



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C12N 9/00	A2	(11) International Publication Number: WO 00/22100 (43) International Publication Date: 20 April 2000 (20.04.00)
(21) International Application Number: PCT/US99/23813 (22) International Filing Date: 14 October 1999 (14.10.99) (30) Priority Data: 60/104,376 15 October 1998 (15.10.98) US (71) Applicant (for all designated States except US): E.I. DU PONT DE NEMOURS AND COMPANY [US/US]; 1007 Market Street, Wilmington, DE 19898 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): CAHOON, Rebecca, E. [US/US]; 2331 West 18th Street, Wilmington, DE 19806 (US). HERRMANN, Rafael [IL/US]; 3120 Naamanns Road, Apt. 405, Wilmington, DE 19810 (US). LU, Albert, L. [CA/US]; 14 Charles Pointe, Newark, DE 19702 (US). (74) Agent: FEULNER, Gregory, J.; E. I. du Pont de Nemours and Company, Legal Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US).		(81) Designated States: AE, AL, AU, BA, BB, BG, BR, CA, CN, CR, CU, CZ, EE, GD, GE, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, US, UZ, VN, YU, ZA, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: ARTHROPOD PROTEIN DISULFIDE ISOMERASES <pre> * * * *+* *+* *+* *+* *+* SEQ ID NO:27 MKFLIC-ALFLAASYVAA-SAAEAEVKKVEEGVLVATVDNFKQLIADNEFVLVEFYAPWCGH SEQ ID NO:28 MAVVRVRAIVALLCLVAALGLAEPLLEEDGVLVLRAANFEQALAAHRHLLVEFYAPWCGH SEQ ID NO:02 M-FIKL--LITTL- IAS-TIADIEIKKENHVLVLTNDNFEGAIKD-KNVLVEFYAPWCGH SEQ ID NO:04 M-YIKL--LILSLC-IYL-CIADIEIKKESVVLVLTNDNFEGAIKD-KSVLVEFYAPWCGH SEQ ID NO:06 MRVI----LFTALALLGT-ALADEVPTEENVLVLSKSNFEGVISANNFILVEFYAPWCGH SEQ ID NO:08 M-LGAV-TLSTILL-VVI-AAADEIKKDGEVLVLNKDNFQKAIQENKHILVEFYAPWCGH 1 60 *+* *+* *+* *+* *+* *+* *+* *+* SEQ ID NO:27 CKALAPEYAKAAQQLAEKESPIKLAKVDATVEGELAEQYAVRGYPTLKFFRSGS---PVE SEQ ID NO:28 CKALAPEYAKAAQQLKAESEIRLAKVDATVEEELAQQFGVGYPTIKFFRNGDKAAPRE SEQ ID NO:02 CKALEPQYAKAAESLAEKSELLAKVDATVETDLAERYGVRGYPTIKFFREGK---IFE SEQ ID NO:04 CKALEPEYAKAAQILEEEKSDLLAKVDATAETDLAEQHGVRGYPTIKFFREGK---VIE SEQ ID NO:06 CKSLAPEYAKAATKLAEESPIKLAKVDATQEQLAEQYGVGYPTLKFFRNGS---PID SEQ ID NO:08 CKALEPEYAKAAQQLKEEQSDIALGKIDATAESELAEYDVRGYPTIKFIRDGK---PSE 61 120 *+* *+* *+* *+* *+* *+* *+* *+* SEQ ID NO:27 YSGGRQAADIIAWVTKKTGPPAKDLTSVADAEQFLKDNEIAIGFFKDLESEEAKTFTKV SEQ ID NO:28 YTAGREADDIVSWLKKRTGPAATTLTDAAAAETLVDSSEVVVIGFFKDVTSDAAKEFLLA SEQ ID NO:02 YNGGRTSDEIIRWLKKKTGPPAVDLSSVEDAKKFVDSNEVAVVGFFKDLESADAKIFKSV SEQ ID NO:04 YSGGRTADDIIRWLKKKTGPPATDLTTVEATKSFIDGGEVVVVGFFKDQNSDQAKIFKNV SEQ ID NO:06 YTGGRTADDIVSWLKKKTGPP----- SEQ ID NO:08 YKGGRTAEDIVRWLKKKVGPPAENLDTVDSVKTFQSSAEVVLVGFFKDQSSDKAKVFLEV 121 180 </pre>		
(57) Abstract This invention relates to an isolated nucleic acid fragment encoding protein disulfide isomerase. The invention also relates to the construction of a chimeric gene encoding all or a portion of the protein disulfide isomerase, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the protein disulfide isomerase in a transformed host cell.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon	KR	Republic of Korea	PL	Poland		
CN	China	KZ	Kazakhstan	PT	Portugal		
CU	Cuba	LC	Saint Lucia	RO	Romania		
CZ	Czech Republic	LI	Liechtenstein	RU	Russian Federation		
DE	Germany	LK	Sri Lanka	SD	Sudan		
DK	Denmark	LR	Liberia	SE	Sweden		
EE	Estonia			SG	Singapore		

TITLE

ARTHROPOD PROTEIN DISULFIDE ISOMERASES

This application claims the benefit of U.S. Provisional Application No. 60/104376, filed October 15, 1998.

5

FIELD OF THE INVENTION

This invention is in the field of molecular biology. More specifically, this invention pertains to nucleic acid fragments encoding arthropod protein disulfide isomerases.

BACKGROUND OF THE INVENTION

Protein folding requires the assistance of folding helpers *in vivo*. The formation or
10 isomerization of disulfide bonds in proteins is a slow process requiring catalysis. In nascent polypeptide chains the cysteine residues are in the thiol form. The formation of the disulfide bonds usually occurs simultaneously with the folding of the polypeptide, in the endoplasmic reticulum of eukaryotes or in the periplasm of Gram-negative bacteria. Cells contain three types of accessory proteins that function to assist polypeptides in
15 folding to their native conformations: protein disulfide isomerases, propyl cis-trans isomerases, and molecular chaperones.

Protein disulfide isomerase (PDI) is a homodimeric eukaryotic enzyme which catalyzes disulfide interchange reactions. PDI is also thought to be the beta subunit of the heterotetramer prolyl hydrolase, the enzyme that hydroxylates the proline residues in
20 Collagen. PDI appears to belong to a family of closely related proteins which have specific functions. PDI (EC 5.3.4.1), also called S-S rearrangase, catalyzes the rearrangement of both intrachain and interchain disulfide bonds in proteins to form native structures. The reaction depends on sulfhydryl-disulfide interchange, and PDI needs reducing agents or partly-reduced enzyme. A family of PDI-like proteins have been
25 identified in mammals, yeasts, fungi, plants, and *Drosophila*.

In *Drosophila*, a PDI precursor was identified by screening a genomic DNA library at reduced stringency hybridization conditions using a rat Phospholipase C alpha cDNA probe. Northern analysis showed that this gene encodes a transcript that is present throughout development, in heads and bodies of adults. The encoded protein contains two
30 domains exhibiting high similarity to thioredoxin, two regions that are similar to the hormone binding domain of human estrogen receptor, and a C-terminal ER-retention signal (KDEL). Overall, this *Drosophila* PDI gene contains a higher similarity to rat protein disulfide isomerase (53% identical) than to rat Phospholipase C alpha (30% identical) (McKay et al. (1995) *Insect Biochem Mol Biol* 25:647-654).

35 Another member of the PDI family is ERp-60, a PDI isoform initially misidentified as a phosphatidylinositol-specific phospholipase C. The human and *Drosophila* ERp-60 polypeptides have been cloned and expressed. These two ERp-60 polypeptides are similar to human PDI within almost all their domains, the only exception being the extreme

C-terminal region. Coexpression in insect cells of the human or *Drosophila* ERp-60 with the alpha subunit of human propyl 4-hydrolase does not result in tetramer formation or prolyl 4-hydroxylase activity in the cells. This lack of tetramer formation is not only due to the differences in the C-terminal region since no prolyl 4-hydroxylase tetramer is
5 formed when a human ERp-60 hybrid containing the C-terminal region of the human PDI polypeptide is used (Koivunen et al. (1996) *Biochem J* 316:599-605). The 5' flanking region of the ERp-60 gene has no TATAA box or CCAAT motif but contains several potential binding sites for transcription factors. The highest levels of expression of the human ERp-60 mRNA are found in the liver, placenta, lung, pancreas, and kidney, and
10 the lowest in the heart, skeletal muscle, and brain. The ERp-60 gene has been mapped by fluorescence in situ hybridization to 15q15, a different chromosome than where the human PDI and thioredoxin genes are found (Koivunen, et al. (1997) *Genomics* 42:397-404).

Full-length cDNA clones encoding two members of the mice PDI family have been cloned, sequenced, and expressed (ERp-59/PDI and ERp-72). ERp-59/PDI has been
15 identified as the microsomal PDI. The ERp-72 amino acid sequence shares sequence identity with ERp-59/PDI at three discrete regions, having three copies of the sequences that are thought to be the CGHC-containing active sites of ERp-59/PDI. ERp-59/PDI has the sequence KDEL at its COOH terminus while ERp72 has the related sequence KEEL (Mazzarella et al. (1990) *J Biol Chem* 265:1094-1101). A cDNA clone containing sequence
20 similarity to the mammalian luminal endoplasmic reticulum protein ERp-72 has been isolated from an alfalfa (*Medicago sativa* L.) cDNA library by screening with a cDNA encoding human PDI. The polypeptide encoded by this cDNA possesses a putative N-terminal secretory signal sequence and two regions identical to the active sites of PDI and ERp-72. This protein appears to be encoded by a small gene family in alfalfa, whose
25 transcripts are constitutively expressed in all major organs of the plant. In alfalfa cell suspension cultures, ERp-72 transcripts are induced by treatment with tunicamycin, but not in response to calcium ionophore, heat shock or fungal elicitor (Shorrosh and Dixon (1992) *Plant J* 2:51-58)

Another member of the PDI family is ERp-5. The amino acid sequence deduced from
30 its cDNA insert contains two copies of the 11-amino-acid sequence Val-Glu-Phe-Tyr-Ala-Pro-Trp-Cys-Gly-His-Cys. Duplicate copies of this sequence are found in the active sites of rat and human PDI and in Form I phosphoinositide-specific phospholipase C. Genomic sequences similar to the cDNA clone are amplified 10-20-fold in hamster cells selected for resistance to increasing concentrations of hydroxyurea, a phenomenon observed earlier with
35 cDNA clones for the M2 subunit of ribonucleotide reductase and ornithine decarboxylase. RNA blots probed with ERp-5 cDNA show two poly(A)+ RNA species which are elevated in hydroxyurea-resistant cells (Chaudhuri et al. (1992) *Biochem J* 281:645-650).

A PDI-like protein from *Acanthamoeba castellanii* contains two highly conserved thioredoxin-like domains, each about 100 amino acids. However, the *A. castellanii* PDI-like protein differs from other members in many aspects, including the overall organization and isoelectric point. Southern and Northern analyses demonstrate that the PDI-like protein is encoded by a single-copy gene which is transcribed to generate a 1500-nucleotide mRNA (Wong and Bateman (1994) *Gene* 150:175-179).

Included in this application are scorpion, spider, lepidoptera, and centepede ESTs with similarities to several of these PDIs. Coexpression in plants or insect cells of an arthropod PDI with a secreted arthropod protein should enhance the yield of the foreign protein by increasing the proper folding of the foreign protein.

SUMMARY OF THE INVENTION

The present invention relates to isolated polynucleotides comprising a nucleotide sequence encoding a protein disulfide isomerase precursor polypeptide of at least 50 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of a spider, a centipede, a moth, and a scorpion protein disulfide isomerase of SEQ ID NOs:2, 4, 6, and 8. The present invention also relates to isolated polynucleotides comprising a nucleotide sequence encoding an ERp60 polypeptide of at least 50 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of a spider, a centipede, and a scorpion ERp60 of SEQ ID NOs:10, 12, and 14. The present invention also relates to isolated polynucleotides comprising a nucleotide sequence encoding an ERp72 polypeptide of at least 50 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of a two scorpion ERp72s of SEQ ID NOs:16 and 18. The present invention also relates to isolated polynucleotides comprising a nucleotide sequence encoding an ERp5 polypeptide of at least 50 amino acids amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of a moth, a worm, and two scorpion ERp5s of SEQ ID NOs:20, 22, 24, and 26. The present invention also relates to an isolated polynucleotide comprising the complement of the nucleotide sequences described above.

It is preferred that the isolated polynucleotide of the claimed invention consists of a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, and 25 that codes for the polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, and 26. The present invention also relates to an isolated polynucleotide comprising a nucleotide sequences of at least one of 40 (preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25 and the complement of such nucleotide sequences.

The present invention relates to a chimeric gene comprising an isolated polynucleotide (such as ERp-60, an ERp-72, an ERp-5, or a PDI-like homolog) of the present invention operably linked to suitable regulatory sequences.

5 The present invention relates to an isolated host cell comprising a chimeric gene of the present invention or an isolated polynucleotide of the present invention. The host cell may be of eukaryotic origin, such as an insect, a yeast, or a plant cell; of prokaryotic origin, such as a bacterial cell. The present invention also relates to a virus, preferably a baculovirus, comprising an isolated polynucleotide of the present invention or a chimeric gene of the present invention.

10 The present invention relates to a process for producing an isolated host cell comprising a chimeric gene of the present invention or an isolated polynucleotide of the present invention, the process comprising either transforming or transfecting an isolated compatible host cell with a chimeric gene or isolated polynucleotide of the present invention.

15 The present invention relates to a protein disulfide isomerase precursor polypeptide of at least 30 amino acids comprising at least 80% homology based on the Clustal method of alignment compared to a polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, and 8. The present invention relates to an ERp60 polypeptide of at least 40 amino acids comprising at least 80% homology based on the Clustal method of alignment compared to a polypeptide selected from the group consisting of SEQ ID NOs:10, 12, and 14. The present invention relates to an ERp72 polypeptide of at least 20 amino acids comprising at least 80% homology based on the Clustal method of alignment compared to a polypeptide selected from the group consisting of SEQ ID NOs:16 and 18. The present invention relates to an ERp5 polypeptide of at least 50 amino acids comprising at least 80% homology based on the Clustal method of alignment compared to a polypeptide selected from the group consisting of SEQ ID NOs:20, 22, 24, and 26.

25 The present invention relates to a method of selecting an isolated polynucleotide that affects the level of expression of a protein disulfide isomerase polypeptide in a host cell, the method comprising the steps of:

30 constructing an isolated polynucleotide of the present invention or an isolated chimeric gene of the present invention;

introducing the isolated polynucleotide or the isolated chimeric gene into a plant cell;

measuring the level an enzyme polypeptide in the plant cell containing the isolated polynucleotide; and

35 comparing the level of an enzyme polypeptide in the plant cell containing the isolated polynucleotide or an isolated chimeric gene with the level of an enzyme polypeptide in a plant cell that does not contain the isolated polynucleotide or an isolated chimeric gene.

The present invention relates to a method of obtaining a nucleic acid fragment encoding a substantial portion of a protein disulfide isomerase polypeptide gene, preferably an arthropod polypeptide gene, comprising the steps of: synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of 40 (preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, and the complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a portion of a protein disulfide isomerase amino acid sequence.

The present invention also relates to a method of obtaining a nucleic acid fragment encoding all or a substantial portion of the amino acid sequence encoding a protein disulfide isomerase polypeptide comprising the steps of: probing a cDNA or genomic library with an isolated polynucleotide of the present invention; identifying a DNA clone that hybridizes with an isolated polynucleotide of the present invention; isolating the identified DNA clone; and sequencing the cDNA or genomic fragment that comprises the isolated DNA clone.

The instant invention relates to isolated nucleic acid fragments encoding arthropod protein disulfide isomerases. Specifically, this invention concerns an isolated nucleic acid fragment encoding an ERp-60, an ERp-72, an ERp-5, or a PDI-like homolog. In addition, this invention relates to a nucleic acid fragment that is complementary to the nucleic acid fragment encoding ERp-60, ERp-72, ERp-5, or PDI-like homolog.

Another embodiment of the instant invention pertains to a method for expressing a gene encoding a protein disulfide isomerase in the genome of a recombinant baculovirus in insect cell culture or in viable insects wherein said insect cells or insects have been genetically engineered to express an ERp-60, an ERp-72, an ERp-5, or a PDI-like homolog.

BRIEF DESCRIPTION OF THE DRAWINGS AND SEQUENCE LISTING

The invention can be more fully understood from the following detailed description and the accompanying drawings and Sequence Listing which form a part of this application.

Figure 1 shows a comparison of the amino acid sequences of the protein disulfide isomerase precursors of the instant invention (SEQ ID NOs:2, 4, 6, and 8) with the sequences of protein disulfide isomerase precursors from *Drosophila melanogaster* (NCBI General Identifier No. 1709616; SEQ ID NO:27) and *Gallus gallus* (NCBI General Identifier No. 2144546; SEQ ID NO:28). The top row indicates with asterisks (*) the amino acids which are conserved among all the sequences from the instant invention and one or both of the art sequences. The top row indicates with plus signs (+) the sequences which are conserved only among the sequences from the instant invention.

Figure 2 shows a comparison of the amino acid sequences of the ERp60 of the instant invention (SEQ ID NOs:10, 12, and 14) with the sequence of ERp60 from *Drosophila melanogaster* (NCBI General Identifier No. 1699220; SEQ ID NO:29). The top row indicates with asterisks (*) the amino acids which are conserved among all the sequences.

- 5 The top row indicates with plus signs (+) the sequences which are conserved only among the sequences from the instant invention.

- Figure 3 shows a comparison of the amino acid sequences of the ERp72 of the instant invention (SEQ ID NOs:16 and 18) with the sequence of ERp72 from *Homo sapiens* (NCBI General Identifier No. 2507460; SEQ ID NO:30). The top row indicates with asterisks (*) the amino acids which are conserved among all the sequences and with plus signs (+) the sequences which are conserved only among the arthropod sequences.

- Figure 4 shows a comparison of the amino acid sequences of the ERp5 of the instant invention (SEQ ID NOs:20, 22, 24, and 26) with the sequences of ERp5 from *Medicago sativa* (NCBI General Identifier No. 729442; SEQ ID NO:31) and *Rattus norvegicus* (NCBI General Identifier No. 2501206; SEQ ID NO:32). The top row indicates with asterisks (*) the amino acids which are conserved among all the arthropod sequences and one or both of the art sequences. The top row indicates with plus signs (+) the sequences which are conserved only among the arthropod sequences.

- Table 1 lists the polypeptides that are described herein, the designation of the cDNA clones that comprise the nucleic acid fragments encoding polypeptides representing all or a substantial portion of these polypeptides, and the corresponding identifier (SEQ ID NO:) as used in the attached Sequence Listing. The sequence descriptions and Sequence Listing attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent applications as set forth in 37 C.F.R. §1.821-1.825.

25

TABLE 1
Protein Disulfide Isomerases

Protein	Clone Designation	SEQ ID NO:	
		(Nucleotide)	(Amino Acid)
Spider Protein Disulfide Isomerase Precursor	aot1c.pk016.k11	1	2
Centipede Protein Disulfide Isomerase Precursor	asc1.pk033.i19	3	4
Moth Protein Disulfide Isomerase Precursor	ihv1c.pk001.h12	5	6
Scorpion Protein Disulfide Isomerase Precursor	iks1c.pk0004.c12	7	8
Spider ERp-60	aot1c.pk011.n11	9	10
Scorpion ERp-60	ibj1c.pk015.k8	11	12

Protein	Clone Designation	SEQ ID NO:	
		(Nucleotide)	(Amino Acid)
Centipede ERp-60	isc1c.pk009.m16	13	14
Scorpion ERp-72	ibj1c.pk008.d11	15	16
Scorpion ERp-72	ibj1c.pk014.c1	17	18
Scorpion ERp-5	ibj1c.pk015.o22	19	20
Moth ERp-5	ihv1c.pk001.a7	21	22
Scorpion ERp-5	iks1c.pk010.i14	23	24
Worm ERp-5	iselc.pk002.m4	25	26

The Sequence Listing contains the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IUBMB standards described in *Nucleic Acids Research* 13:3021-3030 (1985) and in the *Biochemical Journal* 219 (No. 2):345-373 (1984) which are herein incorporated by reference. The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822.

DETAILED DESCRIPTION OF THE INVENTION

In the context of this disclosure, a number of terms shall be utilized. As used herein, a "polynucleotide" is a nucleotide sequence such as a nucleic acid fragment. A polynucleotide may be a polymer of RNA or DNA that is single- or double-stranded, that optionally contains synthetic, non-natural or altered nucleotide bases. A polynucleotide in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA, or synthetic DNA. An isolated polynucleotide of the present invention may include at least one of 40 contiguous nucleotides, preferably at least one of 30 contiguous nucleotides, most preferably at least one of 15 contiguous nucleotides, of the nucleic acid sequence of the SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 29, 21, 23 and 25.

"NPV" stands for Nuclear Polyhedrosis Virus, a baculovirus. "Polyhedrosis" refers to any of several virus diseases of insect larvae characterized by dissolution of tissues and accumulation of polyhedral granules in the resultant fluid. "PIBs" are polyhedral inclusion bodies. "AcNPV" stands for the wild-type *Autographa californica* Nuclear Polyhedrosis Virus.

As used herein, "substantially similar" refers to nucleic acid fragments wherein changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the polypeptide encoded by the nucleotide sequence. "Substantially similar" also refers to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotides that do not substantially affect the functional properties of the resulting protein molecule. It is therefore

understood that the invention encompasses more than the specific exemplary nucleotide or amino acid sequences and includes functional equivalents thereof.

Substantially similar nucleic acid fragments may be selected by screening nucleic acid fragments representing subfragments or modifications of the nucleic acid fragments of the instant invention, wherein one or more nucleotides are substituted, deleted and/or inserted, for their ability to affect the level of the polypeptide encoded by the unmodified nucleic acid fragment in a host or host cell. For example, a substantially similar nucleic acid fragment representing at least one of 30 contiguous nucleotides derived from the instant nucleic acid fragment can be constructed and introduced into a host or host cell. The level of the polypeptide encoded by the unmodified nucleic acid fragment present in a host or host cell exposed to the substantially similar nucleic acid fragment can then be compared to the level of the polypeptide in a host or host cell that is not exposed to the substantially similar nucleic acid fragment.

Consequently, an isolated polynucleotide comprising a nucleotide sequence of at least one of 40 (preferably at least one of 30, most preferably at least one of 15) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11,13, 15, 17, 19, 21, 23, 25 and the complement of such nucleotide sequences may be used in methods of selecting an isolated polynucleotide that affects the expression of a polypeptide in a host cell. A method of selecting an isolated polynucleotide that affects the level of expression of a polypeptide (such as a disulfide isomerase, Erp 60, Erp 72, or Erp 5) in a host cell (eukaryotic, such as plant, insect, or yeast; prokaryotic such as bacterial; viral such as baculovirus) may comprise the steps of: constructing an isolated polynucleotide of the present invention or an isolated chimeric gene of the present invention; introducing the isolated polynucleotide or the isolated chimeric gene into a host cell; measuring the level of a polypeptide in the host cell containing the isolated polynucleotide; and comparing the level of the polypeptide in the host cell containing the isolated polynucleotide with the level of the polypeptide in a host cell that does not contain the isolated polynucleotide.

Moreover, substantially similar nucleic acid fragments may also be characterized by their ability to hybridize. Estimates of such homology are provided by either DNA-DNA or DNA-RNA hybridization under conditions of stringency as is well understood by those skilled in the art (Hames and Higgins, Eds. (1985) *Nucleic Acid Hybridisation*, IRL Press, Oxford, U.K.). Stringency conditions can be adjusted to screen for moderately similar fragments, such as homologous sequences from distantly related organisms, to highly similar fragments, such as genes that duplicate functional enzymes from closely related organisms. Post-hybridization washes determine stringency conditions. One set of preferred conditions uses a series of washes starting with 6X SSC, 0.5% SDS at room temperature for 15 min, then repeated with 2X SSC, 0.5% SDS at 45°C for 30 min, and then repeated twice with

0.2X SSC, 0.5% SDS at 50°C for 30 min. A more preferred set of stringent conditions uses higher temperatures in which the washes are identical to those above except for the temperature of the final two 30 min washes in 0.2X SSC, 0.5% SDS was increased to 60°C. Another preferred set of highly stringent conditions uses two final washes in 0.1X SSC,
5 0.1% SDS at 65°C.

Substantially similar nucleic acid fragments of the instant invention may also be characterized by the percent identity of the amino acid sequences that they encode to the amino acid sequences disclosed herein, as determined by algorithms commonly employed by those skilled in this art. Suitable nucleic acid fragments (isolated polynucleotides of the
10 present invention) encode polypeptides that are 80% identical to the amino acid sequences reported herein. Preferred nucleic acid fragments encode amino acid sequences that are 85% identical to the amino acid sequences reported herein. More preferred nucleic acid fragments encode amino acid sequences that are 90% identical to the amino acid sequences reported herein. Most preferred are nucleic acid fragments that encode amino acid sequences that are
15 95% identical to the amino acid sequences reported herein. Suitable nucleic acid fragments not only have the above homologies but typically encode a polypeptide having at least 50 amino acids, preferably 100 amino acids, more preferably 150 amino acids, still more preferably 200 amino acids, and most preferably 250 amino acids. Sequence alignments and percent identity calculations were performed using the Megalign program of the
20 LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS* 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS
25 SAVED=5.

A "substantial portion" of an amino acid or nucleotide sequence comprises an amino acid or a nucleotide sequence that is sufficient to afford putative identification of the protein or gene that the amino acid or nucleotide sequence comprises. Amino acid and nucleotide sequences can be evaluated either manually by one skilled in the art, or by using computer-
30 based sequence comparison and identification tools that employ algorithms such as BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol.* 215:403-410; see also www.ncbi.nlm.nih.gov/BLAST/). In general, a sequence of ten or more contiguous amino acids or thirty or more contiguous nucleotides is necessary in order to putatively identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene.
35 Moreover, with respect to nucleotide sequences, gene-specific oligonucleotide probes comprising 30 or more contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., *in situ* hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12 or

more nucleotides may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises a nucleotide sequence that will afford specific identification and/or isolation of a nucleic acid fragment comprising the sequence. The instant
5 specification teaches amino acid and nucleotide sequences encoding polypeptides that comprise one or more particular plant proteins. The skilled artisan, having the benefit of the sequences as reported herein, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, the instant invention comprises the complete sequences as reported in the accompanying Sequence Listing, as
10 well as substantial portions of those sequences as defined above.

"Codon degeneracy" refers to divergence in the genetic code permitting variation of the nucleotide sequence without effecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment comprising a nucleotide sequence that encodes all or a substantial portion of the amino acid
15 sequences set forth herein. The skilled artisan is well aware of the "codon-bias" exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. Therefore, when synthesizing a nucleic acid fragment for improved expression in a host cell, it is desirable to design the nucleic acid fragment such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

"Synthetic nucleic acid fragments" can be assembled from oligonucleotide building blocks that are chemically synthesized using procedures known to those skilled in the art. These building blocks are ligated and annealed to form larger nucleic acid fragments which may then be enzymatically assembled to construct the entire desired nucleic acid fragment. "Chemically synthesized", as related to nucleic acid fragment, means that the component
25 nucleotides were assembled *in vitro*. Manual chemical synthesis of nucleic acid fragments may be accomplished using well established procedures, or automated chemical synthesis can be performed using one of a number of commercially available machines. Accordingly, the nucleic acid fragments can be tailored for optimal gene expression based on optimization of nucleotide sequence to reflect the codon bias of the host cell. The skilled artisan
30 appreciates the likelihood of successful gene expression if codon usage is biased towards those codons favored by the host. Determination of preferred codons can be based on a survey of genes derived from the host cell where sequence information is available.

"Gene" refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding
35 sequences) the coding sequence. "Native gene" refers to a gene as found in nature with its own regulatory sequences. "Chimeric gene" refers any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that

are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature.

“Endogenous gene” refers to a native gene in its natural location in the genome of an organism. A “foreign” gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A “transgene” is a gene that has been introduced into the genome by a transformation procedure.

“Coding sequence” refers to a nucleotide sequence that codes for a specific amino acid sequence. “Regulatory sequences” refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

“Promoter” refers to a nucleotide sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. The promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an “enhancer” is a nucleotide sequence which can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic nucleotide segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. Promoters which cause a nucleic acid fragment to be expressed in most cell types at most times are commonly referred to as “constitutive promoters”. New promoters of various types useful in a variety of cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro and Goldberg (1989) *Biochemistry of Plants* 15:1-82. It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, DNA fragments of different lengths may have identical promoter activity.

The “translation leader sequence” refers to a nucleotide sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect processing of the primary transcript to mRNA, mRNA stability or translation efficiency. Examples of translation leader sequences have been described (Turner and Foster (1995) *Mol. Biotechnol.* 3:225-236).

The "3' non-coding sequences" refer to nucleotide sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht et al. (1989) *Plant Cell* 1:671-680.

"RNA transcript" refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from posttranscriptional processing of the primary transcript and is referred to as the mature RNA. "Messenger RNA (mRNA)" refers to the RNA that is without introns and that can be translated into protein by the cell. "cDNA" refers to a double-stranded DNA that is complementary to and derived from mRNA. "Sense" RNA refers to RNA transcript that includes the mRNA and so can be translated into protein by the cell. "Functional RNA" refers to sense RNA, ribozyme RNA, or other RNA that may not be translated but yet has an effect on cellular processes.

The term "operably linked" refers to the association of two or more nucleic acid fragments on a single nucleic acid fragment so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense orientation.

The term "expression", as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from the nucleic acid fragment of the invention. Expression may also refer to translation of mRNA into a polypeptide. "Overexpression" refers to the production of a gene product in transgenic organisms that exceeds levels of production in normal or non-transformed organisms.

"Altered levels" refers to the production of gene product(s) in transgenic organisms in amounts or proportions that differ from that of normal or non-transformed organisms.

A "signal sequence" is an amino acid sequence that is covalently linked to an amino acid sequence representing a mature protein. The signal sequence directs the protein to the secretory system (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol.* 42:21-53). "Mature" protein refers to a post-translationally processed polypeptide; i.e., one from which any pre- or propeptides, including signal sequences, present in the primary translation product have been removed. "Precursor" protein refers to the primary product of translation of mRNA; i.e., with pre- and propeptides still present. Pre- and propeptides may be but are not limited to intracellular localization signals.

“Transformation” refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as “transgenic” organisms. Examples of methods of plant transformation include *Agrobacterium*-mediated transformation (De Blaere et al. (1987) *Meth. Enzymol.* 143:277) and particle-accelerated or “gene gun” transformation technology (Klein et al. (1987) *Nature (London)* 327:70-73; U.S. Patent No. 4,945,050, incorporated herein by reference).

It is understood that “an insect cell” refers to one or more insect cells maintained *in vitro* as well as one or more cells found in an intact, living insect.

Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook et al. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989 (hereinafter “Maniatis”).

Nucleic acid fragments encoding at least a portion of several protein disulfide isomerases have been isolated and identified by comparison of cDNA sequences to public databases containing nucleotide and protein sequences using the BLAST algorithms well known to those skilled in the art. The nucleic acid fragments of the instant invention may be used to isolate cDNAs and genes encoding homologous proteins from the same or other arthropod species. Isolation of homologous genes using sequence-dependent protocols is well known in the art. Examples of sequence-dependent protocols include, but are not limited to, methods of nucleic acid hybridization, and methods of DNA and RNA amplification as exemplified by various uses of nucleic acid amplification technologies (e.g., polymerase chain reaction, ligase chain reaction).

For example, genes encoding other ERp-60s, ERp-72s, ERp-5s, or PDI-like homologs, either as cDNAs or genomic DNAs, could be isolated directly by using all or a portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired arthropod employing methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis). Moreover, the entire sequences can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primer DNA labeling, nick translation, or end-labeling techniques, or RNA probes using available *in vitro* transcription systems. In addition, specific primers can be designed and used to amplify a part or all of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding

homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the instant nucleic acid fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA

5 precursor encoding arthropod genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:8998-9002) to generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be
10 designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems (BRL), specific 3' or 5' cDNA fragments can be isolated (Ohara et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:5673-5677; Loh et al. (1989) *Science* 243:217-220). Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman and Martin (1989) *Techniques* 1:165). Consequently, a polynucleotide
15 comprising a nucleotide sequence of at least one of 40 (preferably one of at least 30, most preferably one of at least 15) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25 and the complement of such nucleotide sequences may be used in such methods to obtain a nucleic acid fragment encoding a substantial portion of an amino acid sequence of a
20 polypeptide such as a protein disulfide isomerase. The present invention relates to a method of obtaining a nucleic acid fragment encoding a substantial portion of a polypeptide of a gene (such as ERp-60, an ERp-72, an ERp-5, or a PDI-like homolog) preferably a substantial portion of an arthropod polypeptide of a gene, comprising the steps of : synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of
25 40 (preferably at least one of 30, most preferably at least one of 15) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25 and the complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a
30 portion of a polypeptide such as a protein disulfide isomerase.

Availability of the instant nucleotide and deduced amino acid sequences facilitates immunological screening of cDNA expression libraries. Synthetic peptides representing portions of the instant amino acid sequences may be synthesized. These peptides can be used to immunize animals to produce polyclonal or monoclonal antibodies with specificity
35 for peptides or proteins comprising the amino acid sequences. These antibodies can be then be used to screen cDNA expression libraries to isolate full-length cDNA clones of interest (Lerner (1984) *Adv. Immunol.* 36:1-34; Maniatis).

The nucleic acid fragments of the instant invention may be used to create transgenic plants in which the disclosed protein disulfide isomerases are expressed. This would be useful as a means for controlling insect pests by producing plants that are more insect-tolerant than the naturally occurring variety.

5 Expression in plants of the proteins of the instant invention may be accomplished by first constructing a chimeric gene in which the coding region is operably linked to a promoter capable of directing expression of a gene in the desired tissues at the desired stage of development. For reasons of convenience, the chimeric gene may comprise promoter sequences and translation leader sequences derived from the same genes. 3' Non-coding
10 sequences encoding transcription termination signals may also be provided. The instant chimeric gene may also comprise one or more introns in order to facilitate gene expression.

Plasmid vectors comprising the instant chimeric gene can then be constructed. The choice of plasmid vector is dependent upon the method that will be used to transform host plants. The skilled artisan is well aware of the genetic elements that must be present on the
15 plasmid vector in order to successfully transform, select and propagate host cells containing the chimeric gene. The skilled artisan will also recognize that different independent transformation events will result in different levels and patterns of expression (Jones et al. (1985) *EMBO J.* 4:2411-2418; De Almeida et al. (1989) *Mol. Gen. Genetics* 218:78-86), and thus that multiple events must be screened in order to obtain lines displaying the desired
20 expression level and pattern. Such screening may be accomplished by Southern analysis of DNA, Northern analysis of mRNA expression, Western analysis of protein expression, LC-MS, or phenotypic analysis.

The instant polypeptides (or portions thereof) may be produced in heterologous host cells, particularly in the cells of microbial hosts, and can be used to prepare antibodies to the
25 these proteins by methods well known to those skilled in the art. The antibodies are useful for detecting the polypeptides of the instant invention *in situ* in cells or *in vitro* in cell extracts. Preferred heterologous host cells for production of the instant polypeptides are microbial hosts. Microbial expression systems and expression vectors containing regulatory sequences that direct high level expression of foreign proteins are well known to those
30 skilled in the art. Any of these could be used to construct a chimeric gene for production of the instant polypeptides. This chimeric gene could then be introduced into appropriate microorganisms via transformation to provide high level expression of the encoded protein disulfide isomerase. An example of a vector for high level expression of the instant polypeptides in a bacterial host is provided (Example 9).

35 Insecticidal baculoviruses have great potential to provide an environmentally benign method for agricultural insect pest control. However, improvements to efficacy are required in order to make these agents competitive with current chemical pest control agents. One approach for making such improvements is through genetic alteration of the virus. For

instance, it may be possible to modify the viral genome in order to improve the host range of the virus, to increase the environmental stability and persistence of the virus, or to improve the infectivity and transmission of the virus. In addition, improving the rate at which the virus acts to compromise the infected insect would significantly enhance the attractiveness of insecticidal baculoviruses as adjuncts or replacements for chemical pest control agents. One method for increasing the speed with which the virus affects its insect host is to introduce into the baculovirus foreign genes that encode proteins that are toxic to the insect wherein death or incapacitation of the insect is no longer dependent solely on the course of the viral infection, but instead is aided by the accumulation of toxic levels of the foreign protein. The results are insecticidal recombinant baculoviruses.

Recombinant baculoviruses expressing the instant protein disulfide isomerase (or portions thereof) may be prepared by protocols now known to the art (e.g., Tomalski et al., U.S. Pat. No. 5,266,317, exemplifying neurotoxins from the insect-parasitic mites; McCutchen et al. (1991) *Bio/Technology* 9:848-852; Maeda et al. (1991) *Virology* 184:777-780, illustrating construction of a recombinant baculovirus expressing AaIT; also see O'Reilly et al. (1992) *Baculovirus Expression Vectors: A Laboratory Manual*, W. H. Freeman and Company, New York; King and Possee (1992) *The Baculovirus Expression System*, Chapman and Hall, London; U.S. Patent No. 4,745,051). These methods of gene expression provide economical preparation of foreign proteins in a eukaryotic expression vector system, in many instances yielding proteins that have achieved their proper tertiary conformation and formed the proper disulfide bridges necessary for activity.

Commonly, the introduction of heterologous genes into the baculovirus genome occurs by homologous recombination between viral genomic DNA and a suitable "transfer vector" containing the heterologous gene of interest. These transfer vectors are generally plasmid DNAs that are capable of autonomous replication in bacterial hosts, affording facile genetic manipulation. Baculovirus transfer vectors also contain a genetic cassette comprising a region of the viral genome that has been modified to include the following features (listed in the 5' to 3' direction): 1) viral DNA comprising the 5' region of a non-essential genomic region; 2) a viral promoter; 3) one or more DNA sequences encoding restriction enzyme sites facilitating insertion of heterologous DNA sequences; 4) a transcriptional termination sequence; and 5) viral DNA comprising the 3' region of a non-essential genomic region. A heterologous gene of interest is inserted into the transfer vector at the restriction site downstream of the viral promoter. The resulting cassette comprises a chimeric gene wherein the heterologous gene is under the transcriptional control of the viral promoter and transcription termination sequences present on the transfer vector. Moreover, this chimeric gene is flanked by viral DNA sequences that facilitate homologous recombination at a non-essential region of the viral genome. Recombinant viruses are created by co-transfecting insect cells that are capable of supporting viral replication with viral genomic DNA and the

recombinant transfer vector. Homologous recombination between the flanking viral DNA sequences present on the transfer vector and the homologous sequences on the viral genomic DNA takes place and results in insertion of the chimeric gene into a region of the viral genome that does not disrupt an essential viral function. The infectious recombinant virion
5 consists of the recombined genomic DNA, referred to as the baculovirus expression vector, surrounded by a protein coat.

In a preferred embodiment, the non-essential region of the viral genome that is present on the transfer vector comprises the region of the viral DNA responsible for polyhedrin production. Most preferred is a transfer vector that contains the entire polyhedrin gene
10 between the flanking sequences that are involved in homologous recombination. Recombination with genomic DNA from viruses that are defective in polyhedrin production (due to a defect in the genomic copy of the polyhedrin gene) will result in restoration of the polyhedrin-positive phenotype. This strategy facilitates identification and selection of recombinant viruses.

In another embodiment, baculoviral genomic DNA can be directly modified by introduction of a unique restriction enzyme recognition sequence into a non-essential region of the viral genome. A chimeric gene comprising the heterologous gene to be expressed by the recombinant virus and operably linked to regulatory sequences capable of directing gene expression in baculovirus-infected insect cells, can be constructed and inserted directly into
20 the viral genome at the unique restriction site. This strategy eliminates both the need for construction of transfer vectors and reliance on homologous recombination for generation of recombinant viruses. This technology is described by Ernst et al. (Ernst et al. (1994) *Nuc. Acid Res.* 22: 2855-2856), and in WO 94/28114.

Recombinant baculovirus expression vectors suitable for delivering genetically encoded insect-specific protein disulfide isomerases require optimal gene expression for maximum efficacy. A number of strategies can be used by the skilled artisan to design and prepare recombinant baculoviruses wherein protein disulfide isomerase gene expression results in sufficient quantities of protein disulfide isomerase produced at appropriate times during infection in a functional form and available for binding to target cells within the
30 insect host.

The isolated protein disulfide isomerase gene fragment may be digested with appropriate enzymes and may be inserted into the pTZ-18R plasmid (Pharmacia, Piscataway, NJ) at the multiple cloning site using standard molecular cloning techniques. Following transformation of *E. coli* DH5 α MCR, isolated colonies may be chosen and plasmid DNA
35 prepared. Positive clones will be identified and sequenced with the commercially available forward and reverse primers.

Spodoptera frugiperda cells (Sf-9) may be propagated in ExCell® 401 media (JRH Biosciences, Lenexa, KS) supplemented with 3.0% fetal bovine serum. Lipofectin®

(50 μ L at 0.1 mg/mL, Gibco/BRL) may be added to a 50 μ L aliquot of the transfer vector containing the toxin gene of interest (500 ng) and linearized polyhedrin-negative AcNPV (2.5 μ g, Baculogold[®] viral DNA, Pharmigen, San Diego, CA). Sf-9 cells (approximate 50% monolayer) may be co-transfected with the viral DNA/transfer vector solution. The supernatant fluid from the co-transfection experiment may be collected at 5 days post-transfection and recombinant viruses may be isolated employing standard plaque purification protocols, wherein only polyhedrin-positive plaques will be selected (Granados and Lawler (1981) *Virology* 108:297-308).

To propagate the recombinant virus of interest, isolated plaques may be picked and suspended in 500 μ L of ExCell[®] media supplemented with 2.5% fetal bovine serum. Sf-9 cells in 35 mM petri dishes (50% monolayer) may be inoculated with 100 μ L of the viral suspension, and supernatant fluids collected at 5 days post infection. These supernatant fluids will be used to inoculate cultures for large scale propagation of recombinant viruses.

Expression of the encoded protein disulfide isomerase gene by the recombinant baculovirus will be confirmed using a bioassay, LCMS, or antibodies.

EXAMPLES

The present invention is further defined in the following Examples, in which all parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

EXAMPLE 1

Composition of cDNA Libraries: Isolation and Sequencing of cDNA Clones

cDNA libraries representing mRNAs from various spider, caterpillar, centipede, moth, and scorpion tissues were prepared. The characteristics of the libraries are described below.

TABLE 2

cDNA Libraries from Scorpion, Caterpillar, Centipede, Moth, and Spider

Library	Tissue	Clone
aot1c	Ceplalothorax of orb spider collected from Texas	aot1c.pk011.n11
aot1c	Ceplalothorax of orb spider collected from Texas	aot1c.pk016.k11
asc1	<i>Scolopendra canidens</i> DS milked venom glands	asc1.pk033.i19
ibj1c	<i>Buthatus judaicus</i> Telsons 48-Hours Post Milking	ibj1c.pk008.d11
ibj1c	<i>Buthatus judaicus</i> Telsons 48-Hours Post Milking	ibj1c.pk015.k8
ibj1c	<i>Buthatus judaicus</i> Telsons 48-Hours Post Milking	ibj1c.pk015.o22

Library	Tissue	Clone
ibj1c	<i>Buthatus judaicus</i> Telsons 48-Hours Post Milking	ibj1c.pk014.c1
ihv1c	Tobacco budworm (<i>Heliothis virescens</i>) Whole Insect	ihv1c.pk001.a7
ihv1c	Tobacco budworm (<i>Heliothis virescens</i>) Whole Insect	ihv1c.pk001.h12
iks1c	Kentucky scorpion Telsons 48-Hours Post Milking	iks1c.pk0004.c12
iks1c	Kentucky scorpion Telsons 48-Hours Post Milking	iks1c.pk010.i14
isc1c	<i>Scolopendra canidnes</i> Telsons 48-Hours Post Milking	isc1c.pk009.m16
iselc	Fall armyworm (<i>Spodoptera exigua</i>) Whole Insect	iselc.pk002.m4

cDNA libraries may be prepared by any one of many methods available. For example, the cDNAs may be introduced into plasmid vectors by first preparing the cDNA libraries in Uni-ZAP™ XR vectors according to the manufacturer's protocol (Stratagene Cloning Systems, La Jolla, CA). The Uni-ZAP™ XR libraries are converted into plasmid libraries according to the protocol provided by Stratagene. Upon conversion, cDNA inserts will be contained in the plasmid vector pBluescript. In addition, the cDNAs may be introduced directly into precut Bluescript II SK(+) vectors (Stratagene) using T4 DNA ligase (New England Biolabs), followed by transfection into DH10B cells according to the manufacturer's protocol (GIBCO BRL Products). Once the cDNA inserts are in plasmid vectors, plasmid DNAs are prepared from randomly picked bacterial colonies containing recombinant pBluescript plasmids, or the insert cDNA sequences are amplified via polymerase chain reaction using primers specific for vector sequences flanking the inserted cDNA sequences. Amplified insert DNAs or plasmid DNAs are sequenced in dye-primer sequencing reactions to generate partial cDNA sequences (expressed sequence tags or "ESTs"; see Adams et al., (1991) *Science* 252:1651-1656). The resulting ESTs are analyzed using a Perkin Elmer Model 377 fluorescent sequencer.

EXAMPLE 2

Identification of cDNA Clones

ESTs encoding protein disulfide isomerases were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol.* 215:403-410; see also www.ncbi.nlm.nih.gov/BLAST/) searches for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences obtained in Example 1 were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly

available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish and States (1993) *Nat. Genet.* 3:266-272) provided by the NCBI. For convenience, the P-value (probability) of observing a match of a cDNA sequence to a sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as

- 5 "pLog" values, which represent the negative of the logarithm of the reported P-value. Accordingly, the greater the pLog value, the greater the likelihood that the cDNA sequence and the BLAST "hit" represent homologous proteins.

EXAMPLE 3

Characterization of cDNA Clones Encoding Protein Disulfide Isomerase Precursor

- 10 The BLASTX search using the EST sequences from clones listed in Table 3 revealed similarity of the polypeptides encoded by the cDNAs to protein disulfide isomerase precursor from *Drosophila melanogaster* or *Gallus gallus* (NCBI General Identifier Nos. 4262594, 1709616, and 2144546, respectively). Shown in Table 3 are the BLAST results for individual ESTs ("EST"), or the sequences of the entire cDNA inserts comprising
- 15 the indicated cDNA clones ("FIS"):

TABLE 3

BLAST Results for Sequences Encoding Polypeptides Homologous to Protein Disulfide Isomerase Precursor

Clone	Status	NCBI General Identifier No.	BLAST pLog Score
aot1c.pk016.k11	FIS	1709616	166.00
asc1.pk033.i19	FIS	2144546	168.00
ihv1c.pk001.h12	EST	1709616	56.00
iks1c.pk0004.c12	EST	2144546	165.00

20 The data in Table 4 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:2, 4, 6, and 8 and the *Drosophila melanogaster* and *Gallus gallus* sequences (NCBI General Identifier Nos. 1709616 and 2144546, respectively).

TABLE 4

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to Protein Disulfide Isomerase Precursor

SEQ ID NO.	Percent Identity to	
	1709616	2144546
2	54.8	53.8
4	54.6	55.8
6	65.4	60.2
8	53.2	54.1

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS*. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments and BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode a substantial portion of a spider, a centipede, a moth and a scorpion PDI precursor. These sequences represent the first arthropod sequences encoding protein disulfide isomerase precursor.

EXAMPLE 4

Characterization of cDNA Clones Encoding ERp-60

The BLASTX search using the EST sequences from clones listed in Table 5 revealed similarity of the polypeptides encoded by the cDNAs to ERp-60 from *Drosophila melanogaster* (NCBI General Identifier No. 1699220). Shown in Table 5 are the BLAST results for the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"):

TABLE 5

BLAST Results for Sequences Encoding Polypeptides Homologous to ERp-60

Clone	Status	BLAST pLog Score 1699220
aot1c.pk011.n11	FIS	170.00
ibj1c.pk015.k8	FIS	153.00
isc1c.pk009.m16	FIS	172.00

The data in Table 6 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:10, 12 and 14 and the *Drosophila melanogaster* sequence (1699220).

TABLE 6

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to ERp-60

SEQ ID NO.	Percent Identity to 1699220
10	56.2
12	55.4
14	55.3

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS*. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments and BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode a substantial portion of a spider, a scorpion, and a centipede ERp-60. These sequences represent the first arthropod sequences encoding ERp-60.

EXAMPLE 5

Characterization of cDNA Clones Encoding ERp-72

The BLASTX search using the EST sequences from clones listed in Table 7 revealed similarity of the polypeptides encoded by the cDNAs to ERp-72 from *Homo sapiens* (NCBI General Identifier No. 2507460). Shown in Table 7 are the BLAST results for the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"):

TABLE 7

BLAST Results for Sequences Encoding Polypeptides Homologous to ERp-72

Clone	Status	BLAST pLog Score 2507460
ibjlc.pk008.d11	FIS	161.00
ibjlc.pk014.c1	FIS	160.00

The data in Table 8 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOS:16 and 18 and the *Homo sapiens* sequence (NCBI General Identifier No. 2507460).

TABLE 8

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to ERp-72

SEQ ID NO.	Percent Identity to 2507460
16	53.8
18	53.6

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS*. 5:151-153) with the default

parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments and BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode two entire scorpion ERp-72. These sequences represent the first scorpion sequences encoding ERp-72.

EXAMPLE 6

Characterization of cDNA Clones Encoding ERp-5

The BLASTX search using the EST sequences from clones listed in Table 9 revealed similarity of the polypeptides encoded by the cDNAs to ERp-5 from *Medicago sativa* or *Rattus norvegicus* (NCBI General Identifier Nos. 729442 and 2501206, respectively). Shown in Table 9 are the BLAST results for individual ESTs ("EST"), or the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"):

TABLE 9

BLAST Results for Sequences Encoding Polypeptides Homologous to ERp-5

Clone	Status	NCBI General Identifier No.	BLAST pLog Score
ibj1c.pk015.o22	FIS	729442	38.00
ihv1c.pk001.a7	FIS	2501206	105.00
iks1c.pk010.i14	EST	729442	39.15
ise1c.pk002.m4	FIS	2501206	147.00

The data in Table 10 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:18, 20, 22, and 24 and the *Medicago sativa* and *Rattus norvegicus* sequences (NCBI General Identifier Nos. 729442 and 2501206, respectively).

TABLE 10

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to ERp-5

SEQ ID NO.	Percent Identity to	
	729442	2501206
18	27.9	22.5
20	19.4	59.2
22	24.2	19.2
24	26.4	55.6

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal

method of alignment (Higgins and Sharp (1989) *CABIOS*. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments and BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode a substantial portion of a caterpillar, a moth, and two scorpion ERp-5. These sequences represent the first arthropod sequences encoding ERp-5.

EXAMPLE 7

Expression of Chimeric Genes in Monocot Cells

A chimeric gene comprising a cDNA encoding the instant polypeptides in sense orientation with respect to the maize 27 kD zein promoter that is located 5' to the cDNA fragment, and the 10 kD zein 3' end that is located 3' to the cDNA fragment, can be constructed. The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites (Nco I or Sma I) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the digested vector pML103 as described below. Amplification is then performed in a standard PCR. The amplified DNA is then digested with restriction enzymes Nco I and Sma I and fractionated on an agarose gel. The appropriate band can be isolated from the gel and combined with a 4.9 kb Nco I-Sma I fragment of the plasmid pML103. Plasmid pML103 has been deposited under the terms of the Budapest Treaty at ATCC (American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209), and bears accession number ATCC 97366. The DNA segment from pML103 contains a 1.05 kb Sal I-Nco I promoter fragment of the maize 27 kD zein gene and a 0.96 kb Sma I-Sal I fragment from the 3' end of the maize 10 kD zein gene in the vector pGem9Zf(+) (Promega). Vector and insert DNA can be ligated at 15°C overnight, essentially as described (Maniatis). The ligated DNA may then be used to transform *E. coli* XL1-Blue (Epicurian Coli XL-1 Blue™; Stratagene). Bacterial transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence analysis using the dideoxy chain termination method (Sequenase™ DNA Sequencing Kit; U.S. Biochemical). The resulting plasmid construct would comprise a chimeric gene encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding the instant polypeptides, and the 10 kD zein 3' region.

The chimeric gene described above can then be introduced into corn cells by the following procedure. Immature corn embryos can be dissected from developing caryopses derived from crosses of the inbred corn lines H99 and LH132. The embryos are isolated 10 to 11 days after pollination when they are 1.0 to 1.5 mm long. The embryos are then placed with the axis-side facing down and in contact with agarose-solidified N6 medium (Chu et al., (1975) *Sci. Sin. Peking* 18:659-668). The embryos are kept in the dark at 27°C. Friable

embryogenic callus consisting of undifferentiated masses of cells with somatic proembryoids and embryoids borne on suspensor structures proliferates from the scutellum of these immature embryos. The embryogenic callus isolated from the primary explant can be cultured on N6 medium and sub-cultured on this medium every 2 to 3 weeks.

5 The plasmid, p35S/Ac (obtained from Dr. Peter Eckes, Hoechst Ag, Frankfurt, Germany) may be used in transformation experiments in order to provide for a selectable marker. This plasmid contains the *Pat* gene (see European Patent Publication 0 242 236) which encodes phosphinothricin acetyl transferase (PAT). The enzyme PAT confers resistance to herbicidal glutamine synthetase inhibitors such as phosphinothricin. The *pat* gene in p35S/Ac is under the control of the 35S promoter from cauliflower mosaic virus (Odell et al. (1985) *Nature* 313:810-812) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*.

10 The particle bombardment method (Klein et al. (1987) *Nature* 327:70-73) may be used to transfer genes to the callus culture cells. According to this method, gold particles (1 μ m in diameter) are coated with DNA using the following technique. Ten μ g of plasmid DNAs are added to 50 μ L of a suspension of gold particles (60 mg per mL). Calcium chloride (50 μ L of a 2.5 M solution) and spermidine free base (20 μ L of a 1.0 M solution) are added to the particles. The suspension is vortexed during the addition of these solutions. After 10 minutes, the tubes are briefly centrifuged (5 sec at 15,000 rpm) and the supernatant removed. The particles are resuspended in 200 μ L of absolute ethanol, centrifuged again and the supernatant removed. The ethanol rinse is performed again and the particles resuspended in a final volume of 30 μ L of ethanol. An aliquot (5 μ L) of the DNA-coated gold particles can be placed in the center of a Kapton™ flying disc (Bio-Rad Labs). The particles are then accelerated into the corn tissue with a Biolistic™ PDS-1000/He (Bio-Rad Instruments, Hercules CA), using a helium pressure of 1000 psi, a gap distance of 0.5 cm and a flying distance of 1.0 cm.

20 For bombardment, the embryogenic tissue is placed on filter paper over agarose-solidified N6 medium. The tissue is arranged as a thin lawn and covered a circular area of about 5 cm in diameter. The petri dish containing the tissue can be placed in the chamber of the PDS-1000/He approximately 8 cm from the stopping screen. The air in the chamber is then evacuated to a vacuum of 28 inches of Hg. The macrocarrier is accelerated with a helium shock wave using a rupture membrane that bursts when the He pressure in the shock tube reaches 1000 psi.

30 Seven days after bombardment the tissue can be transferred to N6 medium that contains glufosinate (2 mg per liter) and lacks casein or proline. The tissue continues to grow slowly on this medium. After an additional 2 weeks the tissue can be transferred to fresh N6 medium containing glufosinate. After 6 weeks, areas of about 1 cm in diameter of actively growing callus can be identified on some of the plates containing the glufosinate-

supplemented medium. These calli may continue to grow when sub-cultured on the selective medium.

Plants can be regenerated from the transgenic callus by first transferring clusters of tissue to N6 medium supplemented with 0.2 mg per liter of 2,4-D. After two weeks the tissue can be transferred to regeneration medium (Fromm et al., (1990) *Bio/Technology* 8:833-839).

EXAMPLE 8

Expression of Chimeric Genes in Dicot Cells

A seed-specific expression cassette composed of the promoter and transcription terminator from the gene encoding the β subunit of the seed storage protein phaseolin from the bean *Phaseolus vulgaris* (Doyle et al. (1986) *J. Biol. Chem.* 261:9228-9238) can be used for expression of the instant polypeptides in transformed soybean. The phaseolin cassette includes about 500 nucleotides upstream (5') from the translation initiation codon and about 1650 nucleotides downstream (3') from the translation stop codon of phaseolin. Between the 5' and 3' regions are the unique restriction endonuclease sites Nco I (which includes the ATG translation initiation codon), Sma I, Kpn I and Xba I. The entire cassette is flanked by Hind III sites.

The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the expression vector. Amplification is then performed as described above, and the isolated fragment is inserted into a pUC18 vector carrying the seed expression cassette.

Soybean embryos may then be transformed with the expression vector comprising sequences encoding the instant polypeptides. To induce somatic embryos, cotyledons, 3-5 mm in length dissected from surface sterilized, immature seeds of the soybean cultivar A2872, can be cultured in the light or dark at 26°C on an appropriate agar medium for 6-10 weeks. Somatic embryos which produce secondary embryos are then excised and placed into a suitable liquid medium. After repeated selection for clusters of somatic embryos which multiplied as early, globular staged embryos, the suspensions are maintained as described below.

Soybean embryogenic suspension cultures can be maintained in 35 mL liquid media on a rotary shaker, 150 rpm, at 26°C with florescent lights on a 16:8 hour day/night schedule. Cultures are subcultured every two weeks by inoculating approximately 35 mg of tissue into 35 mL of liquid medium.

Soybean embryogenic suspension cultures may then be transformed by the method of particle gun bombardment (Klein et al. (1987) *Nature* (London) 327:70, U.S. Patent

No. 4,945,050). A DuPont Biolistic™ PDS1000/HE instrument (helium retrofit) can be used for these transformations.

5 A selectable marker gene which can be used to facilitate soybean transformation is a chimeric gene composed of the 35S promoter from cauliflower mosaic virus (Odell et al. (1985) *Nature* 313:810-812), the hygromycin phosphotransferase gene from plasmid pJR225 (from *E. coli*; Gritz et al.(1983) *Gene* 25:179-188) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*. The seed expression cassette comprising the phaseolin 5' region, the fragment encoding the instant polypeptides and the phaseolin 3' region can be isolated as a restriction fragment. This fragment can then
10 be inserted into a unique restriction site of the vector carrying the marker gene.

To 50 µL of a 60 mg/mL 1 µm gold particle suspension is added (in order): 5 µL DNA (1 µg/µL), 20 µl spermidine (0.1 M), and 50 µL CaCl₂ (2.5 M). The particle preparation is then agitated for three minutes, spun in a microfuge for 10 seconds and the supernatant removed. The DNA-coated particles are then washed once in 400 µL 70%
15 ethanol and resuspended in 40 µL of anhydrous ethanol. The DNA/particle suspension can be sonicated three times for one second each. Five µL of the DNA-coated gold particles are then loaded on each macro carrier disk.

Approximately 300-400 mg of a two-week-old suspension culture is placed in an empty 60x15 mm petri dish and the residual liquid removed from the tissue with a pipette.
20 For each transformation experiment, approximately 5-10 plates of tissue are normally bombarded. Membrane rupture pressure is set at 1100 psi and the chamber is evacuated to a vacuum of 28 inches mercury. The tissue is placed approximately 3.5 inches away from the retaining screen and bombarded three times. Following bombardment, the tissue can be divided in half and placed back into liquid and cultured as described above.

25 Five to seven days post bombardment, the liquid media may be exchanged with fresh media, and eleven to twelve days post bombardment with fresh media containing 50 mg/mL hygromycin. This selective media can be refreshed weekly. Seven to eight weeks post bombardment, green, transformed tissue may be observed growing from untransformed, necrotic embryogenic clusters. Isolated green tissue is removed and inoculated into
30 individual flasks to generate new, clonally propagated, transformed embryogenic suspension cultures. Each new line may be treated as an independent transformation event. These suspensions can then be subcultured and maintained as clusters of immature embryos or regenerated into whole plants by maturation and germination of individual somatic embryos.

EXAMPLE 9

Expression of Chimeric Genes in Microbial Cells

35 The cDNAs encoding the instant polypeptides can be inserted into the T7 *E. coli* expression vector pBT430. This vector is a derivative of pET-3a (Rosenberg et al. (1987) *Gene* 56:125-135) which employs the bacteriophage T7 RNA polymerase/T7 promoter

system. Plasmid pBT430 was constructed by first destroying the EcoR I and Hind III sites in pET-3a at their original positions. An oligonucleotide adaptor containing EcoR I and Hind III sites was inserted at the BamH I site of pET-3a. This created pET-3aM with additional unique cloning sites for insertion of genes into the expression vector. Then, the Nde I site at the position of translation initiation was converted to an Nco I site using oligonucleotide-directed mutagenesis. The DNA sequence of pET-3aM in this region, 5'-CATATGG, was converted to 5'-CCCATGG in pBT430.

Plasmid DNA containing a cDNA may be appropriately digested to release a nucleic acid fragment encoding the protein. This fragment may then be purified on a 1% NuSieve GTG™ low melting agarose gel (FMC). Buffer and agarose contain 10 µg/ml ethidium bromide for visualization of the DNA fragment. The fragment can then be purified from the agarose gel by digestion with GELase™ (Epicentre Technologies) according to the manufacturer's instructions, ethanol precipitated, dried and resuspended in 20 µL of water. Appropriate oligonucleotide adapters may be ligated to the fragment using T4 DNA ligase (New England Biolabs, Beverly, MA). The fragment containing the ligated adapters can be purified from the excess adapters using low melting agarose as described above. The vector pBT430 is digested, dephosphorylated with alkaline phosphatase (NEB) and deproteinized with phenol/chloroform as described above. The prepared vector pBT430 and fragment can then be ligated at 16°C for 15 hours followed by transformation into DH5 electrocompetent cells (GIBCO BRL). Transformants can be selected on agar plates containing LB media and 100 µg/mL ampicillin. Transformants containing the gene encoding the instant polypeptides are then screened for the correct orientation with respect to the T7 promoter by restriction enzyme analysis.

For high level expression, a plasmid clone with the cDNA insert in the correct orientation relative to the T7 promoter can be transformed into *E. coli* strain BL21(DE3) (Studier et al. (1986) *J. Mol. Biol.* 189:113-130). Cultures are grown in LB medium containing ampicillin (100 mg/L) at 25°C. At an optical density at 600 nm of approximately 1, IPTG (isopropylthio-β-galactoside, the inducer) can be added to a final concentration of 0.4 mM and incubation can be continued for 3 h at 25°. Cells are then harvested by centrifugation and re-suspended in 50 µL of 50 mM Tris-HCl at pH 8.0 containing 0.1 mM DTT and 0.2 mM phenyl methylsulfonyl fluoride. A small amount of 1 mm glass beads can be added and the mixture sonicated 3 times for about 5 seconds each time with a microprobe sonicator. The mixture is centrifuged and the protein concentration of the supernatant determined. One µg of protein from the soluble fraction of the culture can be separated by SDS-polyacrylamide gel electrophoresis. Gels can be observed for protein bands migrating at the expected molecular weight.

EXAMPLE 10

Expression of Chimeric Genes in Insect Cells

The cDNAs encoding the instant polypeptides may be introduced into the baculovirus genome itself. For this purpose the cDNAs may be placed under the control of the polyhedron promoter, the IE1 promoter, or any other one of the baculovirus promoters. The cDNA, together with appropriate leader sequences is then inserted into a baculovirus transfer vector using standard molecular cloning techniques. Following transformation of *E. coli* DH5 α , isolated colonies are chosen and plasmid DNA is prepared and is analyzed by restriction enzyme analysis. Colonies containing the appropriate fragment are isolated, propagated, and plasmid DNA is prepared for cotransfection.

Spodoptera frugiperda cells (Sf-9) are propagated in ExCell[®] 401 media (JRH Biosciences, Lenexa, KS) supplemented with 3.0% fetal bovine serum. Lipofectin[®] (50 μ L at 0.1 mg/mL, Gibco/BRL) is added to a 50 μ L aliquot of the transfer vector containing the PDI gene (500 ng) and linearized polyhedrin-negative AcNPV (2.5 μ g, Baculogold[®] viral DNA, Pharmingen, San Diego, CA). Sf-9 cells (approximate 50% monolayer) are co-transfected with the viral DNA/transfer vector solution. The supernatant fluid from the co-transfection experiment is collected at 5 days post-transfection and recombinant viruses are isolated employing standard plaque purification protocols, wherein only polyhedrin-positive plaques are selected (O'Reilly et al. (1992), *Baculovirus Expression Vectors: A Laboratory Manual*, W. H. Freeman and Company, New York.). Sf-9 cells in 35 mM petri dishes (50% monolayer) are inoculated with 100 μ L of a serial dilution of the viral suspension, and supernatant fluids are collected at 5 days post infection. In order to prepare larger quantities of virus for characterization, these supernatant fluids are used to inoculate larger tissue cultures for large scale propagation of recombinant viruses. Expression of the instant polypeptides encoded by the recombinant baculovirus is confirmed by bioassay.

CLAIMS

What is claimed is:

1. A composition comprising an isolated polynucleotide sequence comprising a nucleic acid sequence encoding a first polypeptide of at least 50 amino acids that has at least 80% identity based on Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 16, 18, 20, 22, 24 and 26; or
an isolated polynucleotide comprising the complement of the nucleic acid sequence.
2. The composition of Claim 1 wherein the isolated polynucleotide sequence consists of a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, and 25 that codes for the polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, and 26.
3. The composition of Claim 1 wherein the isolated polynucleotide is DNA.
4. The composition of Claim 1 wherein the isolated polynucleotide is RNA.
5. A chimeric gene comprising the isolated polynucleotide of Claim 1 operably linked to suitable regulatory sequences.
6. An isolated host cell comprising the chimeric gene of Claim 5.
7. An isolated host cell comprising an isolated polynucleotide of Claim 1.
8. The isolated host cell of Claim 7 wherein the isolated host is selected from the group consisting of plant, insect, yeast, bacteria, and virus.
9. A recombinant baculovirus expressing all or at least one of 10 contiguous amino acids of a polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, or 26.
10. A virus comprising the isolated polynucleotide of Claim 1.
11. A composition comprising a polypeptide of at least 50 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, or 26.
12. A method of selecting an isolated polynucleotide that affects the level of expression of a polypeptide in a host cell, the method comprising the steps of:
 - (a) constructing an isolated polynucleotide comprising a nucleotide sequence of at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, and 25;
 - (b) introducing the isolated polynucleotide into a host cell;
 - (c) measuring the level of a polypeptide in the host cell containing the polynucleotide; and
 - (d) comparing the level of polypeptide in the host cell containing the isolated polynucleotide with the level of polypeptide in a host cell that does not contain the isolated polynucleotide.

13. The method of Claim 12 wherein the isolated polynucleotide consists of a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, and 25 that codes for the polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, and 26.

5 14. A method of selecting an isolated polynucleotide that affects the level of expression of polypeptide in a host cell, the method comprising the steps of:
(a) constructing an isolated polynucleotide of Claim 1;
(b) introducing the isolated polynucleotide into a host cell; and
(c) measuring the level of polypeptide in the host cell containing the
10 polynucleotide.

15 15. A method of obtaining a nucleic acid fragment encoding a polypeptide comprising the steps of:

(a) synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected
15 from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25 and the complement of such nucleotide sequences; and

(b) amplifying a nucleic acid sequence using the oligonucleotide primer.

16. A method of obtaining a nucleic acid fragment encoding the amino acid sequence encoding a polypeptide comprising the steps of:

20 (a) probing a cDNA or genomic library with an isolated polynucleotide fragment comprising a nucleotide sequence of at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25 and the complement of such nucleotide sequences;

(b) identifying a DNA clone that hybridizes with the isolated polynucleotide;

25 (c) isolating the identified clone; and

(d) sequencing the cDNA or genomic fragment that comprises the isolated polynucleotide clone.

Figure 1

	*	*	*	*+	***	**	*	*****	*
SEQ ID NO:27	MKFLIC-ALFLAASYVAA-SAEAEVKVEEGVLVATVDNFKQLIADNEFVLVEFYAPWCGH								
SEQ ID NO:28	MAVVRVRAIVALLCLVAALGLAEPLLEEDGVLVLRAANFEQALAAHRHLLVEFYAPWCGH								
SEQ ID NO:02	M-FIKL--LITTL-C-IAS-TIADEIKKENHVLTNDNFEGAIKD-KNVLVEFYAPWCGH								
SEQ ID NO:04	M-YIKL--LILSLC-IYL-CIADEIKKEKSVLVLTKDNEFEGAIKD-KSVLVEFYAPWCGH								
SEQ ID NO:06	MRVI----LFTALLGT-ALADEVPTEENVLVLSKSNFEGVISANNFILVEFYAPWCGH								
SEQ ID NO:08	M-LGAV-TLSTILL-VVI-AAADEIKKDGCVLVLNKDNFQKAIQENKHILVEFYAPWCGH								
	1								60

```

** * * * * ** * * * * ** * * * * ** * * * *
SEQ ID NO: 27 CKALAPEYAKAAQQLAEKESPIKLAKVDATVEGELAEQYAVRGYPTLKFFRSGS----PVE
SEQ ID NO: 28 CKALAPEYAKAAQQLKAESEIRLAKVDATATEAAELAQQFVGVRGYPTIKFFRNGDKAAPRE
SEQ ID NO: 02 CKALEPQYAKAAESLAEKSELLAKVDATVETDLAERYGVRGYPTIKFFREGK---IFE
SEQ ID NO: 04 CKALEPEYAKAAQILEEKSDLLAKVDATAETDLAEQHGVRGYPTIKFFREGK---VIE
SEQ ID NO: 06 CKSLAPEYAKAATKLAEESPIKLAKVDATQEQELAESEYGVRGYPTLKFFRNGS---PID
SEQ ID NO: 08 CKALEPEYAKAAKQLKEEQSDIALGKIDATAESELAEYDVRGYPTIKFIRDGK---PSE
61
120

```

```

* * ID NO: 27 YSGRQAAIIAWVTKKTGPPAKDLTSVADAEQFLKDNEIAIIGFFKDLSESEAKTFTKV
SEQ ID NO: 28 YTAGREADDIVSWLKKRTGPAAATLTDAAAETLVDSSEVVVGFFKDVTSDAAKEFLLA
SEQ ID NO: 02 YNGRTSDEIIRWLKKKTGPPAVDLSVEDAKKFVDSDNEAVVGGFKDLESADAKIFKS
SEQ ID NO: 04 YSGRTAADIIRWLKKKTGPPATDLTVEATKSFIDGGEVVVGFFKDQNSDAQAKIFKN
SEQ ID NO: 06 YTGGRQADDIVSWLKKKTGPP-----
SEQ ID NO: 08 YKGRTAEDIVRWLKKKVGPAAENLTDVDSVKTFQSAAEVVLVGFFKDQSSDKAKVFLEV
121 180

```

Figure 1

```

SEQ ID NO:27      * + ** ***+ + * ** *** ***** *** ** * ****
ANALDSFVFGVSSNADVIKYEAKDNGVVLFKPFDDKKSVEGELNEENLKKFAQVQSLP
SEQ ID NO:28      AESVDDIPFGISSADVFSKYQLSQDGVVLFKKFEDEGRNFEGLTKDNLNFIKSNQLP
ASEMDDFVFGITDDDDVVYSELKASKDGVILFKKFEDEGRNEYEGELKEEDLKKFLKSNSLP
SEQ ID NO:04      AAEMDDFVFGITSTDEVYNELKATQDGVVLFKKFEDEGRNVYEGELSEEKLLKKFLKSNSLP
SEQ ID NO:06      -----
SEQ ID NO:08      ALESDDYAFGITSQDDVFKAYNVEKDGI VLFKQFDEGRNDFEGEITADALKEFINANSLP
181

```

```

SEQ ID NO:27      ***** ** * ** ***** *
LIVDENHESASKIFGGSIKSHLLFFVSRE-GGHIEKYVDPLKEIAKKYRDDILFVTISSD
SEQ ID NO:28      LVIEFTEQTAPKIFGGEIKTHILLFLPKS-VSDYEGKLDNFKTAAGNFKGKILFIFIDSD
SEQ ID NO:02      LVVEFSHETAQKIFGGDIKAHNLLFISKE-SSDYESRVDVFRKVAKEFKGVLFVTINTD
SEQ ID NO:04      LVVEFTHESAQKIFGGDIKAHNLLFISKG-TSDYESKIEAFRKAKEFKGVLFVTIDTD
SEQ ID NO:06      -----
SEQ ID NO:08      LVVEFNQDTAQKVFGEIKAHNLLFVSKQQSEYEKLLLEVFRKVAKDFKNKVLFTIDID
241

```

```

SEQ ID NO:27      ***+ ** *** *** * + *** ** ** **+*** + ** *+ **+ *** *
EEDHTRIFEEFFGMNKEEVPTIRLIKLEEDMAKYKPESDDLSAETIEAFLKKFLDGKIKQH
SEQ ID NO:28      HSDNQRILEFFGLKKEECPAVRLITLEEEMTKYKPESDDLTADKIKEFCNKFELEGKIKPH
SEQ ID NO:02      DEDHEKIMDFFGLKKEDTPTMRLIKLEEEEMAKFKPPTEGNSESEIRDFVNGVLEGGIKQH
SEQ ID NO:04      DEDHERIMEFFGLKKEEAPTMRLIKLEDEMTKFKPTTTGIEESDIRGFVTGVLEGGIKQH
SEQ ID NO:06      -----
SEQ ID NO:08      EEDHERIMEFFGMKKEDAPDMRLIRLEEEMTKFKPPSPGLSEENIRSFVQGVLDGKIKRH
301

```

Figure 1

SEQ ID NO:27	***+ * *** ** *** ** * *** * *****+*****+*****	361
SEQ ID NO:28	LLSQELPEDWKNPVKVLVSSNFESVALDKSKSVLVEFYAPWCGHCKQLAPIYDQLAEKY	420
SEQ ID NO:02	LMSQDLPEDWDKQPVKVLVGKNFEEVAFDENKNVFEFYAPWCGHCKQLAPIWDKLGETY	
SEQ ID NO:04	LLSEDIPEGWDKEPVKVLVGKNFDEVAFDKSKNVLVEFYAPWCGHCKQLAPIYDELGEKY	
SEQ ID NO:06	LLSEDVPEGWDKEPVKILVGKNFDEVVFDKTKNVLVEFYAPWCGHCKQLAPIYDELGEKY	
SEQ ID NO:08	LLSESVPDWDKGAVKVLVGQNFDDVAFDKSKDVLVEFYAPWCGHCKQLAPIYEELGEKY	
		420
SEQ ID NO:27	** *****+* *** + ** * *****+ +* +* +* ***** *	
SEQ ID NO:28	KDNEDIVIAKMDSTANELESIKISSFTTIKYFRKEDN-KVIDFNLDRTLDDFVKFLDANG	
SEQ ID NO:02	RDHENIVIAKMDSTANEVEAVKIHSFTLKFPPAGSGRNVLDYNGERTLEGFKFLES GG	
SEQ ID NO:04	KDRDDVVIKMDATANELEHTKINSYPTIKLYKKGTN-EVVEYNGERTLEGINKFTETDG	
SEQ ID NO:06	KDQADIVIAKMDATANELEHTKINSFPTIKLYKKDTN-EVVDENGERTLEGISRFDITGG	
SEQ ID NO:08	KDQSSIVIAKMDATTNELDHVKIHSFPTIKLFKKDTN-EVIDENGERTLEGLTKFIDSGG	
		480
SEQ ID NO:27	* +* +* ** + ****	
SEQ ID NO:28	E----VADSEPV-----EETEEEEAPKKDEL	
SEQ ID NO:02	QDGAAADDDLEDLETDEETDLEEGDDDEQKIQKDEL	
SEQ ID NO:04	EYGKAAPDEEEA-----EEAVREDAQAHRDEL	
SEQ ID NO:06	VDGAAVKEEEED-----EEEEKDDEQAKRDEL	
SEQ ID NO:08	VDGASPKEEEID-----EEEEKDDDEKKRDEL	
		516

Figure 2

```

*      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
SEQ ID NO:29 MMWRLAGVLLLGFLA--ISSGADEDVLELGDDDFATTLKQHETTLVMFYAPWCGHCKRLK
SEQ ID NO:10 M--KL--LFLSSILSCTLSVILCSDVLDGADFDRIAEHDAIILVEFFAPWCGHCKRLA
SEQ ID NO:12 -----TRLG
SEQ ID NO:14 M-WKI--V----AFSCFFVATIASDVLEFTSDSDFERIKEHDTYLVEFYAPWCGHCKRLA
1
*      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
SEQ ID NO:29 PEYAKAAEIVKDDPPPIKLAKVDCT-EAGKETCSKYSVSGYPTLKIIFRQDEVSDYNGPR
SEQ ID NO:10 PEYDKASTILKKADPPPIPLAKVDCTSDNGKDTCSKYGVSGYPTLKIIFRGGESSEYNGPR
SEQ ID NO:12 PKYEEAATILKKNDPPVPLAKVDCTSDAGKETCSKYGVSGYPTLKIIFRNGEFSSEYSGGR
SEQ ID NO:14 PEYEKAATILKDNDDPPPIPLVKVDCI-ESGKETCGKFGVSGYPTLKIIFRNGDFSQEYNGPR
61
*      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
SEQ ID NO:29 DSSGI AKYMR AQVGPASKTVRTVAELKKFLDTKDTTLFGYFSDSDSKLAKI FLKFADKNR
SEQ ID NO:10 DADGIVKYMKAQVGPSSKELQSLDAEKIL-KDDIVVIGYFADSSNKLKEEFLKAADKLR
SEQ ID NO:12 ETDAIVKYMKSQVGPSSVEIKTPADAKKLLSRIEVIIGFFKDEKSQKKEEFLKVADKMR
SEQ ID NO:14 EANGIVKYMAAQVGPSSKEFQNVKEVQQFLEKEEVAIIGFFESEDAKLKATFQKVADKLR
121
*      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
SEQ ID NO:29 EKYRFGHSSEKEVLDKQGETDKIVLIRAPHLNKFESSIKFEG--SSESDLSTFVKENF
SEQ ID NO:10 ESVSEFHTSSKDILDKYGYSDIEIVLYRPKIFWSKFEPQEIKYTG-ADKGEISQFIKDNY
SEQ ID NO:12 ETTSFGHTSNQEVLDLYGYKDQIVLFRPQHLQSKFEEKELKYEGG-AEKSIEDFIRENY
SEQ ID NO:14 ETARFGHSYNSLVILKEYGYTNQVVLFRPKHLQSKFEDSQVVYDGDKSDKQEELEEFVNKNY
181
*      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
SEQ ID NO:29 EKYRFGHSSEKEVLDKQGETDKIVLIRAPHLNKFESSIKFEG--SSESDLSTFVKENF
SEQ ID NO:10 ESVSEFHTSSKDILDKYGYSDIEIVLYRPKIFWSKFEPQEIKYTG-ADKGEISQFIKDNY
SEQ ID NO:12 ETTSFGHTSNQEVLDLYGYKDQIVLFRPQHLQSKFEEKELKYEGG-AEKSIEDFIRENY
SEQ ID NO:14 ETARFGHSYNSLVILKEYGYTNQVVLFRPKHLQSKFEDSQVVYDGDKSDKQEELEEFVNKNY
240

```

Figure 2

```

***** ++ * * * + * * * * * * * * * * * * * * * * * * * * * *
HGLVGHRTQDSVKDFQNP LITAYYSVDYQKNPKGTNYWRNRVLKVAKEFVGVQINF A I A S K
HGLVGHRTHDNDHDFKAP L I V V Y D V D V K N V K G T N Y W R N R V M K V A Q N Y K G Q V N F A I S N K
HGLVGHRTSDNFQDFKNPLV V A Y D V D V K N T K G T N Y W R N R I M K V A Q H F K D K L N F A V S N I
HGLVGHRTTDNTNQSPPLI V S Y K V D V K N T K G T N Y W R N R I M K V A S E F K G R L N F A I S N K
241 300

* * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
DDFQHELNEYGYDFV-GDKPVV LARDEKNLKYAL KDEF S V E N L Q D F V E K L L A N E L E P Y I K
DKFSAEVEDEFLK-ATGDKPVV A A R N D K N Q K F N M K E E F S V E N F E T F V K K F L D G S L E P H L K
NQFSAEIEEFGLT-VKGDKPA I A V R N E K Q Q K F R M T D E F S M D A F E K F L K D F L D G K L E A H V K
DEFTHELSEYGFNYVAGDKPVV A A R N A K S E K F V M E G E F S I P S F E K F I K D F L D E K L K P Y L K
301 360

* * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
SEPIPESNDAPVKV A V A K N F D D L V I N N G K D T L I E F Y A P W C G H C K K L T P I Y E E L A Q K L Q D E
SEVPPEKNDGPVKV A V A Q N F E E L V M E N D K D V L I E F Y A P W C G H C K K L A P T Y E E L G Q K L E G E
SEPIPENNDGPVKV A V A S N F D D I V T N N D K D I L L E F Y A P W C G H C K K L A P T Y E E L G T E M K Q E
SEPIPEKNEEPVKV A V A Q N F E E L V T K S D K D V L I E F Y A P W C G H C K K L A P V Y D E L G K A L E G E
361 420

* * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
D-VAIVKMDATANDVPPEFNV R G F P T L F W L P K D A K N K P V S Y N G G R E V D D F L K Y I A K E A T T
D-VEIVKMDATANDVPPTFEVHGFP T L Y W V P K T H K S S P K K Y E G G R E I K D F I N Y I A K H A T N
D-VEIVKMDATANDVPPPYEVHGFP T L Y W V P K S N N P K K Y D G G R E L D D L I K Y I S K H A T N
TTVEIVKMDATANDVPSPYEVHGFP T L Y W A P R D K K D K P V R Y D G G R E L D D D F I K Y I A K H S T D
421 480

```

Figure 2

SEQ ID NO:29	***	*	*	+	*	*	*	*
SEQ ID NO:10	ELKGFDRSGKPK--KTEL							
SEQ ID NO:12	ELKQYDRSGKKKS-KEEL							
SEQ ID NO:14	ELKGWDRKGTTKKSEKTEL							
	ELKTYNRNGKKK--KVEL							
	481							
								498

Figure 3

SEQ ID NO:30	*****	1
SEQ ID NO:16	MLRRALLCLAVAAALVRADAPEEEDHVLVRKSNFAEALAAHKYLLVEFYAPWCCHCKALA	60
SEQ ID NO:18	MNFGNLLIF-FSFLIVVLGEVREDNVLVLNKENFDHSIKNNKYILVEFYAPWCCHCKALA	

SEQ ID NO:30	*****	
SEQ ID NO:16	PEYAKAAGKILKAEGSEIRLAKVDATEESDLAQYGVRGYPTIKFFRNGDTASPKKEYTAGR	61
SEQ ID NO:18	PEYAKAAGKLLLEEKSEIQAKIDATEETELAEKHKVKGYPTIKFFREGD---PIDYTGGR	

SEQ ID NO:30	*****	
SEQ ID NO:16	EADDIVNWLKKRTGPAATTLPGAAAESLVESEVAVIGFFKDVESDSAKQFLQAAEAID	120
SEQ ID NO:18	TGDDIVTWLKKKTGPPATLLSTVDEATNFKESKDVVIIGFFKQESNQAKEYLNAAYMTD	

SEQ ID NO:30	*****	
SEQ ID NO:16	DIPFGITSDVFSKYQLDKDGVVLFKKFDEGRNFEGETKENLLDFIKHNLPLVIEF	181
SEQ ID NO:18	DHPFGITSDENVYKHFNVEKDTIFLKKFDEGKNEFEGETKDNIIKFIKLNPLVIEF	

SEQ ID NO:30	*****	
SEQ ID NO:16	TEQTAPKIFGGEIKTHILLFLPKSVSDYDGKLSNFKTAAESFKGKILFIFIDSDHTDNQR	240
SEQ ID NO:18	SQENAAQKIFGGDIKMHNLLFISKKSKDFDEIVKTRIVAKYKKNQILFVINTDDEGNGQ	

Figure 3

```

*+*****+*****+*****+*****+*****+*****+*****+*****+*****+
SEQ ID NO:30 ILEFFGLKKECPAVRLITLEEEMTKYKPESEELTAERITEFCHRFLEGIKIPHLMSQEL
SEQ ID NO:16 ITEFFGLKKDEQPSIRLIKLEEGMSKYKPETIEISEENVRFVKGVLDGTVKQHLLSQEL
SEQ ID NO:18 ITEFFGLKKDEQPSIRLIKLEEGMSKYKPETIEISEENVRFVKGVLDGTVKQHLLSQEL
301
*****+*****+*****+*****+*****+*****+*****+*****+*****+
SEQ ID NO:30 PEDWDKQPVKVLVGKNFEDVAFDEKKNVFEFYAPWCGHCKQLAPIWDKLGETYKDHENI
SEQ ID NO:16 PEDWDKHPVKVLVNKNFDEVAFDKTKDVIVEFYAPWCGHCKQLAPIYEELGEKYKNRNDI
SEQ ID NO:18 PEDWDKHPVKVLVNKNFDEVAFDKTKDVIVEFYAPWCGHCKQLAPIYEELGEKYKNRNDI
361
*****+*****+*****+*****+*****+*****+*****+*****+*****+
SEQ ID NO:30 VIAKMDSTANEVAVKVHSFPTLKFFPASADRTVIDYNGERTLDGFKKFLSEGGQDGAGD
SEQ ID NO:16 IIAKMDATANELEHTKINSFPTIKLYKKGTN-EVIDYDGKHSLEGLVNFIDSGGKIT---
SEQ ID NO:18 IIAKMDATANELEHTKINSFPTIKLYKKGTN-EVIDYDGKHSLEGLVNFIDSGGKIT---
421
*****+*****+*****+*****+*****+*****+*****+*****+*****+
SEQ ID NO:30 DDDLEDLEEAEEPDMEEDDDDQKAVKDEL
SEQ ID NO:16 -----KEPEDEKSKPEPAK--GDEL
SEQ ID NO:18 -----KEPEDEKSKPEPAK--RDEL
481
*****+*****+*****+*****+*****+*****+*****+*****+*****+
508

```

Figure 4

SEQ ID NO:31	MK--MEMHQIWSRIALASFAFAILFVSVSADDDVVVLTEENFEKEVGHDKGA-LVEFYAP	*	*	***
SEQ ID NO:32	S-C-----TFFLAVS-----ALYS--SSDDVIELTPSNFNREVIQSDSLWLVVEFYAP			
SEQ ID NO:20	MGCYLLVLLIFLF-FLRDSQSSDLYT-----DNSIKYDEEGFRRNIGN-IVH-FVKFYAP			
SEQ ID NO:22	H-----			
SEQ ID NO:24	-----TRLSSVFSFNVAIYASSDLYV-----DNSLKYDEDEGFRENVGK-LTL-FVKFYAP			
SEQ ID NO:26	MLH-----TYFLGILLCVGSGLALYD-----SSSDVVDLTPDNFYQLVTD RDDVWLVEFYAP			
	1			60
	***** * * *	++	*	* * * *
SEQ ID NO:31	WCGHCKKLAPEYEKLPNSFK--KAKSVLIAKVDCDEHKSVCSKYGVSGYPTIQWFPKGS			
SEQ ID NO:32	WCGHCQRLTPEWKKAASALK-----DVVKGAVNADKHQSLGGQYGVQGFPTIKIFGANK			
SEQ ID NO:20	WCGHCKRLAPIWDELAEKYN-KPGEQKLVIAKIDCTTETALCSEQGITGYPTLKFFKKGT			
SEQ ID NO:22	-----			
SEQ ID NO:24	WCGHCKRLAPTWDELAEKYNIQPEKQQVIAKIDCTSETALCSEQGITGYPTLKFFKKGE			
SEQ ID NO:26	WCGHCKNLVPEYKKAALK-----GIVKVGAIADAKHRSAKDYGVSGFPTIKIFTGRK			
	61			120
	* * *		+	* * *
SEQ ID NO:31	LEPKKFEGPRTAES-----LAEFV-----NTEGG--TNVKIATAP---SHVVVLTP			
SEQ ID NO:32	NKPEDYQGGRTGEAIVDAALSAALRQLVKDRLGGRSGGYSSGKQGRGDSSSKKDVELTDD			
SEQ ID NO:20	TEGHKYRGPRDITS-----LEAFIANSLGHEEAI--K--KSPEPPKFINEIIQLSDN			
SEQ ID NO:22	-----			
SEQ ID NO:24	TEGTKYRGPRDITS-----LEAFIANSLGKEEAV--EDLKPPEP---VNGLIELTDE			
SEQ ID NO:26	HVP--YKGARSADAFVDAALSAVKSKAYERLGKRS-----DSSHKSSDSDVITLTDD			
	121			180

Figure 4

SEQ ID NO: 31	LEGSASRYGKIYLVSKK-----YLEKGS DYAKNEIQRLERLLE-----K	
SEQ ID NO: 32	LENALGIGGGFGYPAMAAINARKMKFALLKGSFSEQINEFLREL SFGRGSTAPVGGGSFP	
SEQ ID NO: 20	-----	
SEQ ID NO: 22	LEESLELGGFGYPAMAVVNNAKKLKFSTLRGSFSETGINEFLRDL SFGRGQTAPVKGAEMP	
SEQ ID NO: 24	VKFFAPWCCH-CRKLAPI-----WDELSWEFIDNENKIAQ-----	
SEQ ID NO: 26	LEESLELGGFGYPAMAVVNNAKKLKFSTLRGSFSETGINEFLRDL SFGRGQTAPVVRGAEMP	
	361	420

SEQ ID NO: 31	SISPA-----KADELTLKKNI-LSTYA-----	
SEQ ID NO: 32	NITPREPWDGKDGE L PVEDDIDLSDVELDDLEKDEL	
SEQ ID NO: 20	-----	
SEQ ID NO: 22	KIVSTDPWDGKDGE L PQEEDIDLSDV---DLEKDEL	
SEQ ID NO: 24	-VDCS-----SQESLCSK-----	
SEQ ID NO: 26	KIVSTDAWDGKDGE L PQEEDIDLSDV---DLEKDEL	
	421	456

SEQUENCE LISTING

<110> E. I. du Pont de Nemours and Company

<120> Arthropod Protein Disulfide Isomerases

<130> BB-1253 PCT

<140>

<141>

<150> 60/104,376

<151> 1998-10-15

<160> 32

<170> Microsoft Office 97

<210> 1

<211> 2149

<212> DNA

<213> Argiope sp.

<400> 1

```

gctcagagttt tcttttctct tgcggcggtg gtgttttcag tgtcaagagt agcagttttc 60
atataccggc tttctattta acacggagca atgtttatta aattactaat aactacgcta 120
tgtattgcat ccactatcgc agatgaaatt aagaaagaaa atcatgtttt agttttgaca 180
aatgataatt tcgaagggtgc aatcaaagat aaaaacgttc tggtcgaatt ctatgctcca 240
tgggtgtgggc attgtaaggc tcttgaaccc caatatgcca aagccgcaga gagtctagct 300
gaagaaaaat ctgaactact cttggctaaa gttgatgcca cagttgaaac tgatcttgct 360
gaacggtatg gagttcgtgg atacccaacc atcaaattct tccgtgaagg aaagatcttc 420
gaatataatg gaggacgaac ttcagatgaa attatcagat ggcttaaaaa gaaaactgga 480
cctcccgagc ttgatttatc atctgtagaa gatgccaaaa agtttggtga tagtaacgaa 540
gttgctgttg ttgggttttt caaggatctt gaaagtgcag atgcaaagat tttcaagagt 600
gttgcatcag aaatggatga cttcgtattc ggtattacag atgacgatgt tgtttattct 660
gaacttaagg cctcaaagga tggagtcatt ctttttaaaa agtttgatga aggacgaaat 720
gaatatgaag gtgaattgaa ggaagaggat ctgaaaaaat tcttgaaatc gaacagcttg 780
ccattagtag ttgaattcag ccatgaaaca gcacaaaaga tctttggcgg agacattaaa 840
gcacacaatc ttctgttcat cagtaaagaa tcttcagatt atgaaagccg tgtagatgtc 900
ttccgtaagg tagcaaagga attcaaaggc aagggtttgt ttgttacaat caataccgat 960
gatgaagatc atgaaaagat catggacttc tttggcttga agaaagaaga tactccaact 1020
atgagattga tcaagcttga agaagaaatg gctaaattca agcctcctac cgaaggaaaac 1080
agcgaaaagt aaatgagaga cttcgtaaat ggtgttttgg aaggaaaagt aaagcaacat 1140
ttactttctg aagacattcc tgaaggatgg gataaagaac ctgtaaaagt tctttagtag 1200
aagaattttg atgaagtggc ttttgacaaa tccaagaatg ttcttgttga attctatgct 1260
ccatggtgtg gtcactgcaa gcagttggct cccatttatg atgagcttgg tgaaaagtac 1320
aaagacagag atgatgttgt tattgccaaa atggacgcta cagcaaatga attggaacat 1380
actaaaatca atagctatcc tactattaaa ttgtacaaaa aaggcacaaa tgaggttggt 1440
gagtacaatg gagaacgtac tttagaaggc attaataaat tcaactgaaac tgatggagaa 1500
tatggaaaag ctgctcccga tgaggaggaa gctgaagaag cagtcagggg agatgcgcag 1560
gcacatcgtg atgaactata agcatcgcaa cttaataggt cattcatttc atactcctca 1620
tacacgcatt gtgtaccaa gtcaagcgca atcagttgtt aaactcattt ttccattcaa 1680
gccaatggct ggtctccagg ggttaaaaata aatcggacat tttgtgttgt ggttgtgtgt 1740
aacaaaattg atttaatgat actaataaaa aaaaaattgt tctgtaaaat atttttctta 1800
tcagatttgt tagagattat atacaaagca ggcattttta gtttcaatta ttccattatt 1860
tttttatgct gatgttgtgt tcaaaaacgg tgtgaacaaa gatgttatgc tgtttttata 1920
ttttttttat ttaagttaaa gttaaatgat aacttttgaa gatttttcat ttattacgtt 1980
atttaaaatc atctgttcat acttttattt ttaatcaa atattggcaat agaataaaat 2040
attttttata taaaaaacat agttcatttt gaattaaatg ctcgctctcc ttgtgtgtat 2100
taatattaca ggtgtaataa aatacttggt aaaaaaaaaa aaaaaaaaaa 2149

```

<210> 2

<211> 496

<212> PRT

<213> Argiope sp.

<400> 2

```

Met Phe Ile Lys Leu Leu Ile Thr Thr Leu Cys Ile Ala Ser Thr Ile
 1           5           10           15

Ala Asp Glu Ile Lys Lys Glu Asn His Val Leu Val Leu Thr Asn Asp
      20           25           30

Asn Phe Glu Gly Ala Ile Lys Asp Lys Asn Val Leu Val Glu Phe Tyr
      35           40           45

Ala Pro Trp Cys Gly His Cys Lys Ala Leu Glu Pro Gln Tyr Ala Lys
      50           55           60

Ala Ala Glu Ser Leu Ala Glu Glu Lys Ser Glu Leu Leu Leu Ala Lys
      65           70           75           80

Val Asp Ala Thr Val Glu Thr Asp Leu Ala Glu Arg Tyr Gly Val Arg
      85           90           95

Gly Tyr Pro Thr Ile Lys Phe Phe Arg Glu Gly Lys Ile Phe Glu Tyr
      100           105           110

Asn Gly Gly Arg Thr Ser Asp Glu Ile Ile Arg Trp Leu Lys Lys Lys
      115           120           125

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

Phe Val Asp Ser Asn Glu Val Ala Val Val Gly Phe Phe Lys Asp Leu
      145           150           155           160

Glu Ser Ala Asp Ala Lys Ile Phe Lys Ser Val Ala Ser Glu Met Asp
      165           170           175

Asp Phe Val Phe Gly Ile Thr Asp Asp Asp Val Val Tyr Ser Glu Leu
      180           185           190

Lys Ala Ser Lys Asp Gly Val Ile Leu Phe Lys Lys Phe Asp Glu Gly
      195           200           205

Arg Asn Glu Tyr Glu Gly Glu Leu Lys Glu Glu Asp Leu Lys Lys Phe
      210           215           220

Leu Lys Ser Asn Ser Leu Pro Leu Val Val Glu Phe Ser His Glu Thr
      225           230           235           240

Ala Gln Lys Ile Phe Gly Gly Asp Ile Lys Ala His Asn Leu Leu Phe
      245           250           255

Ile Ser Lys Glu Ser Ser Asp Tyr Glu Ser Arg Val Asp Val Phe Arg
      260           265           270

Lys Val Ala Lys Glu Phe Lys Gly Lys Val Leu Phe Val Thr Ile Asn
      275           280           285

Thr Asp Asp Glu Asp His Glu Lys Ile Met Asp Phe Phe Gly Leu Lys
      290           295           300

Lys Glu Asp Thr Pro Thr Met Arg Leu Ile Lys Leu Glu Glu Glu Met
      305           310           315           320

Ala Lys Phe Lys Pro Pro Thr Glu Gly Asn Ser Glu Ser Glu Ile Arg
      325           330           335

Asp Phe Val Asn Gly Val Leu Glu Gly Lys Ile Lys Gln His Leu Leu
      340           345           350

```

Ser Glu Asp Ile Pro Glu Gly Trp Asp Lys Glu Pro Val Lys Val Leu
 355 360 365

Val Gly Lys Asn Phe Asp Glu Val Ala Phe Asp Lys Ser Lys Asn Val
 370 375 380

Leu Val Glu Phe Tyr Ala Pro Trp Cys Gly His Cys Lys Gln Leu Ala
 385 390 395 400

Pro Ile Tyr Asp Glu Leu Gly Glu Lys Tyr Lys Asp Arg Asp Asp Val
 405 410 415

Val Ile Ala Lys Met Asp Ala Thr Ala Asn Glu Leu Glu His Thr Lys
 420 425 430

Ile Asn Ser Tyr Pro Thr Ile Lys Leu Tyr Lys Lys Gly Thr Asn Glu
 435 440 445

Val Val Glu Tyr Asn Gly Glu Arg Thr Leu Glu Gly Ile Asn Lys Phe
 450 455 460

Thr Glu Thr Asp Gly Glu Tyr Gly Lys Ala Ala Pro Asp Glu Glu Glu
 465 470 475 480

Ala Glu Glu Ala Val Arg Glu Asp Ala Gln Ala His Arg Asp Glu Leu
 485 490 495

<210> 3
 <211> 1843
 <212> DNA
 <213> Scolopendra canidens DS

<400> 3
 gcacgagtat aaacgcctgc ctctccgttt gcttctgctt gccacgtttg acttttccctc 60
 taaccagcgg cattgggtgca tgttttagtgt caggatgatag gcttttcatt taccggcttg 120
 aacttaaaatt tataaaatgt atataaagct tcttattctt agtttatgca tatatttatg 180
 tatagctgat gaaattaaaa aagaaaagtc agtttttagtg ttgaccaaag acaattttga 240
 aggagcaatc aaagataaaa gtgtactcgt cgagttttat gtccttggt gtggctcattg 300
 taaggcccta gaacctgaat atgctaaagc cgctcagatc ttggaagagg aaaagtcga 360
 cttactgttg gctaaagttg atgctacagc ggaaactgat ttagctgaac agcatggcgt 420
 tcgtgggtat ccaactatta agtttttccg agagggtaaa gtgatagaat actctgggtgg 480
 acgaactgct gatgacatta tacgctgggt gaaaaagaag actgggcctc cagcaactga 540
 tttaactaca gtagaggcaa caaaatcttt tattgatggt ggtgaggtag tagttgtagg 600
 cttcttttaa gaccaaatt cagatcaggc aaagattttc aagaatgttg ctgcagaaat 660
 ggatgacttt gttttcggca ttacttcaac tgatgaggtg tacaatgaat taaaggctac 720
 acaagatggc gttgttttgt ttaaaaagtt tgatgaagga agaatgttt atgaaggtga 780
 attatcagaa gaaaaattga aaaaattctt gaaatctaag agcctgcctc tagttgttga 840
 gtttacctat gaatcagctc agaaaatctt tgggggtgac attaaagccc ataatttact 900
 cttcatcagc aaaggaaact cggattatga aagcaaaatt gaagccttcc gtaaaagtcgc 960
 taaagaattc aaaggcaaaag ttctttttgt tactatcgat actgatgatg aagatcatga 1020
 gagaatcatg gaattctttg gtcttaaaaa agaagaggcg cctaccatga gactgatcaa 1080
 actagaagat gaaatgacta aattcaaacc tactactaca ggaattgagg aaagtgatat 1140
 tagaggattt gttactgggtg ttttagaagg aaagataaag caacacttgc tttctgaaga 1200
 tgttcctgaa ggatgggata aagagcctgt gaaaattcta gttggaaaaa attttgatga 1260
 agtagtattt gataaaacaa agaattgttct agttgaattt tatgctccat ggtgtggcca 1320
 ttgtaaacag ctagctccta tttatgatga attgggtgag aaatacaagg atcaagctga 1380
 tattgttaatt gctaagatgg atgctactgc aaatgaattg gagcatacca agattaacag 1440
 ttttcccaca atcaagttgt acaaaaaaga tacaatgaa gtggttgatt tcaatggaga 1500
 acgcacactt gaaggaatta gcaggtttat tgatactggt ggtgttgatg gggcagctgt 1560
 taaagaagag gaggaagatg aggaagaaga aaaagatgac gaacaggcca agcgtgatga 1620
 gctttaaagg cattatcaca agacttaagt catcatctcc atttttctca tacaacattg 1680
 tgtaccaaag ttgagtgcga tcagttcttg taaccattaa tttcattcat gccaatggct 1740
 ggattccagg ggttgtaaca aattaaggac ctttttcttg ttcagtgaat gtgccgaagc 1800
 agttctaata aaatatttgt tttttaaaaa aaaaaaaaaa aaa 1843

<210> 4
 <211> 496
 <212> PRT
 <213> Scolopendra canidens DS

<400> 4

```

Met Tyr Ile Lys Leu Leu Ile Leu Ser Leu Cys Ile Tyr Leu Cys Ile
 1           5           10           15

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

Asn Phe Glu Gly Ala Ile Lys Asp Lys Ser Val Leu Val Glu Phe Tyr
      35           40           45

Ala Pro Trp Cys Gly His Cys Lys Ala Leu Glu Pro Glu Tyr Ala Lys
      50           55           60

Ala Ala Gln Ile Leu Glu Glu Glu Lys Ser Asp Leu Leu Leu Ala Lys
      65           70           75           80

Val Asp Ala Thr Ala Glu Thr Asp Leu Ala Glu Gln His Gly Val Arg
      85           90           95

Gly Tyr Pro Thr Ile Lys Phe Phe Arg Glu Gly Lys Val Ile Glu Tyr
      100          105          110

Ser Gly Gly Arg Thr Ala Asp Asp Ile Ile Arg Trp Leu Lys Lys Lys
      115          120          125

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

Phe Ile Asp Gly Gly Glu Val Val Val Val Gly Phe Phe Lys Asp Gln
      145          150          155          160

Asn Ser Asp Gln Ala Lys Ile Phe Lys Asn Val Ala Ala Glu Met Asp
      165          170          175

Asp Phe Val Phe Gly Ile Thr Ser Thr Asp Glu Val Tyr Asn Glu Leu
      180          185          190

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

Arg Asn Val Tyr Glu Gly Glu Leu Ser Glu Glu Lys Leu Lys Lys Phe
      210          215          220

Leu Lys Ser Asn Ser Leu Pro Leu Val Val Glu Phe Thr His Glu Ser
      225          230          235          240

Ala Gln Lys Ile Phe Gly Gly Asp Ile Lys Ala His Asn Leu Leu Phe
      245          250          255

Ile Ser Lys Gly Thr Ser Asp Tyr Glu Ser Lys Ile Glu Ala Phe Arg
      260          265          270

Lys Val Ala Lys Glu Phe Lys Gly Lys Val Leu Phe Val Thr Ile Asp
      275          280          285

Thr Asp Asp Glu Asp His Glu Arg Ile Met Glu Phe Phe Gly Leu Lys
      290          295          300

Lys Glu Glu Ala Pro Thr Met Arg Leu Ile Lys Leu Glu Asp Glu Met
      305          310          315          320

```

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

Gly Phe Val Thr Gly Val Leu Glu Gly Lys Ile Lys Gln His Leu Leu
 340 345 350

Ser Glu Asp Val Pro Glu Gly Trp Asp Lys Glu Pro Val Lys Ile Leu
 355 360 365

Val Gly Lys Asn Phe Asp Glu Val Val Phe Asp Lys Thr Lys Asn Val
 370 375 380

Leu Val Glu Phe Tyr Ala Pro Trp Cys Gly His Cys Lys Gln Leu Ala
 385 390 395 400

Pro Ile Tyr Asp Glu Leu Gly Glu Lys Tyr Lys Asp Gln Ala Asp Ile
 405 410 415

Val Ile Ala Lys Met Asp Ala Thr Ala Asn Glu Leu Glu His Thr Lys
 420 425 430

Ile Asn Ser Phe Pro Thr Ile Lys Leu Tyr Lys Lys Asp Thr Asn Glu
 435 440 445

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

Ile Asp Thr Gly Gly Val Asp Gly Ala Ala Val Lys Glu Glu Glu Glu
 465 470 475 480

Asp Glu Glu Glu Glu Lys Asp Asp Glu Gln Ala Lys Arg Asp Glu Leu
 485 490 495

<210> 5
 <211> 497
 <212> DNA
 <213> Heliothus virescens]

<220>
 <221> unsure
 <222> (452)

<400> 5
 gtttcgtctt cggagcaggc ttgtgaagtg ccgtattttc caacatgaga gtgatoctat 60
 ttacggcggt agcccttctg ggcaccgctt tggccgatga agtaccact gaagaaaacg 120
 tactcgtttt aagcaaactt aacttcgaag gagtcatttc agcaaacaac ttcattattag 180
 tggaattcta tgcgccatgg tgcggtcact gcaagtcctt cgctccggag tacgccaagg 240
 ccgccaccaa gctggccgag gaggagtctc ccatcaaact ggccaagggt gatgccacgc 300
 aagagcaaga gctcgctgag agctacggcg tcaggggata cccgaccctc aagttcttca 360
 gaaacggcag ccctattgat taaactgggt gtcgccaagc tgacgacatt gtctcctggc 420
 tgaagaagaa gactggtcct cccgcccttg anggtgcctc ctgctgagca agccaaggaa 480
 ctcaattgcc gccaca 497

<210> 6
 <211> 133
 <212> PRT
 <213> Heliothus virescens]

<400> 6
 Met Arg Val Ile Leu Phe Thr Ala Leu Ala Leu Leu Gly Thr Ala Leu
 1 5 10 15

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

Asn Phe Glu Gly Val Ile Ser Ala Asn Asn Phe Ile Leu Val Glu Phe
 35 40 45

Tyr Ala Pro Trp Cys Gly His Cys Lys Ser Leu Ala Pro Glu Tyr Ala
 50 55 60

Lys Ala Ala Thr Lys Leu Ala Glu Glu Glu Ser Pro Ile Lys Leu Ala
 65 70 75 80

Lys Val Asp Ala Thr Gln Glu Gln Glu Leu Ala Glu Ser Tyr Gly Val
 85 90 95

Arg Gly Tyr Pro Thr Leu Lys Phe Phe Arg Asn Gly Ser Pro Ile Asp
 100 105 110

Tyr Thr Gly Gly Arg Gln Ala Asp Asp Ile Val Ser Trp Leu Lys Lys
 115 120 125

Lys Thr Gly Pro Pro
 - 130

<210> 7
 <211> 1859
 <212> DNA
 <213> *Vaejovis carolinianus*

<400> 7
 gcacgagttt tagtagaaaa atgttggttg cggttacttt atctacaatt ttactagttg 60
 ttatagcagc agcagatgaa atcaagaaaag acggcgaaagt tctagtttta aacaaagaca 120
 atttccaaaa agctattcaa gaaaacaaac atatcttggt tgaattttat gcaccatggt 180
 gtggccactg taaagcactt gagccagaat atgccaaagc agccaagcag cttaaagaag 240
 aacagtctga tattgctctt ggtaagattg atgccacagc agagagtgaag ttagcgggaag 300
 agtatgatgt ccgaggatat cctacaataa aattcatcag ggatggcaaa ccttcagagt 360
 acaaagggtg tcgaacagcg gaagatattg ttcgctggct gaagaagaaa gttggtcctc 420
 cagctgaaaa tcttgacaca gttgacagtg tcaaaacatt ccaatcaagt gcagaagttg 480
 tattagttgg tttctttaaa gatcaatcat cagataaagc taagggtgtt ttggaggtag 540
 ctttagaatc tgatgactat gcttttgga tccatcaca ggatgatgtg ttttaaggcat 600
 ataagtgtga aaaagatgga attgttttgt ttaaaccagt tgatgaagga agaaatgact 660
 ttgaaggaga aataacagca gatgctttaa aagagtttat taatgctaac agcttgccat 720
 tgggtgttga gttcaatcaa gatactgcac agaaagtctt tgggtggtgaa atcaaagctc 780
 acaatttact tttgttcagc aaacagcaga gtgaagagta tgagaaatta cttgaagtct 840
 tccgtaaagt tgccaaagat tttaagaata aggtattggt tgttacaatt gacatagatg 900
 aagaggacca tgaaagaata atggaatttt ttggaatgaa gaaagaagat gcaccagata 960
 tgcgtttgat cagattagaa gaggagatga caaagttaa accaccatct cctggactct 1020
 cagaagaaaa tatacgtctt tttgttcagg gagttttaga tggaaaaatc aagaggcact 1080
 tgctgtctga aagtgttcca gatgactggg acaagggagc agttaagggt ctggttgga 1140
 aaaacttcga tgatgttgca tttgacaaat caaaagatgt gcttgttgag ttttatgcac 1200
 cttggtgtgg acattgcaaa cagcttgcac ccatctatga agaacttgga gagaagtaca 1260
 aagatcagtc atctattggt attgcaaaaa tggatgccac taccaatgaa ctggaccatg 1320
 taaaaatcca tagttttccc acaattaaat tattcaaaaa agataccaat gaggtcatag 1380
 acttcaatgg tgaacgcaca cttgaaggac tgacaaaatt tattgattca ggaggagtgg 1440
 atggcgcttc accaaaggaa gaggagattg acgaagagga ggaaaaggat gatgatgaaa 1500
 agaaaaggga cgaactgtga tttttcactg aaaactaatt tacacatggt gcttcattgc 1560
 agcagatttt gtccattcct gaattttcct gtgtacagaa aagggttgtg tgaacaatag 1620
 gttttggcaa catttaacgt aacaaacaag gcttgctaag tattgccatt tatcaaatgt 1680
 taaaatgcca aagcatattt gtaggaagtc tttttaattt aaaatatttt tcccctaact 1740
 ttttattttt tatcttttat taaaatgaat gactttttaa tcatcggtgg tgcaaatgct 1800
 ggctcgtgga attatttgga atgctaaaac aaagcaaaac aaattgcgat ggtcgtact 1859

<210> 8
 <211> 499
 <212> PRT
 <213> *Vaejovis carolinianus*

<400> 8
Met Leu Gly Ala Val Thr Leu Ser Thr Ile Leu Leu Val Val Ile Ala
1 5 10 15
Ala Ala Asp Glu Ile Lys Lys Asp Gly Glu Val Leu Val Leu Asn Lys
20 25 30
Asp Asn Phe Gln Lys Ala Ile Gln Glu Asn Lys His Ile Leu Val Glu
35 40 45
Phe Tyr Ala Pro Trp Cys Gly His Cys Lys Ala Leu Glu Pro Glu Tyr
50 55 60
Ala Lys Ala Ala Lys Gln Leu Lys Glu Glu Gln Ser Asp Ile Ala Leu
65 70 75 80
Gly Lys Ile Asp Ala Thr Ala Glu Ser Glu Leu Ala Glu Glu Tyr Asp
85 90 95
Val Arg Gly Tyr Pro Thr Ile Lys Phe Ile Arg Asp Gly Lys Pro Ser
100 105 110
Glu Tyr Lys Gly Gly Arg Thr Ala Glu Asp Ile Val Arg Trp Leu Lys
115 120 125
Lys Lys Val Gly Pro Pro Ala Glu Asn Leu Asp Thr Val Asp Ser Val
130 135 140
Lys Thr Phe Gln Ser Ser Ala Glu Val Val Leu Val Gly Phe Phe Lys
145 150 155 160
Asp Gln Ser Ser Asp Lys Ala Lys Val Phe Leu Glu Val Ala Leu Glu
165 170 175
Ser Asp Asp Tyr Ala Phe Gly Ile Thr Ser Gln Asp Asp Val Phe Lys
180 185 190
Ala Tyr Asn Val Glu Lys Asp Gly Ile Val Leu Phe Lys Gln Phe Asp
195 200 205
Glu Gly Arg Asn Asp Phe Glu Gly Glu Ile Thr Ala Asp Ala Leu Lys
210 215 220
Glu Phe Ile Asn Ala Asn Ser Leu Pro Leu Val Val Glu Phe Asn Gln
225 230 235 240
Asp Thr Ala Gln Lys Val Phe Gly Gly Glu Ile Lys Ala His Asn Leu
245 250 255
Leu Phe Val Ser Lys Gln Gln Ser Glu Glu Tyr Glu Lys Leu Leu Glu
260 265 270
Val Phe Arg Lys Val Ala Lys Asp Phe Lys Asn Lys Val Leu Phe Val
275 280 285
Thr Ile Asp Ile Asp Glu Glu Asp His Glu Arg Ile Met Glu Phe Phe
290 295 300
Gly Met Lys Lys Glu Asp Ala Pro Asp Met Arg Leu Ile Arg Leu Glu
305 310 315 320
Glu Glu Met Thr Lys Phe Lys Pro Pro Ser Pro Gly Leu Ser Glu Glu
325 330 335
Asn Ile Arg Ser Phe Val Gln Gly Val Leu Asp Gly Lys Ile Lys Arg
340 345 350

His Leu Leu Ser Glu Ser Val Pro Asp Asp Trp Asp Lys Gly Ala Val
 355 360 365
 Lys Val Leu Val Gly Gln Asn Phe Asp Asp Val Ala Phe Asp Lys Ser
 370 375 380
 Lys Asp Val Leu Val Glu Phe Tyr Ala Pro Trp Cys Gly His Cys Lys
 385 390 395 400
 Gln Leu Ala Pro Ile Tyr Glu Glu Leu Gly Glu Lys Tyr Lys Asp Gln
 405 410 415
 Ser Ser Ile Val Ile Ala Lys Met Asp Ala Thr Thr Asn Glu Leu Asp
 420 425 430
 His Val Lys Ile His Ser Phe Pro Thr Ile Lys Leu Phe Lys Lys Asp
 435 440 445
 Thr Asn Glu Val Ile Asp Phe Asn Gly Glu Arg Thr Leu Glu Gly Leu
 450 455 460
 Thr Lys Phe Ile Asp Ser Gly Gly Val Asp Gly Ala Ser Pro Lys Glu
 465 470 475 480
 Glu Glu Ile Asp Glu Glu Glu Glu Lys Asp Asp Asp Glu Lys Lys Arg
 485 490 495

Asp Glu Leu

<210> 9
 <211> 2170
 <212> DNA
 <213> Argiope sp.

<400> 9
 gcacgagaaa aagtataagc atcagtgaac cgctgaagtc tattgatttt taccaaaaaga 60
 ttacacaatga agctgttatt tctgtcatca atacttagtt gtacattatc tgtaataactt 120
 tgtagtgatg tcctagattt ttccggtgcc gatttcgaag atcgaatagc agaacatgat 180
 gcaatttttag ttgaattctt cgctccttgg tgtggacatt gtaagagatt agcacctgaa 240
 tatgacaaaag catctacgat attaaaaaaa gctgacccac ctataccact agcaaagggt 300
 gactgcacat cagataatgg taaagataca tgttcaaaat atgggtgtaag tgggtatcct 360
 acttttaaaga tatttagagg tggagaattt tcttcagaat ataattggtcc tctgtatgca 420
 gatggtattg ttaagtatat gaaggctcaa gttgggtccaa gttcaaaaaga acttcaatct 480
 ttggaagatg cagagaaaat tttaaaagat gatattgttg tgattgggta ctttgctgat 540
 tcttcaaata aattaaagga agaattctta aaagcagctg ataaactcag agaattctgta 600
 tcttttgcac atacatctag taaagacatt tttagacaaat atgggtattc tgatgaaatt 660
 gttttataacc gtcccaagat cttctggagt aaatttgaac cccaagaaat aaaatataca 720
 ggtgatgcag ataaaggaga gattttctcaa ttatttaaag acaactacca tgggtcttgtt 780
 ggtcatagaa cacatgacaa tcacgatgac tttaaggctc ctcttattgt tgtatattat 840
 gatgtagact atgtaaagaa tgtaaaaggc acaactact ggcgtaacag agtaatgaag 900
 gttgcacaga actacaaggg acaagtcaac tttgtatta gtaacaaaga caaattttct 960
 gctgaagttg aagatttttg tttaaaagct acaggagata aacctgtagt tgctgccaga 1020
 aatgataaaa accagaaatt taatatgaag gaagaattca gtgttgaaaa ttttgaaact 1080
 tttgtgaaaa aatttttggg tggcagttta gaaccacact tgaaatctga acctgttctt 1140
 gaaaagaacg acgggcctgt taaagtagct gttgcccaga actttgaaga acttgtaatg 1200
 gaaaatgaca aagatgtatt gatcgaattt tatgccccat ggtgtggaca ttgtaagaaa 1260
 ttggctccta cttatgaaga acttggtcaa aagttggaag gtgaagatgt agagattgtg 1320
 aaaatggatg ctacagctaa tgatgttcca cctacatttg aggttcatgg attcccaaca 1380
 ttatatggg tacctaaaac acacaagtct agtccgaaga aatatgaagg tggccgagaa 1440
 atcaaggact ttatcaatta cattgccaa catgctacaa atgaattgaa acaatatgac 1500
 cgatctggca aaaagaagtc caaggaagag ctataagtta atatcactct tttaggaact 1560
 atttttcaat gtgcatagtt tgtgttaagc atcaatcatg ttaattctgt tacatatata 1620
 ttgaacaaat gtggttaggc atgtactgat catctgttca tacgttccaa attgactctg 1680
 tgggacctaa agtgtctttt tgtaataaaa cgttttttta tacttctgct gtttttttct 1740

```

cccttaacgag  tgggaattttt  ttactttttt  cttttattct  ttattaatgg  gtattgagtc  1800
tgaaattatt  ttttagtct  tttattaatc  ttttccttgt  tttttaaga  ttgaatatct  1860
actaaacatt  ttctttaaaa  aaatctatgc  aatttgtaat  atgccatcat  gaatttcatt  1920
gaagttcttg  tatattcatt  agatcaatca  aaatatattt  gcagtgcaga  tatttaaagc  1980
tgaacaattt  cttcgtagag  taatacacat  gtatttaaaa  tgatctgcaa  aagccttaac  2040
tttgaatggt  acttgtagat  ttgttgcaaa  atagcatttg  tcgtatttta  tgtaattttt  2100
ctatttaaat  cgattgaaat  aaaggtagtt  cttttaaaaa  aaaaaaaaaa  aaaaaaaaaa  2160
aaaaaaaaaa  2170

```

<210> 10
 <211> 489
 <212> PRT
 <213> Argiope sp.

<400> 10

```

Met Lys Leu Leu Phe Leu Ser Ser Ile Leu Ser Cys Thr Leu Ser Val
 1           5           10           15

Ile Leu Cys Ser Asp Val Leu Asp Phe Ser Gly Ala Asp Phe Glu Asp
          20           25           30

Arg Ile Ala Glu His Asp Ala Ile Leu Val Glu Phe Phe Ala Pro Trp
      35           40           45

Cys Gly His Cys Lys Arg Leu Ala Pro Glu Tyr Asp Lys Ala Ser Thr
      50           55           60

Ile Leu Lys Lys Ala Asp Pro Pro Ile Pro Leu Ala Lys Val Asp Cys
      65           70           75           80

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

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

Asn Gly Pro Arg Asp Ala Asp Gly Ile Val Lys Tyr Met Lys Ala Gln
      115           120           125

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

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

Asn Lys Leu Lys Glu Glu Phe Leu Lys Ala Ala Asp Lys Leu Arg Glu
          165           170           175

Ser Val Ser Phe Ala His Thr Ser Ser Lys Asp Ile Leu Asp Lys Tyr
          180           185           190

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

Lys Phe Glu Pro Gln Glu Ile Lys Tyr Thr Gly Asp Ala Asp Lys Gly
      210           215           220

Glu Ile Ser Gln Phe Ile Lys Asp Asn Tyr His Gly Leu Val Gly His
      225           230           235           240

Arg Thr His Asp Asn His Asp Asp Phe Lys Ala Pro Leu Ile Val Val
          245           250           255

Tyr Tyr Asp Val Asp Tyr Val Lys Asn Val Lys Gly Thr Asn Tyr Trp
      260           265           270

```

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

<210> 11
 <211> 1584
 <212> DNA
 <213> Hottentotta judiaca

<400> 11
 gcacgagact tgggtccaaaa tatgaagagg cagcaacaat tttgaagaaa aatgatccac 60
 ctgttccttt agctaagggt gattgtacat ctgatgctgg aaaagaaact tgttcaaaaat 120
 atgggtgtcag tggctatcct actctcaaga ttttccgaaa tgggtgaattt tcttcagagt 180
 acagtgggtgg gcgagaaaaca gatgctattg taaaatatat gaaatctcaa gttggaccac 240
 gctctgtaga aattaaaaaca cctgctgatg ctaaaaagtt attaagcaga atcgaagtag 300
 ttataattgg attttttaag gatgaaaaaa gtcagttgaa agaagaattt ttaaaagttg 360
 cagataaaat gagagagacc acttcctttg gacacacatc taatcaagaa gttcttgatt 420
 tatatggata taaagatcaa atagtgtctt tccgtcctca gcatcttcaa agcaaatttg 480
 aagaaaaaga attaaaatat gaaggtgggtg ctgaaaaaatc aaaaatagaa gatttcatta 540
 gagaaaatta tcatggattg gttgggtcatc gaactagtga taatttccaa gatttcaaga 600
 atcctcttgt tgtagcatat tatgatgtag actatgtaaa aaatactaaa ggtacaaatt 660
 attggcgtaa tcgtatcatg aaagtagcac aacatttcaa agacaaattg aactttgctg 720
 tatctaatat aaatcaattt tctgctgaaa ttgaagaatt tggcctaact gttaaagggtg 780
 ataagcctgc aattgcagta cgtaatgaaa agcaacagaa attccgaatg actgatgaat 840
 ttagcatgga tgcatttgag aaatttctta aggacttttt agatggaaag ttagaagcac 900
 acgtaaaatc tgaaccaatt ccagaaaata atgatggacc agttaagggtt gcagttgcat 960
 caaattttga tgaatttgta acaaataatg ataaagacat cttgctagaa ttttatgctc 1020
 catgggtgtgg acattgtaaa aaacttgctc caacatatga agaattaggc acagagatga 1080

```

aacaggaaga tgttgaaata gttaaaatgg atgcaacagc aaatgatgtt cctccacctt 1140
atgaagttca tgggtttcca acactttatt gggttccaaa aaatagcaaa aataatccca 1200
aaaaatatga tgggtggtcga gaattggatg atttaataaa atatatatct aaacacgccca 1260
caaatagaatt gaaaggatgg gacaggaagg gcactaaaaa gtctgagaag acagagctct 1320
gagctgttct gtctaaattt ttcacagttg ttagtgataa attcattttt gtgatgtcca 1380
aaattcattt gatttttagt ttacttggga cttcatccac tgactatcat tcctgcagtt 1440
tgtcattaat agcaactggg ggggttgat taagtgtaat gtttaatgaa tatttgtgaa 1500
ttttgaaggt aaatgttaaa taaatgtcaa ttttgtttac aaaaaaaaaa aaaaaaaaaa 1560
aaaaaaaaaa aaaaaaaaaa aaaa 1584

```

<210> 12
 <211> 439
 <212> PRT
 <213> Hottentotta judiaca

<400> 12
 Thr Arg Leu Gly Pro Lys Tyr Glu Glu Ala Ala Thr Ile Leu Lys Lys
 1 5 10 15
 Asn Asp Pro Pro Val Pro Leu Ala Lys Val Asp Cys Thr Ser Asp Ala
 20 25 30
 Gly Lys Glu Thr Cys Ser Lys Tyr Gly Val Ser Gly Tyr Pro Thr Leu
 35 40 45
 Lys Ile Phe Arg Asn Gly Glu Phe Ser Ser Glu Tyr Ser Gly Gly Arg
 50 55 60
 Glu Thr Asp Ala Ile Val Lys Tyr Met Lys Ser Gln Val Gly Pro Ser
 65 70 75 80
 Ser Val Glu Ile Lys Thr Pro Ala Asp Ala Lys Lys Leu Leu Ser Arg
 85 90 95
 Ile Glu Val Val Ile Ile Gly Phe Phe Lys Asp Glu Lys Ser Gln Leu
 100 105 110
 Lys Glu Glu Phe Leu Lys Val Ala Asp Lys Met Arg Glu Thr Thr Ser
 115 120 125
 Phe Gly His Thr Ser Asn Gln Glu Val Leu Asp Leu Tyr Gly Tyr Lys
 130 135 140
 Asp Gln Ile Val Leu Phe Arg Pro Gln His Leu Gln Ser Lys Phe Glu
 145 150 155 160
 Glu Lys Glu Leu Lys Tyr Glu Gly Gly Ala Glu Lys Ser Lys Ile Glu
 165 170 175
 Asp Phe Ile Arg Glu Asn Tyr His Gly Leu Val Gly His Arg Thr Ser
 180 185 190
 Asp Asn Phe Gln Asp Phe Lys Asn Pro Leu Val Val Ala Tyr Tyr Asp
 195 200 205
 Val Asp Tyr Val Lys Asn Thr Lys Gly Thr Asn Tyr Trp Arg Asn Arg
 210 215 220
 Ile Met Lys Val Ala Gln His Phe Lys Asp Lys Leu Asn Phe Ala Val
 225 230 235 240
 Ser Asn Ile Asn Gln Phe Ser Ala Glu Ile Glu Glu Phe Gly Leu Thr
 245 250 255
 Val Lys Gly Asp Lys Pro Ala Ile Ala Val Arg Asn Glu Lys Gln Gln
 260 265 270

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

<210> 13
 <211> 1759
 <212> DNA
 <213> *Scolopendra canidens* DS

<400> 13
 gcacgaggtg aattgccatt tcaccgggtg gagatagaac ggaatttata ttttttttaa 60
 ttgaaaatgt ggaaaatcgt tgctttttcc tgcttttttg tagcgactat tgctagtgat 120
 gtcttagaat ttaccgattc tgattttgac gaaaggatca aagaacatga tacttattta 180
 gtcgagtttt atgcaccatg gtgtgggcat tgtaaacgct tagctccaga atatgaaaaa 240
 gcagctacaa ttctaaaaga taatgatcca cccattcctc ttgttaaggt tgattgtatc 300
 gaatctggaa agaaaacttg tgggaaattt ggagtttccg gttacccaac tctaaaaatt 360
 tttagaaaatg gagacttttc acaagaatac aatggaccaa gagaagcaa tggaattgta 420
 aaatatatgg ctgcccaagt tgggccaaagt tcgaaaagaat tccagaatgt gaaagaagta 480
 cagcaattcc ttgaaaaaga agaagttgca attattggat tttttgaatc tgaagatgct 540
 aaactcaaag caacattcca aaaagtagca gataaattga gagaaactgc aagatttggt 600
 cattcttaca attcattggg gttgaaggaa tatggttata caaatcaagt tgtattattt 660
 cggcctaaac atcttcaaag taagtttgaa gattctcaag tggatatga tggagacaaa 720
 agtgataaac aggagcttga agaatttggt aacaaaaatt accatgggtt agtgggtcat 780
 aggacaacag acaatactaa tcaattctct cctccattga tagtatcata ttataaagtt 840
 gattatgtta agaacaccaa aggtaccaat tattggcgta atcgtatcat gaaagttgca 900
 tcagaattca aagggcgctt gaattttgcc atttcaaata aagatgaatt tacacatgaa 960
 ttaagcgaat atggttttta ctatgtggct ggagataaac cagttgttgc tgctcgaaat 1020
 gcaaaatctg agaaatttgt aatggaaggc gaattttcaa taccaagctt tgaaaagttc 1080
 attaaggact ttttagatga aaaactaaaa ccatacttaa agtctgagcc aattccagag 1140
 aaaaatgaag agcctgttaa ggttgctggt gctcaaaatt ttgaagagt agtcaccaag 1200
 agtgataaag atgtattgat tgaattctat gctccatggt gtggacattg taaaaaatta 1260
 gcacctgttt atgatgaatt aggcacagcc ttggaaggag aaacaacagt agaaatagtt 1320
 aaaatggatg ccacagcaaa tgatgtgcct tcacctatg aagtgcattg tttccctact 1380
 ttatattggg ctccacgtga taagaaagat aagcctgtcc gatacgatgg tggacgagaa 1440
 ttggatgatt tcattaaata tattgctaag cattccacag atgaacttaa aacttacaac 1500
 agaaatggga aaaagaaaaa agttgaattg taaagtagca atttagaatt taaaatattt 1560

gttcagtaaa agcacaattt tttattttta aggggaataaa aatgtaaaaa tcaattatga 1620
 taatttaaaa ttttagagtgc tttatgttgg tggtagctat tgtctttatt tttttgtaag 1680
 ataaaatgtt gaaaattaat tcgaaataaa atttttttta taaattagtt tttacaaaaa 1740
 gaaaaaaaaa aaaaaaaaaa 1759

<210> 14
 <211> 488
 <212> PRT
 <213> Scolopendra canicens DS

<400> 14
 Met Trp Lys Ile Val Ala Phe Ser Cys Phe Phe Val Ala Thr Ile Ala
 1 5 10 15
 Ser Asp Val Leu Glu Phe Thr Asp Ser Asp Phe Asp Glu Arg Ile Lys
 20 25 30
 Glu His Asp Thr Tyr Leu Val Glu Phe Tyr Ala Pro Trp Cys Gly His
 35 40 45
 Cys Lys Arg Leu Ala Pro Glu Tyr Glu Lys Ala Ala Thr Ile Leu Lys
 50 55 60
 Asp Asn Asp Pro Pro Ile Pro Leu Val Lys Val Asp Cys Ile Glu Ser
 65 70 75 80
 Gly Lys Glu Thr Cys Gly Lys Phe Gly Val Ser Gly Tyr Pro Thr Leu
 85 90 95
 Lys Ile Phe Arg Asn Gly Asp Phe Ser Gln Glu Tyr Asn Gly Pro Arg
 100 105 110
 Glu Ala Asn Gly Ile Val Lys Tyr Met Ala Ala Gln Val Gly Pro Ser
 115 120 125
 Ser Lys Glu Phe Gln Asn Val Lys Glu Val Gln Gln Phe Leu Glu Lys
 130 135 140
 Glu Glu Val Ala Ile Ile Gly Phe Phe Glu Ser Glu Asp Ala Lys Leu
 145 150 155 160
 Lys Ala Thr Phe Gln Lys Val Ala Asp Lys Leu Arg Glu Thr Ala Arg
 165 170 175
 Phe Gly His Ser Tyr Asn Ser Leu Val Leu Lys Glu Tyr Gly Tyr Thr
 180 185 190
 Asn Gln Val Val Leu Phe Arg Pro Lys His Leu Gln Ser Lys Phe Glu
 195 200 205
 Asp Ser Gln Val Val Tyr Asp Gly Asp Lys Ser Asp Lys Gln Glu Leu
 210 215 220
 Glu Glu Phe Val Asn Lys Asn Tyr His Gly Leu Val Gly His Arg Thr
 225 230 235 240
 Thr Asp Asn Thr Asn Gln Phe Ser Pro Pro Leu Ile Val Ser Tyr Tyr
 245 250 255
 Lys Val Asp Tyr Val Lys Asn Thr Lys Gly Thr Asn Tyr Trp Arg Asn
 260 265 270
 Arg Ile Met Lys Val Ala Ser Glu Phe Lys Gly Arg Leu Asn Phe Ala
 275 280 285

Ile Ser Asn Lys Asp Glu Phe Thr His Glu Leu Ser Glu Tyr Gly Phe
 290 295 300

Asn Tyr Val Ala Gly Asp Lys Pro Val Val Ala Ala Arg Asn Ala Lys
 305 310 315 320

Ser Glu Lys Phe Val Met Glu Gly Glu Phe Ser Ile Pro Ser Phe Glu
 325 330 335

Lys Phe Ile Lys Asp Phe Leu Asp Glu Lys Leu Lys Pro Tyr Leu Lys
 340 345 350

Ser Glu Pro Ile Pro Glu Lys Asn Glu Glu Pro Val Lys Val Ala Val
 355 360 365

Ala Gln Asn Phe Glu Glu Leu Val Thr Lys Ser Asp Lys Asp Val Leu
 370 375 380

Ile Glu Phe Tyr Ala Pro Trp Cys Gly His Cys Lys Lys Leu Ala Pro
 385 390 395 400

Val Tyr Asp Glu Leu Gly Lys Ala Leu Glu Gly Glu Thr Thr Val Glu
 405 410 415

Ile Val Lys Met Asp Ala Thr Ala Asn Asp Val Pro Ser Pro Tyr Glu
 420 425 430

Val His Gly Phe Pro Thr Leu Tyr Trp Ala Pro Arg Asp Lys Lys Asp
 435 440 445

Lys Pro Val Arg Tyr Asp Gly Gly Arg Glu Leu Asp Asp Phe Ile Lys
 450 455 460

Tyr Ile Ala Lys His Ser Thr Asp Glu Leu Lys Thr Tyr Asn Arg Asn
 465 470 475 480

Gly Lys Lys Lys Lys Val Glu Leu
 485

<210> 15
 <211> 1498
 <212> DNA
 <213> Hottentotta judiaca

<400> 15
 agatctgttaa ggatcatgaat tttggttaatt tattaatctt tttttctttt ttaatagtcg 60
 tattaggtga agttagagaa gacaatgtat tagttttgaa taaagaaaat tttgatcatt 120
 caattaaaaa caacaagtat atcttagtag aattttatgc tccatggtgt ggacattgta 180
 aagcactagc tccagaatat gctaaagctg caaagctgtt gttagaagaa aaatctgaaa 240
 ttcagttagc aaaaattgat gcaactgaag aaacagaatt agcagagaag cataaagtaa 300
 aaggttatcc aacaattaaa ttcttccgtg aagggtgatcc tattgattat acaggtggcc 360
 gtactggtga tgatattgta acttggttga agaaaaaac tggacctcca gctacattat 420
 taagtacagt tgatgaagca aaaaacttta aagagagtaa agatgtcgta attataggat 480
 ttttcaagga tcaggaaagt aatcaagcta aagaatattt aaatgcagca tatatgactg 540
 atgatcatcc atttggtatt acttcagatg aaaaatttta taaacatttt aatgttgaaa 600
 aggatactat tttcttattt aagaagtttg atgaggggaa gaatgaattc gagggagaat 660
 ttacaaaaga taacattata aaattcatta aactcaacaa tttaccatta gtaattgaat 720
 ttagtcaaga gaatgcacag aagatatttg ggggtgacat aaaaatgcat aatcttcttt 780
 ttattagtaa aaagagtaaa gattttgatg aaatagttaa aacgtttcgt attgtggcaa 840
 aagaatataa aaatcagata ttatttggtg ttattaatac tgatgatgaa gacaatgaaa 900
 aaataactga attctttggt ttaaaaaaag atgagcagcc atcaataaga ttaattaaac 960
 tagaagaagg aatgtctaaa tataaacctg aaactattga aatttctgaa gaaaatgttc 1020
 gaaaatttgt taaaggtgtc ttatagtgaa cagttaaaca acatctactt tctcaagaac 1080
 ttcttgaaga ttgggataag catccagtta aggtattagt aaataagaat ttcgatgaag 1140
 ttgcatttga taaaactaaa gatgttattg tagaattcta tgcaccatgg tgtgggtcatt 1200
 gcaaacagtt agctccaatt tatgaagaac tcggagaaaa atataaaaaat cgaaatgata 1260

ttattattgc aaaaatggat gcaacagcca atgaattaga acatacaaaa attaacagct 1320
 ttcctacaat taaattatat aaaaaaggaa ccaatgaagt gatagactat gatggaaaac 1380
 attcacttga aggacttgtg aattttattg attctggtgg aaaaataaca aaggaacctg 1440
 aagatgagga taaatcaaaa gaaccagatg ccaaaggaga tgaattatga gcggccgc 1498

<210> 16
 <211> 491
 <212> PRT
 <213> Hottentotta judiaca

<400> 16

Met Asn Phe Gly Asn Leu Leu Ile Phe Phe Ser Phe Leu Ile Val Val
 1 5 10 15
 Leu Gly Glu Val Arg Glu Asp Asn Val Leu Val Leu Asn Lys Glu Asn
 20 25 30
 Phe Asp His Ser Ile Lys Asn Asn Lys Tyr Ile Leu Val Glu Phe Tyr
 35 40 45
 Ala Pro Trp Cys Gly His Cys Lys Ala Leu Ala Pro Glu Tyr Ala Lys
 50 55 60
 Ala Ala Lys Leu Leu Leu Glu Glu Lys Ser Glu Ile Gln Leu Ala Lys
 65 70 75 80
 Ile Asp Ala Thr Glu Glu Thr Glu Leu Ala Glu Lys His Lys Val Lys
 85 90 95
 Gly Tyr Pro Thr Ile Lys Phe Phe Arg Glu Gly Asp Pro Ile Asp Tyr
 100 105 110
 Thr Gly Gly Arg Thr Gly Asp Asp Ile Val Thr Trp Leu Lys Lys Lys
 115 120 125
 Thr Gly Pro Pro Ala Thr Leu Leu Ser Thr Val Asp Glu Ala Thr Asn
 130 135 140
 Phe Lys Glu Ser Lys Asp Val Val Ile Ile Gly Phe Phe Lys Asp Gln
 145 150 155 160
 Glu Ser Asn Gln Ala Lys Glu Tyr Leu Asn Ala Ala Tyr Met Thr Asp
 165 170 175
 Asp His Pro Phe Gly Ile Thr Ser Asp Glu Asn Val Tyr Lys His Phe
 180 185 190
 Asn Val Glu Lys Asp Thr Ile Phe Leu Phe Lys Lys Phe Asp Glu Gly
 195 200 205
 Lys Asn Glu Phe Glu Gly Glu Phe Thr Lys Asp Asn Ile Ile Lys Phe
 210 215 220
 Ile Lys Leu Asn Asn Leu Pro Leu Val Ile Glu Phe Ser Gln Glu Asn
 225 230 235 240
 Ala Gln Lys Ile Phe Gly Gly Asp Ile Lys Met His Asn Leu Leu Phe
 245 250 255
 Ile Ser Lys Lys Ser Lys Asp Phe Asp Glu Ile Val Lys Thr Phe Arg
 260 265 270
 Ile Val Ala Lys Glu Tyr Lys Asn Gln Ile Leu Phe Val Val Ile Asn
 275 280 285

Thr Asp Asp Glu Asp Asn Glu Lys Ile Thr Glu Phe Phe Gly Leu Lys
 290 295 300
 Lys Asp Glu Gln Pro Ser Ile Arg Leu Ile Lys Leu Glu Glu Gly Met
 305 310 315 320
 Ser Lys Tyr Lys Pro Glu Thr Ile Glu Ile Ser Glu Glu Asn Val Arg
 325 330 335
 Lys Phe Val Lys Gly Val Leu Asp Gly Thr Val Lys Gln His Leu Leu
 340 345 350
 Ser Gln Glu Leu Pro Glu Asp Trp Asp Lys His Pro Val Lys Val Leu
 355 360 365
 Val Asn Lys Asn Phe Asp Glu Val Ala Phe Asp Lys Thr Lys Asp Val
 370 375 380
 Ile Val Glu Phe Tyr Ala Pro Trp Cys Gly His Cys Lys Gln Leu Ala
 385 390 395 400
 Pro Ile Tyr Glu Glu Leu Gly Glu Lys Tyr Lys Asn Arg Asn Asp Ile
 405 410 415
 Ile Ile Ala Lys Met Asp Ala Thr Ala Asn Glu Leu Glu His Thr Lys
 420 425 430
 Ile Asn Ser Phe Pro Thr Ile Lys Leu Tyr Lys Lys Gly Thr Asn Glu
 435 440 445
 Val Ile Asp Tyr Asp Gly Lys His Ser Leu Glu Gly Leu Val Asn Phe
 450 455 460
 Ile Asp Ser Gly Gly Lys Ile Thr Lys Glu Pro Glu Asp Glu Asp Lys
 465 470 475 480
 Ser Lys Glu Pro Asp Ala Lys Gly Asp Glu Leu
 485 490

<210> 17
 <211> 1498
 <212> DNA
 <213> Hottentotta judiaca

<400> 17
 agatctgtaa ggtcatgaat tttggttaatt tattaatctt tttttctttt ttaatagtcg 60
 tattaggtga agttagagag gacaatgtat tagttttgaa taaagaaaat tttgatcatt 120
 caattaaaaa caacaagtat atcttagtag aattttatgc tccatggtgt ggacattgta 180
 aagcactagc tccagaatat gctaaagctg caaagctgtt gttagaagaa aaatctgaaa 240
 ttcagttagc aaaaattgat gcaactgaag aaacagaatt agcagagaag cataaagtaa 300
 aaggttatcc aacaattaaa ttcttcctg aggtgatcc tattgattat acaggtggcc 360
 gtactggtga tgatattgta acttggttga agaaaaaac tggacctcca gctacattat 420
 taagtacagt tgatgaagca acaaacttta aagagagtaa agatgtcgt attataggat 480
 ttttcaagga tcaggaaaagt aatcaagcta aagaatattt aaatgcagca tatatgactg 540
 atgatcatcc atttggtatt acttcagatg aaaatgttta taaacatttt aatggtgaaa 600
 aggatactat tttcttattt aagaagtttg atgaggggaa gaatgaattc gagggagaat 660
 ttacaaaaga taacattata aaattcatta aactcaacaa tttaccatta gtaattgaat 720
 ttagtcaaga gaatgcacag aagatatttg ggggtgacat aaaaatgcat aatcttcttt 780
 ttattagtaa aaagagtaaa gattttgatg aaatagttaa aacgtttcgt attgtggcaa 840
 aagaatataa aaatcagata ttatttggtt ttattaatac tgatgatgaa ggcaatggac 900
 aaataactga attctttggt ttaaaaaagg atgagcagcc atcaataaga ttaattaaac 960
 tagaagaagg aatgtctaaa tataaacctg aaactattga aatttctgaa gaaaatgttc 1020
 gaaaatttgt taaaggtgtc ttagatggaa cagttaaaca acatctactt tctcaagaac 1080
 ttcctgaaga ttgggataag catccagtta aagtattagt aaataagaat ttcgatgaag 1140
 ttgcatttga taaaactaaa gatgttattg tagaattcta tgcaccatgg tgtggtcatt 1200
 gcaaacagtt agctccaatt tatgaagaac tcggagaaaa atataaaaat cgaaatgata 1260

ttattattgc aaaaatggat gcaacagcca atgaattaga acatacaaaa attaacagct 1320
 ttcttacaat taaattatat aaaaaaggaa ccaatgaagt gatagactat gatggaaaac 1380
 attcacttga aggacttggtg aattttattg attctggtgg aaaaataaca aaggaacctg 1440
 aagatgagga taaatcaaaa gaaccagatg ccaaaagaga tgaattatga gcggccgc 1498

<210> 18
 <211> 491
 <212> PRT
 <213> Hottentotta judiaca

<400> 18
 Met Asn Phe Gly Asn Leu Leu Ile Phe Phe Ser Phe Leu Ile Val Val
 1 5 10 15
 Leu Gly Glu Val Arg Glu Asp Asn Val Leu Val Leu Asn Lys Glu Asn
 20 25 30
 Phe Asp His Ser Ile Lys Asn Asn Lys Tyr Ile Leu Val Glu Phe Tyr
 35 40 45
 Ala Pro Trp Cys Gly His Cys Lys Ala Leu Ala Pro Glu Tyr Ala Lys
 50 55 60
 Ala Ala Lys Leu Leu Leu Glu Glu Lys Ser Glu Ile Gln Leu Ala Lys
 65 70 75 80
 Ile Asp Ala Thr Glu Glu Thr Glu Leu Ala Glu Lys His Lys Val Lys
 85 90 95
 Gly Tyr Pro Thr Ile Lys Phe Phe Arg Glu Gly Asp Pro Ile Asp Tyr
 100 105 110
 Thr Gly Gly Arg Thr Gly Asp Asp Ile Val Thr Trp Leu Lys Lys Lys
 115 120 125
 Thr Gly Pro Pro Ala Thr Leu Leu Ser Thr Val Asp Glu Ala Thr Asn
 130 135 140
 Phe Lys Glu Ser Lys Asp Val Val Ile Ile Gly Phe Phe Lys Asp Gln
 145 150 155 160
 Glu Ser Asn Gln Ala Lys Glu Tyr Leu Asn Ala Ala Tyr Met Thr Asp
 165 170 175
 Asp His Pro Phe Gly Ile Thr Ser Asp Glu Asn Val Tyr Lys His Phe
 180 185 190
 Asn Val Glu Lys Asp Thr Ile Phe Leu Phe Lys Lys Phe Asp Glu Gly
 195 200 205
 Lys Asn Glu Phe Glu Gly Glu Phe Thr Lys Asp Asn Ile Ile Lys Phe
 210 215 220
 Ile Lys Leu Asn Asn Leu Pro Leu Val Ile Glu Phe Ser Gln Glu Asn
 225 230 235 240
 Ala Gln Lys Ile Phe Gly Gly Asp Ile Lys Met His Asn Leu Leu Phe
 245 250 255
 Ile Ser Lys Lys Ser Lys Asp Phe Asp Glu Ile Val Lys Thr Phe Arg
 260 265 270
 Ile Val Ala Lys Glu Tyr Lys Asn Gln Ile Leu Phe Val Val Ile Asn
 275 280 285

Thr Asp Asp Glu Gly Asn Gly Gln Ile Thr Glu Phe Phe Gly Leu Lys
 290 295 300

Lys Asp Glu Gln Pro Ser Ile Arg Leu Ile Lys Leu Glu Glu Gly Met
 305 310 315 320

Ser Lys Tyr Lys Pro Glu Thr Ile Glu Ile Ser Glu Glu Asn Val Arg
 325 330 335

Lys Phe Val Lys Gly Val Leu Asp Gly Thr Val Lys Gln His Leu Leu
 340 345 350

Ser Gln Glu Leu Pro Glu Asp Trp Asp Lys His Pro Val Lys Val Leu
 355 360 365

Val Asn Lys Asn Phe Asp Glu Val Ala Phe Asp Lys Thr Lys Asp Val
 370 375 380

Ile Val Glu Phe Tyr Ala Pro Trp Cys Gly His Cys Lys Gln Leu Ala
 385 390 395 400

Pro Ile Tyr Glu Glu Leu Gly Glu Lys Tyr Lys Asn Arg Asn Asp Ile
 405 410 415

Ile Ile Ala Lys Met Asp Ala Thr Ala Asn Glu Leu Glu His Thr Lys
 420 425 430

Ile Asn Ser Phe Pro Thr Ile Lys Leu Tyr Lys Lys Gly Thr Asn Glu
 435 440 445

Val Ile Asp Tyr Asp Gly Lys His Ser Leu Glu Gly Leu Val Asn Phe
 450 455 460

Ile Asp Ser Gly Gly Lys Ile Thr Lys Glu Pro Glu Asp Glu Asp Lys
 465 470 475 480

Ser Lys Glu Pro Asp Ala Lys Arg Asp Glu Leu
 485 490

<210> 19
 <211> 802
 <212> DNA
 <213> Hottentotta judiaca

<400> 19
 gcacgaggga acatgggatg ttacttggtg gtgttggtga tatttttatt ctttctacga 60
 gatatgcaaa gtagcagtga tttatacacg gataactcga taaaatatga cgagggaagga 120
 tttaggagga atataggaaa tatagtgcac tttgtttaat tttagcggcc ttggtgtgga 180
 cattgtaaaa gattagcacc aatttgggat gaattagcag agaaatataa taaacctgga 240
 gaacagaagc ttgttattgc taaaattgat tgtacaactg aaactgctct ttgttctgaa 300
 caaggaatta ctggttatcc cacattaaag ttttttaaga aaggtacaac tgaaggacat 360
 aaatatagag gtccacgtga cattacttct ttagaagctt ttattgcaa tagcttagga 420
 cacgaagagg ctattaaaaa atctcctgaa cctccaaaat tcataaatga aattattcag 480
 ttaagtgaac atacttttca taaatttgta gcaaaaaggac ttcattttgt taaattttat 540
 gctccttggg gtggtcactg tcagaaactt gttcccattt ggaaagaatt ggcaaatagc 600
 tttaaatttg atacatccat aaaaatatct gagattgatt gcactacaca acatttagta 660
 tgtaaatgaat ttgaagttaa agcatatcca actttattgt ggattgttga tggtaaaaag 720
 attgaaaagt atgaaggaat gagatcccat gaagaactaa aattatttat taataaaatg 780
 aaagaaaaaa aaaaaaaaaa aa 802

<210> 20
 <211> 244
 <212> PRT
 <213> Hottentotta judiaca

<400> 20

Met Gly Cys Tyr Leu Leu Val Leu Leu Ile Phe Leu Phe Phe Leu Arg
 1 5 10 15

Asp Ser Gln Ser Ser Ser Asp Leu Tyr Thr Asp Asn Ser Ile Lys Tyr
 20 25 30

Asp Glu Glu Gly Phe Arg Arg Asn Ile Gly Asn Ile Val His Phe Val
 35 40 45

Lys Phe Tyr Ala Pro Trp Cys Gly His Cys Lys Arg Leu Ala Pro Ile
 50 55 60

Trp Asp Glu Leu Ala Glu Lys Tyr Asn Lys Pro Gly Glu Gln Lys Leu
 65 70 75 80

Val Ile Ala Lys Ile Asp Cys Thr Thr Glu Thr Ala Leu Cys Ser Glu
 85 90 95

Gln Gly Ile Thr Gly Tyr Pro Thr Leu Lys Phe Phe Lys Lys Gly Thr
 100 105 110

Thr Glu Gly His Lys Tyr Arg Gly Pro Arg Asp Ile Thr Ser Leu Glu
 115 120 125

Ala Phe Ile Ala Asn Ser Leu Gly His Glu Glu Ala Ile Lys Lys Ser
 130 135 140

Pro Glu Pro Pro Lys Phe Ile Asn Glu Ile Ile Gln Leu Ser Asp Asn
 145 150 155 160

Thr Phe His Lys Phe Val Ala Lys Gly Leu His Phe Val Lys Phe Tyr
 165 170 175

Ala Pro Trp Cys Gly His Cys Gln Lys Leu Val Pro Ile Trp Lys Glu
 180 185 190

Leu Ala Asn Ser Phe Lys Phe Asp Thr Ser Ile Lys Ile Ser Glu Ile
 195 200 205

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

Tyr Pro Thr Leu Leu Trp Ile Val Asp Gly Lys Lys Ile Glu Lys Tyr
 225 230 235 240

Glu Gly Met Arg

<210> 21

<211> 1495

<212> DNA

<213> *Heliothus virescens*]

<400> 21

gcacgagaaa cccgatcggt catcagaaaa gtcggactct gacgtgatca cgctgacgga 60
 tgagaacttc aagaagctgg tgctggacag cgaagacctg tggctggttg agttctacgc 120
 gccctgggtgc ggtcactgca agaatttgaa gcccagtggt gccaaaggctg ccaaagaact 180
 taagggaag gtgaaactcg gagcattaga cgcgacagtc caccaagcga tggcttcccg 240
 ctaccaagtg caaggctacc ccaccatcaa gctgttccca tctggcaaga agtccagtga 300
 ctccgcagag gactacaatg gaggcaggac cgccagcgac atcgtgactt atgctcttga 360
 caagctcgct gaaaacgtgc ccgctcctga gatcggtcag gttatcgacg aagcgtcaat 420
 gcaggcgtgc agtgaaaaac cgctgtgcgt ggtatcggtt ctgccgcaca tcttgactg 480
 caacgcggcc tgcgcaacg aatacctagc gatactcgca cgactcgggtg acaagtacaa 540
 gagcaagatg tggggatggg tgtgggccga agctggcgcg cagatatctt tgggaagagtc 600
 gctggagctg ggcggtttcg gttaccccg ccatggctgtc gtcaacgcta agaaactcaa 660

```

gttctcaacc ctcaggggat ccttctccga gactggcacc aatgaattcc ttagggatct 720
atcattcggg cgcggacaga ctgcccagc gaagggcgca gagatgccga agatcgtgtc 780
caccgacccc tgggacggca aggacggtga actgccacaa gaagaggaca ttgacctctc 840
tgacgtagac ctcgagaagg acgagttata agtgcagcag ccatgttgct aacagctctg 900
actttataaa acccaacggg tgagtgttct gtaacaagta cgcttctaca caaatcata 960
tcagtaaaaa tctctgattt taaacttaag aaagtgtatc aagttcaagc atttaacagt 1020
ttaggttact atttattttc accagtggag tagtaacttt gtacctata atatgggtca 1080
gtttaaaatt atgctgtttt aaatatcgaa ggagaagctt aattccatca catactatga 1140
attttatttt ctgaaacatt tttaggtgtt tgataatcac aatttagtac cagccatata 1200
tttcgtgtgt agctcggcgc gagcgagtgg tgcaacgact gatctttttg aatcattgtt 1260
atgtgtatgt atatccttca tagtcataaa attgataaca caaactgata cttaatttta 1320
gttggttagg acattaattg gagtgatcat tagctaaacg ccaatcttcc aatattatgt 1380
ttaattttgg taagcattta ttgtgtgag gagatttgga taattttatg aattgataaa 1440
tcgctaataa atttttaata aaaaaaaaaa aaaaagagag agagagagaa ctagt 1495

```

<210> 22

<211> 289

<212> PRT

<213> Heliothus virescens]

<400> 22

```

His Glu Lys Pro Asp Arg Ser Ser Glu Lys Ser Asp Ser Asp Val Ile
  1                      5                      10                      15

```

```

Thr Leu Thr Asp Glu Asn Phe Lys Lys Leu Val Leu Asp Ser Glu Asp
          20                      25                      30

```

```

Leu Trp Leu Val Glu Phe Tyr Ala Pro Trp Cys Gly His Cys Lys Asn
          35                      40                      45

```

```

Leu Lys Pro Gln Trp Ala Lys Ala Ala Lys Glu Leu Lys Gly Lys Val
          50                      55                      60

```

```

Lys Leu Gly Ala Leu Asp Ala Thr Val His Gln Ala Met Ala Ser Arg
          65                      70                      75                      80

```

```

Tyr Gln Val Gln Gly Tyr Pro Thr Ile Lys Leu Phe Pro Ser Gly Lys
          85                      90                      95

```

```

Lys Ser Ser Asp Ser Ala Glu Asp Tyr Asn Gly Gly Arg Thr Ala Ser
          100                     105                     110

```

```

Asp Ile Val Thr Tyr Ala Leu Asp Lys Leu Ala Glu Asn Val Pro Ala
          115                     120                     125

```

```

Pro Glu Ile Val Gln Val Ile Asp Glu Ala Ser Met Gln Ala Cys Ser
          130                     135                     140

```

```

Glu Lys Pro Leu Cys Val Val Ser Val Leu Pro His Ile Leu Asp Cys
          145                     150                     155                     160

```

```

Asn Ala Ala Cys Arg Asn Glu Tyr Leu Ala Ile Leu Ala Arg Leu Gly
          165                     170                     175

```

```

Asp Lys Tyr Lys Ser Lys Met Trp Gly Trp Val Trp Ala Glu Ala Gly
          180                     185                     190

```

```

Ala Gln Ile Ser Leu Glu Glu Ser Leu Glu Leu Gly Gly Phe Gly Tyr
          195                     200                     205

```

```

Pro Ala Met Ala Val Val Asn Ala Lys Lys Leu Lys Phe Ser Thr Leu
          210                     215                     220

```

```

Arg Gly Ser Phe Ser Glu Thr Gly Ile Asn Glu Phe Leu Arg Asp Leu
          225                     230                     235                     240

```

Ser Phe Gly Arg Gly Gln Thr Ala Pro Val Lys Gly Ala Glu Met Pro
245 250 255

Lys Ile Val Ser Thr Asp Pro Trp Asp Gly Lys Asp Gly Glu Leu Pro
260 265 270

Gln Glu Glu Asp Ile Asp Leu Ser Asp Val Asp Leu Glu Lys Asp Glu
275 280 285

Leu

<210> 23
<211> 473
<212> DNA
<213> *Vaejovis carolinianus*

<220>
<221> unsure
<222> (408)

<220>
<221> unsure
<222> (442)

<220>
<221> unsure
<222> (444)

<400> 23
gtgtgcatct gtttttagtt ttaatgttgc aatatatgct agtagtgact tatacgttga 60
caattcgtta aaatatgatg aagatgggtt tagggaaaat gtaggaaaat tgacgctttt 120
tgtgaaattc tacgccctt ggtgtggaca ttgtaaaaga ttggctccta cttgggacga 180
actagctgaa aaatataata ttcaaccaga aaaacaacag gtcataatag ctaagattga 240
ctgtacatca gagacagctc tttgttctga gcaaggaata acaggttatc caacattaaa 300
gttttttaag aaaggtgaaa ctgaaggaaac aaaatacagg ggaccaagag acatcacatc 360
tttagaagct tttattgcta acagcttggg caaagaagag gctgtggnaa gatcttaaac 420
caccaagaac caagtaaag gncnaataag aattaactga tgaaacattc cac 473

<210> 24
<211> 343
<212> PRT
<213> *Vaejovis carolinianus*

<400> 24
Thr Arg Leu Ser Ser Val Phe Ser Phe Asn Val Ala Ile Tyr Ala Ser
1 5 10 15

Ser Asp Leu Tyr Val Asp Asn Ser Leu Lys Tyr Asp Glu Asp Gly Phe
20 25 30

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

Trp Cys Gly His Cys Lys Arg Leu Ala Pro Thr Trp Asp Glu Leu Ala
50 55 60

Glu Lys Tyr Asn Ile Gln Pro Glu Lys Gln Gln Val Ile Ile Ala Lys
65 70 75 80

Ile Asp Cys Thr Ser Glu Thr Ala Leu Cys Ser Glu Gln Gly Ile Thr
85 90 95

Gly Tyr Pro Thr Leu Lys Phe Phe Lys Lys Gly Glu Thr Glu Gly Thr
100 105 110

Lys Tyr Arg Gly Pro Arg Asp Ile Thr Ser Leu Glu Ala Phe Ile Ala
 115 120 125
 Asn Ser Leu Gly Lys Glu Glu Ala Val Glu Asp Leu Lys Pro Pro Glu
 130 135 140
 Pro Val Asn Gly Leu Ile Glu Leu Thr Asp Glu Thr Phe His Lys Thr
 145 150 155 160
 Ile Glu Arg Gly Tyr His Phe Val Lys Phe Tyr Ala Pro Trp Cys Gly
 165 170 175
 His Cys Gln Lys Leu Ala Pro Val Trp Gln Gln Leu Ala Asn Ser Phe
 180 185 190
 Gln His Asp Leu Ser Val Lys Ile Leu Lys Ile Asp Cys Thr Ala His
 195 200 205
 Arg Leu Ser Cys Asn Glu Phe Glu Val Lys Ala Tyr Pro Thr Leu Leu
 210 215 220
 Trp Ile Val Asp Gly Lys Lys Val Glu Ile Tyr Gln Gly Ser Arg Thr
 225 230 235 240
 His Glu Asp Leu Lys Leu Phe Val Asp Lys Met Arg Arg Gln Glu His
 245 250 255
 Glu Thr Asp Ser Gly Gly Glu His Gly Lys Ile Pro Glu Ser Leu Pro
 260 265 270
 Lys Pro Glu Ala Pro Val Ala Gln Leu Val Ala Ser Asn Phe Glu Asp
 275 280 285
 Ser Ile Lys Asn Gly Val Thr Phe Val Lys Phe Phe Ala Pro Trp Cys
 290 295 300
 Gly His Cys Arg Lys Leu Ala Pro Ile Trp Asp Glu Leu Ser Trp Glu
 305 310 315 320
 Phe Ile Asp Asn Glu Asn Gly Lys Ile Ala Gln Val Asp Cys Ser Ser
 325 330 335
 Gln Glu Ser Leu Cys Ser Lys
 340

<210> 25

<211> 2209

<212> DNA

<213> Spodoptera frugiperda

<400> 25

gcacgagata	agttgtgcca	tctcattaaa	aaatagtcgg	tgtttttata	gtttttaaat	60
taagtagaat	ataatcaaca	caatgttaca	cacctatttc	ctgggtattt	tattgtgtgt	120
ggggtctggg	ttggcggtgt	atgactccag	ttcggacgtg	gtggacctga	cacccgacaa	180
tttctatcaa	ctagtcacag	atagagatga	tgtatgggtg	gtggaattct	acgcgccgtg	240
gtgcggtcac	tgcaagaact	tggtgcctga	atacaagaaa	gcggccaaag	ctctgaaggg	300
tattgttaaa	gtgggagcta	tagacgcaga	caagcacaga	agcttcgcaa	aggactatgg	360
agtgctctggc	ttcccacaaa	ttaagatctt	tacgggtcgt	aaacatgttc	catacaaggg	420
cgcaagggtca	gctgatgctt	tcgttgatgc	tgctctaagt	gcagtgaaga	gcaaggctta	480
tgagagactt	ggaaagagat	ccgatgactc	atcacacaag	tcatccgact	ctgacgtgat	540
cacgctgaca	gacgacaact	tcaagaaact	ggtgttggac	agcgatgacc	tgtggttggg	600
ggagttcttc	gccccatggg	gcggacactg	caagaacctc	gagccacact	gggctaaggc	660
agctactgaa	cttaagggca	aggtgaaagt	gggagctctc	gacgctactg	ttcaccagga	720
gatggcaggc	cgcttccaag	tccaaggcta	cccaaccatc	aagtacttcc	catcaggcaa	780
gaagacctac	gactctgctg	aggactacaa	cggaggcagg	acatccagtg	acatcggtgc	840
attcgccctc	gaaaagctgg	ctgagaatgt	acccgctcct	gagattattc	aggttgtcaa	900

```

cgaagcaaca atgcaggcgt gcagcgagaa gccgctgtgt gtgggtatcgg tgctgcctca 960
catcttcgac tgtaacgcgg cctgccgcaa cgactacctc gccatactcg cccgtctcgg 1020
agacaagtac aagaacaaga tgtgggggatg ggtatgggct gaagctgggtg cccaacttgg 1080
tctagaagag tctctggaac tcggcggcctt cggctacccc gccatggctg tggtaacgc 1140
taagaaactc aagttctcaa cacttagggg atccttctcc gaaactggta tcaacgagtt 1200
ccttagggac ctgtcattcg gtgcgggcca gactgcgcca gtcagaggcg ctgagatgcc 1260
caagatagtg tcgacagacg cttgggacgg caaggacggt gaactgcccc aggaagagga 1320
catagacctg tcagacgtgg accttgagaa ggacgagtta taagtgcata cagtacttta 1380
gacaagtgtg gctaggcggg aatgtccttt gtactgagat caacactcaa tacatactaa 1440
aaaaaacatc ataaaagtta tttaatcact tctagaaggt tccaaagcct agctacgcta 1500
caagatactc gtatctcata aactgtacca gtgggtgagt gcatttgaag ttatctgcag 1560
tgagaacaca caaataaatt catatcaaat ctctgcttta tagaaagatt gcagggttcga 1620
gcatttggtc atagtatttt attgaagtga gccagtgaat agttttactg ttagataaat 1680
taatatgtaa catagtttga tactatgctg caatacagga actattttatt ccaagctgga 1740
ttaagcatga gttagggctc gtacgaaat ctacaggcca aaataattaa ggcaatgggtg 1800
attttagtta tgcaatttct actctagcta catggttaat ccagccctga ttccatcaca 1860
tgttatttac tttattttct gatataatta gcgtatctga agatcacaaa ttgaaaacgt 1920
aatgtttgga gctgataagc tcggctctag cgagcaatgt aatgactgat gtttctgaat 1980
cattttactt attatttctt attcatgata caaaatataa aaggactgat acctttttta 2040
gttaatgatt ggaattaatt ctccatcagc cattcttcca ataataattg ttagcgtgg 2100
taagctttta ttgtaatcgt tgtgaggaga tattttggat aattttatga attgtaaaat 2160
cgctaataaa tttttaatat aattaagtca aaaaaaaaaa aaaaaaaaaa 2209

```

<210> 26

<211> 426

<212> PRT

<213> Spodoptera frugiperda

<400> 26

```

Met Leu His Thr Tyr Phe Leu Gly Ile Leu Leu Cys Val Gly Ser Gly
 1             5             10             15

Leu Ala Leu Tyr Asp Ser Ser Ser Asp Val Val Asp Leu Thr Pro Asp
      20             25             30

Asn Phe Tyr Gln Leu Val Thr Asp Arg Asp Asp Val Trp Leu Val Glu
      35             40             45

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

Lys Lys Ala Ala Lys Ala Leu Lys Gly Ile Val Lys Val Gly Ala Ile
      65             70             75             80

Asp Ala Asp Lys His Arg Ser Phe Ala Lys Asp Tyr Gly Val Ser Gly
      85             90             95

Phe Pro Thr Ile Lys Ile Phe Thr Gly Arg Lys His Val Pro Tyr Lys
      100            105            110

Gly Ala Arg Ser Ala Asp Ala Phe Val Asp Ala Ala Leu Ser Ala Val
      115            120            125

Lys Ser Lys Ala Tyr Glu Arg Leu Gly Lys Arg Ser Asp Asp Ser Ser
      130            135            140

His Lys Ser Ser Asp Ser Asp Val Ile Thr Leu Thr Asp Asp Asn Phe
      145            150            155            160

Lys Lys Leu Val Leu Asp Ser Asp Asp Leu Trp Leu Val Glu Phe Phe
      165            170            175

Ala Pro Trp Cys Gly His Cys Lys Asn Leu Glu Pro His Trp Ala Lys
      180            185            190

```

Ala Ala Thr Glu Leu Lys Gly Lys Val Lys Val Gly Ala Leu Asp Ala
195 200 205

Thr Val His Gln Glu Met Ala Gly Arg Phe Gln Val Gln Gly Tyr Pro
210 215 220

Thr Ile Lys Tyr Phe Pro Ser Gly Lys Lys Thr Tyr Asp Ser Ala Glu
225 230 235 240

Asp Tyr Asn Gly Gly Arg Thr Ser Ser Asp Ile Val Ser Phe Ala Leu
245 250 255

Glu Lys Leu Ala Glu Asn Val Pro Ala Pro Glu Ile Ile Gln Val Val
260 265 270

Asn Glu Ala Thr Met Gln Ala Cys Ser Glu Lys Pro Leu Cys Val Val
275 280 285

Ser Val Leu Pro His Ile Phe Asp Cys Asn Ala Ala Cys Arg Asn Asp
290 295 300

Tyr Leu Ala Ile Leu Ala Arg Leu Gly Asp Lys Tyr Lys Asn Lys Met
305 310 315 320

Trp Gly Trp Val Trp Ala Glu Ala Gly Ala Gln Leu Gly Leu Glu Glu
325 330 335

Ser Leu Glu Leu Gly Gly Phe Gly Tyr Pro Ala Met Ala Val Val Asn
340 345 350

Ala Lys Lys Leu Lys Phe Ser Thr Leu Arg Gly Ser Phe Ser Glu Thr
355 360 365

Gly Ile Asn Glu Phe Leu Arg Asp Leu Ser Phe Gly Arg Gly Gln Thr
370 375 380

Ala Pro Val Arg Gly Ala Glu Met Pro Lys Ile Val Ser Thr Asp Ala
385 390 395 400

Trp Asp Gly Lys Asp Gly Glu Leu Pro Gln Glu Glu Asp Ile Asp Leu
405 410 415

Ser Asp Val Asp Leu Glu Lys Asp Glu Leu
420 425

<210> 27
<211> 496
<212> PRT
<213> *Drosophila melanogaster*

<400> 27
Met Lys Phe Leu Ile Cys Ala Leu Phe Leu Ala Ala Ser Tyr Val Ala
1 5 10 15

Ala Ser Ala Glu Ala Glu Val Lys Val Glu Glu Gly Val Leu Val Ala
20 25 30

Thr Val Asp Asn Phe Lys Gln Leu Ile Ala Asp Asn Glu Phe Val Leu
35 40 45

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

Glu Tyr Ala Lys Ala Ala Gln Gln Leu Ala Glu Lys Glu Ser Pro Ile
65 70 75 80

Lys Leu Ala Lys Val Asp Ala Thr Val Glu Gly Glu Leu Ala Glu Gln
 85 90 95
 Tyr Ala Val Arg Gly Tyr Pro Thr Leu Lys Phe Phe Arg Ser Gly Ser
 100 105 110
 Pro Val Glu Tyr Ser Gly Gly Arg Gln Ala Ala Asp Ile Ile Ala Trp
 115 120 125
 Val Thr Lys Lys Thr Gly Pro Pro Ala Lys Asp Leu Thr Ser Val Ala
 130 135 140
 Asp Ala Glu Gln Phe Leu Lys Asp Asn Glu Ile Ala Ile Ile Gly Phe
 145 150 155 160
 Phe Lys Asp Leu Glu Ser Glu Glu Ala Lys Thr Phe Thr Lys Val Ala
 165 170 175
 Asn Ala Leu Asp Ser Phe Val Phe Gly Val Ser Ser Asn Ala Asp Val
 180 185 190
 Ile Ala Lys Tyr Glu Ala Lys Asp Asn Gly Val Val Leu Phe Lys Pro
 195 200 205
 Phe Asp Asp Lys Lys Ser Val Phe Glu Gly Glu Leu Asn Glu Glu Asn
 210 215 220
 Leu Lys Lys Phe Ala Gln Val Gln Ser Leu Pro Leu Ile Val Asp Phe
 225 230 235 240
 Asn His Glu Ser Ala Ser Lys Ile Phe Gly Gly Ser Ile Lys Ser His
 245 250 255
 Leu Leu Phe Phe Val Ser Arg Glu Gly Gly His Ile Glu Lys Tyr Val
 260 265 270
 Asp Pro Leu Lys Glu Ile Ala Lys Lys Tyr Arg Asp Asp Ile Leu Phe
 275 280 285
 Val Thr Ile Ser Ser Asp Glu Glu Asp His Thr Arg Ile Phe Glu Phe
 290 295 300
 Phe Gly Met Asn Lys Glu Glu Val Pro Thr Ile Arg Leu Ile Lys Leu
 305 310 315 320
 Glu Glu Asp Met Ala Lys Tyr Lys Pro Glu Ser Asp Asp Leu Ser Ala
 325 330 335
 Glu Thr Ile Glu Ala Phe Leu Lys Lys Phe Leu Asp Gly Lys Leu Lys
 340 345 350
 Gln His Leu Leu Ser Gln Glu Leu Pro Glu Asp Trp Asp Lys Asn Pro
 355 360 365
 Val Lys Val Leu Val Ser Ser Asn Phe Glu Ser Val Ala Leu Asp Lys
 370 375 380
 Ser Lys Ser Val Leu Val Glu Phe Tyr Ala Pro Trp Cys Gly His Cys
 385 390 395 400
 Lys Gln Leu Ala Pro Ile Tyr Asp Gln Leu Ala Glu Lys Tyr Lys Asp
 405 410 415
 Asn Glu Asp Ile Val Ile Ala Lys Met Asp Ser Thr Ala Asn Glu Leu
 420 425 430

Glu Ser Ile Lys Ile Ser Ser Phe Pro Thr Ile Lys Tyr Phe Arg Lys
 435 440 445

Glu Asp Asn Lys Val Ile Asp Phe Asn Leu Asp Arg Thr Leu Asp Asp
 450 455 460

Phe Val Lys Phe Leu Asp Ala Asn Gly Glu Val Ala Asp Ser Glu Pro
 465 470 475 480

Val Glu Glu Thr Glu Glu Glu Glu Ala Pro Lys Lys Asp Glu Leu
 485 490 495

<210> 28

<211> 515

<212> PRT

<213> Gallus gallus

<400> 28

Met Ala Val Val Arg Val Arg Ala Ile Val Ala Leu Leu Cys Leu Val
 -1 5 10 15

Ala Ala Leu Gly Leu Ala Glu Pro Leu Glu Glu Glu Asp Gly Val Leu
 20 25 30

Val Leu Arg Ala Ala Asn Phe Glu Gln Ala Leu Ala Ala His Arg His
 35 40 45

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

Ala Pro Glu Tyr Ala Lys Ala Ala Ala Gln Leu Lys Ala Glu Gly Ser
 65 70 75 80

Glu Ile Arg Leu Ala Lys Val Asp Ala Thr Glu Glu Ala Glu Leu Ala
 85 90 95

Gln Gln Phe Gly Val Arg Gly Tyr Pro Thr Ile Lys Phe Phe Arg Asn
 100 105 110

Gly Asp Lys Ala Ala Pro Arg Glu Tyr Thr Ala Gly Arg Glu Ala Asp
 115 120 125

Asp Ile Val Ser Trp Leu Lys Lys Arg Thr Gly Pro Ala Ala Thr Thr
 130 135 140

Leu Thr Asp Ala Ala Ala Ala Glu Thr Leu Val Asp Ser Ser Glu Val
 145 150 155 160

Val Val Ile Gly Phe Phe Lys Asp Val Thr Ser Asp Ala Ala Lys Glu
 165 170 175

Phe Leu Leu Ala Ala Glu Ser Val Asp Asp Ile Pro Phe Gly Ile Ser
 180 185 190

Ser Ser Ala Asp Val Phe Ser Lys Tyr Gln Leu Ser Gln Asp Gly Val
 195 200 205

Val Leu Phe Lys Lys Phe Asp Glu Gly Arg Asn Asn Phe Glu Gly Asp
 210 215 220

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

Leu Val Ile Glu Phe Thr Glu Gln Thr Ala Pro Lys Ile Phe Gly Gly
 245 250 255

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

Asp Glu Leu
 515

<210> 29
 <211> 489
 <212> PRT
 <213> *Drosophila melanogaster*

<400> 29
 Met Met Trp Arg Leu Ala Gly Val Leu Leu Leu Gly Phe Ile Ala Ile
 1 5 10 15
 Ser Ser Gly Ala Asp Glu Asp Val Leu Glu Leu Gly Asp Asp Asp Phe
 20 25 30
 Ala Thr Thr Leu Lys Gln His Glu Thr Thr Leu Val Met Phe Tyr Ala
 35 40 45

Pro Trp Cys Gly His Cys Lys Arg Leu Lys Pro Glu Tyr Ala Lys Ala
 50 55 60
 Ala Glu Ile Val Lys Asp Asp Pro Pro Ile Lys Leu Ala Lys Val
 65 70 75 80
 Asp Cys Thr Glu Ala Gly Lys Glu Thr Cys Ser Lys Tyr Ser Val Ser
 85 90 95
 Gly Tyr Pro Thr Leu Lys Ile Phe Arg Gln Asp Glu Val Ser Gln Asp
 100 105 110
 Tyr Asn Gly Pro Arg Asp Ser Ser Gly Ile Ala Lys Tyr Met Arg Ala
 115 120 125
 Gln Val Gly Pro Ala Ser Lys Thr Val Arg Thr Val Ala Glu Leu Lys
 130 135 140
 Lys Phe Leu Asp Thr Lys Asp Thr Thr Leu Phe Gly Tyr Phe Ser Asp
 145 150 155 160
 Ser Asp Ser Lys Leu Ala Lys Ile Phe Leu Lys Phe Ala Asp Lys Asn
 165 170 175
 Arg Glu Lys Tyr Arg Phe Gly His Ser Ser Glu Lys Glu Val Leu Asp
 180 185 190
 Lys Gln Gly Glu Thr Asp Lys Ile Val Leu Ile Arg Ala Pro His Leu
 195 200 205
 Ser Asn Lys Phe Glu Ser Ser Ser Ile Lys Phe Glu Gly Ser Ser Glu
 210 215 220
 Ser Asp Leu Ser Thr Phe Val Lys Glu Asn Phe His Gly Leu Val Gly
 225 230 235 240
 His Arg Thr Gln Asp Ser Val Lys Asp Phe Gln Asn Pro Leu Ile Thr
 245 250 255
 Ala Tyr Tyr Ser Val Asp Tyr Gln Lys Asn Pro Lys Gly Thr Asn Tyr
 260 265 270
 Trp Arg Asn Arg Val Leu Lys Val Ala Lys Glu Phe Val Gly Gln Ile
 275 280 285
 Asn Phe Ala Ile Ala Ser Lys Asp Asp Phe Gln His Glu Leu Asn Glu
 290 295 300
 Tyr Gly Tyr Asp Phe Val Gly Asp Lys Pro Val Val Leu Ala Arg Asp
 305 310 315 320
 Glu Lys Asn Leu Lys Tyr Ala Leu Lys Asp Glu Phe Ser Val Glu Asn
 325 330 335
 Leu Gln Asp Phe Val Glu Lys Leu Leu Ala Asn Glu Leu Glu Pro Tyr
 340 345 350
 Ile Lys Ser Glu Pro Ile Pro Glu Ser Asn Asp Ala Pro Val Lys Val
 355 360 365
 Ala Val Ala Lys Asn Phe Asp Asp Leu Val Ile Asn Asn Gly Lys Asp
 370 375 380
 Thr Leu Ile Glu Phe Tyr Ala Pro Trp Cys Gly His Cys Lys Lys Leu
 385 390 395 400

Thr Pro Ile Tyr Glu Glu Leu Ala Gln Lys Leu Gln Asp Glu Asp Val
 405 410 415
 Ala Ile Val Lys Met Asp Ala Thr Ala Asn Asp Val Pro Pro Glu Phe
 420 425 430
 Asn Val Arg Gly Phe Pro Thr Leu Phe Trp Leu Pro Lys Asp Ala Lys
 435 440 445
 Asn Lys Pro Val Ser Tyr Asn Gly Gly Arg Glu Val Asp Asp Phe Leu
 450 455 460
 Lys Tyr Ile Ala Lys Glu Ala Thr Thr Glu Leu Lys Gly Phe Asp Arg
 465 470 475 480
 Ser Gly Lys Pro Lys Lys Thr Glu Leu
 485

<210> 30
 <211> 508
 <212> PRT
 <213> Homo sapiens

<400> 30
 Met Leu Arg Arg Ala Leu Leu Cys Leu Ala Val Ala Ala Leu Val Arg
 1 5 10 15
 Ala Asp Ala Pro Glu Glu Glu Asp His Val Leu Val Leu Arg Lys Ser
 20 25 30
 Asn Phe Ala Glu Ala Leu Ala Ala His Lys Tyr Leu Leu Val Glu Phe
 35 40 45
 Tyr Ala Pro Trp Cys Gly His Cys Lys Ala Leu Ala Pro Glu Tyr Ala
 50 55 60
 Lys Ala Ala Gly Lys Leu Lys Ala Glu Gly Ser Glu Ile Arg Leu Ala
 65 70 75 80
 Lys Val Asp Ala Thr Glu Glu Ser Asp Leu Ala Gln Gln Tyr Gly Val
 85 90 95
 Arg Gly Tyr Pro Thr Ile Lys Phe Phe Arg Asn Gly Asp Thr Ala Ser
 100 105 110
 Pro Lys Glu Tyr Thr Ala Gly Arg Glu Ala Asp Asp Ile Val Asn Trp
 115 120 125
 Leu Lys Lys Arg Thr Gly Pro Ala Ala Thr Thr Leu Pro Asp Gly Ala
 130 135 140
 Ala Ala Glu Ser Leu Val Glu Ser Ser Glu Val Ala Val Ile Gly Phe
 145 150 155 160
 Phe Lys Asp Val Glu Ser Asp Ser Ala Lys Gln Phe Leu Gln Ala Ala
 165 170 175
 Glu Ala Ile Asp Asp Ile Pro Phe Gly Ile Thr Ser Asn Ser Asp Val
 180 185 190
 Phe Ser Lys Tyr Gln Leu Asp Lys Asp Gly Val Val Leu Phe Lys Lys
 195 200 205
 Phe Asp Glu Gly Arg Asn Asn Phe Glu Gly Glu Val Thr Lys Glu Asn
 210 215 220

Leu Leu Asp Phe Ile Lys His Asn Gln Leu Pro Leu Val Ile Glu Phe
 225 230 235 240
 Thr Glu Gln Thr Ala Pro Lys Ile Phe Gly Gly Glu Ile Lys Thr His
 245 250 255
 Ile Leu Leu Phe Leu Pro Lys Ser Val Ser Asp Tyr Asp Gly Lys Leu
 260 265 270
 Ser Asn Phe Lys Thr Ala Ala Glu Ser Phe Lys Gly Lys Ile Leu Phe
 275 280 285
 Ile Phe Ile Asp Ser Asp His Thr Asp Asn Gln Arg Ile Leu Glu Phe
 290 295 300
 Phe Gly Leu Lys Lys Glu Glu Cys Pro Ala Val Arg Leu Ile Thr Leu
 305 310 315 320
 Glu Glu Glu Met Thr Lys Tyr Lys Pro Glu Ser Glu Glu Leu Thr Ala
 325 330 335
 Glu Arg Ile Thr Glu Phe Cys His Arg Phe Leu Glu Gly Lys Ile Lys
 340 345 350
 Pro His Leu Met Ser Gln Glu Leu Pro Glu Asp Trp Asp Lys Gln Pro
 355 360 365
 Val Lys Val Leu Val Gly Lys Asn Phe Glu Asp Val Ala Phe Asp Glu
 370 375 380
 Lys Lys Asn Val Phe Val Glu Phe Tyr Ala Pro Trp Cys Gly His Cys
 385 390 395 400
 Lys Gln Leu Ala Pro Ile Trp Asp Lys Leu Gly Glu Thr Tyr Lys Asp
 405 410 415
 His Glu Asn Ile Val Ile Ala Lys Met Asp Ser Thr Ala Asn Glu Val
 420 425 430
 Glu Ala Val Lys Val His Ser Phe Pro Thr Leu Lys Phe Phe Pro Ala
 435 440 445
 Ser Ala Asp Arg Thr Val Ile Asp Tyr Asn Gly Glu Arg Thr Leu Asp
 450 455 460
 Gly Phe Lys Lys Phe Leu Glu Ser Gly Gly Gln Asp Gly Ala Gly Asp
 465 470 475 480
 Asp Asp Asp Leu Glu Asp Leu Glu Glu Ala Glu Glu Pro Asp Met Glu
 485 490 495
 Glu Asp Asp Asp Gln Lys Ala Val Lys Asp Glu Leu
 500 505

<210> 31
 <211> 364
 <212> PRT
 <213> Medicago sativa

<400> 31
 Met Lys Met Glu Met His Gln Ile Trp Ser Arg Ile Ala Leu Ala Ser
 1 5 10 15
 Phe Ala Phe Ala Ile Leu Phe Val Ser Val Ser Ala Asp Asp Val Val
 20 25 30

Val Leu Thr Glu Glu Asn Phe Glu Lys Glu Val Gly His Asp Lys Gly
 35 40 45
 Ala Leu Val Glu Phe Tyr Ala Pro Trp Cys Gly His Cys Lys Lys Leu
 50 55 60
 Ala Pro Glu Tyr Glu Lys Leu Pro Asn Ser Phe Lys Lys Ala Lys Ser
 65 70 75 80
 Val Leu Ile Ala Lys Val Asp Cys Asp Glu His Lys Ser Val Cys Ser
 85 90 95
 Lys Tyr Gly Val Ser Gly Tyr Pro Thr Ile Gln Trp Phe Pro Lys Gly
 100 105 110
 Ser Leu Glu Pro Lys Lys Phe Glu Gly Pro Arg Thr Ala Glu Ser Leu
 115 120 125
 Ala Glu Phe Val Asn Thr Glu Gly Gly Thr Asn Val Lys Ile Ala Thr
 130 135 140
 Ala Pro Ser His Val Val Val Leu Thr Pro Glu Thr Phe Asn Glu Val
 145 150 155 160
 Val Leu Asp Gly Thr Lys Asp Val Leu Val Glu Phe Tyr Ala Pro Trp
 165 170 175
 Cys Gly His Cys Lys Ser Leu Ala Pro Ile Tyr Glu Lys Val Ala Ala
 180 185 190
 Val Phe Lys Ser Glu Asp Asp Val Val Ile Ala Asn Leu Asp Ala Asp
 195 200 205
 Lys Tyr Arg Asp Leu Ala Glu Lys Tyr Asp Val Ser Gly Phe Pro Thr
 210 215 220
 Leu Lys Phe Phe Pro Lys Gly Asn Lys Ala Gly Glu Asp Tyr Gly Gly
 225 230 235 240
 Gly Arg Asp Leu Asp Asp Phe Val Ala Phe Ile Asn Glu Lys Ser Gly
 245 250 255
 Thr Ser Arg Asp Ala Lys Gly Gln Leu Thr Ser Glu Ala Gly Ile Val
 260 265 270
 Glu Asp Leu Asp Glu Leu Val Lys Glu Phe Val Ala Ala Asn Asp Glu
 275 280 285
 Glu Lys Lys Ala Val Phe Ala Arg Ile Glu Glu Glu Val Lys Lys Leu
 290 295 300
 Glu Gly Ser Ala Ser Arg Tyr Gly Lys Ile Tyr Leu Lys Val Ser Lys
 305 310 315 320
 Lys Tyr Leu Glu Lys Gly Ser Asp Tyr Ala Lys Asn Glu Ile Gln Arg
 325 330 335
 Leu Glu Arg Leu Leu Glu Lys Ser Ile Ser Pro Ala Lys Ala Asp Glu
 340 345 350
 Leu Thr Leu Lys Lys Asn Ile Leu Ser Thr Tyr Ala
 355 360

<210> 32

<211> 433

<212> PRT

<213> *Drosophila melanogaster*

<400> 32

```

Met Arg Gln Leu Ala Ser Ile Leu Leu Leu Ala Phe Val Val Gly Ser
 1           5           10           15

Val Ser Ala Phe Tyr Ser Pro Ser Asp Gly Val Val Glu Leu Thr Pro
      20           25           30

Ser Asn Phe Asp Arg Glu Val Leu Lys Asp Asp Ala Ile Trp Val Val
      35           40           45

Glu Phe Tyr Ala Pro Trp Cys Gly His Cys Gln Ser Leu Val Pro Glu
      50           55           60

Tyr Lys Lys Leu Ala Lys Ala Leu Lys Gly Val Val Lys Val Gly Ser
      65           70           75           80

Val Asn Ala Asp Ala Asp Ser Thr Leu Ser Gly Gln Phe Gly Val Arg
      85           90           95

Gly Phe Pro Thr Ile Lys Ile Phe Gly Ala Asn Lys Lys Ser Pro Thr
      100          105          110

Asp Tyr Asn Gly Gln Arg Thr Ala Lys Ala Ile Ala Glu Ala Ala Leu
      115          120          125

Ala Glu Val Lys Lys Lys Val Gln Gly Val Leu Gly Gly Gly Gly Gly
      130          135          140

Ser Ser Ser Gly Gly Ser Gly Ser Ser Ser Gly Asp Asp Val Ile Glu
      145          150          155          160

Leu Thr Glu Asp Asn Phe Asp Lys Leu Val Leu Asn Ser Asp Asp Ile
      165          170          175

Trp Leu Val Glu Phe Phe Ala Pro Trp Cys Gly His Cys Lys Asn Leu
      180          185          190

Ala Pro Glu Trp Ala Lys Ala Ala Lys Glu Leu Lys Gly Lys Val Lys
      195          200          205

Leu Gly Ala Leu Asp Ala Thr Ala His Gln Ser Lys Ala Ala Glu Tyr
      210          215          220

Asn Val Arg Gly Tyr Pro Thr Ile Lys Phe Phe Pro Ala Gly Ser Lys
      225          230          235          240

Arg Ala Ser Asp Ala Gln Glu Tyr Asp Gly Gly Arg Thr Ala Ser Asp
      245          250          255

Ile Val Ser Trp Ala Ser Asp Lys His Val Ala Asn Val Pro Ala Pro
      260          265          270

Glu Leu Ile Glu Ile Ile Asn Glu Ser Thr Phe Glu Thr Ala Cys Glu
      275          280          285

Gly Lys Pro Leu Cys Val Val Ser Val Leu Pro His Ile Leu Asp Cys
      290          295          300

Asp Ala Lys Cys Arg Asn Lys Phe Leu Asp Thr Leu Arg Thr Leu Gly
      305          310          315          320

Glu Lys Phe Lys Gln Lys Gln Trp Gly Trp Ala Trp Ala Glu Gly Gly
      325          330          335

```

Gln Gln Leu Ala Leu Glu Glu Ser Leu Glu Val Gly Gly Phe Gly Tyr
340 345 350

Pro Ala Met Ala Val Val Asn Phe Lys Lys Met Lys Phe Ser Val Leu
355 360 365

Lys Gly Ser Phe Ser Lys Asp Gly Ile Asn Glu Phe Leu Arg Asp Ile
370 375 380

Ser Tyr Gly Arg Gly His Thr Ala Pro Val Arg Gly Ala Lys Lys Pro
385 390 395 400

Ala Ile Val Ser Val Asp Pro Trp Asp Gly Lys Asp Gly Gln Leu Pro
405 410 415

Thr Glu Glu Asp Ile Asp Leu Ser Asp Ile Asp Leu Asp Lys Asp Glu
420 425 430

Leu