla;

SEQ ID No. 21 GlnProIleAspAspThrGluSerAlaIleAsnThrThrLeuVal-AsnLeuProGlyAla;

SEQ ID No. 22 GlnProIleAspAspThrGluSerPheAlaThrAsnThrThr-LeuValAsnLeuProGlyAla;

SEQ ID No. 23 GlnProIleAspAspThrGluSerIleAsnThrThrLeuValAsn-LeuMetAlaAspAspThrGluSerArgPheAlaThrAsnThrThr-LeuValAsnLeuProLeu;

SEQ ID No. 24 GlnProIleAspAspThrGluSerIleAsnThrThrLeuVal-AsnLeuMetAlaAspAspThrGluSerArgPheAlaThrAsnThr-ThrLeuAspValValAsnLeuProGlyAla;

SEQ ID No. 25 GlnProIleAspAspThrGluSerAlaAlaIleAsnThrThrLeu-ValAsnLeuProGlyAla;

SEQ ID No. 26 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeu-MetAlaAspAspThrGluSerArgPheAlaThrAsnThrThrLeu-ValAsnLeuAlaAsnValAlaMetAla;

SEQ ID No. 27 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeu-MetAlaAspAspThrGluSerArgPheAlaThrAsnThrThrLeu-AspVal-ValAsnLeuIleSerMetAla;

SEQ ID No. 28 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeu-MetAlaAsnThrThrGluSerArgPheAlaThrAsnThrThrLeu-AspValValAsnLeuIleSerMetAla; and

SEQ ID No. 67 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeu-MetAlaAspAspThrGluSerArgPheAlaThrAsnThrThrLeu-AlaLeuAspValValAsnLeuIleSerMetAlaLysArg.

Particularly preferred leader peptides coded for by the DNA sequence LS are:

SEQ ID No. 15 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeu MetAspLeuAlaValGlyLeuProGlyAla;

SEQ ID No. 16 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeu-MetAlaAspAspThrGluSerIleAsnThrThrLeuValAsnLeu-ProGlyAla;

SEQ ID No. 17 GlnProIleAspAspThrGluSerIleAsnThrThrLeuValAsn-LeuProGlyAla;

SEQ ID No. 18 GlnProIleAspAspThrGluSerAsnThrThrLeuValAsnLeu

ProGlyAla;

SEQ ID No. 19 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeu-MetAlaAspAspThrGluSerArgPheAlaThrAsnThrThrLeu-ValAsnLeuProLeu;

SEQ ID No. 20 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeu-MetAlaAspAspThrGluSerIleAsnThrThrLeuValAsnLeu-AlaAsnValAlaMetAla;

SEQ ID No. 21 GlnProIleAspAspThrGluSerAlaIleAsnThrThrLeuVal-AsnLeuProGlyAla;

SEQ ID No. 22 GlnProIleAspAspThrGluSerPheAlaThrAsnThrThrLeu-ValAsnLeuProGlyAla;

SEQ ID No. 23 GlnProIleAspAspThrGluSerIleAsnThrThrLeuValAsn-LeuMetAlaAspAspThrGluSerArgPheAlaThrAsnThrThr-LeuValAsnLeuProLeu;

SEQ ID No. 24 GlnProIleAspAspThrGluSerIleAsnThrThrLeuValAsn-LeuMetAlaAspAspThrGluSerArgPheAlaThrAsnThrThr-LeuAspValValAsnLeuProGlyAla;

SEQ ID No. 25 GlnProIleAspAspThrGluSerAlaAlaIleAsnThrThrLeu-ValAsnLeuProGlyAla;

SEQ ID No. 26 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeu-MetAlaAspAspThrGluSerArgPheAlaThrAsnThrThrLeu-ValAsnLeuAlaAsnValAlaMetAla; and

SEQ ID No. 28 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeu-MetAlaAsnThrThrGluSerArgPheAlaThrAsnThrThrLeu-AspValValAsnLeuIleSerMetAla.

SEQ ID No. 67 GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeu-MetAlaAspAspThrGluSerArgPheAlaThrAsnThrThrLeu-

AlaLeuAspValValAsnLeuIleSerMetAlaLysArg.

22. An expression cassette according to claim 1, wherein SP is a DNA sequence encoding the α-factor signal peptide, the signal peptide of mouse salivary amylase, the carboxypeptidase signal peptide, the yeast aspartic protease 3 signal peptide or the yeast BAR1 signal peptide.

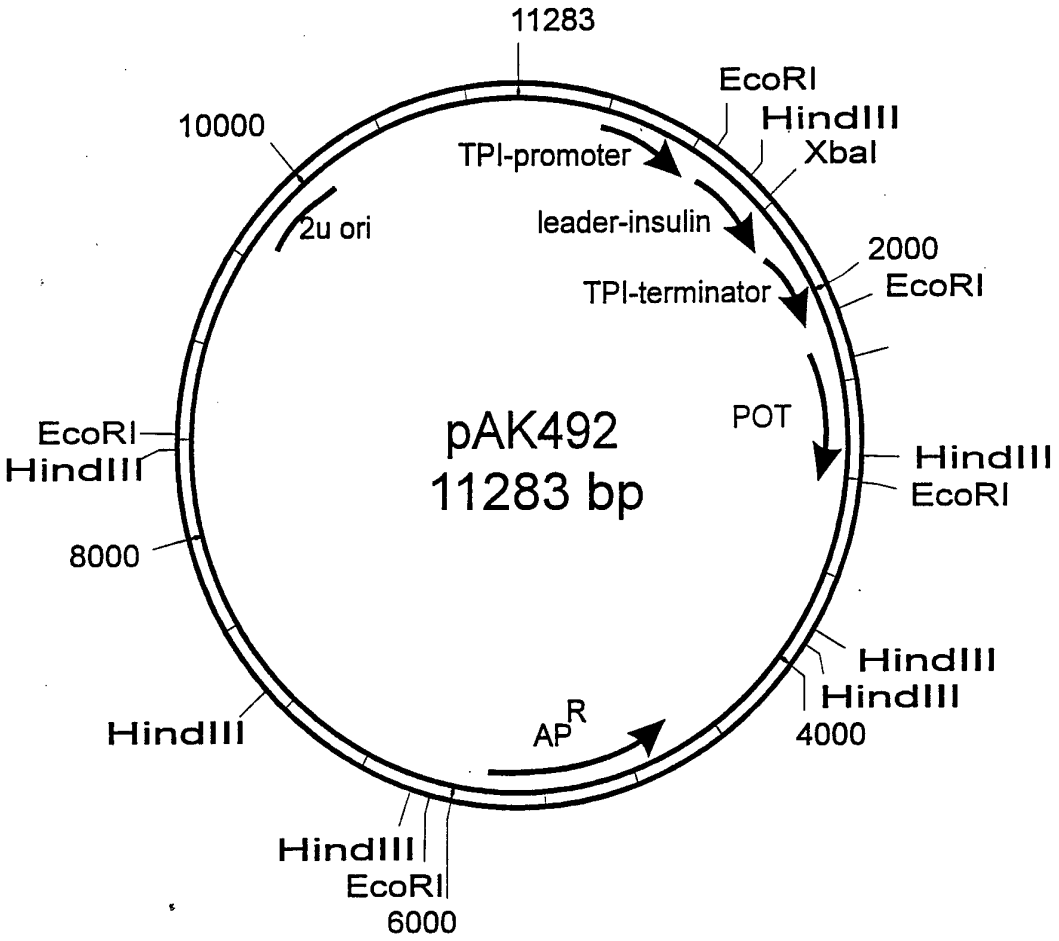
23. An expression cassette according to claim 1, wherein PS is a DNA sequence encoding LysArg, ArgLys, ArgArg, LysLys or Ile-GluGlyArg.

24. An expression cassette according to claim 1, wherein the polypeptide is selected from the group consisting of aprotinin, tissue factor pathway inhibitor, or other protease inhibitors, insulin or insulin precursors, insulin-like growth factor I, insulin-like growth factor II, human or bovine growth hormone, interleukin, glucagon, glucagon-like peptide 1, tissue plasminogen activator, transforming growth factor α or β, platelet-derived growth factor, enzymes, or a functional analogue thereof.

25. A yeast expression vector comprising an expression cassette according to any of the preceding claims.

26. A yeast cell which is capable of expressing a polypeptide and which is transformed with a yeast expression vector according to claim 25.

27. A process for producing a polypeptide in yeast, the process comprising culturing a yeast cell, which is capable of expressing the desired polypeptide and which is transformed with a yeast expression vector according to claim 25, in a suitable medium to obtain expression and secretion of the polypeptide, after which the polypeptide is recovered from the medium.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XbaI
10000
TPI-promoter
2u ori
leader-insulin
2000
EcoRI
TPI-terminator
POT
pAK492
11283 bp
EcoRI
HindIII
HindIII
EcoRI
8000
HindIII
HindIII
4000
HindIII
AP R
HindIII
EcoRI
6000

Fig. 1

Fig. 2

EcoR I

GAATTCATTCAAGAATAGT
CTTAAGTAAGTTCTTATCA

TCAAACAAGAAGATTACAAACTATCAATTTCATACACAATATAAACGACGGGTACCAAAA
AGTTTGTTCTTCTAATGTTTGATAGTTAAAGTATGTGTTATATTTGCTGCCCATGGTTTT

TAATGAAACTGAAAACTGTAAGATCTGCGGTCCTTTCGTCACTCTTTGCATCTCAGGTCCTT
ATTACTTTGACTTTTGACATTCTAGACGCCAGGAAAGCAGTGAGAAACGTAGAGTCCAGGAA
MetLysLeuLysThrValArgSerAlaValLeuSerSerLeuPheAlaSerGlnValLeu

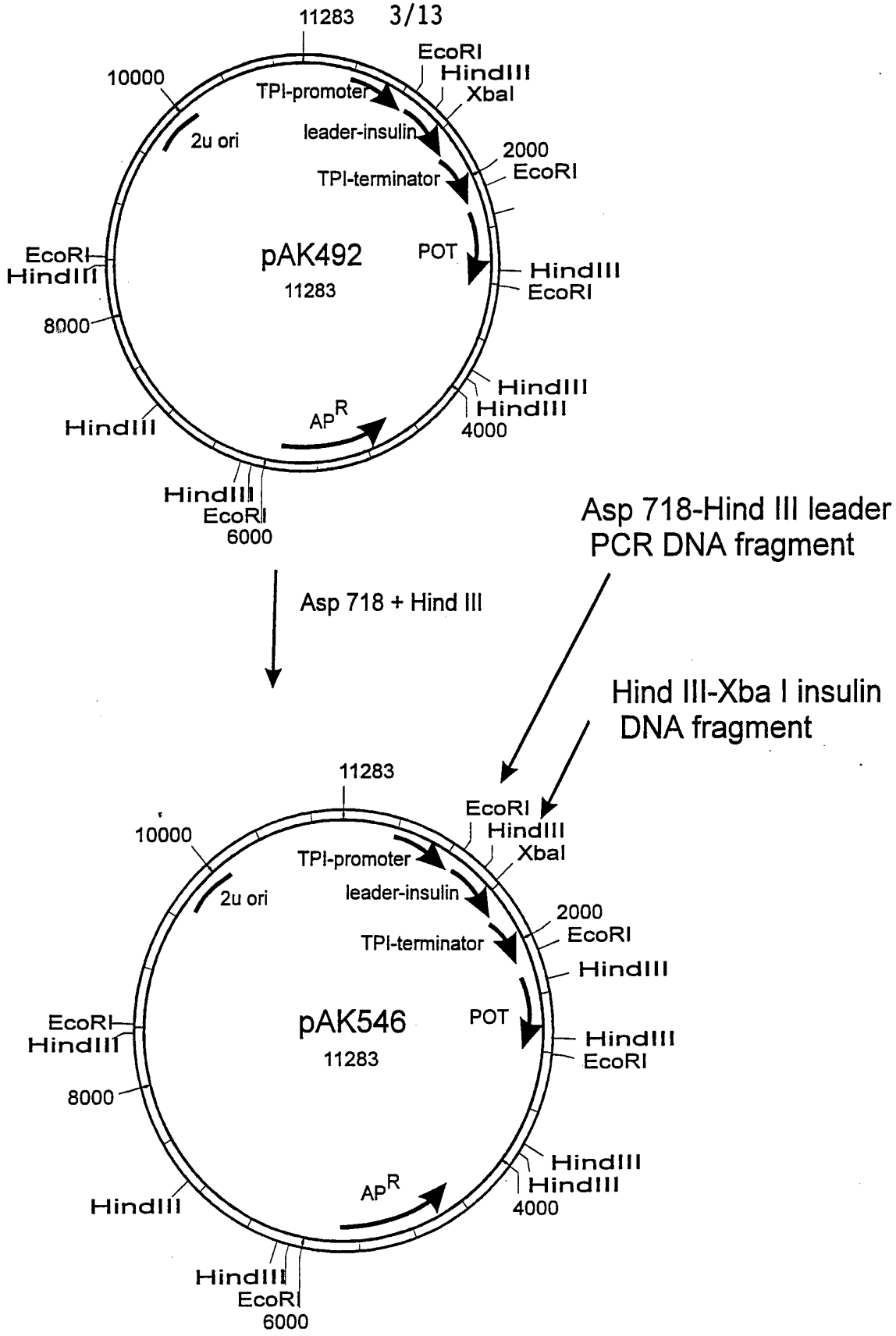
GGCCAACCAATAGACGAAGACAACGACACTTCTTCCATGGCTAAGAGATTCGTTAACCAA
CCGGTTGGTTATCTGCTTCTGTTGCTGTGAAGAAGGTACCGATTCTCTAAGCAATTGGTT
GlyGlnProIleAspGluAspAsnAspThrSerSerMetAlaLysArgPheValAsnGln

CACTTGTGCGGTTCCCACTTGGTTGAAGCTTTGTACTTGGTTTGCGGTGAAAGAGGTTTC
GTGAACACGCCAAGGGTGAACCAACTTCGAAACATGAACCAAACGCCACTTTCTCCAAAG
HisLeuCysGlySerHisLeuValGluAlaLeuTyrLeuValCysGlyGluArgGlyPhe

TTCTACACTCCTAAGGCTGCTAAGGGTATTGTCGAGCAATGCTGTACCTCCATCTGCTCC
AAGATGTGAGGATTCCGACGATTCCCATAACAGCTCGTTACGACATGGAGGTAGACGAGG
PheTyrThrProLysAlaAlaLysGlyIleValGluGlnCysCysThrSerIleCysSer

Xba I

TTGTACCAATTGGAAAACTACTGCAACTAGACGCAGCCCGCAGGCTCTAGA **SEQ ID No.40**
AACATGGTTAACCTTTTGATGACGTTGATCTGCGTCGGGCGTCCGAGATCT
LeuTyrGlnLeuGluAsnTyrCysAsn* **SEQ ID No.41**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EcoRI
HindIII
XbaI
10000
TPI-promoter
leader-insulin
2u ori
2000
EcoRI
TPI-terminator
pAK492
11283
POT
HindIII
EcoRI
EcoRI
HindIII
8000
HindIII
HindIII
HindIII
AP^R
4000
HindIII
EcoRI
6000
Asp 718 + Hind III
Asp 718-Hind III leader
PCR DNA fragment
Hind III-Xba I insulin
DNA fragment
11283
EcoRI
HindIII
XbaI
10000
TPI-promoter
leader-insulin
2u ori
2000
EcoRI
TPI-terminator
HindIII
pAK546
11283
POT
HindIII
EcoRI
EcoRI
HindIII
8000
HindIII
HindIII
HindIII
AP^R
4000
HindIII
EcoRI
6000

Fig. 3

Fig. 4.

CAACCAATTGACGACGAAAACACTACTTCTGTCAACTTGCCAGTT **SEQ ID No. 42**

GTTGGTTAACTGCTGCTTTTGTGATGAAGACAGTTGAACGGTCAA

GlnProIleAspAspGluAsnThrThrSerValAsnLeuProVal **SEQ ID No. 43**

Fig. 5.

ATGAAACTGAAAACTGTAAGATCTGCGGTCCTTTCGTCACTCTTTGCA

TACTTTGACTTTTGACATTCTAGACGCCAGGAAAGCAGTGAGAAACGT

MetLysLeuLysThrValArgSerAlaValLeuSerSerLeuPheAla

TCTCAGGTCCTTGGCCAACCAATTGACGACGAAAACACTACTTCTGTC

AGAGTCCAGGAACCGGTTGGTTAACTGCTGCTTTTGTGATGAAGACAG

SerGlnValLeuGlyGlnProIleAspAspGluAsnThrThrSerVal

AACTTGCCAGTTAAGAGATTCGTTAACCAACACTTGTGTGGTTCTCAC

TTGAACGGTCAATTCTCTAAGCAATTGGTTGTGAACACACCAAGAGTG

AsnLeuProValLysArgPheValAsnGlnHisLeuCysGlySerHis

TTGGTTGAAGCTTTGTACTTGGTTTGCGGTGAAAGAGGTTTCTTCTAC

AACCAACTTCGAAACATGAACCAAACGCCACTTTCTCCAAAGAAGATG

LeuValGluAlaLeuTyrLeuValCysGlyGluArgGlyPhePheTyr

ACTCCTAAGGCTGCTAAGGGTATTGTCGAACAATGCTGTACCTCCATC

TGAGGATTCCGACGATTCCCATAACAGCTTGTTACGACATGGAGGTAG

ThrProLysAlaAlaLysGlyIleValGluGlnCysCysThrSerIle

TGCTCCTTGTACCAATTGGAAAACTACTGCAACTAGACGCAGCCCGCA

ACGAGGAACATGGTTAACCTTTTGATGACGTTGATCTGCGTCGGGCGT

CysSerLeuTyrGlnLeuGluAsnTyrCysAsn* **SEQ ID No. 45**

GGCTCTAGA **SEQ ID No. 44**

CCGAGATCT

Fig. 6.

CAACCAATTGACGACACTGAATCTAACACTACTTCTGTCAACTTGCCAGCT **SEQ ID No. 46**
GTTGGTTAACTGCTGTGACTTAGATTGTGATGAAGACAGTTGAACGGTCGA
GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuProAla **SEQ ID No. 47**

Fig. 7.

CAACCAATTGACGACACTGAATCTAACACTACTTCTGTCAACTTGCCA
GTTGGTTAACTGCTGTGACTTAGATTGTGATGAAGACAGTTGAACGGT
GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuPro

GGTGCT **SEQ ID No. 48**
CCACGA
GlyAla **SEQ ID No. 8**

Fig. 8.

CAACCAATTGACGACACTGAATCTATCAACACTACTTTGGTCAACTTG
GTTGGTTAACTGCTGTGACTTAGATAGTTGTGATGAAACCAGTTGAAC
GlnProIleAspAspThrGluSerIleAsnThrThrLeuValAsnLeu

CCAGGTGCT **SEQ ID No. 49**
GGTCCACGA
ProGlyAla **SEQ ID No. 17**

Fig. 9.

CAACCAATTGACGACACTGAATCTAACACTACTTCTGTCAACTTGATG
GTTGGTTAACTGCTGTGACTTAGATTGTGATGAAGACAGTTGAACTAC
GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMet

GCTGACGACACTGAATCTATCAACACTACTTTGGTTAACTTGCCAGGTGCT **SEQ ID No. 50**
CGACTGCTGTGACTTAGATAGTTGTGATGAAACCAATTGAACGGTCCACGA
AlaAspAspThrGluSerIleAsnThrThrLeuValAsnLeuProGlyAla **SEQ ID No. 16**

Fig. 10.

CAACCAATTGACGACACTGAATCTAACACTACTTCTGTCAACTTGATGGCTGACGACACT
GTTGGTTAACTGCTGTGACTTAGATTGTGATGAAGACAGTTGAACTACCGACTGCTGTGA
GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMetAlaAspAspThr

GAATCTAGATTCGCTACTAACACTACTTTGGTTAACTTGCCATTG **SEQ ID No. 51**
CTTAGATCTAAGCGATGATTGTGATGAAACCAATTGAACGGTAAC
GluSerArgPheAlaThrAsnThrThrLeuValAsnLeuProLeu **SEQ ID No. 19**

Fig. 11

CAACCAATTGACGACACTGAATCTAACACTACTTCTGTCAACTTGATGGCTGACGACACT
GTTGGTTAACTGCTGTGACTTAGATTGTGATGAAGACAGTTGAACTACCGACTGCTGTGA
GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMetAlaAspAspThr

GAATCTATCAACACTACTTTGGTTAACTTGGCTAACGTTGCCATGGCT **SEQ ID No. 52**
CTTAGATAGTTGTGATGAAACCAATTGAACCGATTGCAACGGTACCGA
GluSerIleAsnThrThrLeuValAsnLeuAlaAsnValAlaMetAla **SEQ ID No. 20**

Fig. 12.

CAACCAATTGACGACACTGAATCTGCTATCAACACTACTTTGGTCAAC
GTTGGTTAACTGCTGTGACTTAGACGATAGTTGTGATGAAACCAGTTG
GlnProIleAspAspThrGluSerAlaIleAsnThrThrLeuValAsn

TTGCCAGGTGCT **SEQ ID No. 53**
AACGGTCCACGA
LeuProGlyAla **SEQ ID No. 21**

Fig. 13

pAK527:

TTAAATCTATAACTACAAAAAACACATACAGGAATTCATTCAAGAATAGTTCAAACAA
AATTTAGATATTGATGTTTTTTGTGTATGTCCTTAAGTAAGTTCTTATCAAGTTTGTT

GAAGATTACAAACTATCAATTTCATACACAATATAAACGACGGGTACCAAAATAATGAAA
CTTCTAATGTTTGATAGTTAAAGTATGTGTTATATTTGCTGCCCATGGTTTTATTACTTT
MetLys

CTGAAAACTGTAAGATCTGCGGTCCTTTCGTCACTCTTTGCATCTCAGGTCCTTGGCCAA
GACTTTTGACATTCTAGACGCCAGGAAAGCAGTGAGAAACGTAGAGTCCAGGAACCGGTT
LeuLysThrValArgSerAlaValLeuSerSerLeuPheAlaSerGlnValLeuGlyGln

CCAATTGACGACGAAAACACTACTTCTGTTAACTTGCCAGCTAAGAGATTCGTTAACCAA
GGTTAACTGCTGCTTTTGTGATGAAGACAATTGAACGGTCGATTCTCTAAGCAATTGGTT
ProIleAspAspGluAsnThrThrSerValAsnLeuProAlaLysArgPheValAsnGln

CACTTGTGCGGTTCCCACTTGGTTGAAGCTTTGTACTT **SEQ ID No. 54**
GTGAACACGCCAAGGGTGAACCAACTTCGAAACATGAA
HisLeuCysGlySerHisLeuValGluAlaLeuTyr **SEQ ID No. 55**

Fig. 14

pAK531:

TTAAATCTATAACTACAAAAAACACATACAGGAATTCATTCAAGAATAGTTCAAACAA
AATTTAGATATTGATGTTTTTTGTGTATGTCCTTAAGTAAGTTCTTATCAAGTTTGTT

GAAGATTACAAACTATCAATTTCATACACAATATAAACGACGGGTACCAAAATAATGAAA
CTTCTAATGTTTGATAGTTAAAGTATGTGTTATATTTGCTGCCCATGGTTTTATTACTTT
MetLys

CTGAAAACTGTAAGATCTGCGGTCCTTTCGTCACTCTTTGCATCTCAGGTCCTTGGCCAA
GACTTTTGACATTCTAGACGCCAGGAAAGCAGTGAGAAACGTAGAGTCCAGGAACCGGTT
LeuLysThrValArgSerAlaValLeuSerSerLeuPheAlaSerGlnValLeuGlyGln

CCAATTGACGACACTGAATCTAACACTACTTCTGTCAACTTGCCAGCTAAGAGATTCGTT
GGTTAACTGCTGTGACTTAGATTGTGATGAAGACAGTTGAACGGTCGATTCTCTAAGCAA
ProIleAspAspThrGluSerAsnThrThrSerValAsnLeuProAlaLysArgPheVal

AACCAACACTTGTGCGGTTCCCACTTGGTTGAAGCTTTGTACTT SEQ ID No. 56
TTGGTTGTGAACACGCCAAGGGTGAACCAACTTCGAAACATGAA
AsnGlnHisLeuCysGlySerHisLeuValGluAlaLeuTyr SEQ ID No. 57

Fig. 15

pAK555

TTAAATCTATAACTACAAAAAACACATACAGGAATTCATTCAAGAATAGTTCAAACAA
AATTTAGATATTGATGTTTTTTGTGTATGTCCTTAAGTAAGTTCTTATCAAGTTTGTT

GAAGATTACAAACTATCAATTTCATACACAATATAAACGACGGGTACCAAAATAATGAAA
CTTCTAATGTTTGATAGTTAAAGTATGTGTTATATTTGCTGCCCATGGTTTTATTACTTT
MetLys

CTGAAAACTGTAAGATCTGCGGTCCTTTCGTCACTCTTTGCATCTCAGGTCCTTGGCCAA
GACTTTTGACATTCTAGACGCCAGGAAAGCAGTGAGAAACGTAGAGTCCAGGAACCGGTT
LeuLysThrValArgSerAlaValLeuSerSerLeuPheAlaSerGlnValLeuGlyGln

CCAATTGACGACACTGAATCTAACACTACTTCTGTCAACTTGATGGCTAAGAGATTCGTT
GGTTAACTGCTGTGACTTAGATTGTGATGAAGACAGTTGAACTACCGATTCTCTAAGCAA
ProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMetAlaLysArgPheVal

AACCAACACTTGTGCGGTTCCCACTTGGTTGAAGCTTTGTACTT SEQ ID No. 58
TTGGTTGTGAACACGCCAAGGGTGAACCAACTTCGAAACATGAA
AsnGlnHisLeuCysGlySerHisLeuValGluAlaLeuTyr SEQ ID No. 59

Fig. 16

pAK559:

TTAAATCTATAACTACAAAAAACACATACAGGAATTCATTCAAGAATAGTTCAAACAA
AATTTAGATATTGATGTTTTTTGTGTATGTCCTTAAGTAAGTTCTTATCAAGTTTGTT

GAAGATTACAAACTATCAATTTCATACACAATATAAACGACGGGTACCAAAATAATGAAA
CTTCTAATGTTTGATAGTTAAAGTATGTGTTATATTTGCTGCCCATGGTTTTATTACTTT
MetLys

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CTGAAAACTGTAAGATCTGCGGTCCTTTCGTCACTCTTTGCATCTCAGGTCCTTGGCCAA
GACTTTTGACATTCTAGACGCCAGGAAAGCAGTGAGAAACGTAGAGTCCAGGAACCGGTT
LeuLysThrValArgSerAlaValLeuSerSerLeuPheAlaSerGlnValLeuGlyGln

CCAATTGACGACACTGAATCTAACACTACTTCTGTCAACTTGATGGCTGACGACACTGAA
GGTTAACTGCTGTGACTTAGATTGTGATGAAGACAGTTGAACTACCGACTGCTGTGACTT
ProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMetAlaAspAspThrGlu

TCTATCAACACTACTTTGGTTAACTTGCCAGGTGCTAAGAGATTCGTTAACCAACACTTG
AGATAGTTGTGATGAAACCAATTGAACGGTCCACGATTCTCTAAGCAATTGGTTGTGAAC
SerIleAsnThrThrLeuValAsnLeuProGlyAlaLysArgPheValAsnGlnHisLeu

TGCGGTTCCCACTTGGTTGAAGCTTTGTACTT                    SEQ ID No. 60
ACGCCAAGGGTGAACCAACTTCGAAACATGAA
CysGlySerHisLeuValGluAlaLeuTyr                      SEQ ID No. 61
```

Fig. 17

pAK562:

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  TTAAATCTATAACTACAAAAAACACATACAGGAATTCATTCAAGAATAGTTCAAACAA
  AATTTAGATATTGATGTTTTTTGTGTATGTCCTTAAGTAAGTTCTTATCAAGTTTGTT

GAAGATTACAAACTATCAATTTCATACACAATATAAACGACGGGTACCAAAATAATGAAA
CTTCTAATGTTTGATAGTTAAAGTATGTGTTATATTTGCTGCCCATGGTTTTATTACTTT
                                                        MetLys

CTGAAAACTGTAAGATCTGCGGTCCTTTCGTCACTCTTTGCATCTCAGGTCCTTGGCCAA
GACTTTTGACATTCTAGACGCCAGGAAAGCAGTGAGAAACGTAGAGTCCAGGAACCGGTT
LeuLysThrValArgSerAlaValLeuSerSerLeuPheAlaSerGlnValLeuGlyGln

CCAATTGACGACACTGAATCTATCAACACTACTTTGGTCAACTTGCCAGGTGCTAAGAGA
GGTTAACTGCTGTGACTTAGATAGTTGTGATGAAACCAGTTGAACGGTCCACGATTCTCT
ProIleAspAspThrGluSerIleAsnThrThrLeuValAsnLeuProGlyAlaLysArg
```

TTCGTTAACCAACACTTGTGCGGTTCCCACTTGGTTGAAGCTTTGTACTT **SEQ ID No. 62**
AAGCAATTGGTTGTGAACACGCCAAGGGTGAACCAACTTCGAAACATGAA
PheValAsnGlnHisLeuCysGlySerHisLeuValGluAlaLeuTyr **SEQ ID No. 63**

Fig. 18

CAACCAATTGACGACACTGAATCTAACACTACTTCTGTCAACTTGATG
GTTGGTTAACTGCTGTGACTTAGATTGTGATGAAGACAGTTGAACTAC
GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMet

GCTGACGACACTGAATCTAGATTCGCTACTAACACTACTTTGGATGTT
CGACTGCTGTGACTTAGATCTAAGCGATGATTGTGATGAAACCTACAA
AlaAspAspThrGluSerArgPheAlaThrAsnThrThrLeuAspVal

GTTAACTTGATCTCCATGGCT **SEQ ID No. 66**
CAATTGAACTAGAGGTACCGA
ValAsnLeuIleSerMetAla **SEQ ID No. 27**

Fig. 19

AAGAGAGAAGAAGCTGAAGCTGAAGCTGAACCAAAGTTCGTTAACCAA
TTCTCTCTTCTTCGACTTCGACTTCGACTTGGTTTCAAGCAATTGGTT
LysArgGluGluAlaGluAlaGluAlaGluProLysPheValAsnGln

CACTTGTGTGGTTCTCACTTGGTTGAAGCTTTGTACTTGGTTTGCGGT
GTGAACACACCAAGAGTGAACCAACTTCGAAACATGAACCAAACGCCA
HisLeuCysGlySerHisLeuValGluAlaLeuTyrLeuValCysGly

GAAAGAGGTTTCTTCTACACTCCTAAGGCTGCTAAGGGTATTGTCGAA
CTTTCTCCAAAGAAGATGTGAGGATTCCGACGATTCCCATAACAGCTT
GluArgGlyPhePheTyrThrProLysAlaAlaLysGlyIleValGlu

CAATGCTGTACCTCCATCTGCTCCTTGTACCAATTGGAAAACTACTGC
GTTACGACATGGAGGTAGACGAGGAACATGGTTAACCTTTTGATGACG
GlnCysCysThrSerIleCysSerLeuTyrGlnLeuGluAsnTyrCys

Xba I

AACTAGACGCAGCCCGCAGGCTCTAGA SEQ ID No. 71

TTGATCTGCGTCGGGCGTCCGAGATCT

Asn*** SEQ ID No. 70

Fig. 20

CAACCAATTGACGACACTGAATCTAACACTACTTCTGTCAACTTGATG

GTTGGTTAACTGCTGTGACTTAGATTGTGATGAAGACAGTTGAACTAC

GlnProIleAspAspThrGluSerAsnThrThrSerValAsnLeuMet

GCTGACGACACTGAATCTAGATTCGCTACTAACACTACTTTGGCTTTG

CGACTGCTGTGACTTAGATCTAAGCGATGATTGTGATGAAACCGAAAC

AlaAspAspThrGluSerArgPheAlaThrAsnThrThrLeuAlaLeu

GATGTTGTTAACTTGATCTCCATGGCT SEQ ID No. 69

CTACAACAATTGAACTAGAGGTACCGA

AspValValAsnLeuIleSerMetAla SEQ ID No. 67

Fig. 21

pAK614

TTAAATCTATAACTACAAAAAACACATACAGGAATTCCATTCAAGA

AATTTAGATATTGATGTTTTTTGTGTATGTCCTTAAGGTAAGTTCT

ATAGTTCAAACAAGAAGATTACAAACTATCAATTTCATACACAATATA

TATCAAGTTTGTTCTTCTAATGTTTGATAGTTAAAGTATGTGTTATAT

AACGACGGTACCAAAATAATGAAACTGAAAACTGTAAGATCTGCGGTC

TTGCTGCCATGGTTTTATTACTTTGACTTTTGACATTCTAGACGCCAG

MetLysLeuLysThrValArgSerAlaVal

CTTTCGTCACTCTTTGCATCTCAGGTCCTTGGCCAACCAATTGACGAC

GAAAGCAGTGAGAAACGTAGAGTCCAGGAACCGGTTGGTTAACTGCTG

LeuSerSerLeuPheAlaSerGlnValLeuGlyGlnProIleAspAsp

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ACTGAATCTAACACTACTTCTGTCAACTTGATGGCTGACGACACTGAA
TGACTTAGATTGTGATGAAGACAGTTGAACTACCGACTGCTGTGACTT
ThrGluSerAsnThrThrSerValAsnLeuMetAlaAspAspThrGlu

TCTAGATTCGCTACTAACACTACTTTGGTTAACTTGGCTAACGTTGCC
AGATCTAAGCGATGATTGTGATGAAACCAATTGAACCGATTGCAACGG
SerArgPheAlaThrAsnThrThrLeuValAsnLeuAlaAsnValAla

AACCAACACTTGTGTGGTTCTCACTTGGTTGAAGCTTTGTACTTATGG
TACCGATTCTCTAAGCAATTGGTTGTGAACACACCAAGAGTGAACCAA
MetAlaLysArgPheValAsnGlnHisLeuCysGlySerHisLeuVal

CTAAGAGATTCGTT                                    SEQ ID No. 72
CTTCGAAACATGAA
GluAlaLeuTyr
```

Fig. 22

pAK625

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  TTAAATCTATAACTACAAAAAACACATACAGGAATTCCATTCAAGA
  AATTTAGATATTGATGTTTTTTGTGTATGTCCTTAAGGTAAGTTCT

ATAGTTCAAACAAGAAGATTACAAACTATCAATTTCATACACAATATA
TATCAAGTTTGTTCTTCTAATGTTTGATAGTTAAAGTATGTGTTATAT

AACGACGGTACCAAAATAATGAAACTGAAAACTGTAAGATCTGCGGTC
TTGCTGCCATGGTTTTATTACTTTGACTTTTGACATTCTAGACGCCAG
                  MetLysLeuLysThrValArgSerAlaVal

CTTTCGTCACTCTTTGCATCTCAGGTCCTTGGCCAACCAATTGACGAC
GAAAGCAGTGAGAAACGTAGAGTCCAGGAACCGGTTGGTTAACTGCTG
LeuSerSerLeuPheAlaSerGlnValLeuGlyGlnProIleAspAsp
```

ACTGAATCTAACACTACTTCTGTCAACTTGATGGCTGACGACACTGAA
TGACTTAGATTGTGATGAAGACAGTTGAACTACCGACTGCTGTGACTT
ThrGluSerAsnThrThrSerValAsnLeuMetAlaAspAspThrGlu

TCTAGATTCGCTACTAACACTACTTTGGATGTTGTTAACTTGATCTCC
AGATCTAAGCGATGATTGTGATGAAACCTACAACAATTGAACTAGAGG
SerArgPheAlaThrAsnThrThrLeuAspValValAsnLeuIleSer

ATGGCTAAGAGAGAAGAAGCTGAAGCTGAAGCTGAACCAAAGTTCGTT
TACCGATTCTCTCTTCTTCGACTTCGACTTCGACTTGGTTTCAAGCAA
MetAlaLysArgGluGluAlaGluAlaGluAlaGluProLysPheVal

AACCAACACTTGTGTGGTTCTCACTTGGTTGAAGCTTTGTACTTG **SEQ ID No. 73**
TTGGTTGTGAACACACCAAGAGTGAACCAACTTCGAAACATGAAC
AsnGlnHisLeuCysGlySerHisLeuValGluAlaLeuTyr

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 95/00249

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12N 15/81, C12N 15/79 // C12N 15/17
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN, EDOC, WPI, CLAIMS, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5037743 A (SUSAN K. WELCH ET AL), 6 August 1991 (06.08.91), the whole document, especially column 7, line 13-25	1-27
A	WO 9211378 A1 (NOVO NORDISK A/S), 9 July 1992 (09.07.92)	1-27
A	EP 0324274 A1 (CHIRON CORPORATION), 19 July 1989 (19.07.89)	1-27

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 Sept 1995	02 -10- 1995
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Patrick Andersson Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

28/08/95

International application No.
PCT/DK 95/00249

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A- 5037743	06/08/91	AU-B- 660172	15/06/95
		AU-A- 1498792	21/01/93
		AU-A- 2334088	18/05/89
		CA-A- 1314830	23/03/93
		DE-D,T- 3887425	23/06/94
		EP-A,A,A 0310137	05/04/89
		SE-T3- 0310137	
		ES-T- 2061585	16/12/94
		JP-A- 2016984	19/01/90
WO-A1- 9211378	09/07/92	AU-B- 660161	15/06/95
		AU-A- 9134891	22/07/92
		CZ-A- 9301192	16/02/94
		EP-A- 0563175	06/10/93
		FI-D- 932831	00/00/00
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